

ISOLATION AND MOLECULAR DETECTION OF NEWCASTLE DISEASE VIRUS FROM AN IMPORTED MEAT SAMPLE

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SUMMARY

Infected poultry meat plays an important role in the spread of Newcastle Disease (ND). In this study, an imported meat product was found to be positive for ND by both virus isolation and molecular characterization. Analysis of the deduced amino acid sequences of the F protein cleavage site showed that the isolate was virulent as indicated by the sequence ¹¹²RRQKR¹¹⁶ for the C-terminus of the F2 protein and phenylalanine (F) at the N-terminus of the F1 protein, residue 117. Basic Local Alignment Search Tool (BLAST) analysis showed the isolate was 98% identical with China Hebei ND strain. Though the regulations for the importation of poultry meat for human consumption into Malaysia are strict, the possibility of the persistence of ND virus in imported meat is prevalent. Strict enforcement of importing regulations and screening the disease in imported poultry meat is important to ensure food safety and prevent introducing ND strain from other countries into Malaysia.

Keywords: Newcastle disease, virulent strain, meat product

INTRODUCTION

Newcastle Disease (ND) is a highly contagious disease in chicken where outbreaks can cause flock mortality up to 100% and has been one of the major causes of economic losses in the poultry industry (Aldous and Alexander, 2001). ND is caused by avian paramyxovirus serotype 1 (APMV-1) viruses, a member of genus Rubulavirus, sub-family *Paramyxovirinae*, family *Paramyxoviridae* (Aldous and Alexander, 2001). Methods of introduction and spread of ND virus (NDV) in poultry populations includes movement of live birds, people and equipment; infected poultry products; spread by wild birds and other animals such as flies, poultry endo and ecto-parasites, earthworms, coccidia and rodents; spread by air, water and manure; transmission through hatcheries and etc (Arzey, 1989). Presence of NDV in poultry product has been discussed by several authors and it was believed that infected poultry product was one of the causes of few ND outbreaks in the world (Arzey, 1989). Virulent ND has been detected in poultry meat when kept at low temperature (at -14°C to -20°C) for 270 days (Arzey, 1989). In this case, an imported meat product (boneless chicken breast) from China was submitted to Veterinary Research Institute Ipoh by Department of Veterinary Services of *Wilayah Persekutuan Labuan* for routine screening of Highly Pathogenic Avian Influenza (HPAI). NDV was isolated incidentally from the sample via blind passages. This case report describes isolation and molecular characterization of a virulent strain of NDV from an imported meat product and discussions from the aspect of government enforcement on importing poultry meat, virus persistence characteristic, food safety and potential risk of introducing Chinese NDV strain to Malaysian poultry.

MATERIALS AND METHODS

Specimen preparation and virus isolation

An imported meat product (boneless chicken breast) was submitted to Veterinary Research Institute Ipoh for routine screening of Highly Pathogenic Avian Influenza (HPAI). The specimen was homogenised and diluted 1:10 with tryptose phosphate buffer containing antibiotics prior centrifugation at 2500 g for 5 min at 4°C. The supernatant was collected and filtered through a 0.22 µm syringe filter. Virus isolation was attempted for HPAI virus by inoculating the filtrate sample into 9-11 days old embryonated SPF eggs via intra-allantoic route and incubated for three days at 37°C for two passages. The presence of the virus in the allantoic fluid was detected by Haemagglutination test (HA) (OIE, 2012 and 2014). The virus was then identified by Haemagglutination-Inhibition (HI) test using specific antiserum against HPAI and ND (OIE, 2012 and 2014).

Nucleic acid extraction and Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

For molecular detection and characterisation, the viral RNA was extracted from the infected allantoic fluid by phenol chloroform method using TRI LS Reagent (Molecular Research Centre, Inc) based on manufacturer's instruction. The total RNA was used as template in RT-PCR. RT-PCR was performed using SuperScript III One-Step RT-PCR System with Platinum Taq to amplify the partial matrix and fusion gene of NDV by using primer set MV1/B2 (Herczeg *et al.*, 1999). Primer set of MV1/NDVIR2 (Khairul *et al.*, 2008) was also used to determine the pathotype of the virus. In brief, the RT was carried out at 48°C for 30 min. The reaction mix was then subjected to 94°C for 5 min for initial denaturation, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 68°C for 1 min with a

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final extension for 5 min at 68°C. Non template control was used in RT-PCR to rule out cross contamination. The amplicon was then separated on a 1.5% agarose gel stained with SYBR Safe DNA gel stain.

Purification of PCR product and sequencing analysis

The amplicon generated by primer set of MV1/B2 was cut from the gel and purified using the QIAquick gel extraction kit (Qiagen) according to the manufacturer's instruction. The purified product was submitted to a company (First BASE Laboratories Sdn. Bhd., Malaysia) for sequencing to identify the isolate. Nucleotide sequence was assembled and analysed using Lasergene's SeqMan Pro software. Analysis of protein sequence was done by BioEdit Sequence Alignment Editor (Version 7.1.9). Nucleotide sequence of the isolate was checked and compared with published NDV sequences deposited in the GenBank database using a BLAST (Basic Local Alignment Search Tool) search via the National Center of Biotechnology Information (NCBI).

RESULTS

The isolate presented HA titers of 2048 and was positive for ND by HI test using antiserum against ND. The isolate shows negative result by HI test when tested with antiserum against HPAI. A PCR product of approximately 557 bp was amplified for common detection of NDV (Figure 1). An amplicon of 456bp was detected by using pathotypes determination primer set and the results indicated that the isolate was of a virulent ND strain (Figure 1). Analysis of the deduced amino acid sequences of the F protein cleavage site showed that the isolate was virulent with the presence of multiple basic amino acid sequence ¹¹²RRQKR¹¹⁶ for the C-terminus of the F2 protein and phenylalanine (F) at the N-terminus of the F1 protein, residue 117. The isolate was 98% similar with China Hebei ND genotype VIIId strain via BLAST analysis.

DISCUSSION

In this study, the NDV was isolated incidentally via blind passages during routine screening for HPAI. Both virus isolation, molecular detection and characterization showed that the isolate was NDV. The isolate was identified as virulent ND strain by ND pathotype determination primer set in RT-PCR and the presence of multiple basic amino acid sequence ¹¹²RRQKR¹¹⁶ at the F0 cleavage site. BLAST analysis showed the isolate was 98% identical with China Hebei ND genotype VIIId strain. The BLAST result was in agreement with the case history where the meat product (boneless chicken breast) was imported from China to *Wilayah Persekutuan Labuan*, Malaysia. According to Shohaimi *et al.* (2015), most NDV isolated in Malaysia were NDV genotype VIIId originating from China from year 2000 to 2009. There were no genotype VIIId strain isolated in Malaysia after 2009. In other words, the virulent NDV that was recovered from the imported poultry meat in this study posed a high risk for the potential introduction of this Chinese strain into Malaysian poultry. The finding in this study may highlight the importance of also screening ND in imported poultry meat, besides HPAI as ND is also a disease that has devastating effect on poultry industry worldwide. The importance of infected poultry meat in spreading the disease cannot be neglected as several ND outbreaks in the world caused by this factor have been reported. The original spread of ND in 1926 was believed to be associated with infected poultry carcasses and discarded offal (Arzey, 1989). ND outbreak in several provinces in Austria was also caused by imported frozen poultry (Arzey, 1989). Poultry meat was claim to be the source for the introduction and spread of ND in the United Kingdom in the 1940s to 1960s. 66% of the imported meat causes virus infection and the disease was spread as the poultry waste was use as poultry feed (Animal Health Australia, 2010). Therefore, despite HPAI, screening ND and may be other avian viral diseases is important as imported poultry meat can serves as a vehicle in importing diseases from other country.

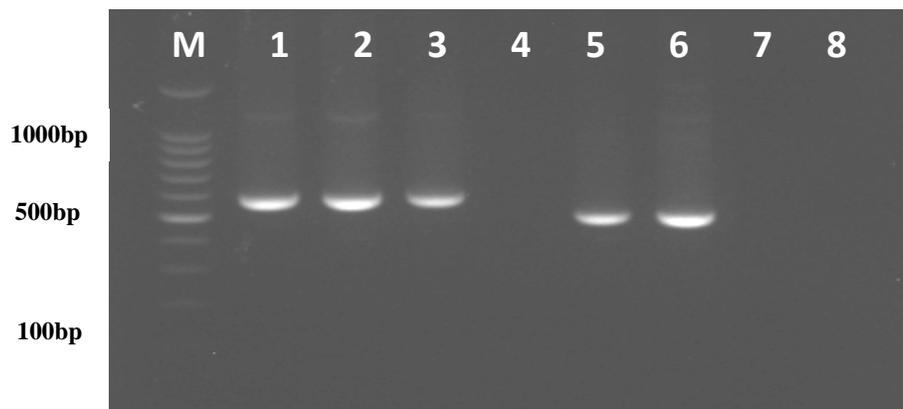


Figure 1. Molecular detection of the NDV isolate by agarose gel electrophoresis. Well 1-4 showed the PCR products for common detection of NDV whereas PCR products for NDV pathotype determination were showed in well 5-8. Well 1: ND Isolate; well 2: positive control (ND virulent strain); well 3: positive control (ND avirulent strain); well 4: non template control; well 5: ND isolate; well 6: positive control (ND virulent strain); well 7: positive control (ND avirulent strain); well 8: non template control, M: 100bp DNA ladder

Strict enforcement on the regulations for the importation of poultry meat for human consumption into Malaysia is also one of the key factors to make sure all imported poultry meat are free of disease. Hence, import risk analysis is important to assess the biosecurity risks associated with importing animals or animal products into Malaysia. Local authorities include Department of Veterinary Services, Section of Quarantine Services, Import and Export Section (SQIE) has primary responsibility for food safety of the imported product.

Despite the importance of import risk analysis, inspection at foreign abattoirs and processing plants that had requested to export meat and product of animal origin to Malaysia also serve as another important checkpoints along the food safety chain as poultry can be contaminated with pathogen during poultry slaughtering and processing. Raw poultry products have been reported to be the cause for certain cases of human food poisoning (Silva, 2013). Thus, controlling and reducing the contamination of the carcasses during poultry processing is important. This can be done by adoption of improved technology and strict hygiene measures (Silva, 2013). Silva (2013) suggested at least three separate sections should be included in the slaughtering facility: a live birds' area; a slaughtering area, including defeathering; and a processing area, starting with evisceration. Poultry product and carcasses should remain frozen throughout the marketing chain to reduce the risk of pathogen multiplying (Silva, 2013). Adopting the Hazard Analysis and Critical Control Points (HACCP) procedure and or Good Manufacturing Practices (GMP) at the farm, slaughter house, processing plants and export packing plants also helps to ensure the quality of final products.

In this case, the retrieval of NDV from the poultry meat were probably due to the persistence characteristic of the virus. According to Arzey (1989), virulent strains of ND, both velogenic and mesogenic ND have been reported to be found in slaughtered poultry. Survival of NDV for 96 days on the skin and 134 days in bone marrow of eviscerated and plucked carcasses at 2°C; 160 days on the skin and 196 days in bone marrow of eviscerated and unplucked carcasses at the same temperature and survival of a mesogenic strain of NDV on skin and meat for 270 days at -14°C to -20°C has been reported (Arzey, 1989). The recovery of the virulent strain of ND may also be due to the slaughtering practices in developed countries, for example in this case, China. There is a higher possibility for the virus to be preserved in infected carcasses as most poultry meat are kept freezing at slaughter (Alexander, 1988). Preservation of meat indirectly helps in virus preservation and survival period of virus in the infected meat has been reported for over 250 days at -14°C to -20°C (Alexander, 1988).

Food safety is one of the issues that has been discussed worldwide recently. Although human are susceptible to all pathotypes of NDV, including lentogenic vaccine strains (Ministry of Agriculture and Forestry, New Zealand, 1999), International Life Sciences Institute (ILSI) expert group (2009) reported that NDV contaminated food is not considered to be a risk for humans. However, the risk of food contamination can be reduced by thorough cooking of the meat product. It is

believed that NDV is relatively sensitive to heat and can be destroyed by cooking (Ministry of Agriculture and Forestry, New Zealand, March 1999). According to an import risk analysis carried out by Biosecurity Australia (Animal Health Australia, 2010), heating of chicken meat to a minimum core temperature at 70°C for at least 8 min and 12 sec is required to ensure the destruction of NDV. Alexander *et al.* (2004) conducted a study on heat inactivation of virulent NDV strain Herts 33/56 in artificially infected homogenised chicken meat reported that heating at 65°C for 2 min, 70°C for 82 sec, 74°C for 40 sec and 80°C for 29 sec reduced the virus titer in meat by 1 log₁₀ or 90%.

CONCLUSION

A virulent ND strain, where the nucleotide sequence is 98% identical with China Hebei ND genotype VIIId strain was isolated from imported poultry meat. Although NDV contaminated meat is not considered to be a risk for human health and the virus can be destroyed thoroughly by cooking; with the increase in international trade in poultry and poultry products, strict enforcement on regulations of importation of poultry products, import risk analysis, inspection at foreign abattoir and processing plants, and screening for the disease are important to ensure food safety and prevent foodborne illnesses as well as prevention of introducing ND strains from other countries into Malaysia.

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CONFLICT OF INTEREST

None of the authors have any potential conflicts of interest to declare.

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