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SHORT COMMUNICATION

ISOLATION OF AVIAN PNEUMOVIRUS IN BROILER CHICKENS IN MALAYSIA

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SUMMARY

A reverse-transcription polymerase chain reaction (RT-PCR), using oligonucleotide primer sets, was set up to detect the presence of avian pneumovirus (APV) associated with swollen head syndrome in broiler chickens. Three field isolates of APV, designated as AP4751/97, J4752/97 and J4753/97, were confirmed by virus isolation in trachea organ culture (TOC) and RT-PCR.

Keywords: Avian pneumovirus (APV), avian rhinotracheitis virus (ARTV), reverse-transcription polymerase chain reaction (RT-PCR).

Swollen head syndrome (SHS) has been associated with either avian pneumovirus (APV) or avian rhinotracheitis virus (ARTV) infection in the upper respiratory tract of chicken. In broilers, this syndrome was first reported in South Africa in 1978. The syndrome was later observed in broilers, broiler breeders and commercial layers in Western Europe, Northern Africa and Middle East (Cavanagh, 1992; Naylor and Jones, 1993). Diseases with similar symptoms have also been reported in chickens and quails in Malaysia (Lim *et al.,* 1994; Asiah *et al.,* 1997). However, the causative virus was not isolated, therefore, the relative importance of the disease in this country was unclear.

It is believed that infection of chickens with APV predisposes the birds to other respiratory infections. In chickens, APV produces clinical signs that are very similar to those produced by some strains of IBV, while both viruses can concurrently grow in chick embryo as well as in trachea organ culture (TOC). The IBV, however, grows more vigorously, making it difficult to isolate the APV *in vitro,* hence, it is difficult to ascertain a sure diagnosis of SHS.

The presence of APV can be demonstrated by a number of methods. The virus isolation in chicken or turkey TOC followed by virus neutralization test, the indirect immunofluorescence antibody (IFA) staining of trachea or nasal turbinate, and the amplification of viral gene using polymerase chain reaction (PCR) from oesophageal or tracheal (OT) samples have successfully been used (Li *et al.,* 1993; Naylor *et al.,* 1997). Demonstration of antibodies in sera collected from infected birds using either IFA staining, virus neutralization or enzyme-linked immunosorbent assay (ELISA), were also used as a tool to diagnose the infection (Cavanagh, 1992; Naylor and Jones, 1993). This paper confirms the presence of SHS in Malaysia by virus isolation and detection of the causative agent by a hemi-nested PCR.

Samples of trachea and head sinus swabs of chickens were collected from two field outbreaks in Kemaman, Terengganu (Table 1). Two sequential samples of ten days interval (J series) were collected from one of the outbreaks. The samples were suspended in phosphate-buffer-saline to give a 10% suspension (w/v) and filtered (0.45 and 0.22μm) before antibiotics were added. The filtered suspension was then inoculated into trachea organ culture and sub-passaged once (Cook *et al.,* 1976). The TOC ring and infected medium were used for RNA extraction using the guanidinium isothiocyanate method with modifications (Chomczynski and Sacchi, 1987). First strand cDNA synthesis was carried out using a 1μL of the RNA template and the reverse primer. The first and the inner hemi-nested PCR were performed according to Li *et al* (1993). The primers used were PCRF.lst with F.GRH for the first PCR and the inner PCR with PCRF.lst and AN17. The latter primer set flanked a 175bp product. A total of 35 cycles were used in a thermocycler (Perkin Elmer, USA). The amplification of cDNA was determined by electrophoresis of 10μL amplicons with 10% loading buffer in 2% agarose gel (Agarose type II: Metaphor in 2:5) in 1x TBE, stained with ethidium bromide and photographed under UV transillumination. Known APV viruses (PL21 strain, Rhone Poulenc, Malaysia; Solvay/Lintrachem, Malaysia) were used to validate

the PCR results. For the negative control, TOC was inoculated with the growth medium.

The hemi-nested PCR produced bands of 175bp from all samples examined, which conformed to that of the reference virus (Fig. 1). The J752/97 and J4753/97 isolates were recovered from the same farm but at different time. This indicated that the virus could persist for more than 10 days after the first recovery. It would be even longer if we consider the time when the clinical signs were first reported. In this case, the viruses were recovered at 22 and 32 days after the clinical signs were first observed. This finding was in agreement with other researchers who detected the virus by PCR from the oesophageal swabs for up to 35 days post-infection (Naylor *et al.*, 1997).

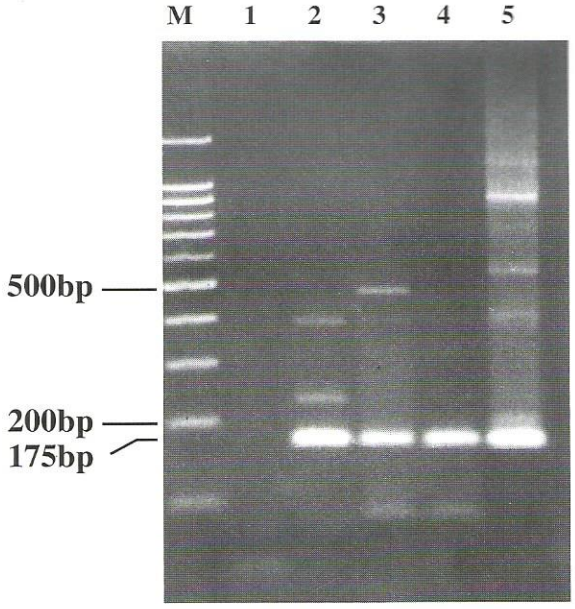

Fig. 1. Hemi-nested PCR diagnosis of avian pneumovirus isolated in trachea organ culture. Lanes M, Marker (100bp DNA ladder); 1, Negative control; 2, Isolate AP4751/97; 3, Isolate J4752/97; 4, Isolate J4753/97; 5, Positive control.

The technique used for diagnosis was very sensitive and specific since the nested PCR was targeted to flank sequences that were specific to the virus. With this advantage, it could then be explored to facilitate diagnosis of the disease (Li *et al.*, 1993). Thus, it is possible to submit OT swabs to the laboratory for diagnosis, either at ambient temperature or preserved in ice packs. PCR can then be performed within 48 hours upon receipt of the samples. While further works are required to characterise the virus, we are now attempting to investigate the prevalence of the virus and its impact in initiating or complicating the respiratory problems/disease in our poultry farms.

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RINGKASAN

PEMENCILAN PNEUMOVIRUS AVIAN DALAM AYAM PEDAGING DI MALAYSIA

Satu tindakbalas rangkaian polimerase pentranskripsian terbalik (RT-PCR) (RT-PCR) mengguna set pritner oligonukleotida telah diguna untuk mengesan avian pneumovirus (APV) yang terkait dengan sindrom kepala bengkak pada ayam pedaging. Tiga isolat APV dinamakan AP4751/97, J4752/97 dan J4753/97 telah disahkan melalui pemencilan virus dalam kultur organ trakea (TOC) dan RT-PCR.