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DETECTION OF AVIAN METAPNEUMOVIRUS (AMPV) FIELD INFECTION VIA REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) AND ELISA IN TWO LAYER FARMS IN JOHOR

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SUMMARY

Avian Metapneumovirus (AMPV) infection which is known as 'swollen head syndrome' has been shown to be prevalent in poultry farms in Malaysia. Two layer farms in Johor denoted as Farm A and Farm B, with previous history of AMPV disease outbreak, were the subjects used for the AMPV field investigation in this study. Thirty chicks from respective treatment groups were monitored at day old, two, four and six weeks of age for AMPV antibody and antigen detection. RT-PCR and ELISA serology indicate that at 2 weeks of age, AMPV field infection had occurred in Farm A. In Farm B, AMPV field infection or lateral spread of vaccine virus was observed as early as 2 weeks. AMPV seroconversion was generally observed at four weeks of age and AMPV subtypes A and B were detected via RT-PCR from both farms in this study. This is the first report of AMPV subtypes A and B by RT-PCR detection in Malaysia.

Keywords: AMPV, ELISA, RT-PCR, AMPV Subtype A and Subtype B Layer Chickens

INTRODUCTION

Avian Metapneumovirus (AMPV) infection previously known as Turkey rhinotracheitis (TRT), Swollen Head Syndrome (SHS) and Avian rhinotracheitis (ART) is directly or indirectly associated with a drop in both egg quality and production and has a significant economic impact on the poultry industry. Avian Pneumovirus was first documented as the causative agent of turkey or avian rhinotracheitis (TRT/ART) in turkeys in South Africa in 1978 (Buys and du Preez, 1980), confirmed in Europe in 1985 in turkeys (Giraud *et al.*, 1986) and subsequently in 1987 in the chicken population in South Africa and Europe (Picault *et al.*, 1987). Avian Metapneumovirus (AMPV) infects the upper respiratory tract of turkeys and chickens; the severity of AMPV infection is aggravated by secondary infections caused by other organisms. The characteristic 'swollen head syndrome' observed occurs as a result of secondary or adventitious bacterial infections with *E. coli* being found to be the most common causative agent (Droual and Woolcock, 1994).

Four subtypes are now recognised for AMPV, that is, sub-types A, B, C and D in chickens, turkeys and ducks (Baxter-Jones *et al.*, 1987; Juhasz and Easton, 1994; Collins & Gough , 1988; Senne *et al.*, 1997; Seal, 1998; Seal, 2000; Toquin *et al.*, 199; Bayon-Auboyer *et al.*, 2000). Variable clinical manifestations have been reported which include the swelling of the head and infraorbital sinuses, periocular oedema, rales, watery eyes, conjunctivitis, head shaking, torticollis, drop in both egg quality and production, and mortality. The virus is shed from the nares and trachea and the disease spreads rapidly. There is, however, no public health risks associated with AMPV infections in poultry.

In Malaysia, serological evidence of AMPV infection in broiler breeder chickens was first reported in 1994 (Lim *et al.*, 1994). Subsequently, a suspected clinical case of AMPV infection in quails was reported (Asiah *et al.*, 1997) and in 1998, AMPV was reported in broiler chickens (Jasni *et al.*, 1998; Ganapathy, 2007). Unpublished evidence of AMPV subtype B detection by reverse transcriptase-polymerase chain reaction (RT-PCR) has been reported by a private veterinary diagnostic laboratory in 2007 (Anon, 2007).

The objective of this study is to report serological findings and nested RT-PCR results from pullets in 2 multi-age layer farms with a previous history of APMV infection (Farms A and B). It is noted that Farm B carried out vaccination with an AMPV vaccine (Nemovac® Merial).

MATERIALS AND METHODS

Farm A is a multi-age layer farm comprising a total of 12 flocks and a total population of approximately 150,000 Hisex layers, previously described with outbreaks of

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Table 1: Seroprevalence of AMPV and detection of AMPV by RT-RCR in the unvaccinated layer pullets in Farm A

Age of layer pullets (weeks)	AMPV antibodies sero-prevalance(%)	RT-PCR				
		Oropharyngeal	Cloacal swab	Trachea swab	Turbinates	Lungs
0	26.7	-	-	-	-	-
2	0	+[A]	-	-	-	-
4	83.3	+[B]	-	-	-	-
6	96.6	+[B]	-	-	-	-

[A] AMPV Subtype A antigen detected by RT-PCR
[B] AMPV Subtype B antigen detected by RT-PCR

Table 2: Seroprevalence of AMPV and detection of AMPV by RT-RCR in the unvaccinated (UV) and vaccinated (Nemovac®) layer pullets in Farm B

Group	Age of layer pullets (weeks)	AMPV antibodies sero-prevalance (%)	RT-PCR				
			Oropharyngeal swab	Cloacal swab	Trachea	Turbinates	Lungs
Unvaccinated	0	30.0	-	-	-	-	-
	2	3.3	+[A]	-	-	-	-
	4	38.7	+[B]	-	-	-	+[A]
	6	33.3	+[B]	-	-	-	-
Nemovac® Vaccinated	0	30.0	-	-	-	-	-
	2	3.3	-	-	-	-	-
	4	35.0	-	-	-	-	-
	6	90.3	-	-	-	-	-

[A] AMPV Subtype A antigen detected by RT-PCR
[B] AMPV Subtype B antigen detected by RT-PCR

AMPV in 2006 and with no history of AMPV vaccination. Farm B is a multi-age layer farm comprising a total of 28 flocks and a total population of approximately 220,000 Hisex layers, previously described with outbreaks of AMPV in January 2006 and had initiated AMPV vaccination – Nemovac® (containing a sub-type B AMPV) in pullets at five to eleven days of age from July 2006.

Thirty sera from birds at day-old, two, four and six weeks of age were collected from both farms and tested using the Flock-Check® TRT Blocking ELISA kit (IDEXX™) for both Farms. In Farm B, thirty chicks, unvaccinated and vaccinated with Nemovac® reared in the same house but in different cages for the unvaccinated birds, were selected randomly and kept in separate cage to serve as unvaccinated control.

Ten dry oropharynx and cloacae swabs were collected, pooled in sets of 5 swabs/pool and tested for the presence of AMPV by RT-PCR as described by Ganapathy *et al.*(2005). Lung tissues, turbinates and trachea from two humanely sacrificed birds were collected from the same group of birds that were bled at day-old, two, four and six weeks of age and also tested for the presence of AMPV by RT-PCR.

The data were statistically analysed using Independent T-test, Chi-Square analysis and Kappa test using SPSS version 15 software.

RESULTS AND DISCUSSION

The results of the field study in relation to (a) seroprevalence and (b) detection of AMPV antigen are shown in Tables 1 and 2 and in Figures 1 and 2.

AMPV maternal antibodies were detected in day-old pullets, with sero-prevalence being 26.7% in farm A and 30.0% in Farm B. RT-PCR did not detect the presence of AMPV in day-old pullets.

At two weeks of age, unvaccinated birds in Farm A were sero-negative for AMPV antibodies whilst in Farm B, 3.3% of samples from both unvaccinated controls and

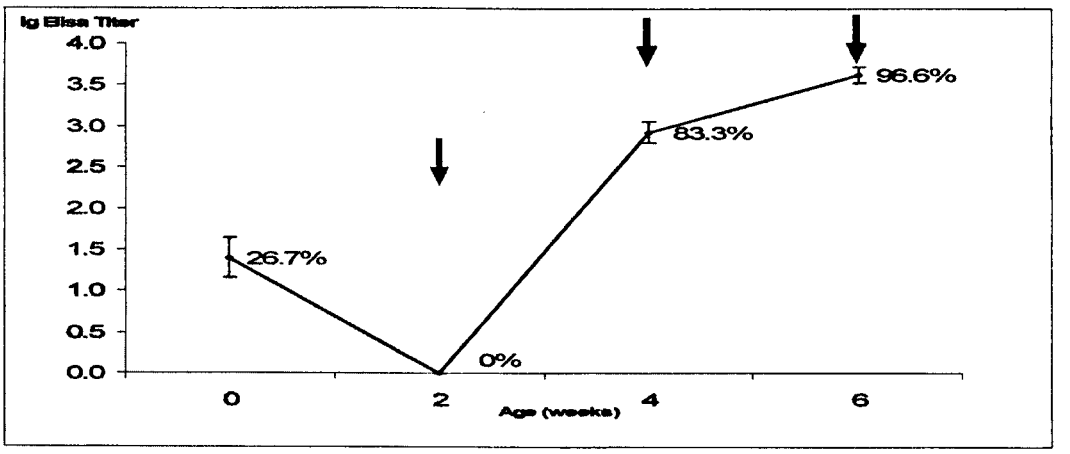

Figure 1: Sero-prevalence to AMPV and detection of AMPV by RT-RCR in layer pullets in Farm A

AMPV Subtype A detected AMPV Subtype B detected
Bars, I = *Mean* ± *Standard Error of Mean*

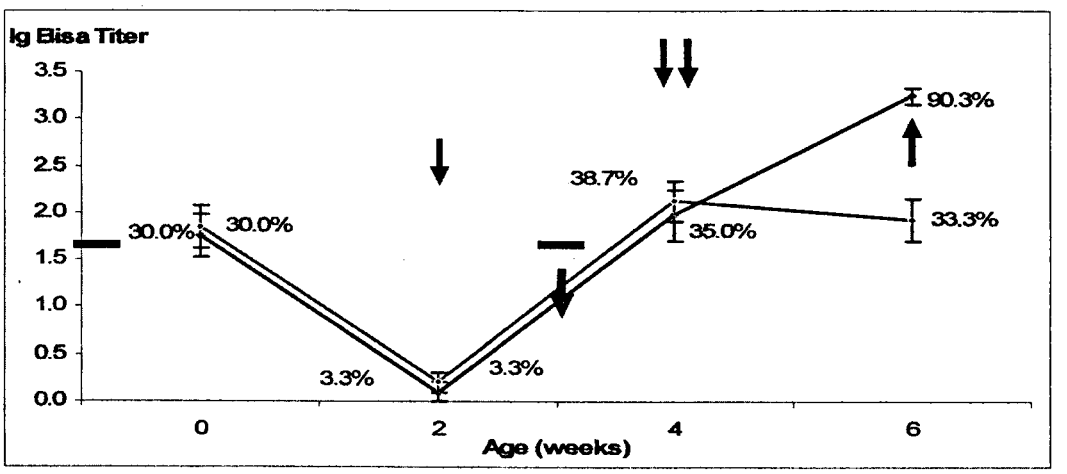

Figure 2: Sero-prevalence to AMPV and detection of AMPV by RT-RCR in unvaccinated and vaccinated layer pullets in Farm B

Vaccinated group Unvaccinated group
AMPV Subtype A detected AMPV Subtype B detected
Bars, I = *Mean* ± *Standard Error of Mean*

AMPV vaccinated groups were sero-positive for AMPV antibodies. The decline in sero-positives in Farm B can be primarily attributed to the decline in maternal antibodies and is consistent with the report by Ganapathy (2006). At two weeks old, AMPV subtype A was detected in oropharyngeaal swabs via RT-PCR in Farm A and from the unvaccinated controls in Farm B. RT-PCR is considered to be more sensitive than ELISA in early detection of AMPV field infection (Ganapathy, 2006).

At four weeks of age, 83.3% of the sampled pullets in Farm A were sero-positive and AMPV subtype B was detected in oropharyngeal swabs by RT-PCR. In Farm B, 35.0% of the vaccinated birds at four weeks of age were seropositive for AMPV antibodies. 38.7% of the unvaccinated control birds were AMPV sero-positive and AMPV subtypes A and B were detected in lung tissues and oropharyngeal swabs respectively by RT-PCR. As the ELISA test kit used detects both antibodies to AMPV subtypes A and B, it was not possible to confirm whether sero-conversion was due to field infection with AMPV subtypes A or/and B or a lateral infection of AMPV subtype B from the field vaccination with Nemovac®.

At six weeks, 96.6% of the sampled birds in Farm A were tested positive for presence of AMPV antibodies and AMPV subtype B was detected in the oropharyngeal swabs by RT-PCR. As no vaccination had been carried out in this farm, it is highly suggestive that the birds sero-converted in response to AMPV field infection. In Farm B, at six weeks of age, 33.3% of the samples from the unvaccinated controls were sero-positive for AMPV

antibodies whilst 90.3% of the vaccinated group was sero-positive. The higher antibody titer in vaccinated birds is most likely due to vaccination with Nemovac®. AMPV antigen (subtype B) was detected in the oropharyngeal swabs of the unvaccinated controls but not in vaccinated birds.

Sero-conversion in the two farms was demonstrated in the layer pullets by four weeks of age from natural infection (Farm A and B) and/or by vaccination (Farm B). AMPV antigen was not detected in the vaccinated birds and oropharyngeal swab was found to be more consistent for the detection of AMPV antigen in both Farms A and B. The frequency for AMPV subtype B detection by RT-PCR was much higher compared to detection of AMPV subtype A. This appears to be in agreement with the findings by Cavanagh *et al.* (1999) who reported that the AMPV subtype A strains grow less vigorously that the subtype B strain. It was not possible to assess the efficacy of vaccination in this study although there were good titres in the vaccinated compared to unvaccinated birds at six weeks of age in Farm B. RT-PCR is more sensitive than ELISA in early detection of AMPV field infection and this is the first report of AMPV subtypes A and B by RT-PCR detection in Malaysia.

The results of this study imply that more studies are required to describe the epidemiology of subtype A and B field infections or with vaccine virus in infected AMPV flocks.

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