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Epidemiological Studies of Avian Paramyxovirus Type 4 and 6 in Commercial Chicken Flocks in Korea

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ABSTRACT Avian paramyxovirus (APMV) type 4 and 6 were isolated during an avian influenza (AI) surveillance program of wild birds. This study also conducted experimental infection of wild-bird-origin APMV type 4 and 6 in specific pathogen free (SPF) chickens to study pathogenicity and transmission within domestic flocks. In addition, serological prevalence data of APMV type 4 and 6 in domestic fowls was conducted with chicken sera collected from 2007 to 2009 in order to understand infection status. The results of the animal experiment showed that APMV type 4 and 6 had the ability to infect chickens with sero-conversion and to transmit the virus from infected birds to contacted birds, but showed low pathogenicity. Serological tests revealed that APMV type 4 was widespread in the poultry industry, especially in layer flocks, but the positive rate for APMV type 6 was very low. This study concluded that wild bird-origin APMV type 4 and 6 could infect the chickens by inter-species transmission and the seroprevalence of APMV type 4 was quite high in Korean poultry. However, since almost all the chicken flocks had a high level of antibody titer against APMV type 1, there was possibility of cross reaction between APMV type 1 and 4, which made the interpretations more complicated. In order to understand infection status in the natural environment, additional study is necessary regarding the seroprevalence of APMV type 4 and 6 in the wild bird population.

(Key words : avian paramyxovirus type 4 and 6, experimental infection, inter-species transmission, seroprevalence, commercial chicken flocks)

INTRODUCTION

Avian paramyxovirus (APMV) belongs to the genus *Avulavirus* of the family Paramyxoviridae and is divided into ten serotypes (APMV type 1 to APMV type 10) by hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests (Alexander and Senne, 2008; Miller *et al*., 2010). Among these serotypes, APMV type 1 is considered an important serotype because it is known as a causative virus of Newcastle disease, which has caused significant economic loss in the poultry industry. Since the Yucaipa strain was isolated in 1956 from turkeys suffering from severe infectious laryngotracheitis and identified as APMV type 2 (Bankowski *et al*., 1968), eight other APMV serotypes have been reported and characterized biologically and molecularly (Alexander *et al*., 1983a; Chang *et al*., 2001; Jeon *et al*., 2008; Kessler *et al*., 1979; Kumara *et al*., 2008; Nayak *et al*., 2008; Nerome *et al*., 1978; Paldurai *et al*., 2009, Samuela *et al*., 2009; Shihmanter *et al*., 1998; Shortridge *et al*., 1980; Tumova *et al*., 1979; Zhang *et al*., 2006). APMV type 10 was the latest virus reported in 2010 from penguins (Miller *et al*., 2010).

All APMV isolates except APMV type 5 (Nerome *et al*., 1978) have been recovered from wild birds, which are recognized as a natural reservoir for avian influenza viruses (AIVs) and APMVs (Deibel *et al*., 1985; Hanson *et al*., 2005; Hinshaw *et al*., 1985; Hlinak *et al*., 2006; Tumova, 2003). Free-range birds, especially wild water birds, can act not only as a reservoir but also as a carrier that has the potential to infect domestic poultry (Hlinak *et al*., 2006).

For domestic flocks, isolates of APMV type 2, 3, 6 and 7 have caused respiratory diseases and/or a drop in egg production, especially in turkeys (Alexander, 1983a; Bankowski *et al*., 1995; Chang *et al*., 2001; Lipkind *et al*., 1995; Saif *et al*., 1997; Shortridge *et al*., 1980), whereas those with APMV

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type 4, 8 and 9 have rarely shown clinical signs (Alexander *et al*., 1983b; Stalknecht *et al*., 1991).

Surveillance programs on different APMV serotypes have been conducted worldwide on poultry farms, wild populations or both (Alkhalaf, 2009; Maldonado *et al*., 1994; Maldonado *et al*., 1995; Stanislawek *et al*., 2002; Warke *et al*., 2008a; Zhang *et al*., 2007). These investigations have been directed at prevalence in order to understand the status of infection in wild birds and domestic flocks and to better understand the role of wild birds in spreading the viruses or infecting domestic birds with the viruses (Hinshaw *et al*., 2003).

Since the outbreak of highly pathogenic AI occurred in Korea in 2003, intensive AI surveillance has been put in place for both poultry and wild birds and from these works, many low pathogenic AI viruses (AIVs) and APMVs were isolated. For APMVs other than APMV type 1, complete sequence analysis of APMV type 4 (Jeon *et al*., 2008) and genetic comparison of fourteen APMV type 4 isolates (Choi *et al*., 2013) were reported in Korea. However, with little reported on the prevalence of APMVs in wild and domestic birds, understanding the immune status of APMVs in Korea is limited.

In this study, wild-bird-origin APMV type 4 and 6 were studied biologically and serologically. Firstly, experimental infection was performed to evaluate the biological characteristics of the APMV isolates and to explore the possible transmission of APMVs between wild birds and domestic flocks. Secondly, a 2007 to 2009 serological survey of chicken flocks was examined to understand the infection status of domestic birds in Korea.

MATERIALS AND METHODS

1. Sample Collection from Wild Birds

The AI surveillance program conducted by the National Institute of Environmental Research (Incheon, Korea) captured wild birds with cannon nets or mist-netting guided by FAO (2007). Oropharyngeal swabs (or tracheal swabs) and cloacal swabs together with blood samples were collected from captured migratory birds, mainly from the order *Anseriformes* and *Charadriiformes*. In addition, environmental fecal samples were also obtained in wild bird habitats. The samples collected from the selected sites were packaged in a Styrofoam® container with chemical coolants and transferred to the Avian Disease Laboratory, College of Veterinary Medicine, Chungbuk National University (Cheongju, Korea) in a day.

2. Virus Isolation and Identification

1) Egg Inoculation and Virus Propagation

Clinical swabs or fecal samples were placed and vortexed in 0.01 M phosphate-buffered saline (PBS) and the fluids were centrifuged to sediment debris. Supernatants were placed at room temperature for one hour after adding antibiotics. Oropharyngeal swab and cloacal swab from each bird were pooled into one sample and five fecal samples were pooled into one sample for convenience and to save time. Pooled samples were inoculated into 9 to 11-day-old SPF embryonated chicken eggs via an allantoic route. After five-day incubation, allantoic fluids were harvested. For positive pooled samples, further inoculation was conducted with the original individual sample to clarify which was the positive sample.

2) Virus Identification

For each harvested fluid, hemagglutination (HA) assays were performed using a microtiter method in a v-shaped 96-well plate with 1% chicken red blood cells (RBCs). Viral ribonucleic acid from fluids with HA activity was extracted using Viral Gene-spin™ (iNtRON Biotechnology, Inc., Seongnam, Korea). The AI and ND virus were initially screened using are verse-transcriptase polymerase chain reaction (RT-PCR) with primers specific to a partial nucleocapsid gene of the AI virus, MMU19 (5'- AGA GCT CTT GTT CTC TGA TAG GTG-3') and MMU39 (5'-CAT CCC AGT GCT GGG AAR GAY CCT AAG AA-3') (Munch *et al*., 2001) and the haemagglutinin-neuraminidase gene of the ND virus, ComHNF (638-657) (5'- CAT CTG CAA CAG GGA GGG TA-3') and ComHNR (757-737) (5'- TMG AGC ACA GCA ATA CAC AAC-3') (Kwon *et al*., 2006). PCR was carried out in a 20 uL reaction mixture manufactured by Maxime™ in a RT-PCR PreMix Kit (iNtRON Biotechnology, Inc., Seongnam, Korea). The general condition for RT-PCR was 45℃ for 30 min (reverse transcription), 94℃ for 5 min, 35 cycles of 94℃ for 30 sec (denaturation), 58℃ (for AI virus) or 50℃ (for ND virus)

(annealing) for 30 sec, 72℃ for 1 min (extension), followed by 72℃ for 5 min (final extension).

HA-positive fluids without bacterial contamination and AI or ND virus were tested using a hemagglutination inhibition (HI) assay with a reference panel of antisera and antigens for 9 serotypes, except for APMV type 5, which was provided by the National Veterinary Service Laboratory (NVSL, Iowa, USA). The HI assay was done using a standard microtiter method (Thayer and Beard, 1998) using 4 HA units of antigens and 1% chicken RBCs.

In addition, PCR was conducted to verify laboratory isolates as APMV type 4 and 6. To design primers, accessible sequences of these viruses were compared to search for conserved regions that could be primer candidates. For APMV type 4, 508 nucleotides (nt 4493-4994) of partial F gene were amplified with forward primer APMV4f (5'- GAG GGG ATT AGG GGT GA -3') and reverse primer APMV4r (5'- ATG GCA GCA CAG GAC AA -3'). For APMV type 6, 525 nucleotides (nt 7154-7661) of a partial HN gene were amplified with forward primer APMV6f (5'- GGC AAC TYT ATA TGA GGG -3') and reverse primer APMV6r (5'- AGG TGG AAT GTA GGA ATG -3'). PCR was carried out in a 20 uL reaction mixture manufactured by Maxime^{TM} in a RT-PCR PreMix Kit (iNtRON Biotechnology, Inc., Seongnam, Korea). The general condition for RT-PCR was 45℃ for 30 min (reverse transcription), 94℃ for 5 min, 35 cycles of 94℃ for 30 sec (denaturation), 50℃ (for APMV type 4 and 6) (annealing) for 40 sec, 72℃ for 1 min (extension), followed by 72℃ for 5 min (final extension).

3. Challenge Experiment of the Isolates in the SPF Chickens

1) Viruses

APMV-4/Green-winged teal/KR/CBU5253/08 (CBU085253) for the APMV type 4 isolate and APMV-6/wild duck/KR/CBU2954/08 (CBU082954) for the APMV type 6 isolate were propagated in 9 to 11-day-old SPF embryonated chicken eggs for 5 days and harvested from the allantoic sac. The allantoic fluids were stored at −70℃ until the experiment. The 50% egg infectious dose of each virus was $10^{7.9}$/mL.

2) Pathogenicity Test

The intracerebral pathogenicity index (ICPI) in one-day-old chicks and mean death time (MDT) in 9 to 11-day-old embryonated eggs were measured for the pathogenicity of the CBU085253 isolate and the CBU082954 isolate. The test methods were according to the ways by Alexander (1998).

3) Experimental Infection

A challenge test was designed to evaluate clinical signs, virus reisolation and transmissibility of wild-bird-origin APMV type 4 and 6 when they infect chickens. Chickens were divided into five groups that were designated as the APMV type 4-infected group and the APMV type 4-contacted group, the APMV type 6-infected group and the APMV type 6-contacted group and the negative control group. Chickens were 1-day-old SPF chicks and the infected groups were inoculated with 0.1 mL of the allantoic fluid by an oronasal route. Infected birds and contacted birds were housed together in a positive-pressure HEPA-filtered isolator (Three-shine Inc., Kumsan, Korea) with feed and water *ad libitum* for 4 weeks. The chickens were observed daily for clinical signs. The chickens in the infected groups were necropsied after euthanasia for tissue sampling and the contacted birds were sampled by cloacal swabbing at 2, 4, 7, 10 and 28 days post inoculation (dpi). Blood sampling for serology was performed at 28 dpi for all the 5 groups by jugular vein puncture. Tissues and swabs were processed and inoculated into 9 to 11-day-old SPF embryonated chicken eggs to test the virus reisolation via the HA test.

This animal experiment was approved and permitted by the Institutional Animal Care and Use Committee at Chungbuk National University with a permission number of CBNUR-170-1001.

4. Serology

1) Antigenic Relatedness between Isolates and Reference Strains

A cross HI test was conducted to test antigenic relatedness between the reference stains and laboratory isolates of APMV type 4 and 6. The reference strains were PMV-4/duck/HK/D3/75 for APMV type 4 and PMV-6/duck/HK/D199/77 for APMV type 6 and their antisera were provided by NVSL.

The CBU085253 isolate, one of the APMV type 4 isolates in this laboratory was recovered from Green-winged Teal (*Anas crecca*) in 2008 and the CBU082954 isolate, an APMV 6 isolate in this laboratory was recovered from fecal samples of wild ducks in 2008. The antisera against these isolates were produced in this laboratory using SPF chickens. In addition, a La Sota strain (ChoongAng Vaccine Laboratories, Daejeon, Korea) as APMV type 1 was also tested for cross-reaction with other serotypes.

2) Genetic Comparisons for Partial Genes between Isolates and Reference Strains

For APMV type 4, 458 nucleotides (nt 4506-4963) of partial F gene in the laboratory isolate, CBU085253, were compared with those in the reference strain (APMV4/Duck/HK/D3/75). For APMV type 6, 463 nucleotides (nt 7175-7639) of a partial HN gene in the laboratory isolate, CBU082954, were compared with those in the reference strain (APMV6/Duck/HK/199/77). Sequencing was performed with Applied Biosystems Automatic Sequencer ABI 3730 xl and the sequencing system was ABI Prism® BioDye™ Terminator version 3.1. Sequence pairwise similarities of nucleotide and amino acid sequences were calculated with default values in the LALIGN program (http://www.ch.embnet.org/software/LALIGN_form.html).

3) Serum Samples

A total of about 1,700 sera from 205 domestic flocks were randomly selected from serum samples submitted for the purpose of regular monitoring of antibody titers or diagnosis of diseases to our laboratory from January 2007 to December 2009. The serum samples were from layers, breeders, and broilers flocks and divided into seven age classes considering the effect of maternal antibodies and growth or production stages (Lee *et al*., 2010).

To reduce non-specific reactions, chicken serum sam.

4) HI Assay

To prepare the antigens for the HI test, the laboratory strains, CBU085253 and CBU082954, were inactivated by the method mentioned in a previous study (Beard *et al*., 1975). A HI titer higher than 3 (log 2) was considered as positive with the antigen of 4 HAU based on the criteria provided by OIE (2009).

RESULTS

1. Virus Isolation from Wild Birds

During the wild bird surveillance campaign for AIVs in Korea, a total of 14,873 wild bird samples were collected and 137 hemagglutinating viruses were isolated and identified as 85 AIVs and 52 APMVs consisting of 44 APMV type 1, seven APMV type 4 and one APMV type 6. There were 20 isolates identified as more than one virus, of which 15 isolates were co-infected with AIV and APMV type 1 and five isolates were co-infected with APMV type 1 and APMV type 4.

In an effort to determine the appropriate isolate for the animal experiment and serology, one field strain, CBU085253, out of the seven APMV type 4 isolates was selected because the host avian species was verified and there was no co-infection.

2. Experimental Infection of APMV Type 4 and 6 in Domestic Chickens

1) MDT and ICPI

The MDT in the 9 to 11-day-old SPF embryonated eggs was 168 hours for both the CBU085253 and CBU082954 isolates. In addition, ICPIs in the one-day-old SPF chicks for CBU085253 and CBU082954 were zero and 0.03, respectively, meaning both strains were lentogenic for chickens.

2) Experimental Infection

Virus reisolation (Table 1) was performed five times for 28 days after virus inoculation and the results of each group were compared to those of other groups. Overall, the reisolation occurred from 2 dpi through 7 dpi and no virus was recovered after 10 dpi. The virus reisolation rate of the APMV type 6-infected group was higher than that of the APMV type 4-infected group and the negative control group did not show virus reisolation. In the APMV type 4-infected group, viruses were reisolated mainly from tracheas and cecal toncils and sometimes from kidneys and small intestines. However, in the APMV type 6-infected group, viruses were isolated mainly

from trachea, small intestine and cecal tonsil from 2 dpi through 7 dpi, isolated from lung and pancreas at 2 dpi, and from kidneys at 2 and 4 dpi. Sero-conversion (Table 1) occurred in all groups except the negative control group. HI titers of the APMV type 6 group were higher than those of the APMV type 4 group and the infected-groups had higher titer than the contacted groups. In addition, clinical signs for APMV type 4 and 6 infected groups were only mild diarrhea at 10 dpi from daily observation.

To test virus transmissibility, cloacal swabs were sampled from contacted-groups of each virus for virus reisolation. The APMV type 6-contacted group at 10 dpi was positive for viral reisolation with sero-conversion. However, APMV type 4-contacted groups did not show the virus reisolation, but had a

Table 1. Comparison of the virus reisolation rate from tissue or swab samples and HI titers of each group (infected and contacted group of APMV type 4, infected and contacted group of APMV type 6 and negative control group) at designated days

Group		Organ or Swab	Virus reisolation[a] at days post inoculation					HI titer[b]
			2	4	7	10	28	
APMV type 4	Infected	Trachea	0/5[c]	2/5	2/5	0/5	0/5	1.9 ± 0.8
		Lung	0/5	0/5	0/5	0/5	0/5	
		Kidney	0/5	0/5	1/5	0/5	0/5	
		Pancreas	0/5	0/5	0/5	0/5	0/5	
		Small intestine	0/5	1/5	1/5	0/5	0/5	
		Cecal tonsil	2/5	1/5	2/5	0/5	0/5	
	Contacted	Cloacal swab	0/2[d]	0/2	0/2	0/2	0/2	1.5 ± 0.8
APMV type 6	Infected	Trachea	5/5	4/5	2/5	0/5	0/5	3.7 ± 0.9
		Lung	3/5	0/5	0/5	0/5	0/5	
		Kidney	2/5	1/5	0/5	0/5	0/5	
		Pancreas	0/5	2/5	0/5	0/5	0/5	
		Small intestine	1/5	2/5	1/5	0/5	0/5	
		Cecal tonsil	3/5	3/5	1/5	0/5	0/5	
	Contacted	Cloacal swab	0/2	0/2	0/2	1/2	0/2	1.2 ± 0.6
Negative control (1XPBS[e])		Trachea	0/3	0/3	0/3	0/3	0/3	0[f]
		Lung	0/3	0/3	0/3	0/3	0/3	
		Kidney	0/3	0/3	0/3	0/3	0/3	
		Pancreas	0/3	0/3	0/3	0/3	0/3	
		Small intestine	0/3	0/3	0/3	0/3	0/3	
		Cecal tonsil	0/3	0/3	0/3	0/3	0/3	

[a] Number of positive / number of tested.
[b] The values show geometric mean HI titer ($\log_2$) ± standard deviation. The samples were collected from all tested groups at 28 dpi.
[c] Virus did not propagate in embryonated chicken eggs (ECEs), but viral genes were detected by polymerase chain reaction with primers designed specific for APMV type 4.
[d] Five pooled cloacal swabs were inoculated in ECEs to examine viral shedding. Number of positive samples / number of tested pooled samples.
[e] 0.01 M Phosphate buffered saline.
[f] The sera of control group were tested with both types of APMV antigens.

positive result for sero-conversion, which was evidence of infection by contact (Table 1).

3. Serology

1) Antigenic Relatedness between Reference Strains and Antigens for Serologic Tests

Antigenic relatedness was evaluated between the reference strains and laboratory isolates of respective serotypes by cross HI tests. In a homologous comparison, HI titers were 16 for La Sota, 32 for D3/HK, 32 for CBU085253, 256 for D199/HK and 16 for CBU082954. Cross HI titers were two or less between the reference strains and the isolates of the respective serotypes. In the heterologous comparison, cross reactions between APMV type 1 and other serotypes showed HI titers of 0 and the cross reactions between APMV type 4 and 6 showed HI titers of 0 or 2 (Table 2).

2) Similarity of Nucleotide and Deduced Amino Acid Sequence between Reference Strains and Antigens for Serologic Tests

For APMV type 4 between CBU085253 and D3/HK, the similarity of nucleotide sequences and predicted amino acid sequences were 91.5% and 98.0%, respectively. For APMV type 6 between CBU085253 and D199/HK, the similarity of nucleotide sequences and predicted amino acid sequences were 92.9% and 98.7%, respectively.

3) Serological Prevalence in Domestic Chicken Flocks

The positive rate was much higher in APMV type 4 at 21.5% in flock (25.4% in birds) than in APMV type 6 at 0% in flock (1.5% in birds) and yearly distributions of positive antibodies were consistent between/among different serotypes, breeds and age groups. The layer flocks had the highest positive rates against APMV type 4 and 6 and the positive results were continuously detected throughout the laying period (Table 3 and 4).

DISCUSSION

During the wild bird surveillance program for AI in Korea, a variety of haemagglutinating viruses were isolated and identified as AIV, APMV type 1, 4 and 6 and their prevalence was comparable to other reports (Deibel *et al*., 1985; Fujimoto *et al*., 2010; Hanson *et al*., 2005; Hinshaw *et al*., 1985; Hlinak *et al*., 2006; Shortridge *et al*., 1980; Stalknecht *et al*., 1991; Stanislawek *et al*., 2002; Tumova, 2003). In addition, there was evidence of co-infection found in 15 viruses in this study, which were reported in the other studies including co-infected isolates with different subtypes of AIVs (Sharp *et al*., 1997), with AIV and APMV type 1 (Dormitorio, 2009),

Table 2. Antigenic relatedness using cross HI tests between reference strains and APMV isolates

Antisera		Antigens				
		APMV type 1	APMV type 4		APMV type 6	
		Ref.[a]	Ref.[b]	Isol.[c]	Ref.[d]	Isol.[e]
APMV type 1	Ref.[a]	16[f]	< 2	< 2	< 2	< 2
APMV type 4	Ref.[b]	< 2	32	16	2	2
	Isol.[c]	< 2	64	32	< 2	< 2
APMV type 6	Ref.[d]	< 2	< 2	< 2	256	256
	Isol.[e]	< 2	2	< 2	32	16

[a] The reference strain of APMV type 1, La Sota.
[b] The reference strain of APMV type 4, D3/HK.
[c] The APMV type 4 isolate, CBU085253.
[d] The reference strain of APMV type 6, D199/HK.
[e] The APMV type 6 isolate, CBU082950.
[f] HI titers are expressed as the reciprocal of the highest dilution of serum causing inhibition of 4 HA units of virus.

Table 3. Comparison of breed-and age-related serological prevalence of APMV type 4 in chicken flocks in Korea from 2007 to 2009

Breeds	Year	APMV type 4 at weeks of age							Total
		≤ 10[a]	11～3[b]	3～10	10～22	22～40	40～60	60～	
Layer	2009	1/5(10/40)[c]	0/5(1/49)	1/3(6/25)	4/5(8/43)	1/3(3/30)	3/5(23/45)	2/5(23/49)	12/31(74/281)
	2008	0/3(2/19)	0/4(0/35)	0/4(5/31)	4/5(36/46)	2/5(21/44)	2/5(22/36)	1/5(15/45)	9/31(101/256)
	2007	0/3(7/28)	0/4(0/47)	1/3(15/31)	1/5(18/55)	1/4(17/44)	0/4(6/27)	1/4(8/23)	4/27(71/255)
Breeder	2009	1/5(18/49)	0/2(0/21)	3/4(8/30)	3/3(23/30)	2/4(9/39)	0/4(4/27)	0/5(10/45)	9/27(72/241)
	2008	0/5(2/30)	0/1(0/6)	2/5(18/42)	2/4(11/33)	1/5(18/43)	0/4(1/20)	1/5(13/35)	6/29(63/209)
	2007	1/5(7/35)	NT[d]	0/4(3/32)	0/3(7/28)	1/4(4/24)	0/5(3/40)	1/4(17/54)	3/25(41/213)
Broiler	2009	0/5(2/52)	1/5(8/43)	0/3(1/20)					1/13(11/115)
	2008	0/3(0/20)	0/5(0/36)	0/3(1/20)			NT[d]		0/11(1/ 76)
	2007	0/3(1/23)	0/4(0/29)	0/4(0/14)					0/11(1/ 66)
Total		3/37(49/296)	1/30(9/266)	7/33(57/245)	14/25(103/235)	8/25(72/224)	5/27(59/195)	6/28(86/251)	44/205(435/1712)

[a] Sera from chickens aged equal to or less than 10 days old.
[b] Sera from chickens aged between 11 days and 3 weeks old.
[c] Number of flocks positive / number of flock tested (number of birds positive / number of birds tested).
[d] Not tested.

Table 4. Comparison of breed-and age-related serological prevalence of APMV type 6 in chicken flocks in Korea from 2007 to 2009

Breeds	Year	APMV type 6 at weeks of age							Total
		≤ 10[a]	11～3[b]	3～10	10～22	22～40	40～60	60～	
Layer	2009	0/5(0/22)[c]	0/5(0/49)	0/3(0/25)	0/5(1/41)	0/3(1/30)	0/5(3/43)	0/5(2/49)	0/31(7/259)
	2008	0/3(0/19)	0/4(0/35)	0/4(0/31)	0/5(2/46)	0/5(1/44)	0/5(0/36)	0/5(0/45)	0/31(3/256)
	2007	0/3(0/29)	0/4(0/47)	0/3(0/31)	0/6(0/62)	0/4(0/36)	0/4(1/30)	0/4(0/19)	0/28(1/254)
Breeder	2009	0/5(0/49)	0/2(0/21)	0/4(0/31)	0/3(4/30)	0/4(0/39)	0/4(0/27)	0/5(0/44)	0/27(4/241)
	2008	0/5(0/30)	0/1(0/ 1)	0/5(0/42)	0/4(0/32)	0/5(0/43)	0/4(0/18)	0/5(1/35)	0/29(1/201)
	2007	0/5(1/35)	NT[d]	0/4(0/32)	0/3(0/37)	0/4(0/21)	0/5(0/40)	0/3(0/26)	0/24(1/191)
Broiler	2009	0/5(0/50)	0/5(5/43)	0/3(0/25)					0/13(5/118)
	2008	0/3(0/21)	0/5(2/29)	0/3(1/20)			NT[d]		0/11(3/ 70)
	2007	0/3(0/23)	0/4(0/33)	0/4(0/13)					0/11(0/ 69)
Total		0/37(1/278)	0/30(7/258)	0/33(1/250)	0/26(7/248)	0/25(2/213)	0/27(4/194)	0/27(3/218)	0/205(25/1659)

[a] Sera from chickens aged equal to or less than 10 days old.
[b] Sera from chickens aged between 11 days and 3 weeks old.
[c] Number of flocks positive / number of flock tested (number of birds positive / number of birds tested).
[d] Not tested.

and with APMV type 1 and 2 or APMV type 1 and 4 (Shihmanter *et al*., 1997). From the results, co-infection between different serotypes of APMVs or with AIVs can occur in wild birds, which are a reservoir for AIVs and APMVs. Similar to the epidemiology of AIV between domestic and wild bird populations, APMVs could spill over to the poultry flocks from the viral reservoir, the wild birds. However, there has been little information on the epidemio-

logy of APMVs other than APMV type 1 in Korea. In this sense, this study focused on 1) a transmission study of APMV type 4 and 6 from feral to domestic birds; and 2) a prevalence study of APMV type 4 and 6 in domestic flocks in Korea.

In experimental infections in the SPF chickens, wild-bird-origin APMV type 4 and 6 infected chickens, replicated in the internal organs, and finally shed viruses that could infect other contacted birds. The virus was mainly isolated from the respiratory and digestive systems and intestinal replication was considered significant as a reservoir of the virus (Hinshaw *et al*., 2003).

ICPI and MDT showed that CBU085253 and CBU082954 were 'asymptomatic' strains based on standard criteria (Alexander, 1998) and this result was consistent with a previous report (Warke *et al*., 2008a). In addition, the experimental infections showed that chickens had no significant signs, which could lead to overlooking infections of APMV type 4 and 6 in chickens. As such, serology is an effective test to understand the immune status of infection of APMV type 4 and 6 in chicken flocks.

To investigate the APMV infection rate for the poultry industry, retrospective serological tests were conducted according to the year, breed and the age in weeks. A three-year survey from 2007 to 2009 showed similar tendencies for each year, which reinforced the reliability of the test results. In addition, an antigenic relatedness test between reference strains and isolates was performed to select appropriate antigens for the HI tests. From the cross reactions in homologous systems, reference strains and isolates for respective serotypes were placed into the same serological groups.

There was a higher infection rate of APMV type 4 than type 6 throughout the studied period, age ranges and breeds, and the layer flocks showed a wide distribution of positive sera against APMV type 4, inferring sustained exposure to the antigen or infection from APMV type 4 viruses. Previous studies indicated that APMV type 1 and 4 fell into the same subgroup (Lipkind *et al*., 1986). Further, there were concerns of possible cross reaction between APMV type 1 and 4 due to the high titer and almost all positive serum rates (Lee *et al*., 2010) of APMV type 1 considering massive vaccination in the poultry industry (Warke *et al*., 2008b). Thus, the non-existence of cross reaction between the La Sota strain, APMV type 1 and the strains selected in this study was considered to be significant and confirmed the reliability of the serological test results. Other studies reported that the positive rate of breeders was higher than that of the layers (Maldonado *et al*., 1994; Warke *et al*., 2008b; Zhang *et al*., 2007), which could reflect different circumstances in the poultry industry. The positive serum rate of APMV type 6 was relatively low, which was similar to the tendency in another study (Warke *et al*., 2008b).

In this study, wild-bird-derived APMV type 4 and 6 were studied for the possibility of inter-species transmission from wild birds to domestic flocks and the serological prevalence of these viruses in chicken farms in Korea. Further studies of these viruses in wild bird populations are needed to clarify overall infection status.

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