Short Communication

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Pathogenicity of H5N8 virus in chickens from Korea in 2014

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In 2014, two genetically distinct H5N8 highly pathogenic avian influenza (HPAI) viruses were isolated from poultry and wild birds in Korea. The intravenous pathogenicity indices for the two representative viruses were both 3.0. Mortality of chickens intranasally inoculated with the two H5N8 viruses was 100% with a mean death times of 2.5 and 4.5 days. Mortality rates of the contact groups for the two H5N8 viruses were 33.3% and 66.6%. Our study showed that transmissibility of the novel H5N8 viruses was different from that of previously identified H5N1 HPAI viruses, possibly due to genetic changes.

Keywords: chicken, H5N8, highly pathogenic avian influenza, pathogenicity

Since 2003, the H5N1 highly pathogenic avian influenza (HPAI) virus has been widely distributed among poultry and wild birds in Asia, Europe, and Africa [2]. This virus has caused considerable damage economically and is a potential public health threat. In Korea, the outbreaks of H5N1 HPAI have occurred four times from 2003 to 2011, causing severe clinical symptoms and mortality in chicken farms [7,8,9,14].

On January 16, 2014, the fifth HPAI outbreak that was the first involving H5N8 occurred in Jeonbuk province in Korea. The H5N8 viruses isolated from wild birds, domestic ducks, and chickens could be divided into two distinct genetic groups [10] with one being predominant in Korea [4]. Unlike the H5N1 HPAI outbreaks that occurred previously, the predominant H5N8 virus did not dramatically increase mortality and infection in chicken farms. Therefore, this particular virus was regarded as comparable to other pathogens such as low pathogenic avian influenza virus or *Salmonella gallinarum* in farms (data not shown). In the present study, we evaluated the pathogenicity of two representative H5N8 viruses that had not previously been studied in chickens.

Two viruses were assessed in this study: A/breeder duck/korea/Gochang1/2014(H5N8) (Gochang1) and A/broiler duck/korea/Buan2/2014(H5N8) (Buan2). The viruses were propagated in embryonated specific pathogen-free (SPF) chicken eggs. To evaluate pathogenicity, ten 5-week-old SPF chickens were intravenously inoculated with 1/10 diluted infective allantoic fluid of Buan2 virus for an intravenous pathogenicity index (IVPI) test according to OIE regulations [15]. For experiment 1, eight 5-week-old SPF chickens were intranasally inoculated with Gochang1 or Buan2 HPAI virus ($10^{6.5}EID_{50}$/0.1 mL). After 8 h, three SPF chickens were co-housed with the infected birds as the contact group. The control group consisted of uninfected chickens. To further assess transmissibility with the Buan2 virus, eight 5-week-old SPF chickens were co-housed as a contact group with three SPF chickens intranasally inoculated with Buan2 virus in experiment 2.

To evaluate the recovery of the two viruses, oropharyngeal (OP) and cloacal (CL) swabs were collected 1, 2, 3, 4, 5, 6, 7, 10, and 14 days post-infection (dpi) along with tissues from the infected chickens. The samples used to inoculate chicken embryo fibroblast (CEF) cell cultures. Virus growth was determined by measuring the cytopathic effect (CPE). Serum samples from live chickens taken 14 dpi were subjected to a hemagglutination inhibition (HI) test to detect antibodies against the AIV H5 HA protein using eight hemagglutination units of homologous antigen according to the OIE protocol [15]. All animal experiments were performed in a biosafety level 3 facility and with permission from the Animal Ethics Committee of the Animal and Plant Quarantine Agency, Korea (approval no. 2014-232).

In a previous study, the IVPI of Gochang1 virus was 3.0 [10]. The IVPI of Buan2 virus in chickens was also found to be 3.0 by classifying the viruses as HPAI according to OIE criteria [15]. This finding is consistent with previous studies showing that there are multiple basic amino acids at HA cleavage sites of the two viruses [10].

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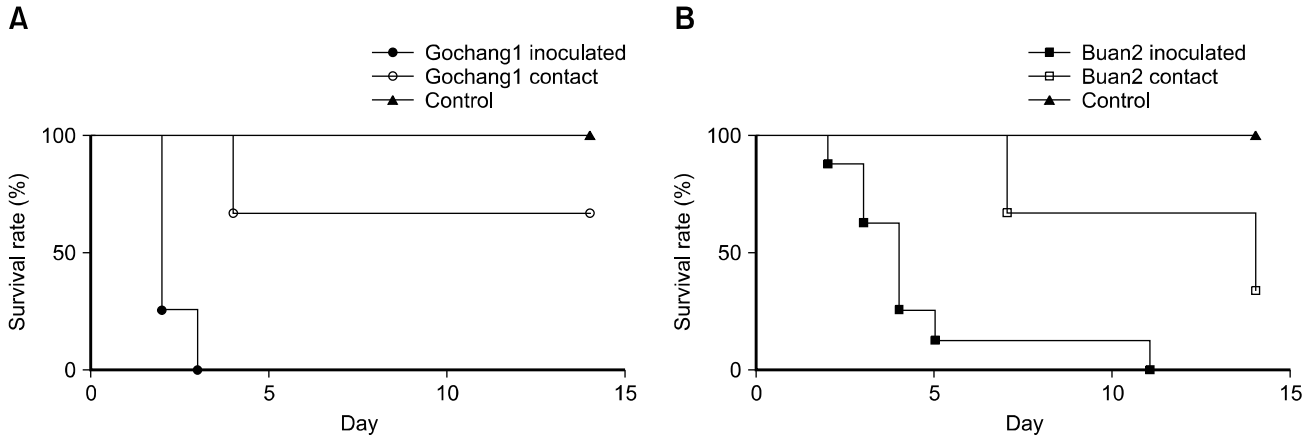

Fig. 1. Survival curves for the experimentally inoculated and contact chickens. Eight 5-week-old chickens were inoculated with $10^{6.5}$ EID_{50}/0.1 mL of A/breeder duck/korea/Gochang 1/2014(H5N8) (Gochang1; A) or A/broiler duck/korea/Buan2/2014(H5N8) (Buan2; B). When comparing the survival curves of the two viruses, mortality rates were 100% for the inoculated chickens. There were significant differences between the survival curves of the two viruses for the contact chickens (log-rank test, $p < 0.05$).

For the intranasal inoculation experiment, mortality rate of the inoculated groups was 100% while those of the contact groups varied: 33.3% for Gochang1 and 66.6% for Buan2 (Fig. 1). This result indicated that although the H5N8 viruses are highly virulent, chicken-to-chicken transmissibility is lower than that of previously described H5N1 HPAI [5,13]. All of the chickens inoculated with the Gochang1 virus died within 3 dpi with a mean death time (MDT) of 2.5 days. In contrast, all chickens inoculated with the Buan2 virus died within 11 dpi, producing an MDT of 4.5 days. One out of eight chickens inoculated with Buan2 did not shed the virus from 1 dpi to 7 dpi and died at 11 dpi. This was probably caused by an inoculation error and the chicken died due to secondary infection (Table 1). In contrast to the Gochang1 virus that was associated with a MDT similar to that of the H5N1 HPAI previously isolated in Asia [3,5,12], the Buan2 virus had a longer MDT compared to H5N1 HPAI. The fact that mortality was not dramatically increased in the chicken farms might be related to the low levels of transmissibility of H5N8 among chickens and long MDT of chickens infected with the Buan2 H5N8 virus, which was the predominant strain on the farms [4].

Similar levels of Gochang1 and Buan2 were shed in the OP and CL swab samples: $10^{4.0} \sim 10^{6.3}$ $TCID_{50}$/0.1 mL and $10^{3.1} \sim 10^{7.5}$ $TCID_{50}$/0.1 mL, respectively, for experiment 1, and $10^{2.3} \sim 10^{3.9}$ $TCID_{50}$/0.1 mL and $10^{0.8} \sim 10^{3.9}$ $TCID_{50}$/0.1 mL, respectively, for experiment 2 (Table 1). These data differ from findings from previous studies in which the majority of H5N1 HPAI viruses isolated after late 2002 were associated with a higher level of oropharyngeal than cloacal virus shedding [1,6,11]. Despite the relatively high degree of virus shedding in the OP and CL samples, the H5N8 viruses showed a low level of transmissibility among chickens: 33.3% for Gochang1 and 66.6% for Buan2 in experiment 1, and 12.5% for Buan2 in experiment 2 (Table 1). This characteristic of H5N8 viruses might contribute to lower mortality than previously H5N1 HPAIVs reported in contact chickens [5,13]. To further understand the reason why the transmissibility of the H5N8 viruses was low, additional experiments, such as assessment of the minimum infectious dose, need to be performed.

Both Gochang1 and Buan2 replicated systemically as indicated by recovery of the virus from various tissues including the brain. This characteristic is similar to the H5 HPAI virus that also replicates systemically [1,2,6]. Virus titers in the tissue samples ranged from $10^{4.3}$ to $10^{7.5}$ $TCID_{50}$/0.1 mL for the Gochang1 group and from $10^{5.1}$ to $10^{7.5}$ $TCID_{50}$/0.1 mL for the Buan2 group (Table 2).

In this study, pathogenicity of H5N8 viruses isolated from chickens from Korea in 2014 was evaluated. Although the H5N8 viruses were highly pathogenic in chickens, they showed lower transmissibility and a longer MDT than those of H5N1 HPAI virus previously isolated in Asia. Due to extensive genetic divergence and re-assortment between other subtypes, influenza viruses undergo evolution and a change of pathogenicity in the host. Our observations suggest that continuous monitoring along with characteristic analysis of influenza viruses is needed to predict future changes of the viruses.

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Table 1. Pathogenicity and virus recovery of the Korean H5N8 HPAI viruses in chickens

Virus	IVPI	Experiment	Route (number)	MDT (day)	Mortality (%)	Sample	Virus titer (log 10 TCID50/0.1 mL, mean ± SD)									HI titer
							1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi	10 dpi	14 dpi	
Gochang1	3.0	Experiment 1	Inoculated (8)	2.5	100%	OP	−(0/8)*	4.0 ± 1.5 (7/8)	6.3 ± 0.2 (2/2)	NT	NT	NT	NT	NT	NT	NT
						CL	−(0/8)	4.6 ± 1.7 (7/8)	7.5 (2/2)	NT	NT	NT	NT	NT	NT	
			Contact (3)	4.0	33.3%	OP	−(0/3)	−(0/3)	−(0/3)	5.3 (1/3)	−(0/2)	−(0/2)	−(0/2)	−(0/2)	−(0/2)	(0/2)
						CL	−(0/3)	−(0/3)	−(0/3)	5.8 (1/3)	−(0/2)	−(0/2)	−(0/2)	−(0/2)	−(0/2)	
Buan2	3.0	Experiment 1	Inoculated (8)	4.5	100%	OP	−(0/8)	5.2 ± 2.3 (2/8)	4.2 ± 1.8 (6/7)	4.6 ± 0.9 (4/5)	6.3 (1/2)	−(0/1)	−(0/1)	5.3 (1/1)	NT	NT
						CL	−(0/8)	3.4 ± 0.9 (3/8)	3.1 ± 1.1 (6/7)	3.8 ± 0.2 (4/5)	7.5 (1/2)	−(0/1)	−(0/1)	1.6 (1/1)	NT	
			Contact (3)	10.5	66.6%	OP	−(0/3)	−(0/3)	−(0/3)	−(0/3)	−(0/3)	−(0/3)	4.2 (1/3)	−(0/2)	5.8 (1/2)	(0/1)
						CL	−(0/3)	−(0/3)	−(0/3)	−(0/3)	−(0/3)	2.2 (1/3)	4.4 (1/3)	−(0/2)	4 (1/2)	
		Experiment 2	Inoculated (3)	4.3	100%	OP	−(0/3)	2.3 (1/3)	2.3 ± 1.6 (2/3)	3.9 ± 2.0 (3/3)	3.1 (1/1)	NT	NT	NT	NT	NT
						CL	−(0/3)	0.8 (1/3)	2.0 ± 1.0 (3/3)	3.9 ± 1.3 (3/3)	1.8 (1/1)	NT	NT	NT	NT	
			Contact (8)	9.0	12.5%	OP	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/7)	−(0/7)	(0/7)
						CL	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/7)	−(0/7)	
Control	NA	−	Non-infection	NA	0%	OP	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	(0/8)
						CL	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	−(0/8)	

*Number of virus isolated/number of tested samples. Virus re-isolation from swab samples from experimentally inoculated and contact chickens. In experiment 1, eight chickens were inoculated intranasally with $10^{6.5}$ EID_{50}/0.1 mL of Gochang1 or Buan2 virus, and three chickens were co-housed with the infected chickens as the contact group. Experiment 2 was conducted to further evaluate transmissibility of the Buan2 virus. Eight 5-week-old SPF chickens were co-housed as a contact group with three SPF chickens intranasally inoculated with Buan2 virus. Oropharyngeal and cloacal swab samples were collected on the indicated day, and the virus titer was measured in chick embryo fibroblasts. Virus titer is the average of the calculable positive samples. IVPI: intravenous pathogenicity index, MDT: mean death time, HI: hemagglutination inhibition, NT: not tested, NA: not applicable. OP; oropharyngeal swab sample, CL; cloacal swab sample.

Table 2. Virus re-isolation from tissue samples from experimentally inoculated and contact chickens

Virus	Route	Virus titer (log 10 TCID50/0.1 mL, mean ± SD)										
		Trachea	Lung	Liver	Spleen	Kidney	Cecal tonsil	Proventriculus	Intestine (pancrease)	Heart	Muscle	Brain
Gochang1	Inoculated	6.2 ± 1.2	7.4 ± 0.3	6.6 ± 1.2	7.5	7.5	7.4 ± 0.4	7.4 ± 0.4	6.1 ± 1.0	7.5	7.3 ± 0.4	4.3 ± 0.9
	Contact	7.5	7.5	7.4	7.5	7.5	7.5	7.5	7.5	7.5	7.5	4.5
Buan2	Inoculated	5.1 ± 2.1	7.1 ± 0.8	5.1 ± 1.4	5.9 ± 1.4	6.5 ± 1.1	6.9 ± 0.8	7.0 ± 0.6	5.5 ± 1.5	7.5	6.8 ± 1.1	6.9 ± 0.9
	Contact	5.9 ± 1.6	7.0 ± 0.3	5.5 ± 0.1	6.0 ± 1.7	6.0 ± 0.7	7.3 ± 0.4	7.5	5.4 ± 1.1	7.5	6.0 ± 2.1	6.7 ± 0.7
Control	Non-infection	0	0	0	0	0	0	0	0	0	0	0

Virus re-isolation from tissue samples from experimentally inoculated and contact chickens. The virus titers of the tissues (10% homogenates) from the dead chickens in the intranasally inoculated and contact groups were measured in chick embryo fibroblasts. Virus titer is the average of calculable positive samples.

Conflict of Interest

There is no conflict of interest.

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