Pathogenicity of H5N8 virus in chickens from Korea in 2014

Byung-Min Song, Hyun-Mi Kang, Eun-Kyoung Lee, Jipseol Jeong, Yeojin Kang, Hee-Soo Lee, Youn-Jeong Lee*

Division of Avian Diseases, Animal and Plant Quarantine Agency, Anyang 430-737, Korea

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
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: $10^{5.0} \sim 10^{6.3}$ TCID$_{50}$/0.1 mL and $10^{5.1} \sim 10^{5.5}$ 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 $10^{7.2}$ 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.

Acknowledgments

This work was supported by a grant (no. I-1541781-2012-15-01) from the Animal and Plant Quarantine Agency, Korea.
Table 1. Pathogenicity and virus recovery of the Korean H5N8 HPAI viruses in chickens

| Virus   | IVPI | Experiment | Route (number) | MDT  | Mortality (%) | Sample | Virus titer (log 10 TCID50/0.1 mL, mean ± SD) |
|---------|------|------------|----------------|------|---------------|--------|-----------------------------------------------|
|         |      |            |                |      |               |        | 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)*  | (0/8)   | 4.0 ± 1.5| 6.3 ± 0.2| NT      | NT      | NT      | NT      | NT      | NT      |
|         |      |            | Contact (3)     | 4.0  | 33.3%         | OP     | (0/3)   | (0/3)   | 5.0 ± 0.9| 3.0 ± 1.7| 7.5     | NT      | NT      | NT      | NT      | NT      |
| Buan2   | 3.0  | Experiment 1| Inoculated (8)  | 4.5  | 100%          | OP     | (0/8)   | (0/8)   | 5.2 ± 2.3| 4.2 ± 1.8| 4.6 ± 0.9| 6.3     | 5.3 (1/1)| NT      | NT      | NT      |
|         |      |            | Contact (3)     | 10.5 | 66.6%         | OP     | (0/3)   | (0/3)   | 3.4 ± 0.9| 3.1 ± 1.1| 3.8 ± 0.2| 7.5     | (0/1)   | (1.6/1)| NT      | NT      | NT      |
|         |      | Experiment 2| Inoculated (3)  | 4.3  | 100%          | OP     | (0/3)   | (0/3)   | 2.3 ± 1.6| 3.9 ± 2.0| 3.1     | NT      | NT      | NT      | NT      | NT      |
|         |      |            | Contact (8)     | 9.0  | 12.5%         | OP     | (0/8)   | (0/8)   | 0.8 ± 0.8| 2.0 ± 1.0| 3.9 ± 1.3| 1.8     | NT      | NT      | NT      | NT      | NT      |
| 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)   |

*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 EID50/0.1 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 (pancreas) | 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 | 7.4 ± 0.4| 7.4 ± 0.4| 6.1 ± 1.0| 7.5 | 7.3 ± 0.4| 4.3 ± 0.9 |
| 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.

References

1. Brown JD, Stallknecht DE, Beck JR, Suarez DL, Swayne DE. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. Emerg Infect Dis 2006, 12, 1663-1670.

2. Capua I, Alexander DJ. Avian influenza: recent developments. Avian Pathol 2004, 33, 393-404.

3. Choi JG, Kang HM, Jeon WJ, Choi KS, Kim KI, Song BM, Lee HS, Kim JH, Lee YJ. Characterization of clade 2.3.2.1 H5N1 highly pathogenic avian influenza viruses isolated from wild birds (mandarin duck and Eurasian eagle owl) in 2010 in Korea. Viruses 2013, 5, 1153-1174.

4. Jeong J, Kang HM, Lee EK, Song BM, Kwon YK, Kim HR, Choi KS, Kim JY, Lee HJ, Moon OK, Jeong W, Choi J, Baek JH, Joo YS, Park YH, Lee HS, Lee YJ. Highly pathogenic avian influenza virus (H5N8) in domestic poultry and its relationship with migratory birds in South Korea during 2014. Vet Microbiol 2014, 173, 249-257.

5. Jeong OM, Kim MC, Kim MJ, Kang HM, Kim HR, Kim YJ, Joh SJ, Kwon JH, Lee YJ. Experimental infection of chickens, ducks and quails with the highly pathogenic H5N1 avian influenza virus. J Vet Sci 2009, 10, 53-60.

6. Kang HM, Choi JG, Kim KI, Kim BS, Batchuluun D, Erdene-Ochir TO, Kim MC, Kwon JH, Kwon JH, Park CK, Lee YJ. Pathogenicity in domestic ducks and mice of highly pathogenic H5N1 clade 2.3.2.1 influenza viruses recently circulating in Eastern Asia. Vet Microbiol 2013, 167, 327-333.

7. Kim HR, Lee YJ, Park CK, Oem JK, Lee OS, Kang HM, Choi JG, Bae YC. Highly pathogenic avian influenza (H5N1) outbreaks in wild birds and poultry, South Korea. Emerg Infect Dis 2012, 18, 480-483.

8. Kim HR, Park CK, Lee YJ, Woo GH, Lee KK, Oem JK, Kim SH, Jeon YH, Bae YC, Yoon SS, Roh IS, Jeong OM, Kim HY, Choi JS, Byun JW, Song YK, Kwon JH, Joo YS. An outbreak of highly pathogenic H5N1 avian influenza in Korea, 2008. Vet Microbiol 2010, 141, 362-366.

9. Lee YJ, Choi YK, Kim YJ, Song MS, Jeong OM, Lee EK, Jeon WJ, Jeong W, Joh SJ, Choi KS, Her M, Kim MC, Kim A, Kim MJ, Lee EH, Oh TG, Moon HJ, Yoo DW, Kim JH, Sung MH, Poo H, Kwon JH, Kim CJ. Highly pathogenic avian influenza virus (H5N1) in domestic poultry and relationship with migratory birds, South Korea. Emerg Infect Dis 2008, 14, 487-490.

10. Lee YJ, Kang HM, Lee EK, Song BM, Jeong J, Kwon YK, Kim HR, Lee KJ, Hong MS, Jang I, Choi KS, Kim JY, Lee HJ, Kang MS, Jeong OM, Baek JH, Joo YS, Park YH, Lee HS. Novel reassortant influenza A(H5N8) viruses, South Korea, 2014. Emerg Infect Dis 2014, 20, 1087-1089.

11. Pantin-Jackwood MJ, Swayne DE. Pathobiology of Asian highly pathogenic avian influenza H5N1 virus infections in ducks. Avian Dis 2007, 51 (Suppl), 250-259.

12. Suzuki K, Okada H, Itoh T, Tada T, Mase M, Nakamura K, Kudo M, Tsukamoto K. Association of increased pathogenicity of Asian H5N1 highly pathogenic avian influenza viruses in chickens with highly efficient viral replication accompanied by early destruction of innate immune responses. J Virol 2009, 83, 7473-7486.

13. Tsukamoto K, Imada T, Tanimura M, Okamatsu M, Mase M, Mizuhara T, Swayne D, Yamaguchi S. Impact of different husbandry condition on contact and airborne transmission of H5N1 highly pathogenic avian influenza virus to chickens. Avian Dis 2007, 51, 129-132.

14. Wee SH, Park CK, Nam HM, Kim CH, Yoon H, Kim SJ, Lee ES, Lee BY, Kim JH, Lee JH, Kim CS. Outbreaks of highly pathogenic avian influenza (H5N1) in the Republic of Korea in 2003/04. Vet Rec 2006, 158, 341-344.

15. World Organisation for Animal Health. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012. Chap. 2.3.4. Avian Influenza. 7th ed. pp. 465-481, OIE, Paris, 2012.