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Experimental infection of highly and low pathogenic avian influenza viruses to chickens, ducks, tree sparrows, jungle crows, and black rats for the evaluation of their roles in virus transmission

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ABSTRACT

Highly pathogenic avian influenza viruses (HPAIVs) have spread in both poultry and wild birds. Determining transmission routes of these viruses during an outbreak is essential for the control of avian influenza. It has been widely postulated that migratory ducks play crucial roles in the widespread dissemination of HPAIVs in poultry by carrying viruses along with their migrations; however close contacts between wild migratory ducks and poultry are less likely in modern industrial poultry farming settings. Therefore, we conducted experimental infections of HPAIVs and low pathogenic avian influenza viruses (LPAIVs) to chickens, domestic ducks, tree sparrows, jungle crows, and black rats to evaluate their roles in virus transmission. The results showed that chickens, ducks, sparrows, and crows were highly susceptible to HPAIV infection. Significant titers of virus were recovered from the sparrows and crows infected with HPAIVs, which suggests that they potentially play roles of transmission of HPAIVs to poultry. In contrast, the growth of LPAIVs was limited in each of the animals tested compared with that of HPAIVs. The present results indicate that these common synanthropes play some roles in influenza virus transmission from wild birds to poultry.

1. Introduction

Influenza A viruses are widely distributed in mammals and birds. Influenza A viruses of each of the known subtypes (H1–16 and N1–9) have been isolated from water birds, particularly from migratory ducks (Kida and Yanagawa, 1979; Fouchier et al., 2005). Therefore, migratory ducks are the natural hosts for influenza A viruses (Webster et al., 1992; Kida, 2008). Since late 2003, H5N1 highly pathogenic avian influenza viruses (HPAIVs) have seriously affected poultry in Eurasia and Africa (World Organisation for Animal Health, 2015; http://www.oie.int/animal-health-in-the-world/web-portal-on-avian-influenza/). HPAIVs are generated when non-pathogenic viruses circulating among water birds are transmitted to chickens via domestic water and terrestrial birds, where they acquire pathogenicity in chickens via multiple infection and replication in the chicken population (Ito et al., 2001). After 2005, H5N1HPAIVs were isolated from dead migratory water birds on the way back to their nesting lakes in Siberia in May (Liu et al., 2005; Okamatsu et al., 2010; Sakoda et al., 2010). The pathogenicity of HPAIVs to migratory ducks varies depending on the virus strain (Sturm-Ramirez et al., 2004; Sakoda et al., 2010; Kajihara et al., 2013); in general, HPAIVs are less pathogenic to ducks compared with chickens (Kishida et al., 2005). Indeed, infected ducks shed viruses without showing any clinical signs (Kida et al., 1980). Thus, it has been widely postulated that

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migratory ducks play crucial roles in the widespread dissemination of HPAIVs in poultry by carrying viruses along with their migrations.

Recently, HPAIVs of various genetic clades derived from A/goose/Guangdong/1/1996 (H5N1) have been circulating in Asian countries (Donis et al., 2015). These HPAIVs have evolved to be genetically and antigenically divergent (Shichinohe et al., 2013; Hiono et al., 2015). HPAIVs of clade 1.1.1 have been detected in Mekong River Delta, 2.1.3 in Indonesia, and 2.2.1 in Egypt. In addition, HPAIVs of clades 2.3.2.1 and 2.3.4 have widely spread mainly in East and Southeast Asia. Moreover, HPAIVs of clade 2.3.4.4 have spread to North American and European continents along with migration of wild ducks (Hall et al., 2015). It is therefore imperative to prepare for the future outbreaks caused by HPAIVs of various clades.

In 2010, two H5N1HPAIVs of clade 2.3.2.1 were isolated from fecal samples of ducks on the migration flyway from Siberia to the south in Hokkaido, which was followed by 63 cases of HPAIV infections in wild birds and 24 sporadic cases in poultry (Kajihara et al., 2011; Sakoda et al., 2012). Monitoring avian influenza viruses in wild bird populations is highly beneficial to prepare for outbreaks of highly pathogenic avian influenza (HPAI). On the other hand, close contacts between wild migratory ducks and poultry are less likely in modern industrial poultry farming settings. Accordingly, there should be some other factors involved in the transmission of HPAIVs from wild migratory ducks to poultry.

Unveiling the routes of virus transmission that cause outbreaks is essential for controlling avian influenza in poultry. Viruses might invade poultry houses via newly introduced birds, feed, equipment, and wild animals. Tree sparrows (*Passer montanus*), jungle crows (*Corvus macrorhynchos*), and black rats (*Rattus rattus*) are characterized as synanthropes, and they are found commonly around poultry houses in Japan. In the present study, we selected seven influenza viruses, which comprised of five HPAIVs isolated recently in Asia, and two low pathogenic avian influenza viruses (LPAIVs) including an H7N9 influenza virus isolated in China, and a non-pathogenic H5N1 strain. We investigated their pathogenicity to these wild animals to evaluate their potential risk for carrying viruses into poultry houses.

2. Materials and methods

2.1. Viruses

A/muscovy duck/Vietnam/OIE-559/2011 (H5N1) (VN/559) was isolated from a muscovy duck in a live bird market of Vietnam in our previous study (Okamatsu et al., 2013). A/whooper swan/Hokkaido/4/2011 (H5N1) (Hok/4) was isolated from dead whooper swan in 2011 (Sakoda et al., 2012). A/peregrine falcon/Hong Kong/810/2009 (H5N1) (HK/810) was kindly provided by Dr. Luk S.M. Geraldine of the Tai Lung Veterinary Laboratory, Hong Kong SAR. A/chicken/Kumamoto/1-7/2014 (H5N8) (Km/1-7) was kindly provided by Dr. Takehiko Saito of the National Institute of Animal Health, Japan (Kanehira et al., 2015). A/chicken/Taiwan/0502/2012 (H5N2) (Tw/0502), which is a classical Taiwanese HPAIV, was kindly provided by Animal Health Institute, Taiwan. A/Anhui/1/2013 (H7N9) (Ah/1) was kindly provided by Dr. Masato Tashiro of the National Institute of Infectious Diseases, Japan (Gao et al., 2013). A/duck/Hokkaido/Vac-1/2004 (H5N1) (Hok/Vac1), which is a reassortant virus from non-pathogenic H5N2 and H7N1 viruses was previously established in our laboratory and used as a representative strain of non-pathogenic avian influenza viruses circulating in wild migratory ducks. (Soda et al., 2008). The viruses were propagated in 10-day-old embryonated chicken eggs at 35 °C for 36–48 h, and the infectious allantoic fluids were used as virus stocks (Table 1).

2.2. Animal experiments

Four-week-old chickens (*Gallus gallus*, Julia) were obtained from Hokkai Starchick, Hokkaido, Japan. Four-week-old domestic ducks (*Anas platyrhynchos*var. *domesticus*,Cherry Valley) were obtained from Takikawa Shinseien, Hokkaido, Japan. Tree sparrows (*Passer montanus*) were captured in Azumino, Nagano, Japan. Jungle crows (*Corvus macrorhynchos*) were captured in Yubari, Hokkaido, Japan. Black rats (*Rattus rattus*) were bred and raised at IKARI Institute of Technology, Chiba, Japan. The sera of chickens, ducks, and crows were collected before the challenge. The absence of specific antibodies against the challenge virus was confirmed by hemagglutination-inhibition (HI) test with 25 μl of collected sera according to the standard protocol. Because of technical problem, serological status of sparrows and black rats were not able to be monitored before the challenge. Eight of each animal were used in the present study and they were randomly divided into two groups. All chickens, ducks, and crows were intranassally inoculated with 100 μl of virus solution containing $10^{6.0}$ 50% egg infectious dose (EID_{50}) of either VN/559, Hok/4, HK/810, Km/1-7, Tw/0502, Ah/1, or Hok/Vac1. Sparrows were intranassally inoculated with 30 μl of virus solution containing $10^{6.0}$ EID_{50} of each virus. Black rats were intranasally inoculated with 30 μl of virus solution containing $10^{6.0}$ EID_{50} of each virus in anesthetized conditions. At 3 dpi, four individuals of each type of inoculated animals were euthanized, and oral and cloacal swabs (chickens, ducks, sparrows, and crows), blood samples (ducks, sparrows, crows, and black rats), as well as brain (all animals), lung (all animals), kidney (chickens, ducks, crows, and black rats), colon (chickens, ducks, and crows), large intestine (sparrows and black rats), and feces (black rats) were collected. Since all of chickens inoculated with VN/559 or HK/810 died at 2 dpi, swabs and tissue samples were collected from the dead birds. To prepare a 10% suspension with Minimum Essential Medium (MEM; Nissui Pharmaceutical, Tokyo, Japan), tissue samples and feces were homogenized using a Multi-Beads Shocker (Yasui Kikai, Osaka,

Table 1
Influenza viruses used in the present study.

Viruses	Abbreviations	Genetic clades	References
A/muscovy duck/Vietnam/OIE-559/2011 (H5N1)	VN/559	1.1	Okamatsu et al. (2013)
A/whooper swan/Hokkaido/4/2011 (H5N1)	Hok/4	2.3.2.1	Sakoda et al. (2012)
A/peregrine falcon/Hong Kong/810/2009 (H5N1)	HK/810	2.3.4	Shichinohe et al. (2013)
A/chicken/Kumamoto/1-7/2014 (H5N8)	Km/1-7	2.3.4.4	Kanehira et al. (2015)
A/chicken/Taiwan/0502/2012 (H5N2)	Tw/0502	NA	–
A/Anhui/1/2013 (H7N9)	Ah/1	NA	Gao et al. (2013)
A/duck/Hokkaido/Vac-1/2004 (H5N1)	Hok/Vac1	NA	Soda et al. (2008)

NA: clade definition is not applicable.
–: references are not available.
DDBJ/EMBL/GenBank accession numbers are KJ720208–KJ720215.