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Infection of chicken H9N2 influenza viruses in different species of domestic ducks

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ABSTRACT

Domestic ducks are considered as the interface between wild aquatic birds and terrestrial poultry and play an important role in the transmission and evolution of avian influenza viruses (AIVs). However, the infectivity of H9N2 AIVs in different domestic duck species has not been systematically evaluated. Here we investigated the infectivity of various genotypes of chicken H9N2 AIVs in Pekin duck *(Anas Platyrhynchos)*, Mallard duck *(Anas Platyrhynchos)* and Muscovy duck *(Cairina Moschata)* through intranasal inoculation. We found that Pekin ducks and Mallard ducks were generally resistant to chicken H9N2 virus infection, while Muscovy ducks were relatively susceptible to H9N2 AIVs. All the tested viruses were isolated from oropharynx, trachea and lung tissues of Muscovy ducks. Additionally, genotype 57 (G57) H9N2 AIVs, which was predominant in chickens since 2010, showed increased virus replication in this duck species, indicating an improved interspecies transmission ability of recent H9N2 viruses from chickens to ducks. Our results demonstrated the role of Muscovy ducks in the ecology of H9N2 AIVs. More attentions should be paid to this host during viral surveillances. Additionally, inactivated H9N2 vaccine may be unnecessarily used in Pekin and Mallard ducks.

1. Introduction

H9N2 AIVs have been detected worldwide from wild and domestic avian species and mammals for several decades (Homme et al., 1970; Kawaoka et al., 1988; Peiris et al., 2001; Yu et al., 2013). In 1994, H9N2 virus was first isolated in chickens in China, and the virus has been predominantly circulating in this species for over 20 years (Sun and Liu, 2015). H9N2 virus replicates mainly in the upper respiratory tract and has low pathogenicity for chickens (Guo et al., 2000). Since 2010, G57 H9N2 viruses have become predominant with enhanced infectivity and changed antigenicity, which leads to widespread outbreaks of H9N2 AIVs in chickens during 2010–2013 in China (Pu et al., 2015). G57 viruses donate internal genes to facilitate the generation of novel reassortants such as H7N9, H10N8 and H5N6 viruses (Bi et al., 2016; Pu et al., 2015; Zhang et al., 2014). A study has been performed to evaluate the pandemic potential of the G57-like H9N2 influenza viruses. Authors found that the viruses can cause disease in mice without pre-adaptation, and some strains were transmissible in ferrets by respiratory droplets (Li et al., 2014). However, the infectivity and pathogenicity of H9N2 AIVs in different duck species have not been systematically evaluated.

Domestic ducks play important roles in AIV ecology. They can be infected by most subtypes of AIVs, but generally do not show obvious signs of disease (Sturm-Ramirez et al., 2005). Therefore, AIVs could circulate silently in this host, allowing them to be transmitted to other hosts or to be reassorted with other subtype viruses (Bi et al., 2016; Chen et al., 2004). Vaccines against highly pathogenic H5 subtype viruses have been used in ducks in China for several years (Zeng et al., 2016). Recently, vaccines against H9N2 viruses have been used in ducks (Teng et al., 2015). Pekin duck *(Anas Platyrhynchos)*, Mallard duck *(Anas Platyrhynchos)* and Muscovy duck *(Cairina Moschata)* are three main species raised in China. In this study, the infection and replication of various genotype H9N2 viruses in Pekin duck, Mallard duck and Muscovy duck were evaluated.

2. Materials and methods

2.1. Ethics statements

All animal work was approved by the Beijing Association for Science

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and Technology (approval ID: SYXK [Beijing] 2007 − 0023) and conducted in accordance with the Beijing Laboratory Animal Welfare and Ethics guidelines, as issued by the Beijing Administration Committee of Laboratory Animals, and in accordance with the China Agricultural University Institutional Animal Care and Use Committee guidelines (ID: SKLAB-B-2010 − 003) approved by the Animal Welfare Committee of China Agricultural University.

2.2. Viruses

Six H9N2 influenza viruses from different genotypes, A/chicken/Beijing/3/1999 (CK/BJ/3/99, G02 genotype), A/chicken/Beijing/7/2005 (CK/BJ/7/05, G51 genotype), A/chicken/Shandong/1/2008 (CK/SD/1/08, G62 genotype), A/chicken/Hebei/YT/2010 (CK/HB/YT/10, G57 genotype), A/chicken/Jiangsu/TS/2010 (CK/JS/TS/10, G57 genotype) and A/chicken/Shandong/qd1013/2012 (CK/SD/qd1013/12, G57 genotype), were described previously (Pu et al., 2015). These genotypes were identified in our previous study and were the representative genotypes in different periods (Pu et al., 2015). These viruses were propagated in 9–days-old Specific Pathogen Free (SPF) embryonated chicken eggs at 35 °C for 72 h. Viral titers were determined by 50% egg infective dose (EID_{50}).

2.3. Virus infection in ducks

Groups of six four-week-old Pekin ducks, Mallard ducks and Muscovy ducks which were seronegative for antibodies against of H5 and H9 subtypes AIVs were inoculated by intranasal routes with 10^7 EID_{50} of H9N2 AIVs in a 200 μL volume. Three ducks from each group were euthanized on 3 day post-infection (dpi) for virus titration detection in the lungs, tracheas and intestines. Oropharyngeal and cloacal swabs from the remaining three ducks of each group were collected on days 1, 3, 5, and 7 dpi for viral detection and titration. All the remaining ducks were survived during 14 days observation period for signs of disease and finally euthanized by exposure in euthanasia chamber connected to carbon dioxide (CO_2) source.

2.4. Statistical analysis

Statistically significant differences between experimental groups were compared by using the GraphPad Prism software (GraphPad Software, La Jolla, CA) ANOVA test. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results and discussion

We investigated the infection of H9N2 AIVs from different genotypes in Pekin ducks, Mallard ducks and Muscovy ducks. During the 14 days experiment period, all the Pekin ducks, Mallard ducks and Muscovy duck showed no obvious clinical signs of disease. At 1, 3, 5 and 7 dpi, oropharyngeal and cloacal swabs were collected for virus detection. As shown in the Fig. 1, no virus was detected from oropharyngeal and cloacal swabs of Pekin ducks and Mallard ducks. In the Muscovy ducks inoculated group, all of the tested viruses were detected in oropharyngeal swabs with 100% isolation rate. The virus titers ranged from $10^{1.5}$ to $10^{4.5}$ EID_{50}/mL. It is noteworthy that the three G57 viruses replicated with significantly higher ($P < 0.05$ or $P < 0.01$) titers than the other genotypes viruses at 1, 3, and 5 dpi. For viral shedding time, the three G57 viruses shed virus for 5 days in oropharynx, and two of them kept significant higher ($P < 0.01$) replication titers in all the inoculated ducks. Similarly, CK/BJ/3/99 (G02) and CK/BJ/7/05 (G51) were also recovered from ducks at 5 dpi, but the isolation rate and viral titers were lower. Genotype G62 virus CK/SD/08 only shed for 1–3 days and could not be detected at 5 dpi. No virus was detected from cloacae of Muscovy duck throughout the experimental period. Additionally, all Muscovy ducks seroconverted by 14 dpi, as measured by hemagglutination inhibition (HI) assay.

To assess virus replication in tissues, tracheas, lungs and intestines were collected at 3 dpi for virus titration (Fig. 2). In the Pekin duck and Mallard duck groups, all the six H9N2 viruses did not replicate in tracheas, lungs and intestines. In the Muscovy duck groups, all the six viruses were recovered from the tracheas at 3 dpi, with titers ranging from$10^{1.5}$ to $10^{5.5}$ EID_{50}/mL, while the G57 viruses replicated to significantly higher ($P < 0.01$) titers. All the G57 viruses were recovered from the lungs with higher isolation rates and significant higher ($P < 0.01$) viral titers. For other genotype viruses, only G51 genotype virus CK/BJ/7/05 was detected in 1 out of 3 lung tissues. All the six H9N2 viruses failed to replicate in the intestines of Muscovy duck.

H9N2 AIVs have been prevalent in multiple terrestrial birds in China since 1994, resulting in great economic loss to poultry industry (Sun and Liu, 2015). China has the largest domestic duck populations all over the world. However, due to low viral isolation rate in ducks in field, the susceptibility of domestic duck species to H9N2 AIVs has not been systematically evaluated. Here, we investigated the infection of various genotypes of chicken H9N2 viruses in Pekin duck, Mallard duck and Muscovy duck. We found that Muscovy ducks are more susceptible to H9N2 AIVs than Pekin ducks and Mallard ducks, showing more effective replication in tracheas and lungs. Additionally, G57 H9N2 viruses showed increasing virus replication in Muscovy ducks.

Our and other previous researches revealed that the H9N2 viruses isolated from chickens since 2010, particularly for G57-like H9N2 virus, which had improved replication ability in chickens and mammals (Li et al., 2014; Pu et al., 2015; Zhu et al., 2018). Here, we further found that in Muscovy ducks, compared with the earlier genotype viruses, the G57 viruses showed enhanced growth property. All of these results indicated that the H9N2 viruses had undergone a widely adaptive evolution from 2010, by which G57-like virus obtained improved ability to expand its host range from terrestrial poultry to aquatic birds and to mammals. Our researches have determined that quail-origin G1-like lineage M gene contribute to the increased infection of G57 H9N2 viruses, which conferred an early surge in progeny virus production and more severe pathology and extrapulmonary virus spread in chickens (Pu et al., 2017). Additionally, duck-origin DK1-like lineage PB2 gene of G57 H9N2 viruses enhanced the polymerase activity and exacerbated inhibition of beta interferon expression in mammalian host (unpublished data). Effect of other viral proteins associated with the increased infectivity of G57 viruses need to be further investigated.

As the major aquatic hosts for AIVs, domestic ducks are considered as an interface between wild aquatic birds and terrestrial poultry and play an important role in the viral transmission and evolution of AIVs (Bi et al., 2016; Chen et al., 2004). All domesticated ducks originate from the Mallard, with the exception of the Muscovy duck which has distinct origins in South America, belonging to *Cairina Moschata*. Muscovy duck is a large, hardy perching duck different from other species of ducks. Previous studies have reported that more severe clinical signs and higher mortality were seen in Muscovy ducks after H7N1 AIV infection compared with other waterfowl species (Capua and Mutinelli, 2001). Additionally, the susceptibility and mortality of Muscovy ducks were similar to chickens when they were infected with different genotypes of H5N1 AIV (Phuong do et al., 2011). Similarly, we demonstrated here that Muscovy ducks are more susceptible to chicken H9N2 viruses than Pekin ducks and Mallard ducks, with effective viral replication in tracheas and lungs. Therefore, Muscovy ducks play more important roles as a possible vector of AIV than Pekin ducks and Mallard ducks. As G57 H9N2 viruses have been involved in the generation of novel reassortants, the enhanced infection ability of G57 viruses in Muscovy ducks might increase the possibility of the viral gene reassortment between H9N2 and other subtype AIVs circulated in waterfowls. Although the virus can only be detected in oropharynx but not cloaca in Muscovy ducks, they might be transmitted among contacted ducks through sharing feed and water troughs, which provide ample opportunities for the efficient horizontal transmission.

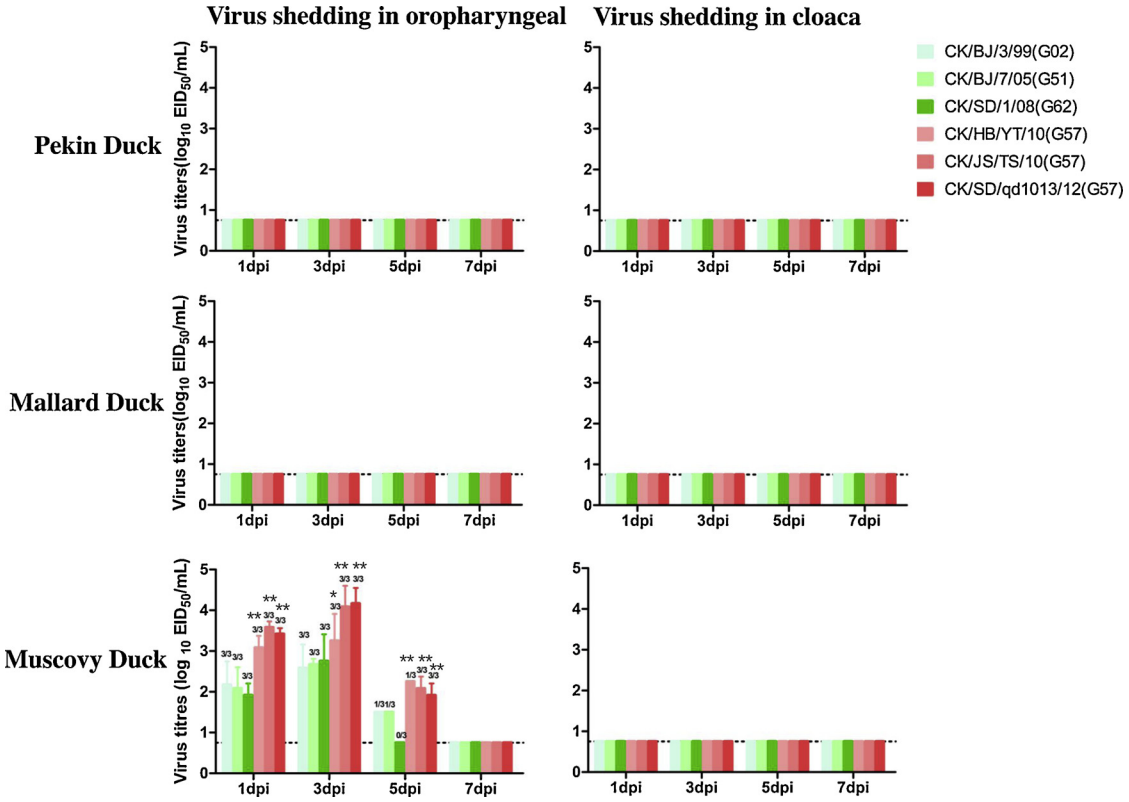

Fig. 1. Virus titers of H9N2 viruses in oropharyngeal swabs of Pekin duck, Mallard ducks Muscovy duck. Six representative H9N2 chicken viruses were selected from G57 or other genotypes to test their infection in ducks. From left to right on each x axis, they are A/chicken/Beijing/3/1999 (G02), A/chicken/Beijing/7/2005 (G51), A/chicken/Shandong/1/2008 (G62), A/chicken/Hebei/YT/2010 (G57), A/chicken/Jiangsu/TS/2010 (G57) and A/chicken/Shandong/qd1013/2012 (G57). Oropharyngeal and cloacal swabs were collected for virus detection and titration. Viral titers are shown as the means ± standard deviation. Numbers of positive ducks/tested ones are shown above each column. Dashed lines indicate the lower limit of virus detection. The titers of the G57 viruses were significantly higher than those of other genotypes of the earlier viruses (*$P < 0.05$, **$P <$ 0.01, one-way ANOVA).

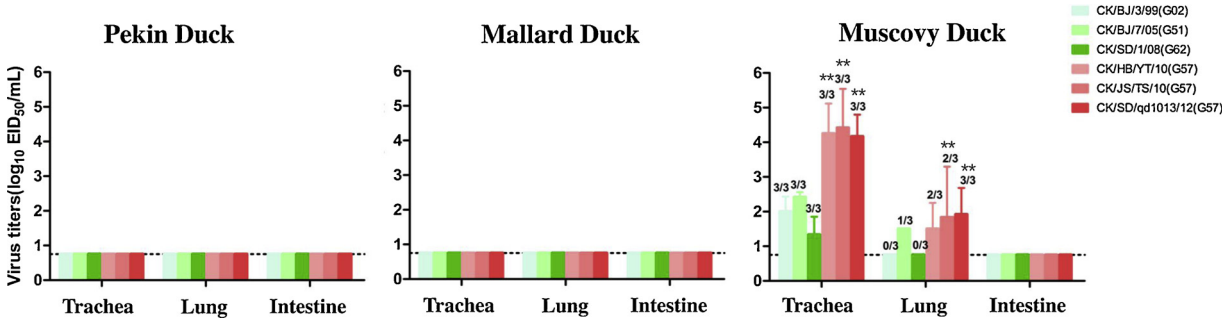

Fig. 2. Virus titers of H9N2 viruses in tracheas, lungs and intestines of Pekin duck, Mallard ducks and Muscovy duck. Six representative H9N2 chicken viruses were selected from G57 or other genotypes to test their infection in ducks. From left to right on each x axis, they are A/chicken/Beijing/3/1999 (G02), A/chicken/Beijing/7/2005 (G51), A/chicken/Shandong/1/2008 (G62), A/chicken/Hebei/YT/2010 (G57), A/chicken/Jiangsu/TS/2010 (G57) and A/chicken/Shandong/qd1013/2012 (G57). Tracheas, lungs and intestines were collected for virus detection and titration at 3 dpi. Numbers of positive ducks/tested ones are shown above each column. Dashed lines indicate the lower limit of virus detection. The titers of the G57 viruses were significantly higher than those of other genotypes of the earlier viruses (*P < 0.05, **P < 0.01, one-way ANOVA).

At present, inactivated H9N2 vaccines have been used in ducks to prevent and control H9 AIVs infection in many farms in China. Previous research found that H9N2 duck isolates could only replicate in ducks by the intravenous infection, could not infect and replicate in ducks efficiently by an intranasal infection route (Teng et al., 2015). Similar results were observed in our study, we found that Pekin ducks and Mallard ducks were generally resistant to H9N2 virus infection. Since various genotype H9N2 viruses studied here failed to replicate in Pekin and Mallard ducks, inactivated H9N2 vaccine may be unnecessary to be used in Pekin and Mallard ducks. The H9N2 AIVs infection caused no clinical signs in Muscovy ducks under experimental conditions. However, co-infection of H9N2 virus and other pathogens is a common case in clinical, resulting in reduced egg production or high mortality. For this reason, vaccination to Muscovy ducks should be taken into comprehensive consideration.

In conclusion, the effective replication of G57 H9N2 AIVs in Muscovy ducks implies that this species should be paid more attentions to their roles in the evolution and transmission of AIVs. Our findings provide guidance for the prevention and control of H9N2 AIVs in waterfowls.

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