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Enhanced replication of swine influenza viruses in dexamethasone-treated juvenile and layer turkeys

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ABSTRACT

Frequent transmission of swine influenza viruses (SIVs) to turkeys has been reported since 1980s. Experimental studies also showed that SIVs can infect turkeys with varying replication and transmission efficiency depending on the strain. However, host factors involved in infection/replication efficiency remain unclear. To investigate whether the immune status of turkeys might play a role in the susceptibility of turkeys to SIVs, we studied the replication efficiency of two recent SIVs (human-like H1N2 and triple reassortant (TR) H3N2) in dexamethasone-treated turkeys. The viruses were inoculated intranasally in both dexamethasone-treated and untreated control juvenile and layer turkeys. Amount of virus shedding was monitored at 2, 4, and 7 days post inoculation (DPI). Additionally, passage of both viruses was attempted in dexamethasone-treated 4-week-old turkeys. In both juvenile and layer turkeys, we were able to detect human-like H1N2 SIV only from dexamethasone-treated turkeys and no virus was detected in untreated birds. The virus shedding of the TR H3N2 SIV was also consistently higher ($\approx$1 Log_{10} EID_{50}/ml) in dexamethasone-treated birds in both tracheal and cloacal swabs compared to untreated birds. Virus passage in dexamethasone-treated turkeys was successful up to the second passage and no virus was recovered from the third passage. These results show that potential immunosuppression due to dexamethasone treatment may enhance the transmission and adaptation of SIVs in turkeys through enhancement of virus replication, prolonged virus shedding, and possible decrease of infectious dose required to initiate infection.

1. Introduction

Influenza virus, a member of the family Orthomyxoviridae, contains segmented single stranded RNA genome of negative polarity. Influenza viruses are classified into 3 types (A, B, and C) based on their major internal proteins, the NP and matrix (M) proteins (Palese and Shaw, 2007). Type A influenza viruses can infect both mammals including humans, pigs, horses, sea mammals and domestic and wild birds. Influenza infections in the wild aquatic birds are usually asymptomatic and the evolutionary rate of influenza virus in these natural reservoir appears to be low (Suarez, 2000). However, introduction of influenza viruses into a new host species initiates rapid evolution to adapt to the new host with the selection of viruses with highest replication and transmission potential (Munier et al., 2010).

Among the avian species, turkeys have been found to be susceptible to many subtypes of influenza viruses including avian influenza viruses of domestic and wild birds

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(Alexander et al., 1978; Alexander and Spackman, 1981; Bos et al., 2010; Giannecchini et al., 2006) and mammalian-origin influenza viruses (Berhane et al., 2010; Mohan et al., 1981; Suarez et al., 2002; Tang et al., 2005). Of the mammalian influenza viruses, swine influenza viruses (SIVs) are the most frequently isolated viruses from turkeys (Pillai et al., 2010). Due to close proximity of both swine and turkey production in the U.S., SIV infection in turkeys has been related to the prevalence and predominant strains circulating in the swine population. For instance, the classical swine H1N1 SIV had been the predominant subtype circulating in the U.S. swine population till the late 1990s and was the most frequently reported influenza subtype transmitted to turkey populations (Andral et al., 1985; Ficken et al., 1989; Ludwig et al., 1994; Mohan et al., 1981). Similarly, a few years after the emergence and predominance of the triple reassortant (TR) H3N2 SIVs in the swine population in the U.S., it has been frequently transmitted to turkey populations causing severe egg production problems (Webby et al., 2000). Experimental studies in turkeys further confirmed that turkeys seem to be naturally susceptible to many SIVs (Yassine et al., 2007). Although experimental infections of TR H3N2 (Pillai et al., 2009; Yassine et al., 2007) and other mammalian origin influenza viruses indicated that all mammalian influenza viruses tested (unpublished data) can replicate in turkeys as indicated by virus detection and/or seroconversion, these viruses vary in replication efficiency. Some mammalian influenza strains induced seroconversion only without detectable virus while others extensively replicated especially in the oviduct of layer turkey hens (Pillai et al., 2009; Yassine et al., 2007).

Several studies have been initiated by our group to identify both the viral and host factors affecting the interspecies transmission of SIVs between swine and turkeys. Studies of the potential molecular determinants indicated the importance of the surface glycoproteins, especially the HA protein in the TR H3N2 virus transmission in turkeys (Yassine et al., 2010, 2011). Studies were also conducted to determine the differences in the distribution of influenza virus receptors (avian and human type) in avian species including turkeys. Though these studies showed that avian species express both types of receptors with variable intensity in different organs, a direct correlation between the influenza receptor distribution and the SIV pathobiology in turkeys could not be established (Pillai and Lee, 2010; Pillai et al., 2009). Hence, other viral, host and environmental components, which remain largely unknown, may influence the transmission and replication of SIVs in turkeys.

The host immune status has been shown to alter the pathogenesis of different bacterial and viral infections (Dobbs et al., 1993; Huff et al., 1998, 2005, 2009). With regard to influenza virus infection, stress was shown to suppress antiviral immunity and diminish resistance to infection in murine influenza viral infections (Dobbs et al., 1993; Sheridan et al., 1998). In this study, the effect of immunosuppression induced by dexamethasone treatment on the transmission and adaptation of two recent SIVs in turkeys was investigated.

2. Materials and methods

2.1. Viruses

The reassortant human-like H1N2 (A/swine/Ohio/FAH10-1/2010) and the TR H3N2 (A/swine/Ohio/FAH11-2/2010) were recently isolated in our laboratory from swine showing respiratory signs in Ohio. These viruses were passaged once in Madin-Darby Canine Kidney (MDCK) cells for initial isolation and passaged one more time in MDCK cells to make working stocks.

2.2. Dexamethasone treatment of juvenile and layer turkey hens

To evaluate the effect of dexamethasone treatment, two groups of both 5–6 week-old juvenile and 48–52 week-old layer turkey hens (4 birds/group) were used. All birds were weighed, and then the birds in group 1 in both ages received three alternative doses of dexamethasone via the intramuscular route with 2 mg/kg and 1 mg/kg per body weight for juvenile and layer turkeys, respectively. The birds of group 2 in both ages were kept as untreated control group. At 4 and 7 days after the last day of dexamethasone treatment, birds were weighed and 2 birds were euthanized. Their spleen, heart and liver in addition to bursa of Fabricius in juvenile turkeys were weighed and the organs relative weights were calculated (Huff et al., 1998).

2.3. Infection of recent SIVs in dexamethasone-treated juvenile and layer turkey hens

For each SIV strain tested; two groups of both 5–6 week-old juvenile and 48–52 week-old layer turkey hens (6 birds/group) were used. All birds were weighed and the first group in both ages was treated with dexamethasone as mentioned above and the other group served as the untreated challenge control group. All birds (dexamethasone-treated and untreated groups) were challenged with 10^6 $TCID_{50}$/0.5 ml of the reassortant H1N2 or the TR H3N2, through the intranasal route one day after the last dexamethasone treatment. All birds were kept in negative pressure poultry isolators. Tracheal and cloacal swabs were collected from the infected birds at 2, 4, and 7 days post inoculation (DPI) in 2 ml of phosphate-buffered saline (PBS) containing gentamycin (10 μg/ml). At 4 and 7 DPI, 2 birds/group were weighed, euthanized and tissues were collected for virus detection. Tissue samples included tracheal tissue, lung, and oviduct in layer turkeys. Additionally organ relative weights were calculated for further confirmation of the effect of dexamethasone in the infection groups. The remaining birds from all groups were bled at 14 DPI and the hemagglutination inhibition (HI) titers were determined.

Swab samples were vortexed and then centrifuged at 2000 rpm for 10 min at 4 °C to pellet the debris. Tissues were homogenized in sterile PBS (1:5 ratio W/V) and then centrifuged at 2000 rpm for 10 min at 4 °C to pellet the tissue debris. The RNA was extracted from 100 μl of the swab samples and tissue supernatants using a Viral RNA