Do We Need Better Influenza Vaccine For Ohio Turkeys?

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ABSTRACT

Since 2003, triple reassortant (TR) swine H3N2 virus have been detected in the U.S turkey population including Ohio flock. The virus has been isolated even from birds that were vaccinated with the currently available swine- and avian- origin vaccines. To better understand this newly emerged TR H3N2 virus that contains gene segments from human, avian and swine origins, we undertook genetic, biologic and antigenic characterization of selected H3N2 viruses isolated from different species in different years. Phylogenetically, all turkey isolates were closely related and grouped together with the TR swine isolates including the vaccine strain. However, the avian-origin vaccine strain shared less than 80% sequence homology with the turkey isolates for the HA1 protein which is directly related to humoral protective immunity. Critical amino acid changes were also detected in the HA1 protein between turkey isolates and swine or avian origin vaccine strains. Our in vivo study demonstrated the successful establishment and replication of H3N2 viruses in turkeys, both in upper respiratory and intestinal tracts. These viruses also transmitted efficiently to the contact control cage mates. Antigenically, all turkey isolates were closely related to each other. However, the turkey isolates showed little or no cross-reactivity with the avian origin or swine origin vaccine strains. These results call for the re-evaluation of currently available vaccines being used in turkey flocks. Our research is currently focused on developing an effective vaccine that can also be used to differentiate vaccinated and infected flocks using an H3N2 virus isolated from Ohio turkeys.

INTRODUCTION

Avian influenza (AI), commonly referred to as bird flu is caused by viruses of the family Orthomyxoviridae. These viruses are segmented single stranded RNA viruses. They have been divided into subtypes A, B and C based on the antigenic properties of two of their internal proteins, the matrix protein and nucleoprotein (1).Type A influenza virus which causes disease in birds has been divided into sixteen hemagglutinin and nine neuraminidase subtypes based on serologic analysis (2). Individual viruses are host-specific depending upon the receptor availability in the host for binding of the HA protein. Turkeys are susceptible to a broad range of influenza viruses including different subtypes of swine influenza viruses. From 2003, a triple reassortant (TR) H3N2 virus that contains gene segments from human, avian and swine origin has been causing serious problems in turkeys in North America. Even turkey flocks that were vaccinated with swine or avian H3 subtype vaccines were not optimally protected. Also, it has been suggested that the virus is evolving in turkeys (3, 4). Hence, to detect the emergence of new strains that may be potentially pathogenic to poultry and humans and to develop effective vaccines, it is important to characterize antigenically and genetically the different H3 subtype viruses and study their pathogenesis. In our present study, we undertook genetic and antigenic characterization of different lineages of H3N2 viruses. We also compared the pathogenecity and transmission characteristics of selected isolates.

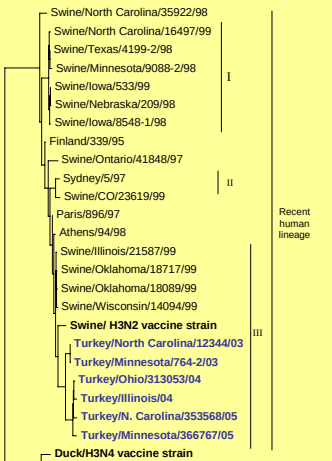

Fig 1. Phylogenetic tree based on nucleotide sequence of the H3 genes. The tree was generated by the maximum parsimony method with the PAUP 4.0 b10 program. The tree is mid-point rooted.

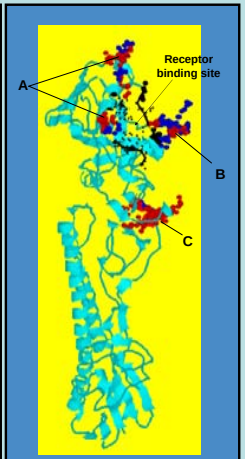

Fig 2. Locations of amino acid changes at the antigenic sites between swine vaccine strain and turkey isolate (TK/IL/04) are labeled in the ribbon diagram of the monomer of HA protein. Color scheme: Black - receptor binding site; Red - conserved amino acids between the two molecules; Blue - amino acid changes at the previously known antigenic sites (A, B, and C).

MATERIALS AND METHODS

Viruses: The virus isolates used in this study were obtained from the repository of the Southeast Poultry Research Laboratory (SEPRL) or Food Animal Health Research Program (FAHRP). Allantoic fluid stocks of the viruses were passaged one or two additional times in embryonating chicken eggs (ECE) to make working stocks of the virus.

Production of hyper-immune sera: For raising antisera, viruses were grown in 10-day old ECE and the infectious allantoic fluid was inactivated with 0.1% beta-propionolactone. One ml of the inactivated virus was mixed with 2.3 ml of montanide ISA adjuvant to produce an oil-emulsion vaccine. 0.5 ml of the vaccine was injected sub-cutaneously into the neck and 0.5 ml into the breast muscle of 2-week-old chickens or turkeys and booster vaccinated at 3-week after first vaccination.

Cross hemaglutination inhibition (HI) test: Cross HI tests were done on micro-titer plates using polyclonal antibodies generated against the selected viruses, 4 HA units of homologous and heterologous virus as antigens and one percent turkey red blood cells.

Antigenic and genetic relatedness: Antigenic relatedness among the different viruses was evaluated using the Hemagglutination Inhibition (HI) test. The antigenic relatedness value was calculated based on Archetti and Horsfall formula (R = square root of (r1 x r2)., where r1 = heterologous titer 2/ homologous titer 1 and r2 = heterologous titer 1/ homologous titer 2). Pairwise sequence alignments were performed with Megalign program (DNASTAR) to determine sequence similarity among the viruses. Phylogenetic analysis of the H3 gene segments of the different viruses was generated using the Maximum parsimony method with the PAUP 4.0 b 10 software. Protein modeling was performed using the RasMol program (version 2.7.1).

Pathogenesis study in turkeys: Three-week-old turkeys were used in the present study. Eleven birds were inoculated with 0.2 ml of 10^6 50% egg infectious dose (EID_{50}) of the virus through intrachoanal route. Four birds were introduced as contact controls one day after infection. Tracheal and cloacal swabs were collected from all the birds on days 2, 4 and 7 days post-infection (1, 3, 6 days for contact controls). Individual swabs were placed in 1.5 ml of sterile phosphate buffered saline (PBS) containing gentamycin (1mg per 100ml). On the third day post-infection, four infected turkeys were euthanized and tissues (lower part of trachea, lungs, kidney, bursa, cloaca, spleen, portions of small and large intestine, caecal tonsils) were collected for histopathology. All the birds were bled for serum collection on days 7 and 14 and euthanized on 14th day post-infection. Viral RNA was extracted from the tracheal and cloacal swabs and subjected to real-time reverse-transcriptase PCR (RRT-PCR) for virus detection and quantitation.

Table 1. Pathogenesis and transmission of H3N2 viruses in turkeys

			VIRUS TITERS[a]			HI TITERS[c]	
VIRUS USED	GROUP	SWAB TYPES	2 DPI[d]	4 DPI	7 DPI	7 DPI	14 DPI
TK/MN/366767/05	INFECTED	TRACHEAL	1.9 + 0.6(11/11)[b]	4.1+ 0.9(7/7)	2.3 + 0.3(7/7)	2.4 + 1.1	1.4 + 0.5
		CLOACAL	1.4 + 1.6(11/11)	2.2 + 0.7(7/7)	2.0 + 0.2(7/7)		
	CONTACT CONTROL	TRACHEAL	2.1 + 0.3(4/4)	4.1 + 0.6(4/4)	2.8 + 0.7(4/4)	1.8 + 0.5	1.8 + 0.5
		CLOACAL	1.6 + 0.8(4/4)	1.7 + 0.4(4/4)	2.2 + 0.3(4/4)		
TK/NC/353568/05	INFECTED	TRACHEAL	1.8 + 0.3(11/11)	1.5 + 0.8(1/7)	0(0/7)	8.6 + 0.5	9.6 + 1.1
		CLOACAL	2.6 + 0.3(11/11)	1.5 + 0.7(6/7)	1.9 + 0.1(7/7)		
	CONTACT CONTROL	TRACHEAL	1.4 + 0.3(4/4)	0(0/4)	0(0/4)	3.8 + 0.5	6.3 + 2.6
		CLOACAL	2.4 + 0.1(4/4)	1.9 + 0.1(4/4)	2.0 + 0.1(4/4)		

[a] RRT-PCR titers expressed as $\log_{10}$ EID_{50} per 0.2 ml of swab fluid
[b] Number of positive swabs / Total number of tested swabs
[c] Log_2 HI titer of the antisera + standard deviation
[d] DPI = days post infection

Table 2. Antigenic and genetic relatedness among H3 viruses

Antigen \ Antisera	Swine/ NC/03	Turkey NC/03	Turkey OH/04	Turkey/ IL/04	Turkey/ NC/05	Turkey/ MN/05	Chicken/ NY/98	DK/ H3N4
Swine H3N2 vaccine strain	100.0	94.5	92.8	92.5	91.3	91.9	80.3	80.9
Turkey/North Carolina/03	0.0	100.0	95.4	95.1	93.6	94.5	80.0	80.0
Turkey/Ohio/04	3.1	8.8	100.0	98.8	97.1	98.0	78.6	78.8
Turkey/Illinois/04	2.2	70.7	100.0	100.0	96.8	97.7	78.3	78.6
Turkey/North Carolina/05	4.4	70.7	70.7	100.0	100.0	95.9	78.3	78.6
Turkey/Minnesota/05	3.1	70.7	70.7	100.0	70.7	100.0	78.3	78.6
Chicken/New York/98	0.0	0.0	0.0	0.0	0.0	1.1	100.0	95.4
Duck/H3N4	0.0	0.0	0.0	0.0	0.0	0.2	100.0	100.0

% genetic relatedness
Antigenic relatedness value, R

RESULTS AND DISCUSSION

Phylogenetic analysis grouped the recent turkey H3 isolates under antigenic subgroup III of recent human lineage together with swine-origin TR H3N2 viruses (Fig 1). The phylogenetic tree revealed that the recent turkey isolates show some differences in HA gene from the swine H3N2 vaccine strain and turkey isolates of 2003, indicating that the turkey H3N2 viruses are genetically evolving. Also, the recent turkey isolates belong to a group distinct from the duck (H3N4) vaccine strain and the chicken influenza viruses.

Experimental infection of turkeys with the two recent turkey H3N2 isolates: TK/NC/353568/05 and TK/MN/366767/05, showed that these viruses replicate well in turkeys and are transmitted efficiently to the contact control birds as shown by the high virus titers in the trachea and cloaca determined by RRT-PCR (Table 1). All the experimental and contact control birds sero-converted. Histopathologically, minor lesions were observed in the trachea, lung, and bursal tissues (Fig 3). Birds in both groups showed hypertrophy of the tracheal epithelia and some birds infected with TK/MN/366767/05 virus had lymphocyte depletion in the bursal follicles. Immunohistochemistry staining results correlated with the lesions observed and minor stainings were observed mainly in the tracheal epithelium and some positive staining in the epithelia of the bursa of TK/MN/366767/05 infected birds. Studies are being conducted to evaluate the pathogenecity and transmission characteristics of these viruses in other poultry species such as chicken and ducks.

The swine H3N2 vaccine strain showed 91.3% and 91.9% genetic relatedness respectively with the recent turkey isolates, TK/NC/05 and TK/MN/05 (Table 2). A decreased genetic relatedness was seen over the years from 2003 to 2005. Similarly, the recent turkey isolates showed very low genetic relatedness to the chicken H3 isolate and the duck (H3N4) vaccine strain. These suggest that the virus is slowly evolving in turkeys.

Cross-HI test was performed to evaluate the antigenic similarity among the different H3N2 isolates. It was found that the turkey isolates were antigenically similar (Table 2). TK/NC/05 and TK/MN/05 shared 100% antigenic relatedness (R) to TK/IL/04. Similarly, the R value for TK/IL/04 and TK/OH/04 was 100%. Although the genetic similarity between TK/IL/04 and swine vaccine strain is still considered high (93%), the alignment of HA1 amino acid sequences of the two viruses showed critical amino acid changes at the antigenic sites (Fig 2). These changes would explain the low degree of relatedness between the two viruses in antigenic studies. The R value among the swine and the avian vaccine strains and the recent turkey isolates was below 10% indicating that these belong to different antigenic subtypes. This raises the question whether the current vaccine strain can optimally protect turkeys in a heterologous infection. The antigenetic and genetic disparity of the vaccine strains from the currently circulating virus strains and the fact that these viruses are well adapted to turkeys call for re-evaluation of the current vaccines being used in field and the identification and use of a better vaccine.

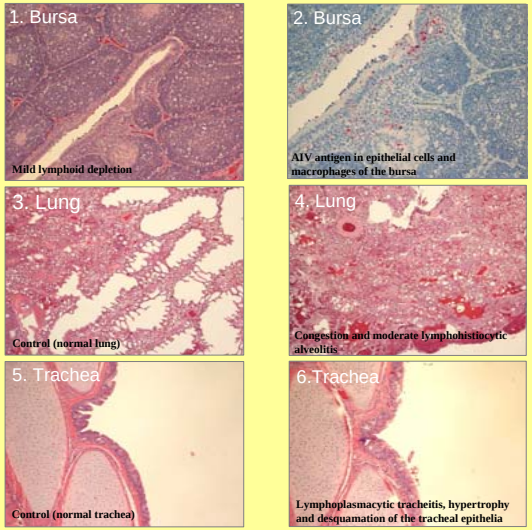

Fig 3. Photomicrographs of hematoxylin and eosin stained tissue sections or sections stained by IHC methods to demonstrate viral antigen in TK/MN/05 (1 & 2) and TK/NC/05 (4 & 6) infected turkeys.

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