Vaccine (2008) **26**, 966–977

available at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/vaccine

Genetic and antigenic relatedness of H3 subtype influenza A viruses isolated from avian and mammalian species☆

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Received 3 July 2007; received in revised form 16 November 2007; accepted 21 November 2007
Available online 26 December 2007

KEYWORDS
H3N2 triple reassortants;
Vaccine;
Turkeys

Abstract In 2004, we isolated triple reassortant H3N2 influenza viruses from turkey breeder hens in Ohio and Illinois. The Illinois flock was vaccinated twice with an inactivated H3N2 vaccine containing a swine origin virus before the outbreak. Additionally, a commercial inactivated vaccine containing an H3N4 virus of duck origin is being used in some turkey breeders. This prompted us to initiate a comparative study on the antigenic and genetic relatedness of various H3 subtype influenza viruses isolated from turkeys, ducks, pigs and humans. The antigenic relatedness between the different viruses was evaluated with the Archetti and Horsfall formula, while nucleotide genetic similarities were calculated using pairwise alignments. Results obtained indicated a high degree of antigenic (>90%) and genetic (>99%) similarities among the turkey-origin H3N2 viruses. However, the turkey viruses were antigenically distantly related to the swine-origin vaccine virus (<30%), although they had approximately 95% genetic similarity in the HA1 gene. Additionally, major genetic and antigenic changes were observed between the turkey viruses and the H3N4 duck vaccine virus as well as the H3N2 human virus. Such genetic and antigenic differences between the turkey-origin viruses and other H3 subtype viruses including vaccine strains could be the reason for the failure in protection in the Illinois turkey breeders vaccinated with swine origin virus. This also emphasizes the importance of using viruses for vaccines that are antigenically similar to the field strains.

☆ The failure of protection in turkeys vaccinated with a swine H3N2 virus against a similar subtype virus prompted us to initiate a study, which the first on antigenic and genetic relatedness of H3N2 triple reassortant viruses isolated from turkeys and swine as well as other H3 subtype influenza A viruses of mammalian and avian origin.

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doi:10.1016/j.vaccine.2007.11.094

Introduction

Influenza A viruses belong to the family *Orthomyxoviridae* that includes four other genera: influenza B, influenza C, Isavirus and Thogotovirus [1]. The influenza A viruses are unusual in this group because they can be highly infectious pathogens to a variety of mammalian and avian species [2,3]. They are usually divided into subtypes based on the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). So far, sixteen HA and nine NA subtypes have been identified [4]. Generally, individual viruses are host specific [3,5]. Although the molecular basis of host range restrictions are not completely defined, the compatibility between the HA protein of the virus and its corresponding receptor, sialic acid, on the host cell is known to determine in part the infection of the virus in a specific host [6,7]. Avian influenza A viruses are generally thought to preferentially bind the *N*-acetylneuraminic acid-α2,3-galactose (NeuAcα2,3Gal) form of sialic receptors which is the predominant form of glycosylation of proteins found in birds [8,9]. However, human influenza A viruses preferentially bind to NeuAcα2,6Gal sialic acid receptors, which is the predominant form of glycosylation expressed on proteins found in the upper respiratory tract in humans [8,9]. Pigs, however, express substantial amount of both forms of sialic acids, and it is believed that both avian and human influenza viruses can attach to the appropriate receptor and infect pigs [7], potentially allowing them to serve as ''mixing vessels'' for the generation of reassortant influenza viruses [10,11]. Before 1998, classic swine H1N1 lineage viruses were the dominant cause of influenza in pigs in the United States (U.S.) [12]. In 1998, a new lineage of H3N2 influenza viruses was isolated from pigs experiencing severe influenza-like illness on a farm in North California [13,14]. Additional outbreaks in pigs were also reported in the same year in Minnesota, Iowa and Texas [15]. Genetic analysis of these viruses showed that they are reassortant viruses [13], with gene segments of swine, human and in some cases avian influenza origin [13,14]. Triple reassortant viruses genes were derived from human (HA, NA and PB1), swine (M, NS and NP) and avian (PA and PB2) viruses [14]. The triple reassortant virus has now become the predominant strain found in U.S. swine, although classical H1N1 viruses and other reassortant viruses (H1N2, H3N1) are also isolated [16].

Turkeys are susceptible to a wide range of influenza A viruses and serve as an important host for these viruses, and as a major exception to the host range restriction rule, are routinely infected with swine-like influenza viruses [4]. Influenza infections in turkeys range from asymptomatic to severe disease, including respiratory tract disease, depression, drop in egg production and high mortality [4,17]. Most infections of turkeys with swine influenza are associated with drop in egg production but with no other clinical signs [18]. Natural and experimental infections of turkeys with classic H1N1 swine viruses were reported for the first time by our lab in 1978 and 1981 [19,20]. In 2004, we isolated two triple reassortant H3N2 viruses from turkey breeder hens in Ohio [18] and Illinois. Infected turkeys showed no clinical signs but underwent complete cessation of egg production. Interestingly, the Illinois turkey flock was vaccinated twice with an inactivated H3N2 virus isolated from swine in North Carolina in 2003. The failure of protection in turkeys vaccinated with a swine H3N2 virus against a similar subtype virus, prompted us to initiate a study on antigenic and genetic relatedness of H3N2 viruses isolated from turkeys and swine. Additionally, an H3N4 duck virus (A/mallard duck/Minnesota/79/79) that is being used as a vaccine for turkey breeders, and a recent H3N2 virus isolated from humans in Ohio in 2006 were also included in this study since the HA and NA genes in the triple reassortant viruses are of human lineage. Antigenic relatedness assessments were done based on the cross reactivity of the different viruses in the hemagglutinin inhibition (HI) and virus neutralization (VN) tests, while genetic comparisons were done based on gene sequences and phylogenetic analysis.

Materials and methods

Viruses

Six viruses were included in this study (Table 1): three turkey viruses, one swine, one duck and one human virus. Two turkey viruses were isolated in our lab, A/turkey/Illinois/04 (H3N2) and A/turkey/Ohio/313053/04 (H3N2) from turkey tracheal swabs on Madin—Darby Canine Kidney (MDCK) cell line maintained in Opti-MEM minimum essential medium (Invitrogen, Grand Island, NY) containing 0.5 μg/ml trypsin. The samples were passaged twice in MDCK cells and then used to inoculate 9—10 days old specific pathogen free (SPF) embryonated chicken eggs (ECE) to make a working stock. One turkey virus, A/turkey/North Carolina/03 (H3N2) (passaged twice (P2) in MDCK), and one swine virus (vaccine strain), A/swine/North Carolina/03 (H3N2) (unknown passage number), were generously provided by Dr. Eric Gonder (Goldsboro Milling Co. Goldsboro, NC), and were propagated in 9—10 days old ECE to make a working stock. The duck vaccine virus, A/mallard duck/Minnesota/79/79 (H3N4) (unknown passage number), was obtained from

Table 1 Viruses included in the study

Virus	Origin	Year of isolation	Source
A/turkey/Illinois/2004 (H3N2)	Turkey	2004	FAHRP/OSU
A/turkey/Ohio/313053/2004 (H3N2)	Turkey	2004	FAHRP/OSU
A/turkey/North Carolina/2003 (H3N2)	Turkey	2003	Dr. Eric Gonder
A/swine/North Carolina/2003 (H3N2)	Swine	2003	Dr. Eric Gonder
A/mallard duck/Minnesota/1979 (H3N4)	Duck	1979	Lohmann Animal Health
A/human/Ohio/2006 (H3N2)	Human	2006	Ohio Department of Health