Letter to the Editor

Increasing Prevalence of Unique Mutation Patterns in H5N1 Avian Influenza Virus HA and NA Glycoproteins from Human Infections in Egypt

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Highly pathogenic avian influenza H5N1 virus (HPAIV) continues to be a candidate of a further influenza virus pandemic. Egypt is the country worst affected by human cases of HPAIV H5N1 infection in 2009. Increased infection of preschool children and decreased mortality rates suggested subtle changes in the epidemiology of the infection. Among other factors, the evolution of several conspicuous viral genetic markers in the HA and NA genes of HPAIV H5N1 viruses of human cases from Egypt and their putative influence on biological virus characteristics described here may contribute to this situation.

From March 2006 to April 9, 2010, 34 human fatalities out of 109 cases of human highly pathogenic avian influenza virus (HPAIV) H5N1 infection have been reported from Egypt [1, 2]. Transmission has mainly been linked to close contact with infected poultry although limited human-to-human transmission within several family clusters was proposed [3]. This has caused considerable concern about the pandemic potential of HPAIV H5N1 in Egypt.

Egypt is one of the countries most severely affected by HPAIV H5N1 in the world in 2009. Despite nationwide poultry vaccination campaigns, endemicity persists and caused 39 human H5N1 infections in 2009; already 19 occurred in 2010 [1, 2]. Most of the human cases in 2009 were reported in toddlers and children less than 6 years old ($n = 32$; 74%). All but one of them recovered. In general, there seems to be a decreasing tendency in both age of the reported human H5N1 patient and fatality rates (2006: 10/18, 56%; 2007: 9/25, 36%, 2008: 4/8, 50%, 2009: 4/39, 10%), especially last year [1]. This has been observed earlier and changes of the HPAIV H5N1 strains infecting humans were suggested which favor a less pathogenic, and therefore

clinically unremarkable, course of infection correlating with decreasing age of the patient [4, 5].

When analyzing GenBank data of HA gene sequences ($n = 310$; 280 used for analysis) of HPAIV H5N1 viruses from Egypt phylogenetically, sequences from human cases of year 2009 form two separate clusters together with a number of poultry viruses, mainly from ducks isolated from several Egyptian provinces (see Figure 1 in Supplementary Material available online at doi:10.1155/2010/450823, red and blue branches; 6-8). Viruses within one of these cluster (Supplementary Figure S1, depicted by red branches) show a conspicuous deletion of amino acid 129 ($\Delta 129$) in the hemagglutinin (H) protein (H5 numbering, Table 1). S129 is part of the receptor binding site and also belongs to an antigenic site [6, 7]. This deletion is not present in the parent H5N1 A/Goose/Guangdong/1/96 virus [8] or in the H5N1 viruses originally introduced into Egypt in 2006 [9, 10]. Isolates from nonhuman mammals (felids and dogs; GenBank, $n = 22$) likewise do not reveal the 129-deletion. Interestingly, $\Delta 129$ viruses accounted for the majority of sequenced human infections in Egypt in 2009. This is

TABLE 1: Marker-like mutations in the hemagglutinin and neuraminidase proteins of highly pathogenic avian influenza virus H5N1 in human and poultry isolates from Egypt compared to other countries with human cases as retrieved from public data bases.

Country	Hemagglutinin			Neuraminidase		
	Isolates	S/L/V129 ¹	Δ 129	Isolates	A/S/V/D46	V/L/S/F339
Vietnam	249 ²	56/193/0	0	160	120/36/4/0	0/0/160/0
Indonesia	162	141/21/0	0	44	43/1/0/0	0/0/40/4
Thailand	161	1/159/1	0	70	70/0/0/0	0/0/70/0
China	475	355/120/0	0	201	145/1/55/0	0/0/199/2
Nigeria	174	172/2/0	0	34	34/0/0/0	0/0/34/0
Egypt	310	202/38/0	70	134	86/0/0/ 48	3/2/81/ 48
Total	1531	927	70	643	498/38/59/ 48	3/2/584/ 54

¹ Variations of amino acid residues at the indicated position of the mature HA5 (D₁Q₂I₃...) and the nascent NA 1(M₁N₂P₃...).

² Numbers of isolates in GenBank.

a significant increase in prevalence among humans: whereas the variant was not detected in 2006 (0/16), human isolates in the years 2007–2009 revealed the Δ 129 deletion in 21% (5/23), 33% (2/7), and 85% (21/25) of sequenced cases, respectively. Interestingly, we found a similar deletion in the H protein at the corresponding position of all human seasonal H1N1 and H3N2 viruses. Previously, it was reported that a mutation at this site (S129L) decreased virulence of HPAIV H5N1 in mice [6]. Two further mutations concurrently present in the HA of Δ 129 HPAIV H5N1 viruses are noteworthy: S120N is unique to Egyptian H5N1 strains, and I141T is also seen in all human seasonal H1N1 and H3N2 viruses. All three mutations are located in the globular head of the HA monomer as shown in Supplementary Figure 2 [6, 7]. Also, the neuraminidase (NA) protein of Egyptian H5N1 viruses of human origin is carrying two unique mutations with as yet undefined functions (D46 and F339, N1 numbering, Table 1) which became grossly enriched in human isolates since 2007 (2006: 0/7, 0%; 2007: 14/24, 58%; 2008: 5/7, 71%; 2009: 23/24, 96%). In parallel, an increasing prevalence of Δ 129 HPAIV H5N1 viruses was also observed in sequenced isolates from poultry in Egypt: in 2006, this variant was not observed among 38 cases sequenced and reported in GenBank while Δ 129 isolates were detected in poultry in 2007 (6/66, 9%), 2008 (4/69, 6%), and 2009 (32/66, 49%). A similar increase in prevalence in poultry is observed for the two NA (D46 and F339) mutations (from 0% in 2006 to 13% [5/39] in 2008; no public NA sequence data available for 2009). It is interesting to note that most of the Δ 129 mutants of the year 2009 originated from domestic waterfowl (23 out of 32, 72%), birds which are considered to be more resistant to disease [11]. These 23 human-like viruses harboring the Δ 129 deletion are derived from a group of 32 sequenced isolates from waterfowl compared to 9 out of 33 sequenced isolates from chickens and turkeys (Fisher's exact test, $P < .0005$). These observations may imply a higher risk of human infections by contact to infected waterfowl. Also, transmission via waterbodies contaminated by subclinically infected waterfowl might explain the higher infection rate of preschool children observed in 2009 (19/22). Children in rural areas of the Nile valley where raising of backyard ducks is common come into close contact to both the ducks and small water ponds during play. Whether, in addition,

these mutations account for biological variations like a less pathogenic phenotype causing milder or asymptomatic courses of infection and/or more efficient transmission of such viruses to or even between humans has to be addressed experimentally using animal models and viruses modified specifically by reverse genetics. Host tropism and pathogenicity of influenza A viruses are polygenic traits and genes encoding the replication complex and the NS-1 protein are involved in host switch and adaptation processes; however, data on these genes are lacking so far for the Egyptian human cases. It can of course not be excluded that further factors such as the immune status of the hosts, or a higher public awareness, earlier detection, and better treatment of human cases in Egypt are responsible for the observed epidemiological changes in human H5N1 cases in Egypt.

Despite intensified public awareness for the new pandemic H1N1 influenza virus, the looming danger of an incursion of HPAIV H5N1 viruses into the human population on a broad front has not ceased in the meantime. Defining the frontier of HPAIV H5N1 transmission from poultry to humans remains one of the most important challenges of HPAIV-control strategies. Based on the observations presented, we hypothesize that the Δ 129 variant appears to represent a valuable molecular marker for further studying epidemiology of HPAIV H5N1 infections in Egypt. Further experiments using the tools of reverse genetics are needed in order to clarify the role of these genetic markers [12].

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