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SHORT COMMUNICATIONS

Serological surveillance reveals widespread influenza A H7 and H9 subtypes among chicken flocks in Egypt

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Abstract Multiple avian influenza viruses' subtypes are circulating worldwide possessing serious threat to human populations and considered key contributors to the emergence of human influenza pandemics. This study aimed to identify the potential existence of H7 and H9 avian influenza infections circulating among chicken flocks in Egypt. Serum samples were collected from chicken flocks that experienced respiratory distresses and/or variable mortality rates. H7 and H9 virus infections were screened by haemagglutination inhibition assay using chicken erythrocytes. Serum samples were collected from 9 broiler, 12 breeder and 18 layer flocks. Out of 1,225 examined sera, 417 (34 %) from 14 flocks and 605 (49.4 %) from 21 flocks were found positive for H7 and H9, respectively. Prevalence of both H7 and H9 antibodies were higher in layer followed by breeder then broiler flocks. Special consideration should be paid to control influenza viruses in Egypt, as pandemic influenza strains may develop unnoticed given the presence of subclinical infections, and the possibility of re-assortment with the prevailing endemic H5N1 virus strains in Egypt do exist.

Keywords Chicken · H7 · H9 · Serology

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Introduction

Avian influenza (AI) is a highly contagious respiratory disease of poultry caused by influenza A viruses of the family *Orthomyxoviridae*. Influenza A viruses are classified into 17 haemagglutinin (HA) subtypes and 10 neuraminidase subtypes (Tong et al. 2012). The disease constitutes a major threat to poultry industry worldwide (Swayne and Halvorson 2003). Influenza A virus infections in poultry comprise two forms: highly pathogenic AI (HPAI) and low pathogenic AI (LPAI). HPAI viruses cause severe systemic disease with mortality reaching 100 % and are associated with some strains of H5 or H7 haemagglutinin subtypes (Senne et al. 1996). LPAI viruses induce manifestations that range from asymptomatic infection to mild respiratory diseases and drop in egg production, however some LPAI strains can cause higher mortality usually due to the co-infection with the secondary pathogens (Alexander 2000).

HPAI H5N1 is endemic in many countries including Egypt that resulted in severe economic losses in poultry and cross to human population. H5N1 and several H5N2 inactivated vaccines are used in poultry flocks in Egypt in a trial to control the H5N1 infections but none were used for other influenza subtypes.

H7 and H9 subtypes pose similar threat to human as H5 viruses. The mild nature of some strains of these viruses may provide such viruses a great opportunity to become more virulent through surreptitious spread, mutation and/or reassortment

with other subtypes of influenza viruses (Horimoto and Kawaoka 2001; Li et al. 2003; Tweed et al. 2004). Epidemiological data suggests that subclinical or mild diseases are more common than detected and there is an increased need for AIV disease surveillance. The haemagglutination inhibition (HI) test is an accurate, inexpensive and potent test, facts that render it the most commonly used test to determine the presence of antibodies in the serum to an influenza virus (Julkunen et al. 1985; Prince and Leber 2003).

This study was designed to investigate the seroprevalence of AIV H7 and H9 subtypes in different chicken flocks in Egypt and to evaluate the prevalence of the circulating virus subtypes in different chicken flocks.

Materials and methods

Chicken flocks

Serum samples (N=1,225) were collected from different Egyptian Governorates from 39 poultry flocks during the period of February 2009–April 2010. A total of 9 broiler, 12 breeder, and 18 layer chicken flocks were examined. All flocks were vaccinated by either H5N1 or H5N2 inactivated vaccines. Broiler chicken flocks suffered from coughing, conjunctivitis, nasal and ocular discharge, depression, and inappetence. Breeder and layer chicken flocks suffered from coughing, nasal discharge, and 10–30 % drop in egg production. Mortality rates in all flocks ranged from 1 to 5 %. Blood samples were collected by veno-puncture of the wing vein. Sera were separated, heat inactivated at 56 °C and stored at −20 °C until used.

Sera and antigens

Inactivated H7 (VLDIA098 HAG-INFH7) and H9 (VLDIA113 HAG-INFH9) antigens produced in the allantoic cavity of specific pathogen free hens' eggs as well as monospecific positive control antisera for H7 (VLDIA043 HAR-INFH7) and H9 (VLDIA150 HAR-INFH9) were obtained commercially (GD Animal Health Service Deventer, The Netherlands). H7 and H9 antigens and antisera were used in HI assay.

Haemagglutination inhibition

Twofold serial dilutions were performed in 25-μl volumes in 96-well HI plates. Equal volumes of 4HA units of H7, or H9 influenza virus antigen were added to diluted serum samples then a 1 % suspension of chicken erythrocytes was dispensed into each well (Beard 1989). HI titers ≥5 $\log^2$ were considered positive. Samples assayed in duplicates and each assay was validated by comparison with positive and negative chicken control sera as well as back titration of the used virus antigen dilutions.

Statistical analysis

Microsoft Excel and Instat® version 3.00 were used to calculate means, standard errors, and possible titers variations.

Results and discussion

Seroprevalence of H7 and H9 avian influenza viruses revealed field exposure of both subtypes (Tables 1 and 2). The positive flocks comprised 2/9 (22.2 %), 9/18 (50 %), and 3/12 (25 %) for H7 and 1/9 (11.1 %), 13/18(72.2 %), and 7/12 (58.3 %) for H9, in the examined broiler, layer, and breeder poultry farms, respectively (Table 1). In broiler flocks, 35 (13.5 %) and 15 (6.4 %) out of 260 samples were found positive for H7 and H9, respectively (Table 2). In breeder flocks, 105 (29.9 %) and 279 (79.5 %) out of the 351 examined samples were found positive to H7 and H9, respectively, 2/12 flocks were found seronegative for both H7 and H9 (Table 2). In layer flocks, 277 (50.1 %) and 311 (50.7 %) out of 614 were found positive for H7 and H9, respectively (Table 2). The seropositivity of H7 and H9 in chickens in different Governorates in the northern and delta areas (Dakahlia Sharkia and Qalubia) as well as the middle of Egypt (Giza, Fayoum, and Beni-Suef; Table 2) provided an evidence of the circulation of such subtypes in Egypt.

The mild clinical disease in birds with antibody titer to AIV H7 or H9 subtype might indicate that these birds were subjected to mild natural field exposure to low pathogenic strains of H7 or H9 . Broiler flocks were found to be less affected with H7 or H9, only 2/9 and 1/9 flocks were found seropositive to H7 and H9 (Table 1). This may be resulted from the short life span of such birds that lead to lower opportunity of virus exposure than breeder and layer flocks. Some breeder and most of layer as well as one broiler flock were found positive to both H7 and H9 (Table 2).

H7 is less predominant to H9 in Egypt, as 14/29 and 21/39 flocks were found positive to H7 and H9, respectively (Tables 1 and 2). Recently, we isolated H9N2 from broiler flock from Alexandria Governorate in the northern part of Egypt (unpublished data) however, only a broiler flock from Beni-Suef was found seropositive to H9 (Table 2). Interestingly, another recent report for the isolation of H9N2 from quail in Egypt was also reported (El-Zoghby et al. 2012). Meanwhile, the isolation of AIV subtype H7 from Egypt was only recorded from migratory birds (Aly et al. 2010; Soliman et al. 2012), on 2004 (H7N1), 2004, 2005, and 2006 (H7N7), 2006 (H7N9), and on 2007 (H7N3). Interestingly, H7 serological results on backyard from 11 villages in the nearby areas were negative (Aly et al. 2008),

Table 1 Chicken flocks that showed positive seroconversion to H7 and H9

Chicken type	Number of flocks	H7			H9		
		Positive	Negative	Positive %	Positive	Negative	Positive %
Broiler	9	2	7	22.2	1	8	11.1
Breeder	12	3	9	25.0	7	5	58.3
Layer	18	9	9	50.0	13	5	72.2
Total	39	14	25	35.9	21	18	53.8

remaining possible that the virus might spread in the field. To the best of our knowledge, no recent report revealed isolation of H7 from commercial or backyard poultry populations. Historically, LPAIV A/turkey/Egypt/88 (H7N1) was isolated (Khafagy et al. 1992); however, they reported the absence of H7 seropositive sera when testing 6,124 chicken and 92 turkey sera (Khafagy et al. 1995) however, the presence of antibodies against AIV H7 were reported (Afifi et al. 1999).

Egypt is located in the pathway of migratory birds and represents a hinge zone of wild bird migration, where the East Africa–West Asia and Black Sea–Mediterranean

Table 2 Seroconversion to H7 and H9 in serum samples from broiler, breeder and layer chicken flocks from different Egyptian governorates

Governorate	Number of flocks	Sample No	H7				H9			
			Positive	Negative	Positive %	Mean Positive	Positive	Negative	Positive %	Mean Positive
Broiler										
Sharkia	1	35	–	35	0	–	–	35	0	–
Qalubia	4	120	–	120	0	–	–	120	0	–
Giza	1	20	20	–	100	5.1±0.05	–	20	0	–
Fayoum	2	50	–	50	0	–	–	50	0	–
Beni-Suef	1	35	15	20	42.9	5.5±0.16	15	20	42.9	5.3±0.16
Total	9	260	35	225	13.5	5.3±0.11	15	220	6.4	5.3±0.16
Breeder										
Dakahlia	1	22	–	22	0	–	–	22	0	–
Sharkia	1	30	–	30	0	–	–	30	0	–
Qalubia	6	164	20	144	13.0	5.8±0.17	144	20	87.8	6.1±0.06
Fayoum	4	135	85	50	63.0	5.8±0.05	135	–	100	5.5±0.05
Total	12	351	105	246	29.9	5.8±0.11	279	72	79.5	5.8±0.04
Layer										
Dakahlia	4	126	23	103	18.3	5.5±0.14	126	–	100.0	5.9±0.07
Sharkia	2	68	52	16	76.5	5.1±0.05	20	48	29.4	5.5±0.14
Qalubia	4	159	110	49	71.4	5.9±0.17	39	120	24.5	6.6±0.09
Giza	3	101	52	49	51.5	5.5±0.13	81	20	80.1	5.7±0.08
Fayoum	4	140	20	120	14.3	5.1±0.05	45	95	32.1	5.3±0.13
Beni-Suef	1	20	20	0	100	5.1±0.05	–	20	0.0	–
Total	18	614	277	275	50.1	5.3±0.13	311	303	50.7	5.8±0.05
Cumulative										
Dakahlia	5	148	23	125	15.5	5.5±0.15	126	22	85.1	5.6±0.31
Sharkia	4	133	52	81	39.1	5.1±0.05	20	113	15.1	5.5±0.28
Qalubia	14	443	130	313	29.3	5.7±0.12	183	260	41.3	6.0±0.37
Giza	4	121	72	49	59.5	5.5±0.13	81	40	66.9	5.7±0.23
Fayoum	10	325	105	220	32.3	5.5±0.14	180	145	55.4	5.3±0.24
Beni-Suef	2	55	35	20	63.6	5.3±0.13	15	40	27.3	5.4±0.12
Total	39	1,225	417	808	34.0	5.4±0.12	605	620	49.4	5.6±0.15

flyways overlap and large diversity of species migrating to and from South Africa, Europe, and Central Asia were detected in Egypt (Soliman et al. 2012). It was recorded that the migratory birds play an important role in the introduction but not the spread of AIV to other wild and domestic species that are present in their migratory pathways (Feare 2010; Soliman et al. 2012). Increased number of seropositives were observed in farms located within the migratory route of wild birds (Al-Natour and Abo-Shehada 2005). In the current study most governorates, from which serum samples were collected harbour or located in their vicinity, migratory birding areas.

In conclusion, the current investigation assumed that the H7 and H9 viruses may have been introduced into chickens from migratory wild birds. The predominance of H7 and H9 in Egypt may aggravate the situation of AIV in Egypt with possible emergence of reassortant from different AIV subtypes. A strict eradication program is recommended to alleviate such possibility and avoid catastrophic consequences.

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