

Short communication

Active surveillance of avian influenza viruses in Egyptian poultry, 2015

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الترصد الفعّال لفيروسات إنفلونزا الطيور عند الدواجن في مصر، 2015

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الخلاصة: إن ترصد فيروسات إنفلونزا الطيور لدى الدواجن في مصر كان يجري منذ عام 2015. وكانت جميع الفيروسات المكتشفة حتى عام 2011: H5N1، بمعدل انتشار عام 5%. وفي عام 2011 لوحظ أن فيروسات H9N2 تشارك فيروسات H5N1 في التجوال وفي العدوى لنفس المضيفين. ومنذ ذلك الحين ازداد معدل اكتشافها إلى ما يقرب من 10%. وفي فصل شتاء 2014-2015 كان H5N1 يحول بكثافة لدى أسراب الدواجن، ولقد تسبب ذلك في حدوث عدد غير مسبوق من حالات العدوى البشرية. وعلى نقيض ذلك أشار الترصد في الربع الأخير من عام 2015 إلى غياب شبه كامل لـ H5N1 لدى الدواجن في مصر. يجب أن يستمر ترصد فيروسات إنفلونزا الطيور في مصر لمراقبة ما يستجد من تطورات في تجوال H5N1 لدى الدواجن.

ABSTRACT Surveillance for avian influenza viruses in Egyptian poultry has been conducted since 2009. Up to 2011, all the detected viruses were H5N1, and the overall prevalence was 5%. In 2011, H9N2 viruses were observed to be co-circulating and co-infecting the same hosts as H5N1 viruses. Since then, the detection rate has increased to around 10%. In the 2014-2015 winter season, H5N1 was circulating heavily in poultry flocks and caused an unprecedented number of human infections. In contrast, surveillance in the last quarter of 2015 indicated a near absence of H5N1 in Egyptian poultry. Surveillance for avian influenza viruses must continue in Egypt to monitor further developments in H5N1 circulation in poultry.

Surveillance active des virus de la grippe aviaire dans les populations de volailles égyptiennes en 2015

RÉSUMÉ La surveillance des virus de la grippe aviaire dans les populations de volailles égyptiennes est en cours depuis 2009. Jusqu'à 2011, tous les virus détectés appartenaient au H5N1, et la prévalence générale était de 5 %. En 2011, on a remarqué que les virus H9N2 circulaient en même temps et co-infectaient les mêmes hôtes que les virus H5N1. Depuis, le taux de détection a augmenté pour atteindre près de 10 %. Pendant la saison hivernale 2014-2015, le virus H5N1 a considérablement circulé dans les élevages de volailles, entraînant un nombre d'infections sans précédent chez l'homme. À l'inverse, la surveillance au cours du dernier trimestre 2015 a constaté la quasi-absence du H5N1 dans les populations de volailles égyptiennes. La surveillance des virus de la grippe aviaire doit se poursuivre en Égypte afin de déceler les futures évolutions de la circulation du H5N1 dans les populations de volailles.

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Introduction

Since 2006, the highly pathogenic avian influenza H5N1 virus has circulated among domestic poultry in Egypt, causing massive economic losses in the poultry production sector (1). Within a few months of the first wave of H5N1 virus in 2006, the veterinary authorities in Egypt implemented a comprehensive response plan to control the spread of the virus in Egypt; this included increasing public awareness through the media, culling infected poultry, placing restrictions on the movement of live poultry, and applying biosecurity measures and emergency vaccination (2,3). However, the H5N1 virus continued to circulate and it became endemic in 2008, which led to genetic drift of the surface immunogenic glycoproteins (4,5). Accordingly, the Egyptian H5N1 viruses diversified into several subclades (classical 2.2.1, 2.2.1.1, 2.2.1.1a and 2.2.1.2), of which at least two subclades co-circulated between 2008 and 2011 (6–8). The subclades of H5N1 viruses in Egypt are antigenically distinct and most vaccines used are no longer antigenically matched (2,9).

Egypt reported more laboratory-confirmed cases of human infection with avian influenza virus H5N1 to the World Health Organization (WHO) between 2003 and 2015 than any other country (346 cases), with 116 deaths, giving a case fatality rate of 33.5% (10).

Weakly pathogenic avian influenza H9N2 viruses have been isolated from chickens, turkeys and quails in Egypt (11–13). All the H9N2 isolates obtained between 2010 and 2013 were closely related to Middle Eastern H9N2 viruses (12,13). Poultry infected with Egyptian H9N2 viruses showed no clinical illness, except when the infection was complicated by other pathogens (14).

Active surveillance of avian influenza viruses among poultry has been

conducted in Egypt since 2009 (15). The details of the surveillance system and findings from the surveillance have been published before; we previously reported that the average infection rate was about 7.7% between 2009 and 2014 (2,16,17). Here, we provide an update on the changing epizootology of avian influenza viruses in Egypt during 1 year of active surveillance in 2015.

Methods

Collection of specimens

Between 1 January 2015 and 31 December 2015, a team of veterinarians collected 2383 cloacal and 1877 oropharyngeal swab samples from commercial poultry farms, backyard flocks, abattoirs and live-bird markets. Samples were taken from convenience-selected healthy birds and from sick and dead birds from the same farms in 10 of 27 governorates in Egypt, comprising 5 Nile Delta governorates (Daqahliya, Gharbiya, Menofiya, Qalubiya and Sharqiya), 1 in mid-Egypt (Fayyoun) and 4 in Upper Egypt (Assiut, Beni Suef, Menia and Sohag). The governorates selected cover the areas where the bulk of poultry growing is done. The tip of each individual swab was placed in a collection vial containing 1 mL of transport medium [50% glycerol, 50% phosphate-buffered saline, penicillin (2×10^6 U/L), streptomycin (200 mg/L) and amphotericin B (250 mg/L)]. The specimens were stored on ice and transported to the laboratory with 24 h for processing.

Virus isolation and subtyping

After viral RNA extraction, viruses were detected by M gene real-time polymerase chain reaction (18). Positive samples were subtyped as H5, H9 or co-infection with both H5 and H9 viruses, as described previously (17).

Statistical analysis

Percentages were used to summarize data. The chi-squared test was used to analyse differences within the variables examined (sample type, governorate, species and production source). A P -value < 0.05 was considered statistically significant.

Results

Of 4260 samples collected, 192 (4.5%) were positive for influenza A; the prevalence was significantly higher in oropharyngeal swabs (6.2%) than in cloacal swabs (3.2%) ($P < 0.001$) (Table 1). Of the governorates in the Nile Delta region, Daqahliya showed the highest prevalence (10.1%), followed by Sharqiya (5.2%). In Upper Egypt, the highest prevalence was found in Beni Suef governorate (10.0%) followed by Assiut (5.2%) and Sohag (4.4%). In the targeted species, the prevalence was highest in chickens (3.7%), followed by ducks (2.7%) and pigeons (0.9%); no virus was detected in samples from turkeys. The prevalence was lowest in abattoirs (1.3%) compared to commercial farms, backyards and markets (4.6–4.9%).

The prevalence by month is shown in Figure 1; most positive samples were detected at the start of the year with a second peak in May. The distribution of H5N1, H9N2 and co-infected samples isolated by month are shown in Figure 2. Both H5N1 and H9N2 were detected between January and August. H5N1 virus was highly prevalent in January and August, while the prevalence of H9N2 was high in February, April and July. From October, H9N2 predominated, and there were no H5N1-positive samples except one in November. No influenza A viruses were detected in samples collected in September.

Table 1 Epizootological data on avian influenza viruses isolated during 2015, Egypt

Variable	No. (%) of samples collected (n = 4260)	No. (%) of influenza A-positive samples	P-value ^a
Sample type			< 0.001
Cloacal	2383 (55.9)	76 (3.1)	
Oropharyngeal	1877 (44.1)	116 (5.8)	
Governorate			< 0.001
Gharbiya	129 (4.5)	0 (0)	
Daqahliya	670 (15.7)	68 (10.1)	
Qalubiya	417 (9.8)	10 (2.4)	
Menofiya	22 (0.52)	0 (0)	
Sharqiya	747 (17.5)	39 (5.2)	
Fayyoun	717 (16.8)	18 (2.5)	
BeniSuef	20 (0.46)	2 (10.0)	
Menia	530 (12.4)	6 (1.1)	
Assiut	557 (13.0)	29 (5.2)	
Sohag	451 (10.6)	20 (4.4)	
Species			0.096
Chickens	3767 (88.4)	138 (3.7)	
Ducks	292 (6.9)	8 (2.7)	
Pigeons	110 (2.6)	1 (0.9)	
Turkeys	91 (2.1)	0 (0)	
Production sector			0.270
Abattoir	154 (3.6)	2 (1.3)	
Commercial farm ²	2671 (62.7)	122 (4.6)	
Backyard flock	777 (18.2)	36 (4.6)	
Live-bird market	658 (0.2)	32 (4.9)	

¹ P-value generated by Chi Square test 2; ²Farms with more than 1000 birds raised for commercial purposes.

Discussion

In 2015, we detected an infection rate of 4.5%, and the most commonly isolated subtype was H9N2. Most of detected H9N2 viruses were from apparently healthy poultry, which reflects the widespread prevalence of this weakly pathogenic subtype among poultry in Egypt. During our previous active surveillance, the H5N1 and H9N2 subtypes were both commonly detected, with a rate of infection between August 2009 and July 2010 of 5% (exclusively H5N1 infection), increasing to 10% (H5N1, H9N2 and co-infection) during August 2010–January 2013 (17). Between February

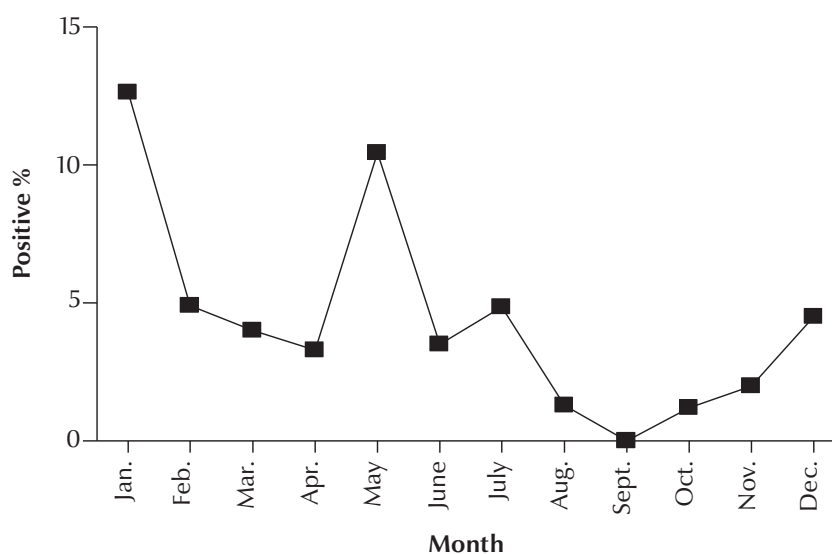


Figure 1 Percentage of samples positive for avian influenza viruses in Egyptian poultry, by month

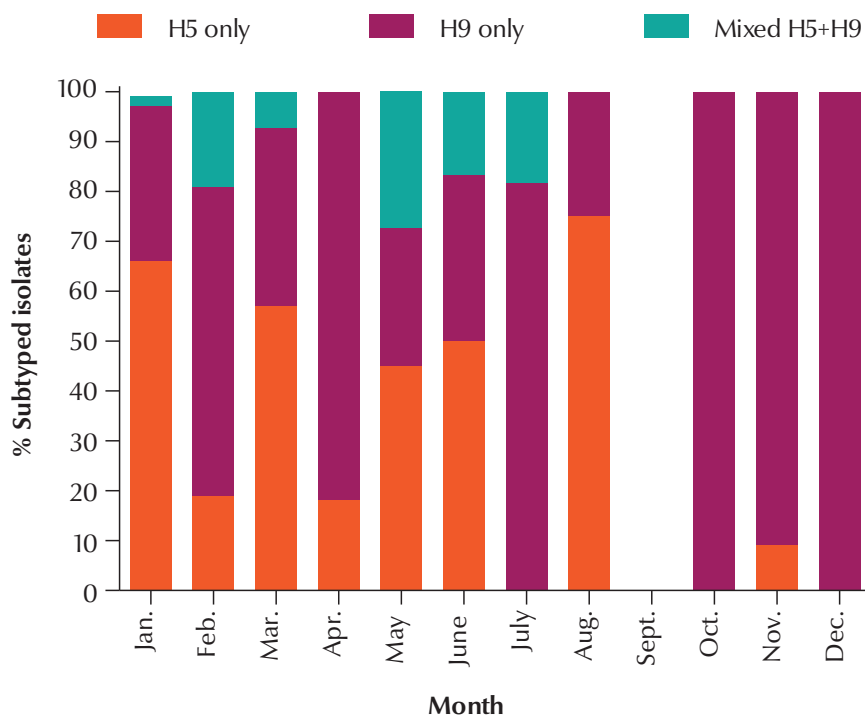


Figure 2 Subtypes of influenza A virus detected in Egyptian poultry, by month

2013 and December 2014, the infection rate was about 4.7% (2).

In the last quarter of 2015, a near absence of avian influenza subtype H5N1 was observed (in 1/32 isolates), for the first time since the initiation of surveillance in 2009. This finding represents a sharp change from the 2014/15 season when many cases of human infection with H5N1 were reported. The reason for the decrease is unknown, as, to our knowledge, no interventions were introduced. Information from the field indicates that the production of eggs and subsequently chicks decreased due to heavy circulation of the velogenic Newcastle disease virus. Another possible

explanation for the absence of H5N1 virus is economic. In previous years, poultry growers were allowed to purchase chicks on credit. After the events of the 2014/15 season, many growers lost their flocks and were thus unable to pay their instalments, and the credit system was stopped in 2015; thus, many growers did not raise flocks. In both scenarios, poultry density decreased, hence reducing H5N1 circulation.

It is nevertheless important to remain vigilant and continue surveillance to monitor whether the trend seen in 2015 will continue and to monitor the genetic and antigenic evolution of avian influenza in Egypt.

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