

Avian Influenza in Live Bird Markets in the Suez Canal region, Egypt

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Abstract

Avian influenza causes severe economic losses in poultry industry and endangers human life. This study aimed to detect avian influenza viruses (AIVs) in live bird markets (LBMs) in the Suez Canal region. Tracheal and cloacal swabs were collected from apparently healthy birds (152 chickens, 119 ducks, 44 geese and 60 turkeys) from live bird markets in Ismailia, Portsaid and Suez Governorates during the period from January to December 2014. Our results revealed that AIVs prevalence was 4.3% in the surveyed markets. The H9 low pathogenic (LPAI) positive birds (56.3%) were higher than H5 highly pathogenic (HPAI) infected cases (43.8%), while no H7 positive cases were detected. The positive cases in turkeys, chickens, geese, and ducks were 6.7%, 5.3%, 4.6%, and 1.7% respectively. Additionally, the highest frequencies were recorded in cold weather during the winter season 2.4%. Our investigation verified that live bird markets in the Suez Canal region continue to be high risk locations for AIVs due to the existence of various AIV subtypes (H5 and H9) in poultry species from different breeding sectors in Egypt. This mixing permits transmission of the disease from infected areas to non-infected ones. In addition, the coexistence of both H5 and H9 subtypes in the same poultry population may provide an opportunity for genetic reassortment and emergence of novel viruses. Consequently, birds in LBMs are incriminated in the continuous circulation of AIVs, therefore representing a main source of AI infection to commercial poultry and householders. Thus, control actions towards AIVs should include live bird markets as a critical threat source of the disease transmission.

Keywords: Avian Influenza, Live Bird Markets, Suez Canal.

Introduction

Avian influenza virus (AIV) is a devastating virus causing enormous losses in the poultry industry worldwide [1]. AIVs are segmented negative-sense ssRNA viruses belonging to the family Orthomyxoviridae that is divided into five genera, including influenza types A, B, and C, Isavirus and Thogotovirus [2]. Only influenza type A viruses infect poultry, and they are subdivided into subtypes based on the antigenic relationships of the surface glycoprotein hemagglutinin (HA) and neuraminidase (NA). There are 18 haemagglutinin (HA) and 11 neuraminidase (NA) subtypes; the recent subtypes H17N10 and H18N11 were detected in bats [3,4].

In Egypt, highly pathogenic AIV (HPAIV) H5N1 has been circulating in domestic poultry

since February 2006 [5] and was declared endemic in July 2008 [6]. The recent wave of H5N1 AIVs in Egypt during 2014-2015, showed a dramatic increase of H5N1 infections in poultry and humans. The H5N1 incidence increased over 400 outbreaks in both commercial and backyard poultry [7]. An alarming increase of H5N1 human cases was also reported from November 2014 to March 2015, where, 47 out of 159 new human cases were fatal. Therefore, Egypt recorded the first highest number of confirmed human infections and deaths during 2014 and 2015 [8]. Additionally, as of July 2016, HPAIV H5N1 caused 117 fatal human cases out of 354 infected individuals [9]. The first report in Egypt described the isolation and identification of H9N2 virus was in May 2011

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from commercial quail flock in Giza Province [10]. The H9N2 AIV is common in chickens, ducks and other poultry species. It can sometimes cross the species barrier and cause human infections, which has raised public health concerns [11]. The disease in humans is usually subclinical [12], and H9N2 interspecies transmission from avian to mammalian hosts could happen due to the presence of human virus-like receptor specificity in H9N2 from poultry [13].

Live bird markets (LBMs) are considered the most serious points in the poultry value chain, as they link commercial, small-scale household farms, slaughter houses, producers, traders and consumers [14]. In Egypt poultry meat trade depends mainly on LBMs due to insufficient slaughterhouses, lack of marketing infrastructure, and cultural preference for the consumption of freshly slaughtered poultry [15]. Nearly, 16000 LBMs in Egypt sell live or freshly slaughtered birds to consumers, besides over 4300 small slaughtering and de-feathering points sell freshly slaughtered or chilled birds [16]. Abdelwhab *et al.* [15] documented the wide circulation of H5N1 AIV in LBMs in Egypt. Backyard waterfowl can act as a reservoir and/or source of A/H5N1 especially in LBMs [17], because these markets provide optimal conditions for

amplifying and sustaining virus circulation and could thus, become viral reservoirs themselves [18].

The goal of this study was to investigate the role of LBMs in the continuous circulation of AIVs in the Suez Canal region. The role of bird species and the season on the AI viruses dissemination was determined.

Materials and Methods

Samples

Tracheal and cloacal swabs were collected from apparently healthy domestic poultry species (chicken, ducks, geese and turkeys) at different LBMs in the Suez Canal region including Ismailia, Suez and Port Said during the period from January to December 2014 (Table 1). All swab samples were collected in 1-2 mL phosphate Buffer Saline (PBS) tubes as transport media (PH 7-7.4) containing antibiotics, according to OIE manual [19]. The tubes were then placed in ice bags and transported quickly to the laboratory and then were preserved at -80 °C until tested [19]. For sample preparation, a pool of tracheal and cloacal swabs (1 to 10 swabs of the same bird species and source) was dispensed in 1.5 – 3 ml plastic screw capped tubes.

Table 1: Collected tracheal and cloacal swabs from live bird markets in the Suez Canal region during 2014

Governorate	Species				Total
	Chicken	Duck	Geese	Turkey	
Ismailia	49	41	15	18	123
Suez	51	40	16	20	127
Port Said	52	38	13	22	125
Total	152	119	44	60	375

Real-time reverse-transcription polymerase chain reaction (RT-qPCR)

RNA was extracted from a mix of tracheal and cloacal samples using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA) following the manufacturer's instructions, then subjected to real time reverse transcription

polymerase chain reaction (RT-qPCR) targeting AIV matrix gene. Positive samples were further subtyped for detection of the H5, H7 and H9 genes. The test was performed in a Stratagene MX3005P real time PCR machine.

Primers and probe used in RRT-PCR for Matrix gene were M24 (5'-AGA TGA GTC

TTC TAA CCG AGG TCG -3') and M25 (5'-TGC AAA AAC ATC TTC AAG TCT CTG-3'), probe (6-FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA) [20]. Primers and probe for H5 subtype were H5 LH1 (5'-ACA TAT GAC TAC CCA CAR TAT TCA G -3') and H5 RH1 (5'-AGA CCA GCT AYC ATG ATT GC - 3'), H5 probe (6-FAM-TCW ACA GTG GCG AGT TCC CTA GCA-TAMRA) [20]. Primers and probe for H7 subtype were LH6 H7(5'-GGC CAG TAT TAG AAA CAA CAC CTA TTG A-3') and RH4 H7 (5'-GCC CCG AAG CTA AAC CAA AGT AT-3'), H7 Probe (6-HEX-CCG CTG CTT AGT TTG ACT GGG TCA ATC T-BHQ) [21]. Primers and probe for H9 subtype were H9F (5'-GGA AGA ATT AAT TAT TAT TGG TCG GTA

C-3') and H9R (GCC ACC TTT TTC AGT CTG ACA TT), H9 probe (6- CY5-AAC CAG GCC AGA CAT TGC GAG TAA GAT CC-TAMRA) [22]. Thermal profile and cycling condition of Matrix gene detection was done by Reverse transcription at 50°C for 30 min, followed by Primary denaturation step at 95°C for 15 min and secondary denaturation at 95°C for 30 second then annealing and extension at 60°C for 20 second. While, thermal profile and cycling condition of H5, H7 and H9 genes detection was done by reverse transcription at 50°C for 30 min, followed by Primary denaturation step at 95°C for 15 min and secondary denaturation at 94°C for 15 second then annealing at 54°C for 30 second and extension at 72°C for 10 second.

Table 2: Avian influenza prevalence in different species in live bird markets in the Suez Canal region during 2014

Species	RRT-PCR results for M gene (positive/total tested of each species)	Positive cases subtype (positive/total positive)	
		H5	H9
Chickens	8/152 (5.3%)	1/8 (12.5%)	7/8(87.5%)
Ducks	2/119 (1.7%)	2/2 (100%)	0/2(0%)
Geese	2/44 (4.6%)	2/2 (100%)	0/2(0%)
Turkeys	4/60 (6.7%)	2/4 (50%)	2/4(50%)
Total	16/375 (4.3%)	7/16(43.8%)	9/16(56.3%)

Results and Discussion

Live bird markets (LBMs) facilitate the spread of avian influenza viruses to poultry and humans particularly women selling birds in traditional markets and children participating in removal of wastes and offal in retail shops [15]. Consequently, LBMs have played a key role in the persistence of infection [23]. In this study, low AIV prevalence was recorded in the examined birds at LBMs (4.3%) (Table 2); this might be attributed to that poultry sellers do not present the diseased poultry for selling at markets. Abdelwahab *et al.* [15] recorded that 5.6% of the examined LBMs were positive for AI in the Canal region from January to April 2009.

In the examined markets, the H9 positive cases were higher than H5 (56.3% and 43.8%, respectively) (Table 2). The H9 positive cases are usually associated with subclinical infection since the H9 AIV was reported to be of low pathogenicity in chickens [24] causing mild clinical signs [25]. Moreover, H9 AIV is known to negatively affect poultry health generally and increase the danger of infections of H5N1 HPAI which is already endemic in Egypt [26]. This ascertains that only apparently healthy birds are presented in markets, while, those with H5 subtype infection show clinical signs rendering them from sale in markets.

Table 3: Seasonal prevalence of avian influenza viruses in live bird markets in the Suez Canal region during 2014

Season*	AIVs prevalence (positive/total tested cases)
Winter	9/375 (2.4%)
Spring	2/375 (0.5%)
Summer	2/375 (0.5%)
Autumn	3/375 (0.8%)

*Winter: December, January, and February; Spring: March, April, and May; Summer: June, July, and August; Autumn: September, October, and November

Regarding poultry species, the highest positive cases were detected in turkeys and chickens 6.7% and 5.3% respectively (Table 2). These results might be due to turkey is more susceptible to AIV than chickens and ducks [27]. Additionally, it is worth mentioning that chickens are the predominant poultry species in LBMs. In the surveyed markets, the H9 positive cases in chickens (87.5%) were higher than H5 (12.5%). All positive cases in ducks and geese were related to HP H5 AIV, however, in turkeys the H5 and H9 positive cases were equal (Table 2). Kayali *et al.* [28] recorded that the incidence of H5 was higher than H9 in chickens and ducks but in geese and turkeys all positive cases were H5. Infection of ducks as an important waterfowl reservoir with HPAI is well documented worldwide either wild or domesticated ducks [29-31]. Moreover, waterfowl can be silently infected with H5N1 [32-36] which can maintain the virus in these markets for longer times. It is worth pointing out that, ducks are capable of excreting H5N1 HPAIV for at least 17 days via the cloacal and

respiratory routes [37]. In addition, a close relationship of viruses from backyard ducks and humans was recorded [15, 17, 38-40]. Consequently, domestic ducks have been implicated in the spreading and evolution of H5N1 HPAIVs, and their inclusion in disease control programs is essential [23,37,41]. As a result, keeping different species of birds together in LBMs provides appropriate conditions for inter- and intra-species transmission [15,42,43].

Regarding the role of season on the AIVs spread, the highest prevalence was identified during the winter season (2.4%) (Table 3). Similarly, Abdelwahab *et al.* [15] recorded higher incidence of positive LBMs (40.8%) during the cold month of February. Our results might be attributed to that the influenza A virus survival and viability are known to increase at lower environmental temperatures [44]. The virus spread decrease in summer due to hot weather and dryness. Thus, the disease is associated with cold weather due to favorable conditions for propagation and spread of the virus [44-48].

Table 4: Avian influenza positive cases in live bird markets concerning bird source (backyard flocks - commercial farms)

Species	Backyard flocks			Commercial farm		
	positive cases	AIV subtypes		positive cases	AIV subtypes	
		H5	H9		H5	H9
Chickens	2/8	0/8	2/8	6/8	1/8	5/8
Ducks	0/2	0/2	0/2	2/2	2/2	0/2
Geese	2/2	2/2	0/2	0/2	0/2	0/2
Turkeys	2/4	1/4	1/4	2/4	1/4	1/4
Total	6/16 (37.5%)	3/16 (18.8%)	3/16 (18.8%)	10/16 (62.5%)	4/16 (25%)	6/16 (37.5%)

Concerning the geographical distribution of AIVs in the Suez Canal region, the highest positive cases were recorded in Ismailia Governorate (9.8%) followed by Port Said (3.2%), no positive cases were detected in Suez Governorate (Figure 1). These results might be due to high poultry production density in Ismailia Governorate. In relation to bird source/origin, the surveyed LBMs contain birds from both commercial farms (mostly chickens) and backyard flocks (mainly waterfowl). Higher positive cases were

recorded in birds from commercial farms (62.5%) than that from backyard flocks (37.5%) (Table 4). Moreover, the H9 positive cases (37.5%) in birds from commercial farms were higher than H5 (25%). On the other hand, birds coming from backyard flocks, H5 and H9 positive cases were equal (18.8%). The H9 positive cases in birds coming from commercial farms (37.5%) were higher than those from backyard (18.8%). No H7 positive cases were detected in the examined markets (Table 4).

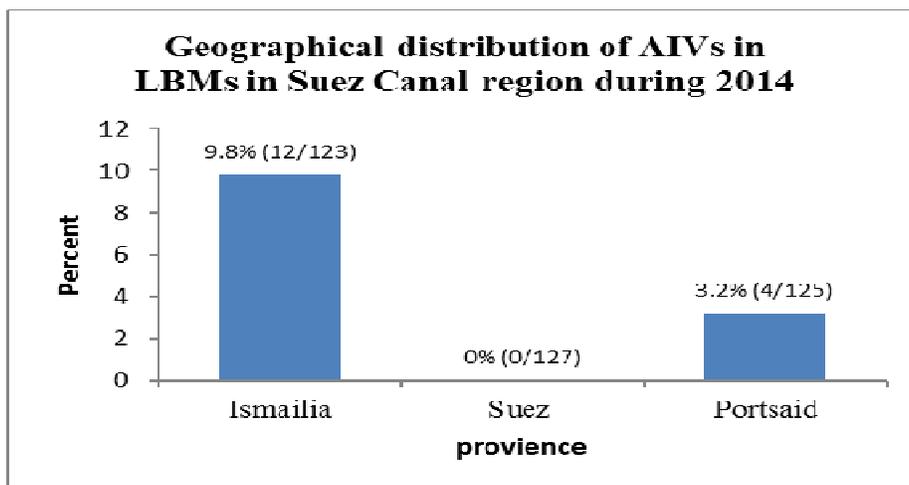


Figure 1: Geographical distribution of AIVs in LBMs in the Suez Canal region during 2014

Consequently, the wide spread of LP H9 AIV in birds coming from commercial farms might be due to the H9 AI vaccines are used in narrow scale in commercial poultry farms because some poultry producers thought that H9 vaccine is not essential. In addition to the low pathogenic nature of the virus, which permits its silent spread in commercial chickens [26]. Therefore, continuous surveillance in LBMs and incorporation of multifaceted strategies and global cooperation are required to control the virus spread in Egypt [17]. Birds in these markets came from different localities in Sharkia, Qlubiya, El Menya, and Ismailia Governorates. As a result, LBMs act as a mixing vessel due to the existence of various birds coming from different sources/origin (backyard flocks and commercial farms) also from different localities which permit transmission of the disease from infected areas to non-infected ones. Furthermore, LBMs are considered a

high risk location of AIVs due to the coexistence of both H5 and H9 subtypes in the same poultry population which may provide an opportunity for genetic reassortment and emergence of novel viruses similarly to what has happened in Pakistan and Southern China in the recent past [49,50].

The circulation of the AIVs in LBMs may be attributed to absence of biosecurity measures or any veterinary supervision in these markets. Moreover, the illegal transportation of birds between different localities.

Conclusion

Our findings revealed that LBMs in the Suez Canal region represent a high risk location of potential AIV transmission to commercial poultry and householders and thus are incriminated in the continuous dissemination of AIVs. Therefore, the authorities must make temporary or permanent

closure of AI positive markets. In addition, veterinary supervision, biosecurity measures, prevention of the illegal transportation of poultry among different provinces, along with routine surveillance must be enforced in these markets.

Conflict of interest

The authors have any conflict of interest to declare.

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أنفلونزا الطيور في أسواق بيع الطيور الحية في منطقة قناة السويس , مصر

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تسبب إنفلونزا الطيور خسائر اقتصادية حادة في صناعة الدواجن كما انها تعرض حياة الإنسان للخطر. هدفت هذه الدراسة إلى الكشف عن فيروسات أنفلونزا الطيور في أسواق بيع الطيور الحية في منطقة قناة السويس. حيث تم جمع مسحات زور ومجمع من طيور سليمة ظاهريا (١٥٢ دجاج و ١١٩ بط و ٤٤ اوز و ٦٠ رومي) من أسواق بيع الطيور الحية في محافظات الإسماعيلية وبورسعيد والسويس خلال الفترة من يناير إلى ديسمبر ٢٠١٤. وكشفت نتائجنا أن نسبة انتشار فيروس أنفلونزا الطيور في الأسواق التي شملتها الدراسة كانت ٤.٣٪ (٣٧٥/١٦). وكانت الحالات الإيجابية ل LPAI H9 (٥٦.٣%) أعلى من حالات H5 HPAI (٤٣.٨٪) في حين لم نجد أي حالات إيجابية ل H7. وكانت الحالات الإيجابية في الرومي والدجاج والإوز والبط ٦.٧٪، ٥.٣٪، ٤.٦٪، ١.٧٪ على التوالي. بالإضافة إلى ذلك، تم تسجيل أعلى نسب اصابه خلال فصل الشتاء ٢.٤٪ (٣٧٥/٩). لذلك وجدنا أن أسواق بيع الطيور الحية تعتبر موقعا شديدا لانتشار فيروس أنفلونزا الطيور بسبب وجود أنواع مختلفة من الدواجن تأتي من قطاعات تربية مختلفة (تربيته منزليه ومزارع تجارية) من عدة محافظات أيضا. حيث يسمح هذا الاختلاط لانتقال المرض من المناطق المصابة إلى غير المصابة. بالإضافة إلى ان تواجد كل من H9 و H5 في نفس المكان قد توفر فرصة لإعادة التكوين الجيني للفيروس وبالتالي ظهور فيروسات جديدة. ونتيجة لذلك، تعتبر الطيور المتواجده في تلك الأسواق سببا في استمرار انتشار فيروس أنفلونزا الطيور ، وبالتالي تمثل مصدرا رئيسيا للعدوى لكلا من مزارع الدواجن التجارية والمربيين. ومن ثم، ينبغي أن تشمل إجراءات السيطرة على المرض، الطيور المتواجده في أسواق بيع الطيور الحية لانها تمثل مصدرا خطيرا لانتشار المرض.