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A SURVEY STUDY FOR DETECTION OF AVIAN INFLUENZA VIRUS IN QALUBEYA GOVERNORATE

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A B S T R A C T

A total 100 suspected samples (80 chicken, 15 ducks, and 5 geese) collected from poultry farms and human houses and throat swabs collected from persons having respiratory manifestations and in contact with infected birds were examined by rapid test and Reverse transcription real time-PCR (rRT-PCR) test for detection of avian influenza virus. Rapid antigen detection test detect avian influenza virus type A in seven out of 100 tracheal swabs collected from poultry while, rRT-PCR detect avian influenza virus H5 in two out of 100 samples collected from poultry and one out of 100 throat swabs collected from human in contact with poultry. Reverse transcription real time-PCR gives accurate result with fresh samples than stored samples.

KEY WORDS: Avian influenza, PCR, Poultry, Survey.

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1. I N T R O D U C T I O N

Avian influenza is an infectious disease caused by influenza type A belongs to family Orthomyxoviridae, and Occur naturally among birds. Wild birds worldwide carry the viruses in their intestines, but usually do not showing clinical signs. However, avian influenza is very contagious among birds and can makes some domesticated birds, including chickens, ducks, and turkeys, very sick and kill them [7]. Infected birds shed influenza virus in their saliva, nasal secretions, and feces, susceptible birds become infected when they have contact with contaminated secretions or excretions or with surfaces that are contaminated with secretions or excretions from infected birds. Domesticated birds may become infected with avian influenza virus through direct contact with infected waterfowl or other infected poultry, or through contact with surfaces such as dirt or cages or materials such as water or feed that have been contaminated with the virus [5]. Close contact with dead or sick birds is the principal source of human infection with H5N1 virus .Most human cases had occurred in rural or peri urban areas where many household keep small poultry flocks, which often roam freely [4]. Prevention of such disease can be attained by the early diagnosis of the virus. This study conducted for detection of influenza virus from samples collected from poultry and human in contact having respiratory manifestations for determining disease prevalence in Qalubeya governorate.

2. MATERIAL AND METHODS

2.1. *Collection and preparation of poultry samples*:

A total of 100 suspected samples (80 chicken, 15 ducks and 5 geese) were collected from poultry farms (60 chickens) and human houses (20 chickens, 15 ducks

and 5 geese. according to previous author [12], birds collected from infected farms and houses were sent to the laboratory for tracheal swab collection and postmortem examination. Tracheal swabs placed in phosphate buffer saline with antibiotics and antimycotic, samples for rapid detection of viral antigen processed within one-two hours, while samples for reverse transcription real- time PCR (rRT-PCR) were transferred immediately to the testing laboratory (molecular biology research unit, assiut university) on ice bags and stored at -20 ˚C.

2.2. *Human samples*:
A total number of 100 throat swabs were collected from persons having respiratory manifestations and in contact with infected birds [3]. Swabs transferred to the laboratory as soon as possible in phosphate buffer saline with antibiotics and antimycotic in ice bags and stored at -20 ˚ C for rRT-PCR examination.

2.3. *Chemicals, kits and equipment*:
1-Phosphate buffer saline PH 7.4 containing antibacterial and antimycotic.
2-Rapid avian influenza antigen detection kit type A (Anigen, korea) [2].
3- Reverse transcription real time –PCR for detection of Influenza A1H5/ N1 [1]:

2.3. *Extraction of RNA from samples*
2.3.1. *QIAamp viral RNA kit* (Qiagene, , Germany) consist of: QIAamp MiniSpin columns,Collection tubes (2ml), Buffer AVl, Buffer AW1 (concentrated), Buffer AW2 (concentrated), Buffer AVE, Carrier RNA

2.3-2. *Equipments and reagents*:
Ethanol (96-100%), Microcentrifuge tubes (1.5 ml), sterile RNase-free pipet tips, Microcentrifuge tubes with rotor (1.5 and 2ml).

2.3.3. *PCR reaction mix*:
Reaction components of TaqMan Influenza A1H5/ N1 detection kits are: Assay beads containing d NTPs, Taq Man probes and primers, DNAse-RNAse free water, Mgcl2, PCR buffer, Taq Man DNA polymerase, Sample (RNA), RNA positive and negative control, Each kit provides reagents for 90 reactions

2.4. *One step reverse transcription real-time PCR*:
Instrument in amplification PCR room contain: Program for real- time PCR and Program for ABI PRISM7000SDS.

3. RESULTS AND DISCUSSION

The rapid antigen detection method is a primary field screening of influenza type A virus was successful to monitor avian influenza infection (Table 1), it is evident that the total detection of influenza virus by rapid antigen detection test from chicken farms was (2.3%) in layer chickens and (23.5%) in broiler breeder chickens, while (5%) in chickens collected from human houses. The highest infection rate was (6.7%) in ducks which in contact with infected chickens, this indicate the role of ducks as a carrier of a influenza A virus, ducks that are not show any symptoms of disease continue to circulate the virus representing a pandemic threat. That result lower than those reported by [13] who detect avian influenza virus type A antigen in 12 out of 15 (80%) chicken and one (25%) out of four ducks.

Table 1): Detection of avian influenza virus in samples collected from poultry by rapid antigen detection test.

Sample			Number of examined samples	Number of positive	%
Chicken	Farms	Layer	43	1	2.3
		Broiler breeder	17	4	23.5
		Total	60	5	8.3
	House reared		20	1	5
Ducks			15	1	6.7
Geese			5	0	0
Total			100	7	7

The difference in the detection rates may be due to environmental conditions and seasonal variations. The recurrence of influenza A H5N1 has highlighted the need for a highly sensitive, accurate, and rapid diagnostic test for detection of the infection. Such test is important, not only for infection control but also to facilitate early antiviral therapy. Molecular diagnosis of influenza A virus by reverse transcription real time PCR can be a rapid assay, results including sub typing may be available in less than one day [6]. It is obvious from the results obtained in Table (2) that samples collected from houses had significantly higher detection rate for avian influenza virus (100%) than samples collected from farms (25%). This due to that poultry farms may be vaccinated and the workers in farms more experienced in controlling the disease, they rapidly get rid of any suspected cases from the farm. In addition, total detection of avian influenza virus from all samples was (28.5%), this result lower than that obtained by [12] who detect avian influenza virus in (94.3%) of samples collected from birds during an outbreak of avian influenza A (H5N1) in China in 2005. The difference in the detection rate may be explained in 2005 the disease was undefined and no vaccine was available, so the disease was spread rapidly and the infection rate was high.

Table 2 Detection of avian influenza virus in samples collected from poultry by rRT-PCR.

Sample			Number of examined samples	Number of positive	%
Chicken	Farms	Layer	1	0	0
		Broiler breeder	4	1	25
	House reared		1	1	100
Ducks			1	0	0
Geese			0	0	0
Total			7	2	28.5

The results in Table (3) proved the lower sensitivity of real time-PCR test with the stored specimens (zero%) compared to those in the fresh specimens (100%) due to degradation of virus RNA from long storage of specimens at - 20 ◦C, this temperature is not favorable for long term stability of the avian influenza virus, for this reason it is necessary to keep samples at - 70 ◦C to obtain accurate results [10]. Human infection by avian influenza virus is relatively rare but fatal, of the 373 cases of confirmed H5N1 infection recorded up to March 2008, 236 were fatal [14]. Most human infections were acquired through contact with infected poultry, as this virus has evolved rapidly, however there is concern that it may re-assort with other human influenza viruses or become adapted to humans and cause a pandemic influenza [9]. The present study showed that out of 100 throat swabs collected from humans in contact with infected poultry one (1%) was positive for avian influenza virus (Table 4). The obtained results lower than the results recorded by [3] who found that 112 out of 133 respiratory specimens collected from patients in Australia during the period between 2000 and 2001 give positive results for avian influenza infection. Live bird markets play an important role in infection spread among poultry as well as human because these markets is the place at which different bird species from different sources were sold. In this occasion, sellers become at risk of infection with the virus as they collect birds from farms and houses without following preventive measures, so one out of four specimens collected from live bird market sellers give positive result for avian influenza virus with percentage (25%).

The clinical features of the patient with confirmed infection with influenza A (H5N1) virus are fever, shortness of breath, cough, sputum production, in some cases the sputum was blood stained, myalgia, diarrhea, sore throat, conjunctivitis, rash, runny nose, respiratory distress. Physical chest examination revealed rapid respiratory rate, crackles, wheeze, and dyspnea. The estimated time between the exposure to poultry and the onset of illness

suggests an incubation period of two to four days [8]. Table (5) revealed that the most common symptoms of suspected human cases were fever, cough, sore throat, runny nose, headache, malaise, and dyspnea. From this study it is important to early diagnosis of avian influenza disease to reduce disease prevalence and disease morbidity.

Table 3 Number of avian influenza virus detection by rRT-PCR in fresh and stored samples collected from poultry.

Sampling	Fresh			Stored			Total		
	No. of exam samples	No. of +ve	%	No. of exam. samples	No.of +ve	%	No. of exam. samples	No. of +ve	%
Chicken	2	2	100	4	0	0	6	2	33.3
Duck	0	0	0	1	0	0	1	0	0
Geese	0	0	0	0	0	0	0	0	0
Total	2	2	100	5	0	0	7	2	28.5

Table 4 Avian influenza virus detected in examined human samples.

Sampling	Examined persons	Number of samples	Number of positive	%
Farms	Workers	24	0	0
	Employee	5	0	0
	Veterinarians	8	0	0
Houses	Children	15	0	0
	Adult	44	0	0
Live bird markets	Seller	4	1	25
Total		100	1	1

Table 5 Symptoms of suspected human cases.

Clinical finding	Yes	No
Fever	√	
Cough		√
Sore throat		√
Runny nose	√	
Body aches	√	
Headache	√	
Tachypnea		√
Vomiting		√
Diarrhea		√
Myalgia	√	
Malaise		√
Sputum production		√
Dyspnea	√	
Conjunctivitis		√

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مجلة بنها للعلوم الطبية البيطرية
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دراسة استقصائية للكشف عن فيروس انفلونزا الطيور فى محافظة القليوبية

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الملخص العربى

تم تجميع عدد 100 عينة من الدواجن (80 دجاج , 15 بط , 5 أوز) من الطيور المشتبه اصابتها بانفلونزا الطيور و تم اكتشاف فيروس انفلونزا الطيور type A بالإختبار السريع فى سبع عينات من 100 عينة (مسحة من القصبة الهوائية) أخذت من الطيور المشتبه إصابتها بالمرض بنسبة (7%) , بينما تم اكتشاف الفيروس (H5) باختبار rRT–PCR فى عدد 2 عينة من الدجاج. و أعطت العينات التى لم يتم تخزينها نتيجة أفضل من العينات المخزنة و الأشخاص المخالطين للطيور المصابة أعطت نتيجة ايجابية واحدة للفيروس باختبار rRT–PCR من 100 عينة (مسحة من الزور) بنسبة (1%).

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