HUMAN PROTECTION FROM AVIAN INFLUENZA

Ahmed A. El bassiouny [a], *Wael F. El-Tras* [a], *Ahmed A. Tayel* [b] *and Doaa M. Elkordy* [c]

[a] Department of Hygiene and Preventive Medicine (Zoonoses), Faculty of Veterinary Medicine, Kafrelsheikh University

[b] Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt

[c] Department of preventive medicine, Veterinary Medicine Directorate, Gharbeya Governorate

ABSTRACT

Avian influenza (AI) is among the most emerging diseases that threatened human worldwide. The aim of current study was to investigate the seasonal emergence of H5N1 in poultry backyards and contact humans in Gharbia governorate to assess the human protection from the disease. A total of 480 poultry backyards and 102 human contacts were examined in different seasons for the presence of H5N1 using Real time PCR. The results revealed that out of 480 examined poultry backyards, 42 (8.75%) backyards were positive for the disease and the higher risk was recorded in winter. While, out of 102 humans, 4 (3.92%) were positive for the disease and the higher risk was in summer. The study concluded that the protection measures as biosecurity, quarantine, and vaccination must be applied for the protection of the disease.

INTRODUCTION

Avian influenza is a public health challenge because of its ongoing spread and pandemic potential. ***(De zwart et al., 2010)***. AI is also known as fowl plague as a zoonotic viral disease characterized by respiratory, gastrointestinal and nervous system finding with high morbidity and mortality in the avian species ***(Jordan, 1996)***. The birds, especially water birds are the natural reservoir of AI viruses and many species of birds, domesticated and wild, can be infected with this virus ***(Swayne, 1997).***

In Egypt, H5N1 virus has been emerged in February 2006 after an incursion by wild ducks ***(Saad et al., 2007)***. Until 8th April 2011, the epidemic among poultry leads to culling of more than 30 million birds ***(Meleigy, 2007)***. Long-term endemic AI virus infections in poultry increase the exposure risk to humans ***(Matrosovich et al., 1999).***

Backyard chickens, ducks and geese are mostly reared together and roam freely in the vicinity of the house in close contact with human, particularly children. More than 70% of the Egyptian poultry production from commercial or backyard sectors is marketed through live bird markets ***(Abdelwhab and Hafez, 2011).***

The majority of outbreaks have been reported in the backyard poultry sector ***(Sonaiya, 2007)***. Given the importance of backyard poultry as a source of proteins and cash income for rural families, the need to contain HPAI in backyard poultry population is great ***(Alders and Pym, 2009)***. Employees in commercial farms usually maintain their own household birds.

Furthermore, selling of remaining feed, utensils and equipment from commercial farms to the rural family poultry often occurs in Egypt. ***(El-Zoghby et al., 2012)***.

Basic biosecurity measures are rarely implemented in traditional farming systems; the backyard poultry population may perpetuate virus circulation and become a perpetual virus source ***(Capua and Marangon, 2007).***

Greiner et al., 2007 recorded that H5N1 transmitted from poultry-to-human via direct routes including contact with infected blood or bodily fluids via food preparation practices (e.g., slaughtering, boiling, de-feathering, cutting meat, cleaning meat, removing and/or cleaning internal organs of poultry).

In Egypt, efforts to control highly pathogenic H5N1 avian influenza virus in poultry and in humans have failed despite increased biosecurity, quarantine, and vaccination at poultry farms. ***(Kim et al., 2011)***.

To reduce the risk of H5N1 infection in humans, the seasonal emerging of H5N1 in poultry backyards and human contacts in Gharbia governorate was investigated in current study.

MATERIALS AND METHODS

The collected swabs were kept in 1-2 ml of viral transport media (VTM), which contained 0.5% (w/v) bovine plasma albumin, penicillin G (2x 10^6 U/l), streptomycin (200mg/l), gentamicin (250mg/l), nystatin 66 (0.5X10^3 U/l, polymyxin B (0.5X10^6 U/l), ofloxacin (60mg/l),and sulfamethoxazole (0.2g/l). All specimens were transported, chilled (at approximately 4°C) using ice boxes, and delivered to the laboratory within 48hr.

Laboratory analysis:

The extraction of the RNA from the samples was performed using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif. and USA) Cat. No.52904. The kit contains QIAamp mini spin columns, collection tubes (2ml), Buffer (AVL), Buffer AW1, Buffer AW2, Buffer AVE and Carrier RNA. Real-Time PCR was applied according to ***OIE manual, (2008)***.

Statistical analysis:

Odds Ratios (OR) was calculated to assess the risk of H5N1 infections in poultry and humans. Statistical analysis was performed using MedCalc-version 12.1.4.0 statistical software (MedCalc Software bvba, Mariakerke, Belgium).

RESULTS

Table (1): Seasonal Emerging of H5N1 in Poultry Backyards of Gharbia Governorates

Seasons	Examined Backyards				Rural Locations of Positive Backyards
	District	Number	Positives	Percentage	
Winter	Basyoun	20	3	15%	*Basyoun - Kafr Gafar*
	El Santa	20	1	5%	*Meet Yazed*
	Kafr Elzayat	20	4	20%	*Kafr Elzayat - Ibiar - Eldalgamoun*
	Kotor	20	4	20%	*Damat - Mahalet Sad - Beltag*
	Tanta	20	3	15%	*Shabsher Elhssa - Kafr Elhama*
	Zefta	20	8	40%	*Farses - El Smalwya - Kafr Ismaeil - Kafr Ghazy - Hanoot - Shubra Malas*
Sub-total		**120**	**23**	**19.2%**	
Spring	Basyoun	20	4	20%	*Mahalet Ellaban - Kafr Kransho - San Elhagar - Meet Sharaf*
	El Santa	20	0	0%	
	Kafr Elzayat	20	0	0%	
	Kotor	20	0	0%	
	Tanta	20	0	0%	
	Zefta	20	2	10%	*Tafhna Elazab - El Smalwya*
Sub-total		**120**	**6**	**5%**	
Summer	Basyoun	20	2	10%	*Shubratana - Kranshou*
	El Santa	20	5	25%	*El Monshaa Elkobra - Meet El Leet*
	Kafr Elzayat	20	0	0%	
	Kotor	20	0	0%	
	Tanta	20	1	5%	*Ikhnawai*
	Zefta	20	1	5%	*Kafr El Gezeira*
Sub-total		**120**	**9**	**7.5%**	
Autumn	Basyoun	20	1	5%	*Kafr Gafar*
	El Santa	20	0	0%	
	Kafr Elzayat	20	0	0%	
	Kotor	20	1	5%	*Damat*
	Tanta	20	0	0%	
	Zefta	20	2	10%	*Elsmalawia - Hanoot*
Sub-total		**120**	**4**	**3.3%**	
Total		**480**	**42**	**8.7%**	

Table (2): Seasonal Risk of H5N1 in Poultry Backyards of Gharbia Governorates

Seasons	Number of Examined Backyards	Positive Backyards	OR	95% CI	P-Value
Winter	120	23	Base Line		
Spring	120	6	4.50	1.76 to 11.51	P = 0.002*
Summer	120	9	2.92	1.29 to 6.62	P = 0.01*
Autumn	120	4	6.88	2.30 to 20.56	P = 0.0006*

Table (3): Mortality Rate of Poultry in Positive H5N1 Backyards of Gharbia Governorate

Seasons	Rural Locations of Positive Backyards		No of Backyards	Number of Poultry		Mortality Rate
	District	City/Village		Total	Dead	
Winter	Basyoun	*Basyoun*	2	55	15	27.3%
		Kafr Gafar	1	22	2	9.1%
	El Santa	*Meet Yazed*	1	13	10	76.9%
	Kafr Elzayat	*Kafr Elzayat*	1	39	15	38.5%
		Ibiar	1	18	16	88.9%
		Eldalgamoun	2	50	0	0%
	Kotor	*Damat*	2	65	60	92.3%
		Mahalet Sad	1	25	3	12%
		Beltag	1	42	4	9.5%
	Tanta	*Shabsher Elhssa*	1	6	0	0%
		Kafr Elhama	2	69	27	39.1%
	Zefta	*Farses*	1	25	23	92%
		El Smalwya	1	15	0	0%
		Kafr Ismaeil	1	16	0	0%
		Kafr Ghazy	1	10	0	0%
		Hanoot	3	82	27	32.9%
		Shubra Malas	1	34	4	11.8%
Sub-total				**552**	**202**	**36.6%**
Spring	Basyoun	*Mahalet Ellaban*	1	13	8	61.5%
		Kafr Kransho	1	10	9	90%
		San Elhagar	1	12	2	16.7%
		Meet Sharaf	1	38	0	0%
	Zefta	*Tafhna Elazab*	1	35	0	0%
		El Smalwya	1	10	2	20%
Sub-total				**118**	**21**	**17.8%**
Summer	Basyoun	*Shubratana*	1	40	15	37.5%
		Kranshou	1	25	10	40%
	El Santa	*El Monshaa Elkobra*	4	141	0	0%
		Meet El Leet	1	20		0%
	Tanta	*Ikhnawai*	1	10	2	20%
	Zefta	*Kafr El Gezeira*	1	30	20	66.7%
Sub-total				**266**	**47**	**17.7%**
Autumn	Basyoun	*Kafr Gafar*	1	19	2	10.5%
	Kotor	*Damat*	1	14	2	14.3%
	Zefta	*Elsmalawia*	1	20	0	0%
		Hanoot	1	26	5	19.2%
Sub-total				**79**	**9**	**11.4%**
Total				**1015**	**279**	**27.5%**

Table (4): Seasonal Emerging of H5N1 in Suspected Human Cases in Gharbia Governorates

Seasons	Suspected Humans				Locations	Gender	Age	Status
	District	No.	+ ve	%				
Winter	El Mahala Elkobra	7	0	0				
	Samanoud	3	1	33.3	*Mahlt Kalf*	Male	7.5	Recovered
	El Santa	6	0	0				
	Kafr Elzayat	1	0	0				
	Kotor	3	0	0				
	Tanta	15	1	6.7	*Shabsher Elhssa*	Female	3	Recovered
	Zefta	3	0	0				
Sub-total		38	2	5.3				
Spring	El Mahala Elkobra	3	0	0				
	Samanoud	1	0	0				
	Kafr Elzayat	1	0	0				
	Kotor	6	0	0				
	Tanta	8	0	0				
Sub-total		19	0	0				
Summer	El Mahala Elkobra	5	1	20	*Kafr Hegazy*	Male	2	Recovered
	Samanoud	2	0	0				
	El Santa	2	0	0				
	Tanta	4	0	0				
	Zefta	1	1	100	*Shobramles*	Female	30	Died
Sub-total		14	2	14.3				
Autumn	El Mahala Elkobra	21	0	0				
	Samanoud	2	0	0				
	El Santa	3	0	0				
	Kotor	4	0	0				
	Tanta	1	0	0				
Sub-total		31	0	0				
Total		102	4	3.9				

Table (5): Seasonal Risk of H5N1 in Suspected Human Cases in Gharbia Governorates

Seasons	Number of Suspected Human Cases	Positive Backyards	OR	95% CI	P-Value
Summer	14	2	Base Line		
Autumn	31	0	12.60	0.56 to 281.46	$P = 0.11$
Winter	38	2	3.00	0.38 to 23.68	$P = 0.30$
Spring	19	0	7.80	0.34 to 176.35	$P = 0.20$

DISCUSSION

Table (1) revealed that out of 120 examined backyard in winter in Gharbia governorate, 23 were positive to H5N1, with percentage 19.2 %; while in spring, out of 120 examined backyard, 6 were positive, with percentage 5 %; in summer, out of 120 examined backyard, 9 were positive, with percentage 7.5 % & in autumn, out of 120 examined backyard, 4 were positive, with percentage 3.3 %. The overall percentage of positivity in the governorate was 8.7%.

From the result achieved, it is clear that more positive backyards were detected in winter followed by summer, then spring and finally autumn. The results are in agreement with ***Soda et al. (2013)*** who mentioned that many highly pathogenic avian influenza (HPAI) outbreaks occurred in Japan during the 2010-2011 winter and ***Newman et al. (2009)*** who recorded that the prevalence of H5N1 outbreaks among poultry in eastern Asia during 2003-2007 peaked during winter.

Table (2) assessed the seasonal risk of H5N1 in poultry backyards of Gharbia governorate. The highest risk was in winter and the risk is decreased in spring than winter by 4.5 OR (CI: 1.76 to 11.51) with a significant difference at P = 0.002. Also, in summer, the risk was decreased up to 3-fold than winter where OR = 2.92 (CI: 1.29 to 6.62) with a significant difference at P = 0.01. Moreover, lower risk was shown in autumn than winter with up to 7-folds, CI was between 2.30 to 20.56 with a significant difference at P = 0.0006 .

Our result was in agreement with ***Aly et al.(2008)*** who mentioned that climatic condition were critical for increasing incidence of H5N1 cases reported during winter season. Also, ***Si et al. (2009)*** mentioned that H5N1 outbreaks showed a clear seasonal pattern, with a high density of outbreaks in winter and early spring (i.e., October to March).

Table (3) showed the mortality rate of poultry in positive H5N1 backyards of Gharbia Governorate, in winter out of 552 birds 202 birds died, with a mortality rate of 36.6 %. In spring, out of 118 bird 21 is died, the mortality rate was 17.8 %; while in summer out of 266 bird 47 died, the mortality rate was 17.7 % and in autumn out of 79 birds 9 birds died, the mortality rate was equal to 11.4 %. The overall mortality rate was equal to 27.5.%

Our result was in agreement with the result of ***Aly et al. (2008)*** who mentioned that mortality and morbidity in the affected flocks or household cases were varied and commonly reached 100 % within a few days.

Table (4) revealed out of 38 suspected human cases in winter, 2 were positive (1 male, age 7.5 year. and recovered the other case was female, 3 years old, and recovered) with percentage 5.3 %. In spring, out of 19 suspected human cases, 0 was positive, with percentage 0 %. In summer, out of 14 suspected human cases 2 were positive (1 male, age 2 year, and recovered, the other case was female, 30 years old, and died) with percentage of 14.3 %; and in autumn, out of 31 suspected human cases, no cases were positive, with percentage 0 %. The overall percentage of positivity of H5N1 in suspected human cases in the governorate was 3.9 %. The case fatality was equal to 25%.

Our result was in agreement with ***Lohiniva et al.(2013)*** who recorded that 28% households had at least one contact that involved a child <2 years old, small children were often observed joining adult females during feeding. In households with unconfined poultry, children frequently fed poultry .

Our result was, also, in agreement with ***Kayali et al.(2011)*** who recorded that most H5N1 positive cases were less than 18 years old (62%) and 60% from them were females. Out of 119 confirmed cases, 40 were died, putting the overall case fatality rate at 34%.

Table (5) indicated seasonal risk of H5N1 in suspected human cases in Gharbia governorate. The higher risk was in summer. The risk is decreased in winter than summer by 3-folds, CI was between 0.38 to 23.68 with no significant difference at P = 0.30. Then, the risk was decreased in spring by up to 8 folds,where O.R=7.8 (C.I: 0.34 to 176.35) with no significant difference at P= 0.20. The lower risk was in autumn where O.R = 12.6, C.I between 0.56 to 281.46 with no significant difference at P = 0.11.

These results were disagreed with the result of ***Kayali et al. (2011)*** who recorded that the onset of new human H5N1 influenza virus infections cases peaked annually during the winter and spring months .From March 2006 to December 2010, 119 human infected cases with H5N1 were reported in Egypt. The first infected case was detected during the spring of 2006, and then during the winter and spring months of 2007, 2008, 2009, and 2010. Ten cases were reported in March 2007 and May 2009. The occurrence of cases in the summer months was rare, with only 17 cases reported in summer 2006 (1 case), summer 2007 (4 cases), summer 2009 (9 cases), and summer 2010 (3 cases).

CONCLUSION

The current study concluded that the higher risk of infection with H5N1 was in winter for the poultry and in summer for humans. Biosecurity, quarantine, and vaccination must be applied for human protection from the disease.

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حماية الإنسان من أنفلونزا الطيور

احمد احمد البسيونى – وائل فوزى التراس – أحمدعبد الفتاح طايل* – دعاء محمد الكردى**

قسم الصحة والطب الوقائي (أمراض مشتركه)- كليه الطب البيطرى – جامعه كفر الشيخ

*معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية ، جامعة مدينة السادات – مصر

**إدارة الوقاية – مديرية الطب البيطري – الغربية

يعتبر مرض انفلونزا الطيور من أهم الأمراض المستجدة التى هددت العالم نظرا لإتساع انتشاره و خطورته

تم فى الدراسة الحالية تقييم الظهور الموسمى لمرض أنفلونزا الطيور H5N1 فى طيور التربية المنزلية والادميين المخالطِين لها فى محافظة الغربية بمصر وذلك لتقييم كيفية حماية الإنسان من هذا المرض.

- تم فحص عدد 480 حظيرة طيور وكذلك 102 عينة ادمية من المخالطين للطيور فى المواسم المختلفة للكشف عن وجود مرض أنفلونزا الطيور H5N1 باستخدام اختبار " Real time PCR".
- وقد أظهرت النتائج ايجابية 42 حظيرة طيور للإصابة بالمرض من اجمالى 480 حظيرة طيور تم فحصها وكانت نسبة الايجابية بشكل عام هى 8,75٪ وتبين ارتفاع نسبة الاصابة فى فصل الشتاء.
- تبين ايجابية 4 حالات آدمية للإصابة بالمرض من اجمالى 102 حالة تم فحصها وكانت نسبة الايجابية بشكل عام 3,92 ٪ وتبين ارتفاع نسبة الاصابة فى فصل الصيف.
- خلصت هذه الدراسة إلى انه لابد من تطبيق إجراءات الأمان الحيوى والحجر الصحى وتحصين الدواجن للتحكم فى انتشار المرض.