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THE ZOONOTIC IMPACT OF SWINE ERADICATION STRATEGY ON THE OCCURRENCE OF AVIAN INFLUENZA H5N1

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ABSTRACT

This study was carried out to assess the impact of swine eradication strategy on the occurrence of Avian Influenza H5N1 in poultry backyards, poultry farms and human population, to identify risk factors for H5N1 around swine colonies in Egypt. The studied regions included three Governorates (Giza, Cairo and Qalyoubya).

A total of 73 poultry backyard, 95 poultry farms and 168 human samples were examined for the presence of H5N1 before the eradication of swine. Moreover, a total of 67 poultry backyard, 85 poultry farms and 152 human samples were examined for the presence of H5N1 after the eradication of swine. All samples were examined by using real time PCR.

The results revealed that 16 poultry backyards were positive before the eradication of swine while, 5 backyards were positive after applying this strategy. Also, 6 poultry farms were positive before *applying the strategy and 3 farms were positive after the eradication of swine. In suspected humans; 22 and 9 cases were confirmed for the presence of H5N1 before and after the eradication of swine respectively.*

In conclusion, the applied swine eradication strategy had a significant impact on the control of H5N1 in backyard poultry, and human population. Conversely, there is no significant impact of the swine eradication on farm poultry.

INTRODUCTION

Avian influenza (AI) viruses are highly contagious, widely spread in birds, particularly wild waterfowl and shorebirds. Most of these viruses are usually carried asymptomatically by wild birds and only mild symptoms may appear in poultry. AI viruses are classified according to pathogenicity into low pathogenic avian influenza (LPAI) and high pathogenic avian influenza (HPAI). Some AI viruses can also infect mammals including humans. The severity of zoonotic AI varies with the virus strain. Although the disease symptoms in human infections are limited to conjunctivitis or mild respiratory symptoms, some strains may cause severe disease and death. Moreover, some viruses may become adapted to a new species and cause epidemics or pandemics ***(Aamir et al., 2009; Abbott, 2003; Alexander 2004).***

The term “influenza” originally referred to epidemics of acute, rapidly spreading catarrhal fevers of humans caused by viruses of the family Orthomyxoviridae ***(Kilbourne, 1987).***

The first outbreak of HPAI H5N1 in poultry was recorded in Egypt in February, 2006 ***(Aly, et al., 2008)*** and there are still reported outbreaks in most Egyptian Governorates.

Swine influenza viruses are belonging to the family Orthomyxoviridae. Although the main host is the aquatic bird, these viruses also infect and cause disease with varying severity in domestic poultry and mammals including humans, horses, swine, and dogs ***(Wright and Webster 2001; Crawford et al., 2005).*** Influenza viruses were first isolated from swine in 1930 ***(Shope, 1931)*** and those viruses were the initial examples of classical H1N1 lineage of swine influenza viruses.

The role of swine in the generation of new influenza viruses is well documented ***(Kida et al., 1994).***

During the crisis of H1N1 (swine flu) the Egyptian Veterinary Authorities has adopted a strategy of swine eradication to mitigate the impact of swine flu and avian flu spreading within the poultry and human population.

So, the aim of this work was to assess the impact of swine eradication strategy on the prevalence of H5N1 in Egypt.

MATERIALS AND METHODS

Study Area:

The study area included three Governorates; Cairo, Giza and Qalyoubya. These three Governorates have been classified into two main regions, exposed to swine colonies and non-exposed to swine colonies.

Samples:

Sample size has been calculated by using of Epidemiological software (Win EpiScope Version 2.0) using disease detection module, with approximate prevalence 0.25, confidence level 97.5%.

The collection and preparation of samples was done according to ***OIE manual, (2008)*** and ***WHO*** guidelines for specimen collection and laboratory testing for H5N1 diagnosis.

Poultry sampling:

Pooled cloacal and tracheal samples were collected from backyard and farm poultry before and after swine eradication.

The trachea of live or freshly dead birds is swabbed by inserting dry cotton or polyester swab into the trachea and gently swabbing the wall. The cloacae of live or freshly dead birds are swabbed by inserting a swab deeply into the vent and vigorously swabbing the wall, swabs were collected for clinically infected birds.

The collected swabs were kept in 1-2ml of viral transport media (VTM), which contained 0.5% (w/v) bovine plasma albumin, penicillin G (2x 106 U/l), streptomycin (200mg/l), gentamicin (250mg/l), nystatin 66 (0.5310U/l, polymyxin B (0.5X106 U/l), ofloxacin (60mg/l),and sulfamethoxazole (0.2g/l). All specimens were transported, chilled (at approximately 4^{0}C) using ice boxes, and delivered to the laboratory within 48hr ***(Siengsanan, et al. 2009).***

Human sampling:

Nasal and pharyngeal swabs have been collected before and after swine eradication from suspected patients. Respiratory specimens placed into virus transport medium, specimens appropriately labeled and transported on ice and tested fresh upon receipt in the laboratory.

Laboratory analysis:

Extraction of the RNA from swab samples was done using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA) Cat. No.52904. The kit combines the selective binding properties of a silica-gel-based membrane with the speed of microspin technology by using Real-Time PCR (RT-PCR).

Statistical analysis:

Odds Ratios (OR) was calculated to assess the risk of H5N1 before and after the eradication of swine colonies. Statistical analysis was performed using MedCalc-version 12.1.4.0 statistical software (MedCalc Software bvba, Mariakerke, Belgium).

RESULTS AND DISCUSSION

Table (1): HPAI H5N1 risk divergence in exposed poultry backyards before and after applying the eradication strategy

	Before swine eradication strategy				After swine eradication strategy				OR	95% Cl	p-Value
	No. of poultry samples	No. of backyards	No. of positive backyards	Percentage	No. of poultry samples	No. of backyards	No. of positive backyards	Percentage			
Giza	175	35	8	23	200	40	3	7.5	3.7	0.89-15.07	0.07
Cairo	80	16	2	12.5	60	12	1	8.3	1.6	0.13-19.67	0.72
Qalyoubya	110	22	6	27.3	75	15	1	7	5.3	0.56-49.08	0.15
Total	365	73	16	22	335	67	5	7.5	3.5	1.19-10.11	0.021

Table 1 showed the risk of **HPAI H5N1** exposure in poultry backyards **exposed** to swine colonies before and after applying the eradication strategy. The p-Value of the total results was significantly represented (0.021). The OR showed that the risk of the disease was lower after applying the eradication strategy. That means, the swine eradication strategy had a positive effect to control the **HPAI H5N1** in backyard poultry. This result was supported by ***Kida et al., (1994)*** who documented the role of swine in the generation of new influenza viruses**.**

Table (2): HPAI H5N1 risk divergence in exposed poultry farms before and after applying the eradication strategy

	Before swine eradication strategy				**After swine eradication strategy**				**OR**	**95% Cl**	**p-Value**
	No. of poultry samples	**No. of Farms**	**No. of positive Farms**	**Percentage**	**No. of poultry samples**	**No. of Farms**	**No. of positive Farms**	**Percentage**			
Giza	550	55	3	4.5	550	55	2	3.6	1.53	0.25-9.53	0.65
Cairo	80	8	1	12.5	120	12	0	0	5	0.18-139.17	0.34
Qalyoubya	320	32	2	6.3	180	18	1	5.6	1.13	0.096-13.44	0.92
Total	950	95	6	6.3	850	85	3	3.5	1.8	0.45-7.60	0.39

Table 2 indicated the risk of HPAI H5N1 exposure in poultry farms exposed to swine colonies before and after applying the eradication strategy. The p-Value of the total results was not significantly different (0.39). So, the risk of the disease hasn’t any significant difference before and after applying the eradication strategy. That means, the eradication strategy hasn’t an important role in the control of HPAI H5N1 in farm poultry. Our results were supported by ***Sims et al, 2005; Songserm et al, 2006*** who stated that, although most poultry sectors were affected, the low-intensity village smallholder flocks were more susceptible than larger commercial farms.

Our results also supported by ***Tombari et al., 2013***, who said that there is a potential risk of AI to avian health in the commercial farms and strict enforcement of biosecurity measures and possible vaccination of all poultry flocks with continuous monitoring of poultry stations may ensure reduction of AI prevalence and avoid emergence of more pathogenic strains.

Comparing the effect of eradication strategy on backyard and poultry farm, poultry backyards were more susceptible to catch the HPAI infections more than poultry farms, this may be attributed to the strict biosecurity measures, vaccination programs, and hygienic measures applied to protect their investments.

Table (3): HPAI H5N1 risk divergence in exposed humans before and after applying the eradication strategy

	Before swine eradication strategy			**After swine eradication strategy**			**OR**	**95% Cl**	**p-Value**
	Suspected cases.	**Confirmed**	**Prevalence**	**Suspected cases.**	**Confirmed**	**Prevalence**			
Giza	90	11	0.12	95	5	0.05	2.51	0.83-7.52	0.1
Cairo	24	3	0.13	24	2	0.08	1.57	0.24-10.37	0.64
Qalyoubya	54	8	0.15	33	2	0.06	2.7	0.54-13.55	0.23
Total	168	22	0.13	152	9	0.06	2.4	1.07-5.38	0.03

Table 3 illustrated the positive human cases before and after application of swine eradication strategy. The obtained results indicated a significant difference between the rate of infection in human before and after the eradication (p- Value = 0.03). The risk of exposure to HPAI H5N1 showed a remarkable decrease in the human infection after the application of swine eradication strategy. The prevalence of the disease among human population of our study was very high before application of swine eradication strategy 0.13, and significantly lowered after application of swine eradication strategy 0.06. These results were supported by ***Ludwig et al., 1995, Ma, et al., 2007*** who said that the swine may be an intermediate host for interspecies spread; the replication of all avian viruses in swine supports this notion, and also the presence of avian-type and mammalian-type virus receptors in swine. Also **Ma, et al., 2007** stated that the periodic transmission of avian influenza viruses to swine in the absence of disease and the spread of human H1N1 and H3N2 viruses to swine are also consistent with the "mixing-vessel" hypothesis.

Table (4): Prevalence of HPAI H5N1 in human in different examined regions after applying the eradication strategy

	Giza			**Cairo**			**Qalyoubya**			**Total**		
	Suspected cases.	**Confirmed**	**Prevalence**	**Suspected cases.**	**Confirmed**	**Prevalence**	**Suspected cases.**	**Confirmed**	**Prevalence**	**Suspected cases.**	**Confirmed**	**Prevalence**
Exposed regions	95	5	0.05	24	2	0.08	33	2	0.06	152	9	0.06
Non exposed Regions	97	3	0.03	22	0	0	50	2	0.04	169	5	0.03
Total	192	8	0.04	46	2	0.04	83	4	0.05	321	14	0.04

This table 4 showed the prevalence of **HPAI H5N1** in human in different studied Governorates. The prevalence of the disease in the exposed regions was very high compared with the non-exposed regions. That means that the presence of swine colonies has an important role in the spreading of the disease. These results were supported by ***Rabinowitz, et al., 2012 and Kayali, et al., 2010. Rabinowitz, et al., 2012***, who said that integration of data streams of surveillance for human and animal cases of zoonotic disease holds promise for better prediction of disease risk and identification of environmental and regional factors that can affect risk, and can also point out gaps in human and animal surveillance systems and generate hypotheses regarding disease transmission. Also, our results supported by ***Kayali, et al., 2010,*** who stated that the circulation of pandemic H1N1 viruses in HPAI H5N1 endemic areas raises fears of emergence of a highly pathogenic virus efficient at human to human transmission; given the zoonotic nature of influenza, such an event is most likely to occur at the human-animal interface.

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REFERENCES

- ***Alexander, DY. (2004):*** A review of avian influenza [online]. Available at: http://www.esvv.unizh.ch/gent_abstracts/Alexander.html. Accessed 30 Aug 2004.
- ***Abbott, A. (2003):*** Human fatality adds fresh impetus to fight against bird flu. Nature; 423:5.

- ***Aamir, UB; Naeem, K; Ahmed, Z; Ober,t CA; Franks, J; Krauss, S; Seiler, P and Webster, RG; (2009***): Zoonotic potential of highly pathogenic avian H7N3 influenza viruses from Pakistan Virology. 390(2):212-20.
- ***Crawford, P. C; Dubovi, E. J and Castleman, W. L. (2005):*** Transmission of equine influenza virus to dogs. Science 310: 482-5; Gray, G. C., T.
- ***Kayali G, Webby RJ, Xiong X, Sherif LS, El-Ghafar E, Ali MA. (2010):*** Prospective study of avian influenza transmission to humans in Egypt. BMC Public Health. Nov 9; 10:685. PubMed PMID: 21062486; PubMed Central PMCID: PMC2991296.
- ***Kida, H.; Shortridge, K. F and Webster, R. G. (1988):*** Origin of the hemagglutinin gene of H3N2 influenza viruses from swine in China. Virology 162: 160–166.
- ***Rabinowitz, PM; Galusha, D; Vegso, S; Michalove, J; Rinne, S; Scotch, M and Kane, M. (2012):*** Comparison of Human and Animal Surveillance Data for H5N1 Influenza A in Egypt 2006-2011. PLoS One.7(9):e43851.
- ***Siengsanan, J.; Chaichoune, K.; Phonaknguen, R.; Sariya, L.; Prompiram, P.; Kocharin, W.; Tangsudjai, S.; Suwanpukdee, S.; Wiriyarat, W.; Pattanarangsan, R.; Robertson, I.; Stuart, D. Blacksell, and Parntep Ratanakorn (2009):*** Comparison of outbreaks of H5N1 highly pathogenic avian influenza in wild birds and poultry in Thailand.

- ***WHO (2006) World Health Organization:*** Avian Influenza. Available: http://www.who.int/csr/disease/avian_influenza/en/.

- ***Kida, H.; Ito, T.; Yasuda, J.; Shimizu, Y.; Itakura, C.; Shortridge, K. F.; Kawaoka, Y. and Webster. R. G. (1994):*** Potential for transmission of avian influenza viruses to swine. J. Gen. Virol. 75:2183–2188.

- ***Kilbourne, E. D. (1987):*** Influenza. Plenum; NY, 1-359.

- ***Kim JK, Kayali G, Walker D, Forrest HL, Ellebedy AH, Griffin YS, Rubrum A, Bahgat MM, Kutkat MA, Ali MA, Aldridge JR, Negovetich NJ, Krauss S, Webby RJ, Webster RG. (2010):*** Proc Natl Acad Sci U S A Jun 15; 107(24):11044-9. Epub 2010 Jun 1.

- ***Ludwig S, Stitz L, Planz O, Van H, Fitch WM, Scholtissek C. (1995):*** European swine virus as a possible source for the next influenza pandemic? Virology. ; 212:555–561.

- ***Ma, W; Vincent, AL; Gramer, MR; Brockwell, CB; Lager, KM; Janke, BH; Gauger, PC; Patnayak, DP; Webby, RJ and Richt, JA (2007):*** Identification of H2N3 influenza A viruses from swine in the United States. Proc. Natl. Acad. Sci. USA; 104: 20949–20954.

- ***Tombari W, Paul M, Bettaieb J, Larbi I, Nsiri J, Elbehi I, Gribaa L, Ghram A. PLoS One. (2013):*** 8 (1):e53524. Journal. Pone. 0053524. Epub Jan 11.

- ***Sims, L. D.; Domenech, J.; Benigno, C.; Kahn, S.; Kamata, A.; Lubroth, J.; Martin, V. and Roeder, P. (2005):*** Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. Vet. Rec. 157, 159–164.
- ***Shope, R.E., (1931):*** Swine influenza. III. Filtration experiments and aetiology. J. Exp. Med. 54, 373–380.
- ***Songserm, T., Jam-on, R., Sae-Heng, N., Meemak, N., Hulse-Post,D. J., Sturm-Ramirez, K. M. &Webster, R. G. (2006):*** Domestic ducks and H5N1 influenza epidemic Thailand. Emerging Infect. Dis. 12, 575–581.
- ***Wright PF and Webster RG. (2001):*** Orthomyxoviruses. In: Fields Virology, 4th ed. (D.M. Knipe and P.M. Howley, eds.). Lippincott Williams & Wilkins, Philadelphia, pp1533-1579.
- ***Aly, M. M.; Arafa, A. and Hassan, M. K. (2008):*** Epidemiological Findings of Outbreaks of Disease Caused by Highly Pathogenic H5N1 Avian Influenza Virus in Poultry in Egypt during 2006. Avian Diseases: June, Vol. 52, No. 2, pp. 269-277.
- ***OIE.2008b.Avian influenza. In: Terrestrial Animal Health Code (2008):*** hapter10.4.Available at http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

اثر تطبيق خطة التخلص من الخنازير على انتشار أنفلونزا الطيور في الدواجن والإنسان

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- تم إجراء هذه الدراسة لتقييم أثر تطبيق إستراتيجية التخلص من الخنازير على وجود و انتشار أنفلونزا الطيور H5N1 في تجمعات حظائر الدواجن المنزلية ومزارع الدواجن وكذلك الآدميين في محافظات الجيزة والقاهرة والقليوبية.
- تم اختبار عدد 73 حظيرة دواجن منزلية وعدد 95 مزارعة دواجن وعدد 168 عينة بشرية قبل التخلص من الخنازير.
- بعد تطبيق إستراتيجية التخلص من الخنازير تم اختبار عدد 67 حظيرة دواجن منزلية وعدد 85 مزرعة دواجن وعدد 152 عينة بشرية.
- تم اختبار جميع العينات باستخدام " Real time PCR " للكشف عن وجود مرض أنفلونزا الطيور H5N1.
- أظهرت النتائج قبل التخلص من الخنازير إيجابية 16 حظيرة دواجن منزلية و 6 مزارع دواجن.
- بعد تطبيق إستراتيجية التخلص من الخنازير أظهرت النتائج إيجابية عدد 5 حظيرة دواجن منزلية و 3 مزارع دواجن.
- أوضحت نتائج العينات البشرية ايجابية 22 و 9 من الآدميين للإصابة بمرض أنفلونزا الطيور قبل وبعد تطبيق إستراتيجية إبادة الخنازير وذلك على التوالي.
- خلصت هذه الدراسة على أن تطبيق إستراتيجية إبادة الخنازير كان لها أثرا هاما وملموسا على السيطرة على مرض أنفلونزا الطيور H5N1 في حظائر الدواجن المنزلية و كذلك الآدميين ولم يكن هناك تأثيرا ملموسا على مزارع الدواجن.