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

Ahmed A. El-Bassyouni¹, Wael F. El-Tras¹, Mona Mehrez A.², Yasser M. Bassyouni³

¹ Department of Hygiene and Preventive Medicine (Zoonoses), Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
² Animal Health Research Institute, National Laboratory for Quality Control of Poultry Production, Giza, Dokki, Egypt
³ General Organization for Veterinary Services, Undersecretary of Preventive Medicine, Department of Epidemiology, Giza, Dokki, Egypt

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°C) using ice boxes, and delivered to the laboratory within 48hr (Siengsanan, et al. 2009).
The Zoonotic Impact Of Swine Eradication Strategy On …

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

<table>
<thead>
<tr>
<th></th>
<th>Before swine eradication strategy</th>
<th>After swine eradication strategy</th>
<th>OR</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of poultry samples</td>
<td>No. of backyards</td>
<td>No. of positive backyards</td>
<td>Percentage</td>
<td>No. of poultry samples</td>
</tr>
<tr>
<td>Giza</td>
<td>175</td>
<td>35</td>
<td>8</td>
<td>23</td>
<td>200</td>
</tr>
<tr>
<td>Cairo</td>
<td>80</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
<td>60</td>
</tr>
<tr>
<td>Qalyoubia</td>
<td>110</td>
<td>22</td>
<td>6</td>
<td>27.3</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>365</td>
<td>73</td>
<td>16</td>
<td>22</td>
<td>335</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th></th>
<th>Before swine eradication strategy</th>
<th>After swine eradication strategy</th>
<th>OR</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of poultry samples</td>
<td>No. of Farms</td>
<td>No. of positive Farms</td>
<td>Percentage</td>
<td>No. of poultry samples</td>
</tr>
<tr>
<td>Giza</td>
<td>550</td>
<td>55</td>
<td>3</td>
<td>4.5</td>
<td>550</td>
</tr>
<tr>
<td>Cairo</td>
<td>80</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
<td>120</td>
</tr>
<tr>
<td>Qalyoubya</td>
<td>320</td>
<td>32</td>
<td>2</td>
<td>6.3</td>
<td>180</td>
</tr>
<tr>
<td>Total</td>
<td>950</td>
<td>95</td>
<td>6</td>
<td>6.3</td>
<td>850</td>
</tr>
</tbody>
</table>

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.
The Zoonotic Impact Of Swine Eradication Strategy On …

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

<table>
<thead>
<tr>
<th></th>
<th>Before swine eradication strategy</th>
<th>After swine eradication strategy</th>
<th>OR</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Suspected cases.</td>
<td>Confirmed</td>
<td>Prevalence</td>
<td>Suspected cases.</td>
<td>Confirmed</td>
</tr>
<tr>
<td>Giza</td>
<td>90</td>
<td>11</td>
<td>0.12</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>Cairo</td>
<td>24</td>
<td>3</td>
<td>0.13</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Qalyoubya</td>
<td>54</td>
<td>8</td>
<td>0.15</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>22</td>
<td>0.13</td>
<td>152</td>
<td>9</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Giza</th>
<th>Cairo</th>
<th>Qalyoubya</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected cases</td>
<td>Confirmed</td>
<td>Prevalence</td>
<td>Suspected cases</td>
<td>Confirmed</td>
</tr>
<tr>
<td>Exposed regions</td>
<td>95</td>
<td>5</td>
<td>0.05</td>
<td>24</td>
</tr>
<tr>
<td>Non exposed Regions</td>
<td>97</td>
<td>3</td>
<td>0.03</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>8</td>
<td>0.04</td>
<td>46</td>
</tr>
</tbody>
</table>

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.

ACKNOWLEDGEMENTS

The authors would like to thank the staff members of Animal Health Research Institute and the National Laboratory for Quality Control of Poultry Production, Giza Dokki, Egypt, and Epidemiology Unit of General Organization for Veterinary Services.

REFERENCES


- 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.


- **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.


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

أحمد أحمد البسيوني¹، وائل فؤزي التراس¹، منى محرز حسانين²، ياسر محمد بسيوني³

¹قسم الصحة والطب الوقائي - كلية الطب البيطري، جامعة كفر الشيخ
²معهد بحوث صحة الحيوان الدقيق – المعامل القومي للرقابة على الإنتاج الداجني
³الهيئة العامة للخدمات البيطرية الإدارة المركزية للطب الوقائي/إدارة التخطيط والرصد الوبائي للأمراض

تم إجراء هذه الدراسة لتقييم اثر تطبيق إستراتيجية التخلص من الخنازير على وجود وانتشار أنفلونزا الطيور H5N1 في تجمعات حظائر الدواجن المنزلية ومزارع الدواجن وكذلك الأدميين في محافظات الجيزة والقاهرة والقليوبية.

تم اختبار عدد 73 حظيرة دواجن منزلية وعدد 95 مزرعة 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.

أظهرت النتائج قبل التخلص من الخنازير إيجابية 16 حظيرة دواجن منزلية و 6 مزارع دواجن.

أظهرت النتائج بعد تطبيق إستراتيجية التخلص من الخنازير إيجابية 5 حظيرة دواجن منزلية و 3 مزرع دواجن.

أوضح نتائج العينات البشرية إيجابية 22 و 9 من الأدميين للإصابة بمرض أنفلونزا الطيور قبل وبعد تطبيق إستراتيجية إبادة الخنازير وذلك على التوالي.

خلصت هذه الدراسة على أن تطبيق إستراتيجية إبادة الخنازير كان له أثرا هاما وللمؤسسة على السيطرة على مرض أنفلونزا الطيور H5N1 في حظائر الدواجن المنزلية، وكذلك الأدميين ولم يكن هناك تأثيرا ملموسا على مزارع الدواجن.