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Incidence of Silent AI Virus in Relation to Vaccination and Biosecurity Level in Some Broiler Farms of Egypt

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Abstract: The frequency distribution of positive avian influenza virus of studied farms was 46.14%, 12.12%, 18.75% in Qualubia, Fayoum and Beni-suef Governorates respectively. The mean value of HI titer was 8.6 % with positive PCR detection avian influenza virus in vaccinated farms, while in non vaccinated farms were 18.3%. Negative avian influenza virus PCR detection show HI titer 6.8 and mortality rate 7.1% in vaccinated farms while in non vaccinated HI titer 1.3 and mortality rate 5.8%. In Fayoum and Beni-suef Governorates, the mean values of HI titer were 11.2, 4.7 and the mean values of mortality rate were 20%, 48.8 % with positive avian influenza virus detection, also the mean value of HI titer were 6.01, 5.8 and the mean values of mortality rate were 6.89%, 6.7 % with negative avian influenza detection.
The frequency distribution of biosecurity levels of farms in Qualubia Governorate were 53.86%, 30.76%, 15.38% as good, fair and poor respectively. In Fayoum, frequency distribution of biosecurity levels were 30.30%, 57.58%, 12.12% and in Beni-suef were 28.12%, 56.26%, 15.26% as good, fair and poor biosecurity level respectively.
The results revealed an association between virus 'silent spread 'and HI-titer level, where HI acted as a protective factor). Generally HI is positively related to the prevention of the virus, that the reduction of virus will be increased when the HI is present of high level but, even in the presence of high titer, unseen transmission between poultry farms can be occurred in the presence of biosecurity faults. The association between biosecurity faults as risk factors in broiler house and their effects on HI titers in chickens vaccinated against AIV revealed that, there was an association between faults of biosecurity level and the low value in HI titer of chicken sera vaccinated against AIV vaccines.
Keywords: HPAV ; Silent spread; Biosecurity faults; HI-titer; Vaccination.

Introduction

Vaccination of poultry against highly pathogenic avian influenza (HPAI) is one of the control measures used in endemically infected countries. The main goal of vaccination is to protect flocks against production losses and mortality, but in case of HPAI it should also be to protect a bird against infection.

Vaccination is increasingly being considered as a possible tool to prevent the successful introduction of the disease in certain high- risk areas in case a highly pathogenic virus has been detected at a certain distance from the area of out-break [1].

AI vaccines are usually evaluated by measuring vaccine-induced haemagglutination inhibition antibody (HI) titres in serum samples, and by measuring the level of protection against clinical signs, virus shedding, and mortality after a challenge infection [2] [3].

It is possible that HPAI virus may spread unnoticed, if the vaccine protects against clinical signs or production losses, but not against virus transmission. Then, eradication of virus might not be achieved and continuous spread might also result in the emergence of new strains [4] [5].
Incomplete vaccination of poultry flocks could make the spread of deadly strains of AI such as H5N1 worse, even

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though the available vaccines were effective on individual birds; the disease was likely to spread unless almost all of a flock has been protected. Vaccination of commercial poultry against HPAI (H5N1) is proving controversial because it is thought that it can lead to unseen transmission between poultry farms, a phenomenon known as 'silent spread'. This unseen transmission occurs because as protection levels rise in a flock, it becomes ever harder to detect the spread of AI quite simply because fewer birds die. The result was increased amounts of bird flu virus contaminating the birds' surroundings without farmers realizing it. International debate on the merits of vaccinating poultry against the H5N1 influenza A virus has raised concerns about the possibility of an increased risk of between-flock transmission before outbreaks are detected [6]. Silent spread' can occur because of incomplete protection at the flock level, even if a vaccine was effective in individual birds. A single vaccination applied under field conditions induced clinical protection, but was insufficient to induce protection against virus transmission, suggesting that silent spread of virus in vaccinated commercial flocks may occur [7].

Vaccination in endemically infected countries should, therefore, be accompanied by a good virus surveillance and sero-monitoring system, and with biosecurity measures to identify and reduce vaccination failure [8].

In practice, it was very hard to protect more than about 90% of the birds in any given flock, and protection levels were usually much lower than this. Protection levels of more than 95% needed to guard against silent spread.

The research underlines that vaccination, if used, should be part of a comprehensive control strategy including biosecurity, surveillance and diagnostics, education, movement restrictions and elimination of infected birds.

Biosecurity is a set of practice applied for limiting the spread of disease causing agents from place to another. Prevention of disease and good bird performance based on application of biosecurity programmes.

This study was carried out to investigate degree of biosecurity level in some broiler poultry farms with especial reference for highly pathogenic avian influenza virus in three major Egyptian Governorates (Qualubia, Fayoum, and Beni-suef) . As well as tried to explain the expected causes and risk factors that leads to silent spread of the disease in poultry flocks in Egypt.

Materials and Methods

For evaluation of biosecurity measures in three major Egyptian governorates (Qualubia, Fayoum, and Beni-suef), a total of seventy eight broiler houses were studied from November 2014 through January 2016. The visited farms were described for their construction, bird type, stocking densities, traffic control, pest control, vaccination programmes of AI, disinfection and other managemental criteria. The evaluation process was carried out through filling out a designed questionnaire, taking blood samples and tracheal & cloacal swabs.

Table 1: showing the number of serum samples and cloacal & tracheal swabs from the three major Egyptian Governorates (Qualubia, Fayoum, Beni-suef).

Vaccination and immune response

HA and HI tests were applied in V-bottomed micro well plastic plates in which the final volume for both types of test is 0.075 ml. The reagents required for these tests are: isotonic PBS (0.1 M), pH 7.0-7.2; and red blood cells (RBCs) taken from a minimum of three SPF chickens (if SPF chickens are not available blood may be taken from birds that are regularly monitored and shown to be free from antibodies to avian influenza). Cells should be washed three times in PBS before use as a 1% (packed cell v/v) suspension and Multichannel pipette with tips [12].

Haemagglutination test (HA)

HA was carried out for standardization of AI antigen used in HI test to 4 Haemagglutination units which recommended by [10].

Serum Sampling

Blood samples were collected from commercial birds representing different ages and vaccination schedules at total number of 1805 samples during May 2014 till October 2015. Blood samples were obtained by sterile syringes from wing vein, or by severing neck blood vessels in case of chicks, then placed in slope position and transported in ice box to the laboratory for separation of serum by centrifugation at 3000 rpm/10 minutes [13].

Biosecurity practices failure as a risk factor concerning avian influenza virus spread in three major Egyptian governorates (Qualubia, Fayoum and Beni-suef).

Analyze a 2x2 contingency table (Fisher's exact test), where $\chi 2\ (1) = 81.93$, $p < .001$.

Biosecurity indicators [9]

Biosecurity parameters	Score (code)
1. Self proofing (bird and house)	(yes = 0.1, no = 0)
2. Rodent and wild bird proofing	(yes = 0.1, no = 0)
3. Ventilation area	(yes = 0.1, no = 0)
4. Adequate distance between farms and other poultry operations	(yes = 0.1, no = 0)
5. Hygienic disposable of carcass	(yes = 0.1, no = 0)
6. Self sufficient (farm equipment)	(yes = 0.1, no = 0)
7. Cleaning and disinfection	(yes = 0.1, no = 0)
8. Foot dips	(yes = 0.1, no = 0)
9. Traffic control	(yes = 0.1, no = 0)
10.Visitor restriction	(yes = 0.1, no = 0)

Biosecurity failure as a risk factor:

Biosecurity Level	AI Infection	
	Present	Absent
Exposed	A	B
Non -exposed	C	D

Relative Risk:

$$R.R. = \frac{\frac{A}{A+B}}{\frac{C}{C+D}}, \quad \text{Attributable Risk} = \frac{A}{A+B} - \frac{C}{C+D},$$

$$\text{Omega} = \frac{A+C}{B+D}, \quad \text{The odd ratio } (\Psi) = \frac{A \times D}{B \times C}$$

Sampling

Cloacal and tracheal samples were taken from poultry (broilers) at total number of 3250 samples from November 2014 to January 2016, The swab is used to collect a tracheal or cloacal sample from poultry According to OIE, 2006 [10] andClement, et.al.2010 [11], an extraction buffer is added to the swab in a tube, and then the swab is removed from the tube and replaced by the test strip. A pink- purple line appears on the test strip if the Influenza A virus is present.

Governorate	Number of serum samples	Number of cloacal and tracheal swabs
Qualubia	560	1010
Fayoum	670	1120
Beni-suef	575	1120
Total	**1805**	**3250**

Risk factor: - is a factor associated with an increase in probability of occurrence of outcome of interest.

Relative Risk (R.R):- is an epidemiologic measure of the strength of the relationship between risk factor and an outcome that can be estimated by Odds ratio.

Biosecurity level	**Avian influenza virus antigen detection by RRT-PCR**	
	positive	negative
Good biosecurity	A	B
Fair to poor biosecurity	C	D

Materials required for RRT-PCR

RNA extraction

Primers used in RRT-PCR of M and H5 genes

Gene	Name	Type	Sequence (5' - 3')	Reference
M	M24	Forward	AGA TGA GTC TTC TAA CCG AGG TCG	(Spackman et al .2002) [17]
	M25	Reverse	TGC AAA AAC ATC TTC AAG TCT CTG	
	SEPRO	Probe	FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA	
H5	H5 LH1	Forward	ACG TAT GAC TAC CCG CAG TAT TCA	(Slomka et al., 2007) [18]
	H5 RH1	Reverse	AGA CCA GCC ACC ATG ATT GC	
	H5PRO	Probe	FAM-TCWAC AGTGGCGTTCCC TAGC A –TAMRA	

RNA extraction done according to QIAamp Viral RNA Mini Kit that supplied from (Qiamp viral RNA mini Kit. GmbH, Hilden, Germany) Commercial licensed kit Catalogno. 52904. MicroAmp® Optical 8-Tube Strip, 0.2 ml, Catalog number 4316567. Reagents and volume of RRT-PCR reaction mix for M and H5 genes of H5N1 subtype according to WHO, 2007, [16].

Attributable Risk factor:-measure of how a risk factor contributes to the overall incidence of disease in a population [14].

Odds Ratio (OR) is a measure of effect size, describing the strength of association or non-independence between two binary data values. It is used as a descriptive statistic, and plays an important role in logistic regression. OR ranged in value from 0 to infinity; values close to 1.0 indicate no relationship between the exposure and the outcome; Values less than 1.0 suggest a protective effect, while values greater than 1.0 suggest a causative or adverse effect of exposure [15]. poor respectively. In Fayoum, frequency distribution of biosecurity levels were 30.30%, 57.58%, 12.12% and in Beni-suef were 28.12%, 56.26%, 15.26% as good, fair and poor biosecurity level respectively. The probability of infection is strongly increases in districts of the same governorates when the poultry farm density of heavy character as in case of Qualubia and Sharkia governorates. Probability of infection is strongly decreased in governorates of low poultry farm density as in case of Quena, Aswan, and Sohage – governorates [1].

Biosecurity level and its relation to mortality rate and HI titer in broilers

Biosecurity levels in broiler farms.

Frequency distribution of biosecurity level in broiler farms

The collected data in Tables (3-a,b and c) revealed that frequency distribution of biosecurity level of farms in Qaliopia G. were 53.86%, 30.76%, 15.38% as good, fair and

Results and Discussion

Relationship between degree of biosecurity, mortality rate and HI titer against AI

The collected data in Tables (4-a, b and c) revealed that the mean values of HI titer were 6.64, 5.72, 3.83 with good, fair, poor biosecurity level in farms ,respectively in Qualubia, Fayoum and Beni-suef Governorates. While the mean values of mortality rate were 4.7%, 5.3%, 8% with good, fair, poor biosecurity level respectively. In Beni-suef Governorate the mean values of HI titer were 8.5, 6.75, and 5.71 with good, fair, poor biosecurity level respectively. While the mean values of mortality rate were 6.28%, 8.26%, 38.75% with good, fair, poor biosecurity level respectively. In Giza Governorates, the mean values of HI titer were 6.57, 9.5, 6.5 with good, fair, poor biosecurity level in vaccinated farms respectively, while in non vaccinated farms were 1.97, 9.15. While the mean values of mortality rate were 6.57%, 13.3%, 18.5% and 15.83%, 28% in good, fair, poor biosecurity level in vaccinated farms and non-vaccinated respectively.

Avian influenza control could be achieved through inclusions and exclusions biosecurity practices, diagnosis and surveillance, elimination of infected bird, increasing host resistance and personnel education [19].

Risk analysis of possible causes and risk factors influencing AI spread

Form Table 5, we found that there was no association concerning level of applied biosecurity in broiler farms and virus detection **(Silent spread)**, where the relative risk concerning virus detection is less than one (0.126). Odds Ratio (OR) = 0.092; its means that the 'silent spread ' will be not reduced significantly *(P<0.05)* even if the satisfactory biosecurity applied to such farms. If the odds ratio for an event deviates substantially from 1.0, the odds ratio for the event's failure to occur will also deviate substantially from 1.0, though in the opposite direction. This indicate that even in the presence of good biosecurity unseen transmission between poultry farms, a phenomenon known as 'silent spread 'can be occurred.

Table 3: showing frequency distribution of biosecurity levels in broilers in investigated Governorates.

Biosecurity levels	Farm number and percentage		
Good	**Qualubia**	**Fayoum**	**Beni-suef**
	70(53.86%)	100 (30.30%)	90 (28.12%)
Fair	40(30.76%)	190 (57.58%)	180 (56.26%)
Poor	20(15.38%)	40(12.12%)	50 (15.62%)
Total	130(100%	330 (100%)	320(100%)

Table (4) showing analysis of variance and least significant difference of biosecurity levels in broilers and their relation to mortality rate and HI titer in the investigated Governorates.

Table 4-a: Qualubia Governorate

Biosecurity level Vaccine application		Mean values	
		Mortality (%)	**HI titer**
Good	Yes	6.75 [c]	6.75 [b]
	No	15.83 [b]	1.97 [c]
Fair	Yes	13.3 [b]	9.50 [a]
	No	28 [a]	9.15
Poor	Yes	18.5 [b]	6.55 [b]

*Means with the same letter in the column are not significantly different *(P<0.05)*

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Table 4-b: Fayoum Governorate.

Biosecurity level	Mean values	
	Mortality %	**HI titer**
Good	4.7 [b]	6.64 [a]
Fair	5.3 [b]	5.72 [a]
Poor	8.0 [a]	3.83 [b]

*Means with the same letter in the column are not significantly different *(P<0.05)*

Biosecurity levels in broiler farms.

Frequency distribution of biosecurity level in broiler farms

The collected data in Tables (3-a,b and c) revealed that frequency distribution of biosecurity level of farms in Qaliopia G. were 53.86%, 30.76%, 15.38% as good, fair and poor respectively. In Fayoum, frequency distribution of biosecurity levels were 30.30%, 57.58%, 12.12% and in Beni-suef were 28.12%, 56.26%, 15.26% as good, fair and poor biosecurity level respectively. The probability of infection is strongly increases in districts of the same governorates when the poultry farm density of heavy character as in case of Qualubia and Sharkia governorates. Probability of infection is strongly decreased in governorates of low poultry farm density as in case of Quena, Aswan, and Sohage – governorates [1].

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Avian influenza control could be achieved through inclusions and exclusions biosecurity practices, diagnosis and surveillance, elimination of infected bird, increasing host resistance and personnel education [19].

Table 4-c: Beni-suef Governorate

Biosecurity level	Mean values	
	Mortality (%)	**HI titer**
Good	6.28 [b]	8.50 [a]
Fair	8.26 [b]	6.75 [a]
Poor	38.75 [a]	5.71 [b]

*Means with the same letter in the column are not significantly different *(P<0.05)*.

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Risk analysis of possible causes and risk factors influencing AI spread

Biosecurity failure as a risk factor concerning avian influenza virus spread in broiler farms

Form Table 5, we found that there was no association concerning level of applied biosecurity in broiler farms and virus detection **(Silent spread)**, where the relative risk concerning virus detection is less than one (0.126). Odds Ratio (OR) = 0.092; its means that the 'silent spread ' will be not reduced significantly *(P<0.05)* even if the satisfactory biosecurity applied to such farms. If the odds ratio for an event deviates substantially from 1.0, the odds ratio for the event's failure to occur will also deviate substantially from 1.0, though in the opposite direction. This indicate that even in the presence of good biosecurity unseen transmission between poultry farms, a phenomenon known as 'silent spread 'can be occurred.

Table 5: showing biosecurity practices failure as a risk factor concerning avian influenza virus spread

Biosecurity level	Avian influenza virus antigen detection		
	positive	negative	
Good biosecurity	10	260	270
Fair to poor biosecurity	150	360	510
	160	620	780

(P<0.05)
Relative risk (R.R.) = 0.126
Attributable risk = 27
Odds Ratio (OR) = 0.092

Table 6: Showing AI virus (Silent spread) as a risk factor concerning avian influenza and mortality rate

Mortality rate	Avian influenza virus antigen detection		
	positive	negative	
High mortality	150	380	530
Low mortality	10	240	250
	160	620	78 0

(P<0.05)
Relative risk (R.R.) =7.075
Attributable risk = 0.243
Odds Ratio (OR) = 10

AI virus (Silent spread) as a risk factor concerning avian influenza and mortality rate in broiler farms

Form Table (6), we found that there was an association concerning virus antigen detection and mortality rate in broiler farms, where the relative risk concerning detection was 7.075 while, the magnitude of this association (attributable risk) was 0.243, this means that 24% of mortality mainly was due presence of virus *(P<0.05)*, in other meaning 24 % of mortality attributable to presence of virus in such farms. OR was 10, this means that the mortality would be increased to more than 10 folds when the virus is present in such farms.

Table 7: showing HI titer of AI as risk factors concerning (Silent spread)

HI titer	Avian influenza virus antigen detection		
	positive	negative	
High titer	70	380	450
Low titer	90	240	330
(P<0.05)	160	620	780

Relative risk (R.R.) = 2.02
Attributable risk = - 0.17
Odds Ratio (OR) = 2

Hi titer of AI as risk factors concerning (Silent spread) in broiler farms:-

The HI titre is often used in the field to determine whether a flock is sufficiently protected against infection or should be revaccinated. Titres above a value of 4 log 2 [20] and a coverage of 80%, i.e. the percentage of vaccinated birds in the flock, is supposed to be sufficient [21] [22].

Form Table 7, we found that there was an association concerning virus 'silent spread 'and HI-titer level, where the relative risk concerning detection was 2.02 and the magnitude of the association (attributable risk) risk was -0.17; this means that HI acted as a protective factor). Generally HI is positively related to the prevention of the virus, because the Odds ratio was greater than 1. The OR was 2; this means that the reduction of virus will be increased to more than 2 folds when the HI is present of high level in such farms (if the odds ratio for an event deviates substantially from 1.0, the odds ratio for the event's failure to occur will also deviate substantially from 1.0, though in the opposite direction). This indicates that even in the presence of high titer unseen transmission between poultry farms can be occurred. The association between biosecurity faults as risk factors in broiler house and their effects on HI titers in chickens vaccinated against AIV revealed that, there was an association between faults of biosecurity measurements and the low value in HI titer of chicken sera vaccinated against AIV vaccines [9] [23].

Conclusion

Titres above a value of 4 log 2 and a coverage of 80%, i.e. the percentage of vaccinated birds in the flock, is supposed to be sufficient. A single vaccination applied under field conditions induced clinical protection, but was insufficient to induce protection against virus transmission, that silent spread of virus in vaccinated commercial flocks may occur, so 'silent spread ' will be not reduced significantly even if the satisfactory biosecurity applied to such farms.

The research underlines that vaccination, if used, should be part of a comprehensive control strategy including biosecurity, surveillance and diagnostics, education, movement restrictions and elimination of infected birds.

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