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Epidemiological Studies on Avian Influenza in Behera Province, Egypt

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Abstract: The current study was conducted to monitor avian influenza A viruses using commercially available rapid antigen detection test procedures in small-scale commercial poultry farms (sector III) in selected districts of Behera province and to investigate some biosecurity practices in examined poultry farms through using a specially designed questionnaire directed to Farms' owners. Moreover, estimation of some epidemiological parameters including relative risk (RR), attributable risk (AR), odds ratio (OR) and omega measure was done. A total of 140 cloacae fecal samples were taken from poultry (broilers and layers) during the period extended from January 2010 till December 2011. Chickens were sampled opportunistically from various sources in Behera province. In addition, data were collected using structured questionnaires captured poultry disease prevention measures. The recorded results of rapid testing of collected samples revealed that the overall prevalence of AI infection in investigated backyard poultry farms in 8 districts of Behera Province was 12.85 % (18 out of 140 examined samples). Statistical analysis of these obtained results by Chi-square ($P> 0.05$) showed no significant association ($P = 3.94$) between the prevalence of AI in the investigated districts of Behera Province. Based on the collected data by the used questionnaire in the 140 backyard poultry farms, 5 variables were studied including vaccination, traffic control, precautions taken by visitors, application of routine disinfection programs and control of wild birds using (Yes/No) questions where yes: means the presence of the factor while no: means absence of the factor. Obtained results could make a useful contribution towards preventing AI viruses type A in backyard poultry farms and decreasing losses in the poultry industry. It is suggested that more attention should be paid towards implementing a proper control program for AI and more efforts should be directed towards improving the biosecurity program in Behera Province.

Key words: Avian influenza · Epidemiology · Biosecurity practices · Backyard farms · Egypt

INTRODUCTION

Avian influenza (AI) is a disease of agro economic and public health importance manifesting generally as severe respiratory infection of birds [1] affecting almost every organ and tissue of the body in its severe form known as highly pathogenic AI (HPAI). The virus is excreted in both respiratory and gastro-enteric systems and can be detected in tracheal, cloacal, or fecal matter [2]. Day concerns about avian influenza or 'bird flu' in more informal terms began with a 1997 outbreak in Hong Kong which resulted in six fatalities among the 18 people affected [3]. This was quickly identified as a new, highly pathogenic strain of avian flu, named H5N1, which originated in chickens [4, 5]. Chicken exports were banned, about 1.6 million chickens were slaughtered and the virus seemed to have been eradicated. It re-emerged in 2003 in East-Asia at the same time that another strain infected poultry in the Netherlands, devastated the poultry industry there and killed one veterinarian [6]. Since then bird flu has claimed the lives of 235 people [7]. Governments and health protection agencies have also been on high alert, in line with the World Health Organization's action plans and its warning that avian flu strains may cause a global human flu pandemic, similar to the one that killed tens of millions of people in 1918 [8]. Avian influenza or bird flu is a zoonotic disease that can spread between poultry and humans through direct contact with feces or blood and can cause death in both species. Of specific concern is the possibility of the avian

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influenza virus transmuting into a virus that could be transmitted between humans, causing a world-wide pandemic of human influenza [9]. The first HPAI outbreak in Egypt was announced on 17th February 2006. It started in 3 governorates and spread thereafter to 21 out of the 27 governorates of the country, infecting commercial and backyard systems. 36 million birds have been culled with costs estimated at between 2 to 3 million USD. 1.5 million Individuals whose livelihoods depend on poultry were affected. The disease became endemic and outbreaks were detected from north to south of Egypt but mainly in the areas adjacent to the Nile River [10]. The most common methodologies for the identification of influenza virus strains typically require virus isolation, culture and characterization by immunoassay [11].This method of characterization of cultured virus is considered the "gold standard" for virus identification and generates a large quantity of virus for further characterization [12]. Unfortunately, this method requires 3 to 7 days to culture the virus prior to antigenic testing and can test only a few samples simultaneously [13]. Anigen™ Rapid Avian Influenza Virus Antigen Test Kit is a chromatographic immunoassay for the qualitative detection of avian influenza type A virus antigen where specially selected avian influenza virus antibodies are used in test band as both capture and detector materials to identify avian influenza virus antigen in cloacal swab, tracheal swab or feces with a high degree of accuracy. Since stopping virus spread as early as possible can result in significant savings and minimize disruption to the poultry industry. So, the current study aimed to monitor AI using commercially available rapid antigen detection test procedures in small-scale commercial poultry farms (sector III) in Behera governorate, investigate biosecurity practices in the examined poultry farms using a specially designed questionnaire directed to farms owners and estimation of some epidemiological parameters including relative risk (RR), attributable risk (AR), odds ratio (OR) and omega measure of obtained results.

MATERIALS AND METHODS

Farms under Investigation: Behera province has been facing the problem of spreading of small rural backyard poultry flocks with capacity ranging from the number of 500 to 10,000 birds (Sector 3: Small-Scale Commercial Production System [14].

Samples and Data Collection: Cloacae fecal samples were taken from poultry (broilers and layers) at a total 140 samples during January 2010 till December 2011. Chickens were sampled opportunistically from various sources in 8 different districts of Behera province targeting likely incidences of (highly pathogenic avian influenza type A, HPAI. Sources included small commercial broiler and layer farms. Data were collected using structured questionnaires.

Detection of Avian Influenza Virus Antigen (Antigen Capture) Method: Rapid avian influenza virus antigen test kit (ANIGEN® animal genetics, Inc. Korea) (CAT. No: RG 15-01) was used according to the manual supplied by the manufacture company.

Farm Visit Questionnaire: In order to gain access to poultry farmer's knowledge and understanding of disease risks and determining the role of biosecurity measures in disease prevention, interviews with poultry farmers were carried out in this study providing a platform for the fieldwork. Our corpus is relatively small and does not cover all aspects of the industry. This reflects, in part, difficulties of access, as there were various outbreaks of avian influenza while we were in the process of recruiting. Participants were interviewed between Jan. 2010 and Dec. 2011. Farmers in our sample had farms of small-holdings (farm capacity of less than 10000 birds). Interviews with farmers were carried out in Behera providence, as this region has a high density of poultry farms and is so far a region with high incidence of avian flu in poultry flocks.

Questionnaires Data Analysis: Collected data from poultry farms were incorporated to enrich the analysis and subsequent discussions as it were used to calculate some epidemiological outcomes (measures of association) which will help in better understanding of patterns and behavior of AIV.

Statistical Analysis: Epidemiological Measures and Fischer's exact test was used to evaluate questionnaire data.

Calculation: The most basic data layout in epidemiology is notation for a 2x2 Contingency Table:

	AI		
Risk factors	Positive	Negative	Total
Exposed	a	b	a+ b
Not-Exposed	c	d	c + d
	a+ c	b + d	n

Measures of strength:

- Incidence risk ratio (Relative Risk) (RR)

$$RR = \frac{R_E}{R_O} \tag{7}$$

- Odds Ratio (Cross-Product Ratio, Relative Odds)

$$OR = \frac{O_E}{O_O} = \frac{ad}{bc} \quad (8)$$

Measures of effect in the exposed population:

$$AR = R_E - R_O \quad (10)$$

Attributable Risk (rate): (AR)

Measures of Association: Omega measure:

Omega Risk Measure = a+c/ b+d

Fisher Exact Probability Test (P Value): The degree of disproportion within any array of cell frequencies in effect, the degree of ostensible association between variables within the sample can be measured by the absolute difference:

$$\left| \frac{a}{a+b} - \frac{c}{c+d} \right|$$

RESULTS AND DISCUSSION

AIV continue to circulate in poultry and cause disease and form a threat to human. Both animal and public health sectors at the national, regional and international levels should maintain vigilance in regularly detecting, reporting and characterizing animal influenza viruses and in assessing and managing existing and evolving health risks associated with these viruses. Diagnosis of AI depends on the detection of the viral antigen as early identification and isolation of infected flocks is of most importance in minimizing the risk of further epidemic spread [15].

The prevalence of AI investigated back yard poultry farms in 8 districts of Behera Province in 2010 and 2011 was illustrated in Table 1. Firstly, it was found that the overall prevalence of AI infection in investigated backyard poultry farms was 12.85 % (18 out of 140 examined samples). This result was lower than that recorded by Afifi *et al.* [16] who found that the total prevalence of AI in 1,225 examined serum samples was 34 %. On other hand, it was higher than recorded as 1.17 %, by Aly *et al.* [17], 1.16 % by Fasina *et al*. [18], 8.8% by Ayaz *et al*. [19] and 5% by Ghazi *et al*. [20]. Concerning broiler farms, it was found that the prevalence of AI in examined samples was 12.9 % which was higher than detected by Ayaz *et al*. [19] (9%). Also, it was recorded that the highest prevalence was observed in Damanhour (4 / 20) and Abo Homous (3/15) Districts (20 %) followed by El Rahmania District (3/19; 15.8 %) then Shobrakhet District (3/20; 15 %) then Mahmoudia District (1/10 ;10 %), Etai-Elbarod District (2/25; 8 %), Abo El Matameer District (1/21; 4.7 %) and lastly, Kafr El dawar District where no positive samples were detected in the examined 2 farms. Statistical analysis of these obtained results by Chi-square (P> 0.05) showed no significant association (P = 3.94) between the prevalence of AI in the investigated districts of Behera Province. Concerning layer farms under investigation, only one positive farm was detected in 8 examined layer farms with

Table 1: Prevalence of AI in investigated back yard poultry farms

	Broiler						Layers					
	2010			2011			2010			2011		
District	No.	+ ve	%	No.	+ ve	%	No.	+ ve	%	No.	+ ve	%
Damanhour	12	2	16.7	No.	+ ve	%	-	0	0.00	-	0	0.00
Rahmania	13	2	15.4	8	2	25	-	0	0.00	-	0	0.00
Mahmoudia	5	0	0.00	6	1	16.7	-	0	0.00	-	0	0.00
Kafer-El dawar	1	0	0.00	5	1	20	2	1	50	1	0	0.00
Abo Homos	6	1	16.7	1	0	0.00	-	0	0.00	-	0	0.00
Shobrakhet	11	2	18.2	9	2	22.2	1	0	0.00	1	0	0.00
Etai- El barod	11	1	9.1	9	1	11.1	-	0	0.00	-	0	0.00
Abo El matamer	8	1	12.5	14	1	7.1	1	0	0.00	2	0	0.00
Total	69	9	13.4	13	0	0.00	4	1	25	4	0	0.00
Chi-square value and significance	1.66 (P>0.05) NS			4.72 (P>0.05) NS			1.33 (P>0.05) NS					

Table 2: Distribution of the AI seropositive and seronegative flocks and the relevance with different investigated variables and the magnitude of association between the different risk factors of positive backyard poultry farms for AI in Behera province

	AI results				Epidemiological analysis				
Biosecurity practices (Variables)	Category	Number	Positive No. (%)	Negative No. (%)	Relative risk (RR)	Odds ratio (OR)	Attributable risk (AR)	Omega magnitude	P-value
Vaccination against AI	Yes	97	10 (10.3)	87 (89.7)					
	No	43	8 (18.6)	35 (81.4)	0.55	0.50	-0.08	0.15	0.140
Traffic control	Yes	30	1 (3.3)	29 (96.7)					
	No	110	17 (15.5)	93 (84.5)	0.22	0.19	-0.12	0.15	0.064
Precautions taken to visitors	Yes	23	0 (0.0)	23 (100)					
	No	117	18 (15.4)	99 (84.6)	0.57	0.52	-0.07	0.17	0.319
Routine disinfection programs	Yes	135	17 (12.6)	118 (87.4)					
	No	5	1 (20)	4 (80)	0.63	0.58	-0.07	0.15	0.502
Control of wild birds	Yes	7	0 (0.0)	7 (100)					
	No	133	18 (13.5)	115 (86.5)	0.00	0.00	-0.14	0.15	0.372

Yes: means the presence of the factor

No: Means the absence of the factor

a total prevalence of 12.5 % and it was found in Kafr El dawar District where 3 suspected layer farms were sampled. Statistical analysis of these obtained results by Chi-square (P> 0.05) showed no significant association (P = 1.90) between the prevalence of AI in the investigated districts of Behera Province. This obtained result was higher than that recorded by Ayaz *et al*. [19] as 4%. Moreover, statistical analysis of the obtained results of examined broiler and layer farms by Chi-square (P> 0.05) showed no significant association (P = 0.001) between the prevalence of AI in the investigated broiler and layer back yard poultry farms in Behera Province. In the current study, it was found that the prevalence of AI in broiler was higher than in layers in examined backyard poultry farms that was in harmony with Ayaz *et al*. [19] while it was in disagreement with Afifi *et al*. [16] who found that the prevalence of AI antibodies were higher in layer followed by broiler flocks. From the previously mentioned results, it was clear that AI was detected in both broiler and layer back yard poultry farms in Behera Province throughout the current study extended from 2010 to 2011 that emphasized the continuous spreading of the disease in investigated province that threatening poultry industry and human health in Behera Province. The obtained result was in agreement with WHO [21] who reported that Egypt was currently one of the most infected countries in Africa and in the world.

The recorded data in Table 2 clarified the prevalence of AI in investigated broiler back yard and poultry farms in the 8 districts of Behera Province during the year of 2010. It was found that the overall prevalence of AI infection in investigated broiler farms in 2010 was 13.4 % (9 out of 69 examined samples). The highest prevalence was observed in Shobrakhet District (2/11;18.2 %) followed by both Damanhour (2 / 12) and Abo Homous (1/6) Districts (16.7 %) then El Rahmania District (2/13; 15.4 %), Abo El Matameer District (1/8; 12.5 %) and Etai-Elbarod District (1/11; 9.1 %). On the other hand, AI infection was not detected in examined samples of both Mahmoudia and Kafr El dawar. Statistical analysis of the obtained results by Chi-square (P> 0.05) showed no significant association (P = 1.66) between the prevalence of AI in broiler back yard poultry farms the investigated districts of Behera Province in 2010.

Data presented in table 3 clarified the prevalence of AI in investigated 4 layer back yard poultry farms distributed in only 3 districts of Behera Province including Kafr El dawar, Shobrakhet and Abo El Matameer during the year of 2010. Only 1 out of 4 examined samples was found to be positive with a percentage of 25 % and it was detected in Kafr El dawar District.

Statistical analysis of the obtained results by Chi-square (P> 0.05) showed no significant association (P = 1.33) between the prevalence of AI in examined layer back yard poultry farms the investigated districts of Behera Province in 2010. The obtained results in tables 4, 5 were in harmony with that reported by FAO [22] who reported that a total of 21 H5 HPAI positive cases were reported in 14 governorates during 2010, 4 of them were in Behera Province.

The illustrated data in table 4 clarified the prevalence of AI in investigated broiler back yard poultry farms in the 8 districts of Behera Province during the year of 2011. It was found that the overall prevalence of AI infection in investigated broiler farms in 2011 was 12.3 % (8 out of 65 examined samples). The highest prevalence was observed in Damanhour (2/8; 25 %) followed by Abo Homous (2/9; 22.2 %), Mahmoudia (1/5; 20 %), El Rahmania District (1/6; 16.7 %), Shobrakhet District (1/9; (11.1 %), Etai-Elbarod District (1/14; 7.1 %). On the other hand, AI infection was not detected in examined samples of both Abo El Matameer and Kafr El dawar Districts. Statistical analysis of the obtained results by Chi-square ($P > 0.05$) showed no significant association ($P = 4.72$) between the prevalence of AI in broiler back yard poultry farms the investigated districts of Behera Province in 2011. Data presented in table 6 showed the results of examination of 4 layer back yard poultry farms distributed in only 3 districts of Behera Province including Kafr El dawar (1), Shobrakhet (1) and Abo El Matameer (2) during the year of 2011. It was found that all examined samples were tested negative for AI. The obtained results in Tables 6, 7 were in harmony with that reported by FAO [23] who reported that a total of 103 H5 HPAI positive cases were reported in 15 governorates during 2011, 18 of them were in Behera Province. The significance of backyard poultry farms in transmission of AI was confirmed by the reports obtained from the Egyptian Ministry of Health that showed that, between March 2006 and March 2009, a sum of 6360 suspected human cases were reported to the local authorities, where 63 cases were confirmed to be H5N1 positive, of these 24 died. Among these deaths, 20 reared poultry in their backyards and 4 worked in poultry farms or poultry distribution.

The epidemiology of influenza is complex. This complexity is driven by a number of factors including the number of possible hosts and the intrinsic capacity of the virus for genetic and antigenic change. Although we understand some of the factors driving these properties of the virus, a number remain undefined. Some of the areas that need addressing include the effect of vaccination on virus change, the different effects of different host species on virus evolution and the possibility that other unidentified hosts may be present. Until we understand more of these issues, we will be continually playing catch up with the virus and acting in response to rather than in advance of diversification of emerging lineages.

Data presented in table 7 represent distribution of the AI seropositive and seronegative flocks and the relevance with different investigated variables. Based on the collected data by the used questionnaire in the 140 backyard poultry farms, 5 variables were studied including vaccination, traffic control, precautions taken by visitors, application of routine disinfection programs and control of wild birds using (Yes/No) questions where yes: means the presence of the factor while no: means absence of the factor. The first studied variable was vaccination, 97 farms informed that they followed a vaccination program against AI of whom 10 (10.3 %) farms were tested positive by rapid test while 87 (89.7%) farms were tested negative. On the other hand, 43 farms informed that they did not apply any vaccination program against AI of whom only 8 (18.6%) farms were tested positive and 35 (81.4%) farms were tested negative. The second variable under investigation was traffic control, 30 farms informed that they followed a traffic control regulations of which only 1 (3.3 %) farm was tested positive by rapid test while 29 (96.7%) farms were tested negative. On the other hand, 110 farms informed that they did not apply any regulation for traffic control of whom only 17 (15.5 %) farms were tested positive and 93 (84.5 %) farms were tested negative. The third variable under investigation was precaution taken to visitors, 23 farms informed that they applied precaution to visitor of where none of them were tested positive. On the other hand, 117 farms informed that they did not apply any precautions to visitors whom only 18 (15.4 %) farms were tested positive and 99 (84.6 %) farms were tested negative. The forth variable was application of routine disinfection programs, 135 farms informed that they applied routine disinfection program of which 17 (12.6 %) farm was tested positive by rapid test while 118 (87.4 %) farms were tested negative. On the other hand, 5 farms informed that they did not apply any disinfection program of whom nly 1 (20 %) farm was tested positive and 4 (80 %) farms were tested negative. The last variable to be studied was control of wild birds, only 7 farms informed that they had a program for control of wild bird through keeping the windows of the farm closed by using wire nets that routine maintenance practice. Moreover, all farms were tested negative. On the other hand, 133 farms informed that they had no program of eradication against wild birds, of which 18 (13.5 %) farms were tested positive and 115 (86.5 %) were tested negative. Poultry production practices are largely risky, especially in the wake of emerging zoonotic diseases. The main risk factors for disease are poultry markets, inadequate preventive measures at the household level, the free-range management system; poultry's sharing of housing with humans and improper disposal of dead chickens. Vaccination coverage is inadequate across all districts.

Relative risk used as a measure to determine if an association exists and whether there is an excess risk of the disease in populations who have been exposed to

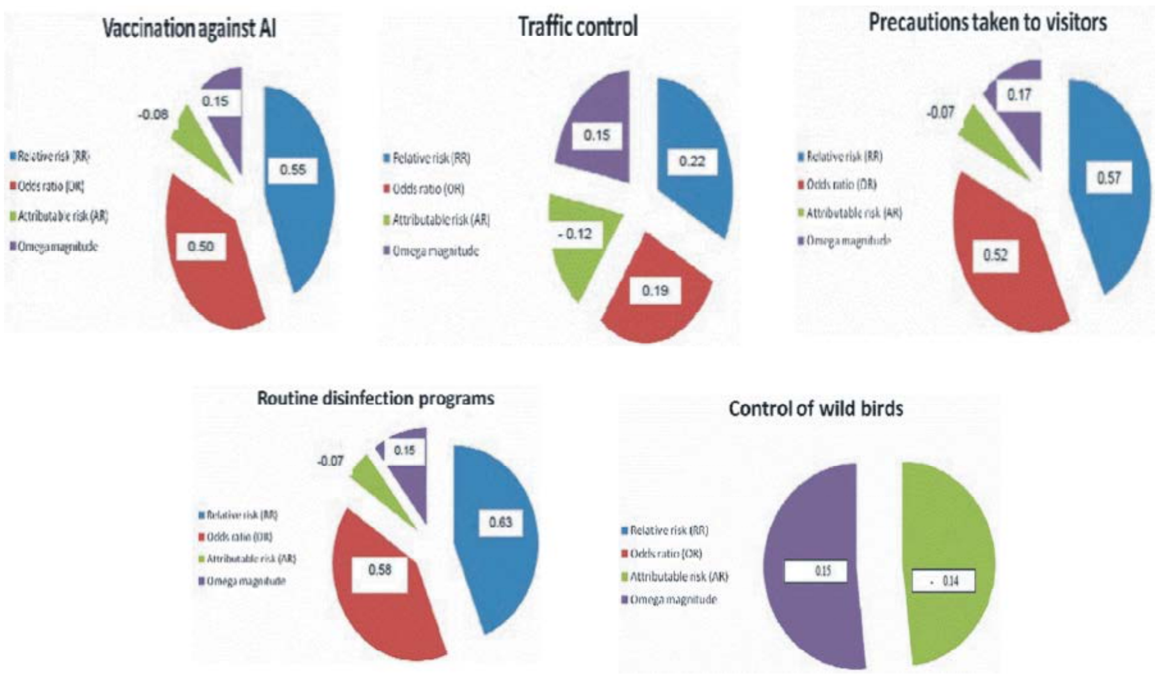

Fig 1: The magnitude of association between the different risk factors of positive backyard and poultry farms for AI in Behera province

disease [24]. Figs (1-5) indicated that there was an association concerning the applied biosecurity practices in back yard farms and seropositive reactors of AI, where the relative risk of vaccination against AI, traffic control, precautions taken to visitors, routine disinfection programs and control of wild birds were 0.55, 0.22, 0.57, 0.63 and 0.0, respectively. The presence of good sanitary measures in farms considered as a protective factor where R.R was less than 1(negative association) and the attributable risk was - 0.01, this means that the sanitary measures play a minor role in preventing the introduction of infection, while its role in preventing the spread of the infection inside the farms or flocks has a major property. These results indicated that vaccination against AI, traffic control, precautions taken to visitors, routine disinfection programs and control of wild birds were considered as very important risk factors for the introduction and spread of AI infection among poultry farms. Odds are commonly used as measures in epidemiology (the odds of AI infection is the ratio of the number of ways the infection can occurred to the number of ways the infection cannot occurred). The chance and the probability of AI occurrence in poultry farms and the degree of association between risk factors and AI occurrence were calculated by omega measure. According to the association by this measure, the risk factors were arranged as following: precautions taken to visitors (0.17), routine disinfection programs (0.15), vaccination against AI (0.15), traffic control (0.15) and control of wild birds (0.15). Odds ratio measures the relative frequency of the risk factors for AI to be occurred in farms or the degree of association between the risk factors with AI. According to this measure, the frequency of risk factors was arranged as following: routine disinfection programs (0.58), precautions taken to visitors (52), vaccination against AI (0.50), traffic control (0.19) and control of wild birds (0.0). The OR value showed that the farms that routine disinfection programs, precautions taken to visitors, vaccination against AI, traffic control and control of wild birds were about 0.58, 52, 0.50, 0.19 and 0.0 times more at risk to AI than those no disinfection, made a strict biosecurity measures against visitors, no vaccination against AI, absence of traffic control and absence of control of wild birds was reported by many researchers to be a risk factor for AI transmission. Attributable risk is a measure of how much of the disease risk is attributable to a certain exposure and is useful in answering the question of how much can be prevented. Risk factors (vaccination against AI, traffic control, precautions taken to visitors, routine disinfection programs and control of wild birds) were - 0.08, - 0.12, - 0.07, - 0.07 and - 0.14, respectively. In this study, vaccination against AI, traffic control, precautions taken to visitors, routine disinfection programs and control of wild birds were identified as the risk factors associated with seropositivity to AI antigen. Prevention or at least the reduction of AI can be achieved through the satisfactory or strict application of biosecurity measures in poultry farms. The use of disinfectants and the presence of adequate veterinary services were identified as the factors that protect against AI. Decrease of association (loss of association) between vaccination against AI as a risk factor and exposure to AI may be due to incompatibility of the used vaccines, in both commercial sector and governmental mass vaccination plan. These vaccines lacks strong antigenic relation with the field strains of AI exists in Egypt, where there is no reference lab for virus isolation found, thus we do not reserve our own seeds of AI virus for further vaccine production.as consequences Egypt became under the mercy of vaccine production companies which always interested in financial benefits resulting in AI persistence in Egypt since 2006.

CONCLUSION

In conclusion, Obtained results could make a useful contribution towards preventing AI viruses type A in backyard poultry farms and decreasing losses in the poultry industry. More attention should be paid towards implementing a proper control program for AI and more efforts should be directed towards improving the biosecurity program in Behera Province that are large in size. In addition, controlling AI in backyard poultry farms will indirectly reduce the prevalence of the disease. Control progress should be monitored serologically and evaluated epidemiologically; veterinary extension should be played a major role to guarantee the application of the sanitary procedures and measures in rearing, raising and breeding places and education of personnel and dissemination of awareness as well as veterinary public health culture through various multimedia.

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