

INVESTIGATING THE BIOSECURITY MEASURES' APPLICATIONS IN POULTRY FARMS AND ITS RELATIONSHIP WITH THE OCCURENCE OF AVIAN INFLUENZA

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Abstract: Biosecurity application in poultry farms means measures or practices which limit or prevent the spread of many harmful pathogens onto or out of the farm. When teamed with disinfection and sanitation procedures, biosecurity practices can eradicate or reduce pathogens to noninfectious levels and this illustrate that prevention is better than cure. Such preventive measures as vaccination and serologic monitoring also help to ensure good flock health. Inadequate biosecurity can contribute to creation of wide epidemics of highly pathogenic diseases such as avian influenza virus. For data collection, a predesigned questionnaire was created, and two visits were applied for investigating the application of biosecurity measures in the selected farms. Results summarize the response of the farm workers regarding the application of biosecurity measures in 244 farms under investigation. Regarding the number of farms with footbath, it was 150 (61%) in the first visit, although only 133 (56%) were filled with disinfectant. While, in the second visit, footbath number was significantly increased to 190 (78%) ($\chi^2 = 12.68$, $P > 0.01$) and 169 (69%) were filled with disinfectant ($\chi^2 = 7.39$, $P > 0.01$). The increased cleanliness of the area between the farm gate and poultry house was significantly increased from 68% in the first visit to 85% after the second visit ($\chi^2 = 19.1$, $P > 0.01$). As a result of the improved biosecurity measures observed in the second visit, the occurrence of avian influenza infection has significantly reduced from 36% to 20% in the farms under investigation. This in turn highlights the important role of biosecurity measures in the prevention and control of avian influenza infection. There is a need for more consideration on biosecurity measures targeting dead birds' disposal, changing shoes before entrance to the farm, presence of clothes for visitors, disinfection of injection tools, disinfection machines and veterinary supervision.

Key words: biosecurity; poultry farms; avian influenza; warning sings; motor using; changing shoes

Introduction

Biosecurity means limitation of transmission of infectious diseases between and within farms. Mainly, three biosecurity issues were defined: segregation, cleaning, and disinfection (1). To apply biosecurity in farms, previous studies recommended training and awareness of farm workers (2). Another explanation of biosecurity is a set of preventive measures designed to reduce the risk of transmission of infectious diseases especially

in organized poultry sector throughout the world (3). Biosecurity practices applied to limit the spread of infectious diseases' transmission within and between farms and are an important constituent of modern flock health programs (4). Poultry constitutes an important sector in animal production, with small commercial and backyard systems which are often extensive dominating the industry especially in the developing countries (5). High levels of baseline deaths due to infectious diseases occurs because of poor disease

control strategies and low or inadequate biosecurity measures (6). The movement of farm workers was linked with the probability of farm infection as declared by McQuiston et al. (7). Food and Agriculture Organization (FAO) and Office International des Epizooties (OIE) declared that to prevent and control HPAI infections, it is indispensable to improve the biosecurity at all stage, especially in the long-term infection (1).

Prevention and control of AI also depend on awareness and protective behaviors of the general population as well as high risk-groups (8). In Egypt, Radwan et al. (9) concluded that sufficient awareness does not necessarily lead to behavior change. Behavior change is a complex process that should involve comprehensive and multidisciplinary intervention, which combines risk perception, communication, and feasible and practical recommendations, including economic considerations. However, Abbate et al. (10) recommended improving knowledge of transmission, application of preventive measures and coordination between employers and health professionals for decreasing the effects of avian influenza. This study aimed to investigate the biosecurity measures application in poultry farms and its relationship with the occurrence of Avian Influenza.

Material and methods

Biosecurity application in farms and its relationship with the occurrence of Avian Influenza was investigated in the present study. A pre-designed questionnaire was designated for collection of demographic data from 244 farms in the city of Aga, Dakahlia Governorate. Forty-one villages were enrolled in the study. Two visits were conducted for asking about the application of biosecurity measures in the selected farms. During the first visit, if some applications were not applied, the farm owners were advised to apply them. In the second visit, the measures applied were recorded (Supplementary file 1).

The first visit was during the period from January to June 2017, several factors were recorded during the visit as described in Table (1). Based on the observation of the biosecurity measures in the farms under investigation, we advised the farmers to apply the measures to decrease the

probability of avian influenza infection in the reared birds. After three months, we paid the second visit to re-observe and record the response to the biosecurity measures.

Statistical Analysis

Descriptive statistics for counts and percentages was used to describe and summarize categorical data. Chi-square test was used to test the association between categorical variables. Data were analyzed using statistical package for social sciences (SPSS) version 25.0. The level of significance was equal to or less than 0.05.

Results

The results in Table (1) summarize the response of the farm workers regarding the application of biosecurity measures in 244 farms under investigation. Out of 244 farms, 143 (59%) had fence around the farm, this proportion was not changed after the second visit. Closure of farm gate at the first visit was only applied in 138 (57%) of the farms, while in the second visit there was significant increase to 70% ($\chi^2 = 8.64$, $P = 0.003$). In the current study, only 53 (22%) farms used warning signs in the first visit, this percentage was significantly doubled to 41% in the second visit ($\chi^2 = 26.3$, $P > 0.01$). The number of farms with footbath was 150 (61%) in the first visit, although only 133 (56%) were filled with disinfectant. While, in the second visit, footbath number was significantly increased to 190 (78%) ($\chi^2 = 12.68$, $P > 0.01$) and 169 (69%) were filled with disinfectant ($\chi^2 = 7.39$, $P > 0.01$). The wire net of poultry breeding barn windows was fit and secured in 82% (201/244) of the farms at the first visit, which increased significantly to 91% (223/244) in the second visit. The presence of dogs or cats near the farm at the first visit was reported in 141 (58%) of the farms which significantly decreased to 97 (40%) in the second visit ($\chi^2 = 16.22$, $P > 0.01$). In the current study, 75 (31%) of the farms stored food in special well-prepared place, which significantly increased to 99 (41%) in the second visit ($\chi^2 = 5.09$, $P > 0.05$). The increased cleanliness of the area between the farm gate and poultry house was significantly increased from 68% in the first visit to 85% after the second visit ($\chi^2 = 19.1$, $P > 0.01$).

Table 1: Impact of follow up(second visit) on response of farm workers and level of biosecurity in (244) investigated poultry farms

Variables under study	Positive (No.) Proportion	Total	χ^2	P-value
Presence of fence at first visit	143 (0.59)	244	0.009	0.92
Presence of fence after second visit	144 (0.59)	244		
Presence of one inlet and one gate for the farm at first visit	125 (0.51)	244	0.009	0.923
Presence of one inlet and one gate for the farm after second visit	124 (0.51)	244		
The gate always closed at first visit	138 (0.57)	244	8.64	0.003**
The gate always closed after second visit	172 (0.70)	244		
Warning sign in the farm at first visit	0.22 (53)	244	26.30	2.937 E -***
Warning sign in the farm after second visit	101 (0.41)	244		
Footbath at first visit	150 (0.61)	244	12.68	0.0004***
Footbath after second visit	190 (0.78)	244		
Filling footbath with disinfectant at first visit	133 (0.56)	244	7.39	0.007**
Filling footbath with disinfectant after second visit	169 (0.69)	244		
The wire net of poultry breeding barn windows fit at first visit	201 (0.82)	244	8.82	0.003**
The wire net of poultry breeding barn windows fit after second visit	223 (0.91)	244		
Presence of household birds around the farm at the first visit	64 (0.26)	244	2.41	0.12
Presence of household birds around the farm after the second visit	50 (0.20)	244		
Presence of dogs or cats near the farm at the first visit	141 (0.58)	244	16.22	0.00006***
Presence of dogs or cats near the farm after the second visit	97 (0.40)	244		
Cleanliness of the area between the farm gate and poultry house at first visit	166 (0.68)	244	19.10	0.00001***
Cleanliness of the area between the farm gate and poultry house after second visit	208 (0.85)	244		
Presence of piles of litter and birds' droppings in front of the farm at first visit	56 (0.23)	244	7.93	0.005**
Presence of piles of litter and birds droppings in front of the farm after second visit	33 (0.14)	244		
Presence of tree and plants between the gate and the poultry house at first visit	181 (0.74)	244	0.009	0.92
Presence of tree and plants between the gate and the poultry house after second visit	180 (0.74)	244		
The water storage sources are clean and covered at first visit	233 (0.95)	244	#	0.23
The water storage sources are clean and covered after second visit	238 (0.98)	244		
Covering of poultry breeding barn windows with fit wire before first visit	205 (0.84)	244	8.51	0.004**
Covering of poultry breeding barn windows with fit wire before after second visit	226 (0.93)	244		
Soft roof, tightly closed without cracks at first visit	145 (0.59)	244	5.28	0.02*
Soft roof, tightly closed without cracks after second visit	170 (0.70)	244		
Walls are smooth without cracks at first visit	152 (0.62)	244	3.81	0.05*
Walls are smooth without cracks after second visit	173 (0.71)	244		
Using disinfection machine under pressure at first visit	117 (0.48)	244	23.22	0.000001****
Using disinfection machine under pressure after second visit	169 (0.69)	244		
Storage of Food & egg in special well prepared place at first visit	75 (0.31)	244	5.09	0.02*
Storage of Food & egg in special well prepared place after second visit	99 (0.41)	244		
Workers always stay in the farm at first visit	213 (0.87)	244	9.50	0.002**
Workers always stay in the farm after second visit	233 (0.95)	244		
Changing shoes before entrance to the farm at first visit	191 (0.78)	244	28.2	0.00007***
Changing shoes before entrance to the farm after second visit	226 (0.93)	244		
The workers had household birds at first visit	39 (0.16)	244	13.92	0.0002***
The workers had household birds after second visit	15 (0.06)	244		

Table 1 (continuation): Impact of follow up(second visit) on response of farm workers and level of biosecurity in (244) investigated poultry farms

Presence of a worker for each poultry breeding barn at first visit	215 (0.88)	244	10.62	0.001**
Presence of a worker for each poultry breeding barn after second visit	235 (0.96)	244		
Presence of clothes for visitors at first visit	23 (0.09)	244	22.91	0.000002***
Presence of clothes for visitors after second visit	66 (0.27)	244		
Asking for help by external injector at first visit	75 (0.31)	244	12.70	0.0004***
Asking for help by external injector after second visit	40 (0.16)	244		
at first visit Changing clothes while ask for help by external injector	42 (0.17)	244	10.59	0.001
Changing clothes while ask for help by external injector after second visit	73 (0.30)	244		
Disinfection of tools used for injection at first visit	225 (0.92)	244	#	0.001**
Disinfection of tools used for injection after second visit	241 (0.99)	244		
Permission of entrance of vehicle to the farm at first visit	228 (0.93)	244	33.47	7.2383E-9***
Permission of entrance of vehicle to the farm after second visit	(180) 0.74	244		
Presence of vehicle bath at first visit	159 (0.65)	244	9.59	0.002**
Presence of vehicle bath after second visit	187(0.77)	244		
Filling of vehicle bath with disinfectant at first visit	136 (0.56)	244	18.68	0.00002****
Filling of vehicle bath with disinfectant after second visit	178 (0.73)	244		
Using motor For disinfection of vehicle before entrance at first visit	51 (0.21)	244	9.56	0.002**
Using motor For disinfection of vehicle before entrance after second visit	81 (0.33)	244		
Borrowing tools from another farms at first visit	1 (0.004)	244	#	1
Borrowing tools from another farms after second visit	0 (0.0)	244		
Disinfection of borrowed tools before and after using at first tools	84 (0.34)	244	45.78	1.3216E-11***
Disinfection of borrowed tools before and after using after second tools	153 (0.63)	244		
Occurrence of avian influenza at first visit	88 (0.36)	244		
Occurrence of avian influenza after second visit	49 (0.20)	244	16.23	0.0006***

#Binomial distribution used; χ^2 : chi-square statistic; *P< 0.05: denotes significant difference; **P< 0.01 and ***P< 0.001 denote highly significant difference

Discussion

In the present study, out of 244 farms, 143 (59%) had fence around the farm, this proportion was not changed after the second visit. This is consistent with Wang *et al.* (11) who reported that most farms in poultry production clusters do not have fences, gates, or barriers. Another study conducted by Mustafa and Ismail (12) mentioned that farm fence was available for all farms in both close and open systems. The obtained result in the current study may be due to difficulties in extraction of building license. Closure of farm gate at the first visit was only applied in 138 (57%) of the farms, while in the second visit there was significant increase to 70% ($\chi^2 = 8.64$, $P = 0.003$) which reflects the effect of the visits and easy application of this biosecurity measure. Closure of farm gates aids in preventing any pathogen from entering the farm by visitors and restricts access

to poultry facilities (13). The mechanisms of subsequent spread and transmission of AIVs may occur through movements of humans (visitors, servicemen and farm personnel), vectors (wild birds, rodents, insects), air- and dust related routes and other fomites (e.g., delivery trucks, visitors' clothes and farm equipment) which have all been hypothesized to be decreased through closure of the farm gates and application of fit wire net(14-18).

In the current study, only 53 (22%) farms used warning signs in the first visit, this percentage was significantly doubled to 41% in the second visit ($\chi^2 = 26.3$, $P > 0.01$). This highlights the increased response rate and the obvious change in the attitude of poultry farm workers and owners to the advises recommended in the first visit. A study by Mustafa and Ismail (12) reported that only 7 (15.6%) of farms used warning signs in poultry farms. The presence of warning signs

helps in stopping or controlling the movement to the farm which in turn aid in increasing biosecurity level.

The number of farms with footbath was 150 (61%) in the first visit, although only 133 (56%) were filled with disinfectant. While, in the second visit, footbath number was significantly increased to 190 (78%) ($\chi^2 = 12.68$, $P > 0.01$) and 169 (69%) were filled with disinfectant ($\chi^2 = 7.39$, $P > 0.01$). Uddin *et al.* (19) stated that footbath facilities was absent in 53 (75.71%) and was present in 17 farms (24.29%). Continuous usage of footbath and its filling with disinfectant aid in the control of introducing infectious agents to the poultry farm which in turn increases the biosecurity level in these farms

The wire net of poultry breeding barn windows was fit and secured in 82% (201/244) of the farms at the first visit, which increased significantly to 91% (223/244) in the second visit. Mohammed and Helal (20) reported that the secured wire netting on windows help to protect the sheds from predators and pest birds.

Pets are vectors which could transport contaminated material between locations as well as the possibility of cross-species transmission. AI viruses are known to affect hobby birds and other animal species such as pigs, horses, and cats (21-23). If pets were infected, there could be a chance of infecting poultry or feed contamination due to access of pets to poultry houses and storage rooms on some farms. The presence of dogs or cats near the farm at the first visit was reported in 141(58%) of the farms which significantly decreased to 97 (40%) in the second visit ($\chi^2 = 16.22$, $P > 0.01$). This has an influence in decreasing the probability of cross infection from pets to birds reared in the farms.

In the current study, 75 (31%) of the farms stored food in special well-prepared place, which significantly increased to 99 (41%) in the second visit ($\chi^2 = 5.09$, $P > 0.05$). This has an influence in lowering the possibility of introduction of infection with food or spreading of infection from the farms with contaminated eggs. AL haji and Odetokun (24) reported that the risk of introduction of AI is mostly associated with sources of water and feed, poor handling of litter, drinkers, feeders, and environment which could create favorable conditions.

The increased cleanliness of the area between the farm gate and poultry house was significantly increased from 68% in the first visit to 85% after the second visit ($\chi^2 = 19.1$, $P > 0.01$). Wakawa (25) stated that lack of cleaning and hygiene could predispose birds to external parasites, which cause harm, discomfort, stress, and act as intermediate hosts for various diseases. Carey (13) stated that effective cleaning and disinfection measures can substantially decrease disease transmission by reducing pathogens in the environment to noninfectious levels. Facilities and equipment should be cleaned from top to bottom, inside to out and with the natural drain of effluent water to prevent recontamination of cleaned facilities. Mccrea and Bradley (26) mentioned that sanitation is crucial in poultry farms to eliminate disease agents. Using disinfectant footbaths may aid in decreasing the dose of organisms on boots. Banshi (27) reported that efficient cleaning of feeding and drinking was urgent. Poultry farmers were expected to have periodic clean-out, clean-up and disinfection of houses and equipment, at least once in every production cycle of poultry birds. Shane (28) noted that the effective cleaning and disinfecting methods can mitigate disease transmission by decreasing pathogens in the environment below infection level. Similarly, Sharma (29) stated that clean poultry farm will reduce foul smelling to neighbors and disease spread.

Overall, other factors have been observed to be significantly improved in the second visit. These factors include disinfection machines, changing shoes before entrance to the farm, presence of clothes for visitors, disinfection of injection tools, presence of vehicle bath with disinfectant. Consequently, because of the improved biosecurity measures observed in the second visit, the occurrence of avian influenza infection has significantly reduced from 36% to 20% in the farms under investigation. This in turn highlights the important role of biosecurity measures in the prevention and control of avian influenza infection.

Conclusion

It could be concluded that application of biosecurity measures reduces the occurrence of diseases such as AIV. The advices during the first

visit improved the awareness of farm workers towards the application of biosecurity measures. The present study confirmed that biosecurity is considered as an indispensable tool to mitigate the spread of infectious diseases.

The authors declare that they have no competing interests.

References

1. FAO 2008. Biosecurity for highly pathogenic avian influenza: Issues and options. FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy.
2. Tabibdi M, Mustafa E, et al. Data analysis of biosecurity measures for poultry farms registration in Khartoum state, Sudan. *Int. J. Curr. Res* 2014; 6, 9714-9718.
3. Newell D, Elvers K, et al. Biosecurity based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. *Appl. and Environ. Microbiol.* 2011; 77, 8605-8614.
4. Dorea F, Berghaus R, et al. Survey of biosecurity protocols and practices adopted by growers on commercial poultry farms in Georgia, U. S. A. *Avian Dis.* 2010; Sep; 54 (3):1007-15.
5. Conan A, Goutard F. L, et al. Biosecurity measures for backyard poultry in developing countries: a systematic review *BMC Veterinary Research*, 2012.
6. Abdelqader A, Wollny C, et al.. Characterization of local chicken production systems and their potential under different levels of management practice in Jordan. *Trop Anim. Health Prod.*, 2007. 39 (3):155-164.
7. McQuiston J, Garber L, et al. Evaluation of risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial poultry farms. *J. Am. Vet. Med. Assoc.* 2005.
8. Neupane D, Khanal V, et al. Knowledge, attitudes and practices related to avian influenza among poultry workers in Nepal: a cross sectional study. *BMC Infectious Diseases* 2012, 12:76.
9. Radwana G, Wafaa Y, et al. Knowledge, attitudes, and practices of avian influenza among backyard poultry breeders in Fayoum Governorate, Egypt . *Journal of the Egyptian Public Health Association* 2011, 86:104–110
10. Abbate R, Di Giuseppe G, et al. Knowledge, attitudes, and practices of avian influenza, poultry workers, Italy. *Emerging infectious diseases*, 2006; 12(11), 1762–1765. <https://doi.org/10.3201/eid12.11.060671>.
11. Wang L, Basuno E, et al. An Eco health assessment of poultry production clusters (PPCs) for the livelihood and biosecurity improvement of small poultry producers in Asia. *Infectious Diseases of Poverty* 2015; 4:6 (1-9).
12. Mustafa M.E, Ismail H.M. Evaluation of Biosecurity Measures in Layer Farms in Khartoum State, Sudan. *Journal of Applied Science And Research*, 2017; 5 (6):23-31.
13. Carey J.B. 2005. Poultry Facility Biosecurity. Texas Agri Life Extension Service
14. Halvorson D, Karunakaran D et al. Avian influenza in caged laying chickens. *Avian Dis.* 1980; 24, 288–294.
15. Webster R, Bean W, et al. Evolution and ecology of influenza A viruses. *Microbiol. Mol. Biol. Rev* 1992; 56, 152–179.
16. Sawabe K, Hoshino K, et al. Detection and isolation of highly pathogenic H5N1 avian influenza A viruses from blow flies collected in the vicinity of infected poultry farm in Tokyo, Japan, 2004. *Am. J. Trop. Med. Hyg.* 2006; 75, 327–332.
17. Sievert K, Alvarez R, et al. House flies and the avian influenza threat. *Int. Poult. Prod.* 2006; 14, 7–9.
18. Ssematimba A, Hagensars T, et al. Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms. *PLoS ONE.* 2012b; 7, e31114.
19. Uddin S, Juli S, et al. Investigation of biosecurity in commercial poultry farms of Dinajpur district. *International Journal of Natural and Social Sciences*, 2020, 7(1): 14-20.
20. Mohammed A , Helal H. Current situation assessment of biosecurity measures of some poultry sectors and hatcheries in Egypt. *Journal of Veterinary Medical Research.* 2016 : 23 (2): 143 – 154.
21. Röhm C, Horimoto T, et al . Do hemagglutinin genes of highly pathogenic avian influenza viruses constitute unique phylogenetic lineages? *Virology* 1995; 209, 664–670.
22. Loeffen W, de Boer-Luijtz E, et al. Transmission of highly pathogenic avian influenza virus to swine in The Netherlands, Animal production in Europe: the way forward in a changing world. In: *Proc. Congress of the International Society for Animal Hygiene*, 2004:11–13 October, St. Malo, France, pp. 329–330.
23. Belser J, Blixt O, et al. Contemporary North American influenza H7 viruses possess human receptor specificity: implications for virus transmissibility. *Proc. Natl. Acad. Sci.* 2008; U.S.A. 105, 7558–7563.
24. AL haji N and Odetokun I. Assessment of biosecurity measures against Highly Pathogenic Avian Influenza risks in small scale commercial farms and free-range poultry flocks in the North central Nigeria. *Transboundary and Emerging Diseases* 2011; 58, 2 p. 157-161.
25. Wakawa, A. M. Surveillance and evaluation of

risk factors for the occurrence and spread of avian influenza in Kano State, Nigeria. Ph.D Dissertation, Ahmadu Bello University, Zaria. (2012): 95-10.

26. Mccrea BA, Bradley FA. Biosecurity for poultry at community farms. University of California Division of Agriculture and Natural Resources Publication 2008 : 8280. ANR CS Web site, <http://anrcatalog.ucdavis.edu>.

27. Balish A, Davis C, et al. Antigenic and genetic diversity of highly pathogenic avian influenza A

(H5N1) viruses isolated in Egypt. *Avian dis*, 2010: 54: 329-334.

28. Shane B. 1995. Decontamination of housing and equipment in bio-security in the poultry industry. American association of avian pathologist, University of Pennsylvania, New Boltan Center, Kennet Squire. PA.: pp: 120.

29. Sharma B. 2010. Poultry production management and bio-security measures. *J. Food Agric. and Environ*, 1:122.