Serofrequency of Avian Influenza Virus (H5N1) Among Poultry Farms Workers in Khartoum-Sudan

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Abstract:

Background:-Avian influenza is a highly infectious disease caused by type A influenza viruses of the Orthomyxoviridae family, which mainly infect birds. Recently, it was shown to infect all mammalian including humans. This study aimed to determine the serofrequency of a vianflu in poultry farm's workers in Khartoum State, Sudan.

Methods:-This prospective cross-sectional study was conducted in poultry farm workers in Khartoum State, Sudan, during August to September 2016. A total of 60 participants as case group and 30 as control group were enrolled in this study. Blood specimens were collected and serum was obtained. The solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) was used to assay the flu-IgG antibodies in samples. The sociodeogrsphic data and period of their works were gathered by interviewing questionnaire. The obtained data was analyzed by SPSS program.

Results:-The avian influenza virus (H5N1) IgG antibodies were detected in 12% (7/60) among case group and 0% (0/30) in control group. The all seropositive result was observed among age group 40-50 years old 100% (7/7). Individuals working at laying hens farm's had higher serofrequency rate of H5N1 (57%), when compared with those working at meat hens farm's (43%). Furthermore, the serofrequency of H5NI was higher among slaughtering workers (43%) in comparison with cleaner workers (29%) and (14%) in eggs collector and poultry feeder workers.

Conclusion:-The serofrequency of avian flu was frequent in poultry farm workers in Khartoum. Further confirmation with large scale specimen is recommended.

Keywords:-Avianflu; H5N1; Serofrequency; Farm workers; IgG; ELISA.

I. INTRODUCTION

Influenza A virus belongs to the family of orthomyxoviridae that have lipoprotein enveloped, and segmented, negative-sense single-stranded RNA genome 1. The natural reservoir of Influenza A virus is wild birds, however, they have been isolated from a number of avian and mammalian species, including humans 2,3. This virus can overcome species barriers through its ability to adapt to the receptors of a new host, which allows the virus to enters the cell, replicates and, subsequently, spread from animal-to-animal 4.The influenza A viruses (IAV) are divided into subtypes basis on diversity in two surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). At present, 18 HA subtypes and 11 NA subtypes were identified 5.

The H5N1 virus was first known to cross the animal-human species barrier in 1997 when 18 hospitalized, symptomatic cases, six of whom died, were identified in Hong Kong 6. In 2013, WHO reported 628 cases and 374 deaths of H5N1 infected patients from 15 countries with high fatality rate approximately 59% 7. The largest numbers of human cases reported were 192, 172, and 125 cases in Indonesia, Egypt and Vietnam, respectively 7. Most of H5N1 cases are attributed to direct contact with sick poultry, however, it is unclear how many cases were due to human-to-human transmission 8.

In Sudan, H5N1 was reported in poultry with prevalence rate an about (86.4%) 9. Nevertheless, there is no seroepidemiologic data about H5N1 in Sudanese populations. Therefore, this study aimed to determine the serofrequency of influenza A virus (H5N1) in poultry farm workers at Khartoum State, Sudan.

II. MATERIALS AND METHODS

A. Design:

This was cross-sectional study design conducted during a period from August up to September 2016 in Khartoum State, Sudan. The data was gathered by direct interviewing questionnaire. Ethical approval taken from Al Neelain University Research Ethical Board and verbal consent was taken from participants before sample collection.

B. Experimental Work:

a). Collection of Specimens and Processing

Blood specimens were collected from 60 poultry farm's working staff as case group and from 30 healthy volunteers as control that did not work at poultry farm's. Serum was separated from clotted blood by centrifugation and stored at -20° C until processed by enzyme linked immune sorbent assay (ELISA, SunLong Biotech Co.,LTD, China) at research laboratory \AL Neelain University to detect IgG antibodies specific for avian flu (H5N1). All reagents were brought to room temperature before assaying.

Forty microliters of diluents buffer were added to each 10 microliters of the sample and then, the samples were dispensed into their respective wells. Fifty microliters of negative and positive control were also incorporated in their corresponding wells. Next, the microplate was sealed and incubated in 37° C for 30 minutes and then, the plate was washed five times by washer machine. Fifty microliters of horseradish peroxidase (HRP)-conjugate antigens were added to all wells, except blank well. After incubation, the plate was washed, dried, 100 micoliters of substrate was added to every well and incubated at dark place for 15 minutes. Next, 50 micoliters of stop solution was added to wells.

b). Measurement:

The optical density of the specimens was measured photometrical at 450nm within 15 minutes of adding the stop reagent.

C. Calculation and Interpretation of Result:

The results were calculated by cut-off value. This was determined by adding 0.15 to average of absorbance of the negative control values (NC), which was obtained by following equation (cut-off value = NC + 0.15). When the result above than cut-off value were considered positive and lower than that were considered negative.

Data was analyzed by using Statistical Package for Social Sciences (SPSS), version 16.0. Significance of difference was determined using chi-square test. P.

value< 0.05 was supposed to be significant.

III. RESULTS

A total of 90 blood specimens, 60 were obtained from poultry farm's workers as case group and 30 from non-poultry farm's contact were enrolled as control group. Out of the total 60 poultry farm's workers, 7 (12%) were positive and 53 (88%) were negative. While, non of control group was positive (0.0%) (Figure 1 and Table 1), statistical analysis showed significant difference (P = 0.051) between serofrequency of avian influenza among poultry farm's workers and non-exposed individuals.

Regarding age distribution, most of study populations were belonged to age group 50-60 years old. Nevertheless, all seropositive results were found at age group 40-50 years old 100% (7/7), the statistical analysis showed significant relationship (P = 0.001) between the age of participants and avian influenza infection, table 2.

Based on type of farm, the serofrequency rate was higher in laying hens farm's workers (57%) than those working at meat hens farm's (43%) (Figure 2). The serofrequency rate was slightly higher among individuals working for more than 15 years (57%) compared with those who works less than 15 years (43%) (figure 2).

The impact type of work on serofrequency of H5NI, revealed that cleaners had more seropositive 25% (2/8) followed by slaughtering workers 21% (3/14) and 4% (1/24) in eggs collector and 7% (1/14) in hens feeding workers (Table 2).
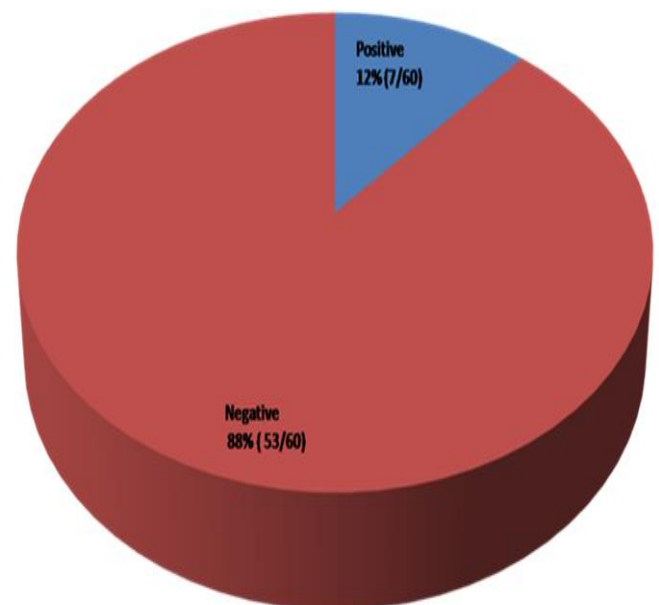
IV. FIGURE AND TABLES

Fig. 1 Serofrequency of Avian Influenza Among Poultry Farm's Worker (No = 60)
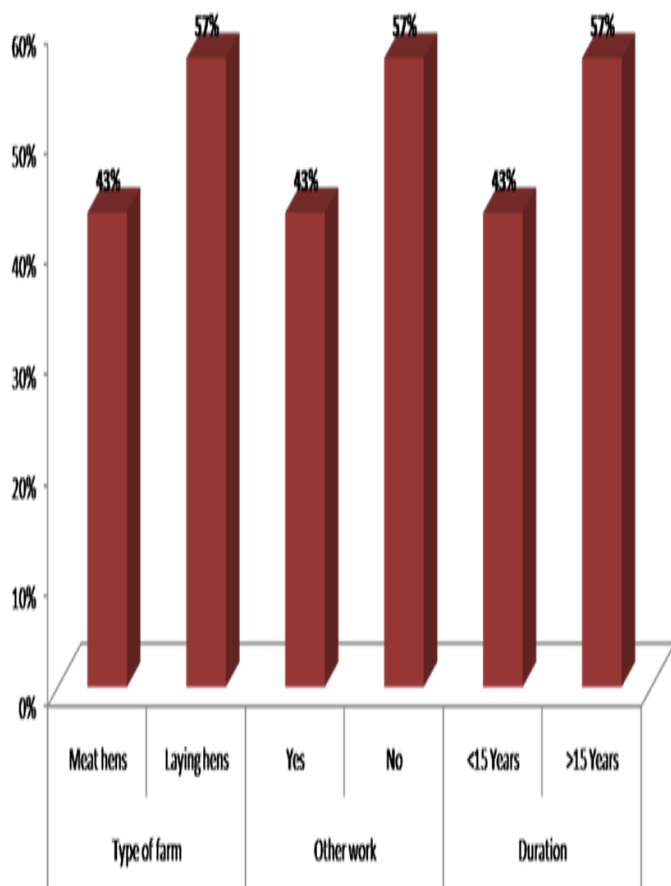
Fig. 2 serofreqrency of H5N1 According to Type of Farm Among Study Population

Results		Group		Total
		Case	Control	
Positive	Count	7	0	7
	(%)	12%	0.0%	7.8%
Negative	Count	53	30	83
	(%)	88%	100.0%	92.2%
Total	Count	60	30	90
	(%)	100.0%	100.0%	100.0%

Table 1: Serofrequency of Avian Influenza Virus Among Study Populations (No = 90)

Type of work	Subjects examined	No of positive	Percentage
Slaughtering	14	3	%21
Egg collecting	24	1	%4
Hens feeding	14	1	%7
Cleaners	8	2	%25
Total	60	7	%12

Table 2: Serofrequency of H5N1 Among Farm Workers in Relation To Type of Work.

Age group		Results		Total
		Positive	Negative	
30-40 Years	Count	0	27	27
	(%)	0.0%	32.5%	30.0%
40-50 Years	Count	7	16	23
	(%)	100.0%	19.3%	25.6%
50-60 Years	Count	0	40	40
	(%)	0.0%	48.2%	44.4%
Total	Count	7	83	90

Table 3: Serofrequency of Avian Influenza Virus Among Study Population in Relation to Age.

V. DISCUSSION

Several studies proved the circulating of avian flu among domestic birds in Sudan[9,10]. However, there was published data about sero-prevalence of avian flu in Sudanese populations. Direct contact with sick poultry was shown to be increased risk of human infection with H5N1. The present study aimed to investigate the sero-frequency of H5N1 in poultry workers at Khartoum State, Sudan.

Our study included a total of 90 blood specimens, 60 were obtained from poultry farm's workers and 30 specimens collected from non-poultry contact were enrolled as control. The seropositive of H5N1 among poultry farm workers were 12% (7/60).The sero-frequency rate of H5N1 among farm poultry workers in the present study appears to be higher than the finding previously reported inNigeria (0%) [11], South Korea (0.3%) [12], Bangladesh (2%) [13]and Viet Nam (6.1%) [14]. However,seropositive H5N1 rate detected in this study was

closely similar to study reported in Hong Kong (10%) [15], but lower than other result reported in Kong Hong (37.8%) [16]. The various rates of H5N1 prevalence reported by investigators in different places could be attributed to several factors, including the population groups, diagnostic techniques used, degree of protective measure, seasonality and the clade or subclade of viruses across the region [17].

In this study, it observed that all seropositive results were among participants age group 40-50 years (100%). This agreed with result obtained by Zhejiang *et al.*, 2013 in China, they found that high seropositive rate among age group (47-62) years [18]. However, different studies conducted in Vietnam and China were found most of seroprevalence H5N1 among age (25–34) years and (18–35) years old, respectively [14,19].Such differences demonstrate that the potential risk for transmission of H5N1 from poultry to humans is not same across age and therefore may not be uniform within or across countries [20].

VI. CONCLUSION

This study revealed that sero-frequency of H5N1 was higher among poultry compared with non–exposed controls. Therefore, good protective measure such use of good hygiene and work practices, personal protective clothing are required to abolished viruses transmission. Further study will be designed with large scale specimens and advanced technique.

VII. ACKNOWLEDGEMENTS

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