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Avian Influenza (H9N2) among poultry workers in Iran

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ABSTRACT

Background and objectives: A number of different subtypes of avian influenza (AI) viruses have emerged in humans including H5N1, H7N2, H7N7 and H9N2. These influenza viruses are excreted in the infected birds and in their respiratory secretions. The aim of this study was to investigate seropositivity against H9N2 and H7N7 viruses among poultry and slaughter-house workers with occupational risk of exposure to poultry in Tehran province.

Materials and Methods: A cross-sectional seroprevalence study was performed using two types of HI assay among 127 poultry and slaughter-house workers and 25 controls with the regular consumer related exposure to poultry against H9N2 and H7N7 avian influenza viruses. Data were analyzed using SAS 9.1.

Results: There was no evidence of previous H7N7 infection among subjects. Both poultry workers had elevated antibodies against H9N2 Influenza viruses compared to controls. Slaughter-house workers who self-reported eviscerating poultry with their bare hands had markedly increased evidence of previous H9N2 infection (15.7%) compared to controls (0%) (OR=18.241, 95% CI ([6.802-48.914]).

Conclusions: Our results suggest poultry-to-human transmission of avian influenza A H9N2 can occur in poultry workers. Eviscerating section workers, in contrast to others had the highest risk of H9N2 infection. It is emphasized that eviscerating sections of poultry work is the most contaminated part of poultry industry that could increase the likelihood of poultry-to-human transmission.

Keywords: Avian influenza virus, H9N2, H7N7, HI assay, Poultry workers, Slaughter-house workers, Iran.

INTRODUCTION

A number of different subtypes of avian influenza (AI) viruses have emerged in humans including H5N1, H7N2, H7N7 and H9N2. These influenza viruses are excreted in the infected birds and in their respiratory secretions. Transmission to humans can result from close contact with infected (dead or live) poultry, droppings, or contaminated surfaces. The suspected organs of influenza (INF) virus entry to humans are assumed to be the mouth, nose, eyes and lungs. Avian influenza H9N2 infections have been reported in the Middle East causing widespread outbreaks in commercial chickens in Iran, Saudi Arabia, and Pakistan (1). An outbreak of H9N2 infection in poultry farms was first reported in 1998 in Iran (2) which is now endemic and vaccination against this subtype is practiced, routinely. So far, there has been no report of H7N7 infection in Iran, although there was an outbreak in Pakistan in 1995 (1). The aim of this study was to investigate seropositivity against H9N2 and H7N7 viruses among poultry and slaughter-house workers with occupational risk of exposure to poultry in Tehran province.

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MATERIALS AND METHODS

A seroepidemiological investigation was conducted in November 2006 by taking 4-ml blood samples from workers in two layer and breeder poultry farms and two slaughter-houses of Tehran province, the most populated province with the highest number of farms and slaughter-house plants in Iran. Samples were accompanied with questionnaires on demographic information and data on vaccination history, occupational type exposure, employment history and recent influenza symptoms (Table 1).

Blood samples were taken from minimally-exposed

Table 1. Demographics and clinical data of exposed workers and minimally-exposed people, Tehran, 2006.

Variable	Exposed workers (no.127)	Minimally-exposed people (no. 25)
Age group(y)		
15-30	36(28.3%)	12(48%)
30-45	58(45.6%)	7(28%)
45-60	33(25.9%)	6(24%)
Mean	35	33.4
Gender		
Male	127(100%)	10(40%)
Female	-	15(60%)
Occupational Exposures		
Chicken Breeder	19(14.9%)	
Chicken Layer	46(36.2%)	
Eviscerating poultry	24(18.8%)	
Feather removals	38(29.9%)	
Vaccination with Human influenza vaccine in the previous year		
Yes	42(33%)	19(76%)
No	85(66.9%)	6(24%)
Employment History (Year)		
<10	79(62.2%)	
10-20	34(26.7%)	
20-30	14(11%)	
Mean	8±0.15	
Diagnosed with INF Disease in the past year		
Yes	42(33%)	
No	85(66.9%)	

people as negative controls. Aliquots were stored at -20˚C for future work. In total, 62 and 65 workers from slaughter-houses and poultry farms; respectively, and 25 minimally-exposed people were examined in the study. The workers were all men in a 16-59 age range with average of 35, while the minimally-exposed people consisted of 60% women at an age range of 23-55. The average employment period was 8 ± 0.15 years. Workers in farms were categorized into two sections; layer and breeder. Workers in slaughter-houses were categorized by type of work into eviscerating poultry and feather removal sections. All slaughter-houses were semi-automatic using bare hands for removing viscera without wearing masks or glasses. The european trivalent commercial influenza vaccine recommended since 2006 had been administered to 33% (42/127) of all workers and 76% (19/25) of minimally-exposed controls within the past year.

Sera were tested using the hemagglutination inhibition (HI) assay, as described by World Health Organization (WHO) (8), against the locally circulating H9N2 strain. H9N2 anti-serum A/tky/wisc/1/66 was obtained from the Veterinary Laboratories Agency, UK, and used as positive control. Anti-H9 was detected in sera using 4HA units of the antigen to perform HI test. HI endpoint titer was determined as the reciprocal of the highest serum dilution that produced complete inhibition of hemagglutinating activity. In order to eliminate cross-reactivity between anti-H3 and anti-H9 a step for serum adsorption was added. In detail, one part of serum was added to two parts of A/panama/2007/99 (H3N2) virus and incubated for one hour at room temperature storage at 4˚C. To confirm absence of the virus, serial dilutions were prepared following incubation with RBCs. Negative hemagglutination suggested successful virus removal.

Table 2. Risk factors H9N2 seropositivity among poultry and slaughter-house workers, in Iran by multiple logistic regression (no. 127).

Variable	N	% seropositive	OR (95% CI)
Age group(y)			
15-30	36	55.5%	2.96 (1.17-7.74)
30-45	58	36.2%	1.41 (0.6-3.34)
45-60	33	21.2%	Reference
Occupational Exposures			
Chicken Breeder	19	21%	Reference
Chicken Layer	46	26%	1.1 (0.35-3.43)
Eviscerating poultry	24	83.3%	18.24 (6.8-48.91)
Feather removals	38	31.5%	1.58 (0.5-4.95)
Vaccination with Human influenza vaccine in the previous year			
	42	16.9%	Reference
Yes	85	83.3%	3.26(1.49-7.16)
No			
Employment History (Year)			
<10	79	45.5%	3.47 (0.96-12.43)
10-20	34	32.3%	1.99(0.5-7.9)
20-30	14	7.1%	Reference
Diagnosed with INF Disease in the past year			
Yes	42	39.6%	1.48(0.73-2.98)
No	85	60.4%	Reference

The samples were tested to investigate antibodies against the A/tky/ENG/647/77 (H7N7) virus using a modified HI adapted by Meijer *et al*. (3). Sera were inactivated at 56˚C and treated with RDE following heme-adsorption with packed horse erythrocytes. HI assay was performed using 2HA units and 1% horse erythrocytes in V-bottom plates. The A/tk/Eng/647/77 (H7N7) positive control antiserum was also obtained from Veterinary Agency Laboratories, UK. Data were analyzed with SAS 9.1. In this study, according to the multinomial response with ordinal levels, proportional odds models were fitted. The ordinal response variable has four levels in which 1:40 and 1:40< levels of response collapse together. All the covariates used in the models are categorical variables. The models were used to analyze the significance of variables to find an optimum model. Also, odds ratios and their 95% Confidence Intervals (C.I) were calculated to interpret the parameters of the models.

RESULTS

In total 37.7 %(48 of 127) of workers were positive (titers≥1:20) by anti H9N2 HI. Distribution of anti-H9 titers in different occupational categories are shown in Fig. 1. Seropositivity in slaughter-house workers (51.6% of eviscerating and feather removal section workers) was 2.09 times higher than poultry farms (24.6% of chicken breeder and chicken layer workers). Seroprevalence in chicken layer (26%) and chicken breeder (21%) poultry workers was not significantly different but seroprevalence in the eviscerating section (83.3%) was 2.64 times higher than the feather removal section (31.5%) (Table 2). The results of proportional odds ratios and the 95% Confidence Interval for proportional odds ratios are presented in Table 2. In calculating the POR, reference categories were selected such that POR is >1.There was no detectable anti H7 response in all sera. It seems that the poultry farms of the Tehran province had not been infected with H7.

DISCUSSION

Our results suggest poultry-to-human transmission of avian influenza A (H9N2) can occur in poultry workers as mentioned in other studies (4, 5, 7). Seropositivity (24.6%) is near to those of Hong Kong (4) and Lebanon (12) workers (30%) and Chinese men in Shenzhen (26%, 19%) (5, 7). Seroprevalence in

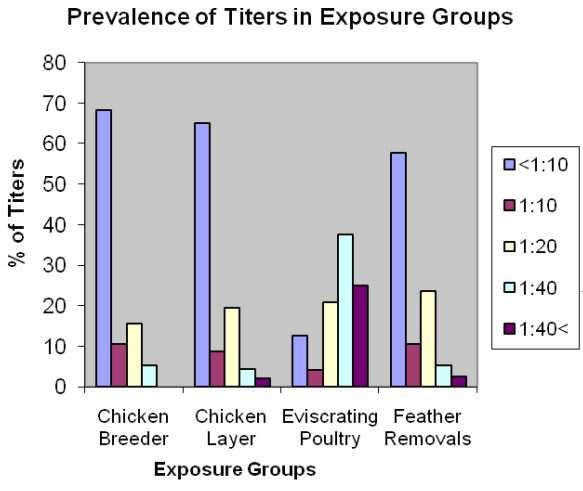

Fig. 1. HI titers (%) in different occupational exposures of the study categorized into four groups: Breeders, Chicken Layers, Eviscerating Poultry, Feather Removals.

slaughter-house workers (51.6%) compared to poultry farm workers (24.6%) might be due to the fact that wearing protective clothing is not routine practice in slaughter-houses. Eviscerating section workers, in contrast to others had the highest risk of H9N2 infection. It is again emphasized that eviscerating sections of poultry work is the most contaminated part of the poultry industry that could increase the likelihood of poultry-to-human transmission. As the administrated influenza vaccine for poultry in Iran is an inactivated vaccine, so, there is no chance for transmission of the killed INF virus from poultry to human. Prevalence of seropositivity was higher in minimally-vaccinated workers and raises the hypothesis that observed cross-reactions might reduce the chance of seropositivity *in-vitro* which could confer cross-protection clinically; however, more work is needed. This study highlights the potential of avian-to-human transmission of H9N2 through occupational exposures. Such a transmission may increase the chance of the adoption of avian INF virus to human, human-to-human transmission and possible future pandemics. In this way management and surveillance in both human and poultry is needed for maintaining occupational health, public health and preventing future pandemics.

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