

ORIGINAL ARTICLE

Evidence of Previous Avian Influenza Infection among US Turkey Workers

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Impact

- Occupational exposure to turkeys increases the risk of infection with low-pathogenicity avian influenza viruses.
- Results from this study show evidence of infection with avian influenza virus types H4, H5, H6, H9 and H10 among backyard and free-range turkey farmers.
- These farmers should be included in surveillance and other preparedness efforts directed at preventing avian influenza infections in man.

Keywords:

Zoonoses; influenza; human; influenza in birds; serology; occupational exposure

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Summary

The threat of an influenza pandemic is looming, with new cases of sporadic avian influenza infections in man frequently reported. Exposure to diseased poultry is a leading risk factor for these infections. In this study, we used logistic regression to investigate serological evidence of previous infection with avian influenza subtypes H4, H5, H6, H7, H8, H9, H10, and H11 among 95 adults occupationally exposed to turkeys in the US Midwest and 82 unexposed controls. Our results indicate that farmers practising backyard, organic or free-ranging turkey production methods are at an increased risk of infection with avian influenza. Among these farmers, the adjusted odds ratios (ORs) for elevated microneutralization assay titres against avian H4, H5, H6, H9, and H10 influenza strains ranged between 3.9 (95% CI 1.2–12.8) and 15.3 (95% CI 2.0–115.2) when compared to non-exposed controls. The measured ORs were adjusted for antibody titres against human influenza viruses and other exposure variables. These data suggest that sometime in their lives, the workers had been exposed to low pathogenicity avian influenza viruses. These findings support calls for inclusion of agricultural workers in priority groups in pandemic influenza preparedness efforts. These data further support increasing surveillance and other preparedness efforts to include not only confinement poultry facilities, but more importantly, also small scale farms.

Introduction

The threat of an influenza pandemic remains real. Outbreaks with avian H5N1 influenza viruses continue to occur among birds in many areas of the world. Human cases continue to occur resulting in severe illness and high mortality rates (Monto and Whitley, 2008). In response, several international and national pandemic influenza response plans were prepared. Epidemiological surveillance, vaccination of susceptible subpopulations

and use of antiviral drugs are the cornerstones of such plans (Monto et al., 2006; Monto and Whitley, 2008). Occupational groups exposed to agricultural animal reservoirs of influenza such as poultry and swine are often ranked with a low priority for pandemic influenza response activities (Gray et al., 2007). Nonetheless, the growing serological evidence that such groups are at risk of infection with avian influenza (AI) viruses highlights the need to include these workers in pandemic response planning as high priority groups to receive potential

vaccines and antivirals (Puzelli et al., 2005; Gill et al., 2006; Myers et al., 2007; Gray et al., 2008). Furthermore, AI surveillance activities should be conducted among such occupational groups, as they constitute a potential first line of contact between humans and AI viruses.

In this study, we investigated serological evidence of previous infection with AI subtypes H4, H5, H6, H7, H8, H9, H10 and H11 among individuals occupationally exposed to turkeys in the US Midwest and we sought to determine the risk factors for infection.

Materials and Methods

Subjects

Between March 2007 and April 2008, 57 turkey growers, 38 turkey meat processing plant workers, and 82 unexposed controls from Iowa and Illinois were enrolled in this cross-sectional study. Participants completed a survey that included questions about the demographic, occupational and general health status of study subjects. A phlebotomist obtained a blood specimen for laboratory analysis from 170 of 177 participants. In our previous reports (Gill et al., 2006; Myers et al., 2007; Ortiz et al., 2007; Gray et al., 2008), a microneutralization (MN) assay and a haemagglutination inhibition (HI) assay were used to assess evidence of previous infection with zoonotic and human influenza A viruses. This study was approved by an institutional review board and all subjects signed informed consent documents.

Viruses, antisera, and cells

Avian influenza viruses and specific chicken, rabbit and goat antisera were kindly provided by Dr Richard J. Webby, St. Jude Children's Research Hospital (Memphis, TN), Dr Alexander I. Klimov, Influenza Branch of the CDC, the Biodefense and Emerging Infections Research Resources Repository (Manassas, VA), or purchased from the National Veterinary Services Laboratory (Ames, IA) (Table 1). Negative sheep serum was obtained from a World Health Organization (WHO) 2005–2006 influenza reagent kit. Viruses were grown in the allantoic cavities of 10-day old embryonated chicken eggs (Charles River Laboratories, Wilmington, MA, USA) for 72 h at 37°C. Eggs were chilled at 4°C overnight then the allantoic and amniotic fluids were harvested. Individual eggs were screened for influenza viruses using a haemagglutination assay. Positive fluids were pooled, aliquoted and frozen at –80°C. Blood specimens from study participants were collected in serum separator tubes, allowed to clot at room temperature, centrifuged for 10 min at 500 g (Allegra 6R, Beckman Coulter, Fullerton, CA, USA), aliquoted and frozen at –80°C, all on the same day.

Table 1. Avian influenza viruses and antisera

Avian influenza virus subtype	Antigen used to produce antisera (host)
A/Duck/CZ/1/56 (H4N6)	A/Duck/Shantou/461/00 (Chicken)
A/Chucker/Minnesota/14591-6/95 (H5N2)	A/Goose/Hong Kong/437/99 (Chicken)
A/Turkey/Massachusetts/65 (H6N2)	A/Turkey/Massachusetts/65 (Goat)
A/Turkey/Virginia/4529/02 (H7N2)	A/FPV/Rostock/34 (Goat)
A/Turkey/Ontario/68 (H8N4)	A/Turkey/Ontario/68 (Goat)
A/Turkey/Minnesota/38391-6/95 (H9N2)	A/Turkey/Minnesota/38391-6/95 (Chicken)
A/Duck/Memphis/546/76 (H11N9)	A/Duck/Hong Kong/M603/98 (Chicken)

Madin-Darby canine kidney (MDCK) cells used for the microneutralization assays were maintained in Dulbecco minimum essential media containing 5% fetal bovine serum (Invitrogen/Gibco, Chicago, IL, USA).

Microneutralization assay

The assay's procedure is described elsewhere (Gill et al., 2006) and was adapted from a previously described protocol (Rowe et al., 1999). Recent trials investigating the safety and immunogenicity of human H5N1 vaccines demonstrated that neutralizing antibodies against the vaccine strains drop nearly to pre-vaccination titres over a period of time as short as 6 months (Nolan et al., 2008; Zangwill et al., 2008). In one of these trials, an antibody titre $\geq 1:20$ was considered protective (Nolan et al., 2008). Unpublished reports suggest that AI infections in man similarly rapidly decline. Thus, in this study, we used a low threshold of evidence of infection as others have used (Puzelli et al., 2005). In our study, sera were tested in duplicate and were considered positive if titres were positive at $\geq 1:10$ dilutions. All assays included a positive control antiserum (Table 1).

Guinea pig RBC haemagglutination inhibition assay

In an effort to control for cross-reactivity between antibodies against human influenza viruses and AI, antibody titres against human influenza were determined using a guinea pig red blood cell (RBC) HI assay. Sera were treated with receptor destroying enzyme (Denka Seiken, Tokyo, Japan), heat inactivated at 56°C for 30 min, and hemadsorbed on guinea pig RBCs. Serum samples were tested against two human influenza A viruses A/New Caledonia/20/99 (H1N1) and A/Panama/2007/99 (H3N2), using a 0.5% guinea pig blood solution. Diluted sera were mixed with a known concentration of virus and

incubated for 15 min. A suspension of guinea pig RBCs was then added and incubated for 1 h. Positive H1 and H3 antisera and uninfected sheep serum from a recent WHO Influenza Reagent Kit were included in all assays as positive and negative controls. The plates were manually read to determine the titre at which haemagglutination was inhibited. Sera were considered positive at a HI titre of $\geq 1 : 40$ or greater.

Statistical methods

Pearson's Chi square and Fisher's exact tests were used to compare categorical variables. Student's *t*-test was used to compare continuous variables. Geometric mean MN titres were calculated for each influenza subtype under study and were compared by exposure type using the Wilcoxon rank-sum test. Odds ratios and associated confidence intervals (CI) were calculated by traditional method or by Fisher's exact method, where data were sparse. A manual, backward elimination logistic regression was then used to determine risk factors associated

with positive titres. Covariates with bivariate *P*-values < 0.1 were considered for inclusion in the regression model. Possible interaction effects between covariates were also studied. Analysis was performed using the *SPSS* 15.0 (The Predictive Analytics Company, Chicago, IL, USA) and WinPepi 1.8 (Brixton Health, <http://www.brixtonhealth.com>) softwares.

Results

Growers were further classified by the type of agricultural practice they conduct. Backyard or free range growing was practiced by 21 growers who kept seasonal flocks of turkeys numbering less than 1000 birds (median number of turkeys = 20). The other 36 farmers raised larger flocks of turkeys in confinement facilities year round (median number of turkeys = 40 000). Data in Table 2 show the demographic characteristics of the study subjects. Turkey growers were older and more likely to be of the male sex than other exposed groups or controls. Most turkey growers were white, non-Hispanic, while most processing

Table 2. Demographic characteristics of the study subjects

Variable	Controls (<i>n</i> = 82)	Meat processing plant workers (<i>n</i> = 38)	Turkey growers (<i>n</i> = 57)	Backyard growers (<i>n</i> = 21)	Confinement growers (<i>n</i> = 36)	<i>P</i> -value
Age						
18–32	32 (39.0%)	14 (36.8%)	15 (26.3%)	1 (4.8%)	14 (38.9%)	0.001
33–44	28 (34.1%)	14 (36.8%)	10 (17.5%)	4 (19.0%)	6 (16.7%)	
45–89	22 (26.8%)	10 (26.3%)	32 (56.1%)	16 (76.2%)	16 (44.4%)	
Mean Age	37.0 (13.0%)	36.0 (10.5%)	45.6 (15.3%)	50.5 (10.5%)	42.7 (16.9%)	
Gender						
Male	43 (52.4%)	23 (60.5%)	46 (80.7%)	10 (47.6%)	36 (100.0%)	<0.001
Female	39 (47.6%)	15 (39.5%)	11 (19.3%)	11 (52.4%)	0 (0%)	
Race/Ethnicity						
White, non-Hispanic	34 (41.5%)	1 (2.6%)	54 (94.7%)	19 (90.5%)	35 (97.25%)	<0.0005
Hispanic	43 (52.4%)	33 (86.8%)	3 (5.3%)	2 (9.5%)	1 (2.8%)	
African American	2 (2.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Asian	3 (3.7%)	4 (10.5%)	0 (0%)	0 (0%)	0 (0%)	
Current user of tobacco products						
Yes	6 (7.3%)	11 (28.9%)	16 (28.1%)	1 (4.8%)	15 (41.7%)	<0.001
No	76 (92.7%)	27 (71.1%)	41 (71.9%)	20 (95.2%)	21 (58.3%)	
Influenza-like illness						
Yes	13 (15.9%)	10 (26.3%)	2 (3.5%)	0 (0%)	2 (5.6%)	0.015
No	69 (84.1%)	28 (73.7%)	55 (96.5%)	21 (100.0%)	34 (94.4%)	
Chronic disease						
Yes	15 (18.3)	8 (21.1%)	11 (19.3%)	4 (19.0%)	7 (19.4%)	NS
No	67 (81.7%)	30 (78.9%)	46 (80.7%)	17 (81.0%)	29 (80.6%)	
Received seasonal human flu vaccine in the previous 5 years						
Yes	48 (58.5%)	16 (43.2%)	24 (42.2%)	12 (57.1%)	12 (33.3%)	NS
No	32 (41.5%)	21 (56.8%)	33 (57.8%)	9 (42.9%)	24 (66.7%)	

NS, not significant.

P-value calculated comparing all three main exposure categories (all turkey growers, meat processing plant workers and non-exposed controls) and was considered significant when < 0.05 . Significant *P*-values in bold font.

plant workers were Hispanic. Use of tobacco products was more frequent among growers. More influenza-like illness (ILI) in the previous year was reported by processing plant workers. Among all groups, there was no significant difference in the reporting of chronic conditions including cancer, heart disease, diabetes and other conditions that affect the immune system. Fewer turkey workers reported receiving a seasonal human influenza vaccine in the previous years than non-exposed controls (42%, 43%, and 58% among growers, processing plant workers, and controls respectively). The use of such vaccine was the lowest among confinement growers (33.3%).

Fourteen of the 95 exposed individuals had elevated antibody titres against any AI virus (15%) as compared to seven of the 82 (8.2%) non-exposed controls. Eight of the 21 study subjects with evidence of past infection with AI had elevated antibody titres against two or more AI viruses. Antibody titres against an AI H5 virus were significantly higher for growers compared with the non-exposed control group (P -value = 0.003). Small scale growers showed elevated titres of antibodies against the H4, H5, H6, H8, H9, and H10 subtypes (P -value <0.05). Processing plant workers had a slightly elevated titre against an avian H4 virus (P -value = 0.057). There was no difference in antibody titres against avian H7 or H11 among the study groups (Table 3).

Further analysis was conducted focusing on small scale growers as this group had the highest antibody titres compared with the control group. In bivariate analysis among small scale growers and non-exposed controls, exposure to chickens, wild birds and swine were significantly associated with elevated antibody titres against the H4, H5, H6, H9 and H10 AI subtypes (Table 4). Age was not associated with antibody titres against any of these viruses.

Several potential risk factors for infection with AI were examined including age, gender, race and ethnicity, smoking, chronic diseases and ILI. A set of occupational risk factors such as the use of gloves, masks, aprons, boots and eye protection were also studied. None of these factors was associated with serological outcomes. Due to potential cross-reactivity between avian and human influenza virus strains, we measured antibody titres against human influenza viruses H1N1 and H3N2 using a HI assay. Growers had significantly lower titres against these viruses compared with the control group. There was no difference in self-reporting of receipt of a human influenza vaccine in the last 5 years between the occupational groups.

Multivariable logistic regression was used to control for potential confounders. After adjusting for antibody titres against human influenza H3N2, we detected an odds ratio (OR) of 4.5 (95% CI 1.5–13.3) for being seropositive for avian H5 among all growers compared to the unexposed

controls. After adjusting for antibody titres against human influenza H1N1, small growers had greater adjusted ORs for being seropositive for avian H4, H5, and H9 viruses (OR = 3.9, 6.2, 3.9; 95% CI 1.2–12.8, 2.0–19.6, 1.2–12.8 respectively). The adjusted OR for seropositivity for avian H6 was also greater among small growers after controlling for the effects of age and antibody titres against the human H1N1 virus (OR = 15.3, 95% CI 2.0–115.2). After adjusting for antibody titres against the human H1N1 virus, exposure to chicken and exposure to swine, small growers had a higher OR for infection with an avian H10 virus compared with controls (OR = 5.8, 95% CI 1.2–27.7) (Table 5). Interactions between final covariates in the logistic regression models for the H6 and H10 influenza viruses were not associated with elevated antibody titres. There was no significant association between exposure to turkeys and H8 seropositivity in logistic regression analysis for any one of the three turkey exposure categories.

Discussion

Evidence provided by this study suggests that occupational exposure to turkeys increases the risk of infection with AI viruses. This finding was more pronounced among small scale farmers who care for seasonal small flocks of turkeys using a backyard or free ranging agricultural approach. To our knowledge, this is the only study that has investigated infection with a wide range of AI viruses among individuals exposed to turkeys.

Small numbers of subjects were seropositive to each AI subtype under study; this could have affected the results of the subgroup analyses and might explain our inability to detect significant risk or protective factors associated with infection. Our findings should be tempered with the knowledge that we used a low threshold for evidence of previous AI virus infections. This low threshold could be confounded by cross-reacting antibody due to previous infections with human influenza virus or receipt of human influenza vaccines. However, we performed considerable efforts to control for such cross-reactivity through controlled, laboratory assessments of antibodies against human influenza virus and advanced statistical analyses. Thus, we believe that our data suggest that sometime in their careers, the turkey workers with elevated antibody titres were infected with low pathogenicity avian influenza (LPAI) viruses. Cross-reactivity between antibodies against different AI subtypes cannot be ruled out; however, such cross-reactivity would not negate our finding that turkey workers may become infected with LPAI viruses.

Among the participants exposed to turkeys, we found no evidence of AI infection among processing plant

Table 3. Distribution and geometric mean antibody titres against avian influenza viruses by exposure group

Influenza virus titre	Controls (n = 78)	Meat processing plant workers (n = 36)	All growers (n = 57)	Backyard growers (n = 21)	Confinement growers (n = 36)
Avian H4^{*,‡}					
<1 : 10	77 (98.7%)	33 (91.7%)	51 (92.7%)	16 (80.0%)	35 (100.0%)
1 : 10	1 (1.3%)	2 (5.6%)	0 (0%)	0 (0%)	0 (0%)
1 : 20	0 (0%)	1 (2.8%)	4 (7.3%)	4 (20.0%)	0 (0%)
Geometric mean titre	5.04	5.40	5.53	6.60	5.00
Avian H5^{†,‡}					
<1 : 10	77 (98.7%)	36 (100.0%)	47 (85.4%)	14 (70.0%)	33 (94.3%)
1 : 10	1 (1.3%)	0 (0%)	4 (7.3%)	2 (10.0%)	2 (5.7%)
1 : 20	0 (0%)	0 (0%)	4 (7.3%)	4 (20.0%)	0 (0%)
Geometric mean titre	5.04	5.00	5.82	7.07	5.20
Avian H6[‡]					
<1 : 10	77 (98.7%)	36 (100.0%)	51 (92.7%)	16 (80.0%)	35 (100.0%)
1 : 10	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1 : 20	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1 : 40	0 (0%)	0 (0%)	3 (5.5%)	3 (15.0%)	0 (0%)
1 : 80	1 (1.3%)	0 (0%)	1 (1.8%)	1 (5.0%)	0 (0%)
Geometric mean titre	5.18	5.00	5.89	7.85	5.00
Avian H7					
<1 : 10	78 (100.0%)	36 (100.0%)	55 (100.0%)	20 (100.0%)	35 (100.0%)
Geometric mean titre	5.00	5.00	5.00	5.00	5.00
Avian H8[‡]					
<1 : 10	77 (98.7%)	35 (97.2%)	53 (96.4%)	18 (90.0%)	35 (100.0%)
1 : 10	1 (1.3%)	1 (2.8%)	1 (1.8%)	1 (5.0%)	0 (0%)
1 : 20	0 (0%)	0 (0%)	1 (1.8%)	1 (5.0%)	0 (0%)
Geometric mean titre	5.04	5.10	5.19	5.55	5.00
Avian H9[‡]					
<1 : 10	77 (98.7%)	36 (100.0%)	51 (92.7%)	16 (80.0%)	35 (100.0%)
1 : 10	1 (1.3%)	0 (0%)	3 (5.5%)	3 (15.0%)	0 (0%)
1 : 20	0 (0%)	0 (0%)	1 (1.8%)	1 (5.0%)	0 (0%)
Geometric mean titre	5.09	5.00	5.33	5.95	5.00
Avian H10[‡]					
<1 : 10	76 (97.4%)	35 (97.2%)	50 (90.9%)	15 (75.0%)	35 (100.0%)
1 : 10	1 (1.3%)	1 (2.8%)	2 (3.6%)	2 (10.0%)	0 (0%)
1 : 20	0 (0%)	0 (0%)	2 (3.6%)	2 (10.0%)	0 (0%)
1 : 40	0 (0%)	0 (0%)	1 (1.8%)	1 (5.0%)	0 (0%)
1 : 80	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1 : 160	1 (1.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Geometric mean titre	5.27	5.30	5.60	6.83	5.00
Avian H11[§]					
<1 : 10	75 (96.2%)	35 (97.2%)	53 (96.4%)	19 (95.0%)	34 (97.1%)
1 : 10	3 (3.8%)	0 (0%)	2 (3.6%)	1 (5.0%)	1 (2.9%)
1 : 20	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1 : 40	0 (0%)	1 (2.8%)	0 (0%)	0 (0%)	0 (0%)
Geometric mean titre	5.14	5.00	5.13	5.18	5.10

^{*}Wilcoxon sum rank test *P*-value comparing plant workers to controls = 0.057, titres ≥1 : 10 were considered positive.

[†]Wilcoxon sum rank test *P*-value comparing growers to controls = 0.003, titres ≥1 : 10 were considered positive.

[‡]Wilcoxon sum rank test *P*-value comparing small growers to controls = 0.001, <0.001, 0.001, 0.043, 0.001, 0.001 for influenza subtypes H4, H5, H6, H8 H9 and H10 respectively, titres ≥1 : 10 were considered positive.

[§]Wilcoxon sum rank test *P*-value not significant, titres ≥1 : 10 were considered positive.

Geometric mean titres of exposed groups significantly higher than that of the control group are in bold font.

workers. This could be attributed to their minimal exposure to live turkeys and to the enhanced biosecurity levels at large turkey processing plants.

It is important to note that turkeys are potentially susceptible to all types of AI viruses including highly pathogenic AI (HPAI) (Alexander, 2000). Low pathogenicity

Table 4. Risk factors associated with a positive antibody titre against avian H4, H5, H6, H9 and H10 influenza viruses among backyard growers

Risk factor	No. of subjects	No. with H4 titre >1 : 10	OR (95% CI)	No. with H5 titre >1 : 10	OR (95% CI)	No. with H6 titre >1 : 10	OR (95% CI)	No. with H9 titre >1 : 10	OR (95% CI)	No. with H10 titre >1 : 10	OR (95% CI)
Exposure to chicken											
Yes	18	4 (22.2%)	22.6 (2.3–217.1)	6 (33.3%)	39.5 (4.4–357.3)	4 (22.2%)	22.6 (2.3–217.1)	4 (22.2%)	7.3 (1.5–36.4)	4 (22.2%)	22.6 (2.3–217.1)
No	80	1 (1.3%)		1 (1.3%)		1 (1.3%)		3 (3.8%)		1 (1.3%)	
Exposure to wild birds											
Yes	3	2 (66.7%)	61.3 (4.3–878.3)	3 (100.0%)	–	2 (66.7%)	61.3 (4.3–878.3)	2 (66.7%)	36.0 (2.8–467.5)	2 (66.7%)	61.3 (4.3–878.3)
No	95	3 (3.2%)		4 (4.2%)		3 (3.2%)		5 (5.3%)		3 (3.2%)	
Exposure to swine											
Yes	19	4 (21.1%)	20.8 (2.2–199.3)	5 (26.3%)	13.8 (2.4–78.0)	4 (21.1%)	20.8 (2.2–199.3)	4 (21.1%)	6.8 (1.4–33.3)	4 (21.1%)	20.8 (2.2–199.3)
No	79	1 (1.3%)		2 (2.5%)		1 (1.3%)		3 (3.8%)		1 (1.3%)	

OR, odds ratio; CI, confidence intervals.

Table 5. Odds ratios for a positive serological response against avian influenza viruses among growers with fewer than 1000 turkeys using logistic regression*

Avian influenza virus subtype	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Avian H4 [†]	4.4 (1.4–13.6)	3.9 (1.2–12.8)
Avian H5 [†]	5.7 (1.9–17.2)	6.2 (2.0–19.6)
Avian H6 [‡]	4.4 (1.4–13.6)	15.3 (2.0–115.2)
Avian H9 [†]	4.4 (1.4–13.6)	3.9 (1.2–12.8)
Avian H10 [§]	3.6 (1.5–8.5)	5.8 (1.2–27.7)

OR, odds ratio; CI, confidence intervals.

*Saturated model included age, gender, race, current use of tobacco, reporting an influenza-like illness in the previous year, antibody titres against human influenza H1N1 and H3N2 viruses, and exposure to chicken, wild birds, and swine, titres ≥ 1 : 10 were considered positive.

[†]Adjusted for antibody titres against human influenza H1N1 virus.

[‡]Adjusted for antibody titres against human influenza H1N1 virus and age.

[§]Adjusted for antibody titres against human influenza H1N1 virus and exposure to chickens or swine.

avian influenza in turkeys may cause little or no clinical signs. A number of outbreaks of AI among turkeys in numerous countries have been reported in the literature. These epizootics were caused by different types of viruses including H1, H3, H7 and H9 (Alexander, 2000; Suarez et al., 2003; Elbers et al., 2005; McQuiston et al., 2005; Tang et al., 2005; Mannelli et al., 2006; Velkers et al., 2006). In February 2007, an outbreak of HPAI H5N1 at a turkey farm in Suffolk, England led to the culling of at least 159 000 birds after 2 600 birds died as a result of the infection. Nine months later, another outbreak involving five turkey farms in that same area resulted in the culling of 30 000 birds. The US is thought to be currently free from HPAI, but outbreaks of LPAI among commercial poultry remain sporadic (USDA 2007). Between 1981 and 2004, several reports indicated that turkeys in the US Midwest have been infected with AI virus types H1, H3, H4, and H7 (Pomeroy, 2003; Suarez et al., 2003; Tang et al., 2005). In 1999, Suarez et al. isolated a reassortant H1N2 influenza virus with swine, human and avian lineage genes from turkey hen breeders in Missouri (Suarez et al., 2003). However, no published data on AI infection among turkeys were available for the geographical areas where our study subjects worked. Thus, we are not able to correlate AI strains that infect the turkeys with those used in human serologic assays in this study.

Our findings among large scale turkey farmers support similar evidence among large scale poultry growers. Ortiz et al. found no evidence of infection with AI subtypes H4–H12 among workers in poultry confinement farms in Peru (Ortiz et al., 2007). Moreover, the HPAI H5N1 outbreak

in the English turkey farms did not lead to human cases. These negative findings may be attributed to high biosecurity measures in poultry confinement facilities.

On the other hand, we have previously found a significant association between exposure to poultry and infection with AI viruses H5, H6 and H7 among veterinarians and farmers in Iowa (Myers et al., 2007; Gray et al., 2008). Those farmers were likely small scale growers as a very low percentage (2.1%) reported daily exposure to poultry and most reported rare contact with poultry (37.9%). Dinh et al. reported exposure to sick birds and lack of an indoor water source, indicators of low biosecurity measures, as risk factors for infection with AI (Dinh et al., 2006). Findings from these studies support the evidence provided in our report.

In conclusion, this study suggests that occupational exposure to turkeys is a risk factor for infection with AI especially among small-scale growers who operate using backyard or free range poultry growing practices. These farms are rarely exclusive turkey farms and usually other poultry species and swine are grown in close proximity. In fact, 81% of small scale farmers enrolled in this study reported also having chickens, swine or ducks on their farm. We also found that exposure to chickens, swine and wild birds were significant determinants of elevated antibody titres against AI. This raises the potential for inter-species transmission of influenza. These findings support calls for inclusion of agricultural groups as higher priority groups in pandemic influenza preparedness efforts as well as for receiving the seasonal influenza vaccine annually (Gray et al., 2007; Myers et al., 2007). These data further support increasing worldwide surveillance and other preparedness efforts to include not only confinement poultry facilities, but more importantly, also small scale farms.

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