

Evidence for avian influenza A infections among Iowa's agricultural workers

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Background Identifying risk factors for zoonotic influenza transmission may aid public health officials in pandemic influenza planning.

Objectives We sought to evaluate rural Iowan agriculture workers exposed to poultry for previous evidence of avian influenza virus infection.

Methods In 2004, we enrolled 803 rural adult Iowans in a 2-year prospective study of zoonotic influenza transmission. Their enrollment data and sera were compared with those of 66 adult controls enrolled at the University of Iowa in 2006 by using proportional odds modeling.

Results Of the 803 participants 58.8% were male with a mean age of 55.6 years. Forty-eight percent reported previous poultry exposure. Sera were studied by microneutralization techniques for antibodies against avian H4, H5, H6, H7 and H9 viruses.

Touching live birds was associated (OR 1.2; 95% CI 1.02–1.8) with increased antibody titer against H5 virus. Similarly, participants who reported hunting wild birds had increased antibody titers against H7 virus (OR 2.8; 95% CI 1.2–6.5) and subjects who reported recent exposure to poultry had increased antibody titers against H6 (OR 3.4; 95% CI 1.4–8.5) and H7 viruses (OR 2.5, 95% CI 1.1–5.7). There was no evidence of elevated antibody against avian H4 or H9 viruses.

Conclusions These data suggest that hunting and exposure to poultry may be important risk factors for avian influenza virus infection among rural US populations. Agriculture workers should be included in influenza pandemic plans.

Keywords Agriculture, avian, influenza, influenza A virus, occupational exposure, seroepidemiological studies, zoonoses.

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Introduction

Studies of avian influenza virus transmission among the poultry exposed have been technically difficult to conduct due to the poor performance and complexity of serological assays.^{1–3} Despite other epidemiological data suggesting that subclinical or mild disease is more common than detected,⁴ serological studies of humans exposed to avian influenza-diseased poultry have often been negative.^{5–7} However, a limited number of serological studies demonstrate that infections do occur. Retrospective seroprevalence studies among Hong Kong bird market workers in 1997 and 1998 showed that 10% had evidence of H5N1 infection.⁸ In addition, following the 2003 Netherlands outbreak, 49% of 508 poultry cullers, as well as 64% of 63 persons exposed to H7N7-infected humans, had serological

evidence of H7N7 infection following the 2003 Netherlands poultry outbreak.¹ A recent serological study of US duck hunters and wildlife biologists exposed to ducks and geese identified several subjects with elevated antibody titers against H11 viruses.⁹ A controlled, 2002 cross-sectional study of US poultry-exposed veterinarians revealed serological evidence of previous infections with avian H5, H6 and H7 viruses.¹⁰ Puzelli found evidence of low pathogenic avian influenza infection among 3.8% of Italian poultry workers in 2003.¹¹ Considering the recently emergent highly pathogenic H5N1 viruses, the exposure most commonly implicated has been free-ranging poultry and small poultry flocks.¹² In this study, we sought to examine evidence for avian influenza virus transmission among poultry workers in Iowa, the leading US egg-producing state.

Methods

Study population

According to our recent report,¹³ the study population consisted of 803 rural adults living in 29 counties in Iowa during 2004 selected from the 89 658-person Agricultural Health Study (AHS) cohort¹⁴ for their non-immunocompromised health status and their likely exposure or non-exposure to swine and poultry. Among the 803 adults, swine-exposed persons and their non-exposed spouses had considerable evidence of swine influenza virus infection.¹³ The study was approved by the University of Iowa's institutional review board. After informed consent was obtained, participants completed a questionnaire and permitted collection of serum specimen. Questionnaires and sera were again obtained at 12 and 24 months. At the enrollment and 12-month encounters, participants were given a first class US Postal Service-ready kit with detailed instructions to complete another questionnaire and self-collect gargle and nasal swab specimens within 96 hrs of symptom onset should they meet a case definition of influenza-like illness (fever $\geq 38^{\circ}\text{C}$ and a cough or sore throat).

Data and sera from non-Agricultural Health Study controls from a concurrent cross-sectional study¹⁵ were included for population comparisons at enrollment. These study subjects were generally healthy University of Iowa students, staff and faculty who denied having swine or poultry exposures.

Laboratory methods

Gargle and swab specimens were transported to the University of Iowa via the US Postal Service in Micro Test M4RT Viral Transport Media (Remel, Inc., Lenexa, KS, USA) and preserved at -80°C . These specimens were studied with both culture in MDCK cells and R-Mix FreshCells™ (Diagnostic Hybrids, Inc., Athens, OH, USA) and with molecular techniques.

Following WHO guidelines¹⁶ and other reports,^{2,17} we used the hemagglutination inhibition (HI) assay to study human sera for antibodies against human influenza viruses and the microneutralization (MN) assay for antibodies against avian influenza virus.

Hemagglutination inhibition assay

According to our previous reports,^{9,10,13,15,18} serum samples were tested using the Centers for Disease Control and Prevention (CDC) HI assay protocol against three human influenza A viruses: A/New Caledonia/20/99 (H1N1), A/Nanchang/933/95 (H3N2) and A/Panama/2007/99 (H3N2). The human influenza virus strains were grown in fertilized eggs. Sera were pre-treated with receptor-destroying enzyme and hemabsorbed with guinea pig erythrocytes.

Titer results are reported as the reciprocal of the highest dilution of serum that inhibited virus-induced hemagglutination of a 0.65% (guinea pig) or 0.50% (turkey) solution of erythrocytes.

Microneutralization assay

Avian influenza viruses and antisera were kindly provided Dr Richard Webby of St Jude Children's Research Hospital, Memphis, TN, USA; Alexander Klimov from CDC; and Dennis Senne of the National Veterinary Services Laboratories, Ames, IA, USA. According to our recent reports,^{9,10} a microneutralization assay, adapted from that of Rowe¹⁷ was used to detect antibodies to avian strains thought to be representative of those recently circulating in the USA: A/Duck/Cz/1/56 (H4N8), A/Chucker/MN/14591-7/98 (H5N2), A/Turkey/MA/65 (H6N2), A/Turkey/VA/4529/02 (H7N2) and A/Turkey/MN/38391-6/95 (H9N2). Avian influenza virus strains were grown in fertilized eggs.

As prevalence was expected to be low, sera were first screened at a dilution of 1:10. Positive specimens were then titered out in duplicate by examining twofold serial dilutions from 1:10 to 1:1280 in virus diluent [85.8% minimum essential medium (Invitrogen, Carlsbad, CA, USA), 0.56% BSA, 25 mm HEPES buffer (Invitrogen), 100 mg/l streptomycin (Invitrogen) and 100 000 units/l penicillin (Invitrogen)]. Virus neutralization was performed by adding 100 TCID₅₀ of virus to the sera. The Reed Muench method was used to determine the TCID₅₀/100 μl . MDCK cells in log phase growth were adjusted to 2.0×10^5 cells/ml with diluent. One hundred microliters of cells were added to each well and the plate was incubated at 37°C with 5% CO₂ for 24 hours. Plates were washed twice with PBS, fixed with cold 80% acetone and incubated at room temperature for 10 minutes. The ELISA endpoint titer was expressed as the reciprocal of the highest dilution of serum with optical density (OD) less than X, where $X = [(average\ OD\ of\ virus\ control\ wells) + (average\ OD\ of\ cell\ control\ wells)]/2$. Test cells with an OD >2 times the cell control OD mean were considered positive for virus growth. The back titer was run in duplicate and was only accepted when both replicates had matching results.

Real-time RT-PCR and sequencing methods

These procedures have been reported previously.¹³ Briefly, RNA was extracted from 140 μl of each nasal swab and gargle sample using a QIAamp viral RNA extraction kit (Qiagen Inc., Valencia, CA, USA) and screened via a proprietary real-time RT-PCR (rRT-PCR) protocol developed and kindly provided by the CDC. The protocol was designed to first screen for influenza A, and then through separate reactions, to rapidly determine influenza HA subtype. Samples positive by rRT-PCR for influenza A were

further studied with RT-PCR and cDNA sequencing for phylogenetic analyses to confirm their subtype and, in some cases, for further genotypic analyses.

Cross-reactivity

Realizing that serological cross-reactivity may occur between avian and human viral strains of the same hemagglutinin types, as per our previous seroepidemiological studies,^{10,13,15,18} we adjusted for this potential confounding in each of the risk factor analyses by including human serological results in the multivariable models. They were included in the final models when statistically significant.

Statistical methods

We examined a number of potential risk factors for association with avian influenza virus infection outcomes: age, gender, influenza vaccination (human) history, meat-processing work, years in poultry production, recent poultry exposure and exposure during follow-up, touching live poultry or game birds, hunting wild birds and hunt times/year, smoking tobacco in the last year, frequency of touching poultry, exposure to poultry vaccine, type of domestic bird exposure, use of personal protective equipment, number of birds on the farm, chronic medical conditions, medications, military service and seropositivity for human influenza viruses.

Studies for previous avian influenza virus infection

The distribution and geometric mean titers of MN assay results from enrollment sera were first compared between the exposure groups. Next, MN results for each avian influenza virus were compared with potential risk factors using a proportional odds modeling approach¹⁹ or, in case of very sparse data, an exact logistic modeling approach. Ninety-five percent confidence intervals were computed about odds ratios. Final multivariable models were designed using a saturated model and manual backwards elimination.

Studies for recent avian influenza virus infection

We used bivariate and multivariable logistic regression to examine risk factors for evidence of influenza virus infection in two ways. First, using the classical approach, we examined risk factor associations for any fourfold rise in MN titer (enrollment to 12 months, 12–24 months or enrollment to 24 months) against the avian influenza viruses in a binary logistic regression model. Next, we examined risk factors for any increase in MN titer (using the participants' greatest increase in titers during the periods: enrollment to 12 months, 12–24 months and enrollment to 24 months) to the avian viruses by examining the entire spectrum of HI titer increase (e.g. no increase,

twofold rise, fourfold rise, sixfold rise and eightfold rise) through proportional odds modeling.²⁰

Results

Participants

Among the 3259 AHS subjects contacted by telephone or mail, 1274 (39.1%) were considered eligible and were willing to participate. Among these, 803 (63.0%) attended enrollment sessions, granted informed consent and were enrolled. Of the subjects who attended enrollment, 385 participants were classified as poultry exposed (*AHS poultry exposed*) and 418 as non-poultry exposed (*AHS non-poultry exposed*, Table 1). Their enrollment data were compared with 66 non-poultry-exposed University of Iowa controls (*university controls*, Table 1). Demographically, university controls were younger and more likely to be female.

During the first 12 months of follow-up, three of the enrolled subjects died and two withdrew from participation. Among the remaining 798 subjects, 372 of the AHS poultry exposed and 368 AHS non-poultry exposed participated in the scheduled 12-month and/or 24-month follow-up encounters. An additional 33 farmers, who missed the 12-month and/or 24-month follow-up sessions, completed and submitted the follow-up questionnaire via mail, which increased participation in at least one follow-up to 97%.

Exposures

More than 50% of the participants reported receiving influenza vaccines during the 4 years before enrollment (Table 1). Relatively few participants ever worked in the meat-processing industry and few were recent tobacco smokers. While many AHS poultry exposed had lived for >10 years in a poultry farm, relatively few continued to have frequent contact with poultry.

Seroprevalence findings

The distribution of MN titers from enrollment sera against avian H4, H5, H6, H7 and H9 viruses helped to demonstrate modest serological reactivity among the two AHS groups and lesser activity among the university controls (Table 2). No differences were observed in enrollment sera MN assay geometric mean titer assays against the avian viruses.

In multivariate proportional odds modeling, the ordinal variable frequency of contact with poultry (assigned score of 0 = never, 1 = rarely, 2 = monthly, 3 = weekly and 4 = every day) was statistically associated with an elevated MN assay titer against avian H5 virus (Table 3). However, the magnitude of this odds ratio (OR 1.2; 95% CI 1.02–15) was meager suggesting that this finding might be explained

Table 1. Characteristics of study subjects at enrollment

Variable	Group		
	AHS poultry exposed (<i>n</i> = 385)	AHS non-poultry exposed (<i>n</i> = 418)	University Controls (<i>n</i> = 66)
Age group* (years)			
18–41	31 (8.1)	59 (14.1)	31 (47)
42–50	88 (22.9)	117 (28)	15 (22.7)
51–89	266 (69.1)	242 (57.9)	20 (30.3)
Mean [†]	57.6	53.7	42.2
Gender*			
Male	225 (58.4)	247 (59.1)	21 (31.8)
Female	160 (41.6)	171 (40.9)	45 (68.2)
Received influenza vaccine?*			
Yes, in the last 5 years	221 (57.4)	224 (53.6)	50 (75.8)
Yes, more than 5 years ago	37 (9.6)	34 (8.1)	8 (12.1)
No/unknown	127 (33)	160 (38.3)	8 (12.1)
Work in a slaughterhouse or meat-processing plant?			
Yes	1 (0.3)	5 (1.2)	0 (0)
No	377 (97.9)	387 (92.6)	0 (0)
Years worked in poultry production?*			
Never	0 (0)	410 (98.1)	66 (100)
<1 year	26 (6.8)	0 (0)	0 (0)
1–4 years	53 (13.8)	0 (0)	0 (0)
4–10 years	103 (26.8)	0 (0)	0 (0)
>=10 years	193 (50.1)	0 (0)	0 (0)
Missing	10 (2.6)	8 (1.9)	0 (0)
Ever touched live poultry or game birds?*			
Yes	241 (62.6)	113 (27)	0 (0)
No	140 (36.4)	301 (72)	66 (100)
Missing	4 (1)	4 (1)	0 (0)
Do you hunt wild birds?*			
Yes	64 (16.6)	69 (16.5)	0 (0)
No	318 (82.6)	345 (82.5)	66 (100)
Missing	3 (0.8)	4 (1)	0 (0)
Smoked tobacco products in the last year?			
Yes	14 (3.6)	23 (5.5)	4 (6.1)
No	371 (96.4)	395 (94.5)	62 (93.9)
How long have you lived in this or other poultry farm?‡			
Never lived in poultry farm	62 (16.1)	245 (58.6)	0 (0)
Less than 1 year	13 (3.4)	0 (0)	0 (0)
1–4 years	15 (3.9)	2 (0.5)	0 (0)
5–10 years	45 (11.7)	3 (0.7)	0 (0)
More than 10 years	202 (52.5)	13 (3.1)	0 (0)
Missing	48 (12.5)	155 (37.1)	66 (100)
On average, how often do you see or touch poultry other than the poultry on the farm where you work?‡			
Never	191 (49.6)	220 (52.6)	0 (0)
Rarely	146 (37.9)	61 (14.6)	0 (0)
Monthly	5 (1.3)	2 (0.5)	0 (0)
Weekly	4 (1)	0 (0)	0 (0)
Every day	8 (2.1)	0 (0)	0 (0)
Missing	31 (8.1)	135 (32.3)	66 (100)

Values are expressed as *n* (%). AHS poultry exposed – participants from the Agricultural Health Study who reported working in poultry production. AHS non-poultry exposed – participants from the Agricultural Health Study who denied ever working in poultry production. University controls – faculty, staff and students from the University of Iowa who denied ever working in poultry production.

*Statistically significant considering a 95% confidence level by Fisher's exact for the three groups.

[†]Statistically significant considering a 95% confidence level by analysis of variance test for the three groups.

[‡]Statistically significant considering a 95% confidence level by Fisher's exact for the AHS groups.

Table 2. Geometric mean and distribution of antibody titers against avian influenza viruses

Titer	Agricultural Health Study						
	Controls (n = 66)	AHS poultry exposed (n = 385)			AHS non-poultry exposed (n = 418)		
		Enrollment (n = 385)	Follow-up 12 months (n = 346)	Follow-up 24 months (n = 339)	Enrollment (n = 418)	Follow-up 12 months (n = 327)	Follow-up 24 months (n = 330)
Avian H4							
Missing*	–	–	–	–	3	–	–
<1:10	66 (100)	385 (100)	346 (100)	339 (100)	413 (99.5)	325 (99.4)	330 (100)
1:10	–	–	–	–	1 (14.3)	–	–
1:20	–	–	–	–	1 (16.7)	2 (28.6)	–
Geometric mean titer	5	5	5	5	5	5	5
Avian H5							
Missing*	–	4	–	1	6	–	–
<1:10	66 (100)	347 (91.1)	326 (94.2)	300 (88.8)	381 (112.7)	301 (89.1)	283 (83.7)
1:10	–	31 (8.1)	19 (5.5)	36 (10.7)	28 (8.3)	24 (7.1)	45 (13.3)
1:20	–	3 (0.8)	1 (0.3)	2 (0.6)	3 (0.9)	2 (0.6)	2 (0.6)
Geometric mean titer	5	5.3	5.2	5.4	5.3	5.3	5.5
Avian H6							
Missing*	–	7	–	–	9	–	–
<1:10	64 (97)	365 (96.6)	341 (98.6)	338 (99.7)	403 (118.9)	323 (95.3)	330 (97.3)
1:10	–	10 (2.6)	5 (1.4)	1 (0.3)	3 (0.9)	4 (1.2)	–
1:20	2 (3)	3 (0.8)	–	–	3 (0.9)	–	–
Geometric mean titer	5.2	5.1	5.1	5	5.1	5	5
Avian H7							
Missing*	–	5	–	–	8	–	–
<1:10	66 (100)	359 (94.5)	346 (100)	339 (100)	397 (96.8)	327 (100)	330 (100)
1:10	–	20 (5.3)	–	–	13 (3.2)	–	–
1:20	–	1 (0.3)	–	–	–	–	–
Geometric mean titer	5	5.2	5	5	5.1	5	5
Avian H9							
Missing*	–	6	–	–	10	–	–
<1:10	65 (98.5)	370 (97.6)	342 (98.8)	334 (98.5)	402 (98.6)	323 (95.3)	325 (95.9)
1:10	1 (1.5)	7 (1.8)	3 (0.9)	4 (1.2)	3 (0.7)	2 (0.6)	4 (1.2)
1:20	–	2 (0.5)	–	1 (0.3)	2 (0.5)	1 (0.3)	1 (0.3)
1:40	–	–	1 (0.3)	–	1 (0.3)	–	–
1:80	–	–	–	–	–	1 (0.3)	–
Geometric mean titer	5.1	5.1	5.1	5.1	5.1	5.1	5.1

Values are expressed as *n* (%).

*Missing – not enough sera to test.

by chance alone. Considering avian H6 virus, working with poultry from the year 2000 to the present (OR 3.4; 95% CI 1.4–8.5) and having a chronic medical condition (OR 5.2; 95% CI 1.9–13.9) were both associated with elevated antibody titers. Considering avian H7 virus, hunting wild birds (OR 2.8; 95% CI 1.2–6.5) and working with poultry from the year 2000 to the present (OR 2.5; 95% CI 1.1–5.7) were both associated with elevated antibody titers. Age was not important in each of the three avian models mentioned above. Elevated antibody against human H1N1 influenza virus (HI assays \geq 1:40) was important to the avian H5 and H7 models. No important risk factors were identified through the H4 and H9 modeling.

Influenza-like illness

As indicated in our previous report,¹³ during the 24 months of follow-up, 66 participants developed an influenza-like illness and submitted 74 sets of self-collected nasal and gargle swab specimens. Two of the study participants were culture positive for influenza B virus and 22 were RT-PCR and culture positive for influenza A virus. One isolate was a 'triple reassortant' swine H1N1 virus (GenBank accession numbers DQ889682–DQ889689) and the remaining 21 influenza A isolates were very similar to circulating human H3N2 viruses. No avian viruses were detected among the influenza-like illness specimens.

Table 3. Enrollment, risk factor analyses with proportional odds model (university control and agricultural health study subjects)

Variable	Avian H5			Avian H6			Avian H7			Avian H9*		
	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age continuous	859	1.01 (0.99–1.03)	1.01 (0.99–1.03)	853	1.02 (0.98–1.06)	1 (0.96–1.04)	856	0.99 (0.97–1.02)	0.99 (0.96–1.02)	853	1 (0.96–1.05)	–
Gender												
Male	487	0.8 (0.5–1.4)	–	484	1 (0.4–2.4)	–	485	1.2 (0.6–2.5)	–	483	1 (0.3–3.1)	–
Female	372	Reference	–	369	Reference	–	371	Reference	–	370	Reference	–
Hunt wild birds												
Yes	133	1.7 (0.9–3.1)	–	132	2.1 (1.4–7)	–	132	2.3 (1–5)	2.8 (1.2–6.5)	132	0.4 (0–2.4)	–
No	719	Reference	–	714	Reference	–	717	Reference	Reference	714	Reference	–
Exposure												
AHS poultry exposed	381	–†	–	378	1.1 (0.3–5.2)	–	380	5.4 (0.9–infinity)	–	379	1.6 (0.2–70.4)	–
AHS non-poultry exposed	412	–†	–	409	0.5 (0.1–2.4)	–	410	3 (0.5–infinity)	–	408	1 (0.1–45.3)	–
Controls	66	Reference	–	66	Reference	–	66	Reference	–	66	Reference	–
Worked with poultry since 2000†												
Yes	140	1.4 (0.8–2.7)	–	139	3.6 (1.5–8.7)	3.4 (1.4–8.5)	140	2.5 (1.1–5.5)	2.5 (1.1–5.7)	139	1.7 (0.4–5.8)	–
No	642	Reference	–	638	Reference	Reference	640	Reference	Reference	638	Reference	–
Score for frequency to touch live poultry or game birds‡	842	1.2 (1.01–1.5)	1.2 (1.02–1.5)	837	1.3 (1–1.7)	–	839	1.3 (0.99–1.7)	–	836	1.1 (0.7–1.7)	–
Chronic medical problems§												
Yes	82	0.8 (0.3–2)	–	82	6.3 (2.5–15.7)	5.2 (1.9–13.9)	82	0.6 (0.1–2.5)	–	82	0.6 (0–4.1)	–
No/unknown	777	Reference	–	771	Reference	Reference	774	Reference	–	771	Reference	–
Reported smoking tobacco products in the last year												
Yes	40	1 (0.3–3.4)	–	39	0.69 (0.4–0.84)	–	39	1.3 (0.3–5.7)	–	39	1.1 (0.2–infinity)	–
No	819	Reference	–	814	Reference	–	817	Reference	–	814	Reference	–
Flu vaccination												
Yes, in the last 5 years	489	1.4 (0.8–2.4)	–	486	0.9 (0.4–1.9)	2.3 (0.6–8.6)	488	0.9 (0.4–1.9)	–	487	1.8 (0.5–7.8)	–
Yes, more than 5 years ago	78	1.5 (0.6–3.7)	–	75	1.3 (0.4–4.1)	3 (0.6–15.6)	76	1.4 (0.4–4.4)	–	75	0.7 (0–5.9)	–
No/unknown	292	Reference	–	292	Reference	Reference	292	Reference	–	291	Reference	–
Human H1N1 New Caledonia												
Positive	357	2 (1.2–3.3)	2 (1.2–3.4)	355	2.3 (1.2–4.8)	–	356	2.2 (1.1–4.6)	3.1 (1.4–7)	356	3.1 (1–11.6)	–
Negative	502	Reference	Reference	498	Reference	–	500	Reference	Reference	497	Reference	–
Human H3N1 Nanchang												
Positive	576	1.9 (1–3.4)	–	572	2.4 (1.5–8)	–	574	2.1 (0.9–5.3)	–	573	2.1 (0.6–11.8)	–
Negative	283	Reference	–	281	Reference	–	282	Reference	–	280	Reference	–

Table 3. (Continued)

Variable	Avian H5			Avian H6			Avian H7			Avian H9*		
	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Human H3N1 Panama												
Positive	388	2.2 (1.3–3.7)	–	385	2.3 (1.1–4.7)	–	387	2.2 (1.1–4.6)	–	386	2 (0.7–6.9)	–
Negative	471	Reference	–	468	Reference	–	469	Reference	–	467	Reference	–

Odds ratios in bold are statistically significant.

Avian H4 not included as almost all subjects have antibody titers <1:10 against this virus (see Table 2). Sparse data were grouped when necessary to meet model assumptions. The proportional odds assumption was evaluated with the score test for proportional odds. Final multivariable models were designed using a saturated model including all potential risk factors (see methods) and manual backwards elimination. Age was forced into the final models. Flu shot was forced into the final models when no multicollinearity occurred.

*Exact method.

†Complete separation. Significant odds with exact logistic regression when grouping titers above 1:10. Odds ratio (95%CI) for AHS poultry exposed and AHS non-poultry exposed equal, respectively, to 9.2 (1.61–infinity) and 7.6 (1.33–infinity), comparing with controls.

‡Includes chicken, turkey and others (duck and geese).

§Score: 0 = Never, 1 = Rarely, 2 = Monthly, 3 = Weekly, 4 = Every day.

*Subject who did not answer yes when asked: 'Do you have other chronic medical problems such as diabetes, kidney disease, cancer, blood disease or a disease that weakens the immune system?'

Evidence for influenza infections during follow-up

Like the enrollment sera, the 12- and 24-month follow-up sera revealed no geometric mean titer difference between the AHS poultry-exposed and the AHS non-poultry-exposed participants for the avian influenza viruses (Table 2). Considering the 740 participants who donated sera at least twice and examining each sera pair (enrollment to 12 months, 12–24 months and enrollment to 24 months), there was sparse evidence of incident avian influenza virus infection. Among the subjects with available MN results, six of 740 (0.8%) and two of 737 (0.3%) experienced a \geq fourfold rise in antibodies against avian H5 and H9 viruses respectively (Table 4). Modeling for risk factors for these sparse incident infections was unfruitful (data not shown).

Discussion

In this report, we document serological evidence that Iowans, who self-reported hunting birds or recent poultry work, had elevated antibodies against low pathogenic avian H5, H6 and H7 viruses representing strains recently circulating in the USA. These data are consistent with previous investigations identifying hunting⁹ and poultry agriculture^{1,11,21} as risk factors for avian influenza infections in humans.

These data are unique in that we avoided using a cut-point in titer (binary outcome) approach which we have previously shown severely limits analytical power.²⁰ Instead, we compared the entire distribution of antibody titers against exposures with the proportional odds model. We conjecture that had a proportional odds modeling method been used in other studies that used binary outcome methodology,^{6,22–24} their results would have probably been very different. Our study is also unique in that we had a non-exposed control group. Without such a control group, it is difficult to evaluate titer activity among the exposed.

One might ask 'What do the findings mean?' While these data do not show the magnitude of risk (odds ratios) that our study of US veterinarians who work with poultry demonstrated,¹⁰ these study data support the position that US hunters and poultry workers are at increased risk of recreational or occupational avian influenza virus infections. We posit here, as we have detailed before,^{25,26} that their increased risk merits special public health attention. They should be educated about their increased risk, encouraged to use personal protective equipment and to seek medical attention whenever they develop an influenza-like illness. They should also receive priority access to annual and pandemic influenza vaccines, so that they do not facilitate the reassortment of novel strains of influenza virus^{25,26} and do not accelerate human or avian influenza epidemics.²⁷

Table 4. Serological evidence for influenza infections during the 24 months of follow-up

Period	Avian H5			Avian H9		
	N	≥Fourfold increase		N	≥Fourfold increase	
		n	Reported ILI*, n (%)		n	Reported ILI*, n (%)
From enrollment to 12-month follow-up	665	3	–	662	2	–
From 12- to 24-month follow-up	601	3	3 (100)	602	0	–
From enrollment to 24-month follow-up	660	3	3 (100)	655	1	–
Any increase between pairs of sera [†]	740	6	3 (50)	737	2	–

No serological evidence for influenza infection from H4, H6 or H7 viruses was detected.

*Percentage of the participants who demonstrated a ≥fourfold increase in titer who also self-reported influenza-like illness (ILI) during follow-up.

[†]From enrollment to 12 months, 12–24 months or enrollment to 24 months, among participants who permitted sera collections at least two times during the study.

Our cross-sectional data are limited in that we cannot discern if the increase in antibody reflects infection or simply antigen exposure. However, other reports do seem to shed light on these questions. Hayden and Croisier⁵ considered similar findings among Italian poultry workers and concluded that the low prevalence of antibody and temporal association with avian influenza epizootics argued for human infection with avian viruses as an explanation. We agree and further argue that as vaccine-generated immunity to influenza viruses wanes over time and as inactivated avian virus immunization may require large doses of unadjuvanted antigen²⁸ to cause an increase in antibody, one might point out that positive serological findings are more likely to represent true infection with avian viruses and their replication in tissue. Regarding the question of clinical illness, we can only speculate as relatively few prospective studies of humans exposed to ill birds have been conducted. However, the available data suggest that subclinical avian influenza virus infections may be more common than expected.^{1,5,8,11} Comprehensive, prospective studies of large numbers of poultry-exposed individuals and their contacts are required to better understand the spectrum of illnesses associated with clinical avian influenza virus infections.

Our study data have a number of other limitations. A number of potential biases may have influenced results: voluntary participation, self-reporting exposure data, a younger mean age of the university controls, potential mismatching between study and circulating viruses, possible cross-reacting antibodies against avian viruses, and passive surveillance for humans with acute avian influenza virus infections. However, as previously described¹³ adjustments have been made to examine or reduce these limitations, and study findings are biologically plausible and consistent with previous reports.

In summary, these data suggest that US bird hunters and poultry workers are at increased risk of avian H5, H6 and H7 influenza virus infections. Efforts should be made to include these citizens in influenza pandemic preparedness plans.

Potential conflicts of interest

Dr Gray has helped to conduct vaccine trials for Glaxo-SmithKline Biologicals and has served as a Scientific Advisory Board member for CSL Biotherapies.

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