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Original Article

Avian influenza and poultry workers, Peru, 2006

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Background Currently numerous countries in Asia, Africa and Europe are encountering highly pathogenic avian influenza (AI) infections in poultry and humans. In the Americas, home of the world's largest poultry exporters, contingency plans are being developed and evaluated in preparation for the arrival of these viral strains.

Objectives With this cross-sectional study, to our knowledge the first in its kind in Central or South America, we sought to learn whether Peruvian poultry workers had evidence of previous AI infection and if so, to determine the risk factors for infection.

Methods We performed a cross-sectional seroprevalence study among 149 workers on a Peruvian poultry farm (132 exposed to poultry and 17 non-exposed controls), serum samples were tested for human influenza virus exposure using a hemagglutination inhibition (HI) assay. Microneutralization assays were performed on all serum samples to detect antibodies against prototypic AI strains H4 through H12.

Results Using multivariate proportional odds modeling we found that the prevalence of elevated titers against AI viruses was low in both groups, exposed and non-exposed controls.

Conclusions No evidence of previous AI infection among Peruvian poultry workers was found in this first cross-sectional study performed in South America. This first occupational study of AI in Latin America was encouraging, but it likely reflects the sector of poultry production with higher biosecurity.

Keywords Human, influenza, influenza in birds, occupational exposure, Peru, poultry, zoonoses.

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Currently numerous countries in Asia, Africa and Europe are encountering highly pathogenic avian influenza (AI) infections in poultry and humans. In the Americas, home of the world's largest poultry exporters, contingency plans are being developed and evaluated in preparation for the arrival of these viral strains.[1] In South America, the incidence of avian influenza virus (AIV) outbreaks affecting avian species is not well described. This may reflect the low intensity of viral sampling, low influenza virus endemnicity, or underreporting of influenza-like illnesses among domestic birds. Despite likely limited sampling, recent outbreaks of AI infections have been documented in South America. For example, a highly pathogenic H7N3 outbreak was reported among poultry in Chile during 2002, antibodies to H1N1 and H3N2 have been reported in wild and domestic birds in Brazil, and a H9 influenza strain has been detected among poultry in Colombia.[2,3] In Peru, the presence of AIV has never been reported. In their 2001–2005 report, the Peruvian National Agrarian Health Service (SENASA) reported sampling 19 309 domestic birds among an estimated population of 80 million birds. All of the sera tested were without evidence of previous AI infection.[4] With this cross-sectional study, to our knowledge the first in its kind in Central or South America, we sought to learn whether Peruvian poultry workers had evidence of previous AI infection and if so, to determine the risk factors for infection.

The study

In June 2006, we recruited workers from a large poultry industry farm located in Pacasmayo, Peru to participate in this study. Site selection was based upon the first author's contacts and opportunities to invite poultry workers to participate. The study was approved by the University of Iowa's institutional review board, the US Naval Medical Research Center institutional review board, the Universidad Peruana Cayetano Heredia institutional review board and the Peruvian Ministry of Health. Workers were eligible to participate if they were at least 18 years old, currently worked on the farm and were without immuno-compromising conditions. Poultry farm workers who were in direct

contact with chickens were classified as poultry-exposed (n = 132). Workers who reported no direct occupational or home contact with poultry were classified as non-exposed controls (n = 17). After providing informed consent, participants completed a questionnaire and permitted sera collection. The questionnaire, available in Spanish, captured demographic, medical, and occupational data including influenza immunization history, occupational exposures, and use of protective equipment (gloves, masks, glasses, and aprons).

As per our previous reports,[5,6] serum samples were studied for human influenza virus exposure using a hemagglutination inhibition (HI) assay against three recently circulating human influenza virus strains: A/New Caledonia/20/99 (H1N1), A/Nanchang/933/95(H3N2), and A/Panama/2007/99(H3N2). HI titer results were reported as the reciprocal of the highest dilution of serum that inhibited virus-induced hemagglutination of a 0.65% solution of guinea pig red blood cells.

Microneutralization assays, adapted as per Rowe *et al.*,[7] were performed on all serum samples to detect antibodies against prototypic AI strains H4 through H12. AI viruses and antisera were kindly provided by Dr. Richard Webby of St. Jude Children's Research Hospital, Memphis, TN, USA, Alexander Klimov from US Centers for Disease Control and Prevention, Atlanta, GA, and Dennis Senne of the National Veterinary Services Laboratories, Ames, IA: A/Duck/Cz/1/56(H4N8), A/Chucker/MN/14591-7/98 (H5N2), A/Turkey/MA/65(H6N2), A/Turkey/VA/4529/02(H7N2), A/Turkey/Ontario/68(H8N5), A/Turkey/MN/38391-6/95(H9N2), A/Chicken/Germany/49(H10N7), A/Duck/Memphis/546/76(H11N9), and A/Duck/Alberta/60/76(H12N5).

All serum samples were first screened at a dilution of 1:10, and full titers (sera dilutions 1:10–1:1280) run for all that screened positive. Briefly, sera were heat inactivated at 56°C for 30 min. Two-fold serial dilutions in 50 µl of virus diluent (prepared in-house and containing bovine serum albumin) were performed in flat bottom 96-wells tissue culture plates (Becton Dickinson, Franklyn Lakes, NJ, USA). Virus neutralization was performed by adding 50 µl of virus at 100 $TCID_{50}$ to the sera. The plates were then covered and incubated for 2 h at 37°C and 5% CO_2. Following this incubation, 100 µl of freshly trypsinized MDCK cells diluted to a concentration of 2×10^5cells/ml was added to the plates. The plates were then incubated at 37°C and 5% CO_2 for 24 h. After this incubation, the fluid was discarded and the plates were washed twice with phosphate-buffered saline. The monolayers were fixed with cold 80% acetone for 10 min. ELISA was performed with mouse-derived anti-influenza A as primary antibody and goat anti-mouse IgG conjugated to horseradish peroxidase as secondary antibody. The absorbance was read after 10 min at 450 nm wavelength using an automated reader (VERSAmax; Molecular Devices, Sunnyvale, CA, USA). Sera were tested in duplicate. All assays included a positive control antiserum. The back titer was run in duplicate and was accepted only when it produced positive results in five to seven wells containing the lowest dilution of test virus.

Specimen laboratory results were studied for their statistical association with demographic, immunization, occupational, and other behavioral risk factors. Geometric mean HI titers were calculated for each virus strain. Differences in titer distribution between exposed groups were tested using the Wilcoxon rank-sum test. The Fisher exact test was used for initial bivariate examinations of potential risk factors with antibodies against AI viruses. Proportional odds modeling was used to examine the entire spectrum of serologic results for associations with potential risk factors. Final multivariable models were designed using a saturated model and manual backward elimination.

The distribution of age and ethnicity was similar in both groups – most subjects were male and 'mestizos.' None of the subjects reported ever receiving an influenza vaccine. The exposed subjects reported more influenza-like symptoms during the past 12 months (Table 1). The use of protective equipment (gloves, mask, apron, or glasses) was low. Only 25.2% of the exposed population reported at least sometimes using protective equipment. The prevalence of elevated titers against AI viruses was low in both groups. One poultry-exposed subject had a 1:10 antibody titer against avian H5 influenza and another had a 1:10 antibody titer against avian H12 influenza (Table 2). As microneutralization titers of ≥1:80 are often considered as evidence of previous AI virus infection, these minimally elevated titers could be explained by cross-reactivity with human influenza antibodies, laboratory error, or chance.

Conclusions

A number of studies have examined occupational risk factors for zoonotic influenza virus infections. They have included serosurveys of bird cullers, open bird market workers, swine workers, duck hunters, meat processing workers, veterinarians and poultry workers, concluding that these populations are indeed at greater risk of infection with zoonotic influenza virus.[5,6,8–13]

Our study is unique in that it constitutes the first serologic examination of poultry workers in Central or South America. We recognize that the experience of workers on this one large poultry farm in Peru may be quite different than the experience of other workers in the poultry industry. However, the results were reassuring in that no workers had strong evidence of AI infection. Our findings should be tempered with the knowledge that the study farm is

Table 1. Study populations' characteristics, poultry farm workers, Pacasmayo, Peru, June 2006

Variable	Controls, *n* = 17 (%)	Poultry-exposed, *n* = 132 (%)
Age group		
18–30	6 (35.3)	47 (35.9)
31–41	4 (23.5)	40 (30.5)
42–65	7 (41.2)	44 (33.6)
Gender*		
Male	14 (82.4)	128 (97)
Female	3 (17.7)	4 (3)
Race		
Asian	0 (0)	1 (0.8)
Black	0 (0)	1 (0.8)
Mestizo	17 (100)	111 (84.1)
White	0 (0)	17 (12.9)
Area		
Broilers	0 (0)	68 (51.5)
Hatchery	0 (0)	14 (10.6)
Breeders	0 (0)	41 (31.1)
Office	17 (100)	9 (6.8)
Security	0 (0)	13 (9.9)
Others	0 (0)	9 (6.8)
Raise birds at home		
Yes	0 (0)	6 (4.6)
No	17 (100)	126 (95.5)
Worked with live birds other than poultry in the last 12 months		
Yes	0 (0)	8 (6.1)
No	17 (100)	124 (93.9)
Used gloves when working with sick or dead poultry		
Never	1 (100)	102 (87.2)
Sometimes	0 (0)	9 (7.7)
Most of the time	0 (0)	3 (2.6)
Always	0 (0)	3 (2.6)
Used mask when working with sick or dead poultry		
Never	1 (100)	95 (82.6)
Sometimes	0 (0)	9 (7.8)
Most of the time	0 (0)	3 (2.6)
Always	0 (0)	8 (7)
Used apron when working with sick or dead poultry		
Never	1 (100)	106 (91.4)
Sometimes	0 (0)	5 (4.3)
Most of the time	0 (0)	2 (1.7)
Always	0 (0)	3 (2.6)
Uses glasses when working with sick or dead poultry		
Never	1 (100)	110 (95.7)
Sometimes	0 (0)	3 (2.6)
Always	0 (0)	2 (1.7)
Influenza-like illness symptoms in the last 12 months*		
Yes	1 (6.3)	45 (34.1)
No	15 (93.8)	87 (65.9)
Years working with poultry (current and previous works)		
0–1	1 (100)	40 (32.3)
2–7	0 (0)	41 (33.1)
8–3	0 (0)	43 (34.7)

*Statistically different between groups with Fisher exact test at 95% confidence level.

Table 2. Distribution of antibodies titers and geometric mean titers against avian influenza viruses, poultry farm workers, Pacasmayo, Peru, June 2006

Titer	Controls	Poultry-exposed
Avian H4		
<1:10	17 (100)	132 (100)
Geometric mean titer	5	5
Avian H5		
<1:10	17 (100)	131 (99.24)
1:10	0 (0)	1 (0.76)
Geometric mean titer	5	5.03
Avian H6		
<1:10	17 (100)	132 (100)
Geometric mean titer	5	5
Avian H7		
<1:10	17 (100)	132 (100)
Geometric mean titer	5	5
Avian H8		
<1:10	17 (100)	132 (100)
Geometric mean titer	5	5
Avian H9		
<1:10	17 (100)	132 (100)
Geometric mean titer	5	5
Avian H10		
<1:10	17 (100)	132 (100)
Geometric mean titer	5	5
Avian H11		
<1:10	17 (100)	132 (100)
Geometric mean titer	5	5
Avian H12		
<1:10	17 (100)	131 (99.24)
1:10	0 (0)	1 (0.76)
Geometric mean titer	5	5.03

known to have a good biosecurity program. At this farm, birds are raised from eggs, and the chicks are isolated from any external contact; vehicles are disinfected before entering the farm, and employees in some areas are required to shower and change clothing when they enter and exit the farm. Since 2002, this farm has implemented an active surveillance program that consists of testing poultry for AIV every 3 months, through serologic study of broilers and breeders. Thus far all 3500 test samples have been without evidence of previous AI infection.

Taking into account that much of Latin America has numerous informal poultry businesses and backyard husbandry is a common activity (an estimated 4 million birds in Peru alone),[14] with little biosecurity (Figure 1), there seems much potential for high pathogenic AI transmission in Central and South America. Recognizing the need to include all sectors of poultry production, the National Agrarian Health Service of (SENASA) in Peru is implementing active and passive AI surveillance programs among

Figure 1. Example of one of many informal poultry businesses with poultry, pigs and humans in close contact, Peru, June 2006 (photo courtesy of Dr. Manuel Cumpa).

formal poultry farms, backyard husbandry, and fighting birds.[4]

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Ethical clearance

Prior to entering the study, a free and informed consent was obtained from each subject. The Naval Medical Research Center Institutional Review Board, in compliance with all Federal regulations governing the protection of human subjects, approved the study protocol ''Occupational Risk of Avian Influenza Infection among Peruvian Poultry Workers'' (Protocol #NMRCD.2005.0003).

Conflicts of interest statement

None of the authors has a financial or personal conflict of interest related to this study. The corresponding author had full access to all data in the study and final responsibility for the decision to submit this publication.

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