Published in final edited form as:

J Agromedicine. 2011 January ; 16(1): 52–57. doi:10.1080/1059924X.2011.533612.

No evidence of infection with avian influenza viruses among US poultry workers in the Delmarva Peninsula Maryland Virginia, USA

Jessica H. Leibler, DrPH and Ellen K. Silbergeld, PhD

Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences.

Andrew Pekosz, PhD

Johns Hopkins Bloomberg School of Public Health, Department of Molecular Microbiology and Immunology.

Gregory C. Gray, MD, MPH

University of Florida, College of Public Health and Health Professions, Department of Environmental and Global Health College of Veterinary Medicine, Department of Infectious Diseases and Pathology.

Abstract

Industrial poultry workers may be at elevated risk of avian influenza infection due to intense occupational contact with live poultry. Serum samples from poultry workers and community members in the Delmarva Peninsula, one of the densest regions of poultry production in the United States, were analyzed for antibodies to strains of five avian influenza subtypes using microneutralization assays. No evidence of infection was found, suggesting inefficient transmission to humans or the absence of virus in these premises. Continued serological surveillance of workers in industrial food animal facilities is necessary to prevent the transmission of influenza A viruses.

Keywords

Agricultural workers; influenza A viruses; avian; poultry; occupational exposure; zoonoses

Introduction

Industrial food animal production is characterized by the high-density production of food animals in confinement that are managed to maximize meat production within a short period of time.¹ Industrial production techniques – which now dominate the poultry industry and increasingly swine production as well – were first developed in the United States but have spread around the world, most recently in Asia and Latin America. As evidenced by the 2009 H1N1 pandemic, attention to the animal-human interface in the context of industrial food animal production is critically important in identifying and possibly preventing the emergence and spread of zoonotic influenza A viruses.²

Poultry workers and others in direct contact with domestic fowl are recognized as the front line for transmission of avian influenza viruses to humans, which has been shown in studies of H5N1 and similar viruses in Asia and Europe.^{3,4} While some studies indicate that poultry workers in the industrialized sector have not been infected during the course of work and that background avian influenza seroprevalence is low,^{5,6} others have reported that working in industrial poultry facilities is an important risk factor for human infection with avian influenza, in the context as well as independent of reported outbreaks in poultry.⁷⁻¹⁰

Studies of avian influenza transmission between poultry and workers in industrial facilities in regions of high endemicity for recent outbreaks are limited, due in part to the perception that industrial poultry facilities are biosecure and biocontained.¹¹ Despite these perceptions, low pathogenicity avian influenza strains are periodically detected among US commercial poultry flocks, often resulting in the depopulation of thousands of birds in efforts to control the virus.¹² Between 2002 and 2005, hemagglutinin subtypes H1-H13 and all nine neuraminidase subtypes were detected in US poultry flocks.¹³ In recent years, LPAI H5 viruses were reported in commercial turkeys in Virginia and West Virginia in 2007, resulting in culling over 75,000 birds. Detections of LPAI H7N9 and LPAI H7N3 in Nebraska and Arkansas resulted in the depopulation of 116,000 commercial birds in 2007, and over 20,000 broiler breeders were culled in Kentucky following a detection of a LPAI H7 virus.¹⁴

Despite the documented presence of avian influenza viruses in the commercial poultry flock in the US, little is known about poultry worker exposure to these viruses. Poultry workers in industrial settings where thousands of birds are confined have intense contact with live poultry, frequently in the absence of personal protective equipment or facilities to maintain hygiene. These workers also report taking their work clothing home for laundering, potentially exposing family members to occupational pathogens.¹⁵

In this study, we analyzed serum samples of poultry workers and community residents from the Delmarva Peninsula for antibodies against strains of five subtypes of avian influenza and two subtypes of human influenza to assess frequency of exposure. The Delmarva Peninsula is a region of the US states of Delaware, Maryland, and Virginia that produced more than 7% of the total US broiler chickens in 2007.¹⁶ A low pathogenicity H7N2 virus was detected in the Delmarva Peninsula in 2004, resulting in the depopulation of more than 100,000 broilers in Delaware and Maryland.¹²

This study draws on our previous work in the Delmarva region to assess environmental and occupational health impacts of the poultry industry.^{15,17-21}

Materials and Methods

Sample collection

Serum samples used in this study were taken from a study of poultry workers and community residents in the Delmarva regions of Maryland and Virginia.^{15,19} In this study, a convenience sample (N=99) was obtained to evaluate exposures to bacterial pathogens. We interviewed workers and community residents and collected serum samples in the fall of 2003 and again in the spring of 2005. The study was approved by the Johns Hopkins Medical Institutions Committee on Human Subjects Research.

Subjects were invited to participate through public notices, flyers, and outreach of local organizations. Individuals less than 18 years of age, those employed in the medical industry, those working in a processing plant, and those who had traveled outside the United States in

the past three months were excluded. All data were collected confidentially. Data collection is described in greater detail elsewhere.^{15,19}

Subjects completed a face-to-face questionnaire, which included demographic and employment information, health insurance status and primary source of health care, and non-occupational risks of exposure to zoonotic infections (such as pets in the home). Select demographic and socioeconomic characteristics are presented in Table 1.

Individuals who reported poultry work were asked specific job title (catcher, grower or live hanger), frequency of exposure to poultry, and use of personal protective equipment. Of the poultry workers in this sample (n=24), 16 workers (67%) reported working as chicken catchers (manually collects chickens from chicken houses and transports to processing facility, often visiting multiple farms per day), 7 workers (29%) were growers (owns the chicken houses and responsible for raising the birds), and 1 worker (4%) reported working as a live catcher (unloads the chickens from the trucks at the processing facility and hangs them on the killing line).

Nearly 92% of workers reported working in the poultry industry for more than 5 years, with the remainder working one year or less in the industry. 83% of workers reported working 5 days or more each week in the poultry houses.

Serological analysis

Six to 10 mL of venous whole blood was collected by butterfly catheter from each subject. Blood was kept on ice for up to 1 hour following collection. Blood samples were allowed to clot at room temperature for 15 to 30 minutes then held at 4°C for up to 1 hour. Serum was separated by centrifugation and transferred to 1.5mL Eppendorf tubes. Samples were stored at -20°C until they were analyzed at the University of Iowa.

Serum samples were analyzed for antibodies that recognize the human influenza A viruses A/New Caledonia/20/99 (H1N1) and A/Panama2007/99 (H3N2) using the hemagglutination inhibition (HI) assay protocol. Sera were pre-treated with receptor destroying enzyme and hemabsorbed with guinea pig erythrocytes. Laboratory techniques for the HI assays performed are described elsewhere.²²

Avian influenza viruses and antisera were generously provided by Dr. Richard Webby of St. Jude Children's Research Hospital, Memphis, Tennessee; Dr. Alexander Klimov from CDC; and Dr. Dennis Senne of the National Veterinary Services Laboratories, Ames, Iowa. As per previous reports^{8,22} microneutralization assays adapted from Rowe et al²³ was used to detect antibodies to avian influenza strains believed to be representative of those circulating in poultry the US: A/Duck/Cz/1/56 (H4N6), A/Chucker/MN/14591-7/98 (H5N2), A/Turkey/MA/65 (H6N2), A/Turkey/VA/4529/02 (H7N2), A/Turkey/MN/38391-6/95 (H9N2), and A/Chicken/DE/04 (H7N2). This latter H7N2 strain (A/Ck/DE/04) was recovered from chickens in Maryland and Delaware during an outbreak in commercial poultry in this region in 2004.

Fertilized eggs were used to grow avian influenza viruses for microneutralization assays. Sera were screened at a dilution of 1:10, under the expectation of low titers. Further analyses at higher dilutions were not performed because of the lack of positive specimens at this level of dilution.

Results

We found no evidence of previous infection with any of the avian influenza viruses subtypes among the poultry workers or community residents (Table 2). No individual within our sample had titers to any of the avian influenza subtypes at dilutions greater than 1:10, which we interpreted as evidence against previous infection with these viruses.

In contrast, we found an equally high prevalence of elevated antibodies against both subtypes of human influenza viruses among both the poultry workers and community residents (Table 3).

Discussion

Our findings suggest that poultry workers and community residents in the Maryland and Virginia areas of the Delmarva Peninsula were not exposed to avian influenza viruses prior to our sample collections in 2003 and 2005. We found that seroprevalence to human influenza viruses was similar between poultry workers and community residents, and that more than half of both groups were seropositive to currently circulating human influenza viruses.

Infection with human influenza A viruses among poultry workers is of particular concern to public health as it pertains to 1) the risk of worker co-infection with avian and human influenza subtypes and 2) increasing the probability that an animal influenza A virus can adapt to productively infect a human. Co-infection with avian and human strains provides opportunities for reassortment and the subsequent emergence of a novel influenza strain.²⁴ Infection of humans with animal influenza A viruses can also lead to the selection of virus variants that are better able to infect and spread in the human population.²⁵

The interpretation of the human influenza seroconversion data is limited by our lack of information on influenza vaccination uptake among subjects. However, given that nearly 30% of the poultry workers in our study report having no health insurance (Table 1), it is likely that many individuals in the study had not received seasonal influenza vaccine, and that elevated antibodies reflect natural infection.

We discuss five potential explanations for our negative serological findings for avian influenza infection. First, it is feasible that the workers in our study were not exposed to avian influenza viruses because there was no avian influenza virus present in the poultry houses prior to serum collection. We were unable to confirm the precise poultry houses affected by the LPAI H7N2 virus in Delmarva in 2004, and it is possible that our study population did not include workers working at farms affected by avian influenza viruses.

Second, avian influenza viruses may have been present in the poultry houses but were not transmitted to humans, resulting in a lack of seroconversion. This conclusion would be in accordance with prior studies which documented the importance of the species barrier in preventing easy transmission of some avian influenza strains, including high pathogenicity H5N1.²⁶ The inability to distinguish between a lack of human exposure or limited viral ability to infect humans speaks to the need for increased active surveillance of commercial animal populations and availability of both existing data and animals for routine serological testing.

A third possible explanation is that the workers were exposed to avian influenza viruses, but personal protective equipment prevented infection. Ten workers (42%) reported wearing dust masks during the course of work and five (29%) reported wearing protective glasses.

Given non-universal utilization of respiratory protection, it seems unlikely that the use of personal protective equipment explained the lack of seroconversion observed in this study.

In addition, although we used five antigenically distinct avian influenza viruses to assess a wide number of different virus subtypes, it is possible that slight antigenic differences between the avian influenza viruses used in the assays and the viruses circulating in the poultry (antigenic drift variants) could result in false negative readings. This explanation seems unlikely, however, as other US poultry-exposed adults' sera have been found to be reactive to the same viruses.⁹

We conclude that the most likely explanations for our results are that the workers in our study were either not exposed to avian influenza viruses because none were present in their occupational environments, or that the viruses to which they were exposed were incapable of infecting humans.

The small number of poultry workers in this study may have limited our ability to detect low levels of seroprevalence in this occupational population. Given uncertainty regarding background levels of avian influenza seroprevalence among industrial poultry workers, this concern suggests the need for larger studies of this population.

This study focused on persons with occupational exposure to live poultry in the context of industrial poultry production in the United States, a population with documented exposure risk to zoonotic pathogens. As such, the results do not address the possibility that non-occupational contact – including environmental or household contacts with animals or zoonotic agents – could result in higher risks of seropositivity than those observed here.

Our understanding of the extent and nature of industrial poultry worker exposure to avian influenza viruses in the United States is incomplete. Vaccinating poultry workers against human influenza can reduce one risk factor for the emergence of a novel influenza virus by reducing the risk of co-infection with human and avian influenza A viruses. However, preventing human infection with zoonotic influenza viruses is necessary to reduce risk of pandemic emergence.

Greater attention to the serological status of the industrial food animal worker population is of vital importance. This has been pointed out by recent papers with respect to swine workers.^{22,27} Follow-up studies of poultry workers in the Delmarva region may provide information on temporality of seroconversion in this population. Prospective studies of industrial food animal workers are required to monitor this critical animal-human interface in the United States, and, since they are likely to provide early signals of viral emergence and transmissibility, this should be a central component of pandemic prevention strategies.

Acknowledgments

Funding provided in part by the National Institute of Allergy and Infectious Diseases R01 AI068803-01A1 (Gray). We thank Sharon Setterquist, MT and Dwight Ferguson, MT of the University of Iowa's Center for Emerging Infectious Diseases for their serological laboratory work. We also thank to Lance Price, PhD and Carol Resnick at Johns Hopkins for their key roles in data collection and ensuring serum storage. We are also indebted to the individuals who participated in our study.

References

1. Silbergeld EK, Graham J, Price LB. Industrial food animal production, antimicrobial resistance, and human health. *Annu Rev Public Health* 2008;29:151–169. doi:10.1146/annurev.publhealth.29.020907.090904. [PubMed: 18348709]

2. Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* 2009;459(7249):931–939. doi:10.1038/nature08157. [PubMed: 19525932]
3. Gray GC, Kayali G. Facing pandemic influenza threats: the importance of including poultry and swine workers in preparedness plans. *Poult Sci* 2009;88(4):880–884. doi:10.3382/ps.2008-00335. [PubMed: 19276439]
4. Leibler JH, Otte J, Roland-Holst D, Pfeiffer DU, Soares Magalhaes R, Rushton J, Graham JP, Silbergeld EK. Industrial food animal production and global health risks: exploring the ecosystems and economics of avian influenza. *Ecohealth* 2009;6(1):58–70. doi:10.1007/s10393-009-0226-0. [PubMed: 19437076]
5. Ortiz JR, Katz MA, Mahmoud MN, Ahmed S, Bawa SI, Farnon EC, Sarki MB, Nasidi A, Ado MS, Yahaya AH, Joannis TM, Akpan RS, Vertefeuille J, Achenbach J, Breiman RF, Katz JM, Uyeki TM, Wali SS. Lack of evidence of avian-to-human transmission of avian influenza A (H5N1) virus among poultry workers, Kano, Nigeria, 2006. *J Infect.Dis* 2007;196(11):1685–1691. doi: 10.1086/522158. [PubMed: 18008254]
6. Hinjoy S, Puthavathana P, Laosiritaworn Y, Limpakarnjanarat K, Pooruk P, Chuxnum T, Simmerman JM, Ungchusak K. Low frequency of infection with avian influenza virus (H5N1) among poultry farmers, Thailand, 2004. *Emerg Infect Dis* 2008;14(3):499–501. [PubMed: 18325273]
7. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, Meijer A, van Steenberghe J, Fouchier R, Osterhaus A, Bosman A. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 2009;363(9409):587–593. doi:10.1016/S0140-6736(04)15589-X. [PubMed: 14987882]
8. Gray GC, McCarthy T, Capuano AW, Setterquist SF, Alavanja MC, Lynch CF. Evidence for avian influenza A infections among Iowa's agricultural workers. *Influenza Other Respi Viruses* 2008;2(2): 61–69. doi: 10.1111/j.1750-2659.2008.00041.x. [PubMed: 18941621]
9. Kayali G, Ortiz EJ, Chorazy ML, Gray GC. Evidence of previous avian influenza infection among US turkey workers. *Zoonoses Public Health* 2010;57(4):265–272. doi:10.1111/j. 1863-2378.2009.01231.x. [PubMed: 19486492]
10. Wang M, Fu CX, Zheng BJ. Antibodies against H5 and H9 avian influenza among poultry workers in China. *N Engl J Med* 2009;360(24):2583–2584. doi:10.1056/NEJMc0900358. [PubMed: 19516044]
11. Socialist Republic of Vietnam. Integrative national operational program for avian and human influenza: 2006–2010. Ministry of Health and Ministry of Agriculture and Rural Development; Vietnam: May. 2006 Available at: http://siteresources.worldbank.org/INTVIETNAM/Resources/vn_opi_2006_2010_en.pdf. Accessed 2009 August 28
12. United States Animal Health Association. Proceedings of the Annual Meeting of the US Animal Health Association; 2004–2008. Available at: <http://www.usaha.org/meetings/proceedings.shtml>. Accessed 2009 August 28
13. Senne DA. Avian influenza in North and South America, 2002–2005. *Avian Dis* 2007;51(1 Suppl): 167–173. [PubMed: 17494549]
14. OIE (Organisation for Animal Health). World Animal Health Information Database (WAHID) Interface. Available at: <http://www.oie.int/wahis/public.php?page=home>. Release date 2009 November 20. Accessed 2009 August 28
15. Price LB, Graham JP, Lackey LG, Roess A, Vailes R, Silbergeld E. Elevated risk of carrying gentamicin-resistant *Escherichia coli* among U.S. poultry workers. *Environ Health Perspect* 2007;115(12):1738–1742. doi:10.1289/ehp.10191. [PubMed: 18087592]
16. United States Department of Agriculture (USDA). 2007 Census of Agriculture. Available at: http://www.agcensus.usda.gov/Publications/2007/Full_Report/index.asp Released 2009 February 4; Updated 2009 December. Accessed 2009 August 28
17. Nweke, OC. Agricultural land use determinants of arsenic in drinking water from groundwater in the broiler producing areas of Maryland. Johns Hopkins University; 2006. PhD dissertation
18. Sapkota AR, Ojo KK, Roberts MC, Schwab KJ. Antibiotic resistance genes in multidrug-resistant *Enterococcus* spp. and *Streptococcus* spp. recovered from the indoor air of a large-scale swine-

- feeding operation. *Lett Appl Microbiol* 2006;43(5):534–540. doi:10.1111/j.1472-765X.2006.01996.x. [PubMed: 17032228]
19. Price LB, Roess A, Graham JP, Baqar S, Vailes R, Sheikh KA, Silbergeld E. Neurologic symptoms and neuropathologic antibodies in poultry workers exposed to *Campylobacter jejuni*. *J Occup Environ Med* 2007;49(7):748–755. doi:10.1097/JOM.0b013e3180d09ec5. [PubMed: 17622847]
20. Sapkota AR, Curriero FC, Gibson KE, Schwab KJ. Antibiotic-resistant *enterococci* and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. *Environ Health Perspect* 2007;115(7):1040–1045. [PubMed: 17637920]
21. Graham JP, Price LB, Evans SL, Graczyk TK, Silbergeld EK. Antibiotic resistant *enterococci* and *staphylococci* isolated from flies collected near confined poultry feeding operations. *Sci Total Environ* 2009;407(8):2701–2710. doi:10.1016/j.scitotenv.2008.11.056. [PubMed: 19157515]
22. Myers KP, Olsen CW, Setterquist SF, Capuano AW, Donham KJ, Thacker EL, Merchant JA, Gray GC. Are swine workers in the United States at increased risk of infection with zoonotic influenza virus? *Clin Infect Dis* 2006;42(1):14–20. doi:10.1086/498977. [PubMed: 16323086]
23. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, Fukuda K, Cox NJ, Katz JM. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol* 1999;37(4):937–943. [PubMed: 10074505]
24. Webby RJ, Webster RG. Emergence of influenza A viruses. *Philos Trans R Soc Lond B Biol Sc* 2001;356(1416):1817–1828. doi:10.1098/rstb.2001.0997. [PubMed: 11779380]
25. Malik Peiris JS. Avian influenza viruses in humans. *Rev Sci Tech* 2009;28(1):161–173. [PubMed: 19618624]
26. Kuiken T, Holmes EC, McCauley J, Rimmelzwaan GF, Williams CS, Grenfell BT. Host species barriers to influenza virus infections. *Science* 2006;312(5772):394–397. doi:10.1126/science.1122818. [PubMed: 16627737]
27. Gray GC, McCarthy T, Capuano AW, Setterquist SF, Olsen CW, Alavanja MC. Swine workers and swine influenza virus infections. *Emerg Infect Dis* 2007;13(12):1871–1878. [PubMed: 18258038]

TABLE 1
Select Demographic and Socioeconomic Characteristics of Study Population

	Number of subjects (N=99)	Mean age (min, max)	Number of men (%) [*]	Non-white (%) [*]	High school education or less (%)	Does not have health insurance (%)
Poultry workers	n=24	45 (29,60)	22 (91.6)	18 (75)	19 (79.2)	7 (29.2)
Non-poultry worker community members	n=75	45 (18,60)	33 (44.0)	40 (53.3)	42 (56.0)	24 (32.0)
Total	N=99	45 (18,60)	55 (55.0)	58 (77.3%)	61 (61.6)	31 (31.1)

* Statistically significant differences between poultry workers and non-poultry workers at p<0.05.

TABLE 2

Number and Percentage of Subjects with Observed Antibody Titers to Avian Influenza A Viruses

Avian influenza virus subtype	Number of subjects with titer > 1:10 (%) [*]
A/Duck/CZ/1/56 (H4N6)	0(0)
A/Chucker/Minnesota/14591-7/98 (H5N2)	0(0)
A/Turkey/Massachusetts/65 (H6N2)	0(0)
A/Turkey/Virginia/4529/02 (H7N2)	0(0)
A/Turkey/Minnesota/38391-6/95 (H9N2)	0(0)
A/Chicken/Delaware/04 (H7N2)	0(0)

* Antibody detection to avian influenza A viruses performed using microneutralization assays.

TABLE 3

Number and Percentage of Subjects with Observed Antibody Titers to Human Influenza A Viruses

Number of subjects expressing titers to:			
	Human H3N2 (Influenza A/Panama2007/99) (%) [*]	Human H1N1 (Influenza A/ New Caledonia/20/99) (%) [*]	
Poultry workers (n=24)			
Titer			
1:20	12 (50.0)	18 (75.0)	
1:40	6 (25.0)	4 (16.6)	
1:80	3 (12.5)	1 (4.16)	
≥1:160	3 (12.5)	1 (4.16)	
Geometric mean titer	1:40	1:40	
Non-poultry worker community residents (n=75)			
Titer			
1:20	29 (38.6)	50 (66.6)	
1:40	27 (36.0)	10 (13.5)	
1:80	9 (12.0)	6 (8.12)	
≥1:160	10 (13.3)	9 (12.2)	
Geometric mean titer	1:40	1:80	

* Antibody detection human influenza A viruses performed using hemagglutination inhibition assays.