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Serologic Evidence of Avian Influenza Virus Infections Among Nigerian Agricultural Workers

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Nigeria has had multiple incursions of highly pathogenic avian influenza A (HPAI) H5N1 virus into its poultry population since 2006. This study aimed to determine if Nigerians exposed to poultry had evidence of avian influenza virus transmission to man. Between 2008 and 2010, 316 adult farmers and open market workers and 54 age-group matched, non-animal exposed controls were enrolled in a prospective, population-based study of zoonotic influenza transmission in four towns in southeastern Nigeria. Questionnaire data and sera obtained at the time of enrollment were examined for evidence of previous infection with 10 avian influenza virus strains. Serologic studies on sera collected at the time of enrollment showed modest evidence of previous infection with three avian-origin influenza viruses (H5N1, H5N2, and H11N1) and one avian-like H9N2 influenza virus, with eight (2.4%) of animal-exposed subjects and two (3.7%) unexposed subjects having elevated microneutralization assay antibody titer levels (ranging from 1:10 to 1:80). Statistical analyses did not identify specific risk factors associated with the elevated antibody titers observed for these zoonotic influenza viruses. These data suggested only occasional virus transmission to humans in areas thought to have been enzootic for avian influenza virus. Prospective data from this cohort will help the authors to better understand the occurrence of zoonotic infections due to avian influenza viruses in Nigeria. ***J. Med. Virol. 85:670–676, 2013.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: influenza A virus; avian; zoonoses; occupational exposure; communicable diseases; emerging; agriculture; seroepidemiologic studies

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INTRODUCTION

The Federal Republic of Nigeria is the most populated country in Africa. Home to more than 170 million people within 36 states [CIA, 2012], Nigeria is comprised of diverse groups/ethnicities with many different agricultural practices. Since highly pathogenic avian influenza (HPAI) H5N1 virus was first detected and confirmed in Africa in Kaduna State in February 2006 [Joannis et al., 2006], several other states in Nigeria have reported HPAI virus infections among millions of domestic and wild birds [Henning et al., 2012]. One human case and death due to infection with H5N1 virus occurred in February 2007. The last recorded outbreak of H5N1 virus infection among domestic birds occurred in northern Nigeria in late 2008.

The Central and Northern States of Nigeria are mainly involved in cattle, sheep, and goat production as they have large areas of savannah grassland. Comprised mainly of rain forest, poultry production is concentrated in Southern Nigeria. While larger breeder farms are located in the Southwestern States of Nigeria near Lagos, the Southeast is the second largest poultry and swine producing area of Nigeria. Modern confined animal feeding operations (CAFOs) have not yet fully supplanted small poultry farming in Nigeria. Smaller-scale village/backyard poultry farming and wet markets still exist, especially in

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Southeast Nigeria, where biosecurity measures are not employed and workers do not wear protective clothing. Poultry are not vaccinated against avian influenza virus [Cattoli et al., 2011], and when outbreaks do occur, the "stamp out" policy is employed as their control measure. Nigerians are not commonly vaccinated against seasonal human influenza viruses; although a recent study has demonstrated a willingness to receive pandemic influenza A (H1N1) vaccine by health care workers [Fatiregun et al., 2012].

It is likely that H5N1 virus is enzootic in this densely populated nation; however, influenza surveillance among humans and animals in Nigeria is poor due to its weak public health infrastructure and agriculture assistance programs. This lack of animal and human health control measures, along with inadequate risk perception among poultry workers [Fatiregun and Saani, 2008; Fasina et al., 2009; Musa et al., 2010; Paul et al., 2012], likely help to facilitate HPAI H5N1 virus spread. Identifying subclinical human infections and risk factors for zoonotic influenza virus transmission may help to expand control and prevention strategies in Nigeria [Ortiz et al., 2007; Metras et al., 2012].

OBJECTIVES

The aim of this study was to determine if poultry workers, or people working in the open bird markets, in Nigeria had more evidence of zoonotic influenza virus infections compared to Nigerians not occupationally exposed to poultry.

STUDY DESIGN

A total of four institutional review boards reviewed and approved the study (see Grant Sponsor information). Eligible study participants ($\geq$18 years old and self-reporting no immunocompromising conditions) were recruited in the towns of Nsukka, Udi, and Enugu in Enugu State and the town of Abakaliki in Ebonyi State, all in southeastern Nigeria. Consenting participants were interviewed by University of Nigeria in Nsukka (UNN) staff field workers who completed enrollment forms and collected sera at the participant's place of employment. Animal exposure was classified as exposure to domesticated poultry (including chicken, ducks, geese, turkeys, and pigeons) as part of daily activities for $\geq$5 cumulative hr/week. Animal-exposed participants were enrolled at poultry production facilities, open bird markets, and small backyard poultry farms. Modern biosecurity measures are not aggressively enforced in these settings. Along with demographic information and medical history, community, household, and occupational animal exposures were assessed with the study's enrollment questionnaire. The questionnaire captured flock/herd size for various types of domestic poultry and other animals, well as years of exposure, such that animal exposure could be classified in an continuous or ordinal fashion (e.g., 1,000 chicken-years or 1,000 duck-years). Age group matched non-animal exposed controls with no self-reported household and occupational animal exposure were recruited from UNN.

Laboratory Methods

Whole blood specimens (10 ml red top tube) were transported at 10–15°C to the laboratory at UNN within 24 hr after collection. Upon arrival, specimens were accessioned and blood tubes spun at 3,000*g* for 15 min to separate serum. All collected serum was aliquoted and frozen at −80°C. Frozen sera were transported on dry ice to the University of Florida for testing.

Influenza virus strains were selected by the hemagglutinin (H) type for their best geographic and temporal proximity to the study population (Table I). The hemagglutination inhibition (HI) assay as previously described [Gill et al., 2006; Myers et al., 2006, 2007; Ramirez et al., 2006; Kayali et al., 2008] was conducted to study human sera for antibodies against human influenza A viruses. A microneutralization (MN) assay adapted from previous reports by Rowe et al. [1999], Gill et al. [2006], Myers et al. [2007], Gray et al. [2008], and Khuntirat et al. [2011] was used to

TABLE I. Viruses Used in Serological Studies

Avian viruses	Human viruses
A/Migratory duck/Hong Kong MPS180/2003(H4N6)	A/Brisbane/59/2007(H1N1)[a]
A/Chicken/Nigeria/2007/1132123(H5N1)	A/Mexico/4108/2009(H1N1)[a]
A/Nopi/Minnesota/2007/462960-2(H5N2)	A/Brisbane/10/2007(H3N2)[a]
A/Teal/Hong Kong/w312/97(H6N1)	
A/Water fowl/Hong Kong/Mpb127/2005(H7N7)	
A/Migratory duck/Hong Kong/MP2553/2004(H8N4)	
A/Hong Kong/1073/1999(H9N2)[b]	
A/Migratory duck/Hong Kong/MPD268/2007(H10N4)	
A/Chicken/New Jersey/15906-9/1996(H11N1)	
A/Duck/ALBERT60/1976(H12N5)	

Unless otherwise indicated, serologic study was performed using the microneutralization assay.
[a]Virus studied with hemagglutination inhibition assay.
[b]Virus of avian origin but isolated from a human.

detect antibodies against a large panel of avian and avian-like influenza A viruses.

Due to a low prevalence of elevated antibodies against the various avian influenza viruses, rapidly waning titers [Buchy et al., 2010], and the inability to determine when such an infection might have occurred, a low threshold of antibody titer ($\geq$1:10) was chosen as evidence of previous infection with a strain of avian influenza virus [Capuano et al., 2007]. Because cross-reactions from previous infection with human influenza viruses might confound the serology, potential confounding was controlled by also testing sera for cross-reacting antibodies against human influenza viruses. As the authors have reported previously [Myers et al., 2006, 2007; Ramirez et al., 2006], a HI titer $\geq$1:40 was accepted as evidence of previous human influenza virus infection or vaccination.

Statistical Methods

Questionnaire data were manually entered twice in a relational database (Microsoft, Inc., Redmond, WA) and verified with structured query language. Questionnaire and laboratory data were later merged into a master dataset, using a unique study subject number. Associations between animal exposure and serology results for human and avian influenza viruses were examined using binary logistic regression. An exact conditional method was used for sparse data. Analyses were performed by using SAS v9.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Participants

Between December 2008 and June 2009, field staff enrolled 316 poultry-exposed (open markets and farms) participants in Nsukka, Enugu, Udi, and Abakaliki, all in southeastern Nigeria (Fig. 1). Occupational exposure to domestic poultry and respective median animal-years of exposure among these participants included layer chickens (3,000 animal-years), broiler chickens (1,000 animal-years), pigeons (150 animal-years), ducks (38 animal-years), and turkeys (25 animal-years). Household/community poultry exposures included market chickens (120 animal-years), caged birds at home (120 animal-years), chickens at home (75 animal-years), pigeons at home (58 animal-years), and ducks at home (51 animal-years). Wild birds were not prevalent in these enrollment areas. Median years worked in a specific occupation included veterinarian (8 years), poultry farmer (6 years), poultry market worker (6 years), and poultry industry worker (4 years). In April 2010, 54 age-group matched

Fig. 1. Map of the four enrollment sites in Enugu State and Ebonyi State, Nigeria.

non-animal exposed control subjects were enrolled at UNN. All participants had a mean age of 34.5 years and 53% were female (Table II). Potential risk factors for infection differed between the animal-exposed and unexposed groups. Nearly all of the animal exposed subjects (96%) reported no access to an indoor source or water, while only 76% of the unexposed subjects did not report access (OR = 7.3; 95% CI, 2.9–18.5). Cardiovascular disease (OR = 7.1; 95% CI, 1.2–infinity) and chronic breathing problems (OR = 10.9; 95% CI, 1.8–448) were more prevalent among the exposed group. Animal-exposed participants were also significantly more likely to have developed a respiratory illness in the last 12 months, when compared to the unexposed subjects (OR = 37.3; 95% CI, 9.5–322). Participants self-reported their use of personal protective equipment (PPE) while working with animals. The majority (88%) of animal-exposed subjects reported never wearing gloves, but 69% did report always washing their hands. Eye protection and masks were never used by most participants (95% and 88%, respectively).

Serology

Sparse serological reactivity was found with the MN assay against the 10 avian and avian-like influenza viruses tested, including the HPAI H5N1 virus (Tables III and IV). Four animal-exposed subjects were found to have elevated antibodies against the A/Hong Kong/1073/1999(H9N2) avian-like influenza virus, while 3 exposed subjects had elevated titers against the A/Chicken/New Jersey/15906-9/1996(H11N1) avian influenza virus. One subject in each animal exposure group had elevated titers (1:10 and 1:20) against A/Nopi/Minnesota/2007/462960-2(H5N2). The unexposed subject seropositive for antibodies against the H5N2 influenza virus was also observed to have elevated antibodies (1:80) against the A/Chicken/Nigeria/1132123/2007(H5N1) influenza virus. A secondary interview with this subject revealed approximately 2 years ago he had helped to process 12 live broiler chickens for eating. However, he did not recall developing any signs or symptoms of subsequent influenza-like-illness. Table V details the level of poultry exposure among the seven poultry-exposed subjects with elevated antibody titers against avian influenza viruses. Among subjects exposed to poultry, chronic breathing problems or a history of respiratory illness were not associated with elevated MN titers ($P = 0.34$ and $P = 0.70$, respectively).

The HI assay showed reactivity against two human influenza viruses, with the animal-exposed group significantly less likely than the unexposed group to have elevated antibodies: A/Brisbane/59/2007(H1N1) (OR = 0.04; 95% CI, 0.02–0.08) and A/Brisbane/10/

TABLE II. Characteristics of Study Subjects Upon Enrollment, Nigeria, 2009

Exposure variables	N (n = 370)	Exposed (n = 316) N (%)	Control (n = 54) N (%)	Unadjusted OR (95% CI)
Age (years)				
>=60	16	16 (5.1)	—	3.8 (0.6–infinity)
40–59	92	76 (24.1)	16 (29.6)	0.8 (0.4–1.6)
20–39	262	224 (70.9)	38 (70.4)	Reference
Gender				
Female	195	171 (54.1)	24 (44.4)	1.5 (0.8–2.8)
Male	175	145 (45.9)	30 (55.6)	Reference
Indoor water				
No	344	303 (95.9)	41 (75.9)	7.3 (2.9–18.5)
Yes	26	13 (4.1)	13 (24.1)	Reference
Ever received vaccination for human influenza				
Yes	1	1 (0.3)	—	5.9 (0–228)
No	369	315 (99.7)	54 (100)	Reference
Heart disease, hypertension, or stroke				
Yes	27	27 (8.5)	—	7.1 (1.2–infinity)
No	343	289 (91.5)	54 (100)	Reference
Chronic breathing problems				
Yes	55	54 (17.1)	1 (1.9)	10.9 (1.8–448)
No	315	262 (82.9)	53 (98.1)	Reference
Other chronic medical problems				
Yes	8	8 (2.5)	—	1.9 (0.3–infinity)
No	362	308 (97.5)	54 (100)	Reference
Ever used tobacco products				
Yes	25	24 (7.6)	1 (1.9)	4.3 (0.7–183)
No	345	292 (92.4)	53 (98.1)	Reference
Developed a respiratory illness in the last 12 months[a]				
Yes	187	185 (58.5)	2 (3.7)	37.3 (9.5–322)
No	180	128 (40.5)	52 (96.3)	Reference

Unadjusted odds ratio for animal-exposed participants compared to non-exposed control participants with logistic regression, exact method.
[a]Covariate has some missing data.

TABLE III. Serological Activity Against Avian and Human Influenza Viruses by Microneutralization and Hemagglutination Inhibition Assay, Nigeria Enrollment Sera, 2009

Virus strain	N	Exposed n (%)	Controls n (%)	Unadjusted OR (95% CI)
A/Chicken/Nigeria/2007/1132123(H5N1)[a,b]				
Positive	1	0 (0)	1 (1.9)	–
Negative	368	315 (100)	53 (98.1)	–
A/Nopi/Minnesota/2007/462960-2(H5N2)[a,b]				
Positive	2	1 (0.3)	1 (1.9)	–
Negative	367	314 (99.4)	53 (98.1)	–
A/Hong Kong/1073/1999(H9N2)[a,b]				
Positive	4	4 (1.3)	0 (0)	–
Negative	365	311 (98.4)	54 (100)	–
A/Chicken/New Jersey/15906-9/1996(H11N1)[a,b]				
Positive	3	3 (0.9)	0 (0)	–
Negative	366	312 (98.7)	54 (100)	–
A/Brisbane/59/2007(H1N1)[b,c]				
Positive	65	27 (8.5)	38 (70.4)	**0.04 (0.02–0.08)**
Negative	303	287 (90.8)	16 (29.6)	**Reference**
A/Brisbane/10/2007(H3N2)[b,c,d]				
Positive	113	63 (19.9)	50 (92.6)	**0.4 (0.2–0.9)**
Negative	256	252 (79.7)	4 (7.4)	**Reference**
A/Mexico/4108/2009(H1N1)[b,c,d]				
Positive	6	4 (1.3)	2 (3.7)	3.0 (0.3–21.4)
Negative	363	311 (98.4)	52 (96.3)	Reference

Unadjusted odds ratio for exposed enrollees versus control enrollees with binary logistic regression.
[a]Microneutralization assay, Negative = titer < 1:10, Positive = titer ≥ 1:10.
[b]Covariate has some missing values.
[c]Hemagglutination Inhibition assay, Negative = titer < 1:40, Positive = titer ≥ 1:40.
[d]Fisher exact method used.

2007(H3N2) (OR = 0.4; 95% CI, 0.2–0.9). Little serological reactivity was found for the 2009 pandemic H1N1 influenza virus.

DISCUSSION

Because the kinetics of human infection with avian-like influenza viruses suggest rapid decline in antibodies (within 11 months after subclinical infections with H5N1 influenza A virus), and because for this serosurvey it is unknown when these infections may have occurred, a low threshold for evidence of previous infection was considered [Buchy et al., 2010]. Examinations of sera obtained at the time of enrollment yielded modest evidence of previous infection with avian influenza virus among this Nigerian cohort. The one non-animal exposed subject with elevated antibodies against A/Chicken/Nigeria/1132123/2007(H5N1) had a titer of 1:80, indicating the subject likely experienced a subclinical H5N1 virus infection in recent years. One animal-exposed subject had elevated antibodies against two influenza viruses examined: A/Hong Kong/1073/1999(H9N2) and A/Chicken/New Jersey/15906-9/1996(H11N1). Because of the diverse HA and NA types between the two viruses, serological cross-reactivity is not suspected; at some time in his life, this subject was likely exposed to two avian-like influenza viruses.

Antibody titers against the avian H5N2 and H11N1 influenza viruses, and the avian-like H9N2 influenza virus ranged from 1:10 to 1:40, making it difficult to discern true infections from cross-reacting antibodies in view of such a small sample size. Our results are similar to a previous study conducted in 2006, that

TABLE IV. Distribution of Elevated Microneutralization Titers Against the Four Avian Influenza Viruses Among Study Participants, Nigeria, 2009

	H5N1		H5N2		H9N2		H11N1	
Titer	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control
<1:10	315	53	314	53	311	54	312	54
1:10	0	0	0	**1**	0	0	**2**	0
1:20	0	0	**1**	0	**3**	0	0	0
1:40	0	0	0	0	**1**	0	**1**	0
1:80	0	**1**	0	0	0	0	0	0

TABLE V. Level of Poultry Exposure Among the Seven Poultry-Exposed Subjects With Elevated Antibody Titers Against Avian Influenza Viruses

	Seropositive subject						
Exposure	H5N2	H9N2	H9N2	H9N2	H9N2, H11N1	H11N1	H11N1
Occupation (total years)							
Poultry industry worker	—	15	9	20	13	—	3
Poultry market worker	6	—	—	—	—	—	—
Meat processor	—	—	—	—	—	4	—
Occupational exposure (animal-years since 2003)							
Chickens, broilers	600	25,000	4,000	600	—	4,000	2,400
Chickens, layers	600	25,000	6,000	—	15,000	4,000	3,000

examined the seroprevalence of antibodies against H5N1 avian influenza virus among poultry workers in Kano State. While the authors employed an antibody titer cut-point approach for seropositivity (≥1:80) and reported no evidence of previous infections, workers did have MN antibodies ranging between 1:10 and 1:40 [Ortiz et al., 2007].

As illustrated in Table V, considerable poultry exposure was reported by seropositive subjects. Exposures to other animals does not likely explain the observed elevated antibody titers, as these subjects did not report household or occupational to pigs or horses, save for one subject who had worked for 3 years in the swine industry.

For potential risk factor analyses, unadjusted odds ratios were first calculated with simple bivariate analyses between the animal-exposed and unexposed groups. As illustrated in Table II, there was a significant difference between the groups in regards to indoor water access, history of heart disease, and chronic breathing problems; however, these associations could not be correlated with seropositivity against avian-like influenza viruses. As only eight subjects were seropositive across four viruses, too few outcomes were observed to perform meaningful statistical analyses. A previous case–control study conducted in Lagos and Kano States, Nigeria, identified hand washing as a protective factor for HPAI H5N1 disease occurrence (OR = 0.14; 95% CI, 0.05–0.37) [Metras et al., 2012]; therefore, access to a clean water source may very well play a role in disease transmission. Study results from Metras et al. also suggested that poultry trade practices and farm proximities played a role in HPAI H5N1 transmission; however, these variables were not assessed for this study.

This study had some limitations. Due to political demonstrations and the University's exam schedule, enrollment of the non-animal exposed control group occurred one year after the animal-exposed group was enrolled. This delay made comparing antibody prevalence between groups difficult, as antibodies wane in time and circulating viruses differ over time. In addition, if the viruses used to examine sera reactivity were antigenically different than those circulating in Nigeria, then the negative assays may have been unreliable. While ideally, all viruses used in the serological assays would be sourced from Nigeria, the limited access to active surveillance programs in Nigeria prevented acquisition of influenza virus strains isolated in Nigeria. Therefore, available strains with the closest geographic and temporal proximity to Nigeria were selected. Special efforts were made to ensure that an HPAI H5N1 virus was sourced from Nigeria. Due to the occupational setting of targeted enrollments, only adults at least 18 years of age were enrolled. Many human cases of avian influenza virus infections have been reported in children and therefore this study may have excluded a large subset of the at-risk population [Grose and Chokephaibulkit, 2004; Dudley, 2009]. Finally, some of the reactivity against avian viruses might represent cross-reactivity from non-avian influenza virus strains [Kreijtz et al., 2011].

Despite these limitations, this study established a cohort with distinct exposure to poultry for prospective studies of zoonotic influenza virus transmission. Following enrollment, the subjects were monitored on a monthly basis for influenza-like illness defined as acute onset of a respiratory illness with a measured temperature ≥38°C (100.5°F) and a sore throat or cough for 4 or more hours. Sera samples and questionnaires were also collected at 12 and 24 months post-enrollment to monitor for changes in influenza antibody titers. Future reports will examine this prospective data.

EXPERIMENTAL ETHICS

A total of four institutional review boards (University of Iowa, University of Florida, University of Nigeria, and Human Research Protection Office of the U.S. Army Medical Research and Materiel Command) reviewed and approved the study. All experiments were performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki. Informed consent was obtained for any experimentation with human subjects including human volunteers.

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REFERENCES

Buchy P, Vong S, Chu S, Garcia JM, Hien TT, Hien VM, Channa M, Ha do Q, Chau NV, Simmons C, Farrar JJ, Peiris M, de Jong MD. 2010. Kinetics of neutralizing antibodies in patients naturally infected by H5N1 virus. PLoS ONE 5:e10864.

Capuano AW, Dawson JD, Gray GC. 2007. Maximizing power in seroepidemiological studies through the use of the proportional odds model. Influenza Other Respir Viruses 1:87–93.

Cattoli G, Fusaro A, Monne I, Coven F, Joannis T, El-Hamid HS, Hussein AA, Cornelius C, Amarin NM, Mancin M, Holmes EC, Capua I. 2011. Evidence for differing evolutionary dynamics of A/H5N1 viruses among countries applying or not applying avian influenza vaccination in poultry. Vaccine 29:9368–9375.

CIA. 2012. The World Factbook. Africa: Nigeria. Washington, DC: Central Intelligence Agency.

Dudley JP. 2009. Age-specific infection and death rates for human A(H5N1) avian influenza in Egypt. Euro Surveill 14:1–2.

Fasina FO, Bisschop SP, Ibironke AA, Meseko CA. 2009. Avian influenza risk perception among poultry workers, Nigeria. Emerg Infect Dis 15:616–617.

Fatiregun AA, Saani MM. 2008. Knowledge, attitudes and compliance of poultry workers with preventive measures for avian influenza in Lagelu, Oyo State, Nigeria. J Infect Dev Ctries 2: 130–134.

Fatiregun AA, Adeyemo AA, Olowookere SA. 2012. Willingness to receive pandemic influenza A (H1N1) vaccine among doctors and nurses in public health facilities in Ibadan, Nigeria. Vaccine 30: 2315–2319.

Gill JS, Webby R, Gilchrist MJ, Gray GC. 2006. Avian influenza among waterfowl hunters and wildlife professionals. Emerg Infect Dis 12:1284–1286.

Gray GC, McCarthy T, Capuano AW, Setterquist SF, Alavanja MC, Lynch CF. 2008. Evidence for avian influenza A infections among Iowa's agricultural workers. Influenza Other Respir Viruses 2:61–69.

Grose C, Chokephaibulkit K. 2004. Avian influenza virus infection of children in Vietnam and Thailand. Pediatr Infect Dis J 23:793–794.

Henning J, Bett B, Okike I, Abdu P, Perry B. 2012. Incidence of highly pathogenic avian influenza H5N1 in Nigeria, 2005–2008. Transbound Emerg Dis. PMID: 22530694.

Joannis T, Lombin LH, De Benedictis P, Cattoli G, Capua I. 2006. Confirmation of H5N1 avian influenza in Africa. Vet Rec 158: 309–310.

Kayali G, Setterquist SF, Capuano AW, Myers KP, Gill JS, Gray GC. 2008. Testing human sera for antibodies against avian influenza viruses: Horse RBC hemagglutination inhibition vs. microneutralization assays. J Clin Virol 43:73–78.

Khuntirat BP, Yoon IK, Blair PJ, Krueger WS, Chittaganpitch M, Putnam SD, Supawat K, Gibbons RV, Pattamadilok S, Sawanpanyalert P, Heil GL, Friary JA, Capuano AW, Gray GC. 2011. Evidence for subclinical avian influenza virus infections among rural Thai villagers. Clin Infect Dis 53:e107–e116.

Kreijtz JH, Fouchier RA, Rimmelzwaan GF. 2011. Immune responses to influenza virus infection. Virus Res 162:19–30.

Metras R, Stevens KB, Abdu P, Okike I, Randolph T, Grace D, Pfeiffer DU, Costard S. 2012. Identification of potential risk factors associated with highly pathogenic avian influenza subtype H5N1 outbreak occurrence in Lagos and Kano States, Nigeria, during the 2006–2007 epidemics. Transbound Emerg Dis. PMID: 22469078.

Musa OI, Aderibigbe SA, Salaudeen GA, Oluwole FA, Samuel SO. 2010. Community awareness of bird flu and the practice of backyard poultry in a North-Central state of Nigeria. J Prev Med Hyg 51:146–151.

Myers KP, Olsen CW, Setterquist SF, Capuano AW, Donham KJ, Thacker EL, Merchant JA, Gray GC. 2006. Are swine workers in the United States at increased risk of infection with zoonotic influenza virus? Clin Infect Dis 42:14–20.

Myers KP, Setterquist SF, Capuano AW, Gray GC. 2007. Infection due to 3 avian influenza subtypes in United States veterinarians. Clin Infect Dis 45:4–9.

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. 2007. Lack of evidence of avian-to-human transmission of avian influenza A (H5N1) virus among poultry workers, Kano, Nigeria, 2006. J Infect Dis 196:1685–1691.

Paul AA, Assam A, Ndang TN. 2012. Local poultry biosecurity risks to highly pathogenic avian influenza in Kaduna State, Nigeria. Trop Anim Health Prod. PMID: 22869337.

Ramirez A, Capuano AW, Wellman DA, Lesher KA, Setterquist SF, Gray GC. 2006. Preventing zoonotic influenza virus infection. Emerg Infect Dis 12:996–1000.

Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, Fukuda K, Cox NJ, Katz JM. 1999. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 37:937–943.