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Background. In February 2006, poultry outbreaks of highly pathogenic avian influenza A (H5N1) virus were confirmed in Nigeria. A serosurvey was conducted to assess H5N1 transmission among poultry workers and laboratory workers in Nigeria.

Methods. From 21 March through 3 April 2006, 295 poultry workers and 25 laboratory workers with suspected exposure to H5N1 virus were administered a questionnaire to assess H5N1 exposures, medical history, and health care utilization. A serum specimen was collected from participants to test for H5N1 neutralizing antibodies by microneutralization assay.

Results. The 295 poultry workers reported a median of 14 days of exposure to suspected or confirmed H5N1-infected poultry without antiviral chemoprophylaxis and with minimal personal protective equipment. Among 25 laboratory workers, all handled poultry specimens with suspected H5N1 virus infection. All participants tested negative for H5N1 neutralizing antibodies.

Conclusions. Despite widespread exposure to poultry likely infected with H5N1 virus, no serological evidence of H5N1 virus infection was identified among participants. Continued surveillance for H5N1 cases in humans and further seroprevalence investigations are needed to assess the risk of avian-to-human transmission, given that H5N1 viruses continue to circulate and evolve among poultry.

Since December 2003, an unprecedented epizootic of highly pathogenic avian influenza A (H5N1) virus has affected poultry and wild birds in >50 countries on 3 continents [1]. More than 330 cases of human infections with H5N1 viruses have been reported in 12 countries [2], and direct contact with sick or diseased poultry has been implicated as the major risk factor for human infection [3–7]. Therefore, rapid detection of poultry outbreaks and prompt culling of infected birds are critical to limit human exposures to H5N1 viruses. However, culling of poultry infected with avian influenza A viruses may pose a further risk of human infection [4, 8, 9].

In Nigeria, anecdotal reports of poultry outbreaks with high mortality began in December 2005. H5N1 was first reported on 8 February 2006 among poultry from a commercial farm in Kaduna State, northern Nigeria [10]. This was the first confirmation of H5N1 virus in Africa. Nigeria is the most populous nation in Africa with ~131.9 million people [11], and it has an estimated 140 million poultry, of which nearly 60% is raised in backyard flocks [12]. Shortly after H5N1 was confirmed in Nigeria, agricultural authorities began culling birds at farms with suspected and confirmed H5N1 outbreaks. The earliest outbreaks occurred in north-central Nigeria.
[1], with widespread poultry mortality beginning in Kano State on approximately 20 January. The Nigeria Federal Ministry of Health (FMOH), the Kano State Ministry of Agriculture (KMOA), the Kano State Ministry of Health, and the Centers for Disease Control and Prevention (CDC) conducted a seroepidemiologic survey to assess the risk of H5N1 virus infection in humans who had extensive occupational exposure to poultry infected with H5N1 in Nigeria.

METHODS

Survey sites. The KMOA compiled a list of suspected or confirmed H5N1-affected poultry farms and markets. A suspected H5N1-affected poultry farm or market met the Nigeria Federal Ministry of Agriculture (FMOA) case definition, which required 3 or more of the following: poultry mortality >50% within 2 days; mortality among multiple bird species; mortality among wild birds in the neighborhood within the prior week; mortality among poultry vaccinated against Newcastle disease; poultry mortality in the same village within the prior week; and H5N1 confirmation among poultry in the same state [13]. A confirmed H5N1-affected poultry farm or market met the FMOA case definition and had at least 1 poultry sample that tested positive for H5 at the National Veterinary Research Institute (NVRI) in Plateau State, Nigeria.

The KMOA investigated 117 poultry farms and 4 poultry markets in Kano from 20 January through 31 March 2006 (figure 1). Of these, 115 farms and 4 markets met the Nigeria FMOA suspected H5N1 case definition, and specimens were collected from poultry at 9 of the farms. Specimens collected from 2 farms were unsuitable for laboratory testing, and specimens collected from 1 other farm were not tested. Specimens from poultry at the remaining 6 farms with suspected H5N1-infected poultry were tested for influenza A (H5) by reverse-transcription polymerase chain reaction (RT-PCR) at the NVRI, and all were positive. These results were confirmed by the World Organization for Animal Health/ Food and Agriculture Organization of the United Nations Avian Influenza Laboratory in Padova, Italy. One specimen was shipped to the CDC, where it also tested positive for H5N1 by real-time RT-PCR; the virus was subsequently isolated and sequenced and was confirmed to be a highly pathogenic clade 2 H5N1 virus, subclade 2 lineage (Catherine B. Smith, CDC, personal communication).

Survey recruitment sites included suspected and confirmed H5N1-affected poultry farms and markets in Kano State. Poultry veterinary clinics were also included as recruitment sites in Kano State. In addition, the NVRI was included as a recruitment site because poultry samples with confirmed H5N1 virus were processed, cultured, and tested there.

Enrollment. The survey was conducted from 28 to 31 March 2006 among poultry workers in Kano State and on 3 April 2006 among NVRI laboratory workers in Plateau State. A poultry worker was defined as any poultry-farm worker, poultry-market worker, poultry culler without other occupational farm or market exposure, or poultry veterinarian. A laboratory worker was defined as any employee of the NVRI.

Poultry-farm workers, poultry-market workers, and poultry veterinarians were eligible to participate in the survey if they worked at suspected or confirmed H5N1-affected poultry farms or markets for at least 3 days each week from 20 January 2006 until at least 3 weeks before participation in the survey. Past studies established that H5N1 virus–infected individuals mounted an H5N1 virus neutralizing antibody response by 3 weeks after the onset of symptoms [14].

Poultry cullers were eligible for inclusion in the survey if they had at least 4 h of direct contact with birds from suspected or confirmed H5N1-affected poultry farms or markets from 20 January 2006 until at least 3 weeks before participation in the survey. Short-term poultry cullers without other occupational
poultry contact were included because a prior seroepidemiologic survey in 1997 found H5N1 antibodies among groups with similar exposures [4].

Laboratory workers were eligible to participate if they had spent at least 4 h in rooms where H5N1 virus was cultured or if they had handled poultry specimens from suspected or confirmed H5N1 virus–infected poultry from 20 January 2006 until at least 3 weeks before participation in the survey.

Participation was limited to individuals 12–59 years of age. The minimum age was set at 12 years because of the large number of teenagers employed as poultry workers in Kano and because confirmed H5N1 cases have disproportionately affected children [15]. Persons >59 years were excluded because a prior seroepidemiologic survey found reduced specificity of neutralizing antibody testing for some avian influenza A virus subtypes in that age group [14].

Written informed consent was obtained from participants 18–59 years of age; written assent was obtained from participants 12–17 years of age along with informed consent from a parent or legal guardian. The FMOH and CDC determined that the seroepidemiologic survey constituted a public health response that did not require institutional review board authorization.

**Questionnaire.** A questionnaire was written in English and translated into Hausa, the local language of Kano State, and administered to all participants. Participants were asked about demographic information, poultry and laboratory exposures, symptoms, medical history, and health care utilization. Influenza-like illness (ILI) was defined as reporting subjective fever and either cough or sore throat.

**Blood collection.** A 5-mL blood specimen was collected from each participant via venipuncture. Blood specimens from poultry workers were placed on ice packs, and at the end of each day serum was separated and frozen at −20°C at a local laboratory in Kano. The samples were transported on ice to the Asokoro National Training Laboratory (Asokoro) in Abuja, thawed, split into 2 aliquots, and stored at −70°C. Laboratory worker blood specimens were transported on ice to Asokoro and processed similarly. One serum sample from each participant was shipped to the CDC on dry ice for H5N1 serologic testing, and 1 sample was stored by the FMOH at Asokoro.

**Laboratory testing.** Avian tissue (spleen, trachea, liver, and intestine) and cloacal samples from sick or dying birds taken from farms in Kano were homogenized, inoculated into 10-day-old embryonated hens’ eggs, and incubated at 37°C for 24 h at the NVRI. Allantoic fluid from the eggs were subsequently tested by RT-PCR for the presence of influenza A virus (H5) at the NVRI and CDC using established laboratory protocols and with H5-specific primers provided by the CDC [16].

Human serum samples were tested at the CDC for the presence of antibodies to H5N1 virus by both microneutralization (MN) assay and a modified horse red blood cell hemagglutination-inhibition (horse HI) assay. The procedures for the MN and horse HI assays for H5N1 have been described elsewhere [17, 18]. The MN assay used influenza A/chicken/Nigeria/246/06(H5N1) virus isolated from a chicken specimen collected in Kano State on 6 April 2006 and grown in embryonated eggs at the CDC at 37°C for 24 h. The horse HI assay used influenza A/chicken/Nigeria/42/2006(H5N1) virus isolated from a chicken in Kano State on 30 January 2006, which was grown at the CDC as indicated above and then inactivated by treatment with 0.05% β-propiolactone. To control for specimen quality, the MN assay was also performed for all serum with a virus derived from a recently circulating human influenza A/New York/55/2005(H3N2) virus. Per World Health Organization (WHO) guidelines, an individual was considered to be seropositive for H5N1 antibody if MN titers of ≥1:80 were detected with confirmation by the horse HI assay [19]. The cut-off value of 1:80 for the H5N1 MN assay is based on data obtained by testing serum from nonexposed individuals against clade 2 H5N1 viruses and our previous studies demonstrating that culture-confirmed H5N1 virus infection in humans correlated with titers of ≥1:80 [14].

**Statistical analysis.** An estimated sample size of 297 poultry workers was needed to determine with 95% confidence that the H5N1 antibody seroprevalence was ≤1.5% if all participants were seronegative. Double data entry was performed using Epi Info (version 3.3.2; CDC). Results for laboratory workers were analyzed separately from results for poultry workers. Bivariate analyses were performed using Epi Info (version 3.3.2). The χ² and Fisher’s exact tests were used to compare proportions, and Student’s t test was used to compare means; 2-sided P < .05 was considered to indicate statistical significance.

**RESULTS**

**Poultry worker demographics.** Of 295 poultry workers enrolled in the survey (table 1 and figure 1), 275 were male (93%), and the median age was 28 years (range, 12–58 years). Eight poultry workers (3%) were <18 years old. A total of 141 poultry workers (48%) had at least some secondary schooling, and 68% had average monthly household expenses ≤15,000 Naira (approximately US $115). Poultry workers included poultry-farm workers (76%), poultry-market workers (15%), poultry cullers without other farm or market exposure (5%), and veterinarians (4%).

**Poultry worker exposures.** Poultry worker participants were enrolled from 83 farms and 4 markets in Kano State (figure 1). All 295 poultry workers reported exposure to suspected H5N1-infected poultry, and 22 (7%) had been exposed to poultry from 1 of 4 farms with confirmed H5N1-infected poultry. Poultry workers reported a median of 14 days of exposure (range, 1–69 days) without antiviral chemoprophylaxis to suspected or confirmed H5N1-infected poultry at farms or markets with a median of 2050 birds (range, 8–47,000 birds).
All poultry workers reported occupational exposure to chickens. In addition, 115 (39%) reported occupational exposure to at least 1 other bird species. Occupational exposures included: touching live poultry (95%), cleaning poultry feces (66%), and butchering poultry (57%) (table 2). Of poultry workers, 14% said that they always wore masks when working with live poultry, 9% said that they always wore gloves when touching live poultry, and 85% said that they always washed their hands with soap after touching poultry. Conversely, 66% of respondents said that they never wore masks, 76% never wore gloves, and 11% never washed their hands with soap after touching poultry. One percent of poultry workers reported that they always wore gloves and masks and washed their hands after touching poultry, whereas 8% reported that they never wore gloves or masks or washed their hands after touching poultry. There were no statistically significant associations between monthly household expenses and mask use (P = .24), glove use (P = .11), or hand washing among poultry workers (P = .46).

Table 2. Home and occupational poultry exposures among poultry workers (n = 295).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Participants, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupational exposure</td>
<td></td>
</tr>
<tr>
<td>Touch live poultry</td>
<td>280 (95)</td>
</tr>
<tr>
<td>Feed poultry</td>
<td>241 (82)</td>
</tr>
<tr>
<td>Collect or sell eggs</td>
<td>205 (69)</td>
</tr>
<tr>
<td>Clean poultry stalls or cages</td>
<td>201 (68)</td>
</tr>
<tr>
<td>Clean poultry feces</td>
<td>195 (66)</td>
</tr>
<tr>
<td>Butcher any poultry</td>
<td>168 (57)</td>
</tr>
<tr>
<td>Collect or transport poultry feces for fertilizer</td>
<td>142 (48)</td>
</tr>
<tr>
<td>Clean poultry feathers</td>
<td>116 (39)</td>
</tr>
<tr>
<td>Transport poultry</td>
<td>84 (28)</td>
</tr>
<tr>
<td>Raise hatchlings</td>
<td>10 (3)</td>
</tr>
<tr>
<td>Home exposure</td>
<td></td>
</tr>
<tr>
<td>Has poultry in own house, compound, or yard</td>
<td>159 (54)</td>
</tr>
<tr>
<td>Poultry in the participant’s house, compound, or yard has been sick or died since 20 January 2006</td>
<td>124 (42)</td>
</tr>
<tr>
<td>Touch live or dead poultry in the home</td>
<td>240 (81)</td>
</tr>
</tbody>
</table>

Poultry worker symptoms and medical history. Fifteen poultry workers (5%) reported having experienced symptoms consistent with ILI from 20 January 2006 until the day the survey was administered (table 3). Five poultry workers (2%) reported ILI with difficulty breathing. No poultry workers reported having active ILI symptoms at the time of enrollment. Five poultry workers were hospitalized during the 2-month period prior to the survey, and 1 was diagnosed with pneumonia. Symptoms reported by poultry workers included rhinorrhea (31%), headache (20%), cough (14%), and fever (14%).

Poultry worker health care utilization. More than half of poultry workers (53%) reported that they would first seek care at a hospital for ILI, and 75% reported that they would seek care at a hospital if their symptoms worsened after 2 days. Fewer reported that they would first consult a chemist or pharmacist (26%) or that they would purchase over-the-counter medicines for treatment of ILI (11%). Only 1% reported that they would initially seek medical help for ILI from a traditional healer or...
religious leader. More than half of poultry workers (58%) reported that they would not always go to a hospital or clinic if ill because of the cost of treatment, and 152 poultry workers (52%) reported that if a family member was very sick and might die, they would not always take him or her to the hospital.

**Laboratory worker demographics.** Of 25 laboratory workers participating in the survey (table 1), 70% were male, and the median age was 42 years (range, 28–58 years). Seventy-two percent had matriculated at university/tertiary schools, and 78% had average monthly household expenses approximately US $115 (table 1).

**Laboratory worker exposures.** All laboratory workers had exposure to suspected H5N1 virus in culture or in poultry specimens. Of them, 48% had worked in a room in which H5N1 virus culture was performed. When handling suspected H5N1 virus in culture or in poultry specimens, 2% of laboratory workers reported that they always wore gloves, and 4% reported that they always wore masks. Ninety-six percent of laboratory workers always washed their hands after they handled suspected H5N1 virus in culture or in poultry specimens. Four percent of laboratory workers reported that they always wore gloves and masks and washed their hands after work, whereas 7% reported that they never wore gloves or masks or washed their hands after work.

**Laboratory worker symptoms and medical history.** Seven laboratory workers (28%) experienced ILI, and 3 (12%) experienced ILI and difficulty breathing (table 3). None of the laboratory workers were given a diagnosis of pneumonia or were hospitalized.

**Laboratory testing of all participants.** None of the 320 serum samples collected from poultry workers and laboratory workers tested positive for H5N1 antibody by either serologic assay (figure 2). In contrast, 97% of specimens had neutralizing antibody titers of $\geq 1:80$ against a recently circulating influenza A H3N2 virus. Among poultry workers, there was no statistically significant association between H5N1 MN titers $\geq 1:20$ and never washing hands after work ($P = .90$), never wearing gloves or masks at work ($P = .52$ and $P = .52$), or the top quartile of days with sick poultry exposure ($P = .52$). Furthermore, there was no statistically significant association between H3N2 MN titers $\geq 1:80$ and ILI ($P = .11$) or hospitalization ($P = .46$)

**DISCUSSION**

This is the first serological survey to assess the risk of H5N1 virus transmission to humans in Africa and the first to assess the risk of

![Figure 2](https://academic.oup.com/jid/article-abstract/196/11/1685/1993585)
transmission of clade 2 subclade 2 H5N1 viruses to humans. Clade 2, subclade 2 H5N1 viruses have caused human disease in Turkey, Iraq, and Egypt [2]. None of the 320 Nigerian poultry and laboratory workers had evidence of H5N1 virus infection, despite the minimal use of personal protective equipment (PPE) and lack of antiviral chemoprophylaxis during exposure.

Our finding of very low H5N1 seroprevalence is similar to results from a recent seroepidemiologic survey from Cambodia [21]. In that survey, the authors found no evidence of H5N1 neutralizing antibodies among 351 rural Cambodians exposed to clade 1 H5N1 virus–infected backyard poultry in 2004 [21]. The only other seroepidemiologic survey published to date that assessed the risk of avian-to-human transmission of currently circulating H5N1 viruses was performed in Guangdong, China, in 2006 [22]. Among 110 poultry-market workers enrolled in the study with probable exposure to clade 2, subclade 3 H5N1 virus, only 1 had evidence of H5N1 antibodies. The low risk of infection with H5N1 clade 1 and clade 2, subclade 2 viruses is in contrast to the findings of a study conducted in Hong Kong poultry workers exposed to H5N1 clade 0 viruses. In that study, H5N1 seroprevalence was 3% among poultry cullers who had substantial exposure to infected poultry over a weekend and was ~10% among poultry workers who had months of exposure to infected poultry [4]. Clade 0 H5N1 viruses are no longer circulating and are antigenically distinct from currently circulating H5N1 clade 1 and 2 viruses [23].

The present seroepidemiologic survey suggests that the risk of avian-to-human transmission of the most widespread H5N1 clade 2, subclade 2 virus was very low among our study population. Nevertheless, our findings do not imply the absence of human infection with H5N1 virus in West Africa. On 3 February 2007, the WHO confirmed the first case of human H5N1 virus infection in Nigeria [2]. Genetically similar viruses have caused confirmed H5N1 human disease in other countries [24]. In addition, distinct H5N1 virus lineages have been detected in other regions of Nigeria [25]. Differences between the 10% seroprevalence of H5N1 neutralizing antibodies in Hong Kong poultry workers during 1997 and our findings underscore the need for continued assessment of the risk of avian-to-human transmission as H5N1 viruses evolve and spread to regions in which human genetics, cultural practices, and the extent of exposure to infected poultry may differ.

The high frequency of detectable serum neutralizing antibody titers to human influenza A (H3N2) virus is strongly suggestive of past infection with antigenically related H3N2 viruses among survey participants. The epidemiology of human influenza has not been well described in most tropical regions, including West Africa [26]. Laboratory-based surveillance for human influenza in Nigeria and the region is vital to ascertain the impact of influenza relative to other respiratory diseases and to be able to better differentiate human influenza from human infection with H5N1 virus. Participants with ILI and shortness of breath met the WHO case definition for suspected H5N1 virus infection [27], yet none was referred for evaluation for H5N1 virus infection during a time of heightened awareness about avian influenza. Additionally, our findings that 25% of poultry workers would not go to a hospital for worsening ILI and >50% might not take a dying family member to a hospital indicates that severe respiratory disease could be missed by hospital-based H5N1 surveillance in Nigeria. Similar delay or absence of health care–seeking behavior for respiratory symptoms in Nigeria has been previously reported [28]. These data suggest that hospital-based H5N1 surveillance alone may be insensitive to detect all H5N1 cases that may be occurring.

The present survey’s findings are subject to several limitations. Confirmation of H5N1 virus infection of poultry at the enrollment sites was limited. Nevertheless, we believe that H5N1 virus exposure in participants was substantial before the serosurvey: every poultry worker was exposed to poultry at farms that met the FMOH suspected H5N1 case definition, and every suspected farm that had laboratory assessment had confirmed H5N1 virus in poultry specimens. Participants were not chosen randomly; they represented a convenience sample of market, farm, and laboratory employees who were available on the day of survey enrollment. However, as part of the concurrent outbreak investigation, efforts were made to discover and investigate any absent or ill employees. We may have undetected recent H5N1 virus infections among participants who were tested before they could mount a detectable H5N1 neutralizing antibody response. We addressed this limitation by enrolling participants who had exposure to poultry at suspected or confirmed H5N1–affected farms >3 weeks before participation in the survey. Although the 5 participants who had been hospitalized since 20 January 2006 were well by the time of survey participation, we do not know the dates of their symptoms or hospitalizations. We are therefore unable to determine whether adequate time had elapsed for a detectable antibody response to H5N1 virus infection in those individuals. Furthermore, without paired serum samples, we were unable to assess whether the respiratory symptoms experienced by poultry and laboratory workers were associated with recent H3N2 virus infection.

Since Nigeria reported H5N1 in poultry flocks, other African nations have confirmed H5N1 cases in poultry and humans [1, 2]. Sub-Saharan Africa faces many challenges with controlling and eradicating H5N1 from poultry and implementing surveillance for H5N1 cases in humans. As H5N1 outbreaks among poultry continue in the region, there is a continued risk for human H5N1 virus infections and for mutations or genetic reassortment leading to new virus strains with increased transmissibility to and among people. Improved surveillance for cases of H5N1 in humans and further H5N1 seroprevalence investigations are needed to ascertain the risk of avian-to-human transmission among different exposed populations over time. This is especially important in settings in which poultry workers may
use inadequate PPE and in which people infected with H5N1 virus may not seek medical care from hospitals and medical clinics. Moreover, education regarding the risk of virus transmission, access to PPE, and appropriate health care for poultry workers are essential and should be recommended as public health measures.

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