
Highly Pathogenic Avian Influenza A(H5N1) Virus Infection among Workers at Live Bird Markets, Bangladesh, 2009–2010

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The risk for influenza A(H5N1) virus infection is unclear among poultry workers in countries where the virus is endemic. To assess H5N1 seroprevalence and seroconversion among workers at live bird markets (LBMs) in Bangladesh, we followed a cohort of workers from 12 LBMs with existing avian influenza surveillance. Serum samples from workers were tested for H5N1 antibodies at the end of the study or when LBM samples first had H5N1 virus–positive test results. Of 404 workers, 9 (2%) were seropositive at baseline. Of 284 workers who completed the study and were seronegative at baseline, 6 (2%) seroconverted (7 cases/100 poultry worker–years). Workers who frequently fed poultry, cleaned feces from pens, cleaned food/water containers, and did not wash hands after touching sick poultry had a 7.6 times higher risk for infection compared with workers who infrequently performed these behaviors. Despite frequent exposure to H5N1 virus, LBM workers showed evidence of only sporadic infection.

Human infections and deaths caused by highly pathogenic avian influenza A (H5N1) viruses in several countries (1); by A(H9N2) virus in Bangladesh (2); and by A(H7N2), A(H7N9), A(H9N2), and A(H10N8) viruses in China (3–5) reflect the persistent public health threat posed by different avian influenza A virus subtypes. Subtype H5N1 virus remains endemic among poultry in Bangladesh, China, Egypt, Indonesia, and Vietnam (6). Among these countries the first human cases of H5N1 virus were

identified in China and Vietnam during 2003 (1). The seroprevalence of antibodies against H5N1 virus among poultry workers was 0%–4% in Bangladesh, China, Indonesia, and Vietnam during 2001–2009 (7–13); published data on seroprevalence among poultry workers in Egypt are not available. Beyond the countries where H5N1 is endemic, 0%–10% seroprevalence has been reported among poultry workers in Nigeria; South Korea; Thailand; and Hong Kong, China (14–17). The incidence of H5N1 virus infection among occupationally exposed populations has not been determined in countries where the virus is endemic or nonendemic.

In Bangladesh, a country with a population density of 964/km² and 257 million poultry (18,19), H5N1 virus infection was first detected among poultry in 2007. By the end of 2013, the country had reported 549 outbreaks among poultry to the World Organisation for Animal Health (20). The first human case of H5N1 virus infection in Bangladesh was identified during 2008 (21). Live bird markets (LBMs) are often associated with poultry-to-human transmission of H5N1 virus (22). For example, butchering and exposure to sick poultry were associated with detection of H5 antibody among LBM workers in Hong Kong (17). In one study, workers from 16 LBMs in Bangladesh were rarely observed using personal protective equipment (PPE) or washing their hands during the handling of poultry, suggesting a high likelihood of exposures to H5N1 virus (23). Data are limited on the risk for avian influenza A virus infections among poultry workers in Bangladesh (7).

Seroprevalence studies among humans yield information about how many persons have serologic evidence of infection at a certain point and time, but they do not provide information about when people became infected or the risk for infection with prolonged exposures to contaminated animals or environments. Studies designed to estimate the rate of seroconversion of antibodies to H5N1 virus among poultry workers may also help elucidate the

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risks of poultry-to-human transmission of H5N1 virus in countries, such as Bangladesh, where H5N1 virus is endemic among poultry. Such information may help public health officials develop, prioritize, and reinforce prevention and control strategies. During 2009–2010, a total of 61 H5N1 outbreaks, resulting in the culling of 220,432 birds, were reported among poultry in Bangladesh (24); no human cases were identified during this period. We followed a cohort of LBM workers in Bangladesh to determine the seroprevalence of antibodies to H5N1 virus, the incidence of seroconversion, and risk factors for poultry-to-human transmission of H5N1 virus.

Methods

Study Sites

We conducted this study among workers in 12 LBMs in 4 districts of Bangladesh: 8 in Dhaka, 2 in Chittagong, and 1 each in Netrokona and Rajshahi. We selected these LBMs because they served as sentinel sites for existing avian influenza surveillance throughout the study period; surveillance included the monthly collection of poultry and environmental samples (25,26). The samples were tested for influenza A and subtype H5 by using real-time reverse transcription PCR (27). By April 2009, H5N1 virus was detected from farms in 47 of 64 districts in Bangladesh, including the 4 districts where the LBMs in our study were located (20).

The LBMs in Dhaka, which were open daily from 6:00 AM to midnight, sold chickens, ducks, geese, and quail. The workers slaughtered, defeathered, eviscerated, and sold the poultry. LBMs outside Dhaka were in rural subdistricts and were open once or twice a week. Backyard poultry farmers and, occasionally, commercial poultry farmers sold poultry at these LBMs.

Poultry Worker Enrollment and Baseline Data Collection

We aimed to recruit ≈400 workers. All workers 18–59 years of age were eligible for enrollment. This age limit maximized the specificity of detection of H5N1 virus antibodies by microneutralization assay with confirmatory Western blot because the specificity of these assays is lower among older adults (28). The field team prepared a list of 721 eligible poultry workers present at the LBMs from 8:00 AM to 5:00 PM.

In 2009, we enrolled a convenience sample of consenting workers from rural subdistrict LBMs during May–June and from urban Dhaka LBMs during October–November, when poultry surveillance became operational (Figure). The poultry workers were enrolled as a closed cohort. The field team used a structured questionnaire (online Technical Appendix 1 Figure 1, <http://wwwnc.cdc.gov/EID/>

[article/21/4/14-1281-Techapp1.pdf](http://wwwnc.cdc.gov/EID/article/21/4/14-1281-Techapp1.pdf)) to collect demographic data and information about any history of chronic medical conditions; habits involving frequent hand-to-mouth contact (i.e., smoking, smokeless tobacco use, and betel leaf/nut use); the location of poultry handling; and practices that may have placed the workers at risk for H5N1 virus infection (i.e., not wearing PPE, eating while working with poultry, holding or carrying poultry, and eating raw or undercooked poultry or eggs). Medical technologists collected a 10-mL blood specimen from each study participant.

Follow-up Data Collection

During January–April 2010, which included the peak period of H5N1 virus circulation among poultry (26), we followed up with study participants one time. Follow-up occurred ≥21 days after virus was first detected through poultry surveillance (25) or 1 year after enrollment if H5N1 virus was not detected in an LBM where a study participant worked (Figure). At follow-up, the field team collected information about any history of influenza-like illness (i.e., subjective or measured fever and cough or sore throat) and shortness of breath or difficulty breathing within the 21 days before the follow-up visit and about exposure to sick poultry and precautions taken in the 3 days before respiratory symptom onset (if applicable) or 7 days before collection of the H5N1 virus–positive poultry or environmental surveillance sample (online Technical Appendix 1 Figure 2). In LBMs where H5N1 virus was not detected through poultry surveillance within 1 year after baseline data collection, the field team obtained follow-up data during June 2010, using a questionnaire similar to the one used at baseline. Medical technologists collected a 10-mL blood specimen from all participants during follow-up.

Data Collection from Nonpoultry Workers

In 2010, to get a sense of the baseline seroprevalence rate in a seemingly lower-risk population and to optimize the interpretation of the microneutralization assay results, we obtained samples from a group of nonpoultry workers. We enrolled a convenience sample of nonpoultry workers (18–59 years of age) from 3 accommodating nongovernmental organizations; these persons worked in Dhaka, did not own poultry, and had not participated in studies associated with influenza or other animals since the first detection of H5N1 virus among poultry in Bangladesh during 2007. During July and August 2010, using a structured questionnaire (online Technical Appendix 1 Figure 3), the field team collected demographic data and information about any history of chronic medical conditions; habits involving frequent hand-to-mouth contact (e.g., smoking, smokeless tobacco use, and betel leaf/nut use); and lifetime history of ever handling poultry. Medical technologists collected a 10-mL blood specimen from each nonpoultry worker.

Processing of Blood Specimens and Laboratory Analysis

All blood specimens were transported to the icddr,b laboratory in Dhaka on frozen cold packs at 2°C–8°C. Specimens collected outside Dhaka were centrifuged at the end of each day to separate serum and then transported. Specimens collected in Dhaka were transported to and centrifuged at icddr,b the same day. All serum samples were split into 3 aliquots and stored at icddr,b at –70°C. One aliquot was shipped on dry ice to the Influenza Division at the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) for H5N1 serologic testing.

We performed the microneutralization assay as previously described (28,29), using H5N1 clade 2.2 (A/Bangladesh/3233/2011) virus, the most common strain identified through surveillance in Bangladesh during the study period. Serial 2-fold dilutions of serum (1:10–1:1,280) were tested. Samples that tested positive by microneutralization assay were also tested by a confirmatory Western blot assay against influenza strain recombinant hemagglutinin A/bar-headed goose/Qinghai/1A/2005 (clade 2.2). Samples with positive assay results or that demonstrated evidence of seroconversion against H5N1 virus were also tested by microneutralization and hemagglutination inhibition assays

against pandemic influenza A(H1N1)pdm09 virus strain A/Mexico/4108/2009 (H1N1) to exclude potential serum antibody cross-reactivity. Serum samples that had high titers to A(H1N1)pdm09 virus were adsorbed with A(H1N1)pdm09 virus and then retested by microneutralization for reactivity to H5N1 virus. A seropositive result was defined as an H5N1 virus microneutralization titer ≥ 40 (equivalent to World Health Organization criteria ≥ 80) and confirmation by an H5-specific Western blot (28–30). Seroconversion against H5N1 virus was defined as detection of a ≥ 4 -fold rise in microneutralization antibody titer between the initial serum sample and a paired second serum sample, with the second sample achieving a titer ≥ 40 . Serum samples were tested ≥ 2 times by using the microneutralization assay. Microneutralization titers were expressed as the geometric mean of replicate titers.

Estimating Seroprevalence and Seroconversion

We calculated the proportion of poultry workers and non-poultry workers that were seropositive at baseline, the proportion of poultry workers that seroconverted against H5N1 virus, and 95% CIs of the proportions, assuming binomial distribution. We calculated the incidence of seroconversion against H5N1 virus among workers with

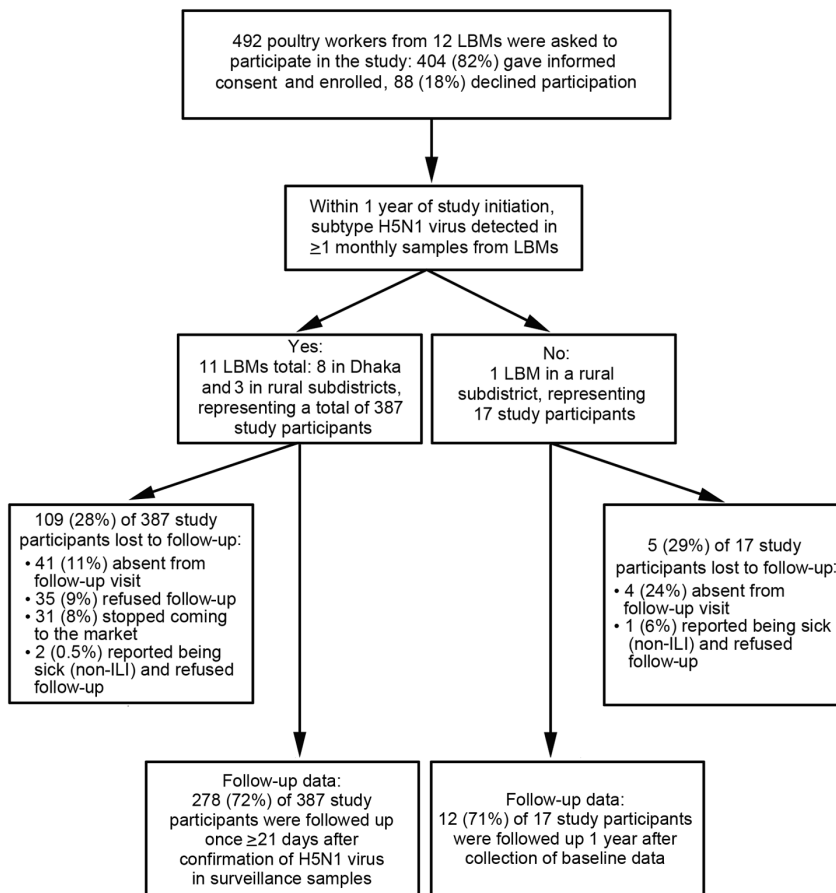


Figure. Enrollment and data for participants in a study of influenza A(H5N1) virus infection among workers at live bird markets (LBMs), Bangladesh, 2009–2010. ILI, influenza-like illness.

paired serum samples who were from LBMs where H5N1 virus was detected through poultry surveillance; workers who were seropositive at baseline were excluded. We calculated the incidence by dividing the number of seroconversions by the person-time each participant contributed to the study between baseline and follow-up data collection and calculated 95% CIs, assuming a Poisson distribution. To be conservative, we assumed that workers were at risk of acquiring H5N1 virus between baseline and follow-up serum collection even though the LBM may have been free of H5N1 virus during some of that period. We extrapolated our calculated incidence of seroconversion among the participating poultry workers to estimate the annual number of poultry workers infected with H5N1 virus among the 721 eligible workers. To compare characteristics between poultry workers and nonpoultry workers, exposure to poultry, and use of PPE between workers who were followed versus those who were lost to follow-up, we performed the 2-sample Wilcoxon rank-sum test and 2-sample test of proportions.

Statistical Analysis of Potential Risk Factors for H5N1 Virus Infection

We assessed risk factors for H5N1 virus infection (seropositivity or seroconversion) only among poultry workers with paired serum samples. Candidate risk factors were collinear, precluding the use of a regression model. Therefore, we performed the Kaiser-Meyer-Olkin test to assess the applicability of factor analysis for this dataset (31) and selected sets of common behaviors that explained >90% of variance among the candidate variables. Using the contribution of individual behavior (factor loading) as the basis, we grouped the behaviors into 3 sets and estimated the factor score for each set. Poultry workers with scores above median and those with scores below median were classified, respectively, as frequently and infrequently engaging in these sets of behaviors. We used a log-linear model, adjusted for clustering at the market level, to calculate risk ratio of serologic evidence of H5N1 virus infection for each set of behaviors between workers who were seropositive or seroconverted and those who were not seropositive and did not seroconvert against H5N1 virus (32). We applied

robust sandwich SE estimation strategy to account for the correlation (33).

Protection of Human Subjects

We obtained written informed consent from all participants before enrollment. Institutional review boards at icddr,b and CDC approved the study protocol.

Results

We enrolled 404 LBM poultry workers in the study: 332 from Dhaka and 72 from rural subdistricts. The percentage of refusals was 18% (71/403) in LBMs in Dhaka and 17% (15/89) in those outside Dhaka. Most refusals were due to an unwillingness to provide a serum sample. We collected data from 101 nonpoultry workers, all of whom were from Dhaka. Overall, compared with nonpoultry workers, poultry workers were younger (median age 28.0 years [interquartile range (IQR) 22.5–38.0 y] vs. 36.0 years [IQR 32–40 y]) and more likely to be male (100% vs. 78%) and to smoke (58% vs. 34%) ($p < 0.001$) (Table 1).

H5N1 Serologic Testing Results

Of 404 poultry workers, 9 (2%) were seropositive for H5N1 virus antibodies at baseline (95% CI 1%–4%). During November 2009–March 2010, routine icddr,b poultry surveillance identified H5N1 virus at 11 (92%) of the 12 LBMs and in 25 (93%) of 27 monthly samples. We obtained a second blood specimen from 278 (72%) of 387 participating poultry workers from the 11 LBMs (online Technical Appendix 2 Table 1, <http://wwwnc.cdc.gov/EID/article/21/4/14-1281-Techapp2.pdf>). Because of a delay in the availability of laboratory results for poultry and environmental surveillance samples, the median interval between detection of H5N1 virus at LBMs and collection of a second blood sample from poultry workers at the corresponding LBM was 56 days (IQR 49–61 days).

Of 9 seropositive poultry workers at baseline, 5 remained seropositive and 1 was seronegative for H5N1 virus antibodies at follow-up (online Technical Appendix 2 Figure); the remaining 3 workers were lost to follow-up. Six (2%) of 284 poultry workers seroconverted during the study period (95% CI 1%–5%) (Table 2). Six other workers

Table 1. Characteristics of live bird market workers and nonpoultry workers, Bangladesh, 2009–2010*

Characteristic	Poultry workers, n = 404	Nonpoultry workers, n = 101	p value
Male sex	404 (100)	79 (78)	<0.001†
Median age, y (IQR)	28 (22–38)	36 (32–40)	<0.001‡
Smoke tobacco	236 (58)	34 (34)	<0.001†
Median duration of smoking, y (IQR)	8 (4–16)	15 (9–20)	0.003‡
Use betel leaf or nut	151 (37)	22 (22)	0.003†
Use smokeless tobacco	15 (4)	1 (1)	0.2
Have chronic medical condition§	28 (7)	11 (11)	0.2

*Data are no. (%) persons except as indicated. IQR, interquartile range.

†Value for 2-sample test of proportion.

‡Value for 2-sample Wilcoxon rank-sum test.

§Conditions such as asthma; diabetes; chronic heart, lung, kidney, and liver disease; immune disorders; and cancer.

Table 2. Characteristics of live bird market workers with evidence of seroconversion against avian influenza A(H5N1) virus, Bangladesh, 2009–2010*

Characteristic	PW1	PW2	PW3	PW4	PW5	PW6
Personal characteristic						
Age, y	28	20	22	24	38	19
Smoke tobacco	+	–	+	–	–	+
Have chronic medical condition	–	–	–	–	–	–
Exposure to poultry or virus						
Handle sick poultry	+	+	+	+	+	+
Perform tasks with sick poultry						
Transport poultry	+	+	+	+	–	+
Feed poultry	–	+	+	+	–	+
Clean feeding tray	–	–	+	+	–	+
Clean water container	–	+	+	+	–	+
Medicate sick poultry	–	–	+	–	–	–
Separate sick poultry	–	+	+	+	+	+
Slaughter poultry	–	+	+	+	–	+
Defeather poultry	–	+	–	+	–	+
Eviscerate poultry	–	+	–	+	–	+
Stuff poultry into bags	+	–	–	+	+	+
Clean feces from pen	–	–	+	–	–	+
Hand-carry sick poultry or held poultry on lap	+	+	+	+	+	+
Carry baskets containing sick poultry on head	–	–	–	–	–	–
Eat raw/undercooked poultry or eggs	–	+	+	–	–	–
Precautions taken when handling sick poultry						
Use personal protective equipment†	–	–	–	–	–	–
Wash hands at the market after working with the poultry	+	+	–	+	–	–
Change clothes upon returning home	+	+	+	+	+	+
Presence of influenza-like illness in past 21 d						
Interval, d, between detection of virus–positive surveillance sample and follow-up collection of serum sample from worker	49	50	54	56	91	26
Neutralizing antibody titer, geometric mean‡						
Baseline	5	10	5	5	5	5
Follow-up	40	61	49	40	66	67

*PW, poultry worker; +, characteristic present; –, characteristic not present.

†Apron, gloves, dedicated coveralls, cloth mask, and boots.

‡By microneutralization assay, using influenza A/Bangladesh/3233/2011 (H5N1, clade 2.2) virus.

met the criteria for seropositivity in the follow-up serum samples, but they were not considered to have seroconverted because baseline titers were >10 and a >4-fold rise in titer was not achieved.

H5N1 virus was not detected by routine poultry surveillance in 1 subdistrict LBM during the study period. We collected follow-up data from 12 (71%) of 17 participating poultry workers at this LBM 1 year after baseline enrollment, and all 12 were seronegative for H5N1 virus at enrollment and follow-up. The overall seroprevalence of antibodies to H5N1 virus among poultry workers from all LBMs during the study period was 5% (20/404, 95% CI 3%–7%). In comparison, none of the 101 nonpoultry workers was seropositive (95% CI 0%–4%).

Incidence of Seroconversion

In LBMs where H5N1 virus was detected through routine poultry surveillance, we followed 278 poultry workers, of whom 266 were H5N1 virus–seronegative at baseline. These 266 workers contributed 30,043 days (≈82 years) of observation between the collection of paired blood samples, resulting in an incidence of 7 cases/100 poultry worker–years (95% CI 3–16). Using this incidence, we

estimate that the annual incidence of H5N1 virus infection after exposure to H5N1 virus at the study LBMs was 50 cases per 721 enlisted poultry workers.

Risk Factors for H5N1 Virus Infection

Seventeen (94%) of the 18 workers who were seropositive or seroconverted against H5N1 virus and 180 (66%) of the 272 seronegative workers reported exposure to poultry through >1 activity. None of the workers who were seropositive or who seroconverted reported exposure to poultry at home, at their farm, or at another place.

Three sets of behaviors explained 95% of the variability among risk behaviors at baseline and follow-up. However, the risk for H5N1 virus infection (risk ratio) was not equal for each set of behaviors (online Technical Appendix 2 Table 2). The set of behaviors with the highest risk ratio consisted of feeding poultry, cleaning feeding trays and water containers, not washing hands after working with sick poultry, and cleaning feces from pens; this set of behaviors was classified as high exposure. The set of behaviors with the second highest risk ratio consisted of slaughtering, defeathering, eviscerating, collecting or transporting feces, and stuffing poultry into bags; this set

Table 3. Risks for testing seropositive or seroconverting against avian influenza A(H5N1) virus among live bird market workers, Bangladesh 2009–2010*

Characteristic/behavior	Poultry workers		Regression model		
	Seronegative, n = 272	Seropositive or seroconverted, n = 18	Simple RR (95% CI)	Multiple RR (95% CI)	p value†
Median age, y (IQR)	27 (23–38)	27 (20–30)	0.9 (0.9–1.0)	0.9 (0.9–1.1)	0.8
Risk behavior					
High exposure			4.8 (0.8–28.2)	7.6 (2.8–20.9)	<0.001
Feed poultry	196 (72)	17 (94)			
Clean feeding tray	156 (57)	15 (83)			
Clean water container	155 (57)	16 (89)			
Clean feces from poultry pen	125 (46)	14 (78)			
Do not wash hands after handling sick poultry	133 (49)	10 (56)			
Medium exposure			3.5 (0.8–14.7)	5.1 (1.8–14.1)	0.002
Slaughter poultry	198 (73)	17 (94)			
Defeather poultry	142 (52)	15 (83)			
Eviscerate poultry	143 (53)	15 (83)			
Collect or transport poultry feces	53 (19)	1 (6)			
Stuff poultry into bags	113 (42)	14 (78)			
Low exposure			1.0 (0.3–3.3)	–	–
Smoke	159 (58)	7 (39)			
Medicate poultry	15 (6)	2 (11)			
Isolate sick poultry	130 (48)	10 (56)			
Eat raw/undercooked poultry or eggs	103 (38)	6 (33)			
Risk of infection from					
Medium-exposure behaviors when frequently performing both medium- and high-exposure behaviors‡	–	–	–	1.4 (0.3–6.2)	0.6
High-exposure behaviors when frequently performing both high- and medium-exposure behaviors‡	–	–	–	2.1 (0.4–12.9)	0.4

*Data are no. (%) except as indicated. IQR, interquartile range; RR, risk ratio; –, not applicable.

†Value for multivariate model.

‡The ratio of RR for interaction between medium- and high-exposure behaviors was 0.3 (1.4/5.1 for medium-exposure behaviors and 2.1/7.6 for high-exposure behaviors (95% CI 0.08–0.88; $p = 0.031$).

of behaviors was classified as medium exposure. The set of behaviors with the lowest risk ratio included smoking, medicating poultry, isolating sick poultry, and eating raw or undercooked poultry or eggs; this set of behaviors was classified as low exposure.

Poultry workers who frequently performed high-exposure behaviors had a 7.6 times higher risk for H5N1 virus infection compared with poultry workers who infrequently performed high-exposure behaviors when they also infrequently performed medium-exposure behaviors ($p < 0.001$) (Table 3). Poultry workers who frequently performed medium-exposure behaviors had a 5.1 times higher risk of H5N1 virus infection compared with poultry workers who infrequently performed medium-exposure behaviors when they also infrequently performed high-exposure behaviors ($p = 0.002$).

Discussion

Our study demonstrates that, despite frequent exposure to infected poultry and low PPE use, LBM workers in Bangladesh had low serologic evidence of H5N1 virus infection. These results also suggest that cross-sectional seroprevalence studies may underestimate the risk for H5N1 virus infection if conducted outside the peak time for H5N1 virus

circulation or if samples are obtained from infected workers long after exposure to the virus (i.e., when antibody titers have declined below the seropositive threshold).

Two percent of poultry workers were H5N1 virus–seropositive at baseline. This finding suggests that previous infection with H5N1 virus was uncommon despite the frequent exposure of workers to poultry. One of the workers who was seropositive at baseline became seronegative at follow-up, possibly because neutralizing antibodies decreased below the threshold for laboratory detection (34). The overall 5% seroprevalence of H5N1 virus antibody among poultry workers in our study is similar to the 4% seroprevalence among LBM workers in Vietnam in 2001 (13) but higher than the <1% seroprevalence reported among LBM workers from Bangladesh, Nigeria, Indonesia, and China during 2005–2009 (7,9,11,14). This finding suggests that human infection with H5N1 virus among heavily exposed workers at LBMs occurs infrequently but may be more common than previously reported. Routine poultry surveillance that included subdistrict LBMs in our study detected H5N1 virus from a higher proportion of poultry and environmental samples collected in 2011 than in 2009 and 2010 (3.8% vs. 0.4% and 0.5%, respectively) (26). Indeed, we would expect an increase in seroprevalence of

H5N1 virus antibodies or seroconversion rates among exposed poultry workers during periods with increased H5N1 virus activity among poultry (35). Nevertheless, it is unclear whether the current proportion represents a substantive opportunity for virus reassortment and the generation of a novel virus with pandemic potential.

We identified 2 sets of correlated behaviors that increased the risk of acquiring H5N1 virus infection among poultry workers. Frequently performing high-exposure behaviors was associated with 1.5 times higher risk of acquiring H5N1 virus infection compared with performing medium-exposure behaviors. Only butchering and exposure to ill poultry were associated with H5 seropositivity among LBM workers performing >1 poultry-related task in Hong Kong (17). The single seropositive LBM worker in China also reported slaughtering birds for 5 years (36). The use of PPE while performing high-exposure behaviors and frequent handwashing may reduce the risk for H5N1 virus infection (37). Nevertheless, because poultry workers handle poultry throughout the workday, it may be challenging for them to use PPE every time they have contact with poultry or their feces (38). Virus exposure and subsequent infection via mucous membranes and the respiratory tract may also be reduced among workers if they avoid touching their eyes, mouth, and nose while at work. Formative research would be helpful to explore if and how environmental controls (e.g., handwashing stands, improved ventilation flow, scalding pots); improved poultry handling techniques (e.g., slaughtering poultry inside plastic bags); and improved PPE (e.g., more accessible, cost-effective, and better tolerated equipment) could help decrease the risk for virus transmission at LBMs.

In Bangladesh, most identified cases of H5N1 virus infection in humans have been asymptomatic or mildly symptomatic (2,21). However, in 2013, the potential for severe and fatal illness from H5N1 virus infection in Bangladesh was highlighted by a fatal case in a child who had been exposed to infected backyard poultry (39). An increase in H5N1 virus infections among occupationally exposed poultry workers could signal the emergence of a virus with increased transmissibility among humans (40).

Our study has several limitations. First, almost 20% of the poultry workers declined to participate, and 28% of those enrolled at baseline were lost to follow-up. The refusals and losses to follow-up may have led to selection bias, resulting in an underestimation of seroprevalence and incidence of seroconversion if some of these workers were infected or in an overestimation if none of them were infected. Second, once H5N1 virus was detected in surveillance samples from an LBM, we conducted a final follow-up with workers at that LBM. Thus, we may have missed seroconversions that occurred after follow-up. Third, because the modified horse erythrocyte hemagglutination

assay is insensitive for the detection of antibody to A/Bangladesh/3233/2011 (H5N1, clade 2.2) virus, we could not use it for confirmation of seropositivity and seroconversion in this study. Fourth, poultry workers in Bangladesh were engaged in multiple activities, making it difficult to identify which specific behavior was the predominant risk factor for H5N1 virus infection. Last, we were unlikely to have accurately ascertained clinical illness associated with H5N1 virus infections because of the lag between collection of H5N1 virus-positive poultry and environmental surveillance samples and the collection of follow-up blood samples from workers.

In conclusion, our study suggests that a low but substantive proportion of LBM poultry workers in Bangladesh become infected with H5N1 virus after unprotected, ongoing sporadic exposures to H5N1 virus-infected poultry and virus-contaminated environments of LBMs. The risk behaviors identified in our study may help public health officials explore interventions to interrupt poultry-to-human transmission of H5N1 virus and other avian influenza A viruses among the poultry workers. The cost of any interventions needs to take into account the anticipated potential modest benefit of decreasing an infrequent event with uncertain pandemic potential.

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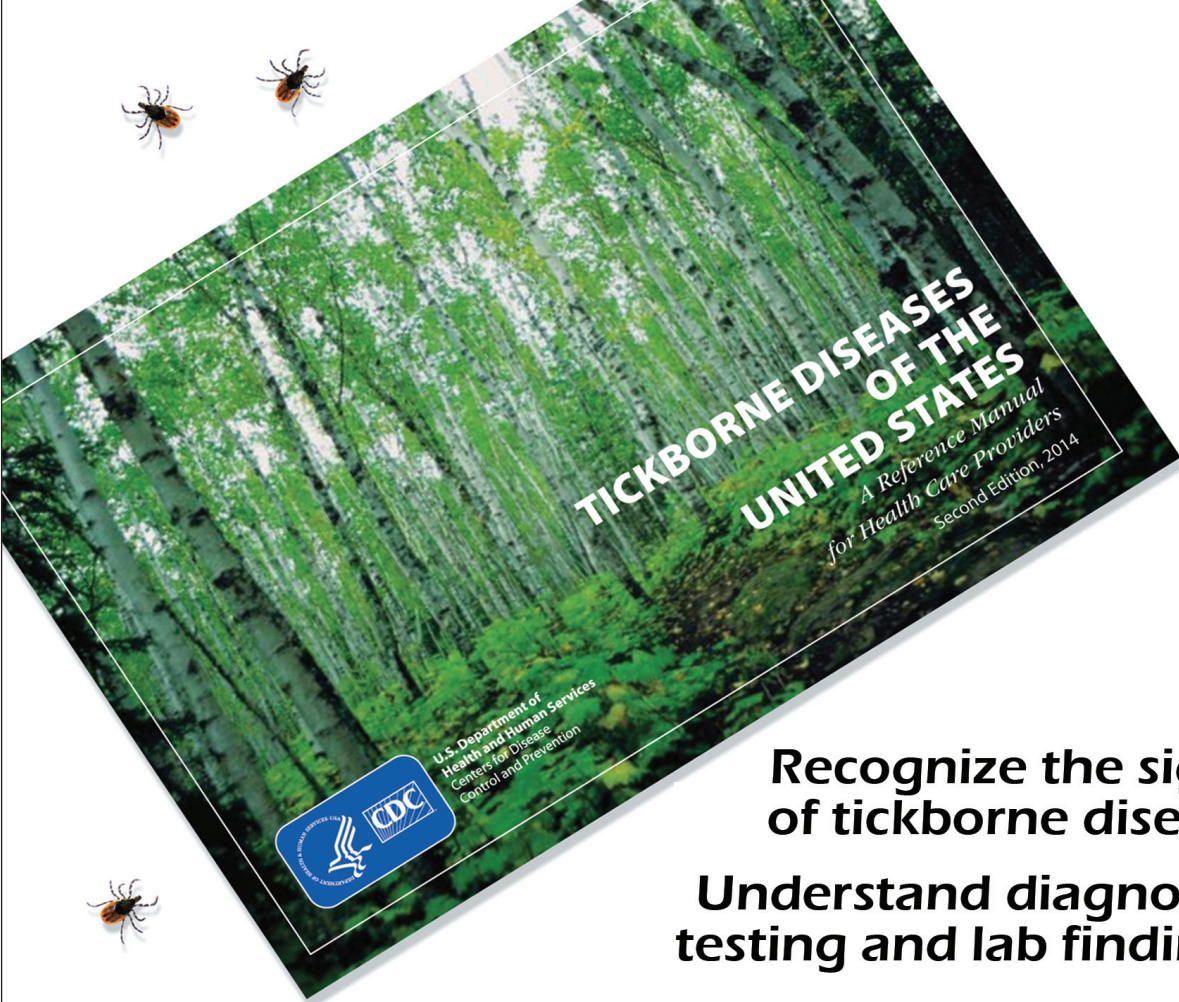
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Highly Pathogenic Avian Influenza A(H5N1) Virus Infection among Workers at Live Bird Markets, Bangladesh, 2009–2010

Technical Appendix 1

International Centre for Diarrhoeal Disease Research, Bangladesh

Sero-prevalence of antibodies to avian influenza A viruses among poultry market workers

ID #						
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1. Name of Interviewer: _____
2. Date: ____ / ____ / ____ (dd /mm /yy)
3. Location: Netrokona Chittagong Rajshahi Dhaka
4. Market ID _____
5. Market worker available (For *follow-up sample collections*)?
 Yes No
6. Initial visit? Yes No (if no, skip to 9)
7. Consent to participate? Yes (if yes, skip to 9) No
8. If refused consent provide reason: _____ (Stop and thank interviewee)

Generic risk factors:

9. How old are you: ____ (years)
10. Sex: Female Male (if male, skip to 11)
 - a. *If female*, Are you pregnant, that you are aware? Yes No
11. What is your ethnicity: _____
12. Height: ____ (meters) (*Use tape measure*)
13. Weight: ____ (Kg) (*Use bathroom scale*)
14. Do you smoke? Yes No (if no, skip to 17)
15. How many sticks a day do you smoke _____
16. How many years have you smoked? _____
17. Do you use: (*read and select all that apply*)
 Betel leaf or betel nuts gul (remains of tobacco-cake mixed with molasses)
 khoini (hand-made tobacco dust) None of the above
18. Has a doctor ever told you that you have any of the following conditions?:
 Asthma Diabetes Chronic heart disease Chronic lung disease

- Chronic kidney disease Chronic liver disease Immune problems
 Cancer None of the above

Environmental risk factors:

19. Is there any hand washing station in the market (interviewer to observe and record)?
 Yes No
20. Do you have running water in the market? Yes No
(skip to Q22)
21. Approximately how far is your water source in the market? ____ (Meters Feet)(should be blank for the skipped ones)
22. Did you wash your hands with soap and water while in the market yesterday? Yes
 No
23. Daily, do you use ash or mud to wash your hands? Yes No
24. If you washed your hands yesterday, when did you do so: (read all the key times)
- | | |
|--|---|
| Before meals? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| After returning home? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| After defecating? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| Before touching your eyes, nose, or mouth? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
25. Daily, how often do wash your hands with ash or mud: (read all the key times)
- | | |
|--|---|
| Before meals? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| After returning home? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| After defecating? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| Before touching your eyes, nose, or mouth? | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |

Poultry worker risk factors:

26. Do you handle poultry? Yes No (if no stop and thank interviewee)
27. Where do you handle poultry (check all that apply)?
 Home (Market Farm Other _____
28. What kind of tasks do you do when you handle poultry? (read and select all that apply)
- | | |
|--|--|
| Transport poultry | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Feed poultry | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Clean feeding tray | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Clean water container | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Slaughter poultry | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Defeather poultry | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Eviscerate poultry | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Collect or transport feces | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
| Cleaning feces from where poultry are kept | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Never |
29. Do you use any personal protective equipment when handling poultry?
- | | |
|------------------|---|
| Protective apron | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| Gloves | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |

Dedicated coveralls Always Often Seldom Never
Mask Always Often Seldom Never
Boots Always Often Seldom Never

30. Do you eat lunch or drink tea during or after working with poultry? Always Often Seldom Never
31. Do you smoke while working with poultry? Always Often Seldom Never
32. Do you carry hand poultry or hold poultry on your lap? Always Often Seldom Never
33. Do you carry baskets containing poultry on your head? Always Often Seldom Never
34. Do you change your clothes upon returning home after working with poultry? Yes No
35. Do you eat raw or undercooked poultry or eggs? Always Often Seldom Never

Thank you for your cooperation and participation in the survey

Technical Appendix 1 Figure 1. Questionnaire administered to poultry workers at live bird markets, Bangladesh, 2009–2010. The questionnaire was administered to all workers at baseline, and 12 months after baseline to workers at the market where avian influenza A(H5N1) virus was not detected through poultry surveillance during the study.

International Centre for Diarrhoeal Disease Research, Bangladesh

Sero-prevalence of antibodies to avian influenza A viruses among poultry market workers

Market worker questionnaire 21 days after animal surveillance recovers influenza

ID #	1					
------	---	--	--	--	--	--

1. Name of Interviewer:
2. Date: ____ ____ ____ (dd mm yy)
3. Location: Netrokona Chittagong Rajshahi Dhaka
4. Market ID _____
5. Market worker available? Yes No

Influenza like illness:

6. Have you been sick in the past 21 days? Yes No (if no, skip to 22)
7. When did you first feel sick? Date ____ ____ ____ (dd mm yy)
8. Did you develop a sudden fever? Yes No
9. Did someone take your temperature? Yes No (if no, skip to 11)
10. What was your highest temperature? ____ F⁰
11. Did you develop:
 - a. Cough? Yes No
 - b. Sore throat? Yes No
 - c. Shortness of breath or difficulty breathing? Yes No
12. Did you seek medical attention? Yes No (if no, skip to 19)
13. Where did you seek medical attention? Local clinic Local hospital Other _____
14. What were you diagnosed with?
 - d. Cold Pharyngitis Bronchitis Pneumonia Dengue Other _____
15. Were you told you needed hospitalization? Yes No
16. Have you taken oseltamavir (show case-patient sample blister pack) for this illness as twice a day for 5 days (or up to the time of the interview)? Yes No
17. Did a doctor obtain a clinical sample?
 - e. From nose or throat Yes No
 - f. Blood Yes No } (if no, skip to 19)
18. Where was this sample obtained?: _____
19. In the 3 days before symptom onset, had anyone **at home** had similar symptoms? Yes No
 - g. If yes, who _____

20. In the 3 days before symptom onset, did you know of anyone with similar symptoms?
 Yes No (if no Skip to 22)
21. In the 3 days before symptom onset, had you been close (< 3 feet/ 2 hands) to anyone you know with similar symptoms outside the home? Yes No (if no Skip to 22)
- h. If yes, where (check all that apply):
 Market School Mosque/church/temple Street Other home
 Other _____

Potential risk factors for present illness

22. In the 3 days before symptom onset/7 days before collecting the animal sample (mention the date), had you been around sick poultry? Yes No
23. Did you handle the sick poultry? Yes No (if no skip to 34)
24. Where did you handle sick poultry (check all that apply)?
 Home (H) Market (M) Farm (F) Other _____
25. What kind of tasks did you do when you handle the sick poultry and where (check all that apply and add location code [i.e. H,M,F])?
- | | | | |
|--|------------------------------|-----------------------------|-----------------|
| Transport poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Feed poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Clean feeding tray | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Clean water container | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Give medicine to the sick poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Separate sick poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Slaughter poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Defeather poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Eviscerate poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Collect or transport feces | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Cull poultry | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Stuff poultry in bags | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Bury poultry carcasses | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Burn poultry products | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |
| Cleaning feces from place where poultry are kept | <input type="checkbox"/> Yes | <input type="checkbox"/> No | Location: _____ |

26. Did you take precautions when handling ill poultry (check all that apply)?
- | | |
|---------------------|---|
| Protective apron | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| Gloves | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| Dedicated coveralls | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| Mask | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |
| Boots | <input type="checkbox"/> Always <input type="checkbox"/> Often <input type="checkbox"/> Seldom <input type="checkbox"/> Never |

27. Did you eat during or after working with ill poultry? Yes No
28. Did you smoke while working with ill poultry? Yes No

29. Did you use: *(read and select all that apply)*

- Betel leaf or betel nuts gul (remains of tobacco-cake mixed with molasses)
 khoini (hand-made tobacco dust) None of the above

30. Did you hand carry sick poultry or hold poultry on your lap? Yes No

31. Did you carry baskets containing sick poultry on your head? Yes No

32. Did you wash your hands at the market after working with ill poultry? Yes No

33. Did you change your clothes upon returning home after working with ill poultry? Yes No

34. Did you eat raw or undercooked poultry or eggs? Yes No

Thank you

Technical Appendix 1 Figure 2. Questionnaire administered to poultry workers at follow-up during a study of avian influenza A(H5N1) virus infection among workers at live bird markets, Bangladesh, 2009–2010.

International Centre for Diarrhoeal Disease Research, Bangladesh

Sero-prevalence of antibodies to avian influenza A viruses among poultry market workers

Non-poultry workers questionnaire

ID #						
------	--	--	--	--	--	--

1. Name of Interviewer:
2. Date: ___/ ___/ ___ (dd/mm/yy)
3. Name of the Organization of control:
4. Consent to participate? Yes (if yes, skip to 7) No
5. If refused consent provide reason: _____ (Stop and thank interviewee)

Generic risk factors:

6. How old are you: ___ ___ (years)
7. Have you owned or handled poultry during the past 2 years? Yes (if yes thank and stop) No
8. Have you worked in influenza field studies during past 2 years? Yes (if yes thank and stop) No
9. Sex: Female Male (if male, skip to 11)
 - a. If female, Are you pregnant, that you are aware? Yes No
10. What is your ethnicity: _____
11. Height: _____ (meters) (Use tape measure)
12. Weight: _____ (Kg) (Use bathroom scale)
13. Do you smoke? Yes No (if no, skip to 18)
14. How many sticks a day do you smoke _____
15. How many years have you smoked? _____
16. Do you use: (read and select all that apply)
 - Betel leaf or betel nuts Gul (remains of tobacco-cake mixed with molasses)
 - Khoini (hand-made tobacco dust) None of the above
17. Do you have any of the following conditions?:
 - Asthma Diabetes Chronic heart disease Chronic lung disease

- Chronic kidney disease Chronic liver disease Immune problems
 Cancer None of the above

Environmental risk factors:

18. Daily, do you use soap to wash your hands? Yes No
19. Daily, do you use ash or mud to wash your hands? Yes No
20. Daily, how often do wash your hands: (read all the key times)
- | | | | | |
|--|---------------------------------|--------------------------------|---------------------------------|--------------------------------|
| Before meals? | <input type="checkbox"/> Always | <input type="checkbox"/> Often | <input type="checkbox"/> Seldom | <input type="checkbox"/> Never |
| After returning home? | <input type="checkbox"/> Always | <input type="checkbox"/> Often | <input type="checkbox"/> Seldom | <input type="checkbox"/> Never |
| After defecating? | <input type="checkbox"/> Always | <input type="checkbox"/> Often | <input type="checkbox"/> Seldom | <input type="checkbox"/> Never |
| Before touching your eyes, nose, or mouth? | <input type="checkbox"/> Always | <input type="checkbox"/> Often | <input type="checkbox"/> Seldom | <input type="checkbox"/> Never |

Poultry risk factors:

21. Have you ever handled poultry? Yes No (*Stop and thank interviewee*)
22. Where have you handled poultry (*check all that apply*)?
- Home Market Farm Other _____

Thank you

Technical Appendix 1 Figure 3. Questionnaire administered to nonpoultry workers during a study of avian influenza A(H5N1) virus infection among workers at live bird markets, Bangladesh, 2009–2010.

Highly Pathogenic Avian Influenza A(H5N1) Virus Infection among Workers at Live Bird Markets, Bangladesh, 2009–2010

Technical Appendix 2

Technical Appendix 2, Table 1. Baseline characteristics among poultry workers, by follow-up status, in a study of avian influenza A(H5N1) virus infection among workers at live bird markets, Bangladesh, 2009–2010*

Baseline characteristic	No. (%) poultry workers		p value
	Followed up, n = 290	Lost to follow-up, n = 114	
Median age, y (IQR)	27 (22–38)	28 (23–35)	0.9†
Smoker	166 (57)	70 (61)	0.4‡
Have chronic medical condition§	18 (6)	10 (9)	0.4‡
Exposure to poultry			
Transport poultry	204 (70)	86 (75)	0.3‡
Feed poultry	226 (78)	72 (63)	0.002‡
Clean feeding tray	177 (61)	66 (58)	0.6‡
Clean water container	168 (58)	63 (55)	0.6‡
Slaughter poultry	229 (79)	75 (66)	0.006‡
Defeather poultry	180 (62)	66 (58)	0.4‡
Eviscerate poultry	178 (61)	65 (57)	0.4‡
Collect or transport feces	61 (21)	21 (18)	0.6‡
Clean feces from pens	137 (47)	51 (45)	0.7‡
Hand-carry poultry or hold poultry on lap during travel	265 (91)	111 (97)	0.03‡
Carry baskets containing poultry on head	4 (1)	8 (7)	0.003‡
Eat raw or undercooked poultry or eggs	84 (29)	47 (41)	0.02‡
Use of personal protective equipment¶			
Protective apron	0 (0)	0 (0)	–
Gloves	0 (0)	0 (0)	–
Dedicated overalls	0 (0)	0 (0)	–
Cloth mask	5 (2)	1 (1)	0.5
Boots	1 (0.37)	0 (0)	0.5

*IQR, interquartile range; –, not applicable.

†p value for 2-sample Wilcoxon rank-sum test.

‡p value for 2-sample test of proportion.

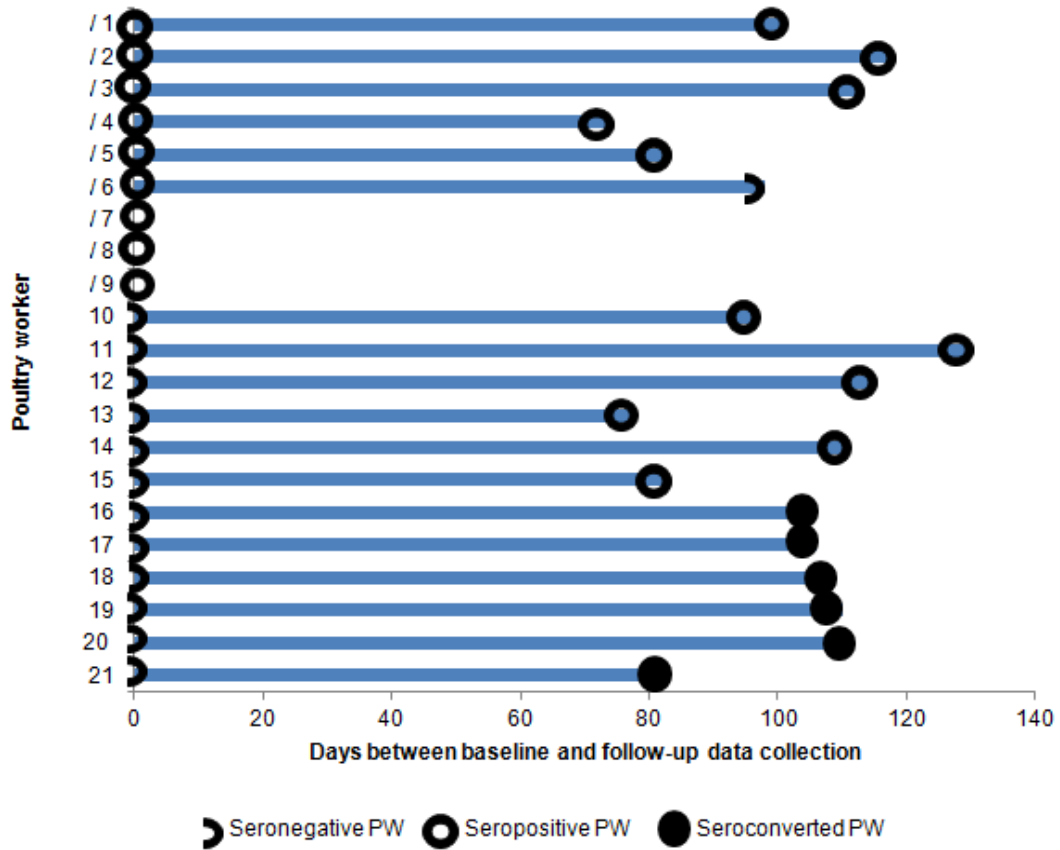
§Conditions such as asthma; diabetes; chronic heart, lung, kidney, liver, and kidney disease; immune disorder; and cancer.

¶During reported handling of poultry at baseline or handling of sick poultry at follow-up for the followed up workers; and handling of poultry at baseline for the lost to follow-up workers.

Technical Appendix 2, Table 2. Contribution of individual behaviors of poultry workers for each set of exposure behaviors (factor loadings) associated with avian influenza A(H5N1) virus infection among live bird market workers, Bangladesh 2009–2010*

Exposure behaviors	Behavior		
	Medium exposure	High exposure	Low exposure
Smoked	–0.0097	–0.0018	0.1193
Fed poultry	0.3628	0.5927	0.0646
Cleaned feeding tray	0.3670	0.8982	0.0150
Cleaned water container	0.3659	0.8958	0.0628
Medicated poultry	0.0693	0.1218	0.2189
Isolated sick poultry	0.1925	0.2104	0.4459
Slaughtered poultry	0.5669	0.3162	0.1107
Defeathered poultry	0.9270	0.3308	0.0413
Eviscerated poultry	0.9179	0.3550	0.0380
Collected or transported feces	0.2626	0.2538	0.1016
Stuffed poultry into bags	0.6055	0.2928	0.0581
Cleaned feces from pens	0.4939	0.4968	–0.0036
Did not wash hands after handling sick poultry	–0.1326	–0.0432	–0.4368
Ate raw or undercooked poultry or eggs	0.0280	0.0766	0.1892

*H5N1 virus infection denotes being seropositive at baseline/follow-up and having evidence of seroconversion to H5N1 virus antibodies at follow-up. Gray shading shows that exposure behaviors with the highest factor loading were grouped together into each set of exposure behaviors that were later classified into high-, medium- and low-exposure on the basis of calculated risk ratios.



Technical Appendix 2, Figure. Timeline for the collection of serum samples for 21 poultry workers (PWs) who were positive for influenza A(H5N1) virus at baseline or who seroconverted at follow-up. The samples were obtained for A(H5N1) virus serologic testing.