The Journal of Infectious Diseases

MAJOR ARTICLE

Serological Evidence of Human Infection With Avian Influenza A(H7N9) Virus: A Systematic Review and Meta-analysis

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Background. The extent of human infections with avian influenza A(H7N9) virus, including mild and asymptomatic infections, is uncertain.

Methods. We performed a systematic review and meta-analysis of serosurveys for avian influenza A(H7N9) virus infections in humans published during 2013–2020. Three seropositive definitions were assessed to estimate pooled seroprevalence, seroconversion rate, and seroincidence by types of exposures. We applied a scoring system to assess the quality of included studies.

Results. Of 31 included studies, pooled seroprevalence of A(H7N9) virus antibodies from all participants was 0.02%, with poultry workers, close contacts, and general populations having seroprevalence of 0.1%, 0.2%, and 0.02%, respectively, based on the World Health Organization (WHO)—recommended definition. Although most infections were asymptomatic, evidence of infection was highest in poultry workers (5% seroconversion, 19.1% seroincidence per 100 person-years). Use of different virus clades did not significantly affect seroprevalence estimates. Most serological studies were of low to moderate quality and did not follow standardized seroepidemiological protocols or WHO-recommended laboratory methods.

Conclusions. Human infections with avian influenza A(H7N9) virus have been uncommon, especially for general populations. Workers with occupational exposures to poultry and close contacts of A(H7N9) human cases had low risks of infection.

Keywords. influenza in humans; influenza A (H7N9); serological evidence.

Since the first human infections with avian influenza A(H7N9) virus were identified in March 2013 [1], 5 epidemic waves of human infections with A(H7N9) virus have been reported in mainland China [2]. In contrast to previous epidemic waves with human infections identified mostly in eastern China, the fifth wave during 2016–2017 began earlier, and led to the highest number of confirmed cases [3]. Human infections with A(H7N9) virus have declined since 2017. As of 30 April 2020, a total of 1568 laboratory-confirmed cases and 616 deaths had been reported to the World Health Organization (WHO) with a case fatality risk of 39% among laboratory-confirmed infections [4].

Laboratory-confirmed cases of influenza A(H7N9) virus infection have been identified mostly in patients with severe illness, especially in those older than 60 years [5]. However, clinically mild illnesses with A(H7N9) virus infection have also been identified through sentinel influenza-like illness surveillance, mostly in young adults, suggesting the existence of many mild cases that are likely underdetected [6, 7]. Seroepidemiological studies are useful to explore the full disease spectrum of infections in nondeceased persons, to allow estimation of the prevalence of clinically mild or asymptomatic cases, and to better inform severity assessments.

It is difficult to understand the public health risk of A(H7N9) virus infection from serological studies because of variations in the study designs and serological assays used. Although the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) developed protocols to standardize seroepidemiological investigations [8], serological methods and the interpretation of serosurveys continue to vary between studies. Additionally, differences in study periods, study participants, and exposure levels of susceptible populations may also contribute to the heterogeneity

Received 5 September 2020; editorial decision 20 October 2020; accepted 23 October 2020; published online October 29, 2020.

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The Journal of Infectious Diseases® 2022;226:70–82

DOI: 10.1093/infdis/jiaa679

between serological studies, which leads to challenges in interpreting the findings. A previously published meta-analysis estimated A(H7N9) virus antibody seroprevalence by different populations, periods, regions, and laboratory methods [9]. However, their analyses did not consider the impact of virus clade-specific antibodies, antigenic similarity between virus strains used in serologic assays and the virus strains circulating among poultry or infected humans, or the prevalence of symptomatic and asymptomatic infections, upon seroprevalence estimates.

This study aimed to perform a systematic and comprehensive assessment of the risk of asymptomatic and clinically mild A(H7N9) virus infections in humans by summarizing serological data in published English-language studies. In addition, we compared the prevalence of A(H7N9) virus–specific antibodies among populations with different levels of exposure to A(H7N9) virus.

METHODS

Search Strategy and Selection Criteria

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (http://www.prisma-statement.org/) [10], we implemented a comprehensive literature review of English-language papers from 1 January 2013 through 30 June 2020 from 3 databases (PubMed, Embase, and Web of Science), using predefined search terms (Supplementary Table 1). Among all potential eligible studies, we excluded studies if they only presented serological evidence for A(H7N9) virus infections in animals or only reported virologically confirmed clinical cases without serologic data. Abstracts of congress meetings or conference proceedings, study protocols, commentaries, reviews, or case reports were also excluded. Initial screening of the titles and abstracts of retrieved articles was done by 2 independent researchers; for potential included abstracts, the full text was scrutinized to assess inclusion and exclusion criteria. A third researcher was consulted when the 2 reviewers disagreed on study inclusion. We modified the scoring system provided by Sikkema et al [11] to assess study quality based on study design and laboratory methods of each eligible study. Studies that utilized an unexposed control group, collected and tested paired sera for participants, and reported influenza vaccination status for study participants were scored higher. Studies that utilized serological assays with laboratory methods to improve specificity of antibody detection to minimize cross-reactivity and validated confirmatory assays were also assigned higher scores. Based on their overall score, each study's quality was classified into 1 of 4 categories: A, B, C, or D. Category A spanned studies with a scores ranging from 15 to 18, category B from 10 to 14, category C from 5 to 9, and category D from 0 to 4. We also described the characteristics, laboratory testing method, and primary outcome for each available study in Supplementary Tables 2–5). The review protocol of this study is available in the International Prospective Register of Systematic Reviews (PROSPERO; identifier CRD42020147759).

Statistical Analysis

From eligible studies, we extracted data for 3 predefined A(H7N9) virus antibody outcomes in humans: (1) seroprevalence; (2) seroconversion; and (3) seroincidence. Seroprevalence was defined as the prevalence of A(H7N9) virus–specific antibodies at or above a designated antibody titer to define a seropositive result in cross-sectional studies. Seroconversion was defined as achieving at least a 4-fold increase in A(H7N9) virus–specific antibody titers detected by hemagglutination inhibition (HAI) assay or microneutralization assay (MN) assay in serum collected at multiple time points. Seroincidence was defined as the number of individuals with serologic evidence of A(H7N9) virus infection divided by total person-time during follow-up visits. For the estimation of seroprevalence, only baseline data were analyzed when there were multiyear follow-up studies or serial cross-sectional studies in order to avoid repeated inclusion of the same study.

Although the WHO has established laboratory procedures for serological confirmation of A(H7N9) human cases with acute febrile illness and respiratory symptom [12, 13], these relatively strict criteria were not suitable for detection of seropositive individuals among non-ill persons in seroepidemiological studies. Therefore, random-effects models were performed using 3 seropositive definitions: the WHO-recommended, modified WHO-recommended, and nonstandardized definitions. The WHO-recommended seropositive definition refers to an HAI titer ≥160 tested by horse erythrocytes or an HAI titer of 20–80 tested by horse erythrocytes with a positive result using a second confirmatory assay (ie, MN [neutralizing antibody titer ≥80] or Western blot assay [WB]) [12, 13].The modified WHO-recommended seropositive definition refers to an HAI titer ≥160 using erythrocytes from other species (eg, chickens, turkeys, and guinea pigs); or an HAI titer of 20–80 using other species' erythrocytes and a positive result by a second confirmatory assay (ie, MN [neutralizing antibody titer ≥80] or WB). The nonstandardized seropositive definition refers to criteria other than the WHO-recommended or modified WHO-recommended criteria used in individual studies to define a seropositive result. All participants involved in this systematic review were reclassified into 3 groups (ie, participants who met WHO-recommended, modified WHO-recommended, or nonstandardized seropositive definition) according to the results of each serological study.

To assess differences in the types and frequency of exposures among populations with potential risk of infection, study populations were categorized into 4 groups: poultry workers (only exposed to poultry), close contacts (exposed to confirmed A[H7N9] cases), mixed exposures (exposed to poultry and confirmed A[H7N9] cases), and

general population (without known exposures to A[H7N9] virus). Virus clade-specific seroprevalence during the study period was also evaluated in this study, based upon 3 distinct A(H7N9) virus clades (ie, W1, W2-1, and W3-2) derived from Wang et al [14]. Clade W1 represents A(H7N9) viruses from the Yangtze River Delta (Anhui, Jiangsu, Shanghai, and Zhejiang provinces) during the first A(H7N9) epidemic wave from February to September in 2013. Clade W2-1 includes primarily A(H7N9) viruses isolated in the Pearl River Delta region, mainly from Guangdong Province and Hong Kong during the second epidemic wave during 2013–2014. Clade W3-3 contains A(H7N9) viruses isolated from a broader area, including viruses from both Yangtze River Delta and other provinces in northwestern China [14]. We also estimated predefined outcomes according to whether an A(H7N9) epidemic occurred during the study period. An epidemic was defined as human infection with A(H7N9) virus or detection of A(H7N9) virus in poultry in the study location during the study period, or both.

Six cross-sectional studies that tested blood samples collected before 2013 and 1 cohort study with baseline serum collected in January 2013 were identified. Because there was no known A(H7N9) virus circulation among poultry before 2013 and the first laboratory-confirmed human case of A(H7N9) virus infection was not identified until February 2013 [1], we estimated seroprevalence with and without these 7 studies.

To assess the true risk of asymptomatic and symptomatic A(H7N9) virus infections among different populations, studies were evaluated according to whether the study reported any acute respiratory illness (ie, fever or respiratory symptoms) among participants shortly before (within 1 month) the time of serum collection. Random-effects models were then performed to estimate the mean prevalence of asymptomatic and symptomatic A(H7N9) virus infections in humans. Additionally, we assessed the impact of antigen used in laboratory assays by comparing the antigenicity between the antigen used and the circulating virus that the study population was exposed to and evaluated the type of red blood cells used in HAI assays and effect upon serological results.

Variability between studies was determined by the heterogeneity tests (χ^2 test) with the Higgins I^2 statistic. We explored the reasons for variations among eligible studies and examined whether prevalence of A(H7N9) virus–specific antibodies varied by year of study, epidemic region, study quality, and level of exposure by multivariable meta-regression models. Subgroup analyses were implemented when assessing seroprevalence of antibodies against A(H7N9) virus for specific populations with higher heterogeneity. Publication bias was qualitatively investigated by funnel plots and assessed statistically by Egger line regression test.

RESULTS

The literature search identified 582 reports, 184 of which were duplicates (Figure 1). After removal of duplicates and initial screening, we reviewed 35 publications in full. Four publications were excluded because they were not serological studies. A total of 31 studies published between 1 January 2013 and 30 June 2020 were included in the final analysis, of which 19 studies involving 25 study populations assessed respiratory illness (Figure 1).

The majority of studies (20/31 [64.5%]) were graded C according to the quality scoring system (Supplementary Tables 8 and 9), with a maximum score of 13 and minimum score of only 2 (Supplementary Figure 1, Supplementary Table 10).

Epidemic curves of the 5 epidemic waves of human infections with A(H7N9) virus and highly pathogenic avian influenza (HPAI) A(H7N9) virus outbreaks in poultry are shown in Figure 2A and 2B, respectively. All included studies were conducted during epidemic waves 1–4 and were focused on infections with low-pathogenic avian influenza (LPAI) A(H7N9) virus circulating among poultry during 2013–2016, with approximately half of studies involving poultry workers and the general population (Figure 2C). Most of the studies were conducted in southeast China, mainly Jiangsu, Zhejiang, and Guangdong provinces where most human cases of A(H7N9) virus infection were identified (Figure 3A), and 3 studies were conducted in India and Cambodia (Figure 3B).

Among 31 studies included in the meta-analysis, the different study populations all had generally low seroprevalence. For poultry workers, the prevalence of H7N9-specific antibodies was 0.1%, 0.4%, and 0.5% when using the WHO-recommended, modified WHO-recommended, and nonstandardized seropositive definitions, respectively (Table 1). The seroprevalence for close contacts (0.2% [95% confidence interval {CI}, 0–.9%]) was higher than that for poultry workers based on the WHO definition, but no significant differences were found between these 2 populations ($P > .05$). For the general population, the seroprevalence was 0% for all 3 seropositive definitions, indicating extremely low infection risk for unexposed populations (Table 1). After excluding data for 7 studies conducted before 1 February 2013 [9], the overall seroprevalence estimates were all very low based on the WHO-recommended seropositive definition (0% [95% CI, 0–.1%]) (Figure 5A). Among the 7 excluded studies, the seroprevalence was 0% (95% CI, 0–.17%]) for poultry workers, except for 1 seropositive individual in the general population based on the nonstandardized seropositive definition (Figure 5B).

Among 19 studies that assessed participant's respiratory symptoms, the seroprevalence of asymptomatic A(H7N9) virus infections was higher for close contacts (0.2% [95% CI, 0–.9%]) and lower for poultry workers (0% [95% CI, 0–.1%]) when utilizing the WHO-recommended seropositive definition ($P > .05$) (Supplementary Figure 5). Seroprevalence was higher in study

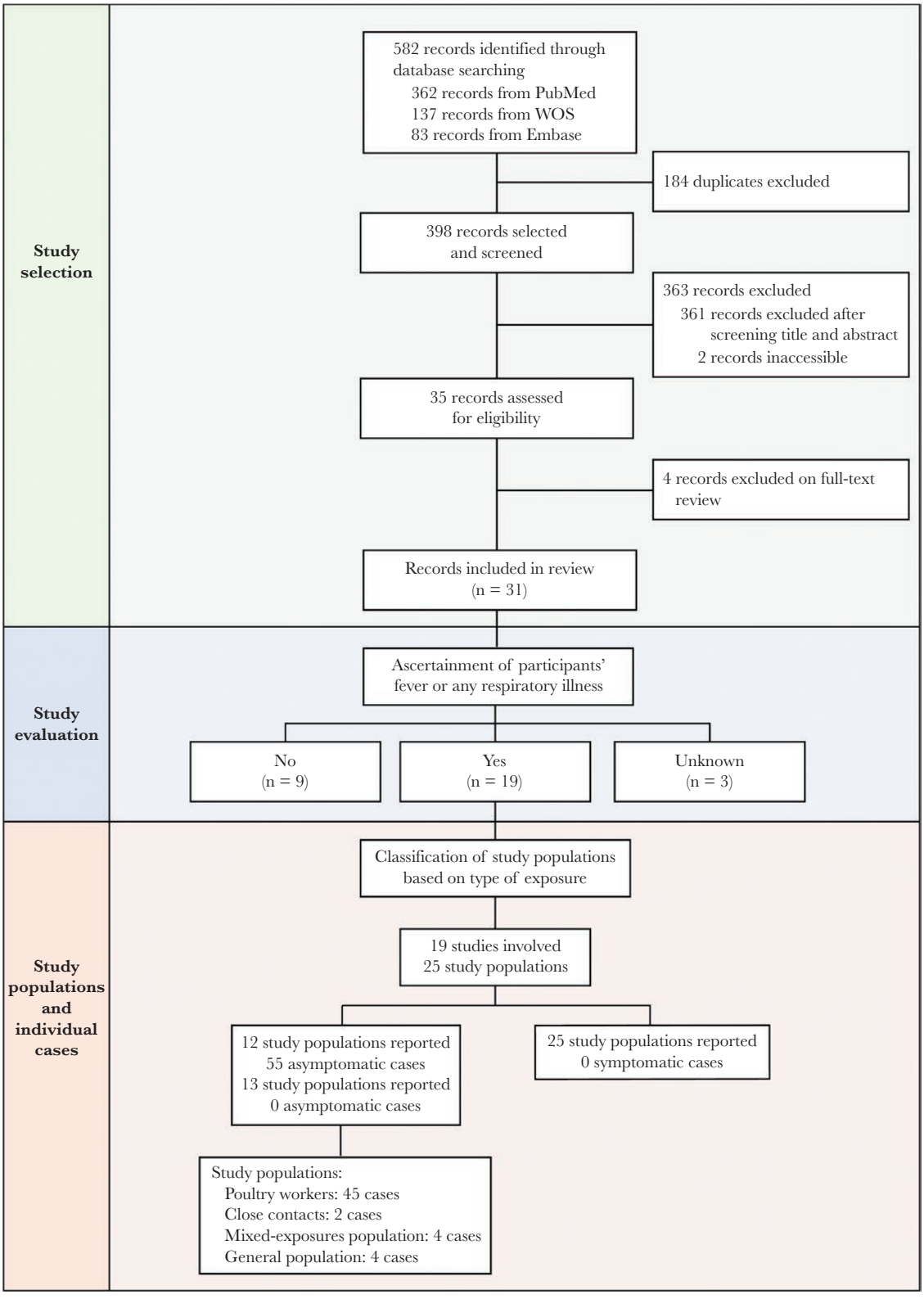

Figure 1. Flowchart of the selection of serological studies of A(H7N9) virus infection, 2013–2020. Abbreviation: WOS, web of Science.

participants exposed to A(H7N9) virus clade W3-3 (range, 0–1.4%) than in participants exposed to other A(H7N9) virus clades (range, 0–.3%), but the differences were not statistically significant on the basis of the WHO-recommended seropositive definition ($P > .05$) (Supplementary Figure 7). Compared to studies without A(H7N9) using viruses that were antigenically

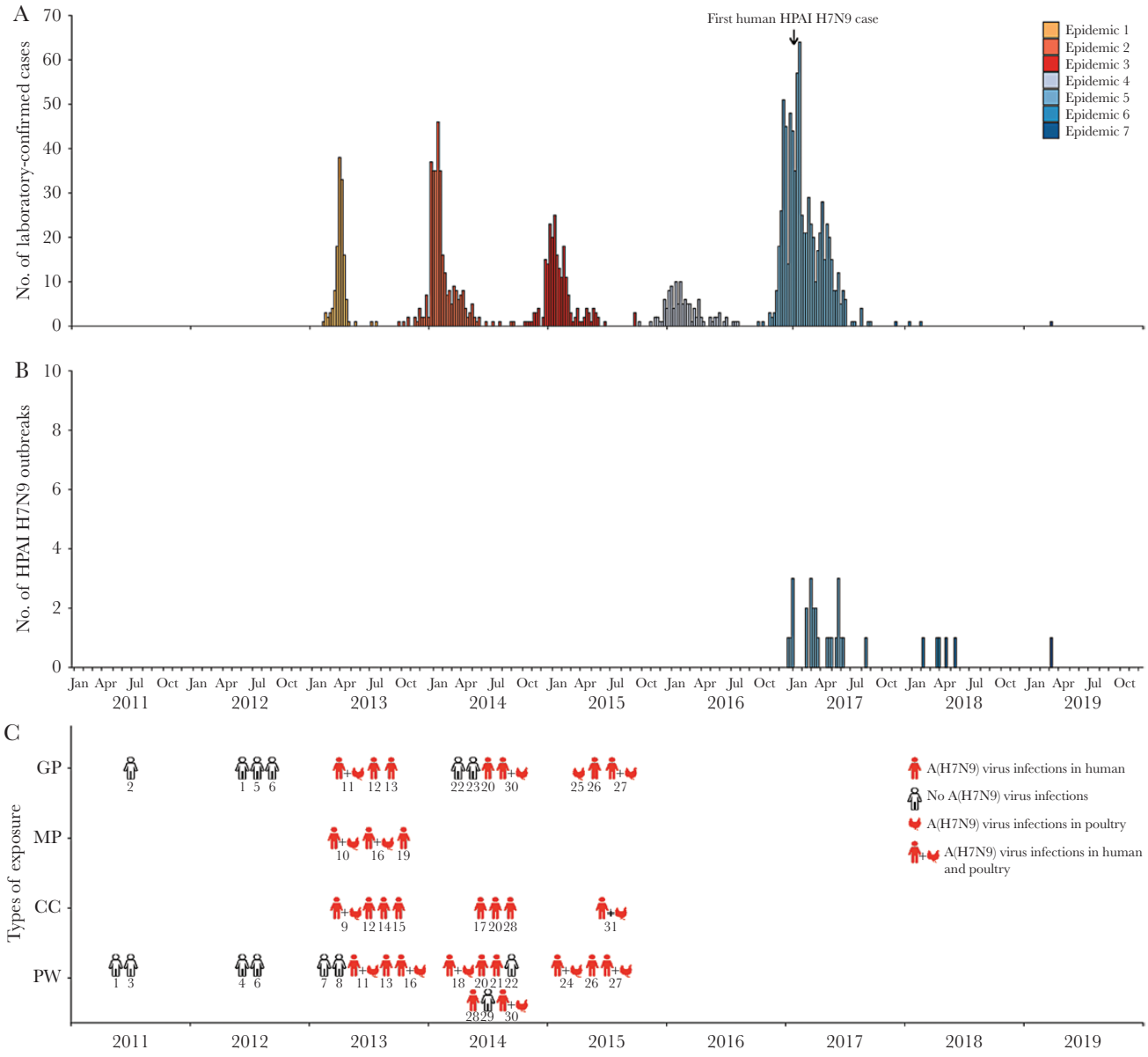

Figure 2. Epidemic curves of virologically confirmed avian influenza A(H7N9) virus infections in humans and animal reservoirs, and temporal distribution of 31 A(H7N9) virus serosurveys in humans by type of exposure, 2013–2020. *A*, Epidemic curve of virologically confirmed human infections with avian influenza A(H7N9) viruses across epidemics since 2013. *B*, Epidemic curve of A(H7N9) virus outbreaks in poultry and wild birds in mainland China. *C*, Temporal distribution of the implementation of 31 A(H7N9) virus serological studies in poultry workers, close contacts, mixed-exposures population, and general population. In (*C*), the color represents whether A(H7N9) virus infections in humans, poultry, or wild birds were occurring (red) or not occurring (white) before or during the implementation of each study. The number below the symbol was the reference number. Part of the serum samples of the general population in the No. 1 study was collected in 2009. Abbreviations: CC, close contacts; GP, general population; HPAI, highly pathogenic avian influenza; MP, mixed-exposures population; PW, poultry workers.

similar to circulating virus strains in poultry, higher seroprevalence was observed in all exposed populations when the antigen used for serological assays was antigenically similar to the local circulating virus in poultry (Supplementary Table 12).

Relatively high heterogeneity in seroprevalence was observed in poultry workers (I^2 = 81.0%, P < .001), while heterogeneity for the other 2 populations was low: close contacts (I^2 = 0%, P = .830) and general population (I^2 = 0%, P =.920) based on the WHO-recommended seropositive definition (Supplementary Table 11). Meta-regression showed that higher seroprevalence was also observed in participants only exposed to poultry than in the general population without any potential exposures to poultry or human A(H7N9) cases (β = .2 [95% CI, .1–.3%, P < .01) (Supplementary Table 15). Live poultry market workers and household contacts were the 2 populations most likely to have detectable A(H7N9) virus–specific antibodies (Supplementary Figures 8 and 9). Publication bias for estimates of seroprevalence based on the WHO-recommended

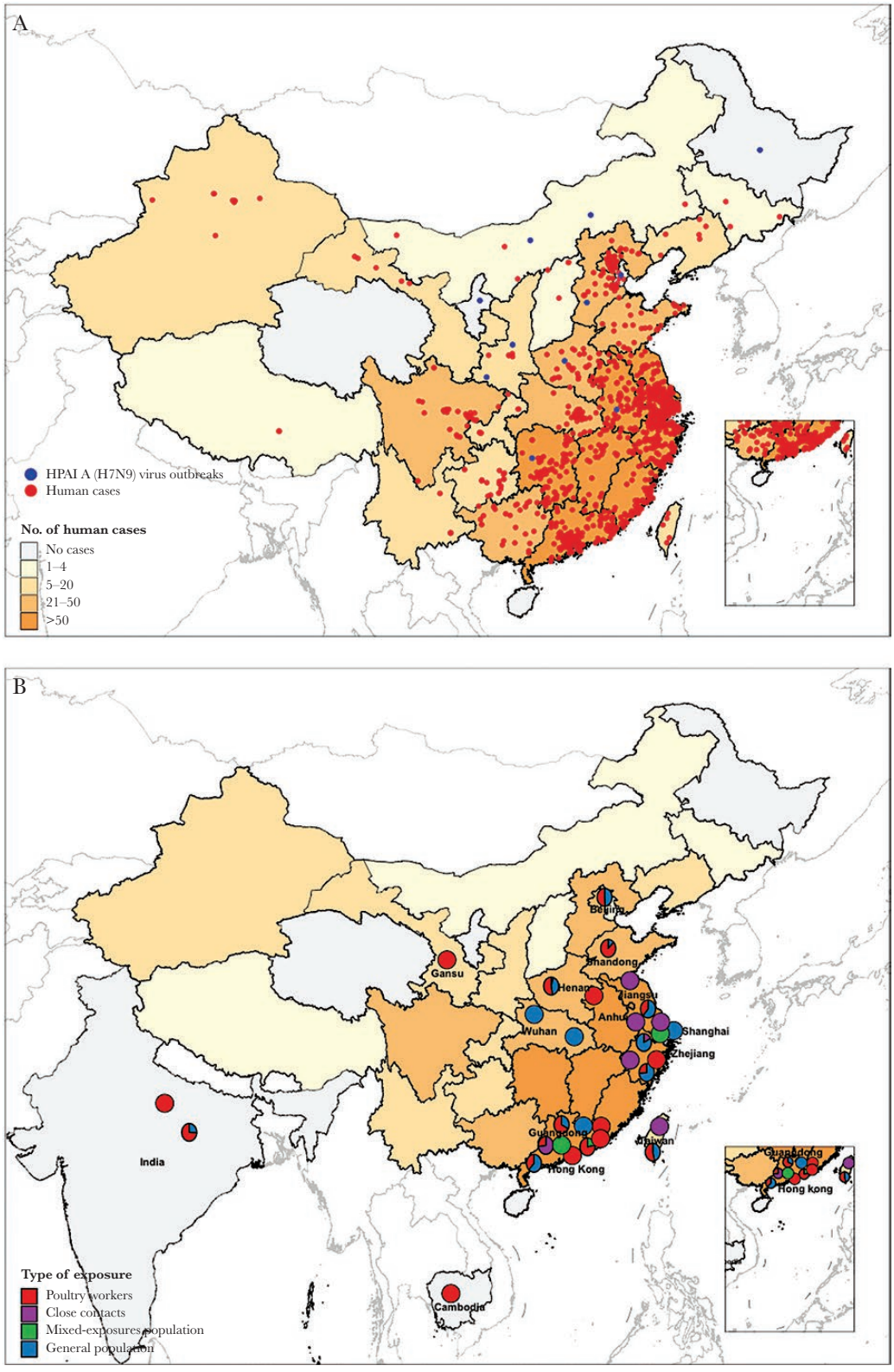

Figure 3. Geographical distribution of virologically confirmed avian influenza A(H7N9) virus infections in human and animal reservoirs, and distribution of 31 A(H7N9) virus serosurveys in humans by types of exposure, 2013–2020. *A*, Geographical distribution of virologically confirmed human cases of A(H7N9) virus infection and outbreaks in domestic poultry and wild birds. *B*, Geographical distribution of 31 A(H7N9) virus serosurveys in humans by types of exposure. Abbreviation: HPAI, highly pathogenic avian influenza.

Table 1. Summary of Estimated Seroprevalence, Seroconversion Rate, and Seroincidence of Antibodies to Avian Influenza A(H7N9) Virus by Type of Exposure, Using 3 Seropositive Definitions (World Health Organization [WHO]–Recommended, Modified WHO-Recommended, and Nonstandardized Seropositive Definitions)

Study Population	Seropositive Definition	No. of Studies	Total No. of Positive	Total No. of Participants	Estimated Result (95% CI)	Figure/Table	References
Seroprevalence (%)							
Poultry workers	WHO	9	39	5746	0.1 (.0–.2)	Figure 4, Suppl Table 11	[15, 32–39]
	Modified WHO	5	21	3340	0.4 (.0–.8)	Suppl Figure 2, Suppl Table 11	[34, 40–43]
	Nonstandardized	19	168	12 052	0.5 (.2–.7)	Suppl Figure 3, Suppl Table 11	[15, 16, 32–48]
Close contacts	WHO	5	2	486	0.2 (.0–.9)	Figure 4, Suppl Table 11	[17, 35, 36, 49, 50]
	Modified WHO	2	0	140	0.0 (.0–1.1)	Suppl Figure 2, Suppl Table 11	[51, 52]
	Nonstandardized	8	2	669	0.2 (.0–.7)	Suppl Figure 3, Suppl Table 11	[17, 35, 36, 49–53]
Mixed-exposures population	WHO	…	…	…	…	…	…
	Modified WHO	…	…	…	…	…	…
	Nonstandardized	3	4	500	0.5 (.0–1.3)	Suppl Figure 3, Suppl Table 11	[18, 32, 48]
General population	WHO	3	1	7665	0.0 (.0-.0)	Figure 4, Suppl Table 11	[15, 35, 54]
	Modified WHO	6	0	3393	0.0 (.0–.1)	Suppl Figure 2, Suppl Table 11	[35, 41–43, 52, 55]
	Nonstandardized	14	9	14 499	0.0 (.0–.1)	Suppl Figure 3, Suppl Table 11	[15, 16, 25, 35, 37, 41–44, 47, 52, 54–56]
Seroconversion (%)							
Poultry workers	Nonstandardized	6	64	1358	5.0 (1.7–8.3)	Suppl Figure 10, Suppl Table 15	[15, 16, 34, 36, 42, 46]
Close contacts	Nonstandardized	4	0	252	0.0 (.0–.9)	Suppl Figure 10, Suppl Table 15	[35, 36, 49, 53]
Mixed-exposures population	Nonstandardized	2	3	375	0.8 (.0–1.8)	Suppl Figure 10, Suppl Table 15	[18, 57]
General population	Nonstandardized	1	1	1030	0.1 (.0–.3)	Suppl Figure 10, Suppl Table 15	[16]
Seroincidence (cases/100 person-years)							
Studies with H7N9 epidemic in human or animal							
Poultry workers	Nonstandardized	3	70	2475	19.1 (12.1–26.1)	Suppl Figure 11, Suppl Table 15	[15, 16, 37]
Close contacts	Nonstandardized	1	0	3	0.0 (.0–32.4)	Suppl Figure 11, Suppl Table 15	[49]
Mixed-exposures population	Nonstandardized	…	…	…	…	…	…
General population	Nonstandardized	2	1	3897	0.0 (.0–.1)	Suppl Figure 11, Suppl Table 15	[16, 37]
Studies with H7N9 epidemic in human or animal							
Poultry workers	Nonstandardized	1	0	19.5	0.0 (.0–6.5)	Suppl Figure 12, Suppl Table 15	[46]
Close contacts	Nonstandardized	…	…	…	…	…	…
Mixed-exposures population	Nonstandardized	…	…	…	…	…	…
General population	Nonstandardized	…	…	…	…	…	…

Abbreviations: CI, confidence interval; Suppl, supplementary; WHO, World Health Organization.

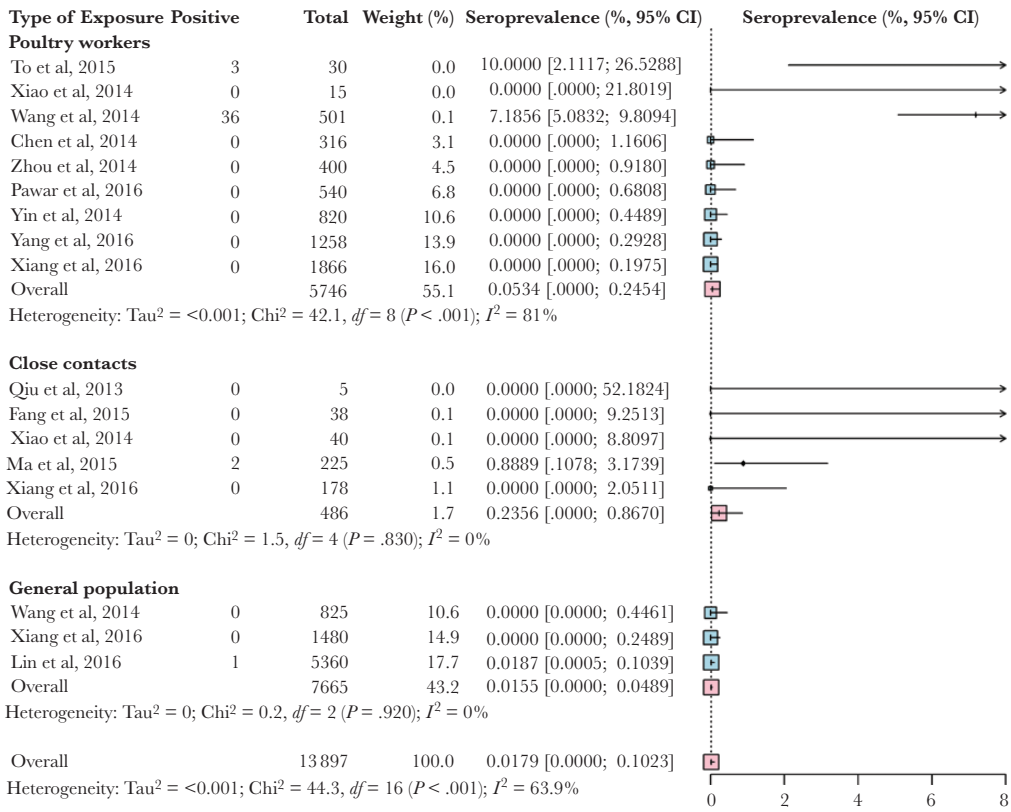

Figure 4. Pooled estimates of seroprevalence of human infections with avian influenza A(H7N9) virus, using the World Health Organization (WHO)–recommended seropositive definition. The WHO-recommended seropositive definition refers to a hemagglutination inhibition (HAI) titer ≥160 tested by horse erythrocytes or an HAI titer of 20–80 tested by horse erythrocytes with a positive result using a second confirmatory assay (ie, microneutralization assay [neutralizing antibody titer ≥80] or Western blot assay). Abbreviation: CI, confidence interval.

seropositive definition was not observed (Egger test $P = .134$) (Supplementary Figure 14).

Among 11 studies that provided data for estimating seroconversion, the median seroconversion rate for A(H7N9) virus infection was 0.1% (range, 0–54.2%), with poultry workers having the highest seroconversion rate of 5.0% (95% CI, 1.7%–8.3%) (Figure 6A, Supplementary Table 15). The mixed exposures population had a higher seroconversion rate of 0.8% (95% CI, 0–1.8%) compared to close contacts and the general population (Figure 6A, Figure 10). Among 5 studies with available data to assess seroincidence, poultry workers had a seroincidence of 19.1 (95% CI, 12.1–26.1) per 100 person-years during an A(H7N9) epidemic (Figure 6B, Figure 11) compared to a seroincidence of 0 (95% CI, 0–6.5) per 100 person-years when no epidemics were occurring (Figure 6C, Figure 12). The general population had the lowest seroincidence of 0 (95% CI, 0–.1) per 100 person-years.

DISCUSSION

Overall, the estimated seroprevalence of A(H7N9) virus–specific antibodies in the unexposed general population was extremely low with a mean seroprevalence of 0.02%, while exposed groups had higher seroprevalence and most infections were asymptomatic (mean seroprevalence of 0.1% and 0.2% for poultry workers and close contacts, respectively, based on the WHO-recommended seropositive definition). Higher seroconversion rates and seroincidence were observed in poultry workers, indicating that new infections occur during ongoing exposures to A(H7N9) viruses circulating among poultry. We found that A(H7N9) virus–specific antibody titers did not vary significantly among study participants exposed to different virus clades. The majority of serological studies were of low to moderate quality, reflecting flaws in study design, incomplete data collection, inconsistent seropositive threshold, antigen-mismatched virus, imperfect laboratory methodology, and less comparable results.

Poultry exposure has long been considered a crucial determinant of human infection with avian influenza A viruses, especially for occupationally exposed populations with daily and prolonged exposures to poultry. One study conducted in Shenzhen, Guangdong province, reported a very high seroconversion rate of 54.2% (52/96) and seroincidence of 81.2% (54/64) for A(H7N9) virus [15]. Another serosurvey with a similar study design and study period [16] that defined seroconversion as detection of a ≥4-fold rise in A(H7N9) virus antibody titer between paired sera, with the second sample

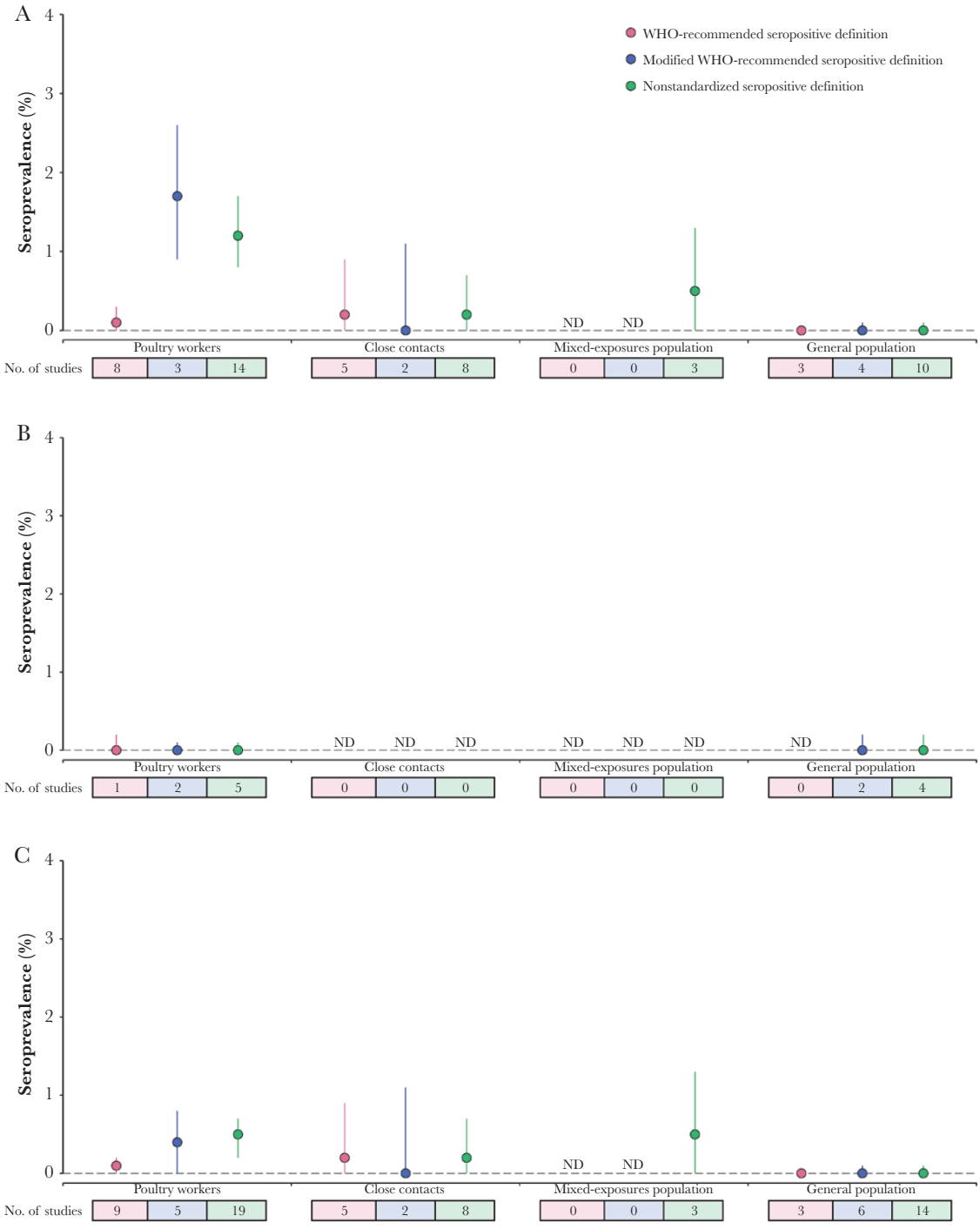

Figure 5. Estimated seroprevalence of human infection with avian influenza A(H7N9) virus, using 3 seropositive definitions (World Health Organization [WHO]–recommended, modified WHO-recommended, and nonstandardized seropositive definitions). The WHO-recommended seropositive definition refers to a hemagglutination inhibition (HAI) titer ≥160 tested by horse erythrocytes or an HAI titer of 20–80 tested by horse erythrocytes with a positive result using a second confirmatory assay (ie, microneutralization assay [MN] [neutralizing antibody titer ≥80] or Western blot assay [WB]). The modified WHO-recommended seropositive definition refers to an HAI titer ≥160 using erythrocytes from other species (eg, chickens, turkeys, and guinea pigs), or an HAI titer of 20–80 using other species' erythrocytes and a positive result by a second confirmatory assay (ie, MN [neutralizing antibody titer ≥80] or WB). The nonstandardized seropositive definition refers to criteria other than the WHO-recommended or modified WHO-recommended criteria used in individual studies to define a seropositive result. *A*, Studies conducted after February 2013. *B*, Studies conducted before February 2013. *C*, All 31 studies. Abbreviations: ND, no data; WHO, World Health Organization.

achieving a titer ≥80, estimated a seroconversion rate of 0.4% (2/468) whereas the Shenzhen study defined a seropositive for the second serum sample as ≥40 and did not utilize any confirmatory serological assay. Due to the limited number of studies with data for estimating such outcomes, the pooled seroconversion results might be very imprecise.

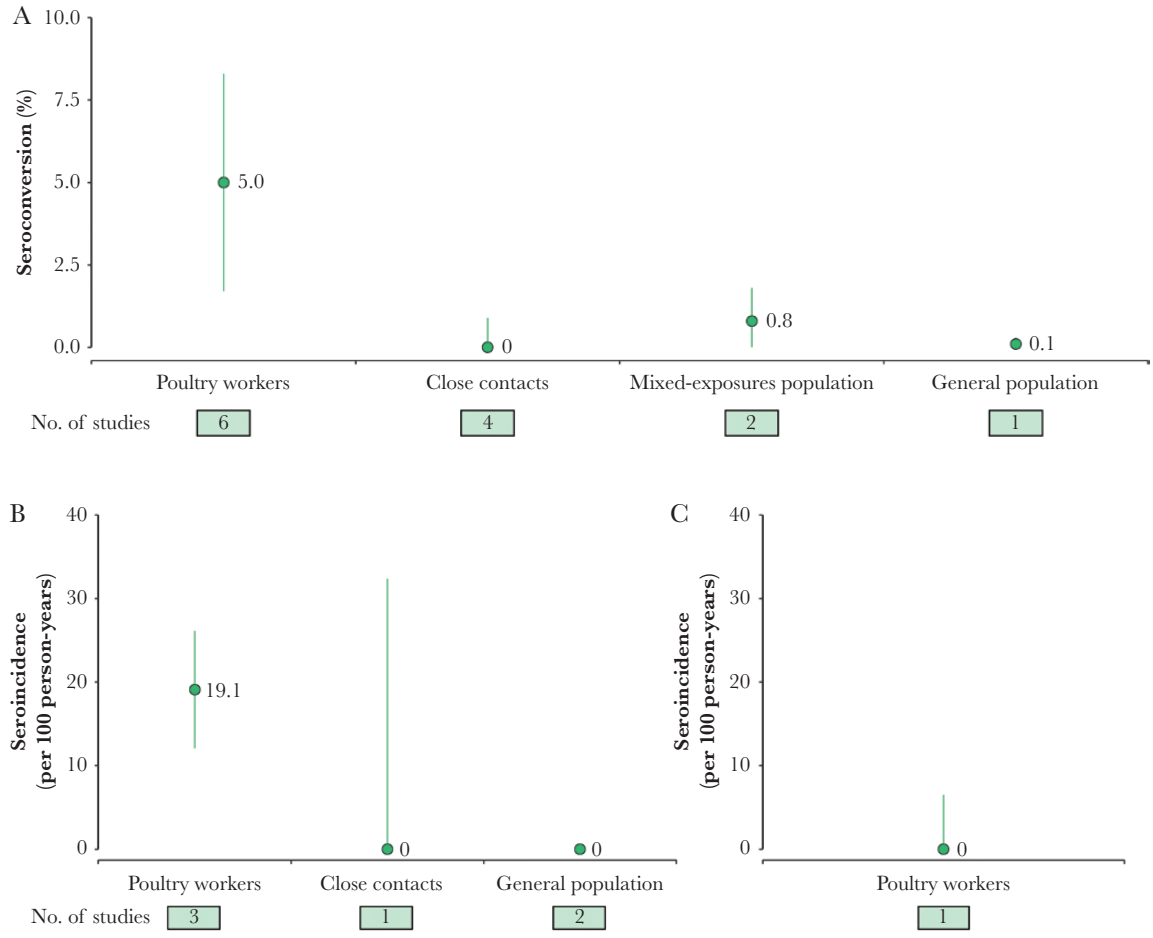

Figure 6. Comparison of seroconversion rate and seroincidence estimates for human infections with avian influenza A(H7N9) virus by types of exposure, using nonstandardized seropositive definition. Data are presented for seroconversion rate for human infections with A(H7N9) virus. The nonstandardized seropositive definition refers to criteria other than the World Health Organization (WHO)–recommended or modified WHO-recommended criteria used in individual studies to define a seropositive result. *A*, Seroincidence of human infections with A(H7N9) virus considering whether A(H7N9) virus outbreaks occurred in humans or poultry (*B*) or did not occur (*C*).

The estimated low seroprevalence of A(H7N9) virus antibodies among close contacts is consistent with limited, nonsustained human-to-human transmission, which has been reported in several studies [17, 18]. However, when compared with serological evidence for A(H5N1) virus infections, the seroprevalence of A(H7N9) virus–specific antibodies among close contacts was higher [17]. From an epidemiological perspective, Qin et al calculated the basic reproduction number for A(H7N9) and A(H5N1) viruses, respectively, estimating 0.27 for A(H7N9) and 0.12 for A(H5N1), suggesting a higher potential pandemic risk for A(H7N9) virus than A(H5N1) virus [19].

Experimental evidence has shown that A(H7N9) virus replicates more efficiently than A(H5N1) virus in ex vivo cultures of the human respiratory tract [20], because A(H7N9) virus can bind to both avian-type (α2,3-linked sialic acid) and human-type (α2,6-linked sialic acid) receptors in the respiratory tract whereas A(H5N1) virus preferentially binds to α2,3 receptors [21]. In addition to the hemagglutinin protein, the polymerase basic protein 2 (PB2) has an important role in the transmission of avian influenza A(H7N9) viruses [22]. Position 627 in PB2, a host-associated genetic signature, has been shown to enhance viral replication, transmission, and host adaptation in A(H7N9) patients [23]. Further identification of the epidemiological and genetic characteristics of A(H7N9) viruses associated with increasing host adaptation and transmission to and among humans is important for ongoing pandemic risk assessment [24].

The establishment of reliable antibody titer thresholds for defining seropositivity is extremely important for standardizing the interpretation of serologic studies. One study, using banked serum collected in 2012, reported a seroprevalence of 0.1% based upon a single seropositive individual with a low neutralizing antibody titer (40), which might be a "false positive" result [25]. Given that the first A(H7N9) virus–infected human case was reported in February 2013, and the virus was not identified until late March, it likely that A(H7N9) virus did not infect humans before 2013 [1]. The limited use of confirmatory

serological assays may increase the likelihood of false-positive results caused by assay error or cross-reactivity with antibodies to other avian or human influenza A viruses [26]. Well-executed and well-controlled serological studies are important for public health, and adherence to protocol and laboratory methodology provided by CONSISE and WHO will help to compare findings across studies [8, 12, 13].

The pooled seroprevalence of A(H7N9) virus antibodies in our study (0.1% [95% CI, 0–.2%]) is consistent with a previous meta-analysis (0.1% [95% CI, 0–.3%]), with the highest seroprevalence in close contacts in both studies, followed by poultry workers and the general population [9]. The estimated seroprevalence in close contacts (1.1% [95% CI, 0–4.4%]) was higher than that in our study (0.2% [95% CI, 0–.9%]), mainly due to the inclusion of a Chinese publication, which reported seroprevalence of 14.3% among 14 close contacts [9, 27]. We chose to exclude Chinese-language studies due to generally low quality, which may affect the accuracy of results. In contrast to the previous meta-analysis, we evaluated the impact of 3 different seropositive definitions on the estimated seroprevalence and conducted subgroup analysis to explore potential factors affecting seroprevalence by controlling for other confounders.

Our study has several limitations. First, the reasons for the apparent heterogeneity for estimating seroprevalence of A(H7N9) virus antibodies observed for poultry workers and in pooled estimates of seroprevalence are unclear. We tried to use meta regression and subgroup analysis to further explore the reasons behind the variations, but analysis was limited by the low number of included studies. Second, misclassification bias may occur due to the limited information on exposures for the study populations that could be extracted from publications.

In conclusion, the risk of A(H7N9) virus infection in the general population was extremely low, and occupationally exposed populations (eg, poultry workers) and close contacts of symptomatic cases (including family members, social contacts, and healthcare workers) also have low risks of infection. Although the risk of human-to-human transmission of A(H7N9) virus was very low, it was nonnegligible and higher than for A(H5N1) virus [28–31]. The overall quality of seroepidemiological studies of A(H7N9) virus infection needs to be enhanced. New seroepidemiologic studies should follow the established guidance on study protocol and laboratory methods (with specific criteria for defining seropositive results) from CONSISE and WHO. Ongoing serologic studies are needed to assess the risk of human infections with LPAI and HPAI A(H7N9) viruses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. H. Y. designed and supervised the study. W. W. and X. C. did the literature search, set up the database, and did all statistical analyses. W. W. and X. C. co-drafted the first version of the article. Y. W. helped with the data collection and did the figures. S. L., J. Y., B. J. C., P. W. H., and T. M. U. provided critical revisions of the manuscript. All authors interpreted the results and critically revised the manuscript for scientific content. All authors approved the final version of the article.

Acknowledgments. We thank Marius Gilbert from the Université Libre de Bruxelles and the Fonds National de la Recherche Scientifique, Qianli Wang, and Junbo Chen from the Fudan University, and Othmar G. Engelhardt from the United Kingdom Medicines and Healthcare products Regulatory Agency for technical support.

Disclaimer. The views expressed are those of the authors and do not necessarily represent the official policy of the Centers for Disease Control and Prevention or other institutions that the authors are affiliated with.

Financial support. This study was funded by the National Natural Science Foundation of China (grant no. 82073613), National Science and Technology Major project of China (grant no. 2018ZX10201001-007, 2017ZX10103009-005, 2018ZX10201001-010), Program of Shanghai Academic/Technology Research Leader (grant no. 18XD1400300), National Science Fund for Distinguished Young Scholars (grant no. 81525023).

Potential conflicts of interest. H. Y. has received research funding from Sanofi Pasteur and Shanghai Roche Pharmaceutical Company; none of this research funding is related to avian influenza viruses. BJC has received honoraria from Roche and Sanofi Pasteur. X. C., W. W., Y. W., S. L., J.Y., B. J. C., P. W. H., and T. M. U. declare no competing interests.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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