Serological evidence of human infection with avian influenza A(H7N9) virus: a systematic review and meta-analysis

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Brief summary:

We performed a systematic review and meta-analysis of sero-epidemiological studies of avian influenza A(H7N9) virus infection. Overall, pooled seroprevalence of A(H7N9) virus antibodies was very low but was highest in close contacts, although most studies were of low-to-moderate quality.

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Abstract

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% based on the WHO-recommended definition, respectively. 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.

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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

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Introduction

Since the first human infections with avian influenza A(H7N9) virus were identified in March 2013, [1] five 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 under detected. [6, 7] Sero-epidemiological studies are useful to explore the full disease spectrum of infections in non-deceased persons, to allow estimation of the prevalence of clinically mild or asymptomatic cases, and to better inform severity assessments.

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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 sero-epidemiological 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 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, 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.

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Methods

Search strategy and selection criteria

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (http://www.prisma-statement.org/),[10] we implemented a comprehensive literature review of English-language papers from Jan 01, 2013 through June 30, 2020 from three databases (PubMed, Embase and Web of Science), using predefined search terms (**Table S1**). 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 two independent researchers; for potential included abstracts, the full text was scrutinized to assess inclusion and exclusion criteria. A third researcher was consulted when the two 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

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higher scores. Based on their overall score, each study's quality was classified into one of four 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 the Supplementary material (**Tables S2-S5**). The review protocol of this study is available in PROSPERO (ID: CRD42020147759).

Statistical analysis

From eligible studies, we extracted data for three predefined A(H7N9) virus antibody outcomes in humans: (i) seroprevalence; (ii) seroconversion; and (iii) 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 four-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 multi-year follow-up studies or serial cross-sectional studies in order to avoid repeated inclusion of the same study.

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Although the WHO 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 sero-epidemiological studies. Therefore, random effects models were performed using three seropositive definitions: the WHO recommended, modified WHO recommended, and non-standardized definitions. The WHO recommended seropositive definition refers to an HAI titer ≥160 tested by horse erythrocytes or a HAI titer of 20-80 tested by horse erythrocytes with a positive result using a 2nd confirmatory assay [i.e. MN (neutralizing antibody titer≥80) or Western blot assay (WB)]. [12, 13] The modified WHO recommended seropositive definition refers to a HAI titer ≥160 using erythrocytes from other species (e.g. chickens, turkeys and guinea pigs); or an HAI titer of 20-80 using other species' erythrocytes and a positive result by a 2nd confirmatory assay [i.e. MN (neutralizing antibody titer≥80) or WB]. The non-standardized seropositive definition refers to criteria other than the WHO or modified WHO recommended criteria used in individual studies to define a seropositive result. All participants involved in this systematic review were reclassified into three groups (i.e. participants who met WHO recommended, modified WHO recommended, or non-standardized seropositive definition) according to the results of each serological study.

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To assess differences in the types and frequency of exposures among populations with potential risk of infection, study populations were categorized into four 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 three distinct A(H7N9) virus clades (i.e. 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 one cohort study with baseline serum collected in January 2013 were identified. Because there was no known A(H7N9) virus circulation among poultry before

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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 seven 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 (i.e. fever or respiratory symptoms) among participants shortly before (within one 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 RBCs used in HAI assays and effect upon serological results.

Variability between studies was determined by the heterogeneity tests (chi-squared test) with 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

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A(H7N9) virus for specific populations with higher heterogeneity. Publication bias was qualitatively investigated by funnel plots and assessed statistically by Egger's line regression test.

Results

The literature search identified 582 reports, 184 that were duplicates (**Fig. 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 Jan 01, 2013 and June 30, 2020 were included in the final analysis, of which 19 studies involving 25 study populations assessed respiratory illness (**Fig. 1**).

The majority of studies (20/31, 64.5%) were graded C according to the quality scoring system (**Table S8-S9**), with a maximum score of 13 and minimum score of only 2 (**Fig. S1**, **Table S10**).

Epidemic curves of the five epidemic waves of human infections with A(H7N9) virus and highly pathogenic avian influenza (HPAI) A(H7N9) virus outbreaks in poultry are shown in **Figures 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

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during 2013-16, with approximately half of studies involving poultry workers and the general population (**Fig. 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 (**Fig. 3A**) and three studies were conducted in India and Cambodia (**Fig. 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 non-standardized seropositive definitions, respectively **(Table 1)**. The seroprevalence for close contacts (0.2%, 95% CI: 0-0.9%) was higher than that for poultry workers based on the WHO definition, but no significant differences were found between these two populations ($p>0.05$). For the general population, the seroprevalence was 0% for all three seropositive definitions, indicating extremely low infection risk for unexposed populations **(Table 1)**. After excluding data for seven studies conducted before Feb 1, 2013, [9] the overall seroprevalence estimates were all very low based on the WHO recommended seropositive definition (0%, 95% CI: 0-0.1%) (**Fig. 5A**). Among the seven excluded studies, the seroprevalence was 0% (95% CI: 0-0.17%) for poultry workers, except for one seropositive

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individual in the general population based on the non-standardized seropositive definition (**Fig. 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: 0-0.9%) and lower for poultry workers (0%, 95% CI: 0-0.1%) when utilizing the WHO recommended seropositive definition (p>0.05) (**Fig. S5**). Seroprevalence was higher in study participants exposed to A(H7N9) virus clade W3-3 (range: 0-1.4%) than in participants exposed to other A(H7N9) virus clades (range: 0-0.3%), but the differences were not statistically significant on the basis of the WHO recommended seropositive definition (p>0.05) (**Fig. S7**). Compared to studies without A(H7N9) using viruses that were antigenically 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 (**Table S12**).

Relatively high heterogeneity in seroprevalence was observed in poultry workers (I^2=81.0%, p<0.001), while heterogeneity for the other three populations was low: close contacts (I^2=0%, p=0.830) and general population (I^2=0%, =0.920) based on the WHO recommended seropositive definition (**Table S11**). Meta regression showed that higher seroprevalence was also observed in

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participants only exposed to poultry than in the general population without any potential exposures to poultry or human A(H7N9) cases (β=0.2, 95%CI: 0.1-0.3%, p<0.01) (**Table S15**). LPM workers and household contacts were the two populations most likely to have detectable A(H7N9) virus-specific antibodies (**Fig. S8, Fig. S9**). Publication bias for estimates of seroprevalence based on the WHO recommended seropositive definition was not observed (Egger's test p-value=0.134) **(Figure S14)**.

Among eleven 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%) (**Fig. 6A, Table S15**). 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 (**Fig. 6A, Fig. S10**). Among five 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 (**Fig. 6B, Fig. S11**) compared to a seroincidence of 0 (95% CI: 0-6.5) per 100 person-years when no epidemics were occurring (**Fig. 6C, Fig. S12**). The general population had the lowest seroincidence of 0 (95% CI: 0-0.1) per 100 person-years.

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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 new infections occur during on-going 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] defined

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seroconversion as detection of a ≥4-fold rise in A(H7N9) virus antibody titer between paired sera, with the second sample achieving a titer ≥80, estimated a seroconversion rate of 0.4% (2/468) whereas the Shenzhen study defined a seropositive for the 2nd 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.

The estimated low seroprevalence of A(H7N9) virus antibodies among close contacts is consistent with limited, non-sustained 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

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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 (HA), the polymerase basic protein 2 (PB2) has an important role in the transmission of avian influenza AH7N9) 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 are 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.

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[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: 0-0.2%) is consistent with a previous meta-analysis (0.1%, 95% CI: 0-0.3%), 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: 0-0.9%), mainly due to the inclusion of a Chinese publication, which reported seroprevalence of 14.3% among fourteen 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 three 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

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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, such as 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 non-negligible and higher than for A(H5N1) virus. [28-31] The overall quality of sero-epidemiological studies of A(H7N9) virus infection need to be enhanced. New sero-epidemiologic 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.

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Contributors

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.

Declaration of interests

H.Y. has received investigator-initiated research funding from Sanofi Pasteur, GlaxoSmithKline, and Yichang HEC Changjiang Pharmaceutical Company; none of this research funding is related to avian influenza viruses. B.J.C. 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.

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Tables

Table 1. Summary of estimated seroprevalence, seroconversion rate and seroincidence of antibodies to avian influenza A(H7N9) virus by type of exposure, using three seropositive definitions (WHO recommended, modified WHO recommended and non-standardized seropositive definitions)

Study populations	Seropositive definition	No. of studies	Total no. of positive	Total no. of participants	Estimated result (95% confidence interval)	Figure/table	References
Seroprevalence (%)							
Poultry workers	WHO	9	39	5746	0.1 [0.0-0.2]	Figure 4/Table S11	[15, 32-39]
	Modified WHO	5	21	3340	0.4 [0.0-0.8]	Figure S2/Table S11	[34, 40-43]
	Non-standardized	19	168	12052	0.5 [0.2-0.7]	Figure S3/Table S11	[15, 16, 32-48]
Close contacts	WHO	5	2	486	0.2 [0.0-0.9]	Figure 4/Table S11	[17, 35, 36, 49, 50]
	Modified WHO	2	0	140	0.0 [0.0-1.1]	Figure S2/Table S11	[51, 52]
	Non-standardized	8	2	669	0.2 [0.0-0.7]	Figure S3/Table S11	[17, 35, 36, 49-53]
Mixed-exposures population	WHO	-	-	-	-	-	-
	Modified WHO	-	-	-	-	-	-
	Non-standardized	3	4	500	0.5 [0.0-1.3]	Figure S3/Table S11	[18, 32, 48]
General population	WHO	3	1	7665	0.0 [0.0-0.0]	Figure 4/Table S11	[15, 35, 54]
	Modified WHO	6	0	3393	0.0 [0.0-0.1]	Figure S2/Table S11	[35, 41-43, 52, 55]
	Non-standardized	14	9	14499	0.0 [0.0-0.1]	Figure S3/Table S11	[15, 16, 25, 35, 37, 41-44, 47, 52, 54-56]
Seroconversion (%)							
Poultry workers	Non-standardized	6	64	1358	5.0 [1.7-8.3]	Figure S10/Table S15	[15, 16, 34, 36, 42, 46]

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Close contacts	Non-standardized	4	0	252	0.0 [0.0-0.9]	Figure S10/Table S15	[35, 36, 49, 53]
Mixed-exposures population	Non-standardized	2	3	375	0.8 [0.0-1.8]	Figure S10/Table S15	[18, 57]
General population	Non-standardized	1	1	1030	0.1 [0.0-0.3]	Figure S10/Table S15	[16]
Seroincidence (cases/100 person-years)							
Studies with H7N9 epidemic in human or animal							
Poultry workers	Non-standardized	3	70	2475	19.1 [12.1-26.1]	Figure S11/Table S15	[15, 16, 37]
Close contacts	Non-standardized	1	0	3	0.0 [0.0-32.4]	Figure S11/Table S15	[49]
Mixed-exposures population	Non-standardized	-	-	-	-	-	-
General population	Non-standardized	2	1	3897	0.0 [0.0-0.1]	Figure S11/Table S15	[16, 37]
Studies with H7N9 epidemic in human or animal							
Poultry workers	Non-standardized	1	0	19.5	0.0 [0.0-6.5]	Figure S12/Table S15	[46]
Close contacts	Non-standardized	-	-	-	-	-	-
Mixed-exposures population	Non-standardized	-	-	-	-	-	-
General population	Non-standardized	-	-	-	-	-	-

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Figure Legends

Figure 1. Flowchart of the selection of serological studies of A(H7N9) virus infection, 2013-2020

Figure 2. Epidemic curves of virologically-confirmed avian influenza A(H7N9) virus infections in humans and animal reservoirs, temporal distribution of thirty-one A(H7N9) virus serosurveys in humans by types 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 thirty-one A(H7N9) virus serological studies in poultry workers (PW), close contacts (CC), mixed exposures population (MP), and general population (GP). In panel C, the color represents whether A(H7N9) virus infections in humans, poultry or wild birds were occurring (red) or not (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.

Figure 3. Geographical distribution of virologically-confirmed avian influenza A(H7N9) virus infections in human and animal reservoirs, and distribution of thirty-one A(H7N9) virus serosurveys in humans by types of exposure, 2013-

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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 thirty-one A(H7N9) virus serosurveys in humans by types of exposure.

Figure 4. Pooled estimates of seroprevalence of human infections with avian influenza A(H7N9) virus, using the WHO recommended seropositive definition. The WHO recommended seropositive definition refers to a HAI titer ≥ 160 tested by horse erythrocytes or a HAI titer of 20-80 tested by horse erythrocytes with a positive result using a 2nd confirmatory assay [i.e. MN (neutralizing antibody titer ≥ 80) or WB].

Figure 5. Estimated seroprevalence of human infection with avian influenza A(H7N9) virus, using three seropositive definitions (WHO recommended, modified WHO recommended, and non-standardized seropositive definitions). The WHO recommended seropositive definition refers to a HAI titer ≥ 160 tested by horse erythrocytes or a HAI titer of 20-80 tested by horse erythrocytes with a positive result using a 2nd confirmatory assay [i.e. MN (neutralizing antibody titer ≥ 80) or WB]. The modified WHO recommended seropositive definition refers to a HAI titer ≥160 using erythrocytes from other species (e.g. chickens, turkeys and guinea pigs); or a HAI titer of 20-80 using other species'

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erythrocytes and a positive result by a 2nd confirmatory assay [i.e. MN (neutralizing antibody titer ≥80) or WB]. The non-standardized seropositive definition refers to criteria other than the WHO or modified WHO criteria used in individual studies to define a seropositive result. (**A**) Studies conducted after February 2013; (**B**) Studies conducted before February 2013; (**C**) All thirty-one studies.

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

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Figure 1

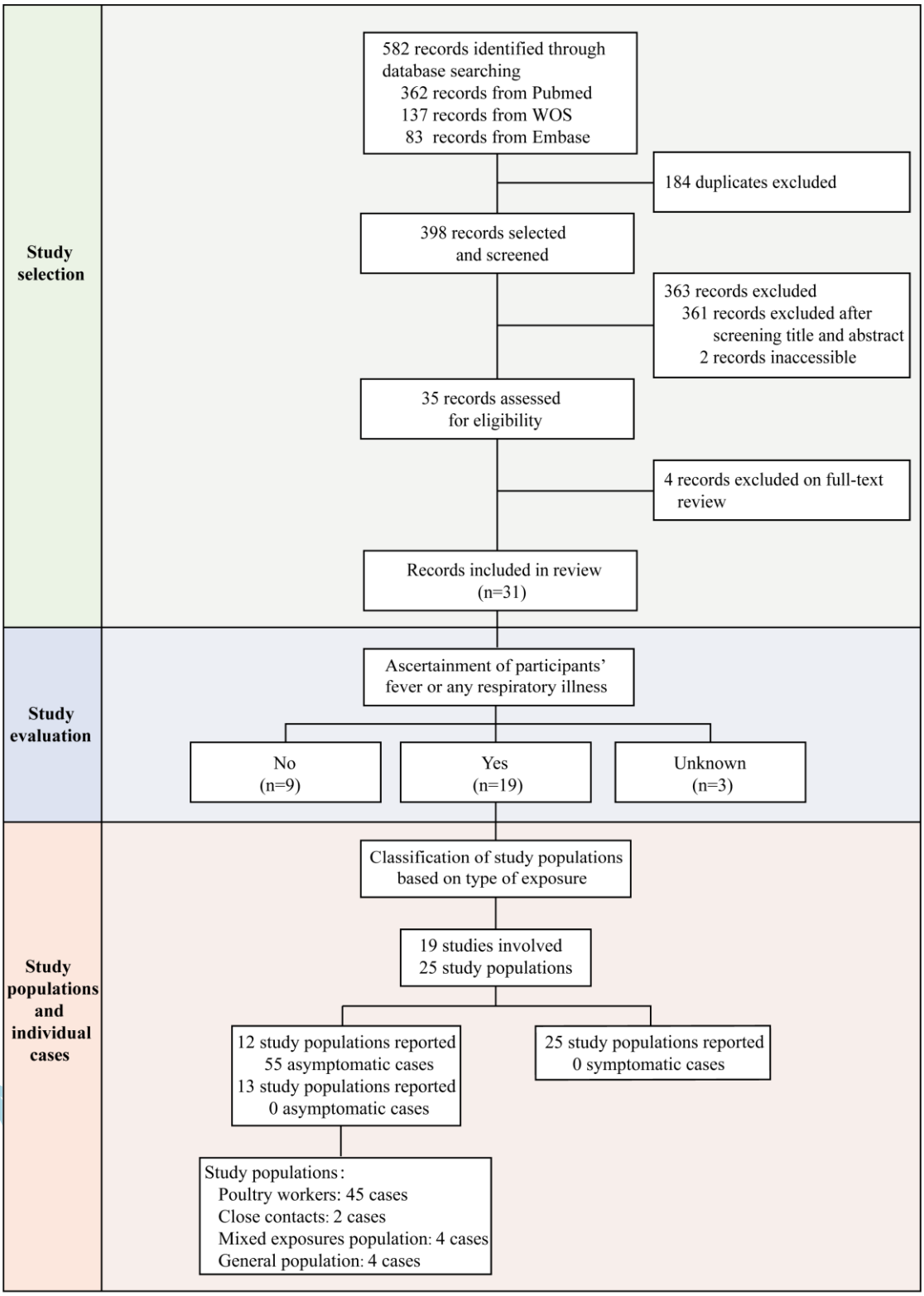

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Figure 2

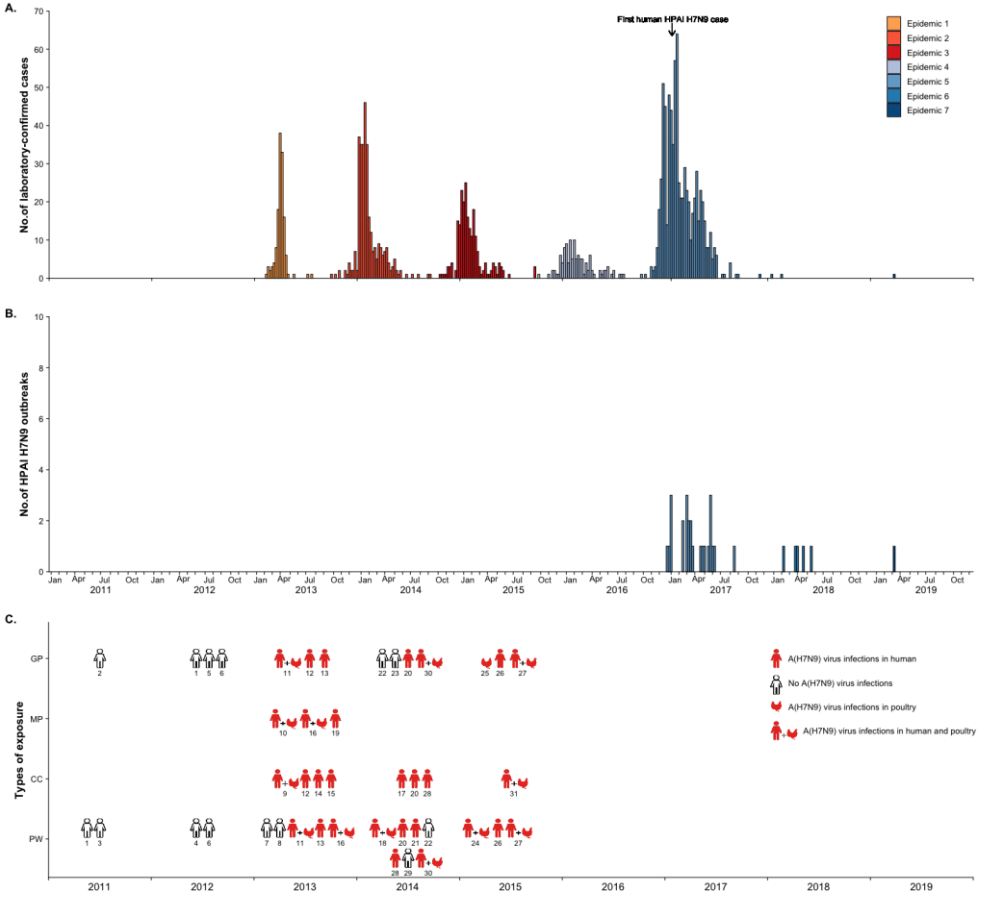

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Figure 3

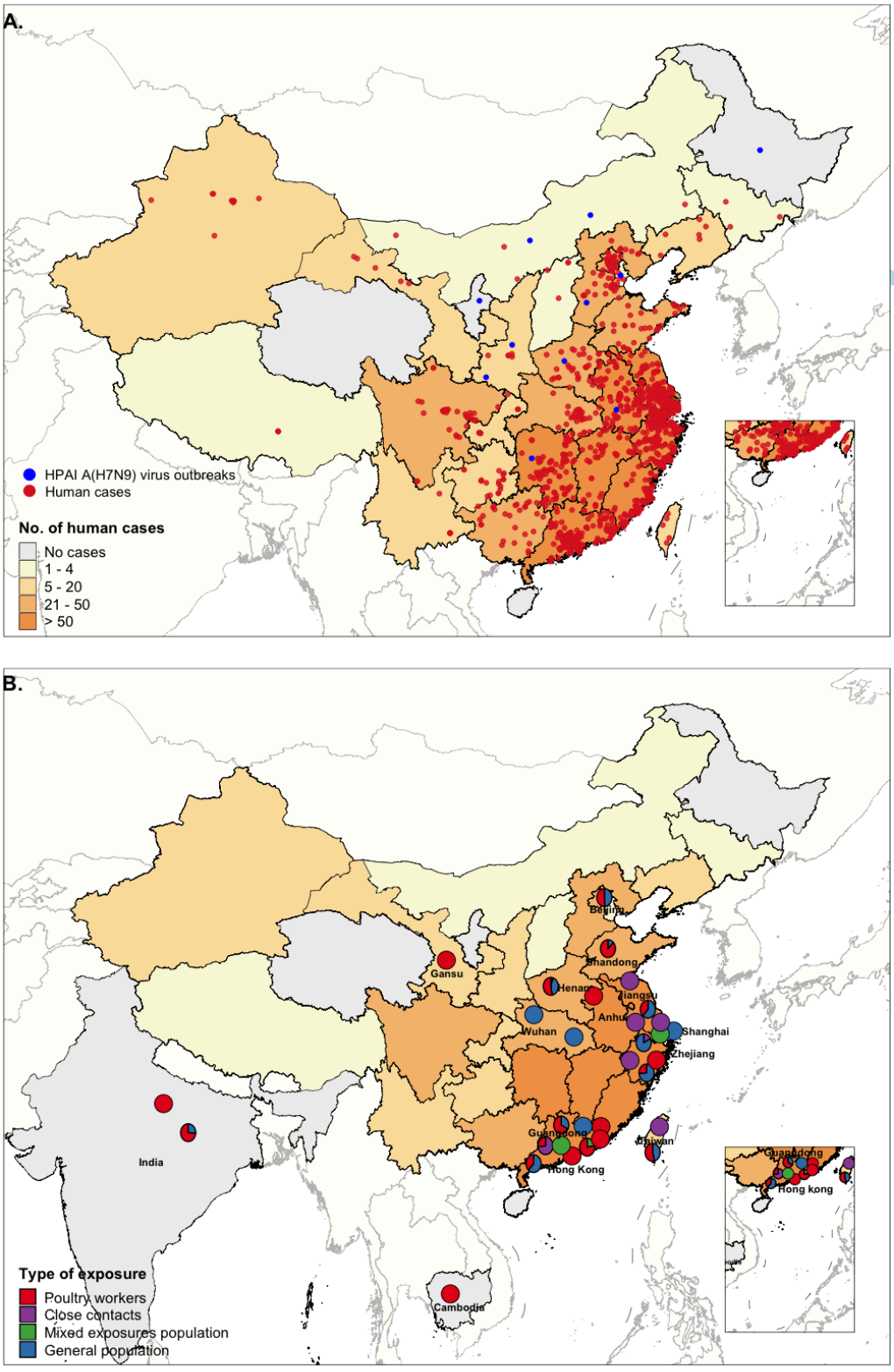

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Figure 4

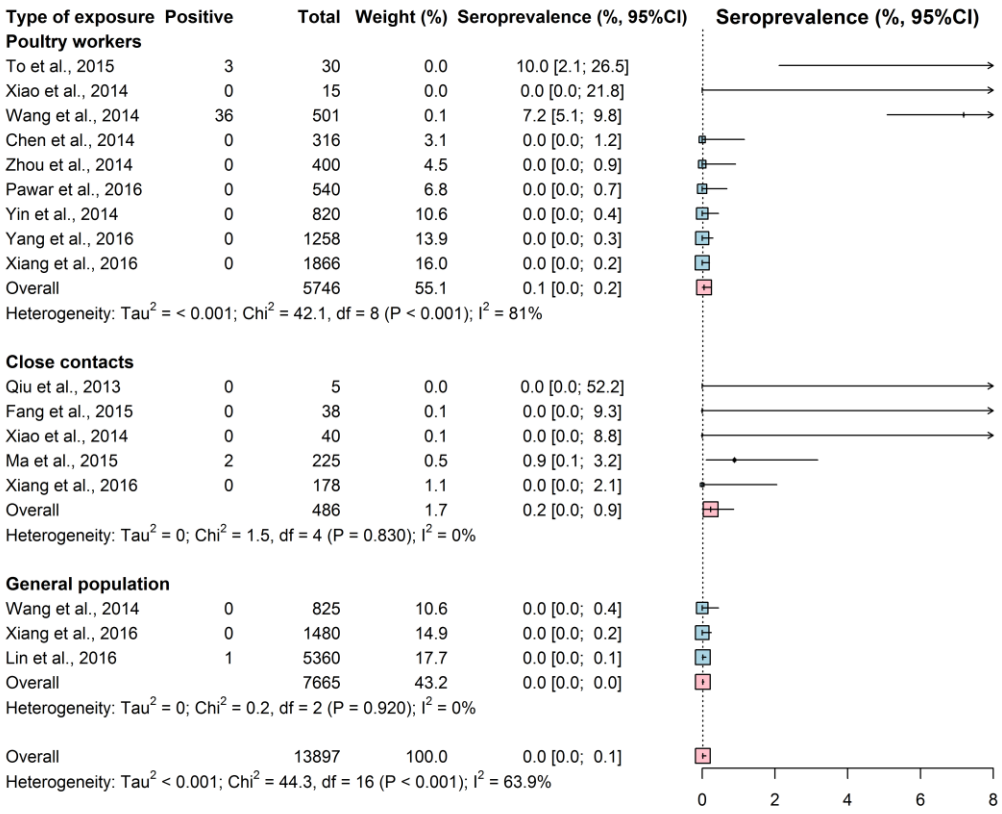

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Figure 5

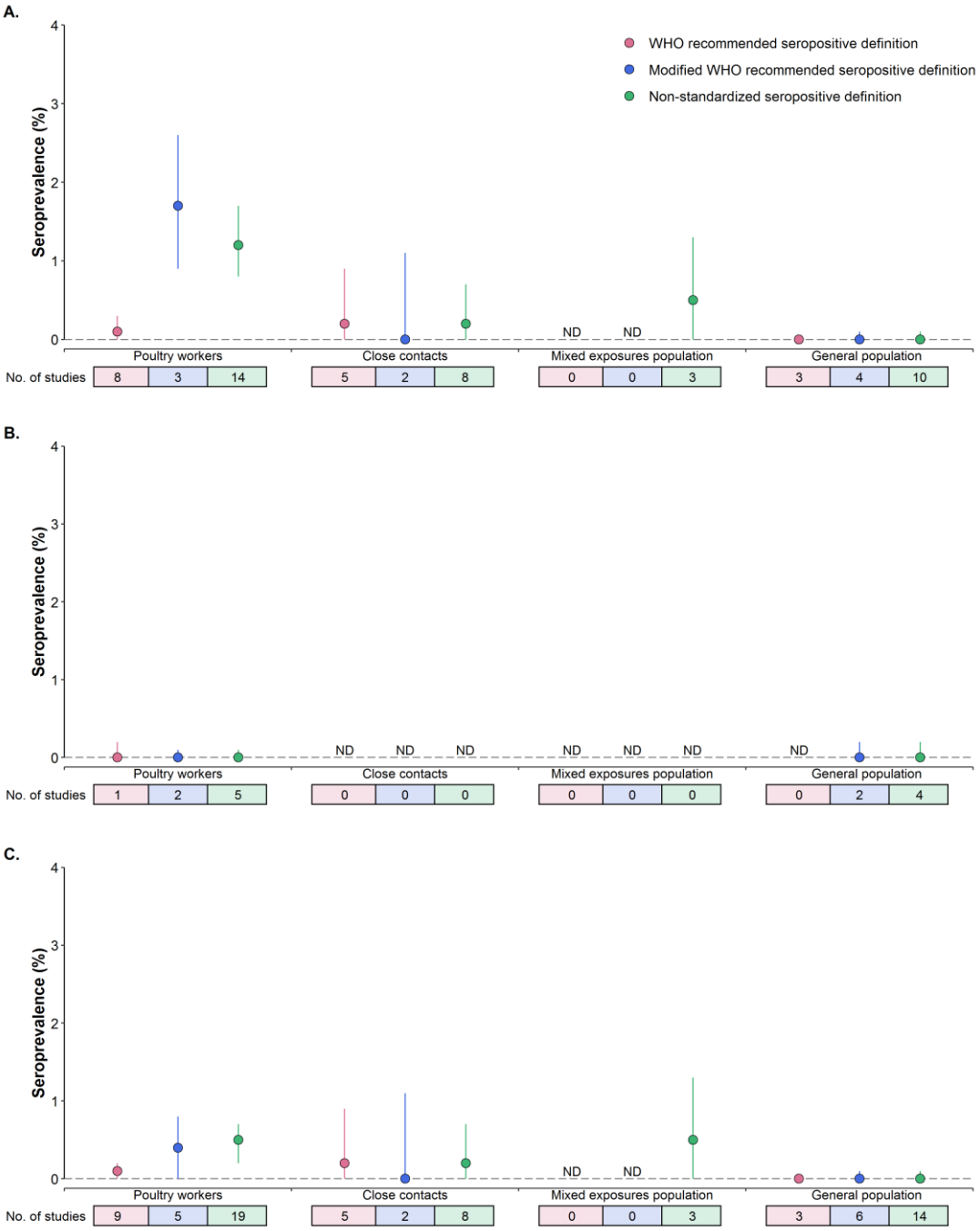

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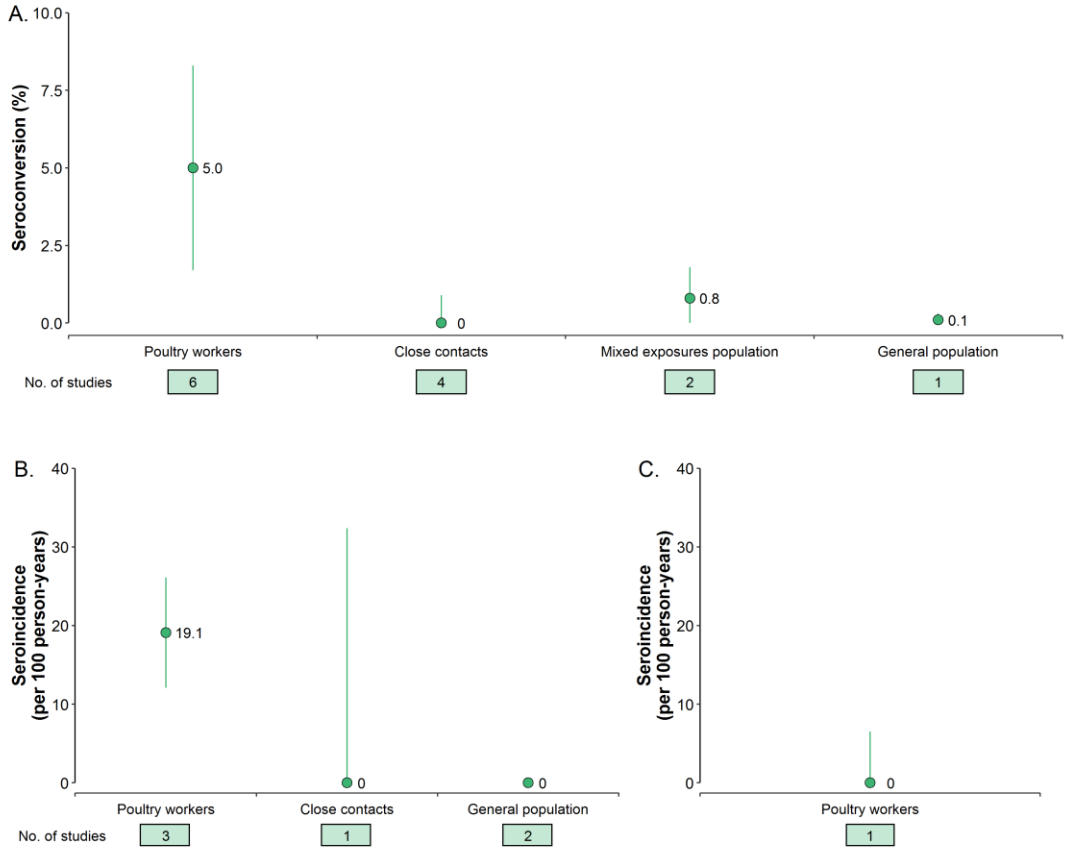
Figure 6



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