Comment on "Seroevidence for H5N1 Influenza Infections in Humans: Meta-Analysis"

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A better understanding of the severity of H5N1 in humans is needed. Wang *et al*. (Brevia, 23 March 2012, p. 1463; published online 23 February 2012) overinterpret the results of seroprevalence studies and take too little account of underlying uncertainties. Although the true risk of death from H5N1 infection will likely be lower than the 60% of reported laboratory-confirmed cases, there is little evidence of millions of missed infections.

A better understanding of the true severity in humans of the H5N1 virus circulating in wild and domestic avian populations is required; however, a recently published meta-analysis (*1*) overinterprets the serological results and takes too little account of underlying uncertainties. Although the true risk of dying from H5N1 infection [case fatality ratio (CFR)] of H5N1 will likely be lower than the 60% found for reported laboratory-confirmed cases, there is little evidence of millions of missed mild or asymptomatic infections (*1*).

The CFR of H5N1 in humans, as reported by the World Health Organization (WHO), is the number of fatal laboratory-confirmed H5N1 cases divided by the total number of laboratory-confirmed H5N1 cases reported to WHO (*2*). The CFR from reported cases is cumulatively 59% (356 of 587) and 55% (34 of 62) in 2011, but it is highly variable by country and age (*3*). Without question, we are missing human H5N1 cases in both the numerator (where cause of death is not investigated or determined) and denominator (where infections never present for care and remain undetected or undiagnosed) of this ratio, due to differences in surveillance and diagnostic capabilities and practices in countries affected by the virus.

We do not understand the extent to which less severely symptomatic (or asymptomatic) human H5N1 infections occur, which may be missed entirely by surveillance systems tuned to detect severe respiratory disease. However, a recent systematic review concluded that infection with H5N1 [as measured by seroprevalence using hemagglutination inhibition (HI) or microneutralization (MN) assays] was rare in populations where the virus had previously been detected in human and/or poultry populations (*4*). Depending on the population under study (e.g., health care workers, poultry workers, or household and/or social contacts of H5N1 confirmed cases), the proportion reported seropositive was <3%, with most studies failing to identify any seropositive individuals (using established criteria for defining seropositivity) [see the supporting information in (*4*)]. In considering the inconsistency of treating serum samples, the existence of antibodies against the N1 subtype of neuraminidase in human populations and the broader antibody repertoire observed with MN, the proportion of seropositive samples may have been overestimated. H5N1 seroprevalence studies have primarily focused on adults, and little is known about the seroprevalence of children in H5N1 endemic countries. That said, even intense serological investigations conducted as part of field investigations of reported severe human H5N1 cases have identified few if any mild or asymptomatic infections (*5*, *6*) and, unlike the studies of fully ascertained acute infections, none of them have virological confirmation such as polymerase chain reaction positivity or isolation of virus from cases. They all rely on serological evidence alone.

The latter review (*4*) and others (*7*) have also highlighted the limitations of influenza seroprevalence studies and cautioned against overinterpretation of low levels of seroprevalence as being indicative of actual infection, particularly when reactive samples have titers close to the threshold level usually used to define seropositivity. Particular laboratory issues that must be rectified include lack of standardization of assay format and performance, variability in criteria used to define seropositivity, cross-reactions in persons exposed to seasonal influenza vaccine or infection (*8*), and virus strains other than the avian H5N1 strain currently circulating. Populations with no known exposure to highly pathogenic H5N1 viruses also manifest low, but detectable, levels of seroprevalence (*9*), and it is unwise to infer asymptomatic H5N1 infection on the basis of such low levels of seroprevalence. Additionally, most of the studies considered by Wang *et al*. were conducted in high-risk populations, which limits the ability to extrapolate to the wider population. Of the 27 groups of individuals for which seropositivity data were available, 23 were either explicitly high risk or categorized as "mixed exposure." The remaining four studies were potentially representative: government workers in Hong Kong (*10*), unexposed health care workers in Hong Kong (*11*), blood donors in China (*12*), and unexposed health care workers in Thailand (*13*). However, the government workers in Hong Kong participated in a poultry culling operation in infected live bird markets (*10*), and groups of unexposed health care workers in both Thailand and Hong Kong were recruited from hospitals in which confirmed H5N1 cases had been treated (*11*, *13*). From all groups included in Wang *et al*., only the 200 samples obtained from blood donors in China in 2009 can reasonably be extrapolated to a population-wide estimate (*12*), and none of these samples tested positive for antibodies to H5N1. Together, the nonrepresentative sampling of populations and the marked heterogeneity in the estimates from the studies analyzed by Wang *et al*. preclude an interpretable summary estimate (*14*). Therefore, we suggest that no data are presented in Wang *et al*. that can reasonably be used to establish a lower bound for the proportion of individuals exposed in the general population of countries affected by H5N1. We also question the appropriateness of combining seroprevalence data from outbreaks in 1997 with data from outbreaks occurring from 2003 to the present because of known H5N1 strain differences that emerge with time. In addition, seroprevalence studies in control populations from countries where H5N1 has not yet been detected are lacking but are needed to determine the extent of reactivity generated by currently available assays.

Thus, we believe that Wang *et al*.'s assertion in their online *Science* Express publication that "if one assumes a 1-2% infection rate in exposed populations, this would likely translate into millions of people who have been infected, worldwide" represents an incautious overinterpretation of limited and uncertain data. We would also note that even if infections were being under-ascertained by a factor of 60 at the current time, natural H5N1

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viruses would still be 100 times as lethal as the 2009 H1N1 pandemic virus (*15*). The precautionary principle dictates that we continue to assume that natural H5N1 infection in humans carries a high risk of death.

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