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Population-level antibody estimates to novel influenza A/H7N9

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Abstract

There are no contemporary data available describing human immunity to novel influenza A/H7N9. Using 1723 prospectively collected serum samples in southern Vietnam, we tested for antibodies to five avian influenza antigens using a protein microarray. General-population antibody titers against subtype H7 virus are higher than antibody titers against subtype H5, and lower than titers against H9. The highest titers were observed for human influenza subtypes. Titers to avian influenza antigens increased with age and with geometric mean antibody titer to human influenza antigens. There were no titer differences between the urban and the rural location in our study.

Introduction

Influenza pandemics typically originate when avian or swine influenza viruses adapt to humans through reassortment or mutation. Not every cross-species jump causes an influenza pandemic, as seen by the differences last decade between the sporadic outbreaks of influenza A/H5N1 and the 2009 A/H1N1 pandemic which spread worldwide in a matter of weeks. The current outbreak of subtype A/H7N9 human cases in China [1,2], with 130 cases confirmed in under three months and no confirmation yet of human-to-human transmission, appears to be more transmissible from poultry to humans than H5N1, but does not yet resemble the transmission patterns of the 2009 pandemic. In any of these epidemiological scenarios, key clinical and epidemiological features are difficult to determine during the early outbreak or epidemic phase, and for this reason pandemic preparedness plans have been put into place globally in an attempt to mitigate or slow down the first stages of the epidemic and to gather early data.

When a pandemic may be imminent, the key knowledge to have in place includes the pattern of population immunity for targeting protective measures and predicting attack rate, virological parameters that enable the development of diagnostics and may inform about the effectiveness of drugs or vaccines, the clinical spectrum of disease, and risk for severe infection. Data gathering will be prioritized differently depending on whether the early epidemiology represents sporadic (H5N1) or consistent (H7N9) animal-to-human transmission, or a rapidly spreading pandemic (2009 H1N1).

One aspect of pandemic preparation that has not received much attention is the analysis of serological data in the early stages of a pandemic, and whether these data can be used to inform the medical and public health communities about the immune status of the general population during this critical period. We address this topic here by presenting general-population

serological data from an ongoing study in southern Vietnam, and we suggest the best way to interpret these results during the context of an emerging pandemic.

Background and Methods

Since 2010, age-stratified serum sample collections have been ongoing in the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam and in Khanh Hoa Provincial Hospital in the city of Nha Trang, 300km northeast of Ho Chi Minh City. Ho Chi Minh City (HCMC) is a densely-populated major urban center with an official population of 7.5 million inhabitants. Nha Trang, with a population of 400,000, is the capital of Khanh Hoa province, and the Khanh Hoa Provincial Hospital serves the city of Nha Trang as well as the surrounding rural areas. Anonymized and unlinked residual serum samples are collected in both hospitals from routine biochemistry and haematology analysis, for the purpose of measuring influenza antibody titers. The serum samples are intended to represent the general population in each hospital's catchment region, as presentation to the hospital should not be correlated with history of influenza infections. Seasonal influenza vaccination in Vietnam is uncommon, and therefore unlikely to influence antibody levels. The research protocol was approved by the Oxford Tropical Research Ethics Committee at the University of Oxford, and the Scientific and Ethical Committee of the Hospital for Tropical Diseases in HCMC.

A total of 1723 samples were collected between 2010 and 2012 – 939 from HCMC and 784 from Khanh Hoa – and were tested for IgG antibodies to the HA1 region of five avian influenza viruses and eleven human influenza viruses (Table S1) by a protein microarray [3–5]. One of the five avian influenza antigens was that of the A/Chicken/Netherlands/1/2003 (H7N7) virus, whose HA1 protein shares 96% homology with the HA1 of the earliest H7N9 strains sequenced in China (A/Shanghai/2/2013, A/Anhui/1/2013 [1]). Only ten amino acid positions differed between these two strains: V38I, T112A, D165N, I170V, T180A, I193V, I227M, E261G, N289D,

and E303R (HA numbering as in [2]). The last two positions do not appear to be in regions associated with binding of virus-neutralizing antibodies, and the remaining changes are largely conservative, but it is difficult to describe the antigenic characteristics of the viruses from the mutations alone. Nevertheless, the high level of homology makes it likely that there would be substantial serological cross reaction between the haemagglutinins from these two viruses.

In addition to the subtype H7 antigen, the microarray includes one subtype H9 antigen and three H5 antigens (H5/04, H5/07, and H5/10; see Table S1). Serology was performed on four-fold dilutions (1:20, 1:80, 1:320, 1:1280), and antibody titers were computed by fitting a four-parameter log-logistic curve to eight luminescence readouts (duplicate spots per antigen) using the curve's point of inflection as the titer measurement for that sample, as described previously [5]. Antibody titer measurements are continuous in this type of analysis and can fall anywhere between 20 and 1280. Titer measurements that fall outside this range were given scores of 10 and 1810. The assay was validated for pandemic 2009 H1N1 influenza by comparing results with haemagglutination inhibition (HI) assays; reactivity of H5, H7, and H9 antigens was confirmed using sera from immunized rabbits and chickens [5]. Statistical analysis was performed with R version 3.0.0 (R Foundation for Statistical Computing, Vienna, Austria) and MATLAB (Mathworks, Natick, MA). Domestic chicken ownership data were obtained from the Government Statistics Office of Vietnam.

Results

Antibodies binding to subtype H7 antigen are at higher titers than antibodies binding to H5; titers to H9 were the highest among the avian antigens. Geometric mean titers (GMT) were 23.1 for H9 (95% CI 21.8-24.4), 19.0 for H7 (18.1-20.0), 13.5 for both H5/10 and H5/07 (13.1-13.9), and 11.1 for H5/04 (10.9-11.3), while GMTs for human influenza antigens ranged from 60 to 200. This indicates that immunity to subtype H7 viruses should be low and comparable to that

for other avian influenza viruses. The microarray assay is more sensitive than HI or microneutralization (MN) tests, but it has not yet been determined whether the binding differences observed among H5, H7, and H9 translate to differences in clinical protection. Because titers calculated from our assay are not directly comparable to HI/MN tests, no cut-off value is chosen to represent positivity or clinical protection. It is not possible to associate these titers with past exposure or past infection, as serological assays have not yet been validated for H7N9. Comparing the H7 GMT with other antigens on the array, it is reasonable to assume that past exposure to avian H7 viruses is similar to that of other avian influenza viruses.

Figure 1 shows quantile-quantile plots for the entire titer distributions as well as their top quartiles; top quartiles are shown as the majority of titers for each antigen were equal to 10 and the upper end of each distribution showed the most variation. Pairwise differences between distributions were significant by both Kolmogorov-Smirnov (KS) test and Wilcoxon Rank-Sum (WR) test (all *P*-values < 10^{-5}), except for the comparison between H5/07 and H5/10 whose titer distributions were very similar. These relative titers among H9, H7, and H5 are consistent with some [6,7] but not all [8,9] past serological investigation in various study populations.

Antibody titers to all avian influenza antigens increase with age as expected (Figure 2), and this increase is largely explained by increased antibody titers to human influenza viruses (Figure S1). If we assume that infections with avian influenza viruses are rare, then the most likely explanation for the titer signals we observe to avian influenza antigens is cross-reactivity of antibodies generated by past infections with human influenza virus [10]. Diversity of influenza antibodies increases with age, as individuals accumulate an antibody repertoire to their different influenza infections, and it becomes more likely that these antibody populations are able to bind antigens from certain avian influenza viruses [11].

No titer differences between locations were detected in the data (KS and WR tests, after generating 100 subsamples without replacement to match age distributions between the two sites; Figure S2), despite the fact that 38% of households in Khanh Hoa province keep domestic chickens, compared to 5.4% of households in HCMC. It is also plausible that in Khanh Hoa human influenza exposure is lower than in HCMC and avian influenza exposure is higher than in HCMC. However, Figure S1 shows that when regressing log-titer of avian influenza antibody onto age and log-GMT of human influenza antibodies, we do not see any differences in the regression coefficients by site. Hence, the data do not show evidence of domestic poultry ownership having an effect on IgG antibody titers to avian subtype haemagglutinins [12].

Discussion

Although validation of serological assays is impossible in the early months of a pandemic due to the lack of positive controls, serological measurements can still be informative when compared across age groups or antigens. The value of comparing antibody titers across antigens in a potentially pre-pandemic scenario is that it may alert us to particularly dangerous situation where cross-reactive antibodies to an emerging virus are lower than we expected; this would have been the case if H7-binding had been weaker than H5-binding in our assays. With pandemic preparedness in mind, antigen-antigen comparisons can also be used to prioritize vaccine development for H7 viruses over H9 viruses, if the higher titers to H9 can be correlated to some level of clinical protection. Comparing antibody titers across age groups can be useful for pandemic response, although these results will not always be available in time, as was the case in 2009 [13].

The perfect early-pandemic seroepidemiological analysis would be able to link quantitative differences in serology to quantitative differences in population transmission rates, but the necessary experimental and analytical methods are many years away from establishing this

link. For pathogens that confer complete immunity, this link can be established because the percentage of completely immune individuals can be equated to the percentage reduction in the basic reproduction number of the pathogen (if mixing patterns are known or assumed to be uniform). For influenza, however, antigenic diversity is high and partial immunity is the norm; thus, it is not currently possible to link the immunity measured in any influenza assay to quantitative reductions in susceptibility, viral replication, or transmissibility.

Prioritizing clinical and epidemiological research is an important component of pandemic response. If patients, contacts, and negative controls from the earliest infections can be enrolled and followed up for serology, validations for positive and negative serological results can be ready after two to three months, depending on rate of spread, case fatality, and enrollment. In 2009, these serological results would have arrived too late, but for the less transmissible H7N9 and H5N1 outbreaks, interpretation of serology could take place well before an outbreak becomes a pandemic.

If the general-population serological responses in Vietnam are representative of other countries in East and Southeast Asia, then the results presented here may tell us something about relative levels of immune-protection in the region. This gives us another reason to seek better understanding of global influenza circulation. If the current belief holds that influenza viruses circulate and mix globally in short time periods [14, and references therein], then the immunological landscapes constructed by influenza epidemics should be similar across countries. Taking national vaccination patterns into account [15], serological studies from a limited number of sites could be used to inform the global response. If a coordinated research response on serology is perceived as too difficult logistically or scientifically, a simpler solution may be to preëmpt this need by maintaining recent serum collections that are either tested or ready for testing for a broad class of important pathogens.

Population-level immunity appears to be low to influenza A/H7N9 and comparable to what we observe for other avian influenza viruses. In southern Vietnam, we do not see evidence that the current H7N9 outbreaks represent a tip-of-the-iceberg observation of widespread H7N9 infection. We observed no differences between two areas with low and high levels of domestic poultry ownership, indicating that poultry ownership does not have an effect on avian influenza exposure or avian influenza antibody levels. If the H7N9 outbreaks develop into a human-transmissible epidemic, the current results will serve as a baseline for interpreting population-level serology after the first wave of infections.

FIGURE LEGENDS

Figure 1. Quantile-quantile plots showing comparisons of titer distributions among five antigens (*n*=1723, upper left). Titer values and specific antigens are labeled on both axes. The ten subplots in the lower-right part of the graph show quantile-quantile plots of the top quartile of individuals (*n*=431) with the highest geometric mean antibody titers across the five avian antigens. All pairwise distribution comparisons show statistical significance that the distributions are different (all $P < 10^{-5}$, Kolmogorov-Smirnov and Wilcoxon Rank-Sum) except for the two panels marked "NS". Antigen abbreviations are A/Vietnam/1194/2004 (**H5/04**), A/Cambodia/R0405050/2007 (**H5/07**), A/Hubei/1/2010 (**H5/10**), A/Chicken/Netherlands/1/2003 (**H7**), and A/Guinea Fowl/Hong Kong/WF10/1999 (**H9**).

Figure 2: Scatter plots of antibody titers by antigen and age group. Red lines show 70th, 80th, and 90th quantiles of the data points. A single red line at 10 indicates that the 70th, 80th, and 90th quantiles of that data set are all equal to 10.

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Competing Interests

All authors report no potential conflicts.

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