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Hiroyu Hatano,1 Peter Bacchetti,2 Priscilla Y. Hsue,3 and Steven G. Deeks1
Departments of 1Medicine and 2Epidemiology and Biostatistics, University of California, San Francisco

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Correspondence: Hiroyu Hatano, MD, MHS, San Francisco General Hospital, Bldg 80, Ward 84, 995 Potrero Ave, San Francisco, CA 94110 (hatanoh@php.ucsf.edu).

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Immunity Status Against Influenza A Subtype H7N9 and Other Avian Influenza Viruses in a High-Risk Group and the General Population in India

TO THE EDITOR—The article by Boni et al reported higher levels of antibodies against avian influenza A virus subtype H7N9 than against influenza A virus subtype H5N1 in Vietnam [1]. The antibodies against influenza A(H9N2) were highest in the study population [1]. As of May 2013, 131 confirmed cases with 32 deaths in humans with influenza A(H7N9) have been reported from China [2]. The epidemiologic and virologic studies have suggested that poultry exposure may be an important risk factor for influenza A(H7N9) infection in humans [3].

India reported outbreaks of highly pathogenic avian influenza (HPAI) A(H5N1) infection among poultry and wild birds but had no reports of human influenza A(H5N1) infections [4, 5]. The isolation of low-pathogenic avian influenza A(H9N2) from poultry and the seroprevalence of influenza A(H9N2) in poultry workers have been reported from India [6, 7]. However, there are no reports of isolation of influenza A(H7N9) from birds or humans from India.

There are few reports of serologic studies of influenza A(H7N9) among high-risk groups in China and Vietnam. In India, no such studies have been conducted to trace influenza A(H7N9) infections. We conducted an exploratory study to evaluate the immunity status against influenza A(H7N9), A(H5N1), and A(H9N2) in the following groups: (1) a high-risk group of poultry workers involved in cleaning and disinfection activities during outbreaks of HPAI A(H5N1) infection among wild birds and (2) the general population in India.

A total of 466 serum samples from poultry workers that were available in the repository and 162 serum samples from the general population (the nonrisk group) in Pune were used in this study. The serum samples were from poultry workers in Pune Maharashtra state (n=338) and Jamshedpur (n=128). The samples from poultry workers in Pune were collected between January and November 2010, and samples from those in Jamshedpur were collected during March 2012. Subjects in the nonrisk group were sampled during 2009. The ratio of males to females was 1.84 to 6.44. All participants were aged 15–75 years. The study was approved by the ethics committee at the National Institute of Virology (Pune), and written informed consent was obtained from all study subjects.

A hemagglutination-inhibition (HI) assay and microneutralization (MN) assays were performed for detection of antibodies [8, 9]. The MN assay was not performed for influenza A(H7N9). The viruses used were A/Chicken/Italy/1067/1999(H7N9) (OIE/FAO, Legnaro, Italy), A/chicken/India/NIV33487/06-RG-2008(H5N1) clade 2.2, A/crow/India/NIV117307/2012(H5N1) clade 2.3.2.1, A/Huebi/1/2010/H5N1-RG30 clade 2.3.2.1 (kindly provided by Dr Ruben Donis, Centers for Disease Control and Prevention, Atlanta, GA), A/chicken/Pune/099321/2009(H9N2). The full-length hemagglutinin gene nucleotide sequence of the A/Chicken/Italy/1067/1999(H7N9) virus antigen (accession no. GU052938GI:26982692) showed 94% nucleotide homology with the recently isolated A/Shanghai/466T/2013(H7N9) virus strain (accession no. KF006369.1GI:491874192) from China. The reference antibodies supplied by the World Health Organization (WHO)
against influenza A(H7N9) were tested with influenza A virus subtype H7N1 antigen to find out its relatedness by means of the HI assay. The H7 antigen reacted with WHO reference antibodies to high titer (HI titer, ≥320), indicating antigenic similarity with influenza A(H7N9) isolated from China. This revealed the appropriateness of using the H7 virus antigen in the study.

All serum samples from the high-risk group and the general population were negative for antibodies against influenza A(H7) strains and influenza A(H5N1). This indicated that there is no population-immunity against influenza A(H7) and influenza A(H5N1), and these viruses did not cause human infections in the study population. Sera from the general population were also negative for antibodies against H9N2 virus. Isolation of influenza A(H9N2) has been reported among poultry from India [6]. It has been shown that 4.7% and 3.8% of poultry workers from Pune were positive by the HI and MN assays, respectively, for antibodies against influenza A(H9N2) [7]. Of the serum samples from high-risk groups in Jamshedpur, 10% (13/128) and 5% (6/128) were positive by the HI and MN assays, respectively, for antibodies against influenza A(H9N2). This could be due to circulation of influenza A(H9N2), in poultry in Jamshedpur. This difference in seroprevalence of antibodies against influenza A(H9N2) in Pune and Jamshedpur could be due to differential environmental exposures. The seropositivity against A(H9N2) virus was similar (P > 0.05) in 15–44 and ≥45 years age-groups. The presence of antibodies against influenza A(H9N2) in poultry workers suggests possible transmission of avian influenza viruses from poultry to humans. The present study showed that antibody levels against influenza A(H9N2) were higher than those against other avian influenza viruses, which is in agreement with findings reported by Boni et al. The limitation of this study is that generalization is not possible from the small number of samples studied.

In summary, animal-human interface studies, together with enhanced clinical and virologic surveillance in high-risk groups, are required to track possible species transfer of novel avian influenza viruses.

Notes

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Shailesh D. Pawar, Babasaheb V. Tandale, Yogesh K. Gurav, Saurabh S. Parkhi, and Sadhana S. Kode
National Institute of Virology, Pune, India

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Reply to Pawar et al

To the Editor—In this issue of the Journal and in a recent study, Pawar et al reported results of serologic tests performed on a high-risk group of 446 poultry workers and 162 individuals from the general population in Maharashtra and Jamshedpur states, India [1, 2]. None of the 608 samples tested positive for antibodies to influenza A virus subtypes H5N1 or H7N1 by hemagglutination-inhibition (HI) or microneutralization (MN) assays. None of the 162 individuals from the general population tested positive for H9N2 antibodies by HI or MN assay. A total of 4.7% and 10% of high-risk individuals in Pune and Jamshedpur, respectively, tested positive by the HI assay for influenza A(H9N2); 3.8% and 4.7%, respectively, had positive results of the MN assay. The authors suggest that this higher rate of seropositivity could be related to the circulation of influenza A(H9N2) in Jamshedpur in poultry, with resultant zoonotic spread to humans, and reference our publication showing presence of antibodies to avian influenza virus antigens in Vietnam.

As noted by the authors, H9 titers were highest among all antibodies to avian influenza virus strains in our general population sample [3]. Other publications looking at high-risk individuals in South East Asia have demonstrated a higher seropositivity rate for influenza A(H9) strains, compared with influenza A(H5) and/or A(H7) strains [4–6], but this is not a consistent finding globally, even in high-risk groups [7, 8]. Vaccine studies conducted in locations thought to have a low risk of avian influenza exposure (ie, the United Kingdom and United States) found that up to a third of