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 titers (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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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
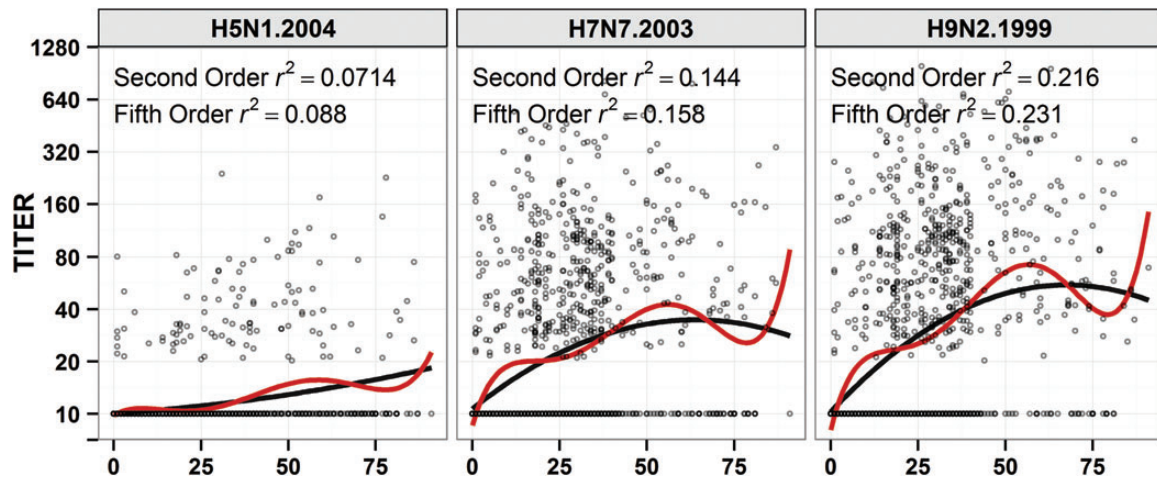
Figure 1. Scatterplot of avian strain titer against age with polynomial regressions lines. The black line denotes the second-order polynomial, and the red line denotes the fifth-order polynomial.

participants were seropositive for H9 [9–11]. Of those positive for H9 antibodies (>1:40 by the HI assay), a greater proportion were born before 1968, and it was postulated that this was related to cross-reactivity from influenza A virus subtype H2N2, which was circulating in humans from 1957 to 1968. Within our data set, there was a significantly higher seropositivity (and, accordingly, mean titer) for all avian strains in individuals born before 1968 (multivariate analysis of variance [MANOVA]: F = 4.7, $P < .0005$, Pillai 0.1256), with the H9 titer being the highest. However, when we investigate other birth year cutoffs (± 20 years), we find the same trend, suggesting that this effect is more related to an increase in age rather than to a specific exposure event.

In the Vietnam sample set (n = 1424; slightly reduced due to quality checks from our original sample set), antibody titers to avian influenza A virus antigens increased with age. The optimal fitted regression curve among nonlinear models was a fifth-order polynomial (ANOVA: $P < .001$), but this curve provided no additional qualitative explanations of the data than the second best fit model, a simple quadratic regression (Figure 1). Titers to H9 antigen increase more rapidly with age than titers to H7 or H5. One

hypothesis is that this difference is caused by varying levels of exposure to avian influenza viruses. A second hypothesis is that this is caused by differences in cross-reactivity between human influenza antibodies and each of the subtypes H9, H7, and H5. Our analysis provides more support for the second hypothesis (supplementary figures 1 and 2 in the article by Boni et al [3]).

As noted in our article, the microarray assay used in this analysis is more sensitive than traditional HI and MN tests when comparing titers for the homologous antigens. A problem in doing studies to evaluate zoonotic exposure is that the exact antigenic composition of the infecting virus may be unknown. Therefore, HI/MN tests may yield false-negative results, a problem that is less evident with the microarray, which measures antibodies to the head of hemagglutinin and is, therefore, more broadly reactive within subtype. The differences we see in response between H9 and H5 avian strains (and to a lesser extent between H7 and H5 strains) are robust even when a much higher titer cutoff is used (up to 1:320, using microarray). With a cutoff titer of 1:20, 76% of individuals aged ≥50 years test positive for H9 antibodies (compared with 33% aged <50 years; $\chi^2 = 89.9$;

$P < .001$). With a more conservative (and probably more appropriate) titer cutoff of 1:80, this percentage is 41% (18% among those aged <50 years; $\chi^2 = 40.6$; $P < .001$), suggesting that age distribution needs to be carefully taken into account when designing seroepidemiologic studies of avian influenza virus in humans. This result is robust for site effects in Vietnam, strengthening the hypothesis that this phenomenon is not related to poultry exposure.

Understanding the best way to interpret avian influenza virus serologic data, including cross-reactions generated by nonavian strains, is crucial for measuring incidence in both high-risk groups and the general population. The results generated by Pawar et al contribute to this understanding, but their study, as with all studies showing H9 positivity, should be interpreted with caution, as these H9-positive signals are possibly cross-reactions.

Supplementary Data

Notes

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Endothelial Activation and Dysfunction in the Pathogenesis of Microvascular Obstruction in Severe Malaria—A Viable Target for Therapeutic Adjunctive Intervention

To THE EDITOR—We read with interest the recent review by White et al [1]. While we agree with the authors' assertion that microvascular obstruction plays a fundamental role in the pathogenesis of lethal falciparum malaria [1], we would like to express a contrasting opinion on the potential impact of innovative adjunctive treatment strategies for severe (and cerebral) malaria.

White et al contend that "malaria researchers have often been distracted by epiphenomena," (p. 193) prompting misguided trials of adjunctive therapeutic strategies in individuals with severe falciparum malaria [1]. Although the authors acknowledge the critical role of inflammation-induced endothelial activation in the binding of parasitized red blood cells to vascular endothelium [1], they neglect to discuss endothelial activation and dysfunction as viable targets for development of novel adjunctive strategies to improve clinical outcomes in life-threatening malaria [2–4].

The angiopoietin-1/2 (Ang-1/2) and Tie2 receptor system plays a key mechanistic role in the regulation of endothelial quiescence and activation. Notably, Ang-1 levels are high and Ang-2 levels are low in the peripheral blood of healthy individuals with quiescent endothelium. In contrast, systemic inflammation causes depressed Ang-1 levels and elevated Ang-2 levels in serum/plasma, contributing to an activated and/or dysfunctional endothelial state [4, 5]. Over the past decade, multiple groups have reported angiopoietin-1/2 dysregulation (ie, low Ang-1/high Ang-2) in the peripheral blood of children and adults with severe and/or cerebral malaria [6–11]. Furthermore, the degree of dysregulation has been shown to correlate with falciparum malaria disease severity and prognosis in multiple populations [6–11]. These observations strongly suggest that Ang-1/2 dysregulation and associated endothelial activation/dysfunction are integral components of the complex pathogenesis of severe and cerebral malaria. Moreover, these observations are consistent with a growing body of evidence supporting a central role for endothelial dysfunction and Ang-Tie2 dysregulation in the pathobiology of other life-threatening infections, including sepsis, multiple organ dysfunction syndrome, acute respiratory distress syndrome, toxic shock syndrome, and hemolytic-uremic syndrome [5, 12–17].

In conclusion, we contend that endothelial activation/dysfunction represents an attractive target for the development of innovative adjunctive strategies to improve clinical outcome in life-threatening infections, including severe and cerebral malaria. Potential interventions for investigation to decrease endothelial activation/dysfunction and improve clinical outcome in severe malaria include administration of Ang-1 agonists, Ang-2 antagonists, Tie2 phosphatase inhibitors, recombinant slit2N, sphingosine-1-phosphate agonists, and nitric oxide [4, 6, 18–20].