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### Serological Survey of Pandemic (H1N1) 2009 Virus, Guangxi Province, China

To the Editor: Since mid-April 2009, a new influenza A virus (H1N1), now called pandemic (H1N1) 2009 virus, has caused influenza outbreaks in humans in North America (1) and a worldwide pandemic (2–4). Human pandemics occur when a new virus subtype emerges that is capable of human-to-human transmission in a population with little or no neutralizing antibodies to the new virus (4).

The current outbreak presents the first opportunity to directly observe this process. We used hemagglutination inhibition (HI) and virus neutralization (VN) assays to detect antibodies in 4,043 serum samples from residents (7–84 years of age) of 2 counties in Guangxi Province, People’s Republic of China, collected during July–August 2008. These persons were mostly farmers who lived in rural areas. Serum samples were obtained, transported, and frozen at −80°C as needed. All samples were tested by HI assay to detect antibodies to the new virus (titers >40), of which 116 (2.9%) had detectable neutralizing antibodies to CA04 (Table). Twelve persons had neutralizing antibody titers ≥160 (Table). The 12 persons from whom the samples were obtained were 30–60 years of age. In contrast with findings from a recent serologic survey of a US population (7), our results showed that none of the 583 persons >60 years of age in our study was VN seropositive for CA04.

All 70 HI-positive samples for CA04 were also screened for neutralizing antibodies against Sw915. Thirteen samples collected from persons 40–84 years of age were VN positive (titers 40–160). Of these 13 samples, 5 were positive (VN titer ≥40) for CA04 and 8 were negative. However, 7 CA04 VN-positive samples were negative for Sw915. These findings suggest that some cross-reactivity exists between CA04 and other Sw915-like H1 subtype viruses circulating in the pig population in southern China, and that sporadic human infection with H1 swine viruses has occurred in rural China, where exposure to pigs is common.

In contrast, screening all 4,043 serum samples with A/Brisbane/59/2007 (H1N1; B59), and A/swine/Hong Kong/915/2004 (H1N2; Sw915). CA04 and B59 were kindly provided by the World Health Organization Collaborating Centers for Reference and Research on Influenza (Atlanta, GA, USA, and Parkville, Victoria, Australia). Sw915 was isolated from pigs by our laboratory. Seven of 8 genomic segments of Sw915 were located in a sister lineage to the current outbreak; this strain is the most closely related swine virus to CA04 identified to date (6). All serum samples were treated with a receptor-destroying enzyme and absorbed with fresh turkey erythrocytes to remove nonspecific inhibitors before the assays. All samples were tested by HI and VN assays according to standard protocols (5).
22 serum samples from vaccinated persons had no neutralizing antibodies against CA04, but all had high seroconversion rates for B59 (Table).

Our results suggest that most persons in our study population from Guangxi, China, are seronegative for pandemic (H1N1) 2009 virus (I). Serum samples from only 0.3% of persons tested neutralized the novel CA04 strain. This finding contrasts with findings from the United States that serum samples from >11% of unvaccinated persons had antibodies against CA04 (7). Furthermore, all CA04-positive persons in our study were <60 years of age; the US study reported a 33% seropositive rate for this age group.

These differences may have been caused by the high proportion of seasonal influenza vaccination coverage in the United States when compared with results form our unvaccinated population from southern China. Therefore, we suggest that vaccination against seasonal influenza, rather than exposure to older, seasonal, influenza viruses (H1N1), which may be genetically and antigenically similar to pandemic (H1N1) 2009 virus, as suggested (7), might have generated partial protection against this new virus. No persons in our vaccinated control group had neutralizing antibodies against CA04.

We hypothesize that the absence of neutralizing antibodies in our control group, all of whom had been vaccinated 3 times, suggests that prolonged and repeated vaccination is required for partial immunity to CA04 or that older vaccines may confer some degree of protection. If these serologic differences are indicative of increased susceptibility, we would expect higher infection attack rates in largely unvaccinated populations than in vaccinated populations in countries such as China.

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