



# Evidence for avian H9N2 influenza virus infections among rural villagers in Cambodia

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Received 5 October 2012; received in revised form 30 November 2012; accepted 30 November 2012

## KEYWORDS

Influenza A virus;  
Avian;  
Zoonoses;  
Occupational exposure;  
Communicable diseases;  
Emerging;  
Cohort studies

## Summary

**Background:** Southeast Asia remains a critical region for the emergence of novel and/or zoonotic influenza, underscoring the importance of extensive sampling in rural areas where early transmission is most likely to occur.

**Methods:** In 2008, 800 adult participants from eight sites were enrolled in a prospective population-based study of avian influenza (AI) virus transmission where highly pathogenic avian influenza (HPAI) H5N1 virus had been reported in humans and poultry from 2006 to 2008. From their enrollment sera and questionnaires, we report risk factor findings for serologic evidence of previous infection with 18 AI virus strains.

**Results:** Serologic assays revealed no evidence of previous infection with 13 different low-pathogenic AI viruses or with HPAI avian-like A/Cambodia/R0404050/2007(H5N1). However, 21 participants had elevated antibodies against avian-like A/Hong Kong/1073/1999(H9N2), validated with a monoclonal antibody blocking ELISA assay specific for avian H9.

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*Conclusions:* Although cross-reaction from antibodies against human influenza viruses cannot be completely excluded, the study data suggest that a number of participants were previously infected with the avian-like A/Hong Kong/1073/1999(H9N2) virus, likely due to as yet unidentified environmental exposures. Prospective data from this cohort will help us better understand the serology of zoonotic influenza infection in a rural cohort in SE Asia.

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## Introduction

Describing the epidemiology of and controlling highly pathogenic avian influenza (HPAI) have been major challenges for many countries in Asia. In Cambodia, HPAI H5N1 virus control has been particularly difficult, as household ownership of backyard poultry is widespread, movement of birds is common, poultry-handling behaviors are difficult to modify, and considerable evidence of human HPAI H5N1 virus exposure exists [1–9].

Detection of HPAI H5N1 in Cambodian poultry first occurred in January 2004 and has continued [1,10,11], with the most recent detection in June 2012, as reported by the World Organization for Animal Health (OIE). Human cases have been sporadic. As of August 2012, Cambodia has reported 21 human HPAI H5N1 infections to the World Health Organization, with 19 fatalities.

As with much of Asia, influenza surveillance in Cambodia chiefly involves assessing ill patients who seek medical attention at large urban medical facilities [12]. Vong et al. [9] have suggested that clinic-based surveillance systems may overlook mild or sub-clinical HPAI H5N1 virus infections among persons residing in rural areas with close contact with sick or dead poultry suspected to be linked to HPAI H5N1 virus. Our report documents the establishment of a prospective cohort study of 800 rural Khmer adults in central Cambodia with the primary objective of describing risk factors for zoonotic influenza infection.

## Materials and methods

### Study location

Six months prior to enrollment, our Cambodian field staff contacted village leaders and local Ministry of Health/Ministry of Agriculture officers to determine the best rural villages in which to conduct enrollment for a cohort study. Considerations included early reports of HPAI H5N1 detection, proximity to the central reference laboratory in Phnom Penh, Cambodia, human population size and density, total

number of homes, number of homes raising poultry and swine, and the variety of poultry.

Based on pre-enrollment assessments, Kampong Cham Province was selected as the study area (Fig. 1). Kampong Cham has a land area of approximately 9000 km<sup>2</sup>, the largest human population of any province in Cambodia (nearly 1.7 million) and had an estimated 1 million chickens and 260,000 ducks at the time of the study (personal communication Kampong Cham Agriculture Department). Within the Kampong Cham province, four districts (Bathay, Cheung Prey, Tboung Khmum, and Ponhea Kraek) were identified as having high poultry counts. Within these districts, eight sites were selected as cohort enrollment field sites (Fig. 1).

### Enrollment

Adults who lived in the study villages were recruited and trained as staff field workers with the responsibility to conduct study enrollments and follow-up encounters. First, houses in the study villages were mapped and sequentially numbered. Then, using a systematic sampling approach with a random-number generated start, staff field workers met with adults  $\geq 20$  years of age in each selected household to explain the study. Study inclusion required that potential enrollees were  $\geq 20$  years of age, resided in the household for 20 or more days each month and had no known immunosuppressive conditions. In addition, potential participants were informed of the prospective nature of the study, which involved an annual revisit to collect a blood specimen, weekly active surveillance for influenza-like illness (ILI), and an additional 'family' study to assess the secondary spread of influenza within a household if a confirmed influenza virus infection occurred. Willing and eligible potential participants were assigned a randomly generated selection number. A random draw of household selection numbers resulted in one adult being selected for study enrollment. All selected participants were then enrolled using informed consent. Study participants were interviewed by staff field workers, who completed enrollment forms, collected sera, trained villagers to use digital thermometers, and

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