Europe (8), resident populations in these countries have been exposed to these virus lineages more frequently than populations in Asia, and therefore may have acquired a greater degree of preexisting cross-reactive immunity to pandemic (H1N1) 2009 virus. A recent review of human swine influenza infections suggests that they may not be uncommon (9), although the true incidence of human infections with swine influenza is unknown because of paucity of swine influenza surveillance data worldwide (8).

In conclusion, partial cross-immunity and cell-mediated immunity may be present but not detected by HI or MN assays. Thus, results of standard serologic assays may not be providing all relevant data (10).

Testing by the Melbourne World Health Organization Collaborating Centre for Reference and Research on Influenza was supported by the Australian Government Department of Health and Ageing.

Julian W. Tang, Paul A. Tambyah, Annelies Wilder-Smith, Kim-Yoong Puong, Robert Shaw, Ian G. Barr, and Kwai-Peng Chan

Author affiliations: National University Hospital, Singapore (J.W. Tang); National University of Singapore, Singapore (P.A. Tambyah, A. Wilder-Smith); Singapore General Hospital, Singapore (K.-Y. Puong, K.-P. Chan); and World Health Organization Laboratory for Influenza Reference and Research, Melbourne, Victoria, Australia (R. Shaw, I. Barr)

DOI: 10.3201/eid1605.091678

References


counties and Taipei City), central (Tai-chung and Changhua counties), southern (Tainan and Kaohsiung counties), and eastern (Hualien County) during 2005–2008. Real-time reverse transcription–PCR (RT-PCR) was used to screen JEV in mosquito pools, pig serum specimens, and human cerebrospinal fluid as described (9). Mosquitoes were pooled by species, location, and collection date in groups of 30–50 mosquitoes. Mosquito pools were homogenized and clarified by centrifugation, and the supernatants were sterilized by filtration and removed for real-time RT-PCR and virus isolation.

We used 3 sets of primers for real-time RT-PCR: flavivirus-specific (FL-F1: 5'-GCCATATGG TACATGTG-GCTGGGAGC-3', FL-R3: 5'-GTKATTCTTGTGTCCCAWCCGGCTGT GTCATC-3', FL-R4: 5'-GTGATGGCRTGTGGTCCCAGCCRGCKTGTCATC-3'), JEV-specific (10) (JE3F1: 5'-CCCTCAGAACCGTCTCGGAA-3' and JE3R1: 5'-CATATGCCAGGTCATATGCGTG-3'), and JEV GIII–specific (E12F: 5'-CTGGGAATGGGCAATCGTG-3' and E325R: 5'-TGTCAATGCTTCCCTTCCC-3'). Samples with positive results by RT-PCR were subjected to virus isolation by using a mosquito C6/36 cell line. A total of 47 JEV isolates were obtained: 38 from mosquitoes, 8 from pig serum samples, and 1 from human cerebrospinal fluid.

Viral RNA was extracted from JEV-infected culture medium, and RT-PCR and DNA sequencing were performed to determine the complete E gene sequences of JEV isolates. Multiple sequence alignment and phylogenetic analysis were conducted by using CLUSTALW software (www.ebi.ac.uk/Tools/clustalw2/index.html) and MEGA version 4 (www.megasoftware.net). The phylogenetic tree was constructed by the neighbor-joining method and the maximum composite likelihood model.

The Figure shows the phylogenetic tree derived from 67 samples of E gene sequences, including 28 representative new sequences in this study (GenBank accession nos. GQ260608–GQ260635), 10 sequences of Taiwanese strains isolated before 2002, and 29 sequences from GenBank. The results show that isolates from Taiwan comprised 2 genotypes, GIII and GI. All of the JEV isolates from Taiwan obtained during 2005–2008, except
2 strains (TPC0806c/M/2008 and YL0806f/M/2008), belonged to GIII and formed into 2 clusters. Cluster 1 contains most new isolates prevalent in different areas of Taiwan. Although cluster 1 isolates are closely related to other JEV strains from Asia, these isolates, together with previously published JEV sequences from Taiwan, form a distinct lineage and may have been continuously evolving and locally adapting in Taiwan. Cluster 2 contains only 2 new isolates, TPC0706a/M/2007 and YL0806f/M/2008, which were isolated from the Culex tritaeniorhynchus mosquito pools in Kuantu Nature Park, Taipei City, and from a pig farm in Wujie Township, Yilan County, respectively.

Notably, the 2 GI strains, TPC0806c/M/2008 and YL0806f/M/2008, were isolated from the same areas as the GII cluster 2 strains. These areas are adjacent to the wetlands, which are stopover sites for migratory birds. These 2 GI strains are most closely related to the strains of the subcluster II JEV strains reported by Nabeshima et al. (7). The TPC0806c/M/2008 GI strain is most closely related to Japan/JanAr13–04/M/2004 and China/Sh03–124/M/2003 strains (99.5% and 99.4% identities, respectively), and the YL0806f/M/2008 GI strain is most closely related to Japan/JanAr13–04/M/2004 and China/JX66/P/2008 strains (99.3% and 99.3% identities, respectively). Therefore, JEV GI strains from Taiwan were likely introduced by water birds migrating back and forth along the Asia–Australia flyway, which passes through many countries, including Indonesia, Malaysia, Australia, the Philippines, Taiwan, China, and Japan (3).

Our results clearly showed that JEV GIII strains remain the most dominant population circulating in Taiwan, although 2 JEV GI strains were isolated from wetland areas in northern Taiwan in 2008. Further studies are needed to continuously monitor the changing epidemiologic pattern of JEV strains endemic in Taiwan and newly introduced viruses.

This study was supported in part by grant 98-0324-01-F-20 from the National Research Program for Genome Medicine, by grant D0197-DC-2002 from Centers for Disease Control, Department of Health, Taipei, Taiwan, Republic of China, and by a grant from Ministry of Health, Labor and Welfare of Japan through the National Institute of Infectious Diseases (Tokyo).


DOI: 10.3201/eid1605.091055

References


