4. Ong CS, Eisler DL, Alikhani A, Fung VW, Tomblin J, Bowie WR, et al. Novel *Cryptosporidium* genotypes in sporadic cryptosporidiosis cases: first report of human infections with a cervine genotype. Emerg Infect Dis. 2002;8:263–8.
5. Ong CSL, Isaac-Renton JL. Molecular epidemiological investigations of waterborne cryptosporidiosis outbreaks in Canada. In: Latham SM, Smith HV, Wastling JM, editors. Workshop on the Application of Genetic Fingerprinting for the Monitoring of *Cryptosporidium* in Humans, Animals and the Environment; Boulder, Colorado, 2003 Aug 3–5. Marlow, Buckinghamshire (UK): Foundation for Water Research; 2004. p. 121–34.
6. Sulaiman IM, Lal AA, Xiao L. A population genetic study of the *Cryptosporidium parvum* human genotype parasites. J Eukaryot Microbiol. 2001;Suppl:24S–7S. DOI: 10.1111/j.1550-7408.2001.tb00441.x
7. Trotz-Williams LA, Martin DS, Gatei W, Cama V, Peregrine AS, Martin SW, et al. Genotype and subtype analyses of *Cryptosporidium* isolates from dairy calves and humans in Ontario. Parasitol Res. 2006;99:346–52. DOI: 10.1007/s00436-006-0157-4
8. Ong CSL, Chow S, So PPL, Chen R, Xiao L, Sulaiman I, et al. Identification of two different *Cryptosporidium hominis* subtypes from cases in the 2001 waterborne cryptosporidiosis outbreak in North Battleford, Saskatchewan. In: Robertson W, Brooks T, editors. Proceedings of the 11th Canadian National Conference and 2nd Policy Forum on Drinking Water; Calgary, Alberta; Apr 2004. Ottawa (Canada): Canadian Water and Wastewater Assoc., Health Canada & Alberta Environment; 2004. p. 628–39.
9. Glaberman S, Moore JE, Lowery CJ, Chalmers RM, Sulaiman I, Elwin K, et al. Three drinking-water–associated cryptosporidiosis outbreaks, Northern Ireland. Emerg Infect Dis. 2002;8:631–3.
10. Zhou L, Singh A, Jiang J, Xiao L. Molecular surveillance of *Cryptosporidium* spp. in raw wastewater in Milwaukee: implications for understanding outbreak occurrence and transmission dynamics. J Clin Microbiol. 2003;41:5254–7. DOI: 10.1128/JCM.41.11.5254-5257.2003

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Two Imported Chikungunya Cases, Taiwan

To the Editor: Chikungunya is a reemerging infectious disease, endemic to Africa and Southeast Asia, caused by a mosquito-borne alphavirus in the family *Togaviridae*. Numerous chikungunya outbreaks have been reported in Africa and Southeast Asia since chikungunya virus (CHIKV) was first isolated in Tanzania in 1953 (*1*). Since 2005, several Indian Ocean islands and India have experienced massive CHIKV outbreaks caused by the East/Central/South African genotype (*2*,*3*), whereas all earlier isolates from India during 1963–1973 were of the Asian genotype (*4*). Other chikungunya outbreaks caused by the Asian genotype were frequently reported during 1960–2003 in many Southeast Asian countries, including India, Malaysia, Indonesia, Cambodia, Vietnam, Myanmar, Pakistan, the Philippines, and Thailand. Epidemics caused by reemerging CHIKV were reported in Indonesia and Malaysia during 2005–2007 (*1*,*5*).

We have previously reported on fever screening at airports in Taiwan as part of active surveillance for a panel of notifiable infectious diseases such as dengue, gastroenteritis caused by enteric bacteria, malaria, and yellow fever (*6*). The activity is carried out by using infrared thermal scanners to measure the body temperature of arriving passengers. Diagnostic testing algorithms for patients being screened for fever were based on evaluation by airport clinicians. The rationale behind this process is to minimize local outbreaks by reducing the number of imported cases. We report 2 imported chikungunya case-patients identified in Taiwan by fever screening at airports; 1 had returned from Singapore in 2006, infected with CHIKV East/Central/South African genotype, and the other had returned from Indonesia in 2007, infected with the Asian genotype.

To assess viremic fever patients with alphavirus infection, a multiplex 1-step SYBR Green I-based real-time reverse transcription–PCR (RT-PCR) was developed. A cocktail consisting of 3 sets of primers was mixed and used for RT-PCR screening. The alphavirus-specific primer set (AL-2: 5′-AAG CTY CGC GTC CTT TAC CAA AG-3′ and AL-3: 5′-GTG GTG TCA AAC CCT ATC CA-3′) targeted a consensus region of the nonstructural protein 1 (*nsp1*) genes to detect all alphaviruses. The CHIKV-specific primer set (F-CHIK: 5′-AAG CTY CGC GTC CTT TAC CAA AG-3′ and R-CHIK: 5′-CCA AAT TGT CCY GGT CTT CCT-3′) targeted a region of the envelope protein 1 (E1) gene of CHIKVs (*7*). The Ross River virus–specific primer set (RRV-1: 5′-GGG TAG AGA GAA GTT YGT GGT YAG-3′ and RRV-2: 5′-CGG TAT ATC TGG YGG TGT RTG C-3′) targeted a region of the envelope protein 2 (E2) gene of Ross River virus. Positive results were then confirmed by gene sequence analysis, virus isolation, and serologic tests. The nucleotide sequences of complete structural polyprotein genes were determined as previously described and submitted to GenBank (accession nos. EU192142 and EU192143) (*3*,*8*). A phylogenetic tree, based on a total of 23 CHIKV partial E1 gene sequences (255 bp), was drawn to trace the origin of 2 CHIKV isolates reported in this study (Figure).

The initial imported chikungunya case was detected at Taiwan Taoyuan International Airport on November, 20, 2006, in a 13-year-old Taiwanese boy who was returning from studying at an international educational training center in Singapore. The second imported case was also detected at Taiwan Taoyuan International Airport on June, 20, 2007, in a 5-year-old boy on his return from visiting relatives in East Kalimantan Province, Indonesia,

with his mother. Both case-patients had fever, fatigue, generalized arthralgia, and rash. Real-time RT-PCR screening showed a high level of alphavirus, but not flavivirus, viremia on day 2 (Singapore imported case) and day 3 (Indonesia imported case) acute-phase samples. Serodiagnosis with immunofluorescent antibody assay (immunoglobulin M + G + A titers ≥640), and ELISA showed positive seroconversions for both patients.

Analysis showed that these 2 imported cases were introduced from Singapore and Indonesia and that the patients were infected with CHIKV of East/Central/South African genotype and Asian genotype, respectively. Unlike dengue, chikungunya is not endemic to Singapore. However, a small chikungunya outbreak caused by an Indian strain of East/Central/South African genotype transmitted by *Aedes aegypti* was reported in January 2008; this occurrence suggests that imported CHIKV may not be detected because of limited transmission and because the signs and symptoms may be mistaken for those of dengue (*9*). In contrast, chikungunya has been endemic to Indonesia since 1973. Indonesia had epidemic outbreaks in 1980, 1983–1984, and yearly outbreaks after 1998. In following the ongoing chikungunya epidemic, we have identified 4 additional imported chikungunya cases from Indonesia since July 2007.

A recent chikungunya outbreak in Italy demonstrated that *Aedes albopictus* is a competent vector that can initiate local transmission of imported CHIKV (*10*). In Taiwan, *Ae. albopictus* is distributed throughout the island, and *Ae. aegypti* is distributed only in southern Taiwan. With increasing numbers of imported CHIKV infections, the risk for local transmission is similar to that of dengue, especially in southern Taiwan. Our results show that CHIKVs of both genotypes are spreading in Southeast Asia. The cocirculation of dengue and chikungunya would likely be increased in many Southeast Asian and African countries because of the rise in international travel and the wide distribution of the competent vectors, *Ae. albopictus* and *Ae. aegypti*.

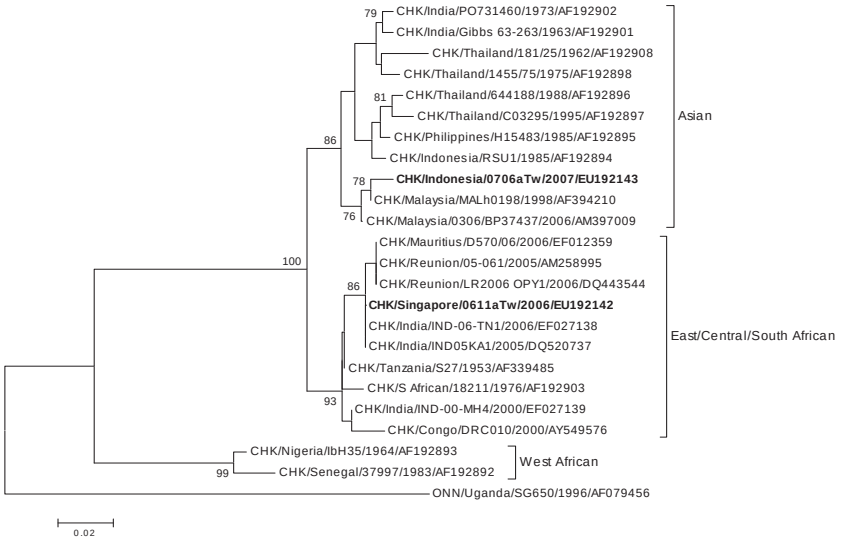

Figure. Phylogenetic relationships of chikungunya virus (CHIKV) isolates from 2 imported cases in Taiwan. The tree was constructed by the neighbor-joining method using partial nucleotide sequences of envelope protein 1 (E1) gene (255 bp) of 23 CHIKV strains. O'nyong-nyong (ONN) virus sequence was used as the outgroup virus. Bootstrap support values >75 are shown. The 2 imported CHIKV strains in Taiwan are designated by **boldface** type. Viruses were identified by using the nomenclature of virus/country/strain/year of isolation/GenBank accession no. Scale bar indicates substitutions per site.

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References

1. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol. 2007;88:2363–77. DOI: 10.1099/vir.0.82858-0
2. Bessaud M, Peyrefitte CN, Pastorino BA, Tock F, Merle O, Colpart JJ, et al. Chikungunya virus strains, Reunion Island outbreak [letter]. Emerg Infect Dis. 2006;12:1604–6.
3. Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med. 2006;3:e263. DOI: 10.1371/journal.pmed.0030263
4. Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep AB, Gandhe SS, et al. Chikungunya outbreaks caused by African genotype, India. Emerg Infect Dis. 2006;12:1580–3.
5. AbuBakar S, Sam IC, Wong PF, MatRahim N, Hooi PS, Roslan N. Reemergence of endemic chikungunya, Malaysia. Emerg Infect Dis. 2007;13:147–9.
6. Shu PY, Chien LJ, Chang SF, Su CL, Kuo YC, Liao TL, et al. Fever screening at airports and imported dengue. Emerg Infect Dis. 2005;11:460–2.
7. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African chikungunya viruses. J Virol Methods. 2005;124:65–71. DOI: 10.1016/j.jviromet.2004.11.002
8. Huang JH, Liao TL, Chang SF, Su CL, Chien LJ, Kuo YC, et al. Laboratory-based dengue surveillance in Taiwan, 2005: a molecular epidemiologic study. Am J Trop Med Hyg. 2007;77:903–9.
9. Ng LC. Chikungunya (07) Singapore. ProMed [cited 2008 Feb 16]. Available from http://www.promedmail.org, archive no.: 20080216.0622.
10. Bonilauri P, Bellini R, Calzolari M, Angelini R, Venturi L, Fallacara F, et al. Chikungunya virus in *Aedes albopictus*, Italy. Emerg Infect Dis. 2008;14:852–4.

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Chikungunya-related Fatality Rates, Mauritius, India, and Reunion Island

To the Editor: During the epidemic of chikungunya virus infection that occurred on Reunion Island in 2005–06, we reported an overmortality corresponding to the epidemic peak, which was estimated by comparing observed and expected deaths (*1*). The excess was similar to the number of deaths related to chikungunya infection reported by death certificates (*2*). The case-fatality rate (CFR) on Reunion Island was estimated to be 1/1,000 population.

According to Beesoon et al. (*3*), the fatality rate attributable to chikungunya infection was much higher on Mauritius: 743 deaths in excess of expected deaths led to a CFR of ≈4.5%, with 15,760 confirmed or suspected cases for 2005 and 2006 as reported in this letter. A similar CFR of 4.9% can be calculated for the city of Ahmedabad, India, during the 2006 chikungunya epidemic (*4*).

This 45- to 49-fold difference could be explained by a greater severity of chikungunya infection in Mauritius or Ahmedabad that could be due to a mutating strain, differences in the preexisting conditions of patients, differences in the management of patients, or by coincident deaths in excess from other causes.

However, the most probable explanation can be attributed to the surveillance systems of chikungunya cases. On Reunion Island, surveillance was highly sensitive and relied either on active case finding or on estimates of suspected cases. Results have been assessed by iterative external studies and serosurveys, and the CFR we found is likely consistent.

If we apply this rate to Mauritius, ≈60% of the population would have contracted chikungunya infection during this epidemic. If so, the risk of epidemic resurgence could be much lower than previously expected. This point raises the need to conduct seroprevalence studies in those territories, the only way to evaluate the herd immunity level of the population.

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References

1. Josseran L, Paquet C, Zehgnoun A, Caillere N, Le Tertre A, Solet JL, et al. Chikungunya disease outbreak, Reunion Island. Emerg Infect Dis. 2006;12:1994–5.
2. Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, Filleul L, et al. A major epidemic of chikungunya virus infection on Réunion Island, France, 2005–2006. Am J Trop Med Hyg. 2007;77:727–31.
3. Beesoon S, Funkhouser E, Kotea N, Spielman A, Robich RM. Chikungunya fever, Mauritius, 2006. Emerg Infect Dis. 2008;14:337–8.
4. Mavalankar D, Shastri P, Bandyopadhyay T, Parmar J, Ramani KV. Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. Emerg Infect Dis. 2008;14:412–5.

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Aquaculture and Florfenicol Resistance in *Salmonella enterica* Typhimurium DT104

To the Editor: In June 2006, the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organisation for Animal Health (OIE) convened an Expert Consultation to consider the risks to human health represented by the use of antimicrobial drugs in aquaculture. This would, therefore, appear to be an opportune time to reexamine some of the arguments that have been presented with respect to the assessment of these risks.

In their contributions to the debate regarding the risks associated with the use of antimicrobial agents in aquaculture, Angulo (*1*), Angulo and Griffin (*2*), Ribot et al. (*3*), and, more recently, Cabello (*4*) have argued that the available molecular evidence suggests that the *flo* gene that encodes chloramphenicol and florfenicol resistance in *Salmonella enterica* serovar Typhimurium DT104 (DT104) originally emerged in Japanese aquaculture and may have transferred horizontally from this host to DT104. This argument also appears in the report of the WHO/FAO/OIE consultation (ftp://ftp.fao.org/ag/agn/food/aquaculture_rep_13_16june2006.pdf). These authors (*1–4*) have based their argument on the assertions that florfenicol was first used in Japan and that *flo* gene–mediated resistance to this agent was first identified in bacteria isolated from Japanese fish farms.

In attempting to identify the date of the emergence of florfenicol resistance in Japanese aquaculture, Angulo and Griffin (*2*) state that florfenicol had been used in this country since the early 1980s. However, Schering Plough, the manufacturer of florfenicol, reports first marketing this