To investigate the mechanism of resistance in the isolates, fragments of *gyrA* (847 bp) and *parC* (822 bp) genes were amplified by using primers gyrA-1F (5′-gttttcccagtcacgacgttgtaATGACCGACGCAACCATCCGCCAC-3′) and gyrA-1R (5′-ttgtgagcggataacaatttcCCAGCTTGGCTTTGTTGACCTGATAG-3′), and parC-1F (5′-gttttcccagtcacgacgttgtaATGAATACGCAAGCGCACGCCCCA-3′) and parC-1R (5′-ttgtgagcggataacaatttcGGAATTGGCGTTCGGCGGCAGCTC-3′), respectively (sequences in lower case letters are adaptors for universal forward and reverse sequences were added for sequencing after amplification). Primers used for amplification of the *parE* gene were as described (*8*).

Sequencing of fragments of *gyrA*, *parC*, and *parE* genes showed a mutation in the *gyrA* gene in the 3 Cip-R isolates resulting in a Thr91 → Ile substitution. Cip-R1 also showed additional alterations of Asn103 → Asp, Ile111 → Val, and Val120 → Ile, which were described for meningococcal isolates (*3*). Sequences of *parC* and *parE* genes were the same as in a ciprofloxacin-susceptible isolate tested. The association of the Cip-R phenotype with mutations in *gyrA* was confirmed by transformation into the susceptible isolate by using appropriate PCR products (*9*). In addition to the common Thr91 → Ile substitution, the 3 Cip-R isolates were distinguishable by additional *gyrA* alterations or phenotypic and genotypic characteristics. This finding suggests independent events and argues against clonal expansion of Cip-R meningococci.

Serogroup A meningococcal isolates in France are rare and mostly imported. Lack of detection of ciprofloxacin resistance among African isolates tested in this study may be caused by the relatively low number of these isolates (n = 246). Therefore, surveillance of antimicrobial drug susceptibility of meningococcal isolates should be enhanced by using molecular approaches that can also be used as nonculture techniques. This molecular approach will be useful in countries with limited access to classic microbiologic culture–based methods. Reports of invasive cases caused by W-135 Cip-R meningococci should alert physicians who use quinolones to treat respiratory infections in elderly persons. This age group is affected most often by invasive meningococcal pneumonia and 54.5% of such cases are caused by W-135 meningococci (*10*).

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Rare *Cryptosporidium hominis* Subtype Associated with Aquatic Center Use

To the Editor: Cryptosporidiosis is the most frequently reported gastrointestinal illness in outbreaks associated with treated (disinfected) recreational water venues in the United States (*1*). In 2003, an increased number of cryptosporidiosis cases occurred in the Tri-Cities area of the Lower Mainland region (near Vancouver), in British Columbia, Canada. Although all cases were associated with the use of a community aquatic

center, their onset dates were spread over a 3-month period, and the link between cases was unclear. The aim of this study was to determine if the cases in this disease cluster were related. Although suitable molecular markers had yet to be defined at the time of the outbreak, recent reports on the use of the gp60 gene for subtyping in molecular epidemiologic studies (*2*,*3*) have enabled us to reanalyze the isolates and report these results.

Fifteen laboratory-confirmed cases were identified from October 15 to December 5, 2003. This number was in excess of the anticipated incidence rate for this community, which averaged 5 reported cryptosporidiosis cases per year. During the period of investigation, an incident of fecal contamination at the aquatic center on October 10, 2003, was documented and remediation involved increasing the free chlorine concentration. Because the regional health authority was concerned about the increased number of cases, the facility closed voluntarily on December 5 for further remediation. However, recorded free chlorine concentrations did not exceed 2.0 ppm at any time during the investigative period (October 5–December 31).

The health authority released a public advisory encouraging those who used the facility to submit fecal specimens for laboratory testing. The health authority also sent letters to family physicians in the area, informing them of the disease cluster and requesting that unpreserved stool specimens be collected, in addition to the formalin-fixed specimens, for routine diagnostic testing. Nine fecal specimens were collected from clinically symptomatic case-patients with histories of exposure to the implicated aquatic center. Five specimens were selected by using the criteria that they were from patients with laboratory-confirmed cryptosporidiosis cases from 5 separate households. The specimens were then coded for anonymity before subsequent molecular analysis. Genomic DNA was extracted from purified *Cryptosporidium* oocysts by freeze-thawing, and the species was determined by PCR amplification and sequencing of the 18S rRNA gene as described previously (*4*). The gp60 gene was also amplified by PCR by using primers described by Ong and Isaac-Renton (*5*). DNA sequences of amplicons were determined by cycle sequencing and assembled as described previously (*4*,*5*). The gp60 allele and subtype were identified by multiple sequence alignment with GenBank reference sequences and phylogenetic analysis that used ClustalX version 1.8 (www.clustal.org) as well as manual quantification of microsatellite repeats.

The 18S rRNA and gp60 genes were amplified successfully from 4 specimens. On the basis of the 18S rRNA gene sequence, all case-patients were infected with *Cryptosporidium hominis*, a species associated primarily with human-to-human transmission. The gp60 sequences from all 4 case-patients were identical and were subtype IdA19, a rarely reported subtype of *C. hominis*. Globally, most reports of the gp60 Id allele, such as 9 reported cases from Australia, have identified the IdA15G1 subtype (*3*). Another subtype, IdA18, was isolated from 5 case-patients in a 1997 foodborne outbreak in Spokane, Washington (*6*). To date, the IdA19 subtype has been identified in only 1 sporadic case, in northern Ontario (*7*), and a subset of cases (seven 1dA19 and 2 mixed IdA19 and IbA10G2) in the 2001 waterborne outbreak in North Battleford, Saskatchewan (*5*,*8*). The IdA19 subtype is identical in sequence to the IdA18 subtype except for 1 extra TCA repeat in the microsatellite region. Neither subtype has been reported anywhere in the world except in Canada and the Pacific Northwest.

Because cases from all previous *C. hominis* outbreaks of cryptosporidiosis in British Columbia have been caused by the IbA10G2 subtype, the most prevalent subtype in sporadic and outbreak cases around the world (*2*,*5*,*9*,*10*), our results indicate the presence of a new subpopulation of *C. hominis* parasites that could cause future disease outbreaks. The identification of the same subtype in all 4 case-patients with cryptosporidiosis associated with the use of a community aquatic center was consistent with their exposure history and confirmed that all cases were linked epidemiologically. However, the association between the single northern Ontario sporadic case and the larger number of Saskatchewan and British Columbia outbreak cases is uncertain. The association with the IdA18 subtype in the Washington foodborne outbreak is also unknown. Further research is needed to determine the distribution and prevalence of gp60 subtypes in Canada as well as other parts of the world before we can more clearly understand the transmission of the IdA19 subtype.

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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,