and rVIV 2 primers, the A-type DNA, respectively. The genus-specific DNA amplified both types at random.

Some colleagues have experienced similar difficulties with the primers pmk 8 and pmk 9 in samples from Vietnam (5); 2 of 5 samples gave false positive results for \( P. \) knowlesi. Unfortunately, their report did not mention which species was actually amplified (5).

Until recently, we had no satisfying explanation for the 3 assumed false-positive results. Then, in 2009, Imwong et al. reported that these \( P. \) knowlesi primers stochastically cross-react with \( P. \) vivax genomic DNA. No polymorphisms alleviating the binding of pmk 8 were found; however, a new PCR for \( P. \) knowlesi was introduced (10).

Given the large distribution of the vector and the natural host of \( P. \) knowlesi in Southeast Asia, it is likely that \( P. \) knowlesi will be found in other parts of Indonesia. As microscopic and molecular diagnosis of this parasite seems difficult, the underestimation of its distribution and clinical relevance can be assumed.

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Ermia Sulistyaningsih, Loeky Enggar Fitri, Thomas Löschner, and Nicole Berens-Riha

Author affiliations: University of Jember, Jember, Indonesia (E. Sulistyaningsih); Brawijaya University, Malang, Indonesia (L.E. Fitri); and Universität München, Munich, Germany (E. Sulistyaningsih, T. Löschner, N. Berens-Riha)

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References


Address for correspondence: Nicole Berens-Riha, Leopoldstrasse 5, 80802 Munich, Germany; email: berens@lrz.uni-muenchen.de

Toscana Virus Infection Imported from Elba into Switzerland

To the Editor: Toscana virus (TOSV) is a serotype of Sandfly fever Naples virus (SFNV) within the family Bunyaviridae and the genus Phlebovirus. TOSV is transmitted to humans by sandflies (Phlebotomus spp.) and is a prominent cause of aseptic meningitis in Mediterranean countries (1). In Italy, for populations living in rural areas and persons engaging in outdoor activities, the highest risk for acquiring TOSV is from August through October (1). TOSV infections should therefore be considered in travelers returning from the Mediterranean area who have fever and signs of meningitis. Laboratory diagnosis of TOSV infections is often limited to the detection of immunoglobulin (Ig) M and IgG because of the short period of viremia and the low amount of virus in the cerebrospinal fluid (CSF) during the acute phase (2). We report a reverse transcription–PCR (RT-PCR)–confirmed TOSV infection acquired on the island of Elba that was then imported into Switzerland.

A 17-year-old man was referred to Basel University Medical Clinic, Liestal, Switzerland, in August 2009 with headache, recurrent episodes of vomiting, photophobia and phonophobia, and an elevated temperature of 38.1°C. The patient had returned to Switzerland from a vacation on the island of Elba, Italy, 14 days before. He recalled that he had received multiple insect bites on the beach. Cardiopulmonary and neurologic examination showed tachycardia and nuchal rigidity. Results of a complete blood count and liver and kidney function tests showed no abnormalities. CSF analysis showed lymphocytic pleocytosis (47 cells/μL), and aseptic meningitis of viral origin was suspected. Empirical treatment with acyclovir (2.3 g/day) was started for the first 48 hours. The results of a
PCR for herpesviruses were negative in the CSF sample, and serologic testing showed no evidence of acute infection with herpesviruses. CSF, urine, and blood cultures showed negative results for fungi and bacteria, including mycobacteria. The patient did not show signs of immune deficiency.

Serum and CSF samples were sent to the Bernhard-Nocht-Institute for Tropical Medicine in Germany for SFNV diagnostics. Results of immunofluorescent assays for TOSV and SFNV were positive with IgM titers of 1,280 and 160, respectively (cut-off 20) and IgG titers of 5,120 and 640, respectively (cut-off 20). Real-time RT-PCRs for detection of TOSV and SFNV were performed using the CSF sample according to a recently published protocol (3). A positive result was obtained for TOSV, and the PCR result was confirmed by sequencing the PCR product. Phylogenetic analysis demonstrated that the TOSV from Elba clustered with the TOSV A lineage (Figure). Attempts to isolate TOSV from the CSF sample in cell culture failed. The patient was afebrile on the second day of hospitalization, headache vanished on the third day, and he was discharged on day 5 restitutio ad integrum (fully recovered).

This report demonstrates the presence of TOSV on the island of Elba by molecular detection and typing. This finding is in agreement with previous serologic reports on imported TOSV infections from this area into central Europe (5,6). However, because of serologic cross-reactivity, serologic tests are usually not able to clearly discriminate between TOSV and other SFNV infections (2).

Real-time RT-PCR is the most appropriate tool for the differentiation of TOSV from other SFNV infections and enables molecular typing of amplified sequences. The Bayesian phylogenetic tree calculated with the short PCR fragment (111 bp, GenBank accession no. GU270841) of the nucleocapsid coding sequence (Figure) shows the same topology of the main clades when compared with trees obtained with the complete N coding sequence (7,8). The assignment of the TOSV from Elba to lineage A (Figure) is consistent with results of previous studies, demonstrating that this is the dominant genotype of TOSV in mainland Italy and the island of Sardinia (8).

The presence of TOSV A on the island of Elba is a major public health issue for the local population and for the >2 million tourists that visit Elba every year (9). Given the incidence of TOSV infections in other surrounding Mediterranean countries, one could assume that the virus is present in other islands of the Mediterranean, posing a public health problem for the resident population and tourists alike. Molecular and serologic surveillance studies in Mediterranean countries could identify potential high risk areas for TOSV infections to help prevent exposure of local residents and tourists to the virus. Moreover, the risk of transfusion-associated transmission of arboviruses in European countries should be addressed.

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Martin Gabriel, Christiane Resch, Stephan Günther, and Jonas Schmidt-Chanasit

Author affiliations: Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (M. Gabriel, S. Günther, J. Schmidt-Chanasit); and Basel University Medical Clinic, Liestal, Switzerland (C. Resch)

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Imported Mollusks and Dissemination of Human Enteric Viruses

To the Editor: The globalization of food production and trade has increased the potential risk for infectious foodborne diseases. Hepatitis A virus (HAV) and norovirus (NoV) constitute the most important foodborne pathogens of humans in terms of numbers of outbreaks and persons affected in industrialized countries (1,2). In these countries, improvement of health conditions and development of specific vaccines are changing the epidemiologic pattern of diseases such as hepatitis A, decreasing their prevalence and increasing the susceptibility of the unvaccinated adult population (I). In recent years, numerous cases of gastroenteritis caused by NoV and hepatitis A linked to imported shellfish have been reported (2–5). In Spain, 2 notable hepatitis A outbreaks associated with clams (Donax sp.) imported from Peru occurred in 1999 and 2008. In both situations, the Spanish Ministry of Health activated the National System of Epidemiologic Surveillance and the European Community Rapid Alert System for Foodstuffs. The implicated shellfish batches were immobilized or removed, and all the shellfish from Peru were banned from the market (1). We present further evidence that imported shellfish from developing countries, where these pathogens are endemic, can be a vehicle for viral gastroenteritis and HAV infections in areas where they are not endemic.

Fifty mollusk samples imported into Spain during September 2006–March 2009 were analyzed for NoV genotype I (GI) and GII, HAV and astrovirus (AsV). Countries of origin were Morocco, Peru, Vietnam, and South Korea (Table). The species studied were clams (Callista chione, n = 25; Transanella pannosa, n = 6; Meretrix lyrata, n = 3; and Donax sp., n = 5), oysters (Crassostrea angulata, n = 1), cockles (Cerastoderma edule, n = 1), and razor clams (Solen marginatus, n = 1 and Ensis sp., n = 8). Digestive tissue was dissected from duplicated samples (10–20 individual mollusks) and homogenized with 0.1% peptone water (pH 7.4), centrifuged at 1,000 × g for 5 min, and supernatant recovered. RNA was extracted by using both Total Quick RNA extraction Cells and Tissue kit (Talent, Trieste, Italy) and Nucleospin RNA Virus Kit (Macherey-Nagel, Düren, Germany).

NoV and HAV were detected by real-time reverse transcription–PCR (RT-PCR) by using the Platinum Quantitative RT-PCR Thermoscript 1-step system (Invitrogen, Carlsbad, CA, USA) (25 μL final volume) with 5 μL of template RNA, and primers, probes, and conditions as described (7). A sample that displayed a cycle threshold value ≤41 was considered positive. AsV was detected by standard RT-PCR (7), coupled with hybridization by using specific biotin-labeled probes with the commercial Kit Hybridowell universal (Argene, Varilhes, France).

Negative and specific positive controls for HAV, NoV, and AsV were introduced in each run. Real-time RT-PCR included appropriate external controls in each analysis to avoid underestimation of viral load. A mutant, nonvirulent, infective strain of mengovirus (vMC0) (10^3 PFU) was used as control for extraction. To calculate the real-time RT-PCR efficiencies, external viral RNA (HAV, 10^3 copies) or synthetic DNA (NoV, 10^6 copies) controls for the respective virus were co-amplified with each template viral RNA as described (8). The number of RNA viral genome copies per gram of digestive tissue (RNA copies/g digestive tissue) was estimated by using standard curves generated from RNA transcripts and synthetic DNA (8) and corrected with the extraction and real-time RT-PCR efficiencies.