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 pmkr 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 pmk8 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.

Acknowledgments

Erma Sulistyaningsih, Loeki Enggar Fitri, Thomas Löscher, and Nicole Berens-Riha

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

References

1. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004;363:10017–24.

2. White NJ. *Plasmodium knowlesi*: the fifth human malaria parasite. Clin Infect Dis. 2008;46:172–3. DOI: 10.1086/524889

3. Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. Emerg Infect Dis. 2004;10:2211–3.

4. Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, et al. Human infections with *Plasmodium knowlesi*, the Philippines. Emerg Infect Dis. 2008;14:811–3. DOI: 10.3201/eid1405.071407

5. Van den Eede P, Van NH, Van Overmeir C, Vythilingam I, Duc TN, Hung LX, et al. Human *Plasmodium knowlesi* infections in young children in central Vietnam. Malar J. 2009;8:249–54. DOI: 10.1186/1475-2875-8-249

6. Cox-Singh J, Singh B. Knowlesi malaria: newly emergent and of public health importance? Trends Parasitol. 2008;24:406–10. DOI: 10.1016/j.pt.2008.06.001

7. Reid MJ, Ursic R, Cooper D, Nazzari H, Griffiths M, Galdikas BM, et al. Transmission of human and macaque *Plasmodium* spp. to ex-captive orangutans in Kalimantan, Indonesia. Emerg Infect Dis. 2006;12:1902–8.

8. Singh B, Divis PCS. Orangutans not infected with *Plasmodium vivax* or *P. cynomolgi*, Indonesia. Emerg Infect Dis. 2009;15:1657–8.

9. Escalante AA, Cornejo OE, Freeland DE, Poe AC, Durrego E, Collins WE, et al. A monkey's tale: the origin of *Plasmodium vivax* as a human malaria parasite. Proc Natl Acad Sci U S A. 2005;102:1980–5. DOI: 10.1073/pnas.0409652102

10. Imwong M, Tanomsing N, Pukrittayakamee S, Day NPJ, White NJ, Snounou G. Spurious amplification of *Plasmodium vivax* small subunit RNA gene using the primers currently used to detect *P. knowlesi*. J Clin Microbiol. 2009;47:4173–5. Epub 2009 Oct 7. DOI: 10.1128/JCM.00811-09

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

EMERGING INFECTIOUS DISEASES

Free Online   RSS Feed

in PubMed Central

Ahead of print

Peer-Reviewed

CME

podcasts

GovDelivery

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 phlyogenetic 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.
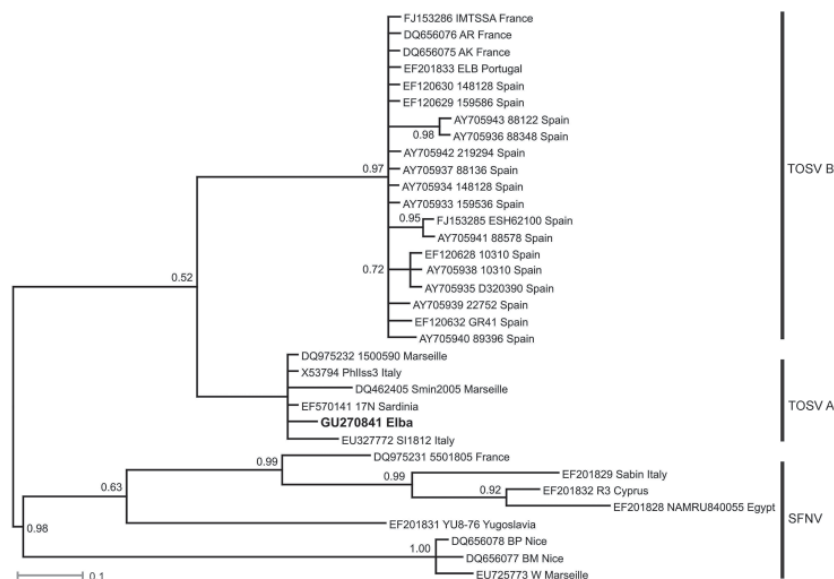

Figure. Bayesian phylogenetic tree of Toscana virus (TOSV) and *Sandfly fever Naples virus* (SFNV) strains. For each sequence used, GenBank accession number, strain designation, and strain origin are shown. Phylognetic analysis was performed by using MrBayes 3.0 program (*4*) with a general time reversible substitution model. Substitution rates were assumed to follow a gamma plus invariants distribution. Three heated chains and a single cold chain were used in all Markov Chain Monte Carlo analyses, which were run for 1,000,000 generations, sampling 1 tree every 100 generations. Trees obtained before convergent and stable likelihood values were discarded (i.e., a 2,500 tree burn-in). Four independent runs, each started from different, randomly chosen trees, were performed to assess convergence. Posterior probabilities for nodes were assembled from all post burn-in trees (i.e., 30,004 trees per analysis). Posterior probabilities are shown on each node. Scale bar indicates nucleotide substitutions per site. The newly described TOSV sequence from Elba is shown in **boldface**.

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)

References

1. Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sánchez-Seco MP, et al. Emergence of Toscana virus in Europe. Emerg Infect Dis. 2005;11:1657–63.
2. Dionisio D, Esperti F, Vivarelli A, Valassina M. Epidemiological, clinical and laboratory aspects of sandfly fever. Curr Opin Infect Dis. 2003;16:383–8. DOI: 10.1097/00001432-200310000-00003
3. Weidmann M, Sanchez-Seco MP, Sall AA, Ly PO, Thiongane Y, Lô MM, et al. Rapid detection of important human pathogenic Phleboviruses. J Clin Virol. 2008;41:138–42.
4. Ronquist F, Huelsenbeck JP. Mrbayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003; 19:1572–4. DOI: 10.1093/bioinformatics/btg180
5. Dobler G, Treib J, Haass A, Frösner G, Woesner R, Schimrigk K. Toscana virus infection in German travellers returning from the Mediterranean. Infection. 1997;25:325. DOI: 10.1007/BF01720413
6. Sonderegger B, Hachler H, Dobler G, Frei M. Imported aseptic meningitis due to Toscana virus acquired on the island of Elba, Italy, August 2008. Euro Surveill. 2009;14:pii=19079.
7. Charrel RN, Moureau G, Temmam S, Izri A, Marty P, Parola P, et al. Massilia virus, a novel Phlebovirus (Bunyaviridae) isolated from sandflies in the Mediterranean. Vector Borne Zoonotic Dis. 2009;9:519–30. DOI: 10.1089/vbz.2008.0131
8. Collao X, Palacios G, Sanbonmatsu-Gámez S, Pérez-Ruiz M, Negredo AI, Navarro-Marí JM, et al. Genetic diversity of Toscana virus. Emerg Infect Dis. 2009;15:574–7. DOI: 10.3201/eid1504.081111
9. Towards regional economic development 2006/2010. Tourism and innovations objectives and instruments [in Italian]. 2006 [cited 2010 Apr 1]. http://www.provincia.livorno.it/economia/turismo/forumturismo2006/FormatLivorno4aprile2006.pdf

Address for correspondence: Jonas Schmidt-Chanasit, Department of Virology, Clinical Virology LaboratoryBernhard Nocht Institute for Tropical Medicine, Bernhard Nocht Strasse 74, D-20359 Hamburg, Germany; email: jonassi@gmx.de

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 (*1*). 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 European Union (*6*). 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 $1,000 \times g$ 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 (vMC_0) (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, 10^5 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.