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Detection of Measles Virus Genotype B3, India

To the Editor: Molecular epidemiologic investigations and virologic surveillance contribute notably to the control and prevention of measles (1). Nearly half of measles-related deaths worldwide occur in India, yet virologic surveillance data are incomplete for many regions of the country (2,3). Previous studies have documented the presence of measles virus genotypes D4, D7, and D8 in India, and genotypes D5, D9, D11, H1, and G3 have been detected in neighboring countries (3,4).

Kerala, India's southernmost state, has high measles vaccination coverage compared with many other states in the country; however, the disease is still endemic in the region. Two districts, Thiruvananthapuram and Malappuram, report the highest numbers of cases (5). Baseline data on circulating measles virus genotypes are needed for measles elimination, but such data are not available for Kerala. In this context, we performed a pilot genetic analysis of the measles virus strains circulating in Thiruvananthapuram, the capital of Kerala. We used throat and nasopharyngeal swab and serum samples from children admitted to Sree Avittom Thirunal Hospital during measles outbreaks occurring March–August 2012.

We used the Vero/human-SLAM cell line (<http://www.phe-culturecollections.org.uk>) for isolation of measles virus from throat and nasopharyngeal swab samples. For serologic confirmation of cases, we used a commercial measles IgM ELISA kit (IBL International GmbH, Hamburg, Germany). Virus genotyping was based on the 450-nt coding sequence for the carboxyl terminus of nucleoprotein (N) of measles virus, as recommended by the World Health Organization (3,6). We extracted

RNA from the samples using TRIzol reagent (GIBCO-BRL, Grand Island, NY, USA). We performed reverse transcription PCR using a SuperScript One-Step RT-PCR kit with a Platinum *Taq* system (Invitrogen, Carlsbad, CA, USA) and previously described primers (3,6). Amplicons were subjected to bidirectional sequencing using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). We edited and aligned nucleotide sequences using Bio Edit 7.1.11 software (7). Phylogenetic analysis was performed by using the maximum-likelihood method implemented in the MEGA5 program (8) to compare the determined N gene sequences with the World Health Organization reference sequences of the 24 known measles genotypes.

PCR products could be amplified from 16 of the 24 samples analyzed. Ten samples provided high quality sequence reads for the N gene coding region, which were used for further analysis. Clinical and demographic data for these 10 cases, virus isolation status, and GenBank accession numbers of the sequences are summarized in the Table.

Phylogenetic analysis revealed 1 of the 10 measles virus strains to be of genotype D8 (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/20/10/13-0742-Techapp1.pdf>), a genotype previously found to be circulating in Kerala and in other regions of India (3,6,9,10). The other 9 virus strains were closely related to B3 genotype reference strains, indicating circulation of the B3 genotype in Kerala (online Technical Appendix Figure 1). The nucleotide sequences of 7 of the 9 strains were identical, indicating a single chain of transmission. The remaining 2 samples showed sequence divergence, indicating independent sources of infection. In a phylogenetic analysis comparing the Kerala B3 genotypes and a dataset of

Table. Details of the clinical samples used for genotype analysis of measles virus strains, Kerala, India, 2012

Strain name*	Clinical details of patients						GenBank accession no.
	Age	Sex	Measles vaccination status	Contact history	Complications		
MVi/Thiruvananthapuram.IND/12.12[B3]	11 mo	F	No	Yes	Pneumonia	KC997602	
MVs/Thiruvananthapuram.IND/14.12/1[B3]	9 mo	M	No	Yes	Pneumonia	KC997603	
MVs/Thiruvananthapuram.IND/14.12/2[B3]	3 y	M	Yes	Yes	None	KC997604	
MVi/Thiruvananthapuram.IND/14.12/3[B3]	6 y	M	No	No	None	KC997605	
MVs/Thiruvananthapuram.IND/14.12/4[B3]	7 mo	M	No	Yes	Pneumonia	KC997606	
MVi/Thiruvananthapuram.IND/15.12[B3]	10 mo	M	Yes	Yes	None	KC997607	
MVs/Thiruvananthapuram.IND/30.12[B3]	7 mo	F	No	No	Pneumonia	KC997608	
MVs/Thiruvananthapuram.IND/31.12/1[B3]	9 mo	F	No	Yes	Pneumonia	KC997609	
MVs/Thiruvananthapuram.IND/31.12/2[B3]	2 y	F	No	Yes	Pneumonia	KC997610	
MVs/Thiruvananthapuram.IND/34.12[D8]	10 y	M	No	No	None	KC997611	

*Named according to World Health Organization nomenclature following the scheme: measles virus (MV), isolate (i) or clinical specimen (s)/place. country/epidemiologic week.year/sample identity [genotype].

global measles B3 genotypes selected from GenBank, the strains from Kerala formed a separate cluster (online Technical Appendix Figure 2). This cluster also contains a strain from Germany (MVs/Regensburg.DEU/37.12/). The strains in this cluster show close identity to a measles strain identified in the state of New York, USA. A search of the MeaNS (Measles Nucleotide Surveillance) database revealed that the Kerala B3 sequences had the closest match to the strain isolated in Germany mentioned above and also to a strain from the Sultanate of Oman (MVs/Muscat.OMN/38.11/).

The B3 genotype identified in this study could be a previously undetected genotype endemic to India or a recent importation. B3 is endemic to many countries in Africa, and its importation into Europe and North America and elsewhere has been described (4). Further studies with samples from a wider geographic area of Kerala are required to determine the spread and genetic diversity of these strains and ascertain their relationship to the global B3 strains. It would also be of interest to determine whether B3 strains co-circulate with D8 strains, whether they will eventually replace D8 as the predominant genotype in Kerala, or if they will cease to exist as the outbreak diminishes. This report underscores the need for systematic nationwide measles virus surveillance in India to

identify all endemic virus genotypes and to monitor importation of new virus strains from other countries.

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O'nyong-nyong Virus Infection Imported to Europe from Kenya by a Traveler

To the Editor: O'nyong-nyong virus (ONNV) is a mosquito-borne RNA virus of the *Togaviridae* family. The virus was first isolated in June 1959 from serum samples from febrile patients in the northern province of Uganda (1). Unlike other members of the alphavirus genus, ONNV is primarily transmitted by anopheline mosquitoes (2). ONNV is genetically and serologically related to chikungunya virus (CHIKV) (1), but is restricted to the African continent. The clinical picture resembles CHIKV infection, i.e., a self-limited febrile illness characterized by headache, rash, and joint pain. In contrast to CHIKV, ONNV is reported to cause

lymphadenopathy more often and affected joints do not show effusions (3).

ONNV caused 2 large-scale epidemics in East Africa during 1959–1962 and in 1996. The first instance had spread from Uganda south to Mozambique and westward to Senegal. Comprising >2 million cases in east Africa alone, this first epidemic ranked among the largest mosquito-borne virus outbreaks recorded (4). After an absence of reported cases for 35 years, a second ONNV epidemic occurred in Uganda (3–4). Patients had fever, a maculopapular rash, pruritis, myalgia, and arthralgia of large joints. Lymphadenitis, most often of the posterior cervical spine region, was also observed (3). Despite the virus' potential to cause large outbreaks and its endemicity in the vast geographic area of East Africa, and at least sporadic occurrence in West Africa, imported cases to other areas have not been reported.

On October 14, 2013, a 60-year-old woman residing in Germany who had returned home 2 days before from a 7-week vacation in East Africa sought medical attention at the University Medical Center, Section of Clinical Tropical Medicine, in Heidelberg for recurring fever and illness that began during her travel. She and her husband had traveled from Kenya to Uganda, Rwanda, Tanzania, and back to Kenya, along the shore of Lake Victoria. Bed nets and malaria prophylaxis were used regularly. On October 9, she had experienced the first episode of fever, general malaise, arthralgia, and nausea while staying at the lake shore near the city of Kisumu, Kenya. Fever had persisted until October 12. Thin and thick blood films, examined in a local hospital and later in Nairobi, did not show malarial parasites.

October 14 was day 5 of symptom onset. Her fever reached 39°C and lasted 3 more days. It was accompanied by cervical spine and nuchal lymphadenopathy, nausea, and arthralgia

of the small joints of her hands and feet. A maculopapular rash developed, which covered her face, hands, feet, and trunk. Her face, hands, and feet were edematous. Laboratory tests on admission to the medical center revealed a slightly elevated C-reactive protein level of 13 mg/L (reference level <5). Full blood count and results of liver function tests were within reference ranges. Thin and thick blood films were examined again and were negative for *Plasmodium* spp. A serum sample from the day of admission showed anti-ONNV IgM and IgG and anti-CHIKV IgM and IgG in the indirect immunofluorescence assay, according to Tappe et al. (5, Table). Serology for dengue virus and generic alphavirus reverse transcription PCR (6) were negative. A 4-fold anti-ONNV IgG titer decrease in the indirect immunofluorescence assay was demonstrated in the second serum sample, which was collected 26 days after disease onset (Table). The presence of ONNV-specific neutralizing antibodies in the second serum sample was confirmed by a virus neutralization test. Cross-neutralizing antibodies against CHIKV were detected also, but with a notably lower titer (1:80) when compared with the ONNV titer (1:1,280) (Table). Ten days after symptom onset, the patient recovered spontaneously. Her husband had no symptoms of illness during travel or after returning.

We report the laboratory-confirmed case of an ONNV infection imported into Europe. This patient most likely was infected in the eastern part of Kenya (Kisumu region), where she had stayed during the 2 weeks before symptom onset. The case highlights the fact that ONNV infections, which occur sympatrically with CHIKV infections in East Africa, lead to symptoms resembling CHIKV infection. The clinical and laboratory findings emphasize the importance of a careful diagnostic and clinical assessment of travelers

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Technical Appendix Figure. Phylogenetic analysis of B3 genotype measles virus sequences detected in India with selected global B3 genotypes. A D8 genotype sequence from Thiruvananthapuram was used as the outgroup. We performed maximum-likelihood analysis with 1,000 bootstrap replications using Kimura2-parameter correction with gamma-distributed settings. Solid red triangles indicate strains from the current study; scale bar indicates nucleotide substitutions per site.