of MRSA. Dr. Hirai is a professor in the department of infectious diseases at Tokyo Medical University Hachioji Medical Center. His primary research interests are infectious diseases and infection control science.

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Address for correspondence: Hidemasa Nakaminami, Tokyo University of Pharmacy and Life Sciences, Department of Microbiology, School of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan; email: nakami@toyaku.ac.jp

Detection of Rocio Virus SPH 34675 during Dengue Epidemics, Brazil, 2011–2013

Marielena V. Saivish, Vivaldo G. da Costa, Roger L. Rodrigues, Valéria C.R. Féres, Eduardo Montoya-Diaz, Marcos L. Moreli

Author affiliations: Federal University of Goiás, Jataí, Brazil (M.V. Saivish, R.L. Rodrigues, M.L. Moreli); University of Brasília, Brasília, Brazil (V.G. da Costa); Federal University of Goiás, Goiânia, Brazil (V.C.R. Féres); Paul-Ehrlich-Institut, Langen, Germany (E. Montoya-Diaz)

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Recent seroprevalence studies in animals detected Rocio virus in regions of Brazil, indicating risk for re-emergence of this pathogen. We identified Rocio virus RNA in samples from 2 human patients for whom dengue fever was clinically suspected but ruled out by laboratory findings. Testing for infrequent flavivirus infections should expedite diagnoses.

Brazil has been affected by outbreaks caused by viruses of the genus *Flavivirus*, such as dengue (DENV), Zika, and yellow fever viruses, along with co-infections with other arboviruses (*1*). The amino acid sequences of polyproteins from viruses of this genus are very similar, which has limited the development of detection methods, often resulting in cross-reactions within serocomplexes during serologic testing (*2*). Therefore, tracking in areas where mosquito-specific flaviviruses co-circulate may have led to underestimated infections because of the detection and the hierarchy of disease based on medical importance.

Rocio virus (ROCV) is a potentially emerging neurotropic flavivirus in Brazil; however, because relatively little is known about the biology of this virus, technologies for its detection are limited (*3*–*5*). In 1975, ROCV was found to be related to the causative agent of a fatal outbreak of human encephalitis in Brazil; the case-fatality rate was 13%, and neurologic sequelae affected 20% of patients (*5*). The unexpected outbreak ended in 1980, but little documentation exists with regard to circulation of ROCV in Brazil.

To determine the extent of ROCV circulation in different areas of Brazil, we screened 647 serum samples collected during an outbreak of dengue fever during 2011–2013. The samples came from patients in care units of the public health system, which offer 24-hour outpatient urgent care, and emergency services in the city of Goiânia, central Brazil. The samples were from patients of all age groups and sexes

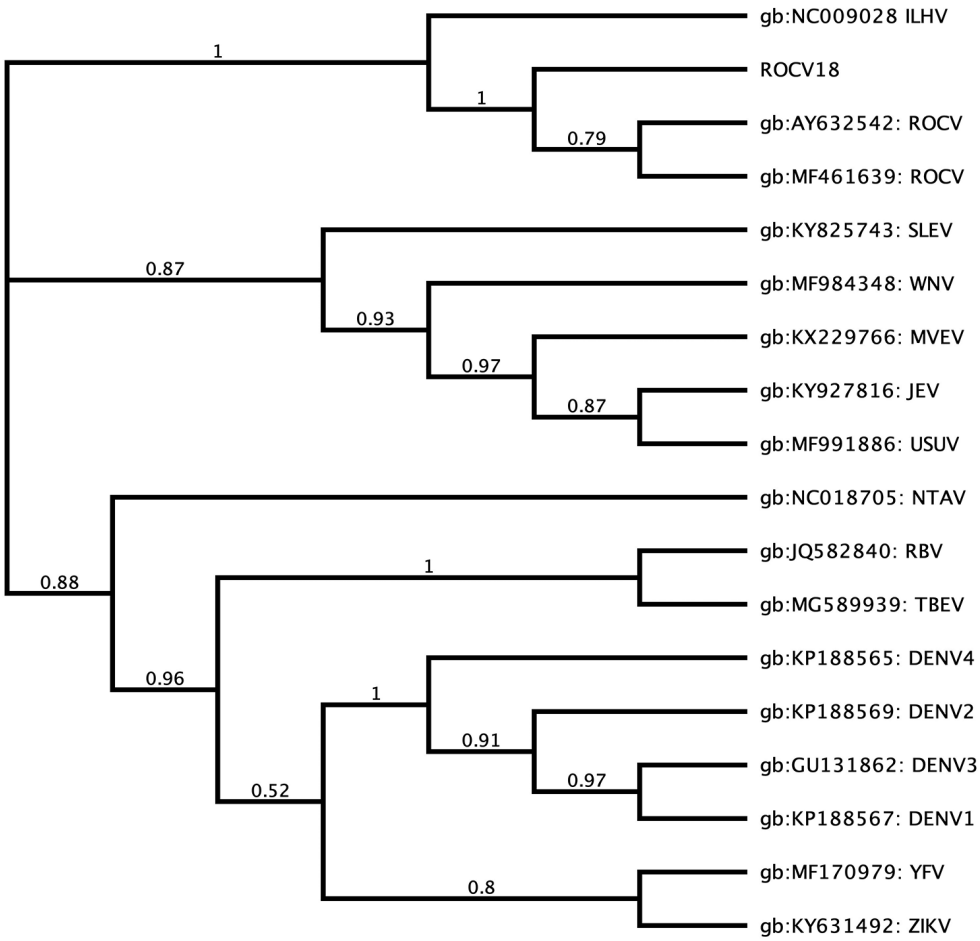

Figure. Phylogenetic analysis of the ROCV nonstructural 5 gene (ROCV18) detected during dengue epidemics in Brazil, 2013, and reference sequences. Tree constructed by using the maximum-likelihood method. Pairwise distances were calculated by using the neighbor-joining algorithm, and node numbers represent bootstrap values (10,000 replicates). GenBank accession numbers are provided. DENV, dengue virus; ILHV, Ilheus virus; JEV, Japanese encephalitis virus; MVEV, Murray Valley encephalitis virus; NTAV, Ntaya virus; RBV, Rio Bravo virus; ROCV, Rocio virus; SLEV, Saint Louis encephalitis virus; TBEV, tick-borne encephalitis virus; USUV, Usutu virus; WNV, West Nile virus; YFV, yellow fever virus; ZIKV, Zika virus.

who exhibited signs and symptoms of suspected dengue infection. During the outbreak, the city reported ≈88,000 cases of DENV infection (*6*). Of the 647 samples screened for DENV by use of serologic and molecular methods, 121 were negative for DENV. We subsequently screened those 121 samples for ROCV.

Using nested PCR with genus-specific primers (*7*), we detected the ROCV nonstructural (NS) 5 gene in 2 of the 121 samples. We used the amplified sequences from the ROCV NS5 gene for phylogenetic analysis, which confirmed 100% identity with the consensus sequence of ROCV NS5 in strain SPH 34675, the strain isolated from the 1975 encephalitis outbreak. Furthermore, the detected NS5 ROCV gene (ROCV 18) is related to Ilheus virus from the Japanese encephalitis virus complex and did not change the topology of the phylogenetic tree with other pathogenic flaviviruses, as previously reported (*3*) (Figure).

The 2 ROCV-positive samples were from a 33-year-old female patient and a 47-year-old male patient. The female patient experienced prostration, abdominal pain, diarrhea, and thrombocytopenia (120,000 platelets/mm^3), and the male patient experienced headache, eye pain, pruritus, nausea, and leukopenia (3,560 cells/mm^3) (Appendix, https://wwwnc.cdc.gov/EID/article/26/4/19-0487-App1.pdf). Both patients had fever, myalgia, and arthralgia, but they denied having had chronic diseases and had been vaccinated against yellow fever virus. No information about patients' residence or travel history was available. The patients received ambulatory care, and their clinical outcome was cure.

The molecular diagnostic result for positive ROCV in humans reported in this study corroborates the results of other studies involving serologic tests for ROCV in animals (*8*,*9*) and demonstrates the high probability that ROCV is circulating in different areas of Brazil. Our findings point out the need for clinicians to clearly establish flavivirus infection diagnoses by testing for various and infrequent regional flaviviruses

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About the Author

Ms. Saivish is a biomedical researcher in the Laboratory of Virology of the Federal University of Goiás–Jataí Regional. Her area of interest is the development of viral diagnostic methods for emergent arboviruses and hantavirus.

Address for correspondence: Eduardo Montoya-Diaz, Veterinary Medicine Division, Paul-Ehrlich-Institut, Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany; email: eduardo.montoyadiaz@pei.de; Marcos Lázaro Moreli, Laboratório de Virologia, Universidade Federal de Goiás-Regional Jataí, Câmpus Jatobá, Cidade Universitária, BR 364, km 195, nº 3800, CEP 75801-615, Goiás, Brazil; email: marcoslmoreli@gmail.com

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Epidemiology of Lassa Fever and Factors Associated with Deaths, Bauchi State, Nigeria, 2015–2018

Mohammed A. Abdulkarim, Sufiyan M. Babale, Chukwuma D. Umeokonkwo, Eniola A. Bamgboye, Adebobola T. Bashorun, Auwal A. Usman, Muhammad S. Balogun

Author affiliations: Gombe State Ministry of Health, Gombe, Nigeria (M.A. Abdulkarim); Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Nigeria (M.A. Abdulkarim, A.T. Bashorun, A.A. Usman, M.S. Balogun); Ahmadu Bello University, Zaria, Nigeria (S.M. Babale); Federal Teaching Hospital, Abakaliki, Nigeria (C.D. Umeokonkwo); University of Ibadan, Ibadan, Nigeria (E.A. Bamgboye)

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We report the epidemiology of Lassa fever in Bauchi State, a disease-endemic region, in Nigeria. Since 2015, major increases in Lassa fever attack rate and in the case-fatality rate have occurred in this state. A delay in seeking care by a case-patient for >7 days after symptom onset was the major predictor of death.

In recent years, Lassa fever (LF) outbreaks in Nigeria have become more frequent and larger in magnitude; the outbreak in 2018 was described as the largest in history (*1*). Bauchi State, which had never reported an LF case before 2012, has quickly become one of the high-risk states for LF in this country (*2*). We report LF epidemiology in Bauchi State and identify factors associated with death.

We retrospectively reviewed data for LF cases during January 2015–December 2018 obtained from the platform for integrated disease surveillance and

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Detection of Rocio Virus SPH 34675 during Dengue Epidemics, Brazil, 2011–2013

Appendix

Appendix Table 1. Clinical data of the acute febrile patients positive for Rocio virus infection in Goiânia county, Central Brazil

	Study patients	
Sign or symptom	ROCV (p18), female	ROCV (p21), male
Myalgia	Yes	Yes
Arthralgia	Yes	Yes
Prostration	Yes	No
Abdominal pain	Yes	No
Diarrhea	Yes	No
Headache	No	Yes
Eye pain	No	Yes
Itching	No	Yes
Nausea	No	Yes
Vomiting	No	No
Dizziness	No	No
Epistaxis	No	No
Ascites	No	No
Palpebral edema	No	No
Neurological	No	No
Exanthema	No	No
Shaking	No	No
Lethargy	No	No
Paresthesia	No	No
Paresis	No	No
Jaundice	No	No
Cyanosis	No	No
Hepatomegaly	No	No
Splenomegaly	No	No
Cholecystitis	No	No
Respiratory distress	No	No

Appendix Table 2. Results of laboratory tests of acute febrile patients positive for Rocio virus in Goiânia county, Central Brazil*

	Study patients					
	ROCV (p18)			ROCV (p21)		
Laboratory tests	T1	T2	T3	T1	T2	T3
Hematocrit (%)	32.7	36.7	34.9	47.4	46.1	45.8
Hemoglobin (g/dL)	10.8	11.9	11.4	15.5	15.4	16.6
Leukocyte (cells/mm3)	6,900	4,900	4,500	3,560	3,400	3,730
Platelets (cells/mm3)	120,000	220,000	147,000	207,000	201,000	198,000
AST (mg/dL)	17	19	19	35	42	29
ALT (mg/dL)	12	78	15	31	51	28
Albumin (mg/dL)	3.4	3.6	3.6	4.3	3.83	4.2
Creatinine (mg/dL)	1.1	1.06	1.05	1.08	1.12	1.19
Direct bilirubin (mg/dL)	0.18	0.27	0.16	0.16	0.29	0.26
Indirect bilirubin (mg/dL)	0.22	0.23	0.24	0.51	0.6	0.62
Activated partial thromboplastin time (seconds)	79.2	76.6	49.9	72.6	48.2	100
International normalized ratio (%)	1.1	1.12	1.38	1.19	1.57	0.53

*T1, sample collected until the seventh day of postdisease onset (PDO); T2, sample collected between the eighth and 15th day PDO; T3, sample collected between the 20th and 30th day PDO.

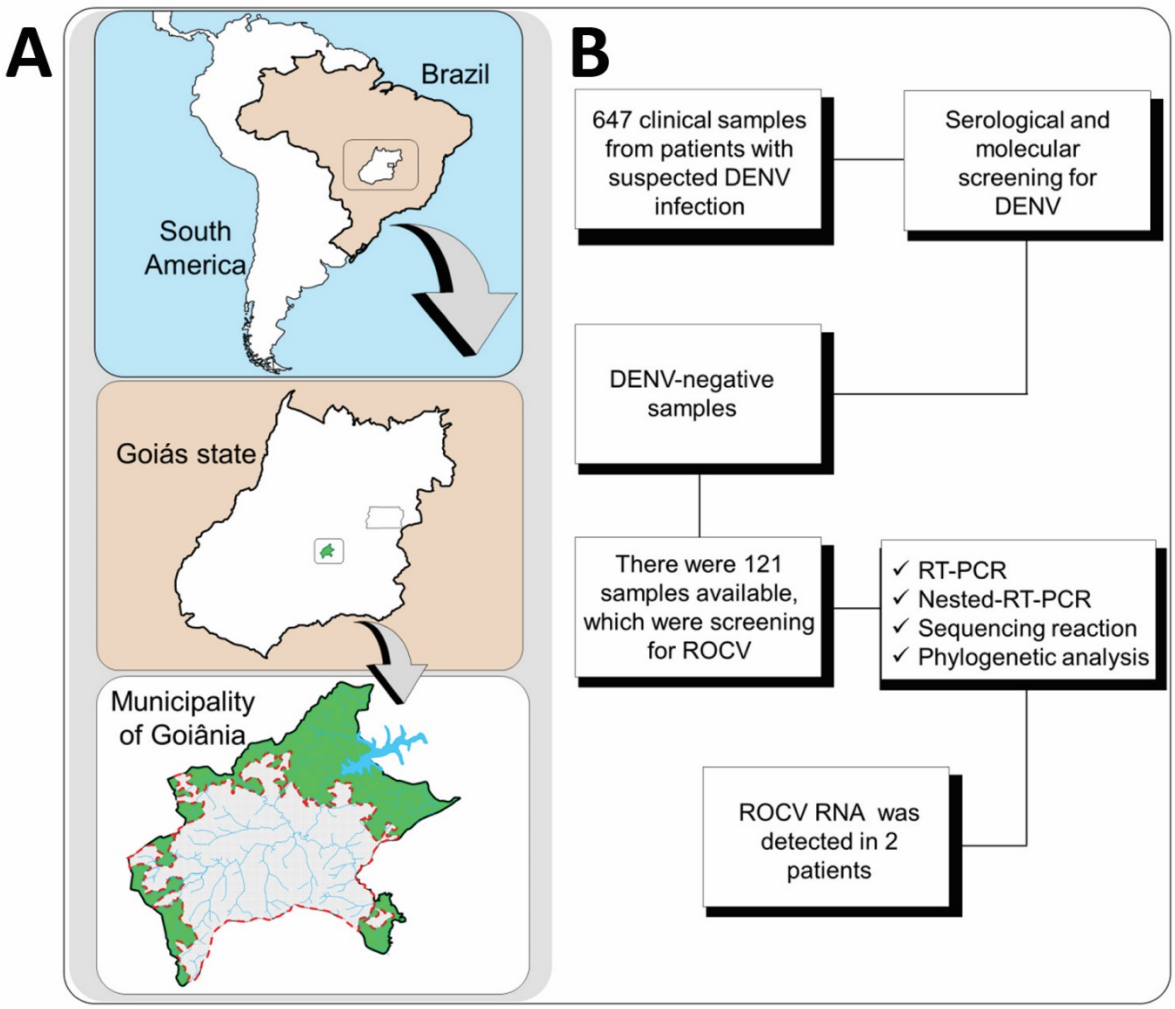
Appendix Table 3. Patient data and background information for the two samples positive for Rocio virus in Goiânia county, Central Brazil

	Study patients	
Patient data/background	ROCV (p18)	ROCV (p21)
Type of patient	Ambulatory patient	Ambulatory patient
Year of blood sample collection?	2012	2013
Has the patient ever had yellow fever?	No	No
Has the patient ever been vaccinated against yellow fever?	Yes	Yes
Has the patients ever had dengue?	-	Yes (2008)
Diabetes mellitus?	No	No
Chronic renal failure?	No	No
Asthma?	No	No
Lupus	No	No
Cancer?	No	No
AIDS?	No	No
Has the patient had hepatitis B or C?	No	No
Has the patient ever had a transplant?	No	No
Does the patient use any medication regularly?	-	No

Appendix Table 4. Results of laboratory tests for DENV in Goiânia county, Central Brazil*

		Study patients					
		ROCV (p18)			ROCV (p21)		
Laboratory tests		T1*	T2†	T3‡	T1	T2	T3
ELISA	IgM anti-DENV	NR[d]	NR	NR	U[e]	U	-
			OD[f]= 0.26			OD= 0.3	
	IgG anti-DENV	NR	NR	NR	R[g]	-	-
			OD= 0.15			OD= 2.1	
NS1 DENV		NR	-	-	NR	-	-
RT-PCR		Negative	-	-	Negative	-	-

*OD, optical density; NR, not reactive; R, reactive; T1, Sample collected until the seventh day after disease onset (ADO); T2, sample collected between the eighth and 15th day ADO; T3, sample collected between the 20th and 30th ADO; U, undetermined.



Appendix Figure. Map of the study site and methodology flowchart. Panel A shows a map of Goiás state (Brazil) with the location of the municipality of Goiânia. Panel B shows the methodology flowchart.