Therefore, several studies were conducted to subdivide the genotypes on the basis of detailed phylogenetic analysis (*7*,*8*). We reported that a large epidemic in Japan in 2013 might have occurred due to the transport of multiple lineages of rubella virus from rubella-endemic countries (*7*). According to the National Epidemiological Surveillance of Infectious Diseases (NESID) of Japan, during 2015–2017, ≈100 cases of rubella, which is a notifiable disease in Japan, were reported annually (*5*), and genotype 1E strains, including a strain closely related to RVs/Osaka.JPN/41.17[1E], were detected. Although these strains might have been transported from countries with endemic rubella, their origin remains unclear because of insufficient genomic information.

Japan has a high risk for subsequent rubella epidemics because the proportion of persons susceptible to rubella virus (≈9.0%) has not changed since 2013. In addition, an epidemic can occur when rubella virus is transported from rubella-endemic countries and the infection occurs in susceptible populations, as happened in Japan in 2013. Of the 11 imported cases of rubella to Japan reported in 2017, 4 were from Indonesia, according to the NESID of Japan. In the case we describe, we identified the rubella-exporting country and clarified the genetic information of the strain, which may contribute to countermeasures for worldwide importation of rubella virus. Rubella control by 2020 is the flagship goal of the World Health Organization South-East Asia region. Indonesia is conducting rubella immunization campaigns targeting ≈70 million children in 2017–2018. Therefore, constructing effective surveillance systems, accumulating genetic information, and promoting immunization in rubella-endemic countries are steps toward the global elimination of rubella.

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Spondweni Virus in Field-Caught *Culex quinquefasciatus* Mosquitoes, Haiti, 2016

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Spondweni virus (SPONV) and Zika virus cause similar diseases in humans. We detected SPONV outside of Africa from a pool of *Culex* mosquitoes collected in Haiti in 2016. This finding raises questions about the role of SPONV as a human pathogen in Haiti and other Caribbean countries.

Spondweni virus (SPONV) and Zika virus are closely related flaviviruses that were first described in Africa in 1952 and 1947, respectively (*1*). Humans infected by these viruses have similar clinical manifestations; asymptomatic infections are common, and illness is generally self-limiting (*1*). In the 6 documented human SPONV infections, fever occurred in all. Other symptoms included headache, nausea, myalgia, conjunctivitis, and arthralgia; only 1 SPONV-infected person had maculopapular and pruritic rash (*1*). The similar clinical presentations for these virus infections and reportedly high serologic cross-reactivity have resulted in frequent misdiagnosis (*1*).

Because of the 2015–2016 epidemic of Zika fever in the Western Hemisphere and the link between microcephaly and Zika virus infection, Zika virus has been studied more comprehensively than SPONV (*1*). SPONV was first isolated from *Mansonia uniformis* mosquitoes during virus surveillance in 1955 in South Africa (*2*). No new reports of SPONV surfaced despite continued mosquito surveillance until 1958, when it was identified in 4 additional mosquito species, including *Aedes circumluteolus*, a tropical sylvatic mosquito found in Africa (*2*). Little is known about possible vertebrate hosts, although SPONV antibodies have been detected in birds, small mammals, and ruminants (*2*). In a recent study by Haddow et al., strains of *Ae. aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* mosquitoes were not susceptible to SPONV infection (*3*).

We detected SPONV from a pool of 7 mixed-sex *Cx. quinquefasciatus* mosquitoes collected in July 2016 during ongoing arbovirus surveillance in Gressier, Haiti. During May–August 2016, we caught 1,756 mosquitoes using Biogents Sentinel traps (BioQuip Products, Rancho Dominguez, CA, USA) within a 10-mile radius in Gressier, a semirural setting. Trap locations were selected based on environmental considerations, low risk for traps being disturbed, and known human arbovirus-caused illnesses in the area (*4*). Trap bags were transported to a field laboratory in Haiti, where mosquitoes were frozen at –20°C, then identified by species and sexed by trained technicians using morphologic keys and identification guides (*5*,*6*). After identification, the mosquitoes were pooled by location, collection date, species (*Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and other), and sex. All pools were screened for chikungunya virus, dengue virus (DENV) serotypes 1–4, and Zika virus RNA by real-time reverse transcription PCR (rRT-PCR) (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/24/9/17-1957-Techapp1.pdf), as we previously have done with human specimens from Haiti (*4*). Mosquito homogenates positive by rRT-PCR were used for sequencing using primer walking and Sanger sequencing methods as previously reported (*4*; online Technical Appendix Table 2). In addition, we confirmed *Aedes* and *Culex* mosquito species by molecular methods (*7*,*8*). In initial screens of a pool of 7 mixed-sex *Cx. quinquefasciatus* mosquitoes (non–blood-fed) collected on July 4, 2016, rRT-PCR results suggested the presence of Zika virus RNA (cycle threshold value 39), but this same pool was negative for chikungunya virus and DENV RNA by rRT-PCR. After unsuccessful attempts to amplify Zika virus–specific amplicons using previously described Zika virus sequencing primers, we used an unbiased sequencing approach after treatment of virions in mosquito homogenate with cyanase (*4*). Because we suspected a closely related virus, we next

Table. Comparison of nucleotide and amino acid identities of representative strains of SPONV and Zika virus, Haiti*

Virus type and nucleotide GenBank accession no. (country of origin, year)	Nucleotide identity, %					
	SPONV, GenBank accession no.			Zika virus, GenBank accession no.		
	MG182017	DQ859064	KX227369	KY989511	KU501215	MF384325
SPONV MG182017 (Haiti, 2016)	100	98.8	96.8	70.7	70.4	70.4
SPONV DQ859064 (South Africa, 1954)		100	97.8	70.9	70.6	70.7
SPONV KX227369 (Nigeria, 1952)			100	71.1	70.8	70.8
Zika virus KY989511 (Uganda, 1947)				100	89.0	89.0
Zika virus KU501215 (Puerto Rico, 2015)					100	99.6
Zika virus MF384325 (Haiti, 2016)						100
Virus type and protein GenBank accession no. (country of origin, year)	**Amino acid identity, %**					
	SPONV, GenBank accession no.			Zika virus, GenBank accession no.		
	AVD68687	ABI54480	AOZ57820	ARM59240	AMC13911	ASF57880
SPONV AVD68687 (Haiti, 2016)	100	98.8	98.3	74.1	74.0	74.1
SPONV ABI54480 (South Africa, 1954)		100	99.1	74.9	74.7	74.8
SPONV AOZ57820 (Nigeria, 1952)			100	74.9	74.8	74.9
Zika virus ARM59240 (Uganda, 1947)				100	96.9	96.9
Zika virus AMC13911 (Puerto Rico, 2015)					100	99.8
Zika virus ASF57880 (Haiti, 2016)						100

*SPONV, Spondweni virus.

tested random hexamers and SPONV-specific primers (online Technical Appendix Table 3), which resulted in formation of virus-specific amplicons (online Technical Appendix). Thereafter, using SPONV primers, we determined a 10,290-nt nearly complete genome and deposited it in GenBank (accession no. MG182017).

The SPONV genome from Haiti shared 10,174 (98.8%) of 10,290 nt identity with a SPONV isolate from mosquitoes in South Africa in 1954 (GenBank accession no. DQ859064) and 9,958 (96.8%) of 10,287 nt identity with the SPONV Chuku strain from blood of a febrile human patient in Nigeria in 1952 (accession no. KX227369) (Table). When compared with the Zika virus reference strain from Uganda (accession no. KY989511), a strain from Puerto Rico (accession no. KU501215), and a strain from Haiti in 2016 (accession no. MF384325), Zika virus and SPONV clearly continue to diverge because the nucleotide and amino acid identities of SPONV are less similar to more recent strains of Zika virus (Table). Few SPONV sequences have been deposited into GenBank, resulting in insufficient information to predict how and when SPONV was introduced in Haiti.

In the Americas and the Caribbean, SPONV is a potential emergent arbovirus and public health threat that manifests clinically with symptoms and signs similar to those of Zika virus infection (*2*,*9*). Misdiagnosis has been documented, and it is possible that SPONV has caused human infection in Haiti but has been misidentified as infection from DENV or other arboviruses (*9*). Little is known about SPONV pathogenesis, host range, and vector competency, especially with vectors present in the Western Hemisphere. Our detection of SPONV in *Cx. quinquefasciatus* mosquitoes raises questions about the role of this species as a vector for this virus and highlights the need for ongoing surveillance for SPONV infection among humans in the Caribbean, combined with studies of potential vector populations.

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Spondweni Virus in Field-Caught *Culex quinquefasciatus* Mosquitoes, Haiti, 2016

Technical Appendix

Methods

Identification of Spondweni Virus Genomic RNA in Mosquito Homogenate

Virus identity was first attained using an unbiased sequencing approach following treatment of virions in mosquito homogenate with cyanase as outlined (*1*). Briefly, 50 μL of mosquito homogenate was diluted with phosphate buffered saline to 500 μL and centrifuged twice at 3,000 × g to pellet debris. The cleared sample was concentrated using an Amicon Ultra-15 centrifugal filter unit with an Ultracel-100 membrane with a molecular mass cutoff of 100 kDa (Millipore, Bedford, MA, USA) at 4,000 × g for 20 min to a volume of ≈80 μl. The volume of the centrifuged fluid was then adjusted to 100 μL by addition of phosphate buffered saline and treated with cyanse nuclease (RiboSolutions, Inc., Cedar Creek, TX, USA) to degrade nucleic acids external to that packaged (and thus protected) in virions (*1*), and as a secondary step, rRNA depletion was performed using a GeneRead rRNA Depletion Kit (QIAGEN, Valencia, CA, USA) to remove residual rRNA. Viral genomic RNA (and/or DNA) were subsequently extracted from virions using a QIAamp Viral RNA Mini Kit (QIAGEN). Because it was not known if there was a DNA or RNA virus, both PCR and reverse transcription PCR (RT-PCR) were performed. For RT-PCR, first-strand synthesis was performed using random 6-mers and an Accuscript High Fidelity 1st strand cDNA kit (Agilent Technologies, Santa Clara, CA, USA). Similarly, PCR was performed using random hexamers and One Taq DNA polymerase (New England Biolabs, Ipswich, MA, USA). PCR amplicons were purified (QIAGEN QIAquick PCR purification kit), and A-tailed with Taq DNA polymerase (New England Biolabs) as a precaution since One Taq, which had been used for PCR, is a mixture of a high-fidelity polymerase and Taq, and therefore, not all amplicons may contain 3′ A-tails. The A-tailed PCR amplicons were then TA-cloned using a commercial kit (pCR2.1 vector with 1 shot TOP10 chemically competent

E. coli cells, ThermoFisher Scientific, Waltham, MA, USA; K204001), and the inserts sequenced using Sanger sequencing. Out of a total of 50 plasmids with TA-cloned inserts, 1 (TA clone 39) contained a 286-bp insert with 98% identity (280/286 nt) with *Spondweni virus* (GenBank accession no. NC_029055.1): 286-bp Insert Derived from Spondweni virus in TA Clone 39:

5′-GCATGAAGGTGTGTTCCAAACAATGTGGCACGTCACAAAAGGTTCGGCCCTTC
GCAGTGGTGAG

GGACGCCTAGATCCATACTGGGGAAACGTGAAGCAGGATTTGATCTCTTACT
GCGGACCATGGAAA

CTGGAGGGGAAATGGGACGGCGTGTCGGAAGTCCAACTGATAGCGGTCGCCC
CAGGTGAGCGCGCCAGAAATGTGCAGACAAAACCAGGAGTGTTCAAGACCACTGAT
GGGGAAATCGGGGCCTTGGCCCTGGACTTCCCAGGCGGAAGTTCAGGC-3′

Sequencing Methods

Sequencing of the complete Spondweni virus genome designated *Culex quinquefasciatus*/Haiti-1/2016 was accomplished using a genome walking strategy based on an approach previously outlined (*1*) using the PCR primers described in Technical Appendix Table 3. Briefly, due to the limited amount of sample material, our preferred strategy of amplifying amplicons of size range 750–800 bp for direct sequencing (*1*) was not possible for the entire sequencing effort. Instead, 3 amplicons of that size range were generated using primer pairs F1 and R1, F2 and R2, and F3 and R3, and 1 that was smaller (289 bp) using primer pairs F7 and R7, and these were directly sequenced to attain consensus sequences. The remaining sequences required amplification of larger amplicons (≈3,000 bp) using primer pairs F4 and R4, F5 and R5, and F6 and R6. Amplicons for sequencing were amplified using Accuscript High Fidelity reverse transcription in the presence of SUPERase-In RNase inhibitor (Ambion, Austin, TX, USA), followed by PCR with Q5 high-fidelity DNA polymerase (New England Biolabs) with denaturation steps performed at 98°C. Smaller amplicons (up to ≈800 bp) were thereafter sequenced directly. The longer amplicons were purified, then smaller amplicons within the size range 750–800 prepared from them using the previously mentioned high-fidelity enzymes, and also directly sequenced using a gene walking approach (primers available upon request). To obtain the 5′ and 3′ ends of the viral genome, we used a 5′ and 3′ system for the Rapid Amplification of cDNA Ends (RACE) in accordance with the manufacturer's protocols (Life

Technologies, Carlsbad, CA, USA) and with primers identified in Technical Appendix Table 3. Bariability at the 5′ and 3′ UTRs made sequence determination impossible using a direct sequencing approach. Sequences were assembled with the aid of Sequencher DNA sequence analysis software v2.1 (Gene Codes, Ann Arbor, MI, USA). The GenBank accession number of Spondweni virus Culex quinquefasciatus/Haiti-1/2016 is MG182017.2.

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Technical Appendix Table 1. Primers and probes used for real-time reverse transcription PCR detection of chikungunya, dengue, and Zika viruses

Protocol reference	Name (fluor)	Sequence
(*2*)	CHIKV_3855F	GAGCATACGGTTACGCAGATAG
	CHIKV3957c1	TACTGGTGATACATGGTGGTTTC
	CHIKV_3957c2	TGCTGGTGACACATGGTGGTTTC
	CHIKV_3886p1	ACGAGTAATCTGCGTACTGGGACGTA
	CHIKV_3886p2	ACGAGTCATCTGCGTATTGGGACGCA
(*3*)	DENV-1F	CAAAAGGAAGTCGYGCAATA
	DENV-1R	CTGAGTGAATTCTCTCTGCTRAAC
	DENV-1Pr (FAM)	CATGTGGYTGGGAGCRCGC
	DENV-2F	CAGGCTATGGCACYGTCACGAT
	DENV-2R	CCATYTGCAGCARCACCATCTC
	DENV-2Pr (HEX)	CTCYCCRAGAACGGGCCTCGACTTCAA
	DENV-3F	GGACTRGACACACGCACCCA
	DENV-3R	CATGTCTCTACCTTCTCGACTTGYCT
	DENV-3Pr (TX Red)	ACCTGGATGTCGGCTGAAGGAGCTTG
	DENV-4F	TTGTCCTAATGATGCTRGTCG
	DENV-4R	TCCACCYGAGACTCCTTCCA
	DENV-4Pr (Cy5)	TYCCTACYCCTACGCATCGCATTCCG
(*4*)	ZIKV_1086F	CCGCTGCCCAACACAAG
	ZIKV_1162R	CCACTAACGTTCTTTTGCAGACAT
	ZIKV_1107FAM (FAM)	AGCCTACCTTGACAAGCAGTCAGACACTCAA

Technical Appendix Table 2. Zika virus sequencing primers*

Primer name	Sequence 5′–3′
ZIKV-F1	ATG AAA AAC CCA AAA AAG AAA TCC
ZIKV-R1	CAA GCG ATG GCA GCT GCT GCT AAC
ZIKV-F2	GAA TAC ACA AAG CAC TTG ATT AGA GTC
ZIKV-R2	GAA CCA CTC CTT GTG AAC CAA CCA GTG
ZIKV-F3	CTT GAT TGT GAA CCG AGG ACA GG
ZIKV-R3	TCC AAA CAA TGA TTT GAA AGC TGC TC
ZIKV-F3A	TGG AAG CCT AGG ACT TGA TTG TGA AC
ZIKV-F4	CTC ATT GGG CAA GGG CAT CCA TC
ZIKV-R4	CCA GTA GCC TAG ATC ACT GTG TAC
ZIKV-F5	GGA ACA GCT GTT AAG GGA AAG GAG
ZIKV-R5	CCA ATT AGC TCT GAA GAT GAA AGA TAC
ZIKV-F6	CAT TCA AAG TCA GAC CAG CGT TGC
ZIKV-R6	GCA CCA CTC CTT TTT CCA GTC TTG A
ZIKV-F7	GCA GCT GGA GCG TGG TAC GTA TAC G
ZIKV-R7	GAG TGG GTG ACA TTG ACT GCT GTT G
ZIKV-F8	GCC CTT AGA GGG CTT CCA GTG CGT TAT ATG
ZIKV-R8	GAG GCC ATC TTG GAG GTA AAT ATT G
ZIKV-F9	CAC ACT GGC TTG AAG CAA GAA TGC T
ZIKV-R9	GCC ATT TGG TTG TCC TGG GGA GAT CTT TG
ZIKV-F10	GGT GGT GCT CAT ACC TGA GCC AG
ZIKV-R10	CCA AGT AAC TTC CCC TAA AAA TGT TAC AC
ZIKV-F11	CTG GAA CTC CTC TAC AGC CAC TTC AC
ZIKV-R11	GTG GTG GAC ACA CTT TTT ATG GTG TTG
ZIKV-F12	CCC GCA ACT CTA CAC ATG AGA TGT AC
ZIKV-R12	CTA GCC ACA TAT ACC AGA TGG CGC
ZIKV-F13	GAA TTT GGA AAG GCC AAG GGC AG
ZIKV-R13	GGT GGC GGC AGG GAA CCA CAA TG
ZIKV-F14	CTC CAT CTC AAG GAC GGG AGG TC
ZIKV-R14	GCG CGT GGG GTT TTT TGA CTC AGT G
ZIKV-F15	CAT GCT GCC TGT GAG CCC CTC AGA GGA C
ZIKV-R15	CCA CTA GTC CCT CTT CTG GAG ATC C
3 UTR F1	CTA CCT ATC CAC CCA AGT TCG CTA C
3 UTR F2	GTG GCG ACC TTC CCC ACC CTT CAA T
ZIKA 5 UTR R1	CAT ATT GAC AAT CCG GAA TCC TCC

*Data from (*1*).

Technical Appendix Table 3. Spondweni virus sequencing primers*

Primer name	Sequence, 5′–3′	Nt position in SPONV reference strain NC_029055.1
5′ RACE roligo	rArGrC rArUrC rGrArG rUrCrG rGrCrC rUrUrG rUrUrG- rGrCrC rUrArC rUrGrG	N/A
5′-RACE-F	TCGTAGCTCAGCCGGAACAACCGGATGACC	N/A
5′-RACE-R	CCACTTTTCCCCATCTGTTGATGAGGCC	217–190
SPONV-F1	ATGAAGAACCCAAAGAAGGCCGGTA	1–25
SPONV-R1	CCACCAACGCATATCCTGGATTGC	778–755
SPONV-F2	CATGCCTCACAAAAGTTGGAGAC	661–683
SPONV-R2	CCAAAGGAGCCAAGTTCCACCTCAAC	1421–1396
SPONV-F3	CACCAACACGACAAGGAGAAC	1339–1359
SPONV-R3	CTCCTCTCATGGTAGCCTC	2122–2104
SPONV-F4	CACAACAAAAATTACCCACC	2046–2065
SPONV-R4	CTGCATGATGGCACTCACGTAGCTTCC	5007–4981
SPONV-F5	GGGGAAATCGGGGCCTTGG	4867–4885
SPONV-R5	CCTCTCACCTCGAGGACTTGC	7883–7863
SPONV-F6	GCAGAGAACCCGCCAGAAGAG	7685–7705
SPONV-R6	GACAGGGGTCTTGTCCTCCATG	10074–10053
SPONV-F7	GGAACAGAGTGTGGATCATAG	10022–10042
SPONV-R7	TTACAACACACCAGCGCTTGGCCC	10290–10267
3′-RACE-F	GGCGAAACTGAAGAATATAGAG	10207–10228
3′-RACE-F (T25)	TTTTTTTTTTTTTTTTTTTTTTTTT	N/A

*N/A, Not applicable; RACE, Rapid Amplification of cDNA Ends.