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Confirmation of Zika virus infection through hospital-based sentinel surveillance of acute febrile illness in Uganda, 2014–2017

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Abstract

Zika virus (ZIKV), transmitted by *Aedes* species mosquitoes, was first isolated in Uganda in 1947. From February 2014 to October 2017, the Uganda Virus Research Institute, in collaboration with the US Centers for Diseases Control and Prevention, conducted arbovirus surveillance in acute febrile illness (AFI) patients at St Francis hospital in Nkonkonjeru. Three hundred and eighty-four serum samples were collected and tested for IgM antibodies to yellow fever virus (YFV), West Nile virus (WNV), dengue virus (DENV), chikungunya virus (CHIKV) and ZIKV. Of the 384 samples, 5 were positive for ZIKV IgM. Of these five, three were confirmed by plaque reduction neutralization test (PRNT) to be ZIKV infections. Of the remaining two, one was determined to be a non-specific flavivirus infection and one was confirmed to be alphavirus-positive by reverse transcriptase polymerase chain reaction (RT-PCR). This study provides the first evidence of laboratory-confirmed ZIKV infection in Uganda in five decades, and emphasizes the need to enhance sentinel surveillance.

Keywords

Arbovirus; Zika virus; hospital-based; sentinel surveillance; plaque reduction neutralization test; confirmation

Zika virus (ZIKV), family *Flaviviridae*, genus *flavivirus*, is transmitted by *Aedes* species mosquitoes, including *Aedes aegypti* and *Aedes albopictus* [1]. ZIKV was first isolated in Uganda from a rhesus monkey in 1947 [2], and a short time later was first identified in humans in Nigeria [3]. Only a few human cases of ZIKV infection were reported before the 21st century [4–9], when in 2007 a large outbreak occurred on the island of Yap [10]. In 2016, a widespread outbreak occurred in South America, marking the first time the virus was known to be transmitted in the Americas [11–13] and resulting in microcephaly in

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Conflicts of interest

The authors declare that there are no conflicts of interest.

newborns [14], Guillain–Barré syndrome [15] and viral meningoencephalitis [16]. Sexual transmission has also been reported for ZIKV infections [17]. Because of its global distribution and ability to cause birth defects, the World Health Organization (WHO) declared the ZIKV epidemic in the Americas to be a Public Health Emergency of International Concern (PHEIC) in 2016 [18].

Studies have reported Uganda as a hotspot for many viral infections [19, 20], and recent studies reported the presence of ZIKV transmission-competent mosquito vectors throughout Uganda [21–23], a nation with environmental conditions conducive to the spread of arboviruses, including ZIKV. Here we report serological evidence of ZIKV infection in patients presenting with acute febrile illness (AFI) in a health facility in central Uganda. This study was facilitated through the arbovirus surveillance programme at the Uganda Virus Research Institute (UVRI), in collaboration with the Division of Vector-Borne Diseases (DVBD) of the US Centers for Disease Control and Prevention (CDC).

The Department of Arbovirology, Emerging and Re-emerging Infectious Diseases at UVRI established sentinel hospital-based surveillance to obtain clinical public health data concerning the causes of acute febrile illness (AFI) throughout the year. All patients enrolled in the study had a documented temperature of ≥37.5 °C or a reported history of fever without an identified source that had persisted for 2–7 days. Other symptoms included headache, chills, or muscle and joint pains. Serum samples were collected from patients at the St Francis Hospital Nkokonjeru sentinel site (Nkokonjeru sentinel site, 0°14′ 22.0″ N, 32° 55′ 23.0″ E; 50 kilometres southeast of Kampala). Samples were collected from February 2014 to October 2017, stored frozen in liquid nitrogen tanks and shipped monthly to the Arbovirology Laboratory at the UVRI in Entebbe. At the UVRI, samples were divided into three aliquots, one each for serological testing, virus isolation and storage.

A total of 384 serum samples were collected and stored as described above. Heat-inactivated sera were tested for IgM antibodies using the CDC immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assay (CDC MAC-ELISA) [24] for ZIKV and YFV and the InBios West Nile Detect IgM Capture ELISA, the DENV Detect IgM Capture ELISA and the CHIKjj Detect IgM ELISA for WNV, DENV and CHIKV, respectively (InBios International, Inc., Seattle, WA, USA). Of the 384 samples tested for IgM antibodies by ELISA, 28 tested positive for antibodies to one virus: CHIKV, 19; ZIKV, 5; YFV, 2; DENV, 1; and WNV, 1 (Table 1, shaded). Note that for the YFV and ZIKV IgM MAC-ELISA, a *P/N* between two and three is normally considered to be equivocal and requires additional testing. For our purpose, since additional testing was performed on all equivocal and positive samples, they are listed as positive. One additional DENV equivocal result obtained from a CHIKV IgM-positive sample was not resolved upon retesting (Table 1, ARBO1208UVRI). Virus isolation on vero cell monolayers was attempted on all samples, but yielded no isolates after 14 days in culture.

None of the five ZIKV IgM-positive samples demonstrated cross-reactivity to the other viruses tested (Table 1). The five ZIKV IgM-positive samples were further tested for specific neutralizing antibodies to ZIKV (MR766), YFV (Couma), WNV (Eg101) and DENV-1–4 (ChimeriVax dengue virus 1–4) by plaque reduction neutralization test (PRNT) as described

previously [25]. Three ZIKV IgM-positive samples were confirmed by PRNT, as determined by fourfold or greater increase in the reciprocal sample dilution resulting in 90 % neutralization relative to all other viruses tested. The neutralizing titres for the acute samples ranged from 1 : 160 to 1 : 2560 (Table 2). A convalescent serum sample was obtained from one ZIKV IgM-positive patient 4 weeks after the first acute sample was taken. The convalescent sample further exhibited a fourfold or greater increase in ZIKV neutralizing antibody titre relative to the acute sample. Clinically, two of these cases presented with intense fatigue, anorexia, muscle pain, joint pain and headache, while one only had fever, abdominal pain and hiccups (Table 3). None had clinical symptoms of vomiting, conjunctivitis, skin rash or retroorbital pain. The cause of infection in two of the five ZIKV IgM-positives could not be distinguished by PRNT. Sample ARB01279 did not neutralize any of the viruses tested and thus was reported as an unspecified flavivirus infection.

Among the 384 specimens tested, a single sample tested positive for more than one virus. ARB01120 demonstrated a fourfold greater neutralization titre against ZIKV than the viruses tested, although the titre was low, suggesting a previous infection. This sample was also positive for alphavirus RNA by reverse transcriptase polymerase chain reaction (RT-PCR) testing using group-specific primers; attempts to further identify the RNA by subsequent RT-PCR testing using CHIKV, onyong-nyong and Semliki Forest virus-specific primers [26] returned negative results. This sample may represent a ZIKV/alphavirus co-infection, but despite the observed fourfold difference, the low ZIKV PRNT titre is more likely indicative of a past infection with ZIKV or another flavivirus than an acute flavivirus infection. Recent surveillance for arboviruses in mosquitoes resulted in the isolation of Babanki virus, genus *Alphavirus*, from Nkokonjeru, and Usutu virus, genus *Flavivirus*, from nearby Jinja [27].

It is likely that ZIKV has circulated continuously at a low level in Uganda, but due in part to a lack of ZIKV surveillance among symptomatic individuals, this is the first time that human ZIKV infection has been reported in Uganda in more than 50 years. ZIKV infection was last reported in the country in 1964 in a 28-year-old European male who had lived in Uganda for 2.5 months before the onset of illness with headache, rash and fever [5]. With the ongoing ZIKV circulation in South and North America, the question has been raised as to whether ZIKV is still circulating in Uganda. This study provides evidence of the continued circulation of ZIKV in Uganda.

PRNT confirmed ZIKV as the cause of infection in three out of five presumptive IgM-positive cases. Neutralizing patient serum exhibited a greater than fourfold increase in the neutralization of ZIKV compared to other flaviviruses. Extensive IgM cross-reactions frequently occur in ELISA testing where patients have been exposed to alternative or previous flavivirus infection or vaccination [28]. This suggests that differential PRNT should be included in the routine laboratory testing algorithm, because ZIKV versus DENV results are crucial for guiding the clinical management of patients and public health prevention efforts. However, we report here confirmed ZIKV infection in a country without a high historical DENV circulation.

This study has some limitations. Most importantly, the number of ZIKV cases identified likely underestimates the true number that occurred. Despite the dramatic birth defects associated with ZIKV infection in newborns, in healthy adults ZIKV infection is generally thought to produce mild illness. Since this was a hospital-based serosurvey, any asymptomatic infections or infections producing symptoms not severe enough to warrant hospitalization would not have been included in the survey. Despite this limitation, the hospital-based surveillance system has proven useful in providing information regarding the occurrence of arbovirus infections in this region, and in establishing a baseline for comparison with future AFI and arbovirus surveillance.

The cause of the infection could not be determined conclusively by PRNT in two of the five ZIKV IgM ELISA-positive cases. Using group-specific RT-PCR, we identified one of the cases as an acute unidentified alphavirus infection. As with other arbovirus infections, RT-PCR can detect acute ZIKV infections, provided that serum is collected within the first few days of illness, and when positive, RT-PCR clarifies interpretation of the results [29]. Molecular methods, however, increase the cost of testing, and serum must be stored at −80 °C immediately after collection to prevent viral RNA degradation. In addition, it is more difficult to establish and maintain the required technical capacity in low- and middle-income hospitals and public health laboratories. Therefore, it would not be possible to perform routine molecular testing for ZIKV surveillance at most African public health laboratories. Similarly, virus isolation testing can provide a clear result, but requires a high-quality patient serum specimen that must be collected relatively early in infection and stored/shipped on liquid nitrogen or dry ice. The majority of hospital and public health laboratories in Uganda do not have the capacity to perform the virus isolation procedure or to characterize any viruses isolated.

The results of our study indicate that the current laboratory-based arbovirus surveillance system in Uganda should be enhanced to include routine laboratory testing confirmation of hospitalized non-malaria AFI cases and enrol more hospitals and health centres in order to further monitor disease incidence and the trends of mosquito-borne viruses. This would provide an opportunity, when possible, to differentiate ZIKV infections from AFIs caused by infections with other viruses. In order to identify regional differences, the most appropriate arbovirus surveillance strategy would be nationwide surveillance with reporting of clinical AFI cases, with selected sentinel hospital sites at which specimens can be collected and sent for arbovirus testing. With limited resources to set up specialized surveillance sites, and with the caveat that cases not requiring hospitalization would be missed, hospitals are the most appropriate choice for sentinel sites.

In conclusion, this study provides evidence of ZIKV infection occurring in patients presenting with AFI at a hospital in Nkokonjeru, Uganda. Future expansion of the hospital sentinel surveillance system will provide continued disease burden information, facilitating the monitoring of trends in arbovirus infections throughout the country.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AFI	acute febrile illness
CDC	Centers for Disease Control and Prevention
CHIKV	chikungunya virus
DENV	dengue virus
DVBD	Division of Vector-Borne Diseases
IgM	immunoglobulin M
MAC-ELISA	immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assay
PHEIC	Public Health Emergency of International Concern
PRNT	plaque reduction neutralization test
RT-PCR	reverse transcriptase polymerase chain reaction
UVRI	Uganda Virus Research Institute
WHO	World Health Organization
WNV	West Nile virus
YFV	yellow fever virus
ZIKV	Zika virus

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Table 1.

Serum specimens with positive IgM results to select arboviruses by ELISA showing *P/N*[*] and ISR[†] values[‡]

Lab ID	Date collected	YFV *P/N*	WNV ISR	DN ISR	ZIKA *P/N*	CHIKV ISR
ARB00357UVRI	03/13/14	1.365	0.857	0.762	1.741	3.658
ARB00578UVRI	11/19/15	1.000	1.073	1.301	0.972	1.392
ARB00588UVRI	11/24/15	1.092	0.953	0.060	1.265	2.091
ARB00634UVRI	02/09/16	0.934	0.324	0.063	0.995	1.175
ARB00692UVRI	03/03/16	0.974	0.955	1.051	0.991	2.684
ARB00699UVRI	03/08/16	1.097	1.076	1.053	1.144	1.534
ARB00705UVRI	03/17/16	0.869	1.054	2.204	0.961	1.955
ARB00710UVRI	03/24/16	1.179	1.027	1.012	0.887	1.178
ARB00712UVRI	04/01/16	1.146	1.150	1.060	0.883	4.085
ARB00714UVRI	04/01/16	0.995	1.164	1.053	1.215	1.154
ARB00872UVRI	05/11/16	1.015	1.319	1.026	0.991	1.236
ARB00975UVRI	08/05/16	0.705	1.558	1.373	0.858	10.745
ARB00990UVRI	09/29/16	1.353	1.261	1.067	1.482	1.836
ARB00995UVRI	10/04/16	2.510	2.741	1.412	1.071	0.284
ARB01027UVRI	11/02/16	0.710	1.128	0.924	0.818	2.248
ARB01028UVRI	11/02/16	0.574	1.143	0.994	0.695	2.066
ARB01034UVRI	11/10/16	0.717	1.728	1.195	**2.821**	0.293
ARB01072UVRI	02/20/17	0.555	5.990	2.704	0.578	0.423
ARB01119UVRI	04/18/17	1.393	1.637	1.398	**2.000**	0.819
ARB01120UVRI	04/18/17	0.979	1.382	1.059	**3.960**	0.147
ARB01125UVRI	04/24/17	0.885	1.212	1.1.5	1.175	1.485
ARB01129UVRI	04/25/17	0.736	1.208	1.054	**2.659**	0.231
ARB01155UVRI	06/16/18	0.799	1.071	0.996	0.811	1.450
ARB01164UVRI	07/26/17	0.823	1.605	4.076	0.986	0.250
ARB01165UVRI	07/27/17	4.442	1.476	1.140	0.694	0.186
ARB01193UVRI	08/21/17	1.048	1.382	0.583	1.234	1.236
ARB01208UVRI	09/12/17	1.025	1.152	1.896	1.117	3.801
ARB01279UVRI	10/25/17	1.457	1.063	0.849	**4.711**	0.269

[*] *P/N*, mean OD450 of patient serum on viral antigen divided by mean OD450 of negative control on viral antigen.

[†] ISR, immune status ratio, ratio of the test sample OD450 to the mean cut-off control value.

[‡] See Table S1 (available in the online version of this article) for result interpretation guidelines.

Shaded only are IgM positives for YFV, WNV and CHIKV.

Shaded and bold are ZIKV only IgM samples.

Table 2.

PRNT results and interpretations for serum samples with positive or equivocal ZIKV IgM ELISA results

Lab ID	YFV titre	WNV titre	DENV-1 titre	DENV-2 titre	DENV-3 titre	DENV-4 titre	ZIKV titre	Final result
ARB01034	5	10	<5	<5	<5	<5	640	ZIKV
ARB01034_C*	10	10	<5	<5	<5	<5	10240	ZIKV
ARB01119	<5	<5	<5	<5	<5	<5	160	ZIKV
ARB01120	<5	<5	<5	<5	<5	<5	20	Unidentified alphavirus infection
ARB01129	<5	<5	<5	<5	<5	<5	2560	ZIKV
ARB01279	<5	<5	<5	<5	<5	<5	<5	Presumptive flavivirus

*Convalescent sample.

Table 3.

Symptoms exhibited by patients confirmed with Zika virus infection The empty cells indicate the patient did not exhibit that symptom.

Symptoms	ARB01034	ARB01119	ARB01129
Fever	X	X	X
Date of illness onset	Not reported	04/01/2017	03/25/2017
Vomiting/nausea			
Intense fatigue/general weakness	X		X
Anorexia/loss of appetite	X		X
Abdominal pain	X	X	X
Chest pain	X		X
Muscle pain	X		X
Joint pain	X		X
Headache	X		X
Cough	X		
Jaundice			X
Conjunctivitis			
Skin rash			
Hiccups		X	
Pain behind eye			