Chikungunya and O’nyong-nyong Viruses in Uganda: Implications for Diagnostics

Tamara L. Clements,1 Cynthia A. Rossi,1 Amanda K. Irish,2 Hannah Kibuuka,3 Leigh Anne Eller,1 Merlin L. Robb,4 Peter Kataaha,5 Nelson L. Michael,6 Lisa E. Hensley,2 and Randal J. Schoepp1

1US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland; 2College of Public Health, University of Iowa, Iowa City, Iowa; 3Makerere University Walter Reed Project, Kampala, Uganda; 4Henry M. Jackson Foundation, Rockville, Maryland; 5Nakasero Blood Bank, Kampala, Uganda; 6Walter Reed Army Institute of Research, Rockville, Maryland; 7National Institute of Allergy and Infectious Diseases–Integrated Research Facility, Frederick, Maryland

Background. A serosurvey of healthy blood donors provided evidence of hemorrhagic fever and arthropod-borne virus infections in Uganda.

Methods. Antibody prevalence to arthropod-borne and hemorrhagic fever viruses in human sera was determined using enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization test (PRNT).

Results. The greatest antibody prevalence determined by ELISA was to chikungunya virus (CHIKV) followed in descending order by West Nile virus (WNV), Crimean-Congo hemorrhagic fever virus (CCHFV), Ebola virus (EBOV), dengue virus (DEN), yellow fever virus (YFV), Rift Valley fever virus (RVFV), Marburg virus (MARV), and Lassa virus (LASV). Further investigation of CHIKV-positive sera demonstrated that the majority of antibody responses may likely be the result of exposure to the closely related alphavirus o’nyong-nyong virus (ONNV).

Conclusions. As the use of highly specific and sensitive polymerase chain reaction–based assays becomes the diagnostic standard without the corresponding use of the less sensitive but more broadly reactive immunological-based assays, emerging and re-emerging outbreaks will be initially missed, illustrating the need for an orthogonal system for the detection and identification of viruses causing disease.

Keywords. chikungunya; CHIKV; diagnostics; o’nyong-nyong; ONNV; serosurvey; Uganda.

Arthropod-borne and hemorrhagic fever viruses are the source of emerging and re-emerging diseases resulting in some of the most significant global outbreaks today. The Ebola virus disease outbreak that began in 2013 was the largest in recorded history and devastated West Africa [1]. Chikungunya virus (CHIKV) emergence in the Caribbean in October 2013 eventually caused >1 million suspected cases in the region [2]. Zika virus disease is the most recent viral disease emerging in Latin America and the Caribbean, prompting the World Health Organization (WHO) to declare a Public Health Emergency of International Concern [3]. Increases in world population, human development into new biomes, and the ease of worldwide travel will make emerging and re-emerging viruses the norm rather than the exception. Diagnostics and surveillance are the most effective mechanisms to identify, control, and prevent disease.

CHIKV, in the genus Alphavirus and family Togaviridae, has caused numerous well-documented outbreaks in sub-Saharan Africa, the Indian subcontinent, South East Asia, and most recently in the Caribbean [4, 5]. The virus was first isolated from a febrile human in Tanzania in 1953 [6]. In West and Central Africa, the virus is thought to be maintained in a sylvatic cycle between nonhuman primates and forest-dwelling Aedes species mosquitoes. In Asia, no sylvatic cycle has been identified, but an urban transmission cycle likely involves the urban mosquito, Aedes aegypti, and humans. In the Caribbean and the Americas, the transmission cycle involves humans and probably Ae. aegypti and Ae. albopictus mosquitoes. Ae. albopictus mosquitoes are of particular concern in the United States and Europe as their geographical range extends into temperate regions [7]. The disease is characterized by 3 symptoms: fever, rash, and arthralgia. Although the acute fever and rash resolve within a few days, the arthralgia can be debilitating and persist for weeks or months. Acute CHIKV disease is similar to other febrile illnesses like dengue fever or malaria that can result in misidentification, but the prolonged, debilitating arthralgia primarily affecting the peripheral small joints is characteristic of CHIKV disease [4].

O’nyong-nyong virus (ONNV) is an alphavirus in the Semliki Forest antigenic complex that is closely related to CHIKV. The virus was initially isolated in 1959 from human blood and anopheline mosquitoes collected in northern Uganda (Gulu) during one of the most extensive arthropod-borne virus epidemics recorded [8]. The ONNV disease spread throughout
southeastern Africa and lasted for more than 4 years, infecting >2 million people [9]. After an apparent 35-year absence, the virus appeared again in southern Uganda, causing another major epidemic. The virus has only been associated with major epidemics in Africa; however, serological evidence suggests that ONNV may be endemic in regions of East and West Africa [10–13]. In contrast to CHIKV and other alphaviruses that are vectored by culicine mosquitoes, ONNV is transmitted by anopheline mosquitoes in a cycle that includes humans and a vertebrate reservoir(s) yet to be identified. Clinically, o’nyong-nyong disease closely resembles chikungunya virus disease, but o’nyong-nyong disease may cause lymphadenopathy more often, and affected joints are without effusions [9].

The clinical similarities between CHIKV and ONNV disease make definitive diagnosis difficult without appropriate diagnostic assays. For acute infections, a virus detection enzyme-linked immunosorbent assay (ELISA) using cross-reactive capture and detector antibodies identifies both CHIKV and ONNV infections. Molecular detection utilizes highly specific and very sensitive CHIKV and/or ONNV reverse transcriptase polymerase chain reaction (RT-PCR) assays. These immunological and molecular assays detecting virus or genomic material must test samples acquired during the transient viremic phase, which is not well characterized in clinical infections.

Diagnosis in the absence of virus utilizes serological testing for immunoglobulin M (IgM) and/or immunoglobulin G (IgG) antibodies. Frequently, only CHIKV assays are used to detect antibodies, but interpretation of results is problematic because of the 1-way antigenic cross-reactivity between the 2 viruses. Antibody to CHIKV reacts equally to both CHIKV and ONNV; however, ONNV antibodies react weakly to CHIKV antigens [14, 15]. Serological confirmation of CHIKV or ONNV infection requires a plaque reduction neutralization test (PRNT) to determine the ability of an antibody sample to neutralize each virus. To confirm an ONNV infection, the neutralization titer for ONNV will be higher than that for CHIKV; the CHIKV titer will typically be lower or undetectable for the same sample.

In the course of this serosurvey of healthy blood donors in Uganda, CHIKV had the highest antibody prevalence, but PRNT results suggested that for a majority of the samples ONNV was the infecting virus. These results illustrate the need for an orthogonal system for the detection and identification of viruses causing disease.

METHODS

Patient Samples

Human blood samples were taken from healthy Ugandan blood bank donors during the course of the Walter Reed Army Institute of Research (WRAIR) study RV-164 (“Determination of Laboratory Reference Data Using Anonymous Healthy Ugandan Blood Bank Donors”) that spanned from 2006 to 2007. The samples were collected from 5 regions distributed throughout Uganda (Figure 1). Fort Portal and Mbale are located in western Uganda, Kampala, the capital in the south-central region, Mabale in eastern Uganda, and Gulu in the north. An aliquot of serum from 1744 donors was obtained for testing by ELISA for IgG antibodies to a variety of arthropod-borne and hemorrhagic fever viruses thought to occur in the regions. All human samples were collected under the approved protocol WRAIR 1194. All testing of human samples was conducted at USAMRIID, under approved human use protocol HP-09-39.

Antigens

Viruses used to produce ELISA antigenic materials included the New Guinea C strain of dengue 2 (DENV-2) virus, a human dengue virus isolate from 1944 [16]; the EG101 strain of West Nile virus (WNV), which was originally isolated in Egypt [17]; the 17D strain (Connaught) of yellow fever virus (YFV) derived by Theiler in 1937 [18]; the Indo23574 strain of CHIKV originally isolated in Thailand in 1962 [19]; the ZH501 strain of Rift Valley fever virus (RVFV), originally isolated from a human patient during a 1977 outbreak in Egypt [20]; the Zaire-76 strain of Ebola virus (EBOV), originally isolated from the clinical material of a patient in the 1976 outbreak [21]; the Musoke strain of Marburg virus (MARV), which was isolated from a human case in 1980 in Kenya [22]; the prototype strain Iba10200 of Crimean–Congo hemorrhagic fever virus (CCHFV), isolated in 1976 from ticks collected in Nigeria [23]; and the Josiah strain of Lassa virus (LASV), isolated from human serum collected in Sierra Leone in 1976 [24].

The ELISA antigens were prepared and optimized as previously described [25]. All viruses were propagated at the appropriate biological safety level (BSL), either BSL-3 or BSL-4. Briefly, the virus-appropriate continuous cell line, at >70% confluence, was infected at a multiplicity of infection (MOI) of approximately 0.01 plaque-forming units (PFU)/cell. Virus-infected cells were harvested when approximately 50% to 75% of the cells exhibited cytopathic effects (CPEs) or, for those viruses that did not cause CPE, at a predetermined day post-exposure. Cell culture supernatants, used in the IgG capture ELISAs, were clarified by a low-speed centrifugation (10,000 × g for 10 minutes), inactivated with 0.3% beta-propiolactone (BPL; Sigma, St. Louis, MO) and 10% tris buffer, pH 8.5, and incubated for 3 days at 4°C before freezing and storing at −70°C until irradiated. Virus-infected cell pellets used to produce cell lysates for the direct IgG ELISAs, were suspended in 0.01 M borate saline, pH 9.0, containing 1% Triton X-100. The suspended pellets were sonicated, clarified by centrifugation (10,000 × g at 4°C for 5 minutes), and the soluble portion was stored at −70°C until irradiated. Virus-infected cell culture supernatants and cell lysates were further inactivated by gamma irradiation and safety-tested to ensure inactivation. Optimal dilutions of antigens were determined by checkerboard titrations against
virus-specific antibodies. Mock antigens, both supernatant and cell lysate, used as negative antigen controls, were prepared from uninfected cell monolayers as described above.

**ELISA**

**Direct IgG ELISA**

Samples tested for LASV, MARV, EBOV, YFV, DENV-2, WNV, and CHIKV antibodies utilized a direct IgG ELISA format. Briefly, 96-well flat bottom polyvinyl chloride (PVC) microtiter plates (Thermo Fisher Scientific, Waltham, MA) were coated with inactivated virus-infected cell lysate or mock antigen overnight at 4°C. Antigen-coated plates were washed, and diluted serum samples (1:100) were added and incubated for 1 hour at 37°C. Samples were tested in duplicate, and at least 1 known positive control sample and 4 known negative control samples were included with every assay. After washing, diluted horseradish peroxidase (HRP)-labeled mouse antihuman IgG (Fc-specific) conjugate (Accurate Chemical, Westbury, NY) was added, and the plates were incubated for 1 hour at 37°C. After washing, diluted serum samples were added, and the assay was completed as described for the direct IgG ELISA above.

**Capture IgG ELISA**

Samples tested for RVFV and CCHFV antibodies used a capture IgG ELISA format, which has been previously described [26, 27]. Briefly, PVC plates were coated with an appropriately diluted virus-specific antibody overnight at 4°C. Antibody-coated plates were washed, and appropriately diluted inactivated virus-infected supernatant or mock control antigen was added and incubated for 1 hour at 37°C. After washing, diluted serum samples were added, and the assay was completed as described for the direct IgG ELISA above.

**Plaque Reduction Neutralization Test**

PRNTs were conducted on selected sera as previously described [28]. Briefly, serum samples were heat-inactivated for 30 minutes.
at 56°C and diluted 4-fold from 1:10 to 1:10 240 in Hank’s balanced salt solution (HBSS) containing penicillin/streptomycin and 5% heat-inactivated fetal bovine serum. Diluted serum samples were tested for their ability to neutralize approximately 100 PFU of the challenge virus. Each sample dilution was tested in duplicate. Both known positive and negative control sera were included with every assay. Serum–virus mixtures were incubated overnight at 4°C and then inoculated onto 75%–90% confluent monolayers of the appropriate cell lines grown in 6-well tissue culture plates. After incubation for 1 hour at 37°C, a nutrient-rich 0.6% agarose overlay was added, and plates were incubated at 37°C for the appropriate number of days for the virus. Plates were then stained with a second overlay containing 5% neutral red, and plaques were counted 24–48 hours later. Titers were recorded as the reciprocal of the highest serum dilution reducing 80% of the plaque assay dose, and a probit titer was calculated using the forecast function in Microsoft Excel. A probit titer was determined using an equation representing the average number of plaques counted per well and the corresponding dilution for each serum sample and then forecasting the exact dilution that would correspond to the number of plaques used as the 80% cutoff. The PRNT virus strains used were ONNV (Gulu), CHIKV (Indo23574), Semliki Forest Virus (SFV-original), and Sindbis Virus (SINV-UgMp6640).

RESULTS

IgG ELISA

IgG antibodies were evident for each of the 9 viruses tested. The arenavirus, LASV , had the lowest antibody prevalence at 0.1% (Table 1). The filoviruses MARV and EBOV had antibody prevalence of 0.3% and 5.7%, respectively. The bunyaviruses, represented by the tick-borne hemorrhagic fever virus CCHFV and the mosquito-borne virus RVFV, were 6.0% and 2.8%, respectively. The flaviviruses were the largest group of viruses tested for IgG antibodies and were represented by YFV, DENV2, and WNV; antibody prevalence was 3.3%, 4.1%, and 8.3%, respectively. The only alphavirus evaluated for IgG antibody was CHIKV, which had the highest antibody prevalence of any of the viruses at 31.7%. Of the CHIKV IgG-positive samples, 5.3% had ODs ranging from 0.20 to 0.50; 8.2% ranging from 0.51 to 1.00; 12.9% ranging from 1.01 to 2.00; and 5.3% had ODs greater than 2.01 (data not shown). The highest antibody prevalence was in the Gulu district, with 63.5%, followed by Mbale at 49.0%, Kampala at 21.2%, Mbarara at 13.7%, and Fort Portal with the lowest CHIKV antibody prevalence at 11.1% (Table 2).

Table 1. Seroprevalence of IgG Against Hemorrhagic Fever and Arthropod-Borne Viruses, Uganda

| Virus Assay       | Positive/Tested | Percent Positive |
|-------------------|-----------------|-----------------|
| Chikungunya       | 552/1744        | 31.7            |
| West Nile         | 144/1744        | 8.3             |
| Crimean-Congo     | 100/1675        | 6.0             |
| Ebola             | 99/1744         | 5.7             |
| Dengue            | 72/1744         | 4.1             |
| Yellow Fever      | 58/1744         | 3.3             |
| Rift Valley Fever | 48/1744         | 2.8             |
| Marburg           | 5/1744          | 0.3             |
| Lassa             | 2/1744          | 0.1             |

Abbreviation: IgG, immunoglobulin G.

Alphavirus PRNT

To confirm the serological ELISA results, a limited number of PRNTs were conducted. To gain a better understanding of the specific virus giving rise to the CHIKV ELISA results, initially 24 of 552 CHIKV IgG-positive samples (4.4%) were tested for their ability to neutralize CHIKV and other related alphaviruses. Representative samples chosen had ELISA OD values spanning from high to low. Comparing samples by PRNT against CHIKV, ONNV (strain Gulu), SINV (strain UgMp6640), and SFV (strain Original), 23 of the samples showed higher titers against ONNV than CHIKV, SINV, or SFV (Supplementary Table 1). A single sample was able to neutralize only SFV at a very low titer, a PRNT 80% probit titer of 33. None of the samples were able to neutralize SINV.

Based on the results of the initial study and to save time and reagents, a larger panel of samples was only tested for the ability to neutralize CHIKV and ONNV. The second panel of 100 samples consisted of 20 samples from each of 5 districts; 15 samples spanned the range of ELISA OD values, and 5 samples were ELISA negative. Of the selected 75 CHIKV ELISA IgG-positive samples, 71 preferentially neutralized ONNV compared with CHIKV; 4 samples failed to neutralize either virus (Supplementary Table 2). There did not appear to be a correlation between the ELISA OD and the PRNT titer. Of the 25 ELISA IgG-negative samples, all but 1 failed to neutralize CHIKV or ONNV when tested by the PRNT. Table 3 is a representative data set of the larger group tested by PRNT.

PRNTs confirmed a selected number of antibody-positive filovirus and RVFV serum samples (data not shown). Due to the lack of neutralizing positive control sera, PRNTs were not done on flavivirus, CCHFV, and LASV serum samples.

Table 2. Seroprevalence of CHIKV IgG Antibodies in Healthy Blood Bank Donors by District, Uganda

| District   | Positive/Tested | Percent Positive |
|------------|-----------------|-----------------|
| Fort Portal| 39/350          | 11.1            |
| Gulu       | 221/348         | 63.5            |
| Kampala    | 74/349          | 21.2            |
| Mbale      | 170/347         | 49.0            |
| Mbarara    | 48/350          | 13.7            |

Abbreviations: CHIKV, chikungunya virus; IgG, immunoglobulin G.
Uganda has always been a region rich in circulating arthropod-borne and hemorrhagic fever viruses that cause human disease. Past serological surveys in Uganda demonstrated that the human populations in the region were constantly infected by a variety of viruses, including the viruses examined in this study [29, 30]. Serological analysis of anonymous healthy blood bank donors from 5 regions throughout Uganda provided an opportunity to assess the antibody prevalence in 9 hemorrhagic fever and arthropod-borne viruses from samples collected from 2006 to 2007 (Table 1). An earlier Uganda serosurvey in 1984 utilized hemagglutination inhibition (HI) for arthropod-borne virus antibodies and an immunofluorescent assay (IFA) for hemorrhagic fever virus antibodies and found comparable serological prevalence [29]. In contrast to our study, Rodhain and colleagues tested serum samples from only northeastern Uganda; however, their findings were remarkably similar to our findings. In that study, the greatest antibody prevalence was to CHIKV (46.9%), followed by WNV (9.8%), LASV (6.0%), ebolaviruses (6.0%), MARV (4.5%), and RVFV (2.8%), with CCHFV, YFV, and DEN2V each having the same antibody prevalence (2.2%). Two notable differences in our findings were LASV and MARV antibody prevalence of 0.1% and 0.3%, respectively, which could be explained by a regional difference in our tested populations and inclusion of regions not covered by the previous study.

The high antibody prevalence in Uganda to CHIKV and the related ONNV from recent or past infections is not unexpected. A serological survey of arthropod-borne viruses in 8 regions in Uganda from 1967 to 1969 found CHIKV antibody prevalence to be 38.6% [30]. However, the HI test used in that study could not distinguish between the 2 viruses. ONNV outbreaks were known to have occurred in northern Uganda in 1959 and in southern Uganda in 1960 [8, 31]. Periodic or endemic CHIKV infections have occurred throughout Uganda at different times [32, 33]. Therefore, the relatively high prevalence of CHIKV antibodies noted in this study was considered the result of infections caused by either virus.

Since the initial isolation of CHIKV in Tanzania in 1953, a number of outbreaks have occurred in East Africa. In 2004, a large epidemic of CHIKV disease started in coastal Kenya and spread over the next 4 years to a number of islands in the Indian Ocean and eventually to India, infecting over a million people [34]. Generally, ONNV is more often associated with large epidemics of disease. In Kenya, the last reported epidemic of o’nyong-nyong disease was in 1961; it spread from northern Uganda [31]. Some serological evidence indicates that the virus continued to circulate in the region as late as 1969 [9, 11, 35]. Recently, a study of CHIKV and ONNV transmission in coastal Kenya demonstrated that of 443 PRNT-confirmed human serum samples, 6% were CHIKV positive, 56% were ONNV, and 38% were equivocal with high titers for both viruses [13]. The significant seroprevalence of ONNV, even though the last known outbreak in the region was in 1961, suggests that the transmission of the virus is high, but cases are not identified as ONNV disease.

This study and previous studies demonstrate that CHIKV and/or ONNV are circulating throughout Africa. Recently, CHIKV disease has emerged in the Caribbean, and due to the abundance of susceptible mosquitoes, this disease can spread to surrounding regions of the world. CHIKV and ONNV are closely related viruses that cause similar human disease presentations that are also similar to dengue fever. Therefore, detection and identification must rely on diagnostic tests. As previously discussed, serological assays suffer from the cross-reactive nature of the antibodies in immunoassays such as HI, IFA, and ELISA, requiring the more labor intensive PRNT to determine antibodies produced in response to the specific virus.

Today, PCR-based molecular assays, such as real-time RT-PCR, are routinely used both in the field and in the clinical laboratory. Once the cause of an outbreak is established, these highly sensitive and specific molecular assays are of the greatest utility. However, early in an outbreak, when the cause is not yet determined, an orthogonal approach using both molecular and immunodiagnostic assays for detection of the virus ensures greater success. Immunodiagnostics are generally less sensitive than PCR-based molecular assays but have a broader specificity. Once the pathogen is known, the greater specificity and sensitivity of the PCR is a diagnostic advantage, though early in an outbreak, the greater or broader specificity of antibody-based immunodiagnostics is an advantage. This was illustrated in
the detection and identification of the newest ebolavirus species, Bundibugyo virus [36]. Initially the hemorrhagic disease appeared to be ebolavirus-like, but none of the molecular assays detected the agent. Only when the less sensitive, more broadly reactive antigen detection ELISA was used was the pathogen identified as an ebolavirus. This illustrates the importance of using an orthogonal diagnostic system when little is known about the cause of an outbreak.

Reliance on only molecular diagnostics in an outbreak of disease will result initially in missed detection and identification of the causative agent when time is of the essence. CHIKV is considered to be endemic in large parts of Africa, infecting humans frequently when they enter into the natural cycle through the bite of an infected mosquito [33]. In contrast, ONNV is thought to cause sporadic epidemics and then disappear during the intervening time periods [35]. Therefore, the cross-reactive nature of CHIKV and ONNV antibodies when no PRNTs are done can result in misinterpretation of serological data. In this study, we performed PRNTs on 22.5% (124 of 522) of the IgG ELISA CHIKV-positive samples and only found antibodies that neutralized ONNV, which would suggest that in Uganda ONNV may be endemic and CHIKV only rarely occurs (Table 3). Obvious caveats are that these samples were collected in a single time frame and that not all 522 samples were PRNT-analyzed; however, as we looked for IgG antibodies that represent the exposure of an individual over a lifetime, it would suggest that there is little to no CHIKV circulating in the regions tested. Possible misinterpretation of alphavirus seroprevalence is not unique to Uganda; results from similar studies that we conducted in Sierra Leone also suggest that ONNV was the greatest cause of alphavirus infection [37]. Using a CHIKV ELISA, we found that 4% of the IgM antibodies were to CHIKV, but after performing PRNTs, the majority were found to be specific to ONNV. After our 2006–2008 study in the area, a CHIKV disease outbreak was detected by a neighboring laboratory in 2012–2013. Although the outbreak was thought to be associated with CHIKV, it was probably ONNV disease. Attempts to detect CHIKV from outbreak samples by RT-PCR were unsuccessful, and PRNTs of the antibody samples were not done [38].

Clearly, previous assumptions that CHIKV disease is endemic whereas ONNV disease is less common and more likely occurs in epidemics in Africa may be incorrect. These assumptions can affect the deployment of surveillance capabilities and diagnostics used to detect and identify these closely related viruses. Expecting to find CHIKV disease in a region experiencing febrile illness with arthralgia and relying on PCR-based diagnostics alone could result in missed diagnoses, costing lost time in treatment, prevention, and control. This study suggests that although historically CHIKV infections were considered more likely in East Africa, ONNV disease surveillance should be a consideration and may be the more likely cause of febrile illness with arthralgia.

**Supplementary Data**

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Acknowledgments**

The authors wish to thank all the dedicated and hardworking employees of the Nakasero Blood Bank, Kampala, Uganda, and the Makerere University Walter Reed Project Laboratory, Kampala, Uganda. Special thanks to Ashley M. Zovanyi, Denise K. Danner, Jim F. Barth, Matthew A. Voorhees, Jose Ramon, Jared Deveraux, Ashley McCormack, Priscilla Williams, Scott Olschner, and Eric M. Mucker at the US Army Medical Research Institute of Infectious Diseases for their expert technical assistance. We thank Laura Bollinger, Integrated Research Facility, National Institute of Allergy and Infectious Diseases, for critically editing this manuscript.

**Disclaimer.** The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army.

**Ethical approval.** Research on human subjects was conducted in compliance with Department of Defense, Federal, and State statutes and regulations relating to the protection of human subjects and adheres to principles identified in the Belmont Report (1979). All data and human subjects research were gathered and conducted for this publication under institutional review board-approved protocols (WRAIR 1194 and USAMRIID HP-09–39).

**Financial support.** The laboratory work was funded in part by the Global Emerging Infections Surveillance Section of the Armed Forces Health Surveillance Branch (ProMIS plans P0023_16_RD and P0017_17_RD) through the US Army Medical Research Institute of Infectious Diseases and US Military HIV Research Program through Walter Reed Army Institute of Research.

**Potential conflicts of interest.** No authors have reported any potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**

1. Bausch DG, Rojek A. West Africa 2013: re-examining Ebola. Microbiol Spectr 2016; 4(3).

2. Patra S, Pandian K. Chikungunya: a threat for Caribbean. Travel Med Infect Dis 2016; 14:419–20.

3. World Health Organization. WHO director-general summarizes the outcome of the emergency committee regarding clusters of microcephaly and Guillain-Barré syndrome. http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/. Accessed 10 February 2017.

4. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol 2007; 88:2363–77.

5. Johansson MA, Powers AM, Pesik N, et al. Nowcasting the spread of chikungunya virus in the Americas. Plos One 2014; 9:e104915.

6. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. Trans R Soc Trop Med Hyg 1958; 49:28–32.

7. Powers AM. Risks to the Americas associated with the continued expansion of chikungunya virus. J Gen Virol 2015; 96:1–5.

8. Haddow AJ, Davies CW, Walker AJ. O’nyong-nyong fever: an epidemic virus disease in east Africa. Trans Royal Soc Trop Med Hyg 1960; 54:157–22.

9. Rweguma EB, Lutwama JJ, Sempala SD, et al. Emergence of epidemic o’nyong-nyong fever in southwestern Uganda, after an absence of 35 years. Emerg Infect Dis 1997; 3:77.

10. Woodruff AW, Bowen ET, Platt GS. Viral infections in travellers from tropical Africa. Br Med J 1978; 1:956–8.
11. Marshall TF, Keenlyside RA, Johnson BK, et al. The epidemiology of o'nyong-nyong in the Kano Plain, Kenya. Ann Trop Med Parasitol 1982; 76:153–8.
12. Rezza G, Chen R, Weaver SC. O'nyong-nyong fever: a neglected mosquito-borne viral disease. Pathog Glob Health 2017; 111:271–5.
13. LaBeaud AD, Banda T, Brichard J, et al. The epidemiology of o'nyong-nyong in the Kano Plain, Kenya. Ann Trop Med Parasitol 1988; 82:153–8.
14. Chanas AC, Hubalek Z, Johnson BK, Simpson DI. A comparative study of o'nyong-nyong virus with chikungunya virus and plaque variants. Arch Virol 1979; 59:231–8.
15. Blackburn NK, Besselaar TG, Gibson G. Antigenic relationship between chikungunya virus strains and o'nyong nyong virus using monoclonal antibodies. Res Virol 1995; 146:69–73.
16. Sabin AB, Schlesinger RW. Production of immunity to dengue with virus modified by propagation in mice. Science 1945; 101:640–2.
17. Melnick JL, Paul JR, Riordan JT, et al. Isolation from human sera in Egypt of a virus apparently identical to West Nile virus. Proc Soc Exp Biol Med 1951; 77:661–5.
18. Theiler M. The virus. In: Strode GK, ed. Yellow fever. New York: McGraw-Hill; 1951; 46–136.
19. Levitt NH, Ramsburg HH, Hasty SE, et al. Development of an attenuated strain of chikungunya virus for use in vaccine production. Vaccine 1986; 4:157–62.
20. Meegan JM. The Rift Valley fever epizootic in Egypt 1977–78. I. Description of the epizootic and virological studies. Trans R Soc Trop Med Hyg 1979; 73:618–23.
21. Ksiazek TG, Rollin PE, Williams AJ, et al. Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis 1999; 179(Suppl 1):S177–87.
22. Smith DH, Johnson BK, Isaacson M, et al. Marburg-virus disease in Kenya. Lancet 1982; 1:816–20.
23. Causey OR, Kemp GE, Madbouly MH, David-West TS. Congo virus from domestic livestock, African hedgehog, and arthropods in Nigeria. Am J Trop Med Hyg 1970; 19:846–50.
24. Auperin DD, Sasso DR, McCormick JB. Nucleotide sequence of the glycoprotein gene and intergenic region of the Lassa virus S genome RNA. Virology 1986; 154:155–67.
25. Chu YK, Rossi C, Leduc JW, et al. Serological relationships among viruses in the Hantavirus genus, family Bunyaviridae. Virology 1994; 198:196–204.
26. Meegan JM, Yedlouetschnig RI, Peleg BA, et al. Enzyme-linked immunosorbent assay for detection of antibodies to Rift Valley fever virus in ovine and bovine sera. Am J Vet Res 1987; 48:1138–41.
27. Watts DM, el-Tigani A, Botros BA, et al. Arthropod-borne viral infections associated with a fever outbreak in the northern province of Sudan. J Trop Med Hyg 1994; 97:228–30.
28. Burke DS, Ramsburg HH, Edelman R. Persistence in humans of antibody to subtypes of Venezuelan equine encephalomyelitis (VEE) virus after immunization with attenuated (TC-83) VEE virus vaccine. J Infect Dis 1977; 136:354–9.
29. Rodhain F, Gonzalez JP, Mercier E, et al. Arbovirus infections and viral haemorrhagic fevers in Uganda: a serological survey in Karamoja district, 1984. Trans R Soc Trop Med Hyg 1989; 83:851–4.
30. Henderson BE, Kirya GB, Hewitt LE. Serological survey for arboviruses in Uganda, 1967–69. Bull World Health Organ 1970; 42:797–809.
31. Williams MC, Woodall JP, Gillett JD. O'nyong-nyong fever: an epidemic virus disease in east Africa. Vii. Virus isolations from man and serological studies up to July 1961. Trans R Soc Trop Med Hyg 1965; 59:186–97.
32. McCrae AW, Henderson BE, Kirya BG, Sempala SD. Chikungunya virus in the Entebbe area of Uganda: isolations and epidemiology. Trans R Soc Trop Med Hyg 1971; 65:152–68.
33. Kalunda M, Lwanga-Ssozi C, Lule M, Mukuye A. Isolation of chikungunya and Pongola viruses from patients in Uganda. Trans R Soc Trop Med Hyg 1985; 79:567.
34. Kariuki Njenga M, Nderitu L, Ledermann JP, et al. Tracking epidemic chikungunya virus into the Indian Ocean from East Africa. J Gen Virol 2008; 89:2754–60.
35. Lanciotti RS, Ludwig ML, Rwaguma EB, et al. Emergence of epidemic o'nyong-nyong fever in Uganda after a 35-year absence: genetic characterization of the virus. Virology 1998; 252:258–68.
36. Towser JS, Sealy TK, Khristova ML, et al. Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. PLoS Pathog 2008; 4:e1000212.
37. Schoepf RJ, Rossi CA, Khan SH, et al. Undiagnosed acute viral febrile illnesses, Sierra Leone. Emerg Infect Dis 2014; 20:1176–82.
38. Ansumana R, Jacobsen KH, Leski TA, et al. Reemergence of chikungunya virus in Bo, Sierra Leone. Emerg Infect Dis 2013; 19:1108–10.