Molecular detection of Zika virus in blood and RNA load determination during the French Polynesian outbreak

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Zika virus (ZIKV) viremia is reported as low and transient; however, these estimates rely on limited data. We report RNA loads in sera collected from symptomatic patients during the 2013-2014 French Polynesian ZIKV outbreak. We performed molecular detection of ZIKV RNA in sera from 747 patients presenting with suspected acute phase ZIKV infection. Among patients with confirmed infection, we analyzed the duration of viremia, assessed viral RNA loads and recorded the main clinical symptoms. A total of 210/747 (28.1%) sera tested positive using a ZIKV-specific RT-PCR. Viral RNA loads in symptomatic patients that ranged from 5 to 3.7 × 10^6 copies/mL (mean 9.9 × 10^4 copies/mL) were not related to a particular clinical presentation, and were significantly lower than those previously obtained from asymptomatic ZIKV infected blood donors. The rate of detection of ZIKV RNA in sera from suspected cases of acute phase ZIKV infection was low. ZIKV RNA loads were lower in symptomatic patients compared to asymptomatic blood donors and were lower than RNA loads usually reported in dengue infections. As there is no abrupt onset of symptoms in ZIKV infections, we suggest that infected patients sought for medical attention when viremia was already decreasing or had resolved.

**KEYWORDS**

arbovirus, French Polynesia, RNA load, RT-PCR, Zika virus

1 | BACKGROUND

Zika virus (ZIKV), an arthropod-borne virus (arbovirus) of the *Flavivirus* genus in the *Flaviviridae* family1 was discovered in Africa in 1947.2 Only sporadic infections have been reported in Africa and Asia until the first ZIKV outbreak that occurred in the Pacific in 2007 in Yap (Federated States of Micronesia).3,4 After, ZIKV caused a second large outbreak in French Polynesia in 2013-2014 and subsequently spread throughout the Pacific islands.5-7 In 2015, ZIKV emerged in the Americas and rapidly spread throughout Latin America and Caribbean, and also caused an outbreak in Africa.7 Between 2007 and September 2016, 72 countries reported evidence of active ZIKV transmission.8 In August 2016, the first ZIKV autochthonous infections within the continental U.S. were reported in Florida (USA).9 In September 2016, the virus was reported circulating in Asia with several autochthonous infections detected in Singapore.10 ZIKV is principally transmitted by the bite of infected *Aedes* mosquitoes but non-vector-borne transmission is possible and includes sexual,11-13 materno-fetal,14,15 and blood transfusion transmissions.16,17 As ZIKV has been detected in humans from urine,18,19 saliva,16 or breast milk,20 other mode of transmission cannot be excluded. Like other arboviruses, most of the ZIKV infections are probably asymptomatic7 but the ratio of asymptomatic/symptomatic infections is not yet established and may vary according to local condition and ZIKV strains. Symptomatic patients usually report a mild disease without acute onset of fever. Clinical manifestations of ZIKV infection mainly consist of maculopapular rash, arthralgia/myalgia, low fever, asthenia, headache, and conjunctivitis,7 as reported in French Polynesia,21 Yap,22 and Brazil.23

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At the time of the study, the recommendation for acute phase ZIKV infection was based on molecular detection of ZIKV RNA during the acute phase of infection using reverse transcription (RT)-PCR in blood samples collected within the first week post-symptom onset (PSO). Since then, this recommendation has been enlarged to the second week PSO, and serodiagnosis is also performed if RT-PCR is negative. Serology-based diagnosis is limited by the cross-reactivity of antibodies developed in response to infections with other flaviviruses, especially dengue virus (DENV) and requires confirmation by sero-neutralization tests which can only be performed by highly trained and specialized laboratories. However, cross-reactivity has been observed even using sero-neutralization assays, especially in secondary arbovirus infections. In French Polynesia, as most of the patients were previously infected by DENV, ZIKV serodiagnosis was not implemented. ZIKV viremia is usually reported as low and transient, making diagnosis by RT-PCR a challenge. We report the data obtained using molecular based detection of ZIKV RNA from suspected cases of ZIKV infections and levels of ZIKV RNA loads observed for confirmed ZIKV infection cases. These viral loads were compared with those previously published for asymptomatic ZIKV RNA positive blood donors in French Polynesia.

2 METHODS

The study was conducted in French Polynesia from October 2013 to March 2014 and was approved by the Ethics Committee of French Polynesia (reference 66/CEPF). Serum samples from 747 patients presenting to health care facilities (hospitals, clinics, private physician, and dispensary) with suspected acute phase ZIKV infections were collected from venous blood puncture according to medical prescriptions. For each patient, a standardized symptom questionnaire inquiring about the number of days PSO and the main clinical symptoms was recorded. A suspected case was defined as a patient presenting with macula-papular rash and/or self reported or measured low grade fever (<38.5°C) and at least two of the following: conjunctivitis, arthralgia, myalgia, hand and/or feet oedema. A confirmed case was a suspected case with ZIKV RNA detected in serum and/or saliva samples. Viral RNA was extracted from serum samples using the NucliSSENS easyMAG® System (BioMérieux) extractor. Detection of ZIKV RNA was performed by RT-PCR with the CFX96 Touch™ Real-Time PCR Detection System thermocycler using the iScript One-Step RT-PCR kit for Probes (Bio-Rad), and two primer and probe sets that specifically bind to partial M/E and E encoding genes, as previously reported. ZIKV RT-PCR results were reported as positive when both genomic regions were amplified, equivocal if only one was amplified, and negative if no amplification occurred. Since DENV was co-circulating in French Polynesia during the ZIKV outbreak, all sera were also tested using a DENV-specific RT-PCR, as previously reported.

ZIKV RNA loads (number of RNA copies/mL) were determined from sera that tested positive by RT-PCR by comparison to a standard curve obtained from serial dilutions of a known concentration of ZIKV RNA synthetic transcript, as previously reported. Viral loads assessed in sera from symptomatic patients were compared using the same quantification method to those previously obtained in sera from asymptomatic blood donors collected by the blood bank center in French Polynesia from November 2013 to February 2014 during the ZIKV outbreak.

All statistical analyses were performed with the GraphPad Prism 6 software, using Mann-Whitney test.

3 RESULTS

Characteristics of the studied population are reported in Fig. 1. Among the 747 serum samples tested by ZIKV-specific RT-PCR, 210 (28.1%) were reported positive, 68 (9.1%) equivocal, and 469 (62.8%) negative (Table 1). Equivocal results were reported for single detection of the partial M/E genes in 38 patients (5.1%) or partial E gene for 30 patients (4%). All sera tested negative for DENV RNA. For patients with suspected ZIKV infections, infection on clinical symptoms and number of days PSO was available for 608 (81.4%), including 180 with confirmed ZIKV infections.

ZIKV RNA loads according to the number of days PSO are reported in Fig. 2. The percentage of positive samples was significantly higher for patients collected from day 1 to 7 PSO [30.8% (176/572)] compared to those collected later [11.1% (4/36)] (P < 0.01).

| TABLE 1 | Results of RT-PCR assays using two primers and probes sets for detection of partial M/E and E partial encoding genes of ZIKV in the 747 serum samples collected from French Polynesian suspected ZIKV infections cases |
|----------|------------------------------------------------|-------------------|-----------------|
|          | M/E partial gene | E partial gene | Total number (%) |
| Neg      | Neg             | 469 (62.8)     |                 |
| Pos      | Neg             | 38 (5.1)       |                 |
| Neg      | Pos             | 30 (4)         |                 |
| Pos      | Pos             | 210 (28.1)     |                 |
| Total    |                  | 747 (100)      |                 |

y/o, years old.
ZIKV RNA loads were assessed in 57 sera found positive by RT-PCR for which remaining sera was available (Supplemental Table S1). Detailed results for number of days PSO, and clinical data (fever, rash, asthenia, conjunctivitis, arthralgia/myalgia) were available for 42 patients.

ZIKV RNA loads that ranged from 5 to 3.7 × 10^6 copies/mL (mean 9.9 × 10^5 copies/mL) were not significantly different between males and females (P = 0.21) and between patients aged under and over 18 years (P = 0.12) (Table 2) and were not significantly different from patients presenting with and without fever, rash, asthenia, conjunctivitis, or arthralgia/myalgia (Table 3). In contrast, ZIKV RNA loads were significantly lower compared to ZIKV RNA loads from 26 asymptomatic French Polynesian blood donors that were previously published (RNA loads ranging from 2.5 × 10^3 to 8.1 × 10^5 copies/mL, mean 7.3 × 10^4 copies/mL) (P < 0.0001) (Supplemental Table S2).28

The analysis of ZIKV RNA loads from five blood donors that became symptomatic post-donation (ranging from 3 × 10^3 to 5.2 × 10^6, mean 1.1 × 10^6 copies/mL) showed no significant difference with those obtained from the 21 blood donors who remained asymptomatic (P = 0.95), but they were significantly higher than those found in symptomatic patients (P = 0.004).

### DISCUSSION

Among the cases suspected to have ZIKV infection, only 28.1% tested positive for detection of both partial M/E and E ZIKV encoding genes. While patients presented with symptoms compatible with a ZIKV infection and tested negative for DENV, the high rate of equivocal and negative results may be explained by the low sensitivity of our method which may have been responsible for false negative results. Another explanation is the timing of symptom development relative to ZIKV viremia in serum with a potential delay between development of viremia and symptom onset. Indeed by the time patients developed symptoms and sought for medical attention, ZIKV viremia may have resolved in blood and RNA cleared in serum.

The sensitivity of our method was the same as previously reported on sera tested in Yap,4 estimated at 25 and 100 copies per assay for partial M/E and E encoding genes, respectively.29 As previously reported, using the same primers and probes, ZIKV RNA was more frequently detected in saliva than in blood collected at the same time during acute phase ZIKV infection, but the use of saliva did not increase the window of detection of ZIKV RNA.31 A study conducted from persons with travel-associated ZIKV disease concluded that urine also increased the rate of ZIKV RNA detection during the acute phase of infection and extended the window of ZIKV RNA detection.32 Our results and those previously published32 suggest that, during the first days PSO, the higher detection rates by RT-PCR in urine and saliva compared to blood are probably related to higher ZIKV RNA loads in these fluids, suggesting that a higher sensitivity could be reached during the acute phase of ZIKV infection by testing urine or saliva instead of blood. Though the most important challenge to ZIKV diagnosis is the short window during which RNA can be detected in serum.

In contrast to other mosquito-borne diseases such as dengue, there is no abrupt onset of clinical symptoms in ZIKV infections.7 In dengue, flushing is common on day 1 or 2 post-onset of fever and rash usually appears between day 2 and 6 after onset of fever.33,34 DENV viremia peaks at day 2 before defervescence of fever, and only one third of infected patients have detectable RNA at the end of the febrile phase.35 Fever is not a prominent feature of ZIKV infection, is low grade, and is not the main cause of consultation as reported in French Polynesia and in Brazil.7,23 In French Polynesia, patients usually sought for medical attention during rash. Consequently, ZIKV infected patients might have sought for medical care later in the acute phase of infection and at that time, viremia was already decreasing or resolved. The finding that ZIKV RNA loads were significantly higher in pre-symptomatic blood donors compared to the 57 symptomatic patients confirms that blood samples from ZIKV infected symptomatic patients were probably collected during the resolving phase of viremia. Data about ZIKV RNA loads in symptomatic patients are scarce and rely on studies conducted in Yap and from case reports,14,20,36–39 with viral loads in blood up to 7.2 × 10^5 copies/mL. In Nicaraguan patients, the mean viral RNA load was 5.4 × 10^3 and 1.1 × 10^5 copies/mL in non pregnant patients and

### TABLE 2

|                  | ≤18 y/o | >18 y/o | Total (range, mean ZIKV RNA load) |
|------------------|---------|---------|----------------------------------|
| Female           | 13      | 23      | 36 (5.0 – 1.4 × 10^6, 4.8 × 10^5) |
| Male             | 6       | 15      | 21 (1.4 × 10^5 – 3.7 × 10^5, 1.9 × 10^5) |
| Total (range, mean ZIKV RNA load) | 19 (5.0 – 3.7 × 10^6, 2.7 × 10^5) | 38 (5.0 – 1.2 × 10^7, 1.3 × 10^6) | 57 (5.0 – 3.7 × 10^6, 9.9 × 10^5) |

y/o: years old.
In symptomatic ZIKV-infected patients, the low rate of detection of ZIKV RNA in serum can be explained by low viral loads, delayed consultation due to mildness of symptoms, and delayed development of symptoms in patients that probably sought for health care provider attention while viremia was resolving in blood. At the acute phase of ZIKV infection, the detection rate of ZIKV RNA by RT-PCR in serum is low compared to urine and saliva. Consequently, ZIKV infection should never be excluded on the basis of a negative RT-PCR in serum, and saliva and urine samples should be also tested.

5 | CONCLUSION

In symptomatic ZIKV-infected patients, the low rate of detection of ZIKV RNA in serum can be explained by low viral loads, delayed consultation due to mildness of symptoms, and delayed development of symptoms in patients that probably sought for health care provider attention while viremia was resolving in blood. At the acute phase of ZIKV infection, the detection rate of ZIKV RNA by RT-PCR in serum is low compared to urine and saliva. Consequently, ZIKV infection should never be excluded on the basis of a negative RT-PCR in serum, and saliva and urine samples should be also tested.

AUTHORS’ CONTRIBUTIONS

D. Musso, M. Lanteri, J. Broult, E. Grange, T. Nhan, and M. Aubry participated to the design of the study and redaction of the manuscript. E. Rouault, A. Teissier, and K. Zisou performed analyses.

CONFLICTS OF INTEREST

Authors disclose any financial and other conflict of interests.
REFERENCES

1. Gubler DJ, Kuno G, Markoff L. Flaviviruses. Field Virology. 5th ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2007;34:1155–1227.

2. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg 1952;46:509–520.

3. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfell M, Holzbauer S, Dubray C, Guillamot L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB. Zika virus outbreak on Yap Island. Federated States of Micronesia. N Engl J Med 2009;360:2536–2543.

4. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Stanfield SM, Duffy MR. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 2008;14:1232–1239.

5. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, Sall AA, Musso D. Zika virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis 2014;20:1085–1086.

6. Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. Clin Microbiol Infect 2014;20:O595–O596.

7. Musso D, Gubler DJ. Zika virus. Clin Microbiol Rev 2016;29:487–524.

8. World Health Organization (WHO). 2016; Situation report Zika virus transmission and impact in travellers to areas of ongoing transmission. http://www.who.int/csr/don/20160729-zika-cases.html

9. Center for disease control and prevention (CDC). 2016;ZIKV Florida. http://www.cdc.gov/media/releases/2016/p0729-florida-zika-cases.html

10. Dyer O. Outbreak of Zika in Singapore sparks warnings in neighbouring countries. BMJ 2016;354:i4740.

11. D’Ortenzio E, Matheron S, de Lamballerie X, Hubert B, Piorkowski G, Maquart M, Descamps D, Diamond F, Leparc-Goffart I. Evidence of sexual transmission of Zika virus. N Engl J Med 2016;374:2195–2198.

12. Hills SL, Russell K, Hennessey M, Williams C, Oster AM, Fischer M, Mead P. Transmission of Zika virus through sexual contact with travelers to areas of ongoing transmission—continental United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:215–216.

13. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerg Infect Dis 2015;21:359–361.

14. Besnard M, Lastère S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, France, 2016. Euro Surveill 2014;19 pii:20751.

15. Miklar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, Mraz J, Kolenc M, Resman Rus K, Vesnaver Vepotnik T, Fabjan Vodusek V, Viziak A, Pižem J, Petrovec M, Avšič Županc T. Zika virus associated with microcephaly. New Engl J Med 2016;374:951–958.

16. Food and Drug Administration (FDA). 2016; Recommendations for donor screening, deferral, and product management to reduce the risk of transfusion-transmission of Zika virus. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM486360.pdf

17. Musso D,斯特拉伯 SL, Bush MP. Zika virus: a new challenge for blood transfusion. Lancet 2016;387:1993–1994.

18. Bonaldo MC, Ribeiro IP, Lima NS, Dos Santos AA, Menezes LS, da Cruz SO, de Mello IS, Furtado ND, de Moura EE, Damasceno L, da Silva KA, de Castro MG, Gerber AL, de Almeida LG, Lourenço-de-Oliveira R, Vasconcelos AT, Brasil P. Isolation of infective Zika virus from urine and saliva of patients in Brazil. PLoS Negl Trop Dis 2016;10:e0004816.

19. Zhang FC, Li XF, Deng YQ, Tong YG, Qin CF. Excretion of infectious Zika virus in urine. Lancet Infect Dis 2016;16:641–642.

20. Dupont-Rouzyel M, Biron A, O’Connor G, Hugueno E, Descloz E. Infectious Zika viral particles in breastmilk. Lancet 2016;387:1051.

21. Mallet HP, Vial AL, Musso D. Bilan de l’épidémie à virus Zika en Polynésie française, 2013–2014, French Polynesia outbreak of Zika in French Polynesia, 2013–2014. BISE 2015;13:1–5.

22. Bel M. 2007; Zika virus infection for clinician and other health professionals. Yap State Department of Health Services. http://www.spc.int/whp/english/publications/information/IA27/Zika-outbreak-Yap-2.pdf

23. Brasil P, Calvet GA, Siqueira AM, Wakimoto M, de Sequeira PC, Nobre A, Quintana Mde S, Mendonça MC, Lapi O, de Souza RV, Romero C, Zogbi H, Bressan Cda S, Alves SS, Lourenço-de-Oliveira R, Nogueira RM, Carvalho MS, de Filippis AM, Jaenisch T. Zika virus outbreak in Rio de Janeiro, Brazil: clinical characterization, epidemiological and virological aspects. PLoS Negl Trop Dis 2016;10:e0004636.

24. World Health Organization (WHO). 2016; Laboratory testing for Zika virus infection. Interim guidance 23 March 2016. http://apps.who.int/iris/bitstream/10665/204671/1/WHO_ZIKV_LAB_16.1_eng.pdf?ua=1

25. Centers for Disease Control and Prevention (CDC). 2016; Guidance for U.S. Laboratories testing for Zika virus infection, June 26 2016. http://www.cdc.gov/zika/pdfs/laboratory-guidance-zika.pdf

26. Aubry M, Finke J, Teissier A, Roche C, Brout J, Paulous S, Després P, Cao-Lormeau VM, Musso D. Seroprevalence of arboviruses among blood donors in French Polynesia, 2011–2013. Int J Infect Dis 2015;41:11–12.

27. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika virus. N Eng J Med 2016;374:1552–1563.

28. Aubry M, Richard V, Green J, Brout J, Musso D. Inactivation of Zika virus in plasma with amotosalen and ultraviolet A illumination. Transfusion 2015;56:33–40.

29. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, Shan Yan A, Cao-Lormeau VM, Brout J. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill 2014;19 pii:20771.

30. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vormdav AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol 1992;30:545–551.

31. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. J Clin Virol 2015;68:53–55.

32. Binguam AM, Cone M, Mock V, Heberlein-Larson L, Stanek D, Blackmore C, Likos A. Interim guidance for Zika virus testing of urine—United States, 2016. MMWR 2016;65:474.

33. Waterman S, Gubler DJ. Dengue fever. Clin Dermatol 1989;7:117–122.

34. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998;11:480–496.

35. Chen LH, Wilson ME. Non-vector transmission of dengue and other mosquito-borne flaviviruses. WHO Dengue Bull 2005;29:18–31.

36. Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, Brugnaro P, Palù G. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016. Euro Surveill 2016;21: DOI: 10.2807/1560-7917.ES.2016.21.10.30159

37. Driggers RW, Ho CY, Kurkomen EM, Kuivanen S, Jääskeläinen A, Smura T, Rosenberg A, Hill DA, DeBiasi RL, Vezina G, Timofeev J, Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika virus. N Engl J Med 2016;374:2142–2151.
38. Wahre T, Maagard A, Tappe D, Cadar D, Schmidt-Chanasit J. Zika virus infection after travel to Tahiti, December 2013. *Emerg Infect Dis* 2014;20:1412–1414.

39. Mansuy JM, Dutertre M, Mengelle C, Fourcade C, Marchou B, Delobel P, Izopet J, Martin-Blondel G. Zika virus: high infectious viral load in semen, a new sexually transmitted pathogen? *Lancet Infect Dis* 2016;16:894–895.

40. Waggoner JJ, Gresh L, Vargas MJ, Ballesteros G, Tellez Y, Soda KJ, Sahoo MK, Nuñez A, Balmaseda A, Harris E, Pinsky BA. Viremia and clinical presentation in Nicaraguan patients infected with Zika virus, chikungunya virus, and dengue virus. *Clin Infect Dis* 2016; pii:ciw589.

41. Appassakij H, Khuntikij P, Kemapunmanus M, Wutthanarungsan R, Silpapojakul K. Viremic pro-fi-lies in asymptomatic and symptomatic chikungunya fever: a blood transfusion threat? *Transfusion* 2013;53:2567–2574.

42. Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. West Nile fever characteristics among viremic persons identified through blood donor screening. *J Infect Dis* 2010;202:1354–1361.

43. Lanteri MC, Lee T-H, Wen L, Kaidarova Z, Bravo MD, Kiely NE, Kamel HT, Tobler LH, Norris PJ, Busch MP. West Nile virus nucleic acid persistence in whole blood months after clearance in plasma: implication for transfusion and transplantation safety. *Transfusion* 2014;54:3232–3241.

44. Murgue B, Roche C, Chungue E, Deparis X. Prospective study of the duration and magnitude of viremia in children hospitalised during the 1996–1997 dengue-2 outbreak in French Polynesia. *J Med Virol* 2000;60:432–438.

45. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000;181:2–9.

46. Wang W-K, Chao D-Y, Kao C-L, Wu H-C, Liu Y-C, Li C-M, Lin S-C, Ho S-T, Huang J-H, King C-C. High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. *Virology* 2003;305:330–338.

47. Fox A, Le NM, Simmons CP, Wolbers M, Wertheim HF, Pham TK, Tran TH, Trinh TM, Nguyen TL, Nguyen VT, Nguyen DH, Farrar J, Horby P, Taylor WR, Nguyen VK. Immunological and viral determinants of dengue severity in hospitalized adults in Ha Noi, Viet Nam. *PLoS Negl Trop Dis* 2011;5:e967.

48. Sudiro TM, Zivny J, Ishiko H, Green S, Vaughn DW, Kalayanarooj S, Nisalak A, Norman JE, Ennis FA, Rothman AL. Analysis of plasma viral RNA levels during acute dengue virus infection using quantitative competitor reverse transcription-polymerase chain reaction. *J Med Virol* 2001;63:29–34.

49. Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev* 2009;22:564–581.

50. Gourinat AC, O’Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerg Infect Dis* 2015;21:84–86.

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