

Acknowledgments

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References

1. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. 2008; 371:1861–71. [http://dx.doi.org/10.1016/S0140-6736\(08\)60800-4](http://dx.doi.org/10.1016/S0140-6736(08)60800-4)
2. Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, et al.; ICTV Report Consortium. ICTV virus taxonomy profile: *Flaviviridae*. *J Gen Virol*. 2017;98:2–3. <http://dx.doi.org/10.1099/jgv.0.000672>
3. Bogovic P, Strle F. Tick-borne encephalitis: a review of epidemiology, clinical characteristics, and management. *World J Clin Cases*. 2015;3:430–41. <http://dx.doi.org/10.12998/wjcc.v3.i5.430>
4. Jääskeläinen A, Tonteri E, Pieninkeroinen I, Sironen T, Voutilainen L, Kuusi M, et al. Siberian subtype tick-borne encephalitis virus in *Ixodes ricinus* in a newly emerged focus, Finland. *Ticks Tick Borne Dis*. 2016;7:216–23. <http://dx.doi.org/10.1016/j.ttbdis.2015.10.013>
5. Jääskeläinen AE, Tonteri E, Sironen T, Pakarinen L, Vaheri A, Vapalahti O. European subtype tick-borne encephalitis virus in *Ixodes persulcatus* ticks. *Emerg Infect Dis*. 2011;17:323–5. <http://dx.doi.org/10.3201/eid1702.101487>

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Zika Virus IgG in Infants with Microcephaly, Guinea-Bissau, 2016

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We analyzed blood samples from infants born with microcephaly and their mothers in Guinea-Bissau in 2016 for pathogens associated with birth defects. No Zika virus RNA was detected, but Zika virus IgG was highly prevalent. We recommend implementing pathogen screening of infants with congenital defects in Guinea-Bissau.

In 2016, the health authorities in Guinea-Bissau reported 14 cases of Zika virus infection and 5 cases of microcephaly (1) to the World Health Organization. The Zika virus strain detected in Guinea-Bissau was the African strain (1) originally detected in Africa in 1947 and in Portuguese Guinea (now Guinea-Bissau) during 1964–1965 (2). As of March 2018, the Asian strain, which has spread throughout the Americas and Cape Verde (2) and is linked to microcephaly and other congenital abnormalities, has not been reported in Guinea-Bissau (3), and the African Zika virus strain has not been linked with microcephaly.

We report an in-depth investigation of pathogens commonly associated with birth defects in 15 infants born with microcephaly in Guinea-Bissau in 2016. Field epidemiologists identified cases of microcephaly through reports from health center personnel across the country and surveillance at Hospital Nacional Simão Mendes in Bissau, Guinea-Bissau (which has 6,000 births/y). Most cases were found in the northern and eastern regions (Gabú, Bafatá, and Oio) of Guinea-Bissau (online Technical Appendix Tables 1, 2, <https://wwwnc.cdc.gov/EID/article/24/5/18-0153-Techapp1.pdf>). Blood samples were collected from the mothers (median age 22 years, range 15–31 years) and infants (median age 5 months, range 1 day–9 months) and sent to Statens Serum Institut (Copenhagen, Denmark) for analysis. Three infants died before sampling, and 1 sample was lost during transport; hence, we analyzed blood samples from 11 of the 15 infants with microcephaly. For comparison, we also analyzed blood samples from 10 mothers (from Tantam Cossé, Bafatá region) of infants born without microcephaly (M.W. Rosenstjerne, unpub. data). We assayed for Zika virus and TORCH pathogens (*Toxoplasma*

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gondii, other [*Treponema pallidum*, varicella-zoster virus, parvovirus B19], rubella virus, cytomegalovirus [CMV], and herpes simplex virus) (online Technical Appendix Tables 1, 2) because these pathogens are most commonly associated with congenital anomalies (4,5).

Zika virus IgG immunofluorescence assay and Zika virus neutralization test (6,7) results revealed that 14 (93%) of the 15 mothers of infants with microcephaly had Zika virus neutralizing antibodies (NAbs) (online Technical Appendix Tables 1, 2) versus 5 (50%) of the 10 mothers of healthy infants (data not shown). We tested blood samples from the 11 infants with microcephaly for Zika virus NAbs, and all were positive (presumably maternal antibodies) (online Technical Appendix Tables 1, 2). We did not perform this assay with samples from the healthy infants. No samples were positive for Zika virus RNA or IgM or had cross-neutralizing antibodies to dengue virus. Thus, the Zika virus seroprevalence among Guinea-Bissau women was surprisingly high and significantly higher in the mothers of infants with birth defects ($p = 0.02$ by Fisher exact test). However, timing of the Zika virus infection and strain could not be determined.

Because of sample volume limitations, we tested only 10 of 15 mothers for TORCH antibodies and all 11 infants with birth defects and available blood samples for TORCH pathogen nucleic acids (online Technical Appendix Tables 1, 2). Four infant blood samples were positive for CMV DNA and IgG but only 2 were positive for CMV IgM (online Technical Appendix Tables 1, 2). Two of these infants' mothers were CMV IgG positive (the other 2 were not tested), and 1 mother tested positive for CMV IgM. Because sampling of infants was mainly performed 5 months postpartum rather than during the first 2–3 weeks postpartum (5,8), determining whether the CMV infections were congenital or acquired perinatally or postnatally (e.g., through breast milk) was not possible.

The mother whose infant died 5 days after birth was positive for *Toxoplasma* IgG (online Technical Appendix Tables 1, 2). However, samples from this child were not collected for analysis, so we could not determine whether the infant died of severe congenital toxoplasmosis. As expected, almost all mothers were positive for antibodies against parvovirus (70%), varicella-zoster virus (90%), rubella virus (90%), CMV (90%), and herpes simplex virus (100%).

Although we found a high prevalence of Zika virus NAbs and TORCH antibodies in mothers and infants, the late sampling of infants and lack of Zika virus RNA-positive samples precludes determination of the cause of microcephaly in these infants. On the basis of our findings, we propose implementing prospective surveillance in Guinea-Bissau for infants with easily identifiable congenital abnormalities, such as microcephaly (i.e., head circumference

2 standard deviations below average for age and sex) (9), microphthalmia, and hearing loss, and screening these infants for Zika virus and TORCH by using blood, saliva, and urine samples collected immediately or within the first 2–3 weeks after birth. The low prevalence (0.6%) of microcephaly reported in 2015 (10) makes this suggestion feasible in resource-poor countries. If the Asian Zika virus strain is detected in Guinea-Bissau, screening of pregnant women during their first trimester should also be implemented. However, the 2-step surveillance and screening model can be applied in countries without reported detection of the Asian Zika virus strain.

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References

- Gulland A. Continued spread of Zika raises many research questions, WHO says. *BMJ*. 2016;354:i4812. <http://dx.doi.org/10.1136/bmj.i4812>
- Musso D, Gubler DJ. Zika virus. *Clin Microbiol Rev*. 2016;29:487–524. <http://dx.doi.org/10.1128/CMR.00072-15>
- Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. *N Engl J Med*. 2016;374:1981–7. <http://dx.doi.org/10.1056/NEJMs1604338>
- Coyne CB, Lazear HM. Zika virus—reigniting the TORCH. *Nat Rev Microbiol*. 2016;14:707–15. <http://dx.doi.org/10.1038/nrmicro.2016.125>
- Devakumar D, Bamford A, Ferreira MU, Broad J, Rosch RE, Groce N, et al. Infectious causes of microcephaly: epidemiology, pathogenesis, diagnosis, and management. *Lancet Infect Dis*. 2018;18:e1–13. [http://dx.doi.org/10.1016/S1473-3099\(17\)30398-5](http://dx.doi.org/10.1016/S1473-3099(17)30398-5)
- De Smet B, Van den Bossche D, van de Werve C, Mairesse J, Schmidt-Chanasit J, Michiels J, et al. Confirmed Zika virus infection in a Belgian traveler returning from Guatemala, and the diagnostic challenges of imported cases into Europe. *J Clin Virol*. 2016;80:8–11. <http://dx.doi.org/10.1016/j.jcv.2016.04.009>
- Van Esbroeck M, Meersman K, Michiels J, Ariën KK, Van den Bossche D. Letter to the editor: specificity of Zika virus ELISA: interference with malaria. *Euro Surveill*. 2016;21:30237. <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.21.30237>
- Centers for Disease Control and Prevention. Identifying congenital cytomegalovirus (CMV) early in life: information for healthcare provider [cited 2018 Jan 30]. <https://www.cdc.gov/cmv/downloads/identifying-cmv.pdf>

9. World Health Organization. Screening, assessment and management of neonates and infants with complications associated with Zika virus exposure in utero. 2016 Aug 30 [cited 2018 Jan 30]. http://apps.who.int/iris/bitstream/10665/204475/1/WHO_ZIKV_MOC_16.3_eng.pdf?ua=1
10. Betunde AG. Casos de microcefalia en Guinea Bissau, 2015–2016. 2016 [cited 2018 Jan 30]. <http://vigisalud.gov.py/muestras/>

VIImuetra/Presentaciones_orales/dia1_LUNES_12-12-2016/PO_tarde_12_12_16/PO11.pdf

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LETTERS

Heterogeneous and Dynamic Prevalence of Asymptomatic Influenza Virus Infections

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To the Editor: We read with interest the article by Furuya-Kanamori et al. on the proportion of influenza virus infections that are asymptomatic or subclinical (1), and we are troubled by a series of fundamental flaws and errors. We were concerned that the authors presented pooled estimates of the asymptomatic fraction, given the massive heterogeneity in estimates (I^2 values of 97%–98% in Table 1). It is not considered good practice to present pooled estimates in instances of massive heterogeneity (2). We were very surprised that the authors included volunteer challenge studies because it is well known that the severity of these infections can be modulated by the route of administration and possibly the infectious dose. We also were surprised that human infections with avian influenza viruses were included because the epidemiology of these infections differs markedly from that of human influenza viruses. These studies were mistakenly labeled as studies of pandemic influenza in online Technical Appendix 1 Table 1 (<https://wwwnc.cdc.gov/EID/article/22/6/15-1080-Techapp1.pdf>). When reviewing serologic studies, the authors did not define a specific antibody titer threshold but relied on the choices made in individual studies; studies that inferred influenza virus infections based on low postepidemic hemagglutination-inhibition titers, such as 10 or 20, may lack specificity because some persons could have preexisting antibodies (3). Measurement error can also be a concern. The authors probably should have excluded such studies.

In another systematic review of the asymptomatic fraction of influenza virus infections (4), we found that study designs could explain a great deal of heterogeneity in the asymptomatic fraction in studies such as outbreak investigations

that used molecular testing to confirm influenza virus infections rather than serologic studies that used antibody titer measurements to indicate infections. Asymptomatic fractions were higher in general, and much more heterogeneous, in studies that followed the latter approach.

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References

1. Furuya-Kanamori L, Cox M, Milinovich GJ, Magalhaes RJ, Mackay IM, Yakob L. Heterogeneous and dynamic prevalence of asymptomatic influenza virus infections. *Emerg Infect Dis.* 2016;22:1052–6. <http://dx.doi.org/10.3201/eid2206.151080>
2. Greenland S, O'Rourke K. Meta-analysis. In: Rothman KJ, Greenland S, Lash TL, editors. *Modern epidemiology*. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008. p. 652.
3. Broberg E, Nicoll A, Amato-Gauci A. Seroprevalence to influenza A(H1N1) 2009 virus—where are we? *Clin Vaccine Immunol.* 2011;18:1205–12. <http://dx.doi.org/10.1128/CVI.05072-11>
4. Leung NH, Xu C, Ip DK, Cowling BJ. The fraction of influenza virus infections that are asymptomatic: a systematic review and meta-analysis. *Epidemiology.* 2015;26:862–72. <http://dx.doi.org/10.1097/EDE.0000000000000340>

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