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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 4 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. Rosenstierne, unpub. data). We assayed for Zika virus and TORCH pathogens (*Toxoplasma*

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.

About the Author

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

Technical Appendix

Technical Appendix Table 1. Epidemiologic characteristics and diagnostics test results of infants with microcephaly and their mothers, cases A-G, Guinea-Bissau, 2016*

Category	Case A		Case B		Case C		Case D		Case E		Case F		Case G	
Patient	Infant†	Mother	Infant	Mother	Infant†	Mother	Infant†	Mother	Infant	Mother	Infant	Mother	Infant	Mother
Age	1 m‡	18 y	4 m	24 y	5 d‡	18 y	9 m	30 y	3 m	20 y	5 m	30 y	5 m	22 y
Image of infant														
Region	Bafatá		Bafatá		Bafatá		Oio		Gabú		Gabú		Bafatá	
Village	Sta Gaulo		Tantam Cossé		Contubel		Nhacra		Buruntuma		Duma		Tantam Cossé	
Symptoms of mother during pregnancy	Periodic headache		Weakness		None		None		Periodic headache		Fever		Fever, headache, weakness, stomach pain	
Month of birth	August		September		August		June		May		June		May	
Birthweight	Not reported		Not reported		Not reported		2,900 g		2,800 g		Not reported		Not reported	
Weight at sampling	2,200 g		3,550 g		Not reported		Not reported		Not reported		Not reported		6,200 g	
Infant sex	M		F		F		F		F		M		M	
Infant head circumference (*1*) at birth Girls: reference range (31.7–36.1 cm) Boys: reference range (32.1–36.9 cm)	25 cm		26 cm		25 cm		Not reported		Not reported		Not reported		Not reported	
Infant head circumference at sampling Girls: reference range (39.0–43.9 cm) Boys: reference range (40.3–44.8 cm)	Not reported		Not reported		Not reported		31.5 cm		Not reported		Not reported		35 cm	
Clinical symptoms	Microcephaly		Microcephaly		Microcephaly		Microcephaly		Microcephaly		Microcephaly		Microcephaly	
Pathogen, diagnostic test														
Zika virus														
RT-qPCR§	NA	–	–	–	NA	–	NA	–	–	–	–	–	–	–
IgM¶	NA	–	–	–	NA	–	NA	–	–	–	–	–	–	–
IgG¶	NA	+	+	+	NA	–	NA	+	+	+	+	+	+	–
Nab#	NA	>640	200	>640	NA	>640	NA	480	240	400	>640	538	80	>640
Dengue virus														
IgM¶	NA	–	–	–	NA	–	NA	–	–	–	–	–	–	–
IgG¶	NA	+	–	+	NA	+	NA	–	+	+	–	–	–	–
Nab#	NA	NA	–	NA	NA	NA	NA	NA	53	NA	NA	NA	–	NA
Chikungunya virus														

Category	Case													
	A		B		C		D		E		F		G	
Patient	Infant†	Mother	Infant	Mother	Infant†	Mother	Infant†	Mother	Infant	Mother	Infant	Mother	Infant	Mother
IgM¶	NA	−	−	−	NA	−	NA	−	−	−	−	−	−	−
IgG¶	NA	+	+	+	NA	+	NA	−	+	+	−	−	−	−
Toxoplasma gondii														
IgM**		NA		−		−		−		−		NA		−
IgG**				−		230		−		−				−
Treponema pallidum														
IgG††		NA		−		−		−		−		NA		−
Parvovirus														
qPCR‡‡	NA		−		NA		NA		−		−		−	
IgG¶¶		NA		0.74		0.90		0.64		0.90		NA		0.89
Varicella-zoster virus														
qPCR‡‡	NA		−		NA		NA		−		−		−	
IgG††		NA		INC		800		800		800		NA		200
Rubella virus														
qPCR‡‡	NA		−		NA		NA		−		−		−	
IgG§§		NA		80		102		87		160		NA		INC
Cytomegalovirus														
qPCR‡‡	NA		−		NA		NA		−		−		−	
IgM††		NA		NA		NA		NA		NA		NA		NA
IgG††		NA		>640		40		-		80		NA		5
Herpes simplex virus														
qPCR‡‡	NA		−		NA		NA		−		−		−	
IgG††		NA		1.98		1.85		2.23		1.82		NA		1.65

*INC, inconclusive; NA, not analyzed; Nab, neutralizing antibody; RT-qPCR, reverse transcription quantitative PCR; qPCR, quantitative PCR.
†Samples not send to Statens Serum Institut (Copenhagen, Denmark) for analysis.
‡Deceased.
§In-house PCR modified from Faye et al (*2*).
¶Arbovirus Fever Mosaic 2 (Zika virus, chikungunya virus, dengue virus) (IgG/IgM) (Euroimmun AG,Luebeck, Germany).
#Neutralization assay with African strain MR766. Positive antibody titers are shown.
**VIDAS TOXO IgG II (bioMérieux, Marcy l'Etoile, France). Positive antibody titers are shown.
††In-house ELISA. Positive antibody titers are shown.
‡‡In-house qPCR. Positive Cq values are shown.
§§ Enzygnost anti-Rubella-Virus IgG (Siemens Healthineers, Erlangen, Germany). Positive antibody titers are shown.
¶¶LIAISON Biotrin Parvovirus B19 IgG (DiaSorin, Saluggia, Italy). Positive antibody titers are shown.
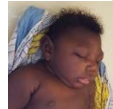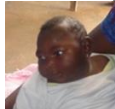
Technical Appendix Table 2. Epidemiologic characteristics and diagnostics test results of infants with microcephaly and their mothers, cases H–O, Guinea-Bissau, 2016*

Category	H Infant	H Mother	I Infant	I Mother	J Infant	J Mother	K Infant	K Mother	L Infant	L Mother	M Infant	M Mother	N Infant	N Mother	O Infant†	O Mother
Patient	Infant	Mother	Infant	Mother	Infant	Mother	Infant	Mother	Infant	Mother	Infant	Mother	Infant	Mother	Infant†	Mother
Age	5 m	18 y	7 m	30 y	4 m	22 y	8 m	25 y	4 m	15 y	5 m	31 y	5 m	18 y	1 d‡	22 y
Image of infant																
Region	Bafatá		Bolama		Bafatá		Gabú		Farim		Bafatá		Bafatá		Gabú	
Village	Tendito		Bolama		Cambadju		Dara		Farim		Xitole		Xitole		Gabú	
Symptoms of mother during pregnancy	Fever, myalgia, headache, weakness, stomach pain		Headache, vaginal discharge		Fever, headache, weakness		Fever, arthralgia, myalgia, headache, weakness, anemia (received blood transfusion)		Headache		Weakness, stomach pain		Fever, headache, weakness, stomach pain		None	
Month of birth	May		March		June		February		June		May		May		August	
Birthweight	Not reported		2,600 g		Not reported		Not reported		2,300 g		Not reported		3,200 g		Not reported	
Weight at sampling	Not reported		Not reported		5,800 g		Not reported		Not reported		6,100 g		No info		Not reported	
Infant sex	F		F		F		M		M		F		F		M	
Infant head circumference (1) at birth Girls: reference range (31.7–36.1 cm) Boys: reference range (32.1–36.9 cm)	Not reported		Not reported		Not reported		Not reported		Not reported		Not reported		Not reported		Not reported	
Infant head circumference at sampling Girls: reference range (39.0–43.9 cm) Boys: reference range (40.3–44.8 cm)	34 cm		35 cm		33 cm		36.5 cm		34 cm		34 cm		33 cm		Not reported	
Clinical symptoms	Microcephaly, malformed umbilicus		Microcephaly, abnormal psychomotor development		Microcephaly		Microcephaly, conjunctivitis, abnormal psychomotor development		Microcephaly		Microcephaly, visual problems		Microcephaly		Microcephaly	
Pathogen, diagnostic test																
Zika virus																
PCR§	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–
IgM¶	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–
IgG¶	+	+	+	+	+	–	–	+	+	+	+	+	+	+	NA	–
Nab#	>640	640	>640	120	140	560	18	>640	>640	>640	240	>640	50	480	NA	–
Dengue virus																
IgM¶	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–
IgG¶	+	–	–	–	–	–	–	+	+	+	+	+	–	+	NA	–
Nab#	–	NA	NA	–	–	NA	–	NA	-	NA	-	NA	–	NA	NA	NA
Chikungunya virus																
IgM¶	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–
IgG¶	–	+	–	–	–	–	–	+	–	–	–	+	–	–	NA	–

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Category	H Infant	H Mother	I Infant	I Mother	J Infant	J Mother	K Infant	K Mother	L Infant	L Mother	M Infant	M Mother	N Infant	N Mother	O Infant†	O Mother
Toxoplasma gondii																
IgM**		NA		–		–		NA		–		NA		–		–
IgG**		NA		–		–				–		NA		–		–
Treponema pallidum																
IgG††		NA		–		–		NA		–		NA		–		–
Parvovirus																
qPCR‡‡	–		–		–		–		–		–		–		NA	
IgG¶¶		NA		–		0.41		NA		1.76		NA		–		INC
Varicella-zoster virus																
qPCR‡‡	–		–		–		–		–		–		–		NA	
IgG††		NA		200		200		NA		400		NA		400		800
Rubella virus																
qPCR‡‡	–		–		–		–		–		–		–		NA	
IgG§§		NA		131		120		NA		101		NA		>200		178
Cytomegalovirus																
qPCR‡‡	36	–	–		31	–	–		–		36	–	34	–	NA	
IgM††	200	–	NA		–	–		NA		NA	–	–	400	200		NA
IgG††	160	NA	160		80	40		NA		320	80	NA	80	40		320
Herpes simplex virus																
qPCR‡‡	–	–	–		–		–		–		–		–		NA	
IgG††		NA		2.01		2.03		NA		1.94		NA	–	2.00		2.24

*INC, inconclusive; NA, not analyzed; Nab, neutralizing antibody; RT-qPCR, reverse transcription quantitative PCR; qPCR, quantitative PCR.
†Samples not send to Statens Serum Institut (Copenhagen, Denmark) for analysis.
‡Deceased.
§In-house PCR modified from Faye et al (*2*).
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#Neutralization assay with African strain MR766. Positive antibody titers are shown.
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