High Prevalence of *Shigella* or Enteroinvasive *Escherichia coli* Carriage among Residents of an Internally Displaced Persons Camp in South Sudan

Jesse Bliss,¹ Malika Bouhenia,² Peter Hale,¹ Brianne A. Couturier,³ Anita S. Iyer,¹ John Rumunu,⁴ Stephen Martin,⁵ Joseph F. Wamala,² Abdinasir Abubakar,² David A. Sack,⁶ Francisco J. Luquero,^{6,7} Marc Roger Couturier,^{3,8} Andrew S. Azman,^{9,10} and Daniel T. Leung^{1,8*}

¹Division of Infectious Diseases, University of Utah School of Medicine, Salt Lake City, Utah; ²World Health Organization, Juba, South Sudan; ³ARUP Laboratories, Salt Lake City, Utah; ⁴Republic of South Sudan Ministry of Health, Juba, South Sudan; ⁵World Health Organization, Geneva, Switzerland; ⁶Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; ⁷Epicentre, Paris, France; ⁸Department of Pathology, University of Utah, Salt Lake City, Utah; ⁹Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; ¹⁰Médecins sans Frontières, Geneva, Switzerland

Abstract. Displaced persons living in camps are at an increased risk of diarrheal diseases. Subclinical carriage of pathogens may contribute to the spread of disease, especially for microbes that require a low infectious dose. Multiplex real-time polymerase chain reaction was performed to detect a panel of 20 bacterial, viral, and protozoal targets, and we report a high prevalence of enteropathogen carriage, including *Shigella* spp. or enteroinvasive *Escherichia coli* in 14%, among a sample of 88 asymptomatic individuals in an internally displaced persons camp in South Sudan. Further studies are needed to determine the contribution of such carriage to the spread of disease.

Enteric infections pose a major health concern for low-resource populations worldwide, especially in young children, for whom diarrheal disease is a leading cause of mortality.¹ There is an increasing number of persons displaced by wars and natural disasters around the world, with over 65 million displaced persons reported in 2015.² Persons living in camps for refugees and internally displaced persons (IDPs) may have an increased risk for diarrheal diseases because of crowded living conditions and inadequate access to safe water and sanitation.^{3,4} This poses risk for pathogen spread among those living and working in camps, as demonstrated by a recent report of *Shigella* infections among refugees and staff in European transit centers.⁵

Studies have demonstrated a high burden of asymptomatic enteropathogen carriage among persons in low-income countries, particular in young children,⁶ although there are few data in adults. Asymptomatic or subclinical carriage of pathogens may contribute to spread of diarrheal diseases, especially for microbes that require a low infectious dose, such as *Shigella* spp.⁷ Little is known regarding the burden of enteropathogen carriage in IDP camps, many of which may have an environment conducive to the spread of enteric pathogens and a large susceptible population, including many children. Recent advances in technology have enabled probing of enteropathogen prevalence among populations using culture-independent methods.^{6,8,9} Our objective was to explore the distribution of enteropathogen carriage of persons living in an IDP camp in South Sudan.

As part of a mass vaccination campaign in an IDP camp in Juba, South Sudan, we obtained stool samples from a convenience sample of 88 healthy vaccine recipients with no obvious or self-reported signs of acute diarrheal illness within the week before their first dose of a killed whole cell oral cholera vaccine.¹⁰ We stored and shipped stool samples at –80°C and dry ice. Stool was diluted in PBS at 1:10 ratio and extracted on the Chemagen II (Perkin Elmer, Baesweiler, Germany) using a 2×

Blood Lysis kit. Multiplex real-time polymerase chain reaction was performed to detect a panel of 20 bacterial, viral, and protozoal targets (Table 1) using a validated laboratory-developed test (ARUP Laboratories, Salt Lake City, UT). The study protocol was reviewed and approved by the ethical review committee of the South Sudan Ministry of Health and the institutional review boards of Johns Hopkins Bloomberg School for Public Health and the University of Utah.

RESULTS

We found a high prevalence of enteropathogen carriage among the 88 participants, across all age groups (Table 1). Overall, 45% (40/88) of subjects had at least one pathogen detected in stool, with 22% (19/88) having two or more. Among those aged 1–5 years ($N = 8$), 6–17 years ($N = 27$), and 18–60 years ($N = 53$), 50%, 48%, and 43% of subjects, respectively, had at least one enteropathogen detected in stool. The most commonly detected pathogens were *Shigella* spp. or enteroinvasive *Escherichia coli* (EIEC) in 14% (12/88), *Dientamoeba fragilis* in 13% (11/88), norovirus (G1/G2) in 11% (10/88), and Shiga toxin–producing *Escherichia coli* in 9% (8/88). We did not find any differences in the prevalence of enteropathogen carriage between age groups or geographic state of origin.

The most prevalent enteropathogens found in our study were *Shigella* spp. or EIEC. Globally, *Shigella* is a leading cause of moderate to severe diarrhea in young children.¹¹ The parasite *Shigella* spreads easily from person to person, particularly in densely populated or overcrowded conditions,¹² including daycare centers¹³ and displaced person camps.^{4,5} This ease of spread may be associated with the low inoculum size necessary to cause infection.⁷ Furthermore, in a camp that draws IDPs from different, sometimes far away, regions, persons may encounter different serotypes than the ones for which they have immunity. Altogether, the high rates of *Shigella* carriage is highly concerning given the crowded living conditions of an IDP camp and the potential introduction of novel serotypes to nonimmune populations.

Despite decreasing diarrhea-related deaths over the past decade,¹ diarrheal diseases are still among the top three causes of under-5 child mortality in sub-Saharan Africa and

* Address correspondence to Daniel T. Leung, Division of Infectious Diseases, University of Utah School of Medicine, 30 North 1900 East, Room 4B319, Salt Lake City, UT 84132. E-mail: daniel.leung@utah.edu

TABLE 1

Enteropathogens detected in fecal samples from asymptomatic subjects living in an internally displaced persons camp in South Sudan

	1–5 yrs (N = 8)	6–17 yrs (N = 27)	18+ yrs (N = 53)	Total (N = 88)
<i>Shigella</i> spp./EIEC	2	1	9	12
<i>Dientamoeba fragilis</i>	1	7	3	11
Norovirus G1/G2	2	2	6	10
Shiga toxin <i>E. coli</i> (STEC)	0	2	6	8
<i>Salmonella</i> spp.	1	4	1	6
<i>Campylobacter jejuni/coli</i>	0	3	2	5
<i>Giardia</i> spp.	1	1	2	4
<i>Cryptosporidium</i> spp.	0	0	2	2
Adenovirus	0	1	0	1
Other <i>Campylobacter</i> spp.*	0	0	1	1
Sapovirus	0	0	1	1
Astrovirus	0	0	0	0
<i>Campylobacter upsaliensis</i>	0	0	0	0
<i>Cyclospora</i> spp.	0	0	0	0
<i>Entamoeba histolytica</i>	0	0	0	0
Hepatitis E virus	0	0	0	0
Rotavirus	0	0	0	0
<i>Vibrio cholerae</i>	0	0	0	0
≥ 1 pathogens	4 (50%)	13 (48%)	23 (43%)	40 (45%)
≥ 2 pathogens	2 (25%)	7 (26%)	10 (19%)	19 (22%)
≥ 3 pathogens	1 (13%)	1 (4%)	3 (6%)	5 (6%)

EIEC = enteroinvasive *Escherichia coli*.* *Campylobacter ureolyticus*, *Campylobacter lari*, *Campylobacter hyointestinalis*.

in 2013 were responsible for an estimated 520,000 deaths globally for those under the age of 5.¹ A recent report demonstrated that young children accounted for the largest burden of infections and deaths during a *Shigella* outbreak in an IDP camp in Papua New Guinea.⁵ Little is known about the asymptomatic carriage of *Shigella* in adults and its impact on children living in their proximity. Regardless of the pathogen type, the contribution of enteropathogen carriage to the transmission of diarrheal diseases among displaced persons living in camps is unknown. Asymptomatic carriers are a potential source for transmission of enteric pathogens in other settings,^{14–16} and prospective cohort studies with microbial strain typing are needed given the unique context of a displaced persons camp. A better understanding of the transmission patterns and the importance of asymptomatic carriage could certainly improve the public health interventions and decrease the diarrheal diseases burden in highly vulnerable populations.

Our study has several limitations. We used a convenience sample from a single IDP camp without controlling for household or regional clustering, so generalizability is limited. Although no mass-treatments had been reported at the camp, we did not collect data on historical antibiotic use among these individuals, and the lack of diarrheal symptoms were self-reported. Third, we used only molecular detection of pathogens, and thus, serotypes and antibiotic susceptibility patterns were not examined, and no isolates were available to further distinguish *Shigella* from EIEC, which causes a syndrome that is clinically indistinguishable from shigellosis. Despite these limitations, we report a high prevalence of asymptomatic *Shigella* spp. or EIEC carriage among IDPs in a camp in South Sudan. With the rise in displaced persons worldwide, the potential for such carriage to contribute to disease transmission needs further examination.

Received April 28, 2017. Accepted for publication September 2, 2017.

Published online December 4, 2017.

Acknowledgments: We would like to thank the study staff, participants, community leaders in the UN House PoC, staff at the WHO

Juba Country Office, and International Medical Corps staff. This work was sponsored by The National Institute of Health (K08 AI100923 grant to D. T. L.), The Bill & Melinda Gates Foundation (OPP1089243 to A. S. A., Delivering Oral Vaccine Effectively (DOVE) Project, OPP153556 to D. A. S., F. J. L., A. S. A., and OPP 1089248 to WHO), and Margaret A Cargill Foundation grants to the WHO.

Authors' addresses: Jesse Bliss, Peter Hale, and Daniel T. Leung, Division of Infectious Diseases, University of Utah, Salt Lake City, UT, E-mails: jesse.bliss@utah.edu, peter.hale@hsc.utah.edu, and daniel.leung@utah.edu. Malika Bouhenia, World Health Organization, Juba, South Sudan, E-mail: malik14@yahoo.fr. Brianne A. Couturier and Marc Roger Couturier, ARUP Laboratories, Salt Lake City, UT, E-mails: brianne.a.couturier@aruplab.com and marc.couturier@aruplab.com. Anita S. Iyer, Division of Infectious Diseases, Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT, E-mail: anita.iyer@hsc.utah.edu. John Rumunu, Department of Preventative Health, Government of the Republic of South Sudan Ministry of Health, Juba, South Sudan, E-mail: jrumunu@yahoo.com. Stephen Martin, World Health Organization, Geneva, Switzerland, E-mail: martins@who.int. Joseph F. Wamala, World Health Organization, Emergency, Juba, South Sudan, E-mail: wamalaj@who.int. Abdinasir Abubakar, World Health Organization, Juba, South Sudan, E-mail: abubakara@who.int. David A. Sack, Department of International Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, E-mail: dsack1@jhu.edu. Francisco J. Luquero, Epicentre, Paris, France, E-mail: francisco.luquero@epicentre.msf.org. Andrew S. Azman, Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, and Medecins Sans Frontieres, Geneva, Switzerland, E-mail: azman@jhu.edu.

REFERENCES

1. Kyu HH et al. Global Burden of Disease Pediatrics Collaboration, 2016. Global and national burden of diseases and injuries among children and adolescents between 1990 and 2013: findings from the global burden of disease 2013 study. *JAMA Pediatr* 170: 267–287.
2. UNHCR, 2016. *Global Trends Forced Displacement in 2015*. Geneva, Switzerland: UNHCR Global Trends.
3. Shultz A, Omollo JO, Burke H, Qassim M, Ochieng JB, Weinberg M, Feikin DR, Breiman RF, 2009. Cholera outbreak in kenyan refugee camp: risk factors for illness and importance of sanitation. *Am J Trop Med Hyg* 80: 640–645.

4. Benny E et al., 2014. A large outbreak of shigellosis commencing in an internally displaced population, Papua New Guinea, 2013. *Western Pac Surveill Response J* 5: 18–21.
5. Lederer I, Taus K, Allerberger F, Fenkart S, Spina A, Springer B, Schmid D, 2015. Shigellosis in refugees, Austria, July to November 2015. *Euro Surveill* 20: pii30081.
6. Taniuchi M, Sobuz SU, Begum S, Platts-Mills JA, Liu J, Yang Z, Wang XQ, Petri WA Jr, Haque R, Houpt ER, 2013. Etiology of diarrhea in Bangladeshi infants in the first year of life analyzed using molecular methods. *J Infect Dis* 208: 1794–1802.
7. DuPont HL, Levine MM, Hornick RB, Formal SB, 1989. Inoculum size in Shigellosis and implications for expected mode of transmission. *J Infect Dis* 159: 1126–1128.
8. Ryan ET, 2013. The intestinal pathobiome: its reality and consequences among infants and young children in resource-limited settings. *J Infect Dis* 208: 1732–1733.
9. Lindsay B et al., 2013. Quantitative PCR for detection of *Shigella* improves ascertainment of *Shigella* burden in children with moderate-to-severe diarrhea in low-income countries. *J Clin Microbiol* 51: 1740–1746.
10. Iyer AS et al., 2016. Immune responses to an oral cholera vaccine in internally displaced persons in South Sudan. *Sci Rep* 6: 35742.
11. Liu J et al., 2016. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. *Lancet* 388: 1291–1301.
12. Mosley W, Adams B, Lyman E, 1962. Epidemiologic and sociologic features of a large urban outbreak of shigellosis. *JAMA* 182: 1307–1311.
13. Arvelo W et al., 2009. Transmission risk factors and treatment of pediatric shigellosis during a large daycare center-associated outbreak of multidrug resistant *Shigella sonnei*: implications for the management of shigellosis outbreaks among children. *Pediatr Infect Dis J* 28: 976–980.
14. Riggs MM, Sethi AK, Zabarsky TF, Eckstein EC, Jump RLP, Donskey CJ, 2007. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic *Clostridium difficile* strains among long-term care facility residents. *Clin Infect Dis* 45: 992–998.
15. Ko CF, Wang LY, Lin NT, Chiou CS, Yeh HC, Renn JH, Lee YS, 2013. Transmission and strain variation of *Shigella flexneri* 4a after mass prophylaxis in a long-stay psychiatric centre. *Epidemiol Infect* 141: 242–250.
16. Thornley CN, Hewitt J, Perumal L, Van Gessel SM, Wong J, David SA, Rapana JP, Li S, Marshall JC, Greening GE, 2013. Multiple outbreaks of a novel norovirus GII.4 linked to an infected post-symptomatic food handler. *Epidemiol Infect* 141: 1585–1597.