LETTER

Reply to Mekalanos et al.: Genomic diversity of *Vibrio cholerae*

We reported non-01/0139 *Vibrio cholerae* prevalence in patients during the early weeks of the Haiti epidemic (1), to which Mekalanos et al. (2) responded. Cholera, a centuries-old worldwide scourge, believed to be caused only by toxigenic *V. cholerae* O1, in 1992 was shown to be more complex, when a non-O1 *V. cholerae* India/Bangladesh epidemic occurred. Non-O1/O139 strains are not routinely assessed (3), but recent studies show significant prevalence of non-O1/O139 infections, up to 30.4% incidence among hospitalized patients (4). We used highly sensitive methods to identify O1 and non-O1/O139 strains (1, 5), because methods used to culture *V. cholerae* O1 are indeed unreliable, particularly under makeshift conditions (2).

Contention that some samples were not outbreak associated (2) is surprising, because we reinforced our microbiological findings with high-resolution phylogenetic analysis. All clinical non-O1/O139 strains formed two closely related clusters within a monophyletic clade—data consistent with an "epidemic genotype" (1). Mekalanos et al. (2) define cholera as "...a diarrheal disease that can be reproduced by oral ingestion of purified cholera toxin," a definition not used by physicians treating cholera. Attending physician and coauthor J.B.P. (1) identified cholera patients as "exhibiting symptoms of profuse watery diarrhea, vomiting, and dehydration with varying severity." Furthermore, the US Centers for Disease Control and Prevention (CDC)'s 1996 cholera case definition states that only confirmed cases should be notified. A confirmed case requires definitive laboratory evidence distinguishing *V. cholerae* O1 from non-O1/O139 strains, no doubt explaining why non-O1/O139 incidence was underreported.

With respect to genomic islands (GIs) in non-O1/O139, examination of 47 Haitian *V. cholerae* O1 strains revealed 18 genetically similar GIs and pathogenicity islands (PIs), whereas 29 clinical and environmental non-O1/O139 strains revealed 40 GIs/PIs encoding genes for virulence, survival, stress response, and metabolism; this large number suggests that the islands may contain additional, unidentified fitness factors associated with human disease. We noted insertion of GIs in VPI–I, -II, and VSP-I, and a not-yet-characterized integrative conjugative element in genomes of several non-O1/O139 strains (figures S2 and S3 in ref. 1). Non-O1/O139 strains can be highly enterotoxic, hemolytic, and/or proteolytic, despite lacking genes encoding cholera toxin and virulence factors of toxigenic *V. cholerae* O1, indicating severe diarrheal disease induced by additional pathogenic mechanisms. We reported diversity and not attribution, as the former was the focus of our study (1, 5). Inclusion of Nepalese strain(s) would have been interesting but, regrettably, up until submission of our manuscript, we did not have access to these strains.

We believe focus should be on cholera spread and intervention strategies during crises—paying critical attention to the etiology of each patient's disease. Before the 2010 cholera epidemic, Haiti listed no reportable waterborne diseases, because water samples were not routinely tested for pathogens. Our recent collaboration with the CDC reported *V. cholerae* non-O1/O139, *V. parahaemolyticus*, human pathogenic viruses, and *Cryptosporidium* spp. in samples collected from river, canal, lake, and marine water in Haiti (6). The dilemma is whether non-O1/O139 in Haitian waters will play a role in establishing O1 as an endemic pathogen in Haiti.

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