Reply to Yue

To the Editor—We thank Dr. Yue for his interest in our study [1]. We investigated reported non-typhoidal *Salmonella* infections in an entire country over 18 years. This design was predicated on the idea that persistent infections of any duration are of epidemiological and clinical interest. For this reason, our study retrospectively selected available exemplars of persistent strains that were observed in this large series.

The correspondent says our results are negative and later says they are confounding relative to studies with much longer times of persistence. On the contrary, for the reasons that the correspondent gives: The actual persistence observed was typically only a few months. Thus, the number of single-nucleotide polymorphisms (SNPs) and gains and losses of plasmids and phages will, inevitably, be less than in cases where persistence proceeds for longer. The results are what one might expect for a shorter period of persistence and are a valuable shorter time point in the process.

The correspondent notes that using short reads for whole genome sequencing (WGS) may limit the ability to "study large structure variations and plasmids". We used paired end reads with an average insert size of about 500 bases and average base coverage of about 175-fold. Thus, all SNPs, all gains and losses of any size, and all recombination involving unique junctions or any repeats less than 500 bases in length could be observed. Papers cited by the correspondent have often used this same approach for studying divergence during persistence [2, 3]. In our study, this approach was successful in identification of gain and loss of both plasmids and prophages as summarized in Supplementary Table 2 in [4]. Furthermore, a WGS-independent approach identified variation in plasmid profile (Figure 2 in [4]). The sequencing data and the independent plasmid profile analysis were found to be fully consistent. We did not investigate recombination centered at

repeats longer than 500 bases, without gain or loss of DNA. When cost effective to do so, it will be interesting to see if such rearrangements are found to be common in short-term persistence, and then determine if they have functional consequences. We previously showed recombination occurs among rRNA clusters in some serovars, although whether this occurs fast, and the phenotypic consequences, remains unknown [5].

Dr Yue suggests that adult CH3 mice are too resistant to be a model and simultaneously argues that pretreatment with streptomycin disrupts the microbiota, which makes the mice more susceptible. Certainly, no currently available model would be perfect. We selected our model based on the fact that 65% of these human patients presented with a symptomatic persistence with relapsing diarrhea. The best currently available mouse model that mimics intestinal inflammation caused by non-typhoidal *Salmonella* (as opposed to a typhoid-like systemic disease) is the streptomycin pretreated mouse model [6, 7]. Infecting young mice, as suggested by the correspondent, causes a systemic or lethal infection and is likely to be a far less appropriate to model the relevant clinical (gastroenteritis) manifestation (and therefore phenotypic changes in this context) that was investigated in our study.

We, and probably all investigators of pathogen persistence in the gut, are united with the correspondent in believing that there is much work to be done, and that the microbiota of the gut plays an important role in the outcome of infection. Nevertheless, this aspect was simply not in the scope of the current study.

Note

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Michael McClelland,[1] Alex Marzel,[2] Prerak T. Desai,[1] and Ohad Gal-Mor[3,4]

[1]Department of Microbiology and Molecular Genetics, University of California, Irvine; [2]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Switzerland; [3]Infectious Diseases Research Laboratory, Sheba Medical Center, Tel-Hashomer, Israel; and [4]Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel

References

1. Yue M. Bacterial persistent infection at the interface between host and microbiota. Clin Infect Dis **2016**; 62:1325–6.
2. Golubchik T, Batty EM, Miller RR, et al. Within-host evolution of *Staphylococcus aureus* during asymptomatic carriage. PLoS ONE **2013**; 8:e61319.
3. Marvig RL, Sommer LM, Molin S, Johansen HK. Convergent evolution and adaptation of *Pseudomonas aeruginosa* within patients with cystic fibrosis. Nat Genet **2015**; 47:57–64.
4. Marzel A, Desai PT, Goren A, et al. Persistent infections by non-typhoidal *Salmonella* in humans: epidemiology and genetics. Clin Infect Dis **2016**; 62:879–86.
5. Kenneth ES, Liu S-L, Hessel A, McClelland M. Genome evolution in the *Salmonella*. In: de Bruijn FJ, Lupski JR, Weinstock GM. Bacterial Genomes. Springer US, **1998**:230–9.
6. Coburn B, Grassl GA, Finlay BB. *Salmonella*, the host and disease: a brief review. Immunol Cell Biol **2007**; 85:112–8.
7. Gal-Mor O, Boyle EC, Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. Front Microbiol **2014**; 5:391.

Correspondence: O. Gal-Mor, Tel-Aviv University and Sheba Medical Center, The Infectious Diseases Research Laboratory, Tel-Hashomer 5262100, Israel (ohad.gal-mor@sheba.health.gov.il).

Clinical Infectious Diseases® 2016;62(10):1326–7
 DOI: 10.1093/cid/ciw137