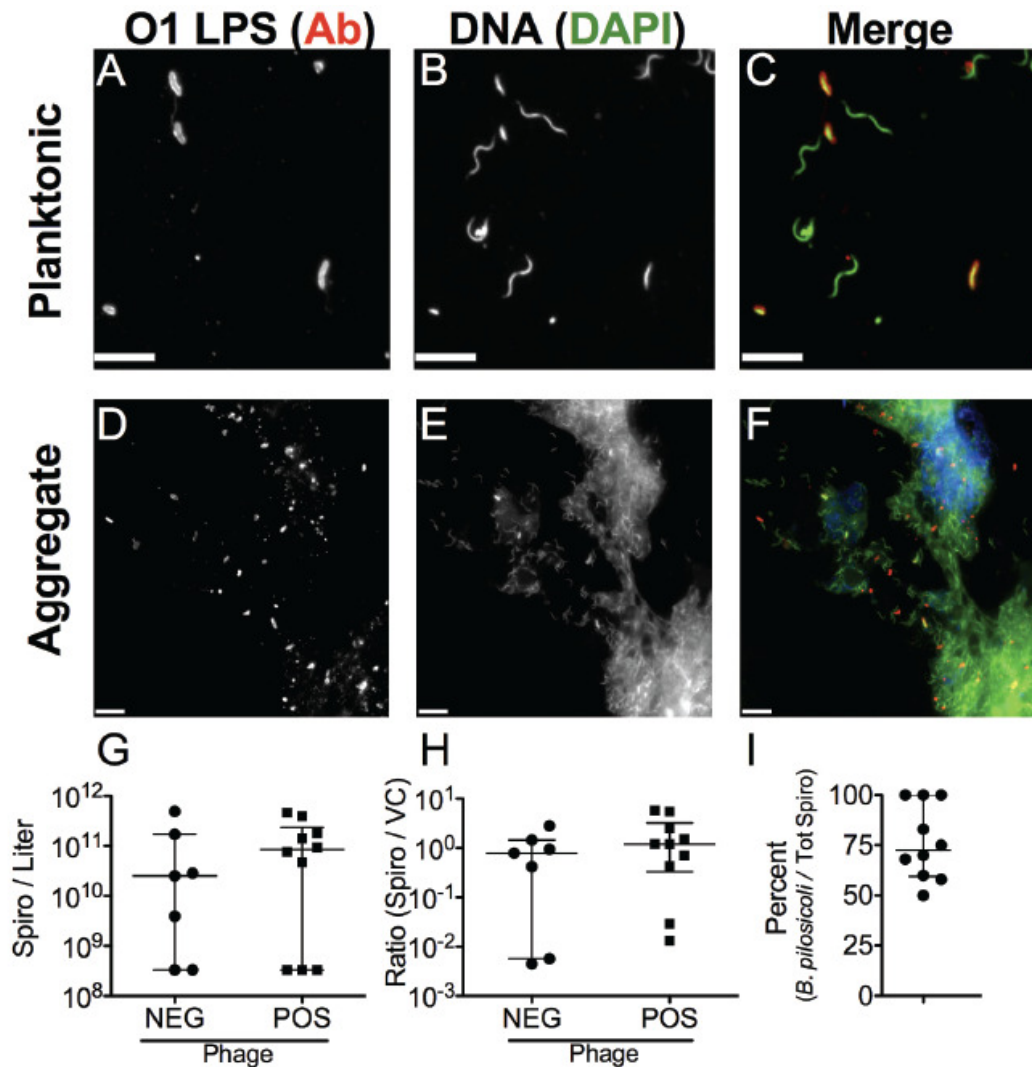


High Prevalence of Spirochetosis in Cholera Patients, Bangladesh

Technical Appendix



Presence of spirochetes (Spiro) in cholera stools. Representative images of planktonic and aggregated spirochetes. *Vibrio cholerae* (VC) were labeled with a fluorescein isothiocyanate–conjugated monoclonal antibody (Ab) to O1 lipopolysaccharide (panels A and D), and nucleic acid was labeled with 4',6-diamidino-2-phenylindole (DAPI) (panels B and E), which showed sigmoidal morphology with tapered ends (4–8 μ m) indicative of spirochetes. The single curve or W shape is indicative of *Brachyspira pilosicoli*. Merged images (panels C and F) showed *V. cholerae* and spirochetes. The aggregate (panel F) was also stained for mucus (blue indicates wheat germ agglutinin lectin) and showed that only a small

portion of the aggregate contained mucus. Panel G shows spirochetes counted by direct count microscopy in rice-water stool samples relative to the presence or absence of lytic vibriophage. Panel H shows the ratio of spirochetes to *V. cholerae* by direct count microscopy relative to the presence or absence of vibriophage. Panel I shows the percentage of spirochetes that were *B. pilosicoli* as determined by using partial sequence analysis of 16S rDNA. PCR amplified products from isolated DNA were cloned and sequenced. Those clones that did not harbor *B. pilosicoli* sequence harbored *B. aalborgi* sequence. NEG, negative; POS, positive; Tot, total. Horizontal bars are median values with interquartile range as whiskers; medians were not significantly different ($p > 0.05$, by Mann-Whitney U test); Scale bars = 10 μm .