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Organelle genome polymorphisms: A molecular epidemiology tool for differentiation of Cyclospora cayetanensis isolates during foodborne outbreaks

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Cyclospora cayetanensis is a coccidian apicomplexan parasite causing large outbreaks in different countries, including the US. The surveillance data collected by the CDC between 1996-2015 indicated that *C. cayetanensis* is the second most common cause of illness and outbreaks in the U.S. associated with imported foods regulated by FDA. Cyclosporiasis outbreak investigations are limited by the absence of molecular epidemiological tools for tracebacks. Due to difficulties in the recovery of the oocysts from produce and clinical samples, the unculturable nature of the organism, and limitations in efficient DNA extraction, until recently very little *C. cayetanensis* genomic information was available. In different apicomplexan parasites, multicopy organellar DNA such as mitochondrial and apicoplast genomes have been used for detection and molecular epidemiology analysis. We developed genomic workflows to obtain complete mitochondrial and apicoplast genome sequences from contaminated foods and clinical samples, for differentiation of *C. cayetanensis* isolates. The 6274 bp *C. cayetanensis* mitochondrial genome was amplified by PCR in four overlapping amplicons from genomic DNA extracted directly from cilantro spiked with oocysts, and from clinical stool samples. DNA sequence libraries of pooled amplicons were prepared using the Ovation Ultralow System library kit (NuGEN technologies) and sequenced using MiSeq. To obtain larger apicoplast genomes (34 kb), oocysts were purified from clinical stool samples. Genomic DNA was extracted from purified oocysts, NGS libraries were prepared using the Ovation Ultralow System library kit and sequenced using Miseq. Sequence reads were assembled using CLC Genomics WorkBench, and Geneious programs, to map to a reference *C. cayetanensis* mitochondria or apicoplast genome. The PCR amplification combined with NGS sequencing approach allowed us to sequence complete mitochondrial genomes directly from produce samples seeded with *C. cayetanensis* oocysts, and from stool samples. We were able to obtain whole apicoplast sequences from purified oocysts isolated from clinical stool samples. SNP profiles of both mitochondria and apicoplast genomes exhibited discriminatory power based on geographical metadata. *C. cayetanensis* isolates from different states grouped together in an evolutionary tree, suggesting that genomic analyses of mitochondria and apicoplast sequences may help to link outbreak cases to the source. The described approaches will facilitate the application of genomics tools to epidemiologically link *C. cayetanensis* identified in clinical and food samples during outbreak investigations.

Biography

Hediye Nese Cinar has completed her MD degree from Dokuz Eylul University in Turkey. After completing her Post-doctoral studies at University of California Santa Cruz she joined Food and Drug Administration as a Research Fellow. She works as a Staff Scientist/ Research Biologist at FDA.

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