

# Nanopore-based rapid sequencing to detect and track protozoan pathogens of concern in the food chain

## AUTHORS & AFFILIATIONS:

Abeer A. Khedr<sup>1,2</sup>, Luis Fernando Valenzuela-Moreno<sup>3</sup>, Claudia Patricia Rico-Torres<sup>3</sup>, Heriberto Caballero-Ortega<sup>3,4</sup>, Zisis Koutsogiannis<sup>1,5</sup>, and Paul W. Denny<sup>1</sup>

<sup>1</sup>Department of Biosciences, Durham University, Durham, DH1 3LE, United Kingdom

<sup>2</sup>Department of Parasitology, Faculty of Veterinary Medicine, New Valley University, El-Kharga 72511, Egypt.

<sup>3</sup>Laboratorio de Inmunología Experimental, Subdirección de Medicina Experimental, Instituto Nacional de Pediatría, CDMX, México.

<sup>4</sup>Programa de Doctorado en Ciencias de la Producción y de la Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, CDMX, México.

<sup>5</sup>Centre for Discovery Brain Sciences, Edinburgh Medical School, Biomedical Sciences, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh, EH8 9XD, Scotland, UK.

## Abstract

Foodborne protozoan parasites, including *Toxoplasma gondii*, *Sarcocystis* spp., and *Cryptosporidium* spp., remain major contributors to global gastrointestinal and systemic disease. Their environmentally resistant stages, broad host ranges, and complex transmission cycles complicate surveillance and outbreak investigations, necessitating the development of a portable, rapid molecular surveillance platform for simultaneous detection and high-resolution genotyping of foodborne protozoan pathogens. We developed a Nanopore-based multi-locus sequence typing (MLST) workflow using Oxford Nanopore Technologies, employing *Toxoplasma gondii* as a representative model to establish a portable, high-resolution genotyping framework for foodborne protozoan surveillance. Initial validation was performed using cultured reference lineages, Type I (RH strain) and Type II (ME49 strain), targeting six loci (*SAG2*, *SAG3*, *ROP5*, *ROP8*, *ROP17*, and *ROP21*). Multi-locus variant analysis revealed distinct strain-specific SNP and INDEL profiles, enabling clear discrimination between Type I and Type II lineages. Notably, long-read sequencing enabled resolution of the paralogous *ROP5* locus, allowing assessment of sequence polymorphisms and repeat copy number variation, a key determinant of strain-specific virulence. Field validation using isolates from Mexico demonstrated that *ROP5* effectively differentiated atypical strains, highlighting its utility as both a genotyping and virulence-associated marker. Ongoing analyses are expanding the genotyping panel to include additional virulence-associated loci (*ROP18*, *GRA7*, and *GRA15*) to further characterize recombinant and geographically diverse isolates. Future development of the platform will expand its diagnostic capacity through the integration of nested and multiplex PCR assays targeting additional apicomplexan parasites within complex biological matrices, including *Cryptosporidium* spp. and *Sarcocystis* spp., thereby enabling comprehensive, field-deployable surveillance of foodborne protozoan pathogens.