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Title: Impact of physicochemical parameters of child or adult digestive tract on *Cryptosporidium parvum* infection

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Abstract:

Cryptosporidium parvum is responsible for a zoonotic disease affecting both human health and livestock. The parasite infects its host through the oral route and develops in ileal epithelial cells, leading to acute and sometimes lethal diarrhoea. The severity of cryptosporidiosis is closely related to the immune status of its host, young ruminants, infants, and immunocompromised individuals being more susceptible. Other non-immune-related factors, such as digestive physicochemical properties, can also vary widely with age but their contribution to infection establishment in young individuals has never been investigated.

In vitro digestion models have been designed and validated to study the fate of orally ingested substances while closely mimicking the physiological processes occurring during digestion. Among the available systems, the dynamic and multicompartmental TNO gastrointestinal model (TIM-1) is currently the most complete simulator of the human upper gastrointestinal tract. The TIM-1 was used for a comparative study of *C. parvum* infection under adult and young children digestive conditions.

The TIM-1 was programmed to reproduce both physicochemical digestive conditions upon simulated ingestion of a glass of mineral water, which was experimentally contaminated with *C. parvum* oocysts. The parasite excystation kinetics throughout the course of the *in vitro* digestion was determined by flow cytometry analysis. Parasite invasion ability was assessed after reinoculation of sporozoites collected from the TIM-1 onto HCT-8 cells. A luciferase reporter gene assay was also used to follow sporozoite activity throughout the digestive process.

Our data show that the parasite excystation rate is almost maximal in the duodenal compartment, one hour after the beginning of digestion in the TIM-1. However, a higher number of parasites reaches the distal ileal compartment while protected in their oocyst shell upon simulation of child compared to adult digestive conditions. This suggests that a lower amount of sporozoites is released in the small intestine in children, nevertheless, the luciferase activity expressed by these free stages in the ileal compartment is significantly higher in children compared to adults. Invasion assay performed on HCT-8 cells suggests that only sporozoites collected in the ileum after three hours of digestion are able to invade host cells.

Our study is the first one exploring the impact of different digestive conditions on *Cryptosporidium* using a sophisticated gastrointestinal model. A global transcriptome analysis by RNA-Seq is also being performed on samples collected during *in vitro* digestion to identify parasite genes that are differentially expressed under child and adult digestive conditions.