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Cryptosporidium

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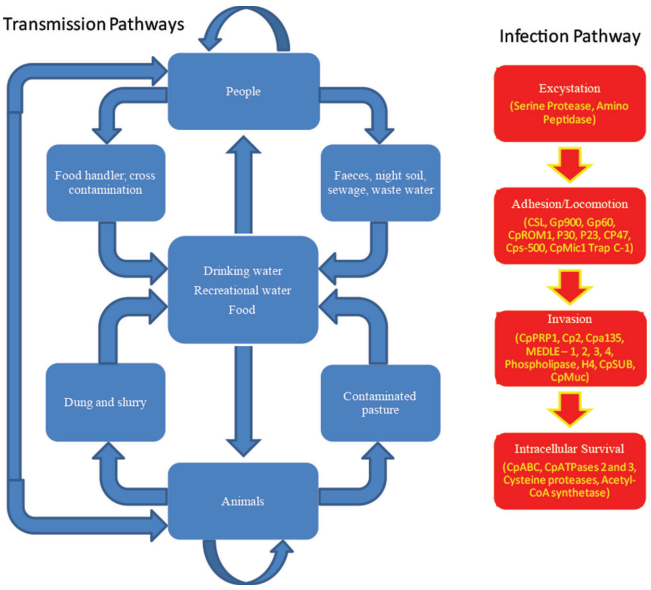

Graphical abstract

Summary of *Cryptosporidium* transmission, infection and putative virulence factors.

The transmission pathway in blue maps the routes oocysts might take from source to vulnerable host. The infection pathway in red shows putative virulence factors - enzymes and genes or gene families,

Abstract

The protozoan *Cryptosporidium* is notorious for its resistance to chlorine disinfection, a mainstay of water treatment. Human infections, mainly of the small intestine, arise from consumption of faecally contaminated food or water, environmental exposure, and person-to-person or animal-to-person spread. Acute gastrointestinal symptoms can be prolonged but are usually self-limiting. Problems arise with immune-deficient, including malnourished, people including chronic diarrhoea, hepato-biliary tree and extra-gastrointestinal site infection, and few options for treatment or prevention exist. Although genomics has enabled refined classification, identification of chemotherapeutic targets and vaccine candidates, and putative factors for host adaption and pathogenesis, their confirmation has been hampered by a lack of biological tools.

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Keywords: *Cryptosporidium*; infection; sequelae.

Abbreviations: AIDS, Acquired immune deficiency syndrome; AT, Adenine and thymine; *C.*, *Cryptosporidium*; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9; FDA, Food and Drug Administration; GC, Guanine and cytosine; HIV, Human immunodeficiency virus; Indel, An insertion or deletion of bases; Mbp, Mega base pairs; SNPs, Single nucleotide polymorphisms; TCA, Tricarboxylic acid cycle; UV, Ultra violet light.

TAXONOMY

A proposed revised taxonomy is given in parentheses. Domain *Eukarya*, phylum *Apicomplexa*, class *Sporozoasida* (*Gregarinomorphea*), order *Eucoccidiorida* (*Cryptogregarida*), family *Cryptosporidiidae*, genus *Cryptosporidium*, species *C. parvum*, *C. hominis* and at least 35 others. Confirmation of laboratory reports of free-living stages would strengthen the gregarine affinity.

PROPERTIES

Cryptosporidium is traditionally considered an obligate gastrointestinal parasite, but free-living stages have been reported in laboratory conditions. Metabolic pathways are minimal as most nutrients are scavenged from the host; energy production is mainly by glycolysis. The life cycle comprises asexual and sexual phases, sequentially in the same host, culminating in oocysts shed in faeces. Each oocyst contains four infective sporozoites and can survive for months in moist, ambient conditions and resists chlorine disinfection enabling food and waterborne transmission. Many species infect humans but *C. parvum* (zoonotic) and *C. hominis* (anthroponotic) are most prevalent. Virulence and pathogenicity factors are poorly described.

GENOME

Next generation sequencing was used to re-sequence and re-annotate the 2004 *C. hominis* TU502 genome, annotate a new reference genome (UdeA01) prepared from oocysts without animal propagation, and re-annotate the original *C. parvum* Iowa II genome. The resulting *C. hominis* UdeA01 and *C. parvum* genomes are, respectively, 9.05 and 9.10 Mbp in length, comprising highly compact coding genes (3819 and 3941; 75.4 and 75.7 %), AT-rich (GC content both 32 mol%), with introns in 10.9 and 10.8 % of genes arranged on eight chromosomes (range 0.88–1.34 Mbp). Sequence similarity is 97 % and synteny is high; 11 protein-coding sequences were identified in *C. hominis* and five in *C. parvum*. Phenotypic differences are potentially due to more subtle sequence divergence (SNPs and indels) and gene expression. Genome evolution was largely reductive and *Cryptosporidium* depends mainly on host cells for basic nutrients.

High-quality genome sequences are increasingly available, analysable via http://cryptodb.org/cryptodb/, including other *C. hominis* genomes, human-adapted *C. parvum*, other zoonotic species including *C. meleagridis*, and host-adapted species rarely found in humans (*C. muris*, *C. andersoni*, *C. baileyi* and *C. tyzzeri*). Comparative genomics has identified several putative virulence factors, mainly in hyperpolymorphic subtelomeric regions, that require biological confirmation.

PHYLOGENY

Historically, *Cryptosporidium* species were described on the basis of host, infection site and oocyst morphology. Mitosome metabolism and invasion-related proteins differ between gastric and intestinal species; gastric *C. muris* and *C. andersoni* retain all enzymes associated with the TCA cycle and a conventional respiratory chain system .

Human infections were identified originally as *C. parvum* but delineated in the 1990s as genotype 1 or H (human-adapted) and genotype 2 or C (cattle/broad host range). Molecular characterization led to re-descriptions and novel species; in 2002 genotype 1 was described as *C. hominis*. Most human pathogens (*C. parvum*, *C. cuniculus* and *C. meleagridis*) form a clade with *C. hominis*. A human-adapted *C. parvum* lineage has been described [1].

It is likely that 'isolates' comprise a population of intraspecies variants arising from sexual recombination. Discriminatory analysis using multi-locus genotyping is required. Single-cell whole genome sequencing has been demonstrated using flow cytometry to separate individual oocysts.

KEY FEATURES AND DISCOVERIES

In 1976 *Cryptosporidium* was identified as the cause of two cases of human gastroenteritis, and veterinary importance in ruminants was recognized, following histological examination. The organism rose to prominence with the AIDS epidemic in the 1980s. These patients were susceptible to severe, intractable and sometimes fatal cryptosporidiosis. Recognition of the impact in the general population followed large drinking water outbreaks, highlighting that supplies compliant with World Health Organization quality standards (based on *Escherichia coli*) could present a risk of cryptosporidiosis. Human infectivity studies and dose–response modelling confirmed the significance of small numbers of oocysts in drinking water; single oocyst infection probabilities could be as high as 72 % [2]. Chlorine resistance necessitates alternative water treatment, chiefly filtration and secondary disinfection using UV light or ozone. Introduction of European guidelines on water quality, promoting improved source water protection, appears to have been effective to some extent in reducing the numbers of water-borne cases and outbreaks. Transmission via food and recreational waters are of concern.

Highly active antiretroviral therapy has reduced the prevalence of life-threatening cryptosporidiosis in people positive for human immunodeficiency virus (HIV), but individuals with other causes of severe T-cell depletion, including primary immunodeficiency or haematopoietic stem-cell transplantation, remain at risk of severe cryptosporidiosis, characterized by biliary involvement, sclerosing cholangitis and, rarely, liver cirrhosis.

In immune-competent people, post-infectious sequelae not dissimilar to those following bacterial gastroenteritis are described. Furthermore, the importance of *Cryptosporidium* in childhood in low-income countries is increasingly recognized. The Global Enteric Multicentre Study identified *Cryptosporidium* among the four most common infectious causes of moderate-to-severe diarrhoea in children aged <24 months

in sub-Saharan Africa and India/Pakistan/Bangladesh/Nepal/Afghanistan, and generated estimates of 2.9 and 4.7 million *Cryptosporidium*-attributable cases respectively annually in that age group, with ~202 000 total *Cryptosporidium*-attributable deaths [3]. Growth stunting and malnutrition can arise in young children, leading to an impaired immune response and chronic infection.

Only nitazoxanide has been shown to be an effective treatment for cryptosporidiosis in a randomized placebo-controlled trial and is licensed by the US Food and Drug Administration (FDA) for immunocompetent cryptosporidiosis patients aged >1 year; there is no European licence. In clinical practice, reducing levels of immunosuppression, where possible, is frequently helpful in controlling severe infection.

Study of *Cryptosporidium* biology and virulence was until recently complicated by an inability to produce oocysts and achieve prolonged culture *in vitro*. A successful application of CRISPR-Cas9 technology to the organism and developments in continuous culture *in vitro* show promise for new approaches in research [4, 5].

OPEN QUESTIONS

(1) To what extent and by what mechanisms does *Cryptosporidium* contribute to sub-clinical environmental enteropathy in low-resource settings? Understanding the role of *Cryptosporidium* in the cycle of mucosal damage, inflammation and malnutrition following recurrent gastrointestinal infections will help to focus improvements for survival and long-term developmental potential.

(2) Which aspects might explain the variable host-specificity or adaption of *Cryptosporidium* species? Host-related features such as the differing gastrointestinal conditions (e.g. pH and body temperature) between and among vertebrates known to be infected by at least one *Cryptosporidium* species might be involved. Host–parasite interactions may differ, for example in the cell-surface molecules involved in host-cell binding and/or invasion. Importantly, the composition and pathological roles of other microbes (mirobiota) in the host gut are only just beginning to be investigated.

(3) Further understanding of the differing innate and acquired immunological responses of the host against *Cryptosporidium* is needed. Understanding of whether (and how) these factors influence the susceptible host range of *Cryptosporidium* species will be of critical importance in the effective control of cryptosporidiosis.

(4) What parasite-related factors influence the clinical impact of infection? These factors are not well understood, and neither is the importance of the population structure of *Cryptosporidium* species during infection. Deep sampling of host and parasite populations in defined geographical areas needs to be undertaken.

(5) If laboratory reports of a free-living stage were to be confirmed independently, is there any public health significance? *Cryptosporidium* control in drinking water is currently based on prevention and removal of oocyst contamination, but if free-living stages were to be confirmed and shown to enable proliferation outside of laboratory conditions, for example in environmental biofilms, a new dimension of control measures would need to be implemented.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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