

FACT SHEET *Genotyping Cryptosporidium*

What is genotyping?

Molecular based method that allows accurate identification to the species or “strain” (i.e., genotypic level). This technology has been used to show that human cryptosporidiosis is predominantly caused by *Cryptosporidium hominis* (synonymous with *C. parvum* genotype 1 and *C. parvum* genotype 2).

Why do it?

To determine if the type present is of pathogenic risk to humans. This data can then support epidemiological studies in assessing disease outbreak and control.

Who can benefit?

Anyone responsible for supply of safe water for human consumption. Catchment authorities, state regulators, water supply authorities, recycling scheme operators, local councils.

Background

Cryptosporidiosis is a diarrheal disease caused by parasitic protozoans belonging to the genus *Cryptosporidium*. These organisms, termed “oocysts” are protected in the environment by a tough outer shell, making them more resistant to both environmental and man-made (e.g. chlorine) stresses. Once ingested, the parasite harbours itself within the intestine wall where it begins a complex reproductive cycle with much of the progeny shed in the faeces. Symptoms are generally experienced approximately 7 days after ingestion and in immunocompetent persons may last for 2 – 3 weeks and include watery or mucoid diarrhoea, nausea, dehydration, anorexia, abdominal pain, fever and vomiting (Palmer and Biffin, 1990). Those who have weakened immune systems as well as children and pregnant women are most at risk

Epidemiological studies have indicated that the main routes of transmission for *Cryptosporidium* are human-to-animal contact, person-to-person spread and waterborne infection (Meinhardt et al., 1996; Rose et al., 1997). Numerous animals are identified as hosts to the protozoa, and at least 15 *Cryptosporidium* sp. and more than 30 genotypes to date have been described (Xiao et al., 2004). Not all of these species are able to cause disease in humans however (Xiao et al., 2004), and through the use of molecular tools it has been shown that human cryptosporidiosis is predominantly caused by *Cryptosporidium hominis* (synonymous with *C. parvum* genotype 1 and *C. parvum* genotype 2) (McLauchlin et al., 2000); (Anon, 2002). It is known that the *C. parvum* genotype 1 has a host range limited almost exclusively to humans, while genotype 2 has a broader host range of humans and animals (Chalmers et al., 2002). However, these tools have also enabled implication of other species capable of infection and associated in clinical disease in both immunocompromised and immunocompetent persons. Species described include *C. melagridis*, *C. felis*, *C. canis* and *C. muris* (Chalmers et al., 2005). Accurate identification of *Cryptosporidium* to the species or “strain” (i.e., genotypic) level is essential for studying transmission patterns and to the control of cryptosporidiosis (Gasser et al., 2003).

Whilst traditional methods of detection such as epifluorescent microscopy and ELISA detect *Cryptosporidium*, these techniques do not allow further discrimination such as species designation and interspecies genetic variation. Since many *Cryptosporidium* spp. can occur simultaneously in environmental samples (Xiao et al., 2001), and traditional methods of identification are not specific enough to determine the source of human infection, molecular techniques are necessary to support epidemiological and environmental investigations. Methods such as SSCP (single strand conformation polymorphism) analysis, allow discrimination of a single nucleotide difference within a targeted genetic marker sequence, are contributing to the knowledge of the epidemiology of the parasite.

Many different genetic markers have been used specifically for the molecular identification and differentiation of genotypes. Studies have demonstrated that a portion of the conserved small-subunit (SSU) ribosomal gene provides the optimal marker for genotyping *Cryptosporidium* as it has the largest genotyping reference database and primers have been designed for all known isolates. To subtype these isolates (e.g. to differentiate between *C. parvum* genotype 1 and 2) the second internal transcribed spacer region of rDNA (ITS-2) has proven to be useful because it is considerably more variable in sequence within a species and is present in sufficient number to increase specificity (Ferguson et al., 2006). The information obtained from genotyping *Cryptosporidium* isolates will allow a greater understanding of the incidence and prevalence of the protozoan in different environments and will permit the potential health hazards of cryptosporidiosis to be considered.

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