

IDENTIFYING

CRYPTOSPORIDIUM
USING DNA METHODS

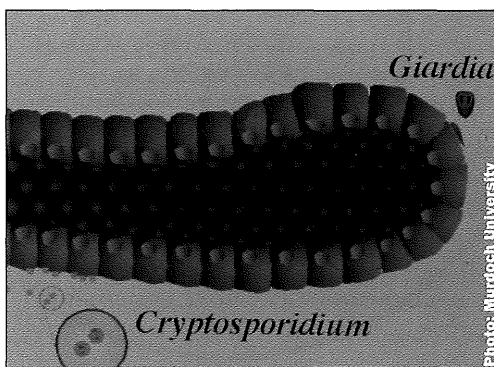
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Cryptosporidium is an intestinal parasite found in humans and animals. It multiplies within the surface layers of the host's small intestine, causing damage which may lead to severe diarrhoea. When this tiny (5 μm) single-celled protozoan parasite leaves its host, a tough outer shell favours its survival during routine water and wastewater disinfection and treatment.

Cryptosporidium has come to prominence in Australia as a result of the recent contamination of Sydney water supplies, but came to world prominence in 1993 when over 400,000 people in Milwaukee, USA became infected with it as a result of drinking contaminated drinking water. Amongst AIDS patients who contracted cryptosporidiosis as a result of this outbreak, the outcome of disease was severe, with 52% to 68% of people dying within six months to a year after the outbreak. Since this epidemic, *Cryptosporidium* has been the subject of an enormous amount of research worldwide and has the unusual distinction of having its own magazine, *Cryptosporidium Capsule* (<http://www.fspubl.com>).

Despite the vast amount of research that has been conducted, there is still a great deal that we do not know about *Cryptosporidium*. At present up to eight species have been recognised: *C. muris* which infects mostly rodents but also cattle and other hosts; *C. wrairi* which infects guinea pigs; *C. meleagridis* and *C. baileyi* in birds; *C. serpentis* in reptiles; *C. nasonum* in fish; *C. felis* in cats and *C. parvum* in humans and other mammals.

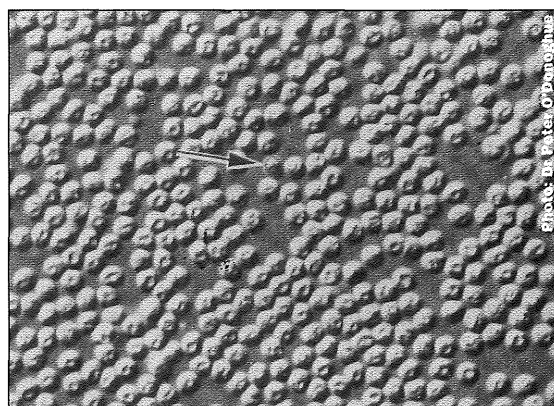
Cryptosporidium parvum is the most widely studied species. In the last couple of years it has become increasingly clear that *C. parvum* is not a single species but is composed of a number of distinct 'strains' (or genotypes) that differ



Cryptosporidium and Giardia in the small intestine

both genetically and biologically. It is possible that these strains will be classed as new species in the near future.

Currently, our research in the Division of Veterinary and Biomedical Sciences at Murdoch University involves characterising the *Cryptosporidium* found in a variety of hosts using sensitive DNA-based detection methods. As part of our role as a World Health Organisation Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, we have been collaborating with a wide variety of National and International Research Centres, including the Centres for Disease Control (CDC) in Atlanta, USA, and we have now



Purified *Cryptosporidium* oocysts (arrow shows single oocyst)

collected over 400 different isolates of *Cryptosporidium*, of which approximately 300 have been characterised genetically or 'genotyped.'

At present, at least seven genotypes of *C. parvum* have been identified: a 'human' genotype which has so far been found only in humans; a 'cattle' genotype found in domestic livestock such as cattle, sheep, goats etc. and which can also infect humans; a 'mouse' genotype that has been found in mice from around the world and more recently in bats; a 'pig' genotype; a 'marsupial' genotype that has been found in koalas and kangaroos; a 'dog' genotype; and a 'ferret' genotype.

So far only the 'human' and 'cattle' genotypes have been found in healthy human beings, and the public health significance of the remaining genotypes is unknown (see Figure 1). Since most of these 'new' genotypes appear to be host-specific, they are unlikely to infect people with intact immune systems. It is possible, however, that individuals with deficient immune systems such as AIDS patients may be susceptible to other *Cryptosporidium* genotypes.

Current methods for identifying *Cryptosporidium* in water supplies generally rely on immunofluorescent microscopy and/or flow cytometry, in which fluorescent antibodies bind to *Cryptosporidium* and diagnosis is made on the basis of morphological characteristics. However, because all the genotypes of *C. parvum* are morphologically identical, they cannot be differentiated using these techniques. This can only be done using DNA-based techniques.

If we can determine the genotype of *Cryptosporidium* found in water supplies, this will not only tell us if parasites that are potentially infectious to humans are present, but it will give us information about their source.

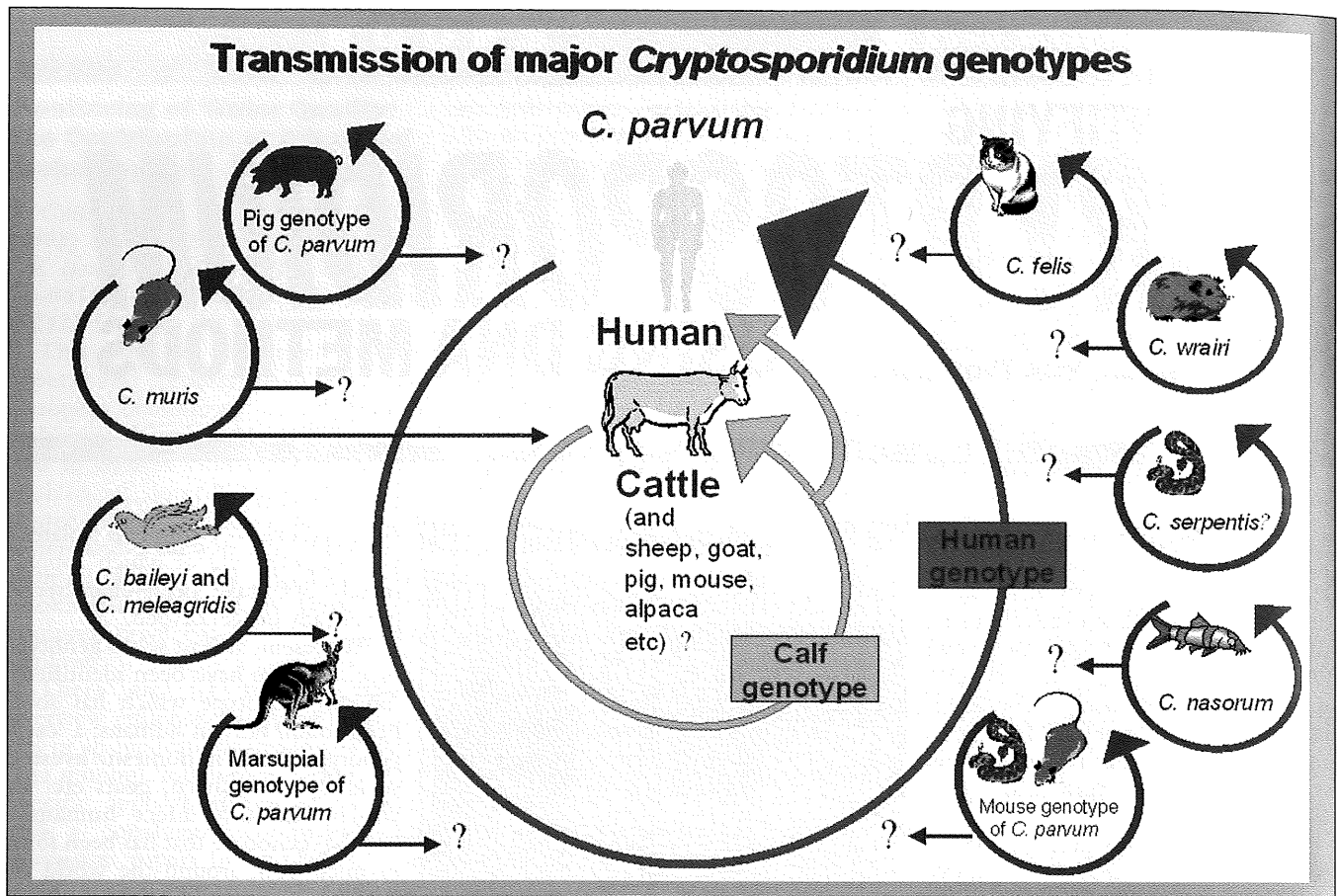


Figure 1 Transmission dynamics of *Cryptosporidium*

For example, if the 'human' genotype is detected, it must have come from human faecal contamination of the water; if it's the 'marsupial' genotype, it may have come from marsupials in the catchment area; and so on.

Research to date on a number of waterborne outbreaks in the UK and USA, including the famous Milwaukee outbreak, has indicated that most waterborne outbreaks have been caused by the 'human' genotype.

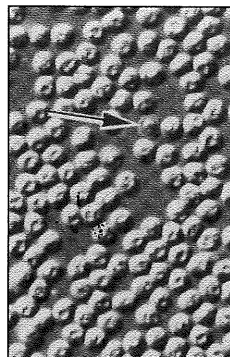
Future genetic and biological characterisation studies are required on a wider range of isolates in order to confirm the distribution of these genotypes and to determine if they are in fact separate species. Ongoing human-infectivity trials in the USA will also provide important information as to the public health significance of the 'mouse', 'pig', 'marsupial', 'ferret', and 'dog' genotypes.

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Cryptosporidium for sale

Purified, freshly passaged, viable *Cryptosporidium* oocysts suitable for standardisation are now available on a regular basis from Murdoch University at a cost of \$500 per 6 million oocysts.



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