

EXECUTIVE SUMMARY

Cryptosporidiosis is a diarrhoeal disease of humans and young animals caused by the protozoan parasite *Cryptosporidium*. Illness in neonatal animals is caused by *Cryptosporidium parvum* and both this and a human-adapted species, *Cryptosporidium hominis*, cause cryptosporidiosis in people. Each species causes approximately half of the reported cases in England and Wales, but the distribution by person, time and place (the epidemiology) differs, as do the risk factors for infection. Further investigation of variants within *C. parvum* has revealed more about the natural history and epidemiology of this species, but genetic variation within *C. hominis* is poorly understood and the distribution of variants unknown. In a previous study, we found that 90% of *C. hominis* isolates from apparently un-linked (sporadic) cases in England and Wales were indistinguishable. A study of the population genetics of *C. hominis* in the north east of Scotland showed isolates were genetically very similar, indicating that the parasite population structure was almost clonal, while in other countries more variation has been observed.

In this project, to investigate whether other typing methods for *C. hominis* isolates could be useful for epidemiological purposes in England and Wales, more genetic loci were studied and any relationship between *C. hominis* variants and epidemiological factors was investigated. We identified sporadic and outbreak cases from the national collection of *Cryptosporidium* oocysts maintained at the UK Cryptosporidium Reference Unit in Swansea. In the first instance, we read the DNA sequence of part of the parasite genome by looking at a large part of the GP60 gene. This is a highly variable gene, and many “families” have been identified in both *C. parvum* and *C. hominis*. The gene also contains variable numbers and forms of a repeating sequence of nucleotides, microsatellite DNA, which can be used to identify variation within these families. Cases in a case control study were investigated for relationships between exposure and GP60 results. Family Ib subtype A10G2 was the most common in sporadic cases, representing over 90% all isolates. However, people with non-IbA10G2 isolates were statistically more likely to have returned from non-European destinations than people with IbA10G2.

Isolates from two drinking waterborne outbreaks that occurred in the Autumn of 2005 were compared with sporadic cases and exposure. In the outbreak in north west Wales, *C. hominis* isolates were exclusively IbA10G2. This was also the predominant type locally in cases during the six years prior to the outbreak. In the outbreak in south east England, IbA10G2 also predominated but two other *C. hominis* families were also present, although there was no difference in the results of the epidemiological analysis of the outbreak when these cases were excluded from the analysis.

We also investigated the development of an alternative typing method for *C. hominis* and *C. parvum* to DNA sequence analysis, which is costly and time consuming. We investigated single strand conformation polymorphisms (SSCP) at two genetic loci. Investigation of the ITS-2 region showed more variation between sample runs than between samples. However, SSCP on the GP60 gene appeared to be much more reliable, and had the advantage of providing direct comparison with the DNA sequence based analysis.

To conclude, this study:

1. tells us that indigenous *C. hominis* in the UK shows little genetic variation in the GP60 gene and supports previous findings at other loci
2. indicates little change in *C. hominis* over the time investigated
3. suggests wider global transmission may be subject to host-related or social factors
4. an international database of *Cryptosporidium* variants with standardised nomenclature would assist in interpretation of studies elsewhere
5. Further method development is required for rapid methods for epidemiological purposes.