

## **Executive summary**

1. The purpose of this project was to investigate *Cryptosporidium hominis* and *Cryptosporidium parvum* subtypes present in two different sample sets: (i) strains from cases implicated in a drinking waterborne outbreak associated with water sourced from Thirlmere and distributed via the Thirlmere aqueduct; (ii) strains from a DWI-funded case-control study of sporadic cryptosporidiosis undertaken in Wales and the North West of England to identify risk factors for sporadic cryptosporidiosis. Analysis with the epidemiological data was performed to identify trends in prevalence of subtypes, clusters of cases, risk factors and sources of infection, and measures for control.
2. Multi-locus microsatellite fragment analysis was chosen on the basis of in-house and international evaluative studies and consultation with funders and the scientific community as the most suitable method for subtyping the required number of samples to a high degree of discrimination.
3. Three microsatellite markers were analysed: microsatellite locus (ML) 1, ML2 and the gp15 surface glycoprotein gene (synonymous with gp60). Microsatellite regions were amplified by PCR and fragment lengths determined using the CEQ<sup>TM</sup> 8000 Genetic Analysis System. Fragment sizes for each locus were combined to produce a multi-locus fragment type for each strain.
4. *C. parvum* was found to be significantly more variable than *C. hominis* in both the Thirlmere and case-control study sample sets. Therefore, subtyping using multi-locus microsatellite fragment analysis is therefore more suitable for studies of *C. parvum* variation than *C. hominis* variation.
5. Multi-locus microsatellite fragment analysis of 190 strains from the case-control study group identified nine subtypes of *C. hominis* and 30 subtypes of *C. parvum*. 90% of *C. hominis* strains were of the same subtype. Three distinct clusters of the *C. parvum* strains were identified (SC1, SC2, SC3). Analysis with the epidemiological data revealed a significant association between *C. parvum* SC1 and animal contact. Associations were also found between ML1, ML2 and gp15 microsatellite sizes and animal contact, ML1 size being the most dramatic and having the potential to be a useful marker for zoonotic transmission. The results support the hypothesis that there may be specific clones of *C. parvum* that are adapted to a human-only life cycle. Phenographic analysis revealed significant associations between *C. parvum* SC1 cases and living in a rural area; this was also the case for the four most common *C. parvum* multi-locus fragment types, which were within SC1.
6. Analysis of 99 Thirlmere outbreak strains identified one subtype of *C. hominis* and 17 subtypes of *C. parvum*. Three clusters of *C. parvum* cases were identified (C1a, C1b, C1c), corresponding to sub-clusters of SC1 from the case-control

study group. Analysis with the epidemiological data revealed significant associations between species (*C. hominis* and *C. parvum*) and the frequency of consuming undercooked meat. The temporal incidence of *C. hominis* and *C. parvum* during the outbreak indicated that *C. hominis* strains were not part of this general outbreak. There was a significant relationship between living in a rural area and cases of *C. parvum* cluster 1a. Clustering of *C. parvum* subtype P36 was observed around Preston and Chorley, whereas there were no cases of P36 strains on the Fylde and only one in the Morecambe Bay area, supporting the epidemiological information which suggested that cases on the Fylde were not related to the Thirlmere supply and the cases in the Morecambe Bay area were probably not waterborne.

7. As frequency of consumption of undercooked meat was identified as a risk factor for *C. hominis* and *C. parvum* infection, control measures should include recommendations to caterers and vulnerable individuals to cook meat thoroughly and adhere to food handling advice from the Food Standards Agency.
8. As animal contact was identified as a risk factor for infection with certain subtypes of *C. parvum*, control measures should include recommendation of comprehensive hygiene and hand washing procedures for implementation during and after animal handling to minimise risk of hand-to-mouth transmission.