## EXECUTIVE SUMMARY

Most cases of the human diarrhoeal disease "cryptosporidiosis" are caused by either *Cryptosporidium parvum* or *Cryptosporidium hominis*. In summer 2008, the *Cryptosporidium* sp. rabbit genotype was identified as the causative agent of an outbreak of diarrhoeal disease (cryptosporidiosis) among the human population. This was linked to the drinking water supply and the source of contamination was a wild rabbit that had entered a treated water tank. Prior to this outbreak, there were no published reports of human infection with this genotype and only one infection was known to the UK Cryptosporidium Reference Unit. The outbreak demonstrates that this genotype is a human pathogen of public health importance. However, little is known about it. This study aims to establish the taxonomic status of the rabbit genotype and improve our understanding of the biological features of, and risks from, this newly-identified human pathogen for better prevention and control via the waterborne route. The objectives are:

1. Establish current knowledge by undertaking a literature review.
2. Describe and measure the morphological features of the rabbit genotype in comparison with *C. hominis.*
3. Undertake genetic characterisation of the rabbit genotype at multiple loci.
4. Investigate the comparative experimental host range of the rabbit genotype and *C. hominis.*
5. Establish and submit for publication the taxonomic status of the rabbit genotype on the basis of the data obtained in objectives 1 to 4.
6. Describe the human epidemiology and pathogenicity of the rabbit genotype in a waterborne outbreak.
7. Estimate the prevalence of the rabbit genotype in human cryptosporidiosis by enhanced typing studies.
8. Compare the human epidemiology and pathogenicity of the rabbit genotype with *C. parvum* and *C. hominis* using *a priori* data.
9. Characterise the human infection risk from drinking water by producing a model using drinking water monitoring and water consumption data.

We reviewed the available knowledge about the occurrence and natural host range of the rabbit genotype, and the prevalence of *Cryptosporidium* spp. in rabbits, using systematic review principles. This showed that the rabbit genotype has only been reported in rabbits and humans so far, and that the prevalence of *Cryptosporidium* spp. in wild rabbit populations ranges from 0 to 5%. This is based on just two large studies of >100 animals. Most studies were too small to estimate prevalence and none of the studies provided age or sex data required for meaningful population analysis. Illness appears to be reported more frequently in neonatal or unweaned rabbits than older rabbits. Only four previous studies, in three continents, identified the infecting species/genotypes, and in all four studies the rabbit genotype was the only one found. However, there is experimental evidence that other human-pathogenic cryptosporidia, *C. parvum* and *C. meleagridis,* could also be carried by rabbits. No previous studies of the host range and biological features of the rabbit genotype were identified, although there is some prior data regarding genetic characteristics. This showed that part of only one gene, coding for small subunit ribosomal RNA, had been analysed in all four studies. Characteristic differences between the rabbit genotype and its closest genetic relation, *C. hominis*, were consistent and, over the part of this gene studied, were 0.51%.

Our morphological studies demonstrated that rabbit genotype oocysts are similar in size, shape and appearance to other human-pathogenic cryptosporidia. Rabbit genotype oocysts can be detected using the most commonly used, approved water testing methods in the UK. It is therefore likely that both raw water and treated water testing would detect but not differentiate the rabbit genotype.

Investigation of the rabbit genotype genome, using isolates obtained from the Northamptonshire outbreak and subsequently identified human sporadic cases, involved DNA sequence analysis of parts of six genes commonly used for the differentiation or characterisation of *Cryptosporidium*. The rabbit genotype is identical to *C. hominis* at the COWP and Lib13 genes and therefore cannot be differentiated from it using assays based on these targets. However, differences between it and *C. hominis* were consistent at the following genes, by the amounts shown in brackets: SSU rRNA (0.51%); HSP70 (0.25%); actin (0.12%), and at multiple loci spanning up to 4469 base pairs (0.27%). These differences are substantially less than the differences between *C. hominis* and *C. parvum*, although it must be acknowledged that only a very small proportion of the genome has been studied. Sequencing the GP60 gene is commonly used to identify subtype families within *Cryptosporidium* spp. The rabbit genotype appears to have its own subtype families, which we have published as subtype families Va and Vb.

Although genetic differences are small, biological differences in terms of host infectivity, are large and sufficient for consideration whether the rabbit genotype would naturally be found in the same hosts simultaneously with *C. hominis*. We therefore propose that this variation be acknowledged with the rabbit genotype named *C. hominis cuniculus*, a subspecies of *C. hominis*. Differences between who, when, where and how people become infected were also explored. It appears that a greater proportion of *C. h. cuniculus* infections occur in adults compared with *C. h. hominis* or *C. parvum.* There is a distinct seasonality, also reflected in *C. h. cuniculus* subtypes, and these epidemiological trends may be more linked to exposure opportunities than parasite infectivity or host susceptibility factors. Risk to public health from *C. h. cuniculus* in drinking water appears to be similar to *C. parvum* and *C. h. hominis*.

Key findings of the research are:

- Pet and wild rabbits are a potential source of human cryptosporidiosis and as such, good hygiene practices are recommended following handling rabbits or exposure to their faeces or potentially contaminated surfaces.
- Water supplies should be protected against access by wildlife, including rabbits.
- To estimate more fully the risks from water contamination by wild mammals, population-based prevalence studies of zoonotic pathogens are required.
- Although the rabbit genotype is genetically very closely related to *C. hominis*, this is based on analysis of only a small proportion of the genome. Biological differences in host infectivity are distinct.
- There is sufficient evidence for the rabbit genotype as a subspecies of *C. hominis*, possibly host-adapted to rabbits. We propose *C. hominis cuniculus*. Evidence for separate species status may become available through further studies.
- There is insufficient clarify in the taxonomic position of *Cryptosporidium* “genotypes” and better algorithms for establishing taxonomic status need to be created.
- Routine clinical diagnostic tests, water sampling and testing by approved methods will detect but not differentiate *C. h. cuniculus* from other *Cryptosporidium* spp.
- There is no significant difference in apparent human infectivity or virulence between *C. h. cuniculus* associated with the Pitsford outbreak and the *C. parvum* associated with the Clitheroe outbreak and is substantially less than the variation in infectivity shown between different *C. parvum* strains.
- Differences in epidemiology, compared with *C. h. hominis* and *C. parvum*, may be linked to exposure opportunities.
- In the absence of human volunteer feeding studies, the currently used dose response model for *Cryptosporidium* would appear to be applicable to Quantitative Microbial Risk Assessment analyses of *C. hominis cuniculus.*