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Molecular characterisation of *Giardia* isolates from clinical infections following a waterborne outbreak

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Summary *Objectives:* During autumn/winter 2004/2005 an outbreak of waterborne giardiasis occurred in Bergen, Norway. Genetic characterisation at 2 genes of *Giardia duodenalis* isolates from samples from the outbreak peak showed significant variations between isolates. Characterisation of further isolates from patients diagnosed in the subsequent months was conducted to determine whether isolates with particular sequences might predominate, or whether the sequence variation would continue.
Methods: Genetic characterisation was conducted on 63 isolates from patients diagnosed in the 12 months subsequent to the outbreak peak.
Results: At the β-giardin gene and glutamate dehydrogenase gene, particular isolate sequences within Assemblage B, gradually predominated over time. These sequences had not been the most frequently identified amongst 21 isolates from the outbreak peak. Nor were they apparently associated with a particular sequence at the triose phosphate isomerase gene.
Conclusions: The predominance of particular sequences at the β-giardin gene and glutamate dehydrogenase gene over time suggests that these sequences may be associated with enhanced transmission characteristics such as higher virulence, greater cyst environmental resistance, increased proliferation, or a combination of these factors. Alternatively greater association with clinical disease may have led to increased submission of samples with these sequences. Whether these sequences may be associated with particular symptom characteristics such as overt clinical disease, infection persistence or unresponsiveness to treatment warrants further study.

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Introduction

During autumn/winter 2004/2005, an extensive outbreak of waterborne giardiasis occurred in Bergen, Norway.[1,2] Genotyping of cysts from 21 randomly selected patient samples taken over an 8 day period during the peak of the outbreak revealed that they were from all Assemblage B, irrespective of the gene used for analysis. However, grouping the isolates by single nucleotide polymorphisms (SNPs) of the two genes (β-giardin and glutamate dehydrogenase (*gdh*)) gave complex results, as a given sequence at one gene might correspond to more than one sequence at the other gene.[1]

The diversity of these data, as well as the large number of samples collected, suggested that this might be an opportunity to follow any evolution of this infection at these genes, and perhaps determine whether particular genotypes were more persistent, or were more likely to be transmitted further through the host population, than others, or whether some genotypes may be associated with particular symptoms or treatment responses. Obtaining information on whether particular genotypes may be associated with particular symptoms or transmission characteristics is important both in terms of increasing our knowledge of this wide-spread infection, and also practically for targeting of therapies and being aware of whether particular infections might be particularly refractory to treatment or more likely to spread throughout a population.

In order to garner further information which might assist in unravelling this complex issue, sequence analyses at these genes were conducted on *Giardia* cysts isolated from faecal samples (from apparently non-persistent infections) submitted to Haukeland University Hospital (HUS) in Bergen in the 12 month period (December 2004 to November 2005) subsequent to the peak months of the outbreak. For some samples, sequence information was also obtained from the triosephosphate isomerase (*tpi*) gene.

Materials and methods

Faecal samples (purification of *Giardia* cysts, DNA isolation, PCR and sequencing)

Giardiasis was diagnosed at the Unit for Infectious Diseases and Parasitology, Department of Medicine, HUS in Bergen, either by detection of cysts in faecal samples by microscopy following standard formalin-ether concentration, or by faecal antigen test (ImmunoCard STAT! Cryptosporidium/Giardia rapid assay; Meridian Bioscience, Inc). Positive samples were forwarded to the Parasitology Laboratory at the Department of Food Safety and Infection Biology at the Norwegian School of Veterinary Science (NVH).

At NVH, samples were further analysed as previously described.[1] In brief, following a simple washing and salt flotation procedure, and immunofluorescent microscopy of stained sub-samples, the cysts were isolated by modified immunomagnetic separation (IMS; Dynal Biotech, ASA). DNA was isolated from the cysts (QIAamp DNA Mini Kit; Quiagen GmbH, Germany) and either stored frozen at −20 °C before further analysis, or PCR was conducted immediately. For this study, between 3 and 9 samples (depending on availability) were chosen at random from each calendar month from December 2004 until end of November 2005 for analysis, however samples known to be from patients with persistent infections were excluded, and, where possible, samples with low cyst numbers were avoided. A summary of the sample/patient details of those selected for genotyping is provided in Table 1. PCR was initially conducted for the β-giardin and *gdh* genes[3,4] on newly isolated or thawed DNA, using published methods modified as previously described.[1] PCR products were visualised by electrophoresis and ethidium bromide staining. However, as PCR was only successful on 39 (62%) of the samples, for selected samples ($n = 25$) PCR was also attempted for the *tpi* gene by a published method

Table 1 Summary of patient details, by month of diagnosis, of samples selected for genotyping

Month of sample submission and diagnosis of giardiasis	No. samples (total number of samples available)[a]	Patient sex ratio (M:F)	Patient age on 1st January 2005 (yrs); mean ± SD (Age range)
December 2004	9 (153)	3:6	37 ± 18 (4–70)
January 2005	5 (47)	2:3	25 ± 1 (24–26)
February 2005	4 (29)	4:0	30 ± 23 (5–61)
March 2005	7 (18)	2:5	21 ± 19 (1–50)
April 2005	5 (34)	3:2	31 ± 17 (13–58)
May 2005	5 (28)	2:3	25 ± 15 (1–42)
June 2005	3 (22)	1:2	25 ± 19 (6–43)
July 2005	4 (9)	3:1	51 ± 18 (28–71)
August 2005	4 (7)	3:1	22 ± 11 (10–37)
September 2005	5 (9)	2:3	28 ± 26 (2–66)
October 2005	6 (10)	1:5	38 ± 20 (8–63)
November 2005	6 (7)	4:2	20 ± 18 (4–54)
December 2004 to November 2005	Total: 63 Monthly mean: 5 Range/month: 4–9	31:34	30 ± 18 (1–71)

[a] The number of samples available listed here also include samples from persistent infections or repeat samples from the same patient, which were avoided for the purposes of this study.