An unexplained but common feature of giardiasis is the broad clinical spectrum of disease, varying from asymptomatic carriage to chronic diarrhoea with malabsorption. Such variation can only partially be explained by differences in host susceptibility. Giardia isolates are known to be heterogenous. Variation in the capacity of Giardia isolates to initiate infection has been demonstrated in animal models. Giardia isolates also differ in their antigenic structure, especially in the surface membrane. Surface antigens may change during the course of infection representing a potential mechanism of immunological evasion. In vitro studies have shown variation in susceptibility to antiangiardial drugs. The importance of this finding in explaining treatment failures remains to be established. Characterization of parasite isolates according to their zymodeme or schizodeme has successfully been used to identify and classify many parasites. This approach has been applied to Giardia isolates. Although many differences have been found no relationship between genetic markers, animal species origin, geographical distribution of isolates or virulence have been found as yet. The significance of the heterogeneity of Giardia lamblia is not completely understood. Variation in parasite virulence is likely to be an important factor in determining the severity of human disease. However, specific virulence factors remained to be identified.

Giardia lamblia is the most common parasite of the gastrointestinal tract. It is endemic in temperate and tropical regions with prevalence rates between 2 and 5% in the industrialized world and up to 20-30% in developing countries. The infection is usually acquired by the ingestion of cysts through contaminated water or more rarely food, although person to person spread also occurs. Giardia can cause acute and chronic diarrhoea with intestinal malabsorption and in children it may be associated with failure to thrive. Asymptomatic cyst excretion is well known and is probably the most common presentation in hyperendemic areas. The reason for such a wide variation in clinical manifestations is...
not completely understood, although there is no doubt that host factors such as nutritional and immunological status are important. Immuno-compromised individuals are particularly susceptible to chronic or recurrent infections notably those with hypogammaglobulinaemia and human immunodeficiency virus infection. The intrinsic characteristics of the parasite are also important but less well understood; no virulence factors have yet been identified.

**Biological variation of *Giardia* isolates**

*Giardia lamblia* cysts isolated from human stools have variable degrees of infectivity. Using the gerbil model of experimental infection Visvesvara et al. demonstrated that only six of ten cyst isolates were able to infect gerbils and only one isolate was able to produce infection in all animals inoculated; the other five produced infection in 11–75% of the animals. Furthermore, certain strains lost their ability to infect gerbils with time, even after repeated animal passage. Variable virulence of *Giardia lamblia* isolates has also been demonstrated in mice. Cyst isolates from patients with well-defined clinical conditions (symptomatic diarrhoea, asymptomatic non-diarrhoeic and asymptomatic cyst carriers) were used. Although the cyst excretion pattern was similar in all groups, significantly higher numbers were excreted by animals which had received cysts from symptomatic and diarrhoeic cases than from asymptomatic cases.

The development of axenic culture of *Giardia lamblia* has facilitated the study of the biology of the parasite. There is now clear evidence which suggests that *Giardia* isolates do vary in terms of DNA restriction fragment length polymorphisms (RFLP), isoenzyme patterns and surface proteins. The analysis of the genotypic and phenotypic characteristics of *Giardia* isolates and their relationship with virulence may allow us not only to identify markers of virulence but also to understand the pathogenetic mechanisms whereby *Giardia* causes diarrhoea and intestinal malabsorption.

*Giardia lamblia* **antigens**

Isolates of *Giardia* show few differences in their total protein profiles when analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Analysis of subcellular fractions however, has shown that there are differences in the protein content of the membrane. Surface iodination of trophozoites indicates that there are differences between isolates and in their excretory-secretory products. Cross-reactivity studies using indirect immunofluorescence have shown that live trophozoites have marked surface fluorescence when probed with homologous antisera, although when heterologous antisera was used fluorescence was reduced or absent. However, with formalin-fixed trophozoites there was diffuse internal fluorescence with both homologous and heterologous antisera. These data confirm that *G. lamblia* isolates have both different and common antigens. The different antigens appear to be on the surface of the organism while the common antigens seem to be predominantly internal. Common antigens include tubulin and the giardins (a group of 29–38kDa cytoskeletal proteins that are specific for the organism). Three giardins have already been cloned; β-giardin, β-1-giardin and α-giardin. In the plasma membranes two major antigens of molecular weight of 82kDa and 56kDa have been identified using hyper-immune sera against surface antigens. These antigens were present in all three isolates studied. These studies of the antigenic structure of *Giardia* trophozoites have been performed with hyper-immune sera prepared by parenteral administration of *Giardia* lysates to experimental animals and thus they do not give information about the role of these antigens *in vivo*.

The antigenic determinants of the human response to *Giardia lamblia* infection have also been studied. The heterogeneity of the antigenic structure of *Giardia* isolates *in vivo* is demonstrated by the great diversity in the pattern of antigenic recognition by immune sera. A 30kDa protein seems to be most prevalent. Edson et al. reported that human immune sera from two patients also recognized a 88kDa protein which probably corresponds to the 83kDa membrane antigen.

The surface antigenic structure of *Giardia* isolates is not static nor isolate-specific. During experimental infection in gerbils with a *Giardia* isolate expressing a 170kDa surface antigen it was shown that during the first 7 days there were changes in the antigenic structure of the isolate with loss of the 170kDa protein. This ability to change antigens expressed on the surface probably constitutes a potential mechanism of immune evasion.

**Biochemical variation in *Giardia lamblia***

Drug resistance is a manifestation of variation between isolates of *Giardia* which is of importance clinically as treatment failures do occur. Boreham et al. induced metronidazole resistance in *Giardia* and demonstrated reduced metronidazole uptake by a resistant clone. This resistance induced in the laboratory was unstable, full susceptibility being regained after 80–100 passages in drug-free media. In addition to this in vitro study, a relationship between metronidazole resistance and genotypic markers has been described in a case of chronic giardiasis which failed to respond to treatment with metronidazole. In this case differences between the metronidazole sensitive pretreatment isolate and the metronidazole resistant posttreatment
isolate were observed in the surface protein profile, metabolic labelling pattern with \([^{35}S]\)-methionine, malic isoenzyme patterns and by DNA RFLP analysis. It was suggested that treatment failure was due to selection of a \textit{Giardia} clone with increased resistance to metronidazole from an existing heterogeneous population of \textit{Giardia} clones with variable sensitivities in the initial infection.

In the search for biochemical markers of a species complex of \textit{Giardia}, attention has focused on the comparative electrophoretic mobilities of a variety of isoenzymes. Isoenzymes are multiple molecular forms of an enzyme occurring in a single individual or in different members of the same species.\textsuperscript{19} Analysis of isoenzyme patterns has proved useful in the classification of other protozoan parasites such as \textit{Entamoeba histolytica}\textsuperscript{20} and \textit{Trypanosoma cruzi}.\textsuperscript{21} This approach has resulted in the subdivision of these parasites into various zymodemes on the basis of the isoenzyme patterns. Zymodeme patterns have been associated with specific biological characteristics, such as severity of disease and geographical distribution.\textsuperscript{22}

Similar studies have been performed with \textit{Giardia} comparing isolates from humans and animals, such as beavers and cats,\textsuperscript{23-25} from different geographical areas and from symptomatic and asymptomatic cases.\textsuperscript{19,26} A variety of common enzymes with multiple forms have been used, such as glucose-6-phosphate dehydrogenase, glucose phosphate isomerase, hexokinase, malate dehydrogenase and malic enzyme.

Zymodemes however, do not distinguish \textit{Giardia} isolates from different host species,\textsuperscript{21,25} nor do they vary consistently in symptomatic and asymptomatic individuals.\textsuperscript{23,26} Analysis of zymodemes does go some way in differentiating isolates from diverse geographic origins. Proctor \textit{et al.}\textsuperscript{23} studied 32 isolates (six beaver, 11 human and 15 others) and described 12 zymodemes, one zymodeme containing 17 of the 32 isolates from the same geographic location. A limited correlation between locality and zymodeme was found by Meloni \textit{et al.}\textsuperscript{24} On further analysis the zymodemes could be divided into two groups; group 1 defined isolates of human and feline origin worldwide and group 2 contained human isolates from western Australia.

Thus, isoenzyme analysis does not provide a useful approach for determining differences in biological activity or geographic region. However, this approach does confirm the genetic heterogeneity of \textit{Giardia} isolates. It is interesting to note that isolates from different hosts and geographically diverse areas may occupy the same zymodeme and so are probably genetically similar.

\textbf{Nucleic acid polymorphisms of \textit{Giardia lambia}}

\textit{Giardia lambia} is a binucleate, possibly diploid\textsuperscript{27} protozoan with 3–4 × 10\textsuperscript{7} base pairs of DNA per nucleus.\textsuperscript{28} This morphologically defined species seems to encompass a wide spectrum of genetic variation\textsuperscript{29} although nothing detectably unique about the human parasite has been shown. There are three main ways of demonstrating this genotypic variation; karyotyping, restriction enzyme analysis and DNA fingerprinting.

Karyotyping, measurement of the number and size of an organism's chromosomes, is carried out using pulsed field\textsuperscript{30} or field inversion\textsuperscript{31} gel electrophoresis; direct microscopic observation is made impossible by the lack of condensed metaphase chromosomes within the organism. So far it has been shown\textsuperscript{32} that within \textit{G. lambia} there are two different karyotypes based on counting the medium size chromosomes. Upcroft \textit{et al.}\textsuperscript{28} have shown geographic variation within the species such that isolates from North America usually have a so-called 3 karyotype pattern, while Australian isolates have a 4 karyotype pattern, regardless of host or isolation techniques.

Restriction enzyme analysis involves the digestion of whole \textit{G. lambia} genomic DNA with restriction enzymes, the fragments of which are then separated on agarose gels and visualized for banding pattern comparison by staining with ethidium bromide. It has been shown that isolates from a wide variety of sources can be divided, on the basis of their banding patterns, into genetically similar schizodemes (groups of isolates with the same banding pattern).\textsuperscript{27,32} Increased sensitivity can be achieved by use of labelled genomic DNA probes (lengths of \textit{Giardia} DNA) which are hybridized to southern blots of the agarose DNA gels. Using this additional technique, differences can be detected within schizodemes.

The third method of demonstrating genetic variation in \textit{G. lambia} is that of DNA fingerprinting. This uses a similar principle to that employed by Jeffreys \textit{et al.}\textsuperscript{34} to develop human DNA fingerprinting. It has been discovered that like most other eukaryotes, \textit{G. lambia} has a form of repetitive minisatellite sequence which in this case seems to be slightly homologous to a repeated sequence in the genome of the M13 virus (a virus of \textit{E. coli} commonly used as a vector in genetic transformation experiments). Therefore, by labelling this virus and using it to probe southern blots of gels similar to those used for restriction analysis, it is possible to produce a sequence of bands which are unique to any one particular isolate.\textsuperscript{32} By this method it is possible to identify a particular strain wherever it may appear.

Despite the demonstration of this great genetic diversity, no relationship between genetic markers,
animal species origin or clinical presentation has yet been described.

Conclusions
Despite morphological similarity between G. lamblia isolates they are clearly heterogeneous on the basis of both phenotype and genotype. The significance of this heterogeneity is not completely understood. Variation in parasite virulence is likely to be an important factor in determining the severity of human disease. A high prevalence of strains of low virulence may explain why in hyperendemic areas a great proportion of cases are asymptomatic although acquisition of host resistance is also likely to be important. Repeated exposure to strains of low virulence might provide some protection when exposed to a virulent strain. Treatment of asymptomatic cases in hyperendemic areas is not recommended because of the high rate of re-infection. Identification of virulent strains may prove important when considering mass eradication programmes.

Well characterized virulent and avirulent isolates will be invaluable for laboratory research into the pathogenesis of giardiasis and host immune response. Study of strain variation may help to identify the pathophysiological mechanisms of diarrhoea and malabsorption in giardiasis. The antigenic profile that results in protective immunity is unknown but may become apparent using isolates of variable virulence in animal models. Lack of specific determinants in some isolates might explain the need for repeated exposure to achieve protective immunity.

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