DIFFERENTIATION OF STRAINS OF *GIARDIA INTESTINALIS* BY IDENTIFICATION OF RESTRICTION FRAGMENT LENGTH POLYMORPHIMS (RFLP's) AND THE CONSTRUCTION OF A GENE LIBRARY.

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ABSTRACT

Giardia intestinalis is a flagellate protozoan which infects the gastrointestinal tract of humans and other mammals such as cats, dogs and farm animals. The organisms involved have been assigned to a single species, which might be taken to suggest the absence of host specificity, yet there is little epidemiological evidence to suggest that human infections are derived from nonhuman sources. This suggests some degree of host specificity which in turn implies the existance of different strains of *Giardia intestinalis*. Consequently, two related questions of public health interest can be raised: do animal strains of *Giardia intestinalis* infect humans and if not, how can cysts of human and animal origin detected in water samples be distinguished? If all or even some strains from animals fail to infect humans then it is probable that water may often be unnecessarily condemned as unsuitable for human use. Conversely, if animal strains infect humans then exclusion of animals from water catchment areas would be desirable. To clarify this situation, it is desirable to be able to distinguish individual strains of *G. intestinalis* and this thesis represents a preliminary attempt to do so using the techniques of molecular genetics.

Initial experiments attempted to detect Restriction Fragment Length Polymorphisms (RFLP's) produced by digests of total genomic DNA using a range of restriction endonucleases. The results were difficult to interpret because an excessive number of bands were produced. However, some denser bands, probably representing repetitive DNA sequences, were relatively well resolved and could allow comparisons between strains to be made. This repetitive DNA is GC-rich and was separated from most of the nonrepetitive genomic DNA by CsCl centrifugation in the presence of Hoescht 33258 stain. Using this approach, GC-rich fractions of DNA from several strains of *G. intestinalis* were compared using a variety of restriction endonucleases. Most did not reveal differences but digestion with some restriction endonucleases revealed minor differences. This demonstrated the potential usefulness of this approach in distinguishing between strains of *Giardia* but the procedure was laborious and required large amounts of DNA which could only be produced by the

culturing of organisms in bulk. This is not yet possible in the case of many strains of *G. intestinalis*, so it was concluded that an alternative approach using digests of total genomic DNA followed by electrophoresis, Southern blotting and hybridisation with specific DNA probes would represent both a better theoretical and practical approach. A desirable preliminary step to facilitate this approach is the production of a *Giardia* gene library and the latter part of this thesis describes this process.

The major problem encountered with the production of the library was that the DNA produced by a range of extraction methods was sheared and was present only in low concentration. This problem was traced to the presence of an excessive amount of polysaccharide which appeared to be strongly associated with unsheared DNA and hence caused it to be trapped at the interphase during phenol/chloroform extractions. As only the supernatant was retained, the high molecular weight DNA was largely lost during such extraction steps. This difficulty was overcome by precipitating the DNA with isopropanol at an early stage in the extraction method. This reagent does not precipitate the polysaccharides present in the cell lysate so that these are removed with the supernatant. The precipitated DNA was redissolved and, following conventional purification procedures, represented a high yield of unsheared DNA. Using this DNA, a library was made using LambdaGEM-11 Xho Half-site arms™ (Promega). Experiments showed that the library is representative of the genome of *G. intestinalis* and exceeded by 6-fold the number of clones required for a 99.9% probability that any particular sequence of interest is present.

The availability of such a library should permit the selection of suitable clones for use as probes to hybridise with total genomic digests to reveal differences between strains of *G. intestinalis*. The ability to distinguish strains would allow investigations of host specificity which may have implications for the formulation of testing procedures designed to prevent human infections of *G. intestinalis* but avoiding the unnecessary condemnation of water supplies containing *Giardia* strains that do not infect humans. The development of strain-specific probes would also serve as a useful epidemiological tool in tracing the source of infection within a community.

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God has made a way where there seemed to be no way
He works in ways we cannot see
He has made a way for me
He is my guide, He holds me closely to His side
With love and strength for each new day
He has made a way. *(Don Moen)*

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