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Genotyping of Human and Animal Isolates of *Giardia intestinalis*

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> Errol Stephen Kwan 2002

ABSTRACT

Giardia intestinalis is an important protozoan parasite that infects humans and animals. It has been suggested that cattle may be a major source of human *Giardia* infection so a dairy farming region of New Zealand was investigated. This thesis uses three molecular methods to genotype *G. intestinalis* isolates obtained from human and animal faecal specimens collected in the Waikato region of New Zealand, to determine if giardiasis is a zoonotic disease.

Random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) fingerprinting techniques were initially assessed for their ability to genotype *G. intestinalis* isolates. "Clear cut" evidence of zoonosis could not be established by either method, due to a low sample number.

To determine the stability of the *G. intestinalis* genome an axenic culture of *G. intestinalis* trophozoites was stressed with toxic levels of metronidazole and the survivors, following a number of passages, were examined using AFLP and RAPD analysis. The DNA fingerprints were compared to those of the original wild-type with the results being indicative of an unstable *G. intestinalis* genome.

A third molecular method was employed, which amplifies a portion of the tandemly repeated ribosomal DNA (rDNA). Each cyst contains 512 head to tail tandem repeat copies of the *rRNA* gene made up of both conserved and variable regions. The use of nested primers increased the sensitivity and specificity of the PCR reaction allowing the amplification of a 505bp rDNA fragment. DNA sequence analysis and alignment of the amplified products facilitated the comparison of *G. intestinalis* isolates. The relationship of the sequence data was generated and displayed using Splitstree software indicating that zoonosis did occur.

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