The Development of Techniques to Distinguish Species and Strains of *Giardia*.

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Abstract

Water supplies, in some rural areas of New Zealand, contain *Giardia* cysts. This is assumed to make the water unsuitable for human consumption. *G. intestinalis* and/or *G. muris* cysts may be present but are not distinguished by the standard test . *G. muris* infects rodents only so it is not infectious for humans. However *G. intestinalis* infects humans and a wide range of animals, but it is unclear if the strains which infect animals also infect humans. If *G. intestinalis* strains are host-specific, then since water in rural areas may contain cysts derived only from animal species it would follow that the water (even if *G. muris* and/or *G. intestinalis* cysts were found) may not be infectious for humans.

Investigation of host-specificity of *G. intestinalis* would be facilitated by a reliable test to distinguish strains of the organism and this thesis investigates the use of PCR for this purpose. A series of random primers were investigated for their ability to distinguish strains of *G. intestinalis* when used with a variety of PCR protocols. We found that several of these primers (especially GC50 at an annealing temperature of 35°C, and GC80 at an annealing temperature of 35°C) had the potential to distinguish strains. The differences seen were not large but this may be because some of the isolates were clonally related. Consequently we concluded that further modifications and extensions of PCR when applied to human and animal strains should distinguish strains and may have the potential to address the question of host-specificity.

The major aim of the thesis however was to produce primers which when used in the PCR are capable of distinguishing *G. muris* from *G. intestinalis* . The same approach, ie the use of a random primer, was used to distinguish *G. muris* from *G. intestinalis* . Clear differences were seen but the non-specificity of the random primer would allow the organisms to be reliably distinguished only in the absence of other organisms. To avoid this lack of specificity an amplified band produced with *G. muris* DNA but not with *G. intestinalis* DNA was sequenced and a primer pair was selected. These primers were, in principle, long enough (21-mer and 23-mer) to be specific for the target DNA and were chosen so as to have matched melting temperatures. The selected primer pair amplified a sequence 307bp long, and the primer sequences were specific for the target species, namely *G. muris* . Thus in our hands using PCR this primer pair amplified DNA from the available strains of *G. muris* but failed to amplify DNA from any of seven *G. intestinalis* strains.

Further work is required to establish both an optimal method for lysing cysts and to estimate the minimum number of cysts required to ensure that DNA is available for amplification. However, the availability of the *G. muris* -specific primers, along with the recently developed genus and *G. intestinalis* -specific primers should allow us to undertake investigations of water supplies to see if *G. muris*, *G. intestinalis* or both species are present. In the case of a small rural supply it would seem reasonable to accept the potability of water supplies containing *G. muris* only, as long as assurance could be given that *G. intestinalis* was not present.

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