Hammondia heydorni oocysts in the faeces of a greyhound

J.T. Ellis*¶ and W.E. Pomroy†

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*Dept. of Cell & Molecular Biology, University of Technology, Sydney, Westbourne Street, Gore Hill, New South Wales 2065, Australia

†Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North,

10 New Zealand

¶Author for correspondence. Email j.ellis@uts.edu.au

15 **AIMS**: To identify oocysts found in faecal material of a greyhound.

METHODS: PCR and DNA sequencing was used to study genomic DNA isolated from oocysts purified from faeces of a greyhound.

20 **RESULTS**: Database searches with the DNA sequences obtained showed they were derived from *Hammondia heydorni*. A species-specific PCR was developed to detect *H. heydroni* DNA.

CONCLUSIONS: Light microscopy in conjunction with PCR and DNA sequencing definitely identified the presence of *H. heydorni* oocysts in faeces of a greyhound.

CLINICAL RELEVANCE: This study confirms the presence of *Hammondia heydorni* in New Zealand and indicates the need to correctly identify oocysts from dogs and cysts in cattle, rather than assume they are *Neospora caninum*.

KEY WORDS: *Hammondia heydorni*, *Neospora caninum*, *Toxoplasma gondii*, PCR, DNA sequencing, ribosomal DNA

Introduction

Hammondia heydorni and *Neospora caninum* are closely related cyst-forming coccidia, both of which are known to possess dogs as definitive hosts (McAllister et al 1998; Mehlhorn and Heydorn 2000; Dubey et al 2002). Thus the detection of "Toxoplasma-like" oocysts in faecal specimens from dogs is not diagnostic for a single parasite species, and additional information is required to identify the species present. Here we report on a case study, which demonstrates the value of PCR for parasite identification.

Materials and Methods

Faecal samples from 9 greyhounds were submitted for routine faecal analysis (at Massey University) in April 1996 and examined by a faecal flotation in 33% zinc sulphate. There is no other history on these dogs, other than two of them had oocysts in their faeces, one with a large number. These oocysts were recovered and sporulated in 2% potassium dichromate. Oocyst measurements were made (n=20) using an eyepiece micrometer on a compound microscope (Olympus). The oocysts (hereafter called NZO) were washed in distilled water and DNA

extracted from them by lysis in SDS and proteinase K followed by phenol chloroform extraction and RNAse treatment as described (Ellis et al 1999b).

50 PCR amplification of the internal transcribed spacer 1 (ITS1) from genomic DNA was performed using primers Tim3 (5'CCGCTGCAGAGGTGAACCTGCGGAAGGATC3') and Tim11 ((5'CACTGAAACAGACGTACC3') and the amplification products were analysed by agarose gel electrophoresis (Payne and Ellis 1996). In addition, sequences from the large subunit (lsu) ribosomal DNA (D2 and D3 domains) were PCR amplified, using primers CR1 (5'-
55 CTGAAATTGCTGAAAAGGAA-3') and CR2 (5'-CCAGCTACTAGATGGTTCGA-3') (Ellis et al 1999b) (Ellis et al 1998). The PCR products obtained were sequenced in both forward and reverse directions. The non-redundant nucleotide sequence database maintained by the National Centre for Bioinformatics (NCBI; http://www.ncbi.nlm.nih.gov/) and the Apicomplexa nucleotide sequence database at the Parasite Genome Blast Server (PGBS;
60 http://www.ebi.ac.uk/parasite/parasite_blast_server.html) were searched with both sequences obtained from the NZO using the program BlastN.

In order to generate a species-specific PCR for *H. heydorni,* primers HheyF (5'ACGGATTCGGCGATTGAAG3') and HheyR (5'CATCGCGTCTTCTGATTC3') were designed from ITS1 data reported previously and used in a standard, non-nested PCR format
65 (Ellis et al 1999b).

Results and Discussion

The mean dimensions of the NZO oocysts (n=20) were 12.88±0.24 by 11.78±0.19 μm, which is in the range expected for either *H. heydorni* or *N. caninum* (Lindsay et al 1999).

70 PCR amplification of ITS1 or lsu rDNA generated PCR products whose sequences are lodged in GenBank under accession numbers AF508030 (ITS1) and AF508029 (partial lsu

rDNA). Database searches with these sequences returned matches to previously described sequences from *H. heydorni* of the dog and the fox (Ellis et al 1999b; Schares et al 2002).

In a PCR the primers HheyF and HheyR produce a 250 bp product (Figure 1) from *H.*
75 *heydorni* genomic DNA whose sequence is identical to ITS1 from both NZO and *H. heydorni* oocysts shed by an Arabian red fox (Ellis et al 1999b) thus confirming the specificity of this PCR. No products were obtained using these primers and genomic DNAs from *N. caninum* or *Toxoplasma gondii*.

A great deal of discussion has occurred in recent times over the relationship between *H.*
80 *heydorni* and *N. caninum* (Dubey et al 2002) (Mehlhorn and Heydorn 2000) and it is unfortunate that no type material of *H. heydorni* exists in order to substantiate claims on its biological properties (Schares et al 2001). Fortunately, the molecular biology of *N. caninum* is now well defined and a number of genetic loci have been isolated and characterised by a variety of PCR methodologies. Thus this study characterized rDNA from NZO. Previous studies have
85 demonstrated that the ITS1 sequence from the rDNA is quite unique to each taxon studied within the Toxoplasmatinae (Ellis et al 1999b). Similarly limited DNA sequencing of the large subunit rDNA was undertaken, since at this locus nucleotide sequence divergence is uncommon within a species (Ellis et al 1998). The weight of evidence presented here from gene sequence data shows the oocysts found in the greyhound faecal material was *H. heydorni*, thereby confirming the
90 existence of this parasite in New Zealand.

Oocysts from *H. heydorni* or *N. caninum* are rarely found in dog faeces (McAllister et al 1998; Slapeta et al 2001; Basso et al 2001). In New Zealand, a survey of 481 dogs in 1974-76 found 2.7% were shedding oocysts of either *H. heydorni* or *N. caninum* (McKenna and Charleston, 1980). A differential identification is impossible without the application of
95 technology other than light microscopy. The story is complicated further because the faeces of

dogs can be contaminated with oocysts from other coccidia such as *T. gondii* (Lindsay et al 1997). The species-specific PCR described here complements an existing test for *N. caninum* (Ellis et al 1999a) and will prove a useful aid for the identification of *H. heydorni* in biological specimens.

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Figure 1. Agarose gel electrophoresis of PCR products obtained using primers HheyF and HheyR from (1) no template control (2) NZO, *N. caninum* isolates Nc-SweB1 (3) Nc-Liverpool (4) Nc1 (5) and *T. gondii* RH strain (6). Only NZO gives a specific 250 bp PCR product with

150 these primers.