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A real-time assemblage-specific PCR assay for the detection of *Giardia duodenalis* assemblages A, B and E in fecal samples

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ABSTRACT

Giardiosis is a common gastrointestinal infection caused by the flagellate *Giardia duodenalis*, and affects both humans and animals, worldwide. Animals are infected with both zoonotic and host-specific *G. duodenalis* assemblages, and their role in the transmission of the infection to humans has been a subject of intense research and debate. Conventional PCR assays are appropriate to determine *G. duodenalis* assemblages, but lack sensitivity for the detection of mixed infections. Previous surveys demonstrated the occurrence of mixed infections with *G. duodenalis* assemblage A and B in humans, and with assemblages A and E in cattle, but are likely to be underestimated. In this study, we designed a set of assemblage-specific primers by exploiting sequence variability in homologous genes from assemblages A, B and E. Primers were designed to amplify fragments of different size that generated different melting curves from each assemblage in real-time PCR (rt-PCR) experiments. The assay has been tested on a large panel of human and farm animal isolates, and shown to possess high specificity (no cross reactions observed) and sensitivity (detection limit close to 20 copies). Therefore, this assay can be useful to detect zoonotic and host-specific *G. duodenalis* assemblages in fecal samples from farm animals, particularly when a large number of samples is to be tested.

1. Introduction

Giardiosis is a common gastrointestinal infection caused by the flagellate *Giardia duodenalis*, and affects both humans and animals, worldwide (Feng and Xiao, 2011). It has been well established that members of this species are morphologically identical, yet can be assigned to at least eight distinct genetic groups (assemblages A–H) based on protein or DNA polymorphisms (Thompson and Monis, 2012). Humans, but also other mammals, are infected with *G. duodenalis* assemblages A and B, whereas the remaining assemblages showed a more restricted host range. The issue of the zoonotic potential of *G. duodenalis* has received considerable attention since the WHO classified human giardiosis as a zoonosis about 30 years ago (WHO, 1981).

A large number of surveys employed molecular methods to determine the assemblages and genotypes of *G. duodenalis* in fecal samples collected mainly from livestock, dogs and cats, non-human primates and some wildlife species. The overall results have indicated that host-specific assemblages are largely more common in their respective hosts than zoonotic assemblages, thus, suggesting a minor role of zoonotic transmission in the epidemiology of human infection (Feng and Xiao, 2011; Ryan and Cacciò, 2013). The few studies carried out in settings characterized by promiscuity between humans and animals, by limited access to safe water and by scarce hygiene, however, have supported the existence of transmission cycles between human and dogs in Asia (Traub et al., 2009), and between humans and cattle in India and Ethiopia (Khan et al., 2011; Wegayehu et al., 2013).

One limitation of the approach based on conventional, end-point PCR is that mixed infections are difficult to detect, as the most abundant parasite population is preferentially amplified.

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Gene: Translation Initiation Factor (locus GL50803_39587)

Test: assemblage A specific rt-PCR

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WB      AGAAGTGTCCTGGACTGGGTCTCCACGGGCGCAACGCAGCTGCAATCGTGTGGCACACTG
GS      ..G...G..TCC......ACAG...T...AA.G..G.....A.....T........T...
P15     ..G...G..TCA......G.GG...T.....T...A.....G.....A........GT..

WB      TACTGTGACGAATCAACCTCTATTCCCCCCAACACCATAATAGGTGGGGACTCGCTAGTG
GS      .....C..T.....T.....G.....T.....T..TG......C...C..T..AT.G..G
P15     ..T.....T..G..............T........T...G...C...A.....A.....A

WB      CACAGAAATTGTACTTTGGAGGACGGTTTAACGATTGACAATTCCACG
GS      .....G..C..C..GC....A...AACC..GTA..A..C..T..G.T.
P15     .....G.....C.A..................A.....T..C......
```

Gene: Cathepsin L precursor (locus GL50581_3714)

Test: assemblage B specific rt-PCR

```
GS      GCGATTTTCCGCGGAAGGTTGTAAACCCTTTCCTCCCAGGAAATTATGCCATAGTATCA
WB      A...C..CT..T.C.....ATC.....................C....T..C...G...
P15     ..A....CT.A..C.....AT.....................C....T..C.A.G..G

GS      TCATGCAAGACATCGCTGGTTTATGTTTATGATGCCCTCT
WB      G.G........C..A..TA.C..CA.C..C..CATA..A.
P15     C....T.....C.....TA....CA.C.......TA..AG
```

Gene: Hypothetical protein (locus GLP15_2932)

Test: assemblage E specific rt-PCR

```
P15     AGATCATGTTCAAGGGTTTGCCAACGGTATCAAAACATTAGCCTTCACACAAAATTCACA
WB      -------------------------.......G.......A........C.G..C.....
GS      ...G..A.A..GG.T.AG.A.TNG..A..AG.T..---.GA...CGNTG.GC...CTG.G

P15     AAGACAGAAACGGAGAAAGTGTAGCGATAGGATGATAGAGTCGGATGACAGCAATTCCGA
WB      GG.C..A.....................................A.....A..T........
GS      ....TCA.......A...A.....TAC......CC.C.GG..C..C..G..T.GCA.T..

P15     AGACACCTCAGATGTGGACCTTAGTGTTGAGAGTAGGCATT
WB      G.................TA..................CC.
GS      ......A......A....AG.GG.A......GA---..CCC
```

Fig. 1. Multiple alignments of the translation initiation factor (*Tif*), cathepsin L precursor (*Cath*) and hypothetical protein (*Hyp1*) homologous sequences from assemblage A, B and E. Dots indicate identical nucleotides; dashes indicate gaps. The primer sequences are shown in bold, underlined characters.

To circumvent this limitation, nested PCR assays that include assemblage-specific primers have been developed (Geurden et al., 2007). In this context, the use of real-time PCR (rt-PCR) assay is expected to provide more reliable results, as assemblage-specific primers can be used in single round amplification protocol, and specificity can be further enhanced by the use of specific probes (e.g., Alonso et al., 2011), or by performing melting curve analysis of the rt-PCR products (Almeida et al., 2010; Zhang et al., 2012).