During the last decade, intestinal microsporidia have become recognized as an important cause of opportunistic infections in immunocompromised patients, especially those with AIDS. Two species are the cause of diarrhoea and other gastrointestinal diseases in HIV infected patients: *Enterocytozoon bieneusi* (Desportes et al., 1985) and *Encephalitozoon intestinalis* (Hartskeerl et al., 1995). Diagnosis of gastrointestinal microsporidiosis can be made by detecting spores of the parasites in stool specimens with Weber's modified trichrome stain or optical brightening agents such as Uvitex 2B (De Girolami et al., 1995). However the identification of microsporidia to the species level currently depends on the observation of cytological characteristics only viewed with the electron microscope. The specific identification of microsporidia is of importance because *E. intestinalis* responds to albendazole therapy. Up to this date, no convincing therapy is available for *E. bieneusi*. Molecular studies have shown that the small-subunit (SSU) rRNA genes of microsporidia shared only limited homology with the SSU rRNA genes of other eukaryotic organisms, pointing out that the PCR procedure proposed herein for the species-specific detection of microsporidia appears to be a simple and time-saving method. Indeed our PCR assay can be performed within one day and its specificity is high since we use two pairs of primers selective of each intestinal species. These qualities indicate that the PCR procedure proposed herein for the species-specific detection of DNA was released from microsporidia by boiling the samples. The PCR reaction was performed under conditions described by Weiss (1994). The primer pair V1 and EB450 was used to amplify DNA from *E. bieneusi* and the internal 30 mer oligonucleotide EB150 was used to confirm by southern blotting that the amplified fragment was from *E. bieneusi*. The primer pair V1 and SI500 was used to amplify DNA from *E. intestinalis*.

In samples containing *E. bieneusi*, amplification of the predicted 350 bp fragment was indisputable on ethidium stained gels (Fig. 1A). Using an internal oligomer, hybridization was apparent in all of these samples (Fig. 1B). In negative stools no amplification was seen by ethidium staining or detected by hybridization. In addition, primer pair V1 and SI500 amplified a 380 bp fragment from stools infected with *E. intestinalis* and from cultured parasite but not from uninfected stools. No amplification was seen with *E. bieneusi* infected stool samples (Fig. 1A). No hybridization was observed when using EB150 the specific probe of *E. bieneusi* (Fig. 1B).

PCR has already been used by Fedorko (1995) to identify intestinal microsporidia in stools. However, the PCR assay proposed by this author required harsh conditions employing both mechanical and chemical disruption and a laborious 4-day procedure. Comparatively, boiling the samples to release DNA from microsporidia appears to be a simple and time-saving method. Indeed our PCR assay can be performed within one day and its specificity is high since we use two pairs of primers selective of each intestinal species. These qualities indicate that the PCR procedure proposed herein for the species-specific detection of
intestinal microsporidia would be very valuable for clinical and epidemiological investigations.

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REFERENCES


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