The Zoonotic Potential of *Enterocytozoon bieneusi* in Thailand

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Abstract

Enterocytozoon bieneusi is the most common microsporidia that causes disease in immunocompromised hosts, especially in AIDS patients. *E. bieneusi* has been reported in a wide range of animals, including domestic, wild and farm animals. Using molecular typing and phylogenetic analysis, the zoonotic potential of *E. bieneusi* to cause disease is indeed a threat. Transmission from animals to humans should be investigated and whether it occurs naturally, before we can address the whole subject of zoonotic transmission of *E. bieneusi* infection.

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บทคัดย่อ

โอกาสของการแพร่กระจายของเชื้อ *Enterocytozoon bieneusi* จากสัตว์สู่คน

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Enterocytozoon bieneusi เป็นเชื้อ **Microsporidia** ที่ทำให้เกิดโรคในผู้ป่วยภูมิคุ้มกันบกพร่อง โดยเฉพาะอย่างยิ่งใน ผู้ป่วย-เอดส์ ที่พบได้บ่อยที่สุด นอกจากในคนแล้วยังสามารถตรวจพบเชื้อนี้ได้ในสัตว์ชนิดต่างๆ รวมถึงสัตว์ป่า สัตว์เลี้ยง ในบ้าน และสัตว์เลี้ยงในฟาร์ม เมื่อนำเชื้อที่แยกได้จากคนและสัตว์มาตรวจแยกสายพันธุ์ในระดับโมเลกุลและศึกษาวิเคราะห์ทาง **phylogenetic** พบว่าเชื้อเหล่านี้มีคุณสมบัติในการติดเชื้อได้ทั้งในคนและสัตว์ อย่างไรก็ตามยังต้องมีการศึกษายืนยันว่า เชื้อนี้มี การแพร่กระจายจากสัตว์มายังคนได้ในธรรมชาติ ก่อนที่จะสรุปได้ว่าเชื้อนี้มีการติดต่อจากสัตว์จริง

Introduction

Microsporidia are a diverse group of obligate intracellular organisms which have recently been reclassified from protozoa to fungi after molecular phylogenic analysis (Keeling and Fast, 2002). The organisms produce unique spores containing a long, coiled tubular extrusion apparatus (polar tube). During host cell invasion, this polar tube protrudes and injects infective material (sporoplasm and nucleus) into the cytoplasm of the host cell through the plasma membrane. The polar tube may pierce through the membrane of the phagosomes if the spores are phagocytosed by the host cells. The infective material then develops to a proliferative merogonic stage, followed by a sporogonic stage, resulting in infective spores (Bigliardi and Sacchi, 2001; Franzen, 2004).

Microsporidia comprise of more than 1,200 species which mostly infect arthropods and fish (Mathis et al., 2005). These organisms rarely caused disease in humans before the AIDS pandemic occurred, with only 8 cases of human microsporidial infections being reported. After 1985, human infection with a significant number of microsporidial

species have been reported in AIDS patients. These include *Enterocytozoon bieneusi, Encephalitozoon intestinalis, Encephalitozoon cuniculi, Encephalitozoon hellem, Vittaforma corneae, Pleistophora ronneafiei, Trachipleistophora spp., Brachiola algerae, Nosema ocularum, Microsporidium ceylonensis* and *Microsporidium africanum* (Didier et al., 2004; Didier, 2005; Mathis et al., 2005). Among these, *E. bieneusi* is the most common microsporidial infection found in humans. *E. bieneusi* infection causes chronic diarrhea and a wasting syndrome in AIDS patients. The main symptoms are chronic nonbloody diarrhea without fever, anorexia, weight loss and bloating. In addition, this organism was identified in the biliary system of patients with cholangitis, cholecystitis, bronchitis, pneumonitis and rhinosinusitis. In Thailand, apart from a report of *Trachipleistophora anthropophthera*, that caused keratitis in an HIV patient (Juarez et al., 2003), only *E. bieneusi* infections have been reported in humans (Wanachiwanawin et al., 1997; Leelayoova et al., 2001; Wanachiwanawin et al., 2002; Leelayoova et al., 2005). This review focuses on this organism.

E. bieneusi was first identified by Desportes and colleagues in 1985 from a 29-year-old Haitian AIDS patient with chronic diarrhea and who lived in France. An *E. bieneusi*-infected case was described in the United States in the same year (Dobbins et al., 1985). Since then, the number of reported cases has steadily increased in all parts of the world. The parasite usually infects intestinal enterocytes of HIV-infected patients but has been also detected in lamina propria cells from small-bowel biopsy specimens, biliary tree, gall bladder, liver cells, pancreatic duct, and tracheal, bronchial, and nasal epithelia.

Diagnostic methods

The diagnosis of *E. bieneusi* infection is dependent on the identification of spores in clinical samples, e.g., stool specimens, duodenal, bile juice or biopsy tissues. The detection of spores in clinical samples, however, is a laborious, challenging, and time-consuming task because the tiny organisms can easily be missed. Initially, definitive diagnosis required transmission electron microscopy to identify the characteristics of *E. bieneusi* spores (Franzen and MÜller, 1999). In Fig. 1, a transmission
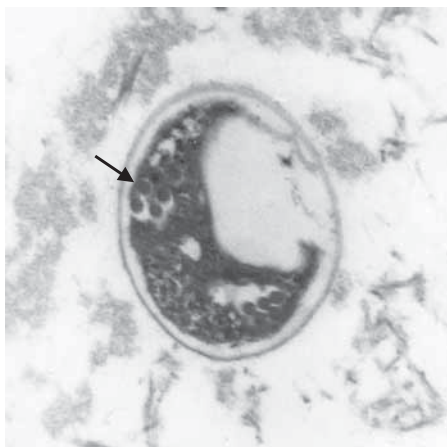

Figure 1 Transmission electron micrograph of a stool specimen showing a spore of *Enterocytozoon bieneusi* with the specific characteristic 4-7 coils of polar tubes, (arrows) arranged in 2 rows (original magnification, X 46200).

electron micrograph of a stool specimen shows a spore of *Enterocytozoon bieneusi* with the specific characteristic 4-7 coils of polar tubes, arranged in 2 rows. During the last few years new staining methods, such as modified trichrome (Weber et al., 1992) and gram-chromotrope (Moura et al., 1996), suitable for light microscopy, have been developed. However, species differentiation and genotypic characterization is usually impossible by these techniques. Immunofluorescent staining techniques have been developed for species differentiation (Garcia, 2002), but the antibodies used in these procedures are available only at research laboratories at the present time.

Recent success in nucleotide sequencing of this organism has now led to the application of new molecular techniques for the diagnosis of *E. bieneusi* infection. Several PCR-based methods have been published to amplify different regions of the small subunit ribosomal RNA gene for species differentiation and genotypic characterization of *E. bieneusi* infecting both humans and animals (Schuitema et al., 1993; Zhu et al., 1993; da Silva et al., 1996; Katzwinkel-Wladarsch et al., 1996; Velasquez et al., 1996). A blinded, externally controlled, multicenter evaluation of light microscopy and PCR has been conducted for the detection of *E. bieneusi* in stool specimens. The sensitivities were reported to be between 71-100%, from 6 different laboratories (Rinder et al., 1998). The difference in sensitivity might be dependent on DNA extraction methods and PCR protocols. We also evaluated the sensitivities and specificities of PCR and light microscopy for the detection of *E. bieneusi* in stool specimens. Sensitive techniques were used to detect *E. bieneusi* i.e. FTA filter paper for DNA extraction and MSP3/MSP4B primers for PCR amplification. The PCR method and light microscopy gave 100% and 87% sensitivity, respectively, for the detection of *E. bieneusi* in stool specimens. Both methods

showed 100% specificity (Subrungruang et al., 2004). Thus PCR is a powerful diagnostic method when dealing with clinical specimens and also meaningful for epidemiological study of this infection. Moreover, the genotype can be characterized by sequence analysis of the PCR product.

The prevalence of human infection

E. biensusi infection has been reported from both developed and developing countries. The majority of cases were HIV-infected patients with a prevalence between 2-50% depending on the population under study and the detection method (Canning and Hollister, 1990; Molina et al., 1993; Rabenek et al., 1993; Kotler and Orenstein, 1994). In Thailand, *E. bieneusi* was detected at a prevalence rate of 11-33.3% in adult AIDS patients (Wanachiwanawin et al., 1998; Waywa et al., 2001). The prevalence of *E. bieneusi* infection ranged from 10.8 to 25.3% in HIV-positive children (Leelayoova et al., 2001; Wanachiwanawin et al., 2002). *E. bieneusi* infections were also reported from HIV-negative patients who had an immunocompromised condition, due to an underlying disease or due to immunonosuppressive drugs given after organ transplantation (Kotler and Orenstein, 1998.). Moreover, self-limiting diarrhea, as well as chronic diarrhea has been seen in immunocompetent patients, particularly in travelers who returned from tropical countries (Sandfort et al., 1994; Weber and Bryan, 1994).

Asymptomatic infections with *E. bieneusi* have also been identified in both HIV-positive and HIV-negative patients. Studies in Africa revealed a high prevalence of asymptomatic infection in healthy HIV-negative children (Bretagne et al., 1993; Cegielski et al., 1999). Our study in a Thai orphanage, showed asymptomatic *E. bieneusi* infection in 5.9% of children and 1.5% of child-care workers (Mungthin et al., 2001).

Animal infection

E. bieneusi has been identified in a wide range of animals. In 1996, detection of this organism was recorded in pigs (Deplazes et al., 1996). A study of *E. bieneusi* infection in pigs from 220 farms in Switzerland showed a prevalence of 35% and a significant higher occurrence in weaned piglets (Breitenmoser et al., 1999). A survey at a slaughterhouse in Massachusetts, United States also revealed a prevalence rate of *E. bieneusi* shedding, in 32% of pigs (Buckholt et al., 2002). Using the PCR method to detect *E. bienusi* in samples from a farm in Nakornpathom, Thailand our preliminary study identified 6 out of 30 fecal samples positive (Leelayoova, unpublished data). Most pigs in both observational and our experimental studies were asymptomatic. This indicates a low pathogenicity of E. bieneusi in pigs (Deplazes et al., 1996; Kondova et al., 1998; Breitenmoser et al., 1999).

The organism has also been detected in other animals, including calves (Rinder et al., 2000; Santin et al., 2004; Sulaiman et al., 2004), dogs (Mathis et al., 1999; Lores et al., 2002), cats (Mathis et al., 1999; Dengjel et al., 2001), a llama (Dengjel et al., 2001) and a goat (Lores et al., 2002). A variety of wild fur-bearing mammals such as beavers, foxes, muskrats, otters and raccoons have also been found to shed *E. bieneusi* (Sulaiman et al., 2003). Screening captive rhesus macaques, showed 16.7% of apparently healthy animals were infected with *E. bieneusi*. While those with simian immunodeficiency virus showed a higher prevalence, 33.8% (Mansfield et al., 1998). It has also been reported in non-mammalian hosts i.e. chickens (Reetz et al., 2002) and pigeons (Haro et al., 2005).

The sources of infection and its transmission

Humans or animals infected with *E. bieneusi* are obvious possible sources of infection. Like other gastrointestinal infections, asymptomatic cases are

considered an important source of infection. Recent studies showed that the spore shedding pattern and intensity, in asymptomatic HIV-positive and HIV-negative children, was variable (Mungthin et al., 2005). Excretion of spores could be too few to be detected by a light microscope. Thus, the role of the asymptomatic group, as the source of infection, is probable and might be underrated.

Since *E. bieneusi* spores are released into the environment via stools and the infection mainly involves the intestinal tract, it is believed that the infection transmits via the faecal-oral route. The possible modes of transmission include human-to-human (Hutin et al., 1998; Gumbo et al., 1999; Leelayoova et al., 2005), animal-to-human (Dengjel et al., 2001; Sulaiman et al., 2003) foodborne and waterborne transmission (Hutin et al., 1998; Dowd et al., 1998; Fournier et al., 2000) and all have been suggested in various studies. The role of human-to-human transmission was highlighted in a case-controlled study which showed that homosexuality was one of the risk factors for intestinal microsporidiosis (Hutin et al., 1998). The study of *E. bieneusi* infection in HIV-positive patients in Zimbabwe also showed that those who had a history of contact with diarrheal patient had a 1.9 times greater risk of getting the infection (Gumbo et al., 1999). In addition, our study revealed that 9 out of 13 infected orphans, who were HIV negative, were confined to 2 houses, while HIV-positive children inhabiting another house were not infected (Mungthin et al., 2001). Further studies at the same institute showed that orphans who were 12-23 months old, were girls and lived in one particular house were independently associated with *E. bieneusi* infection (Mungthin et al., 2005). Genotypic characterization of *E. bieneusi* identified that all of them were genotype A, which might indicate a common source of infection. The study suggests that *E. bieneusi* infection in this orphanage might be transmitted person-to-person.

Due to its resistance to environmental effects, *E. bieneusi* spores could be transmitted via water. A few recent reports suggest the evidences of waterborne transmission of *E. bieneusi* infection (Dowd et al., 1998; Fournier et al., 2000). Using molecular techniques, *E. bieneusi* has been detected in different types of water. Moreover, epidemiological studies suggested that HIV-positive patients who went swimming in pools had a higher risk of getting infected (Hutin et al., 1998). Evidence of foodborne transmission was seen from an epidemiological study that showed eating undercooked beef at least once a month was associated with the infection (Hutin et al., 1998).

The possibility of zoonotic transmission

The zoonotic potential of *E. bieneusi* is supported by evidence that *E. bieneusi* was identified in the faeces of a wide range of animals. In addition, *E. bieneusi* isolated from humans could be successfully transmitted to macaques and pigs (Tzipori et al., 1997; Kondova et al., 1998). Molecular methods have been described which differentiate the genotypes of *E. bieneusi*. Based on nucleotide polymorphisms in the 243-bp internal transcribed spacer (ITS) of the rRNA gene, *E. bieneusi* genotypes have been classified into distinct genotypes (A, B, C, D, etc.) (Katzwinkel-Wladarsch et al., 1996; Rinder et al., 2000; Sulaiman et al., 2003). To date, more than 20 genotypes of *E. bieneusi* have been reported to the Genbank. Of these, genotypes A, B and C, which are mainly reported in HIV-infected patients, have been only found in humans and not in animals. Recent studies show results supporting the zoonotic potential of *E. bieneusi*. Genotypes identified in animals have subsequently been reported in humans (Deplazes et al., 1996; Breitenmoser et al., 1999; Rinder et al. 2000; Dengjel et al., 2001; Buckholt et al., 2002; Sulaiman et al., 2003).

We have studied *E. bieneusi* from faecal samples of 33 HIV-infected Thai patients

(Leelayoova et al., submitted). An analysis of the nucleotide variations of the ITS sequences revealed eleven genotypes. Genotype D and E were identified as being most frequent. These genotypes have been previously reported in animals (Rinder et al. 2000; Buckholt et. al., 2002; Sulaiman et al., 2003). Genotypes O and *Pig*EBITS 7 which were previously found only in pigs were also observed in our study (Dengjel et al., 2001; Buckholt et. al., 2002). Phylogenetic analysis revealed a close genotypic relationship without transmission barriers.

Conclusion

E. bieneusi is now considered as an important opportunistic infection in AIDS patients, including those in Thailand. An understanding of the epidemiology of *E. bieneusi* infection will provide data that will help in the prevention and the control strategies for this disease. However, so far, the data on the risk factors is rather limited. Our knowledge about the source of infection and the mode of transmission is still unclear. The zoonotic potential of *E. bieneusi* has been indicated by several studies. To investigate its role, transmission from animal to human should be determined particularly whether it occurs naturally. Traditional epidemiological studies to address zoonotic transmission of *E. bieneusi* are needed.

Acknowledgements

References

Bigliardi, E. and Sacchi, L. 2001. Cell biology and invasion of the microsporidia. Microbes Infect. 3:373-379.

Breitenmoser, A.C., Mathis, A., Burgi, E., Weber, R., Deplazes, P. 1999. High prevalence of *Enterocytozoon bieneusi* in swine with four genotypes that differ from those identified in humans. Parasitology 118: 447-53

Bretagne, S., Foulet, F., Alkassoum, W., Fleury-Feith, J. and Develoux, M. 1993. Prevalence of *Enterocytozoon bieneusi* spores in the stool of AIDS patients and African children not infected by HIV. Bull. Soc. Pathol. Exot. 86: 351-357

Buckholt, M.A., Lee, J.H. and Tzipori, S. 2002. Prevalence of *Enterocytozoon bieneusi* in swine: an 18-month survey at a slaughterhouse in Massachusetts. Appl Environ Microbiol. 68: 2595-9.

Canning, E.U. and Hollister, W.S. 1990. *Enterocytozoon bieneusi* (Microspora): prevalence and pathogenicity in AIDS patients. Trans. R. Soc. Trop. Med. Hyg. 84:181-186.

Cegielski, J.P., Ortega, Y.R., McKee, S., Madden, J.F., Gaido, L., Schwartz, D.A., Manji, K., Jorgensen, A.F., Miller, S.E., Pulipaka, U.P., Msengi, A.E., Mwakyusa, D.H., Sterling, C.R. and Reller, L.B. 1999. *Cryptosporidium*, *Enterocytozoon*, and *Cyclospora* infections in pediatric and adult patients with diarrhea in Tanzania. Clin. Infect. Dis. 28: 314-21.

da Silva, A.J., Schwartz, D.A., Visvesvara, G.S., de Moura, H., Slemenda, S.B. and Pieniazek, N.J. 1996. Sensitive PCR diagnosis of infections by *Enterocytozoon bieneusi* (microsporidia) using primers based on the region coding for small-subunit ribosomal rRNA. J. Clin. Microbiol. 34: 986-987.

Dengjel, B., Zahler, M., Hermanns, W., Heinritzi, K., Spillmann, T., Thomschke, A., Loscher, T., Gothe, R. and Rinder, H. 2001. Zoonotic potential of *Enterocytozoon bieneusi*. J. Clin. Microbiol. 39: 4495-9.

Deplazes, P., Mathis, A., Muller, C. and Weber, R. 1996. Molecular epidemiology of *Encephalitozoon cuniculi* and first detection of *Enterocytozoon bieneusi* in faecal samples of pigs. J. Eukaryot. Microbiol. 43: 93S.

Desportes, I., Le Charpentier, Y., Galian, A., Bernard, F., Cochand-Priollet, B., Lavergne, A., Ravisse, P. and Modigliani, R. 1985. Occurrence of a new microsporidian: *Enterocytozoon bieneusi* n.g., n. sp., in the enterocytes of a human patient with AIDS. J. Protozool. 32: 250-254.

Didier, E.S., Stovall, M.E, Green, L.C., Brindley, P.J., Sestak, K. and Didier, P.J. 2004. Epidemiology of microsporidiosis: sources and modes of transmission. Vet. Parasitol. 126: 145-166.

Didier, E.S. 2005. Microsporidiosis: an emerging and opportunistic infection in humans and animals. Acta Trop. 94: 61-76.

Dobbins, W.O. and Weinstein, W.M. 1985. Electron microscopy of the intestine and rectum in acquired immunodeficiency syndrome. Gastroenterol. 88: 738-749.

Dowd, S.E., Gerba, C.P. and Pepper, I.L. 1998. Confirmation of the human-pathogenic microsporidia *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Vittaforma corneae* in water. Appl. Environ. Microbiol. 64: 3335.

Fournier, S., Liguory, O., Santillana-Hayat, M., Guillot, E., Sarfati, C, Dumoutier, N., Molina, J. and Derouin, F. 2000. Detection of microsporidia in surface water: a one-year follow-up study. FEMS Immunol. Med. Microbiol. 29: 95-100.

Franzen, C., and MÜller, A. 1999. Molecular techniques for detection, species differentiation, and phylogenetic analysis of microsporidia. Clin. Microbiol. Rev. 12 : 243-285.

Franzen, C. 2004. Microsporidia: how can they invade other cells? Trends Parasitol. 20: 275-279.

Garcia, L.S. 2002. Laboratory identification of the microsporidia. J. Clin. Microbiol. 40: 1892-1901.

Gumbo, T., Sarbah, S., Gangaidzo, I.T., Ortega, Y., Sterling, C.R., Carville, A., Tzipori, S. and Wiest, P.M. 1999. Intestinal parasites in patients with diarrhea and human immunodeficiency virus infection in Zimbabwe. AIDS 13: 819-821.

Haro, M., Izquierdo, F., Henriques-Gil, N., Andr?s, I., Alonso, F., Fenoy, S. and del Aguila, C. 2005. First detection and genotyping of human-associated microsporidia in pigeons from urban parks. Appl. Environ. Microbiol. 71: 3153-3157.

Hutin, Y.J.F., Sombardier, M-N., Liguory, O., Sarfati, C., Derouin, F., Modai, J. and Molina, J.M.1998. Risk factors for intestinal microsporidiosis in patients with human immunodeficiency virus infection: a case-control study. J. Infect. Dis. 178: 904-907.

Juarez, S.I., Ichinose, A., Jongwutiwes, S., Yanagi, T. and Kanbara, H. 2003. A species of microsporidium isolated from an HIV-infected patient and identified as *Trachipleistophora anthropophthera* by transmission electron microscopy. J. Eukaryot. Microbiol. 50 (suppl) : 576.

Katzwinkel-Wladarsch, S., Lieb, M., Helse, W., LÖscher, T. and Rinder, H. 1996. Direct amplification and species determination of microsporidian DNA from stool specimen. Trop. Med. Int. Health 1: 373-378.

Keeling, P.J. nd Fast, N.M. 2002. Microsporidia: Biology and evolution of highly reduced intracellular parasites. Annu. Rev. Microbiol. 56: 93-116.

Kondova, I., Mansfield, K., Buckholt, M.A., Stein, S., Widmer, G., Carville, A., Lackner, A. and

Tzipori, S. 1998. Transmission and serial propagation of *Enterocytozoon bieneusi* from humans and rhesus macaques in gnotobiotic piglets. Infect. Immune. 66: 5515-5519.

Kotler, D.P. and Orenstein, J.M. 1994. Prevalence of enteric pathogens in HIV-infected individuals referred for gastrointestinal evaluation. Am. J. Gastroenterol. 89: 1998-2002.

Kotler, D.P. and Orenstein, J.M.. 1998. Clinical syndromes associated with microsporidiosis. Adv. Parasitol. 40: 321-349.

Leelayoova, S., Vithayasai, N., Watanaveeradej, V., Chotpitayasunondh, T., Therapong, V., Naaglor, T. and Mungthin, M. 2001. Intestinal microsporidiosis in HIV-infected children with acute and chronic diarrhea. Southeast Asian J. Trop. Med. Public Health 32: 33-37.

Leelayoova, S., Subrungruang, I., Rangsin, S., Chavalitshewinkoon-Petmitr, P., Worapong, J., Naaglor, T. and Mungthin, M. 2005. Transmission of *Enterocytozoon bieneusi* genotype A in a Thai Orphanage. Am. J. Trop. Med. Hyg. 73: 104-107.

Leelayoova, S., Subrungruang, I., Suputtamongkol, Y., Worapong, J., Chavalitshewinkoon Petmitr, P. and Mungthin M. Genotypes of *Enterocytozoon bieneusi* isolated from HIV-infected patients in Thailand. (Submitted)

Lores, B., del Aguila, C. and Arias C. 2002. *Enterocytozoon bieneusi* (microsporidia) in faecal samples from domestic animals from Galicia, Spain. Mem. Inst. Oswaldo Cruz 97: 941-945.

Mansfield, K.G., Carville, A., Hebert, D., Chalifoux, L., Shvetz, D., Lin, K.C., Tzipori, S. and Lackner, A.A. 1998. Localization of persistent *Enterocytozoon bieneusi* infection in normal rhesus macaques (Macaca mulatta) to the hepatobiliary tree. J. Clin. Microbiol. 36: 2336-2338.

Mathis, A., Breitenmoser, A.C. and Deplazes, P. 1999. Detection of new Enterocytozoon bieneusi in faecal samples of farm dogs and a cat. Parasite 6:189-193.

Mathis, A., Weber, R. and Deplazes P. 2005. Zoonotic potential of the microsporidia. Clin. Microbiol.Rev. 18: 423-445.

Molina, J.M., Sarfati, C., Beauvais, B., Lemann, M., Lesourd, A., Ferchal, F., Casin, I., Lagrange, P., Modigliani, R., Derouni, F., and Modai, J. 1993. Intestinal microsporidiosis in human immunodeficiency virus-infected patients with chronic unexplained diarrhea: prevalence and clinical and biologic features. J. Infect. Dis. 167: 217-221.

Moura, H., da silva, J.L., Sodre, F.C., Brasil, P., Walmo, D., Wahlquist, S., Wallace, S., Croppo, G.P. and Visvesvara, G.S. 1996. Gram-chromotrope: a new technique that enhances detection of microsporidia spores in clinical specimens. J. Eukaryot. Microbiol. 43: 94S-95S.

Mungthin, M., Suwannasaeng, R., Naaglor, T., Areekul, W. and Leelayoova, S. 2001. Asymptomatic intestinal microsporidiosis in Thai orphans and child-care workers. Trans. R. Soc. Trop. Med. Hyg. 95: 304-306.

Mungthin, M., Subrungruang, I., Naaglor, T., Aimpun, P., Areekul, W. and Leelayoova, S. 2005. Spore excretion pattern of *Enterocytozoon bieneusi* in asymptomatic children. J. Med. Microbiol. 54: 473-476.

Rabenek, L., Gyorkey, F., Genta, R.M., Gyorkey, P., Foote, L.W. and Risser, J.M. 1993. The role of microsporidia in the pathogenesis of HIV-related chronic diarrhea. Ann. Intern. Med. 119: 895-899.

Reetz, J., Rinder, H., Thomschke, A., Manke, H., Schwebs M. and Bruderek, A. 2002. First detection of the microsporidium *Enterocytozoon*

bieneusi in non-mammalian hosts (chickens). Int. J. Parasitol. 32: 785-787.

Rinder, H., Janitschke, K., Asp?ck, H., da Silva, A.J., Deplazes, P., Fedorko, D.P., Franzen, C., Futh, U., HÜnger, F., Lehmacher, A., Meyer, C.G., Molina, J.M., Sandfort, J., Weber, R., LÖscher, T.1998. Blinded, externally controlled multicenter evaluation of light microscopy and PCR for detection of microsporidia in stool specimens. and the diagnostic multicenter study group on microsporidia. J. Clin. Microbiol. 36: 1814-1818.

Rinder, H., Thomschke, A., Dengjel, B., Gothe, R., Loscher, T. and Zahler, M. 2000. Close genotypic relationship between *Enterocytozoon bieneusi* from humans and pigs and first detection in cattle. J. Parasitol. 86:185-188.

Sandfort, J., Hannemann, A., Gelderblom, H., Stark, K., Owen, R.L. and Ruf, B. 1994. *Enterocytozoon bieneusi* infection in an immunocompetent patient who had acute diarrhea and who was not infected with the human immunodeficiency virus. Clin. Infect. Dis. 19: 514-516.

Santin, M., Trout, J.M. and Fayer, R. 2004. Prevalence of *Enterocytozoon bieneusi* in post-weaned dairy calves in the eastern United States. Parasitol. Res. 93: 287-289.

Schuitema, A.R.J., Hartskeerl, R.A., van Gool, T., Laxminarayan, R. and Terpstra, W.J. 1993. Application of the polymerase chain reaction for the diagnosis of microsporidiosis. AIDS 7(suppl. 3): S62-S63.

Subrungruang, I., Mungthin, M., Chavalitshewinkoon-Petmitr, P., Rangsin, R., Naaglor, T. and Leelayoova, S. 2004. Evaluation of DNA extraction and PCR methods for detection of *Enterocytozoon bieneusi* in stool specimens. J. Clin. Microbiol. 42: 3490-3294.

Sulaiman, I.M., Fayer, R., Lal, A.A., Trout, J.M., Schaefer, F.W. 3rd and Xiao, L. 2003. Molecular characterization of microsporidia indicates that wild mammals harbor host-adapted *Enterocytozoon* spp. as well as human-pathogenic *Enterocytozoon bieneusi*. Appl. Environ. Microbiol. 69:4495-501.

Sulaiman, I.M., Fayer, R., Yang, C., Santin, M., Matos, O. and Xiao, L. 2004. Molecular characterization of *Enterocytozoon bieneusi* in cattle indicates that only some isolates have zoonotic potential. Parasitol. Res. 92: 328-334.

Tzipori, S., Carville, A., Widmer, G., Kotler, D., Mansfield, K. and Lackner, A. 1997. Transmission and establishment of a persistent infection of *Enterocytozoon bieneusi*, derived from a human with AIDS in simian immunodeficiency virus-infected rhesus monkeys. J. Infect. Dis. 175: 1016-1020.

Velasquez, J.N., Carnevale, S., Guarnera, E.A., Labbe, J.H., Chertcoff, A., Cabrera, M.G. and Rodriguez, M.I. 1996. Detection of the microsporidian parasite *Enterocytozoon bieneusi* in specimens from patients with AIDS by PCR. J. Clin. Microbiol. 34: 3230-3232.

Wanachiwanawin, D., Manatsathit, S., Thakerngpol, K. and Lertlaituan, P. 1997. Electron microscopic diagnosis of intestinal microsporidiosis in Thailand. Southeast Asian J. Trop. Med. Public Health 28: 347-350.

Wanachiwanawin, D., Manatsathit, S., Lertlaituan, P, Thakerngpol, K. and Suwanagool, P. 1998. Intestinal microsporidiosis in HIV infected patients with chronic diarrhea in Thailand. Southeast Asian J. Trop. Med. Public Health 29: 767-771.

Wanachiwanawin, D., Chokephaibulkit, K., Lertlaituan, P., Ongrotchanakun, J., Chinabut, P. and Thakerngpol, K. 2002. Intestinal microsporidiosis in HIV-infected children with

diarrhea. Southeast Asian J. Trop. Med. Public Health 33: 241-245.

Waywa, D., Kongkriengdaj, S., Chaidatch, S.,Teingrim, S.,Kowadisaiburana, B., Chaikachonpat, S., Suwanagool, S., Chaiprasert, A., Curry, A., Bailey, W., Suputtamongkol, Y. and Beeching, N.J. 2001. Protozoan enteric infection in AIDS related diarrhea in Thailand. Southeast Asian J. Trop. Med. Public Health 32 (suppl2): 151-155.

Weber, R., Bryan, R.T., Owen, R.L., Wilcox, C.M., Gorelkin, L. and Visvesvara, G.S. 1992. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. N. Engl. J. Med. 326: 161-166.

Weber, R. and Bryan, R.T. 1994. Microsporidial infections in immunodeficient and immunocompetent patients. Clin. Infect. Dis. 19: 517-521.

Zhu, X., Wittner, M., Tanowitz, H.B., Kolter, D., Cali, A. and Weiss, L.M. 1993. Small subunit rRNA sequence of *Enterocytozoon bieneusi* and its potential diagnostic role with use of the polymerase chain reaction. J. Clin. Microbiol. 168: 1570-1575.