REVIEW ARTICLE

HUMAN MICROSPORIDIOSIS IN MALAYSIA: REVIEW OF LITERATURES

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ABSTRACT

The purpose of this study is to review the literature on microsporidiosis in various high-risk groups among the Malaysian population, i.e., HIV/AIDS, cancer, hospitalised patients and Orang Asli, and to update information with regards to microsporidia prevalence, diagnosis and association of the disease with gastrointestinal symptoms in Malaysia. Hospitalised patients showed the highest prevalence (28.3%) of microsporidiosis compared to other risk groups. This review did not find any direct correlations between gastrointestinal symptoms and microsporidiosis. Since microsporidiosis is an emerging threat to the high-risk groups, greater awareness should be instilled among clinicians to consider microsporidiosis in their differential diagnosis if no other causes can be defined.

***Keywords**: microsporidiosis, Malaysia, prevalence, Orang Asli, HIV/AIDS, hospitalised patients, cancer, diagnosis, gastrointestinal symptoms*

INTRODUCTION

Microsporidia from phylum Microspora, grouped under the taxonomy of protozoa, consist of more than 140 genera, and 1200 species, and are known to infect a broad range of vertebrates and invertebrates[18, 24]. Microsporidia were discovered in the last century when the destructive pebrine disease in *Bombyx mori* was caused by *Nosema bombycis*. However, Microsporidium was only identified to have caused human infection when it was discovered to have infected members of every animal phylum in the last decade[23].

Microsporidia are obligate intracellular parasites, spore-forming protist with no active metabolic stages. They are protected only by walls of protein (exospore), and chitin (endospore) outside of the host cell[18, 23]. The spores of microsporidia are similar to bacterial spores, which make them resistant and able to survive for long periods of time. The spores are able to retain their infectivity for 6 months in a dry condition and for as long as 10 years in a watery environment[7]. The size ranges of the microsporidia spores that infect mammals are between 1.0 to 3.0 µm and 1.5 to 4.0 µm[13]. The spore will adhere to the host cell's surface before spore activation[26, 47]. The mechanism of microsporidia invasion is by an extrusion apparatus: a polar filament with an anterior attachment complex, which transfers sporoplasms into the host cell. The first human case was reported in 1959, and only ten well-documented human infections with microsporidia were described in immunocompetent and immunocompromised patients without AIDS[17, 22]. However, interest towards this parasite started in 1985, after *Enterocytozoon bieneusi* was discovered to be the reason for chronic diarrhoea and systemic disease in an AIDS patient[10]. Since then, numerous microsporidia-associated infections were reported, and these parasites are now one of the most common pathogens in HIV-infected patients[43]. Currently, there are 17 species in eight genera of microsporidia, known as human-pathogenic microsporidian species[8], such as *Enterocytozoon, Encephalitozoon, Pleistophora, Trachipleistophora, Vittaforma, Brachiola, Nosema* and *Anncaliia*.

Microsporidia have been identified in animals and water sources, and thus, increasing public health concerns regarding their zoonotic and waterborne transmissions [8, 12, 27]. *Enterocytozoon* and *Encephalitozoon* are the most frequently involved genera in cases of human infections[9]. Microsporidia infections have been diagnosed in a broad range among the human population, including in transplant recipients, travellers, children, contact lens wearers, the elderly and immunocompetent persons with no risk factor[38]. Clinical manifestations of microsporidiosis in humans include intestinal, pulmonary, ocular, muscular, and renal diseases[25]. The manifestation depends on the causative agent and the host's immune status[40]. Diarrhoea predominantly is observed as a common symptom, caused by *E. bieneusi* or *E. intestinalis* infection[1, 15]. These organisms may disseminate to other parts of the body, and lead to systemic infections, such as keratoconjunctivitis, myositis, peritonitis, hepatitis, and nephritis[6, 14, 42].

Approximately 1.5 to 50% prevalence rates of microsporidia were reported depending on the

geographic area, the diagnostic method, and the population studied[11, 27]. Nonetheless, in Malaysia, only a few studies related to the prevalence of microsporidia have been reported. Therefore, this article aims to review the available data on the prevalence of microsporidiosis among several high-risk groups among the Malaysian populations, the techniques used to detect the parasites, and the association of gastrointestinal symptoms with the disease. This review is also expected to fill in the knowledge gap and increase awareness among clinicians on the emergence of microsporidia as a silent threat in high-risk groups.

THE PREVALENCE OF HUMAN MICROSPORIDIOSIS IN MOST AT RISK POPULATIONS

HIV/AIDS Patients

Microsporidia emerged as opportunistic pathogens when AIDS became pandemic. The prevalence rate for microsporidiosis was observed to be the highest among HIV-positive patients with diarrhoea and CD4 counts of less than 100 cells/mm^3 blood, worldwide[13]. Lono et al. carried out a preliminary study in 2011 to detect microsporidia in local HIV-positive individuals[28]. Out of 247 stool specimens examined using microscopy and PCR (164 Malays, 33 Indians, 40 Chinese, and 10 unspecified ethnic), 8.5% were positive with microsporidiosis.

Cancer patients

Cancer patients receiving chemotherapy treatments are considered to be immunosuppressed and a high-risk group. In a study to assess microsporidia occurrence among cancer patients in Malaysia, from the 311 and 173 fresh stool specimens collected from oncology clinics at three hospitals, and from among the healthy population, respectively, 21.9% (68/311) were found to be positive for microsporidia, while 2.8% (5/173) were positive for microsporidiosis[29]. Approximately 95.59% of the samples were solely infected by microsporidia, while mixed infections with other gastrointestinal parasites, such as *Blastocystis hominis* were observed in 4.41% (3/68) of the positive specimens. Out of the 68 positive specimens, only seven patients (10.29%) complained of having gastrointestinal discomfort. Chandramathi et al. (2008) reported that cancer patients were found to be positive for microsporidia after chemotherapy treatments. The number of patients infected with microsporidia differed according to treatment cycles[3].

Hospitalised patients

Norhayati et al. (2008) analysed microsporidia detection among hospitalised patients in determining the prevalence, and the spore counts in patients with and without gastrointestinal symptoms. Out of the 893 faecal specimens examined using the Gram-chromotrope kinyoun (GCK) stain, 116 (13%) patients were found to be positive for microsporidia. This study was similar to another study in Thailand, whereby the prevalence of microsporidia among hospitalised HIV-infected individuals was 11%[45]. However, the prevalence varies in other countries[34], depending on the area, the population studied, and the technique used for diagnosis. Norhayati et al. (2008) also found that a third of the cases were among immunocompetent patients[34]. Similarly, Rukman et al. (2008) reported that from 353 samples, 100 of them were tested positive for microsporidiosis (28.3%). This study had shown the highest prevalence of microsporidia in Malaysia with 85% of the infected patients have diarrhoea[37].

Orang Asli

Similar studies were also conducted among the indigenous community, known as the Orang Asli, who resides in the interior regions of Peninsular Malaysia. Norhayati et al. (2007) revealed that 20.7% of 271 Orang Asli children suffered from intestinal microsporidia infections. Only 3 from 56 children who were tested positive had moderate infection, while none showed any symptoms. Lono et al. (2010) reported that 21.2% of 151 faecal specimens were positive for microsporidia via light microscopy, among Orang Asli aged 9 to 60 years old. Six of the 32 individuals with non-diarrheic symptom were solely infected by microsporidia. The prevalence of microsporidia was also significantly higher than normal among the healthy population (2.9%) of this community. Hence, it was concluded that at least one member in each 30 Orang Asli families (60%) was diagnosed with microsprodiosis. Anuar et al. (2013) examined 447 stool samples among the Orang Asli and 67 (15%) of them were found positive for microsporidia. From the 67 individuals, 65 had low spore counts (1-10 per 100 fields/100x), while 2 individuals had moderate spore counts (11-20 per fields/100x).

Association between different high-risk groups and prevalence of microsporidiosis

Based on Table 1, the highest prevalence of microsporidiosis was among hospitalised patients at 28.3%, followed by cancer patients (21.9%). Rukman et al. (2008) highlighted that the difference in prevalence between hospitalised patients and healthy people was quite large, with 28.3% among hospitalised patients, and 2.9% among healthy people[29]. Meanwhile, the prevalence was higher among HIV-infected patients with 8.5%[28, 29]. Children have also been

identified as being at risk[30]. Norhayati et al. (2008) found that 26.1% of microsporidia infections were among hospitalised children aged 0-6 years, while 20.7% were among Orang Asli children, aged 2-12 years[33]. Meanwhile, Anuar et al. (2013) reported that 20 from 194 children, aged <15 years old, were found to be infected.

Table 1 - List of Microsporidia reports in Malaysia

References	Prevalences	Subjects	Percent positive by ethnic groups					Gastrointestinal symptoms for positive samples	Test used	Species identified
			Malays	Chinese	Indians	Orang Asli	Other			
Tengku et al. 2013	15%	Orang Asli (447 samples)	-	-	-	15	-	Not stated	Modified GCK	Not stated
Lono et al. 2011	8.5%	HIV-positive patients (247 samples)	10.4	3	5	-	-	Yes- 100% No- 0%	Modified trichrome stain PCR	*E. intestinalis*
Lono et al. 2010	21.2%	Orang Asli villagers (151 samples)	-	-	-	21.2	-	Yes- 81.25% No-18.75%	Modified trichrome stain PCR	*E. intestinalis*
Lono et al. 2008	21.9%	Cancer patients (311 samples)	28	60	11.7	-	-	Yes- 10.29% No-89.71%	Modified trichrome stain PCR	*E. intestinalis* *E. hellem*
Norhayati et al. 2008	13%	Hospitalised patients (893 samples)	55	34	4	-	-	Yes- 74% No-26%	Modified GCK	Not identified
Rukman et al. 2008	28.3%	Hospitalised patients (353 samples)	57	30	7	-	6	Yes- 85% No- 15%	Modified chromotrope stain	Not stated
Norhayati et al. 2007	20.7%	Orang Asli children (271 samples)	-	-	-	20.7	-	Yes- 0% No- 100%	Modified GCK	Not stated

Microsporidiosis and gastrointestinal symptom

Gastrointestinal microsporidiosis is commonly observed in HIV-infected patients, while immunocompetent patients generally showed symptoms of self-limiting diarrhoea[1, 12]. All faecal specimens from HIV-infected subjects were soft and watery, suggesting diarrhoea[28]. Moreover, 74% of the infected hospitalised patients had gastrointestinal symptoms due to intestinal or systemic infections or induced by immunosuppressive treatment[34]. Another study reported that 85% of the microsporidiosis infected hospital patients have diarrhoea[37]. However, not all of the microsporidiosis cases in Malaysia manifested into gastrointestinal infections. For instance, Norhayati et al. (2008) and Rukman et al. (2008) found that 26% and 15%, respectively, of microsporidia-positive hospitalised patients presented no gastrointestinal symptoms[34, 37]. 89.71% of the infected cancer patients did not complain of gastrointestinal discomfort. This might suggest that most of the infections in cancer patients were asymptomatic[29]. Meanwhile, 18.75% of the Orang Asli did not have diarrhoea[27].

DIAGNOSTIC TECHNIQUES

Transmission electron microscopy (TEM) is the gold standard for microsporidium detection, and was the first technique to allow species identification of microsporidia[17, 42]. However, TEM is relatively

insensitive due to the small amount of sample that can be examined, which could lead to significant sampling error[20]. TEM is also labour intensive, time-consuming, and requires expensive equipment and expertise to detect the spores[43].

Light microscopy is commonly used for the direct visualisation of microsporidia spores using numerous staining techniques, such as Gram stain, Giemsa stain, Gram-chromotrope, and modified trichrome stain [31, 39, 46]. Chemifluorescents reagents, such as Uvitex 2B, and Calcofluor White M2R[44, 49] are used in the identification microsporidian spores. However, these types of staining are non-specific, as fungi or other organisms may also fluoresce, thus generating false-positive results[18, 50]. Therefore, many laboratories tend to use two or more of the above methods concurrently to increase the sensitivity and specificity, especially in patients with light microsporidia infections[17, 18, 50]. However, these techniques could not identify specific species and require experienced microscopists for successful interpretations.

Due to the limitations of microscopy, PCR assays have been established for diagnosis and species differentiation of most human microsporidia. Two primer pairs have been reported to amplify the target gene or the short region of small subunits of rRNA *E. bieneusi* and *Encephalitozoon* spp.[17]. Zhu et al. (1993) reported the first PCR-based diagnosis of *E. bieneusi* which used V1/EB450 primers to amplify cloned *E. bieneusi* SSU rRNA sequences, and DNA from *E. bieneusi*-infected tissues. However, PCR is not routinely used since it is time-consuming, labour intensive, and requires expensive equipment[43, 51], apart from the presence of inhibitors, which needs to be taken into consideration because they may disturb the amplification by PCR[52].

In Malaysia, screening for microsporidial spores is not routinely done in most hospitals, and only a few clinical diagnostic laboratories offer a modified trichrome or Gram-chromotrope staining method on a special request basis[34, 53]. However, there are a few studies on establishing and comparing the techniques used to diagnose microsporidia infection. Salleh et al. (2011) developed a modified Gram-chromotrope staining, known gram-chromotrope kinyoun (GCK), which shortens the staining process and produces blue-pinkish microspiridian spores against the clear background. GCK staining was significantly more sensitive and specific compared to the Weber modified trichrome (WMT) staining. Nur Raihana et al. (2013) compared the GCK staining with Calcofluor White M2R method, and showed that the former had better sensitivity and specificity.

Another study compared the sensitivity and specificity between the WMT staining technique and the immunofluorescence antibody assay with monoclonal antibodies (IFA-MAbs)[50]. The IFA-MAbs was found to be highly sensitive and specific for identifying microsporidia species. However, it was suggested that if the specificity of the IFA-MAbs was low, the presence of microsporidia spores should be confirmed using other methods. Nasarudin et al. (2015) established a loop-mediated isothermal amplification (LAMP) method, which was originally developed by Notomi et al. (2000) for the detection of *E. bieneusi*. LAMP had shown higher sensitivity and specificity in identifying *E. bieneusi* from human stool specimens compared to PCR assays. This molecular technique can be performed rapidly at a constant temperature using a water bath or a heating block, thus it can be used in poorly equipped laboratories.

DISCUSSION

Microsporidiosis cases are not well-documented and can be considered as one of the neglected diseases in Malaysia. Only a few reports highlighted the prevalence of this disease among hospitalised patients, HIV-infected patients, and indigenous communities[2, 27-29, 33, 34]. Based on these studies, most infections had involved immunosuppressed patients and a few immunocompetent individuals.

The prevalence data provided by these studies are limited to certain populations, thus it is difficult to identify the risk factors and causes involved. Nevertheless, it can be assumed that the highest prevalence of microsporidiosis is among hospitalised patients (28.3%) who underwent immunosuppressive treatments. These treatments can increase the chances of acquiring parasitic infection, usually with a high degree of severity[4]. Hospitalised patients are considered as immunosuppressed or relatively immunosuppressed individuals, thus more susceptible to microsporidia infections. Microsporidia were also observed to be more prevalent among patients with haematological malignancy or a combination of malignancy and diabetes mellitus[29]. Usually, these patients have haematological disorders, which makes microsporidiosis more prevalent compared to other types of immunosuppressive illness[4].

The second highest prevalence is among cancer patients, and followed by the Orang Asli. Possible reasons for the Orang Asli having significantly higher cases than other individuals might be malnourishment and poor hygiene. This community bears a high prevalence of protein-energy malnutrition, together with low immune system,

thus, making them more susceptible to microsporidial infection. There is also a relationship between nutrition, and other enteric parasite infections, especially in children[2, 11, 21, 33]. Although the prevalence of microsporidiosis among HIV population is significantly high compared to among healthy individuals, the prevalence of this group is the lowest among other populations studied in Malaysia. This could be because HIV patients receive the highly active antiretroviral therapy (HAART), which helps to halt HIV replication, and restores immune status, thus drastically reduces the occurrence of any opportunistic infection, including those due to microsporidia[36, 54]. The number of microsporidia infections has also significantly decreased in other developed countries since the administration of HAART[1].

The association between microsporidiosis with gastrointestinal symptoms was also examined. In the Orang Asli population, 18.75% of positive samples were taken from non-diarrheic individuals. There were also cases of microsporidiosis that were not associated with gastrointestinal symptoms among hospitalised and cancer patients. These findings showed that the pathogenicity of the microsporidia in causing diarrhoea is still not clear[30]. Several studies even suggested that some persons may be asymptomatic carriers of these microorganisms or that infections may reactivate under conditions of immunesuppression[1, 12, 54]. Moreover, gastrointestinal symptoms may only be caused by some genotypes of microsporidian species[30].

In the case of microsporidiosis among HIV patients, diarrhoea was present in all patients[28]. The mortality of HIV-infected patients and chronic diarrhoea with wasting was reported to be more than 50%[55] with at least 90% of these gastrointestinal infections were caused by *E. bieneusi* and *E. intestinalis*[17]. Nevertheless, some studies found that even though *E. bieneusi*-HIV patients exhibited more diarrhoea symptom, there was less inflammation compared to non-infected HIV patients[30, 57]. Sharpstone et al. (1997) proposed that elevated TNFα levels in the intestine of *E. bieneusi*-infected AIDS patients had contributed to diarrhoeas. However, Lono et al. (2011) discovered that 48% of positive microsporidiosis were co-infected with *B. hominis* and *G. lamblia*. This finding corresponds to the concerns of whether gastrointestinal symptoms may be caused by the direct effect of HIV itself, declining immune status, or the presence of other intestinal pathogens[14].

Similarly, in the Orang Asli community, 81.25% of positive individuals were diarrheic and the symptoms may be the result of other enteric pathogens[27]. Immunosuppressive treatments, such as chemotherapy may also contribute to gastrointestinal symptoms among hospitalized and cancer patients. Co-infection with other parasites could also explain this manifestation[29, 34]. Gastrointestinal symptoms might also be due to alterations to the structure of gut epithelial in cancer patients. Other studies have also demonstrated no significant association between the presence of microsporidia in faecal specimens and patients with diarrhoea[58-60].

There is no current report on disseminated microsporidia in Malaysia, although it has been reported elsewhere. It is believed that the parasites can disseminate, not only in HIV patients but also in HIV-negative individuals, especially in transplant recipients[19]. The lack of reports on disseminated microsporidiosis in Malaysia shows that the diagnosis of this disease might be restricted to localised gastrointestinal infection, by focusing the diagnosis on stool specimen. Thus, cases of disseminated microsporidiosis might have been misdiagnosed or not reported.

The source of most microsporidia infections is still uncertain. However, the genotypes that infect humans have been identified in domestic and wild animals, thus support the finding that microsporidiosis is a zoonotic disease[14]. Previous studies have demonstrated that close contact or living with infected animals could increase the risk[61, 62]. In Malaysia, most Orang Asli keep dogs, cats, and rear chickens[2]. Microsporidia spores may excrete together with the faeces of these animals, and as the spores are resistant to water, possible indirect zoonotic transmission via exposure to contaminated water or food was suggested[12].

The spores of human-pathogenic microsporidian species are relatively small, thus making the filtering process to trap the spores less efficient[11]. In fact, microsporidia have been listed by the Centers for Disease Control and Prevention (CDC) as Category B priority pathogens of concern for waterborne transmission. Consumption of insufficiently cooked meat or fish, and improperly washed raw vegetables could also cause the infection[63, 64]. Tapioca, a staple in the diet of the Orang Asli community, may be one of the sources of infection. Microsporidia were also detected in a variety of fruits and milk[62, 64, 66]. Lack of hygienic practices, such as hand washing can also contribute to the susceptibility to infection[12].

Risk factors associated with microsporidiosis that support transmissions of human-to-human may

include homosexuality, faecal-oral, and direct contact with the infected person. Hospitalised patients may be infected through having direct contact or confined in the same ward with an infected person. Meanwhile, HIV-infected individuals could be infected from practicing homosexual behaviours. Other studies demonstrated a high prevalence of microsporidiosis among homosexual partners compared to other risk groups[5, 61]. It is believed that the parasite can disseminate in both immunocompromised and immunocompetent patients[19], but no cases have been reported in Malaysia. Therefore, increasing the awareness among clinicians on the possibility of the dissemination of microsporidia may aid the diagnosis as well as treatment to patients. Currently, there is no report or article on microsporidia treatment in Malaysia.

These reported findings made it clear that microsporidia infection may be more common than it was originally thought. These parasites may exist as a latent infection, and their numbers may be lower in a healthy individual. However, their numbers may increase when the individual is immunocompromised, but without causing serious illnesses[28]. These organisms are too tiny to be properly identified under a microscope, thus making it difficult to diagnose[40]. Microsporidia infections contribute to a wide range of clinical signs, hence they should be considered when an etiology cannot be determined. Thus, several or at least three consecutive faecal specimens should be evaluated before ruling out microsporidia infection due to intermittent spore shedding[38, 67].

CONCLUSION

In conclusion, the true prevalence of human microsporidiosis in Malaysia might be underestimated as there is limited information on its status. Nonetheless, many cases were reported among immunocompromised patients. The highest prevalence is among hospitalised patients, followed by the cancer patients, Orang Asli, and HIV patients. Some of the infected patients presented with gastrointestinal symptoms, while others did not. The gastrointestinal symptoms, however, may not be related to the infection as they might have been caused by other enteric pathogens, and may also be due to the immunosuppressive treatments they were receiving. A greater awareness amongst clinicians regarding the diversity of the clinical manifestations of microsporidiosis is needed to improve the quality of diagnostic methods and treatment. This review has demonstrated that microsporidia are present in our local settings despite the low detection. Further research is needed to elucidate the epidemiology of microsporidiosis, instigate preventive measures, and improve laboratory diagnosis, and patient treatment.

REFERENCES

1. Anane S, Attouchi H. Microsporidiosis: epidemiology, clinical data and therapy. *Gastroentérologie Clinique et Biologique* 2010; **34**: 450-464.

2. Anuar TS, Al-Mekhlafi HM, Salleh FM, Moktar N. New insights of microsporidial infection among asymptomatic aboriginal populatsion in Malaysia. *PlosOne* 2013; **8** (8): e71870.

3. Botero JH, Castano A, Montoya MN, Ocampo NE, Hurtado MI, Lopera MM. A preliminary study of the prevalence of intestinal parasites in immunocompromised patients with and without gastrointestinal manifestations. *Rev. Inst. Med. Trop. Sao Paulo* 2003; **45**: 197-200.

4. Bergquist R, Morfeldt-Mansson L, Pehrson PO, Petrini B, Wasserman J. Antibody against *Encephalitozoon cuniculi* in Swedish homosexual men. *Scandinavian Journal of Infectious Diseases* 1984; **16** (4): 389-391.

5. Cali A, Kotler DP, Orenstein JM. *Septata intestinalis* n. g., n. sp., an intestinal microsporidian associated with chronic diarrhoea and dissemination in AIDS patients. *J. Euk. Microbiol* 1993; **40** (1): 101- 112.

6. Cali A, Takvorian PM. The microsporidia: pathology in man and occurrence in nature. *Southeast Asian J. Trop. Med. Public Health* 2004; **35** (1): 58-64.

7. Cali A, Neafie RC, Takvorian PM. Microsporidiosis. In Meyers WM, Firpo A, Wear DJ (eds). Topics on the Pathology of Protozoan and Invasive Arthropod Diseases. Washighton, DC: Armed Forces Institute of Pathology; 2011.

8. Candramathi S, Suresh K, Anita ZA, Kuppusamy UR. Infections of blastocystis hominis and microsporidia in cancer patients: are they opprotunistic? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2012; **106**: 267-269.

9. Carlson JR, Li L, Helton CL, Munn RJ, Wasson K, Perez RV, Gallay BJ, Finkbeiner WE. Disseminated microsporidiosis in a pancreas/kidney transplant recipient. *Archives of Pathology & Laboratory Medicine* 2004; **128** (3): e41–43.

10. Desportes I, Charpentier IE, Galian A, Bernard F, Cochand-Priollet B, Lavergne A, Ravisse P, Modigliani R. Occurrence of a new mircosporidian: *Enterocytozoon bieneusi* n.g., n. sp., in the enterocytes of a human patient with AIDS. *J. Protozool* 1985; **32** (2): 250- 254.

11. Didier ES, Stovall ME, Green LC, Brindley PJ, Sestak K, Didier PJ. Epidemiology of microsporidiosis: sources and modes of translation. *Vet. Parasitol* 2004; **126** (1-2): 145-166.

12. Didier ES, Weiss LM. Microsporidiosis: current status. *Curr. Opin. Infect. Dis*. 2006; **19** (5): 485-492.

13. Didier ES, Weiss LM. Overview of microsporidia and microsporidiosis. *Prostitology* 2008; **5** (4): 243–255.

14. Didier ES, Weiss LM. Microsporidiosis: not just in AIDS patients. *Curr. Opin. Infect. Dis*. 2011; **24** (5): 490-495.

15. Didier ES. Microsporidiosis. *State-of-The-Art Clinical Article*1998; **27**: 1-8.

16. Fedorko DP, Hijazi YM. Application of molecular techniques to the diagnosis of microsporidial infection. *Emerging Infectious Diseases* 1996; **2** (3): 183–191.

17. Franzen C, Müller A. Microsporidiosis: human diseases and diagnosis. *Microbes and Infection* 2001; **3** (5): 389–400.

18. Garcia LS. Laboratory identification of the microsporidia. *Journal of Clinical Microbiology* 2002; **40** (6): 1892–1901.

19. George B, Coates T, McDonald S, Russ G, Cherian S, Nolan J, Brealey J. Disseminated microsporidiosis with *Encephalitozoon* species in a renal transplant recipient. *Nephrology* 2012; **12**: 5-8.

20. Ghosh K, Schwartz D, Weiss LM. Laboratory diagnosis of microsporidia. In Weiss LM, Bernel JJ. (eds). Microsporidia: Pathogens of Opportunity. Chichester: John Wiley & Sons, Inc. 2014.

21. Hadju V, Abadi K, Stephenson LS, Noor NN, Mohammed HO, Bowman DD. Intestinal helminthiasis, nutritional status, and their relationship; a cross-sectional study in urban slum school children in Indonesia. *Southeast Asian J. Trop. Med. Public Health* 1995; **26** (4): 719-729.

22. Joseph J, Vemuganti GK, Sharma S. Microsporidia: emerging ocular pathogens. *Indian Journal of Medical Microbiology* 2005; **23** (2): 80-91.

23. Keeling PJ, McFadden GI. Origins of microsporidia. *Trends in Microbiology* 1998; **6** (1): 19–23.

24. Keeling PJ, Slamovits CH. Simplicity and complexity of microsporidian genomes. *Eukaryotic Cell* 2004; **3** (6): 1363-1369.

25. Leder K, Ryan N, Spelman D, Crowe SM. Microsporidial disease in HIV-infected patients: a report of 42 patients and review of the literature. *Scandinavian Journal of Infectious Diseases* 1998; **30** (4): 331–338.

26. Li Y, Wu Z, Pan G, He W, Zhang R, Hu J, Zhou Z. Identification of a novel spore wall protein (SWP26) from microsporidia *Nosema bombycis*. *International Journal for Parasitology* 2009; **39** (4): 391–398.

27. Lono A, Kumar GS, Chye TT. Prevalence of microsporidia in an indigenous Orang Asli community in Pahang, Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2010; **104** (3): 214–218.

28. Lono A, Kumar S, Chye TT. Detection of microsporidia in local HIV-positive population in Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2011; **105** (7): 409–413.

29. Lono AR, Kumar S, Chye TT. Incidence of microsporidia in cancer patients. *J. Gastrointest. Canc*. 2008; **39**: 124–129.

30. Matos O, Lobo ML, Xiao L. Epidemiology of *Enterocytozoon bieneusi* infection in humans. *Journal of Parasitology Research* 2012; 1-19.

31. Moura H, Schwartz DA, Bornay-Llinares F, Sodré FC, Wallace S, Visvesvara GS. A new and improved 'quick-hot Gram-chromotrope' technique that differentially stains microsporidian spores in clinical samples, including paraffin-embedded tissue sections. *Archives of Pathology & Laboratory Medicine* 1997; **121** (8): 888-893.

32. Nasarudin SNS, Zainudin NS, Bernadus M, Mohd Nawi A, Hanafiah A, Osman E. Loop-mediated isothermal amplification for rapid molecular detection of *Enterocytozoon bieneusi* in faecal specimens. *J. Med. Microbiol* 2015; **64**: 1329- 1334.

33. Norhayati M, Al-Mekhlafi HM, Azlin M, Nor Aini U, Shaik A, Sa'iah A, Fatmah MS, Ismail MG, Ahmad Firdaus MS, Aisah MY, Rozlida AR. Intestinal microsporidial infections among Orang Asli (aborigine) children from Malaysia. *Annals of Tropical Medicine & Parasitiology* 2007; **101** (6): 547-550.

34. Norhayati M, Azlin M, Al-Mekhlafi MH, Anisha N, Nor Aini U, Fatmah MS, Rozlida AR. A preliminary study on the prevalence of intestinal microsporidiosis in patients with and without gastrointestinal symptoms in Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2008; **102** (12): 1274–1278.

35. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* 2000; **28** (12): e63.

36. Quihui-Cota L, Valencia ME, Crompton DWT, Phillips S, Hagan P, Diaz-Camacho SP, Tejas AT. Prevalence and intensity of intestinal parasitic infections 2004; **98** (11): 653-659.

37. Rukman AH, Malina O, Noorhayati MI, Marlyn M, Wan Omar A, Roslaini AM, Ngah Z, Norhayati M. Intestinal Microsporidiosis: A New Entity in Malaysia? *Journal of Medicine and Health Sciences* 2008; **4** (2): 11-24.

38. Sak B, Kucerova Z, Kvac M, Kvetonova D, Rost M, Secor EW. Seropositivity for *Enterocytozoon bieneusi*, Czech Republic. *Emerging Infectious Diseases* 2010; **16** (2): 335–337.

39. Salleh FM, Al-Mekhlafi AM, Nordin A, Yasin AM, Al-Mekhlafi HM, Moktar N. Evaluation of gram-chromotrope kinyoun staining technique: its effectiveness in detecting microsporidial spores in fecal specimens. *Diagnositc Microbiology and Infectious Disease* 2011; **69**: 82-85.

40. Suankratay C, Thiansukhon E. Disseminated infection caused by novel species of microsporidium, Thailand. *Emerging Infectious Diseases* 2012; **18** (2): 302-304.

41. Sharpstone D, Rowbottom A, Francis N, Tovey G, Ellis D, Barret M, Gazzard B. Thalidomide: a novel therapy for microsporidiosis. *Gastroenterology 1997;* **112**: 1823-1829.

42. Talabani H, Sarfati C, Pillebout E, van Gool T, Derouin F, Menotti J. Disseminated infection with a new genovar of *Encephalitozoon cuniculi* in a renal transplant recipient. *Journal of Clinical Microbiology* 2010; **48** (7): 2651–2653.

43. Thellier M, Breton J. *Enterocytozoon bieneusi* in human and animals, focus on laboratory identification and molecular epidemiology. *Parasite* 2008; **15**: 349-358.

44. van Gool T, Snijders F, Reiss P, Schattenkerk JKME, van den Bergh Weerman MA, Bartelsman JFWM, Bruins JJM, Canning EU, Dankert J. Diagnosis of intestinal and disseminated microsporidial infections in patients with HIV by a new rapid fluorescence technique. *J. Clin. Pathol*. 1993; **46**: 694-699.

45. Waywa D, Kongkriengdaj S, Chaidatch S, Tiengrim S, Kowadisaiburana B, Chaikachonpat S, Suwanagool S, Chairprasert A, Curry A, Bailey W, Suputtamongkol Y, Beeching NJ. Protozoan enteric infection in AIDS related diarrhoea in Thailand. *Southeast Asian J. Trop. Med. Public Health* 2001; **32** (2): 151- 155.

46. Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, Visvesvara GS. The Enteric Opportunistic Infections Working Group. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. *The New England Journal of Medicine* 1992; **326** (3): 161- 166.

47. Wu Z, Li Y, Pan G, Tan X, Hu J, Zhou Z, Xiang Z. Proteomic analysis of spore wall

proteins and identification of two spore wall proteins from *Nosema bombycis* (microsporidia). *Proteomics* 2008; **8** (12): 2447–2461.

48. Zhu X, Wittner M, Tanowitz HB, Kotler D, Cali A, Weiss LM. Small subunit rRNA sequence of *Enterocytozoon bieneusi* and its potential diagnostic role with use of the polymerase chain reaction. *The Journal of Infectious Diseases* 1993; **168** (6): 1570-1575.

49. Didier ES, Orenstein JM, Aldras A, Bertucci D, Rogers LB, Janney A. Comparison of three staining methods for detecting microsporidia in fluids. *Journal of Clinical Microbiology* 1995; **33** (12): 3138- 3145.

50. Al-Mekhlafi MA, Fatmah MS, Anisah N, Azlin M, Al-Mekhlafi H, Norhayati M. Species identification of intestinal microsporidia using immunofluorescence antibody assays. *Southeast Asian Journal of Tropical Medicine Public Health* 2011; **42** (1): 19-24.

51. Fakruddin M. Loop mediated isothermal amplification (LAMP) – an alternative to polymerase chain reaction (PCR). *Bangladesh Research Publications Journal* 2011; **5** (40): 425- 439.

52. Besetti J. An introduction to PCR inhibitors. Promega Corporation Profiles in DNA 2007; 9-10. http://www.promega.com/~/media/files/resources/profiles%20in%20dna/1001/an%20introduction%20to%20pcr%20inhibitors.pdf (accessed 24 November 2014)

53. Nur Raihana I, Malina O, Fatmah MS, Norhayati M, Eni Juraida AR, Hamat RA. Comparison of calcofluor white M2R fluorescence and modified gram chromotrope kinyoun staining methods for the detection of microsporidial spores from stool samples. *Malaysian Journal of Medicine and Health Sciences* 2013; **9** (2): 63-68.

54. Sriaroon C, Mayer CA, Chen L, Accurso C, Greene JN, Vincent AL. Diffuse intra-abdominal granulomatous seeding as a manifestation of immune reconstitution inflammatory syndrome associated with microsporidiosis in a patients with HIV. *AIDS Patient Care and STDs* 2008; **22** (8): 611-612.

55. Mungthin M, Suwannasaeng R, Naaglor T, Areekul W, Leelayoova S. Asymptomatic intestinal microsporidiosis in Thai orphans and child-care workers. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2001; **95**: 304-306.

56. Weiss LM. Clinical syndromes associated with microsporidiosis. In Weiss, LM., Bernel, JJ. (eds). Microsporidia: Pathogens of Opportunity. Chichester: John Wiley & Sons, Inc., 2014.

57. Bern C, Kawai V, Vargas D, Rabke-Verani J, Williamson J, Chavez-Valdez R, Xiao L, Sulaiman I, Vivar A, Ticona E, Navincopa M, Cama V, Moura H, Secor WE, Visvesvara G, Gilman RH. The epidemiology of intestinal microsporidiosis in patients with HIV/AIDS in Lima, Peru. *The Journal of Infectious Diseases* 2005; **191**: 1658-1664.

58. Sarfati C, Bourgeois A, Menotti J, Liegeois F, Moyou-Somo R, Delaporte E, Derouin F, Ngole EM, Molina JM. Prevalence of intestinal parasites including microsporidia in human immunodeficiency virus-infected adults in Cameroon: a cross-sectional study. *Am. J. Trop. Med. Hyg*. 2006; **74** (1): 162-164.

59. Lobo ML, Xiao L, Antunes F, Matos O. Microsporidia as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and –negative patients. *International Journal for Parasitology* 2012; **42**: 197-205.

60. Chacin-Bonilla L, Panunzio AP, Monslave-Castillo FM, Parra-Cepeda IE, Martinez R. Microsporidiosis in Venezuela: prevalence of intestinal microsporidiosis and its contributions to diarrhoea in a group of human immunodeficiency virus-infected patients from Zulia state. *Am. J. Trop. Med. Hyg*. 2006; **74** (3): 482-486.

61. Mathis A, Weber R, Deplazes P. Zoonotic potential of microsporidia. *Clinical Microbiology Reviews* 2005; **18** (3): 423-445.

62. Lee SY, Lee SS, Lyoo YS, Park HM. DNA detection and genotypic identification of potentially human-pathogenic microsporidia from asymptomatic pet parrots in South Korea as a risk factor for zoonotic emergence. *Applied and Environmental Microbiology* 2011; **7** (23): 8442-8444.

63. Decraene V, Lebbad M, Botero-Kleiven S, Gustavsson AM, Lofdahl M. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. *Epidemiol. Infecto* 2012; **140**: 519-527.

64. Chupp GL, Alroy J, Adelman LS, Breen JC, Skolnik PR. Myositis due to *Pleistophora* (microsporidia) in a patient with AIDS. *Clinical Infectious Diseases* 1993; **16** (1): 15-21.

65. Jedrzejewski S, Graczyk TK, Slodkowicz-Kowalska A, Tamang L, Majewska AC. Quantitative assessment of contamination of fresh food produce of various retail types by human-virulent microsporidian spores. *Applied and Environmental Microbiology* 2007; **73** (12): 4071-4073.

66. Lee JH. Molecular detection of *Enterocytozoon bieneusi* and identification of a potentially human-pathogenic genotype in milk. *Applied and Environmental Microbiology* 2008; **74** (5): 1664-1666.

67. Sokolova OI, Demyanov AV, Bowers LC, Didier ES, Yakovlev AV, Skarlato SO, Sokolova YY. Emerging microsporidian infections in Russian HIV-infected patients. *Journal of Clinical Microbiology* 2011; **49** (6), 2102-2108.