

TRANSMISSION OF MICROSPORIDIA TO HUMANS: WATER-BORNE, FOOD-BORNE, AIR-BORNE, ZONOTIC, OR ANTHROPONOTIC?

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Abstract. Increasing numbers of report suggest different possible modes of transmission of microsporidia to humans. As early as 1997, *Enterocytozoon bieneusi*, the most frequent human-pathogenic microsporidium, was detected in surface water of the river Seine near Paris, France. Subsequent investigations have confirmed the finding of *E. bieneusi* in surface waters, and a second human-pathogenic microsporidium, *Encephalitozoon intestinalis*, was also found in ground water. In the summer of 1995, an outbreak of intestinal microsporidiosis in 200 persons was observed in the city of Lyon, France, which was confined to 1 of 3 independent drinking water treatment systems of that city. The small size of the spores makes them difficult to remove using conventional water filtration techniques and there is growing concern that they may possess increased resistance to chlorine disinfection. It has been demonstrated that microsporidia can survive for more than 3 weeks at 20°C and even longer at lower temperatures. Another conceivable route of transmission is via food, since *E. intestinalis* has recently been found in irrigation waters used for crop production. However, all attempts to detect microsporidia in food products thus far have failed. Infection by inhalation has also been suggested, because *Encephalitozoon* spp and *E. bieneusi* have been found in sputum, bronchoalveolar lavage fluid and the bronchoalveolar epithelium of infected individuals. Finally, there is evidence for a fecal-oral smear infection route, from a case report describing the seroconversion of a 10-year-old girl to another microsporidium, *Encephalitozoon cuniculi*, after contact with an infected dog. It should be noted, however, with the possible exception of the latter report, finding the pathogen without demonstrating subsequent infection is only circumstantial evidence for a particular mode of transmission, and the relative frequencies of these different routes cannot be assessed. In principle, these questions could be addressed by epidemiological investigations, notably from identifying risk factors for infection by case-control studies. Unfortunately, such studies are notably rare also because significant numbers of cases are difficult to assemble. One unmatched case-control study of 30 cases described an association of intestinal microsporidiosis with male homosexuality as well as swimming in pools and suggested sexual and water-borne routes of fecal-oral transmission. Still, further and more extensive studies are needed. Closely related to the route of transmission is the need for elucidation of the reservoir hosts. To do this, fingerprinting techniques are self-suggestive, which are best elaborated for *E. bieneusi*. Here, at least 26 confirmed polymorphic loci exist in the internal transcribed spacer of the rRNA gene. From phylogenetic analyses of these polymorphisms, a zoonotic potential has been demonstrated for *E. bieneusi*, with cats, pigs, and cattle, as reservoirs.

INTRODUCTION

Microsporidia are newly emerging pathogens of humans and animals. Due to the small size of their spores and uncharacteristic staining properties they are difficult to detect by light microscopy. Even if detected, there are no phenotypic features to distinguish strains within species. Therefore, epidemiologic studies to elucidate the reservoirs of human-pathogenic microsporidia and their routes of transmission are difficult to perform. This manuscript reviews the current state of knowledge on these issues, with special attention to the most frequent microsporidial pathogen

in humans, *Enterocytozoon bieneusi*.

MICROSPORIDIA IN THE ENVIRONMENT

The first detection of human-pathogenic microsporidia was reported in 1997 (Sparfel *et al*, 1997) when *E. bieneusi* was found by light microscopy and PCR in a water sample from the river Seine, 15 km downstream from Paris, France. In the following year, *E. bieneusi* was demonstrated in 1 of 4 surface water samples, this time in Arizona, USA (Dowd *et al*, 1998). In a one-year follow-up study of 25 water samples each 300-600 liters, from the river Seine, submitted to sequential filtrations and analysis by light microscopy and PCR, only one sample was positive for *E. bieneusi* (Fournier *et al*, 2000). The authors concluded that the risk of water-borne transmission to humans was limited. Confirmatory to this point was a one-year prospective study on 48 water samples from French swimming pools; no human-pathogenic

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microsporidia could be detected (Fournier *et al*, 2002). The second most commonly found microsporidium in microsporidial infections of humans, *Encephalitozoon intestinalis*, was found in 2 of 4 surface water samples from Arizona, as well as in 1 of 1 sample of raw sewage, and 1 of 3 samples of tertiary effluent from a sewage plant (Dowd *et al*, 1998). Interestingly, this pathogen was also detected in the same study in 1 groundwater sample. In that report, the authors concluded that human-pathogenic microsporidia may be water-borne pathogens. Further indicative of this possibility is the detection of *E. intestinalis* in irrigation water used for food crops (Thurston-Enriquez *et al*, 2002). It was concluded that the presence of human pathogenic parasites in irrigation waters used in the production of crops traditionally consumed raw, suggests that there may be a risk of infection to consumers who come into contact with, or eat, these products.

Infection by inhalation is another theoretic possibility for infection of humans, but evidence for this route is sparse. Case reports exist for respiratory tract infections with *Encephalitozoon hellem* (Scaglia *et al*, 1998), and with *E. bienewisi* (del Aguila *et al*, 1997; Weber *et al*, 1992; Botterel *et al*, 2002). However, it remains unclear whether these pathogens were actually acquired air-borne since all of the patients under investigation suffered from intestinal microsporidiasis. Therefore, direct oro-fecal contamination, regurgitation, and/or hematogenous dissemination from the intestine cannot be ruled out.

ENTEROCYTOZOON BIENEUSI IN HUMANS AND ANIMALS

A prerequisite for considering the relative importance of zoonotic transmissions of *E. bienewisi* from different hosts is elucidation of the host spectrum. The first detection of *E. bienewisi* in an animal was in 1996, when this pathogen was found in pig feces (Deplazes *et al*, 1996). In subsequent years, *E. bienewisi* was reported from increasing numbers of domestic animals, including cats (Mathis *et al*, 1999), cattle (Dengjel *et al*, 2001), and even chickens (Reetz *et al*, 2002), but recently also in wild-living foxes, beavers, muskrats, and raccoons (Sulaiman *et al*, 2003). In summary, the *E. bienewisi* host spectrum known to date is as follows: humans, rhesus macaques, pigs, cattle, cats, llamas, chickens, foxes, beavers, muskrats, and raccoons.

However, mere knowledge of the host range of *E. bienewisi* does not by itself present direct evidence that any of these hosts function as a reservoir for human

infection. To answer this question, epidemiological tools must be employed.

RESERVOIRS AND TRANSMISSION

Elucidation of the reservoirs of *E. bienewisi* and putative transmission routes

Since experimental infections of humans are prohibited for ethical reasons, both traditional as well as molecular epidemiological techniques, at least in principle, may be employed to point to the zoonotic potential of microsporidia and their routes of infection, for which preventive measures could then be proposed. However, non-molecular epidemiological studies, for example, case-control studies to identify risk factors such as contact with certain animals, are hampered by the small number of diagnosed microsporidial infections. Indeed, the only comprehensive case-control study to investigate a sizable number of potential risk factors for infection with microsporidia was done within a population of HIV-infected patients with 200 CD4 cells/ μ l or less, but with only 30 cases and 56 controls (Hutin *et al*, 1998). In this study, swimming in pools was identified as a significant risk factor (OR = 6.4; $p = 0.001$), and, with less significance, male homosexuality (OR = 3.0; $p = 0.19$), and undercooked beef (OR = 2.7; $p = 0.06$). No significant risk was determined for other recreational activities, including swimming in fresh or sea water, foreign travel, or visits to the countryside. The same was true for other undercooked foods, for example, lamb, pork, and poultry, as well as for fruit, honey, or salads. No significant risk was determined for drinking tap water or beverages, including unpasteurized milk or fruit juices. Finally, exposure to cats, dogs, birds, bees, or fish could not be identified as risk factors. The authors concluded that fecal-oral transmission, including sexual and water-borne routes, was associated with intestinal microsporidiosis in persons with HIV infection.

More insight into the zoonotic potential came from molecular epidemiologic studies over the last few years, which were made possible following the detection of intra-specific genotypic polymorphisms within the internal transcribed spacer (ITS) of the rRNA gene (rDNA), in 1997 (Rinder *et al*, 1997). In 2001, based on 27 polymorphic sites within the ITS, which led to the discrimination of 20 *E. bienewisi* genotypes of humans, pigs, cattle, a cat and a llama, the zoonotic potential for *E. bienewisi* was proposed (Dengjel *et al*, 2001), although at that time no genotype was known that was found concurrently in humans and animals, with the exception of one genotype (D) found in a

Table 1
Genotypes of *E. bienersi*.

Genotype	Host
A	human
B	human
C	human
D	human, fox, macaque, pig
E	pig, muskrat, raccoon
F	pig, cattle
G	pig
H	pig
I	cattle
J	cattle, chicken
K	cat, human
L	cat
M	cattle
N	cattle
O	pig
P	llama
Q	human
EbpB	pig
EbpD	pig
WL1	raccoon
WL2	raccoon
WL3	raccoon
WL4	muskrat
WL6	muskrat
WL7	beaver
WL9	beaver
WL10	muskrat
WL11	fox
WL12	beaver
WL14	muskrat
WL15	raccoon, muskrat
III	human
V	human
EBits1	pig
EBits2	pig
EBits3	pig
EBits4	pig
EBits5	pig
EBits6	pig
EBits7	pig
EBits8	pig
EBits9	pig
AF118144	cat
AF502396	human

patient with AIDS and simian-immunodeficiency-virus-infected rhesus macaques. The argumentation was based on phylogenetic analyses, which showed

the absence of monophylogeny, or, in other words, absence of a transmission barrier between *E. bienersi* strains found in humans and those found in animals. Shortly thereafter, this proposal was supported by finding a genotype (K), previously known only from cats, in 5 humans, (Sadler *et al.*, 2002; Tumwine *et al.*, 2002). Furthermore, the a fore-mentioned genotype D was eventually also found in 3 pigs (Buckholt *et al.*, 2002).

To date, there are 95 characterized polymorphic loci in the ITS of *E. bienersi*, giving rise to 44 distinct genotypes. Together with the host species from which they were detected, these genotypes are summarized in Table 1.

These data constitute very good evidence for the zoonotic potential of *E. bienersi*, but the relative significance of each of these hosts as reservoirs for human infection is much less clear. The same is true for the modes of transmission. A major reason for these shortcomings is an apparent scarcity of *E. bienersi* genotyping studies both from human infections and environmental detection. For the latter, no ITS genotypes of *E. bienersi* have been determined. Compared with the number of detections of *E. bienersi* in humans, much larger and more comprehensive surveys have been conducted in animals. Therefore, there is an urgent need for further molecular epidemiologic studies characterizing the entire *E. bienersi* ITS at the molecular level, from human infections and from the environment, in comprehensive, representative surveys. This approach can be expected eventually to provide insight into which animals play a significant role in human infection and which routes of transmission are important, especially the potential for waterborne transmission.

REFERENCES

- Botterel F, Minozzi C, Vittecoq D, Bouree P. Pulmonary localization of *Enterocytozoon bienersi* in an AIDS patient: case report and review. *J Clin Microbiol* 2002;40:4800-1.
- Buckholt MA, Lee JH, Tzipori S. Prevalence of *Enterocytozoon bienersi* in swine: an 18-month survey at a slaughterhouse in Massachusetts. *Appl Environ Microbiol* 2002;68:2595-9.
- del Aguila C, Lopez-Velez R, Fenoy S, *et al.* Identification of *Enterocytozoon bienersi* spores in respiratory samples from an AIDS patient with a 2-year history of intestinal microsporidiosis. *J Clin Microbiol* 1997;35:1862-6.

- Dengjel B, Zahler M, Hermanns W, *et al.* Zoonotic potential of *Enterocytozoon bieneusi*. *J Clin Microbiol* 2001;39:4495-9.
- Deplazes P, Mathis A, Muller C, Weber R. Molecular epidemiology of *Encephalitozoon cuniculi* and first detection of *Enterocytozoon bieneusi* in faecal samples of pigs. *J Eukaryot Microbiol* 1996; 43:93S.
- Dowd SE, Gerba CP, Pepper IL. Confirmation of the human-pathogenic microsporidia *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Vittaforma corneae* in water. *Appl Environ Microbiol* 1998;64:3332-5.
- Fournier S, Liguory O, Santillana-Hayat M, *et al.* Detection of microsporidia in surface water: a one-year follow-up study. *FEMS Immunol Med Microbiol* 2000;29:95-100.
- Fournier S, Dubrou S, Liguory O, Gaussin F, *et al.* Detection of Microsporidia, Cryptosporidia and Giardia in swimming pools: a one-year prospective study. *FEMS Immunol Med Microbiol* 2002; 33:209-13.
- Hutin YJ, Sombardier MN, Liguory O, *et al.* Risk factors for intestinal microsporidiosis in patients with human immunodeficiency virus infection: a case-control study. *J Infect Dis* 1998;178:904-7.
- Mathis A, Breitenmoser AC, Deplazes P. Detection of new *Enterocytozoon* genotypes in faecal samples of farm dogs and a cat. *Parasite* 1999;6:189-93.
- Reetz J, Rinder H, Thomschke A, Manke H, Schwebs M, Bruderek A. First detection of the microsporidium *Enterocytozoon bieneusi* in non-mammalian hosts (chickens). *Int J Parasitol* 2002; 32:785-7.
- Rinder H, Katzwinkel-Wladarsch S, Loscher T. Evidence for the existence of genetically distinct strains of *Enterocytozoon bieneusi*. *Parasitol Res* 1997;83:670-2.
- Sadler F, Peake N, Borrow R, Rowl PL, Wilkins EG, Curry A. Genotyping of *Enterocytozoon bieneusi* in AIDS patients from the north west of England. *J Infect* 2002;44:39-42.
- Scaglia M, Gatti S, Sacchi L, *et al.* Asymptomatic respiratory tract microsporidiosis due to *Encephalitozoon hellem* in three patients with AIDS. *Clin Infect Dis* 1998;26:174-6.
- Sparfel JM, Sarfati C, Liguory O, *et al.* Detection of microsporidia and identification of *Enterocytozoon bieneusi* in surface water by filtration followed by specific PCR. *J Eukaryot Microbiol* 1997;44:78S.
- Sulaiman IM, Fayer R, Lal AA, Trout JM, Schaefer FW 3rd, Xiao L. Molecular characterization of microsporidia indicates that wild mammals harbor host-adapted *Enterocytozoon* spp as well as human-pathogenic *Enterocytozoon bieneusi*. *Appl Environ Microbiol* 2003;69:4495-501.
- Thurston-Enriquez JA, Watt P, Dowd SE, Enriquez R, Pepper IL, Gerba CP. Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. *J Food Prot* 2002;65:378-82.
- Tumwine JK, Kekitiinwa A, Nabukeera N, Akiyoshi DE, Buckholt MA, Tzipori S. *Enterocytozoon bieneusi* among children with diarrhea attending Mulago Hospital in Uganda. *Am J Trop Med Hyg* 2002;67:299-303.
- Weber R, Kuster H, Keller R, *et al.* Pulmonary and intestinal microsporidiosis in a patient with the acquired immunodeficiency syndrome. *Am Rev Respir Dis* 1992;146:1603-5.