



Encephalitozoon spp. as a potential human pathogen

Review

Anna Wilczyńska^{*1}, Jerzy Ziętek¹, Oliwier Teodorowski², Łukasz Adaszek¹

¹Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine of the University of Life Sciences in Lublin, Poland

²"Teodorowski" Veterinary Clinic, Mikołów, Poland

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Abstract

Encephalitozoon spp. are microsporidia, and intracellular opportunistic pathogens. The hosts of these pathogens include vertebrates, invertebrates, and certain protozoa. In people microsporidia may be opportunistic pathogens for immunocompromised patients (with AIDS or after organ transplantation). Infection with these microorganisms was also described in persons with diarrhea and corneal diseases.

The species causing rare infections in humans, *Encephalitozoon cuniculi*, had previously been described from animal hosts. However, several new microsporidial species, including *E. intestinalis* and *E. hellem*, have been discovered in humans, raising the question of their natural origin. Vertebrate animals are now identified as hosts for all three microsporidial species infecting humans, implying a zoonotic nature of these microorganisms. Molecular studies have identified phenotypic and/or genetic variability within these species, indicating that they are not uniform, and have allowed the question of their zoonotic potential to be addressed. The focus of this review is to present the zoonotic potential of *E. intestinalis*, *E. cuniculi*, and *E. hellem*.

Keywords

Encephalitozoon spp • zoonosis • immunodeficiency

1. Introduction

Encephalitozoon spp. are microsporidia and intracellular opportunistic pathogens. They are characterized by a polar cannula by which the sporoplasm and the nucleus are introduced into the cell of the host. A phylogenetic analysis of microsporidia proved that they are related to fungi, but some researchers suggest that microsporidia should be classified as a sister group of fungi [1, 2, 3] and related to *Cryptomycota* [4, 5].

The hosts of microsporidia include vertebrates, invertebrates, and certain protozoa [6]. In beekeeping and fish farming, the microorganisms can contribute to high commercial losses (*Nosema apis* and *N. ceranae* infect the honeybee, *N. bombycis* infect the silk moth [7], whereas *Loma salmonae* infect salmon fish [8, 9, 10, 11, 12].

At least 70 species of microsporidia are known to be capable of infecting humans and the highest pathogenicity potential is with 3 species of the genus *Encephalitozoon* – *E. intestinalis*, *E. cuniculi*, and *E. hellem* [13].

National Institute of Allergy and Infectious Diseases classified them as Category B Priority Pathogens. The classification means that these organisms spread moderately easily in the environment and feature an average pathogenicity index. The Environmental Protection Agency qualified the organisms as hazardous water pollutants [14].

Since they are able to infect a wide range of animal species, microsporidial spores are widely distributed in the environment. Spores are excreted in the feces, urine, or sputum; therefore people become infected by ingestion of food or water contaminated with spores, by inhalation of spores or by contact with infected animals [15, 16, 17, 18, 19, 20].

In humans infected with *Encephalitozoon* spp. the onset of infection is concomitant to diarrhea and loss of body mass. The later stages of infection may develop certain other symptoms: keratitis, urinary tract inflammation, hepatitis, encephalitis, peritonitis, prostatocystitis, sinusitis, inflammation of the rhinal mucosa, urethritis, and cholangitis [18].

* E-mail: ania.osinska@eysys.pl



The individuals at the highest risk of infection with the pathogen include immunodeficient patients after organ transplantation, immunodeficient patients with HIV, and immunosuppressant therapy subjects [21, 22, 23, 24, 25, 26]. Other maximum-risk groups include owners of pets such as rabbits, dogs, cats, rodents, and birds, since these animals can be carriers of microsporidia and release their spores in urine and stool [18, 27].

The objective of this work was to trace the pathogenicity of selected species of *Encephalitozoon* spp. isolated from humans and animals.

2. *Encephalitozoon cuniculi*

The first described and reported infection with *E. cuniculi* was in laboratory rabbits in the year 1922 [28]. Currently, the disease is prevalent in the population of rabbits. The seroprevalence of *E. cuniculi* in the domestic rabbit (*Oryctolagus cuniculus domesticus*) ranges widely from 7.7% [29] to 71% [30]. Among other animal species, the infection with this microorganism was found in rats, mice, muskrats, caviars, gerbils, shrews, birds, horses, goats, sheep, pigs, foxes, dogs, panthers, cats, and primates, including humans [16, 30, 31, 32, 33, 34]. Based on the ITS (internal transcribed spacer) regions recurrent in the genome of *E. cuniculi*, four strains of these pathogenic fungi were identified in different animal species (strain I is isolated from rabbits and mice, strain II is only isolated from rodents, strain III is isolated from dogs, and strain IV was found in humans, cats, and dogs) [33, 35, 36]. Cuniculosis in animals is spread horizontally (orally, in feces, and by inhalation) and vertically through the placenta. The spores in their chitinous capsule are extremely resistant to external factors and may persist in the environment for a long time. Rabbits can expel the spores in urine for approximately 63 days, from day 21 post-infection. In the first stage of infection (the first 30 days post-infection), *E. cuniculi* attacks the airways, the lungs, the liver, and the spleen, followed by (from day 98 post-infection) the heart, the central nervous system (CNS), the kidneys, and the eyes [37, 38, 39, 40]. The afflicted organs are inflamed and their surfaces may form characteristic cocci. The neurological symptoms related to the damage inflicted by the fungus to the vestibulum are manifested as torticollis. Other symptoms include manège movement, scrapie, pareses, strabismus, nystagmus, and fits. Some infected animals are observed to manifest incontinence, uveitis of the eyes, and cataract, where both of the latter lead to loss of vision [41]. The recovery of infected individuals depends on the severity of cerebral and nephral damage. If the course of invasion with the pathogen results in kidney damage and growing kidney failure, the prognosis is poor and becomes a rationale for euthanasia of the animals [42].

All genotypes of *E. cuniculi* characterized so far can infect humans [14, 16, 18, 19, 20, 35, 43]. The first case of *Encephalitozoon* infection in humans was described in 1959 [44]. Microsporidial infection was recognized based in spore morphology only, so

the sensitivity and specificity of this diagnosis was very low, and didn't allow differentiation of *E. cuniculi* with other *Encephalitozoon* species. Currently the diagnosis is based on immunological and/or molecular methods, and the clinical cases of encephalitozoonosis are recorded primarily in immunocompromised patients (with AIDS or after organ transplantation).

Most often in the course of a human infection, hepatitis, peritonitis, urethritis, prostatocystitis, nephritis, keratitis, conjunctivitis, and cystitis occur. It must be noted that *E. cuniculi* in humans may also cause infection of the respiratory system and generalized microsporidiosis [45].

Documented microsporidial infections with respiratory involvement are rare; to date, these have only been shown for immunosuppressed patients. Among transplant recipients, seven such cases have been documented [24, 45, 46, 47, 48, 49, 50]. Four of these patients died as a result of cardiorespiratory failure, which suggests that microsporidia infection in the respiratory tract might be life-threatening. Moreover, the only data concerning the prevalence of microsporidia in respiratory samples showed 14.2% of iatrogenically immunosuppressed patients to be positive for microsporidia in bronchoalveolar lavage, including two out of the six (33.3%) tested [51]. Respiratory microsporidial infection might ensue directly after the inhalation of spores or might develop as a result of dissemination after spore ingestion [34].

The clinical diagnosis of respiratory microsporidiosis is challenging. Clinical symptoms are nonspecific and no consensus facilitating the recognition of microsporidial infection has been proposed. Moreover, respiratory localized microsporidia might coexist with other opportunistic pulmonary pathogens, such as *Pneumocystis jirovecii*, making it more difficult to diagnose.

Kicia et al. (2018) detected periprosthetic infection caused by *E. cuniculi* in 39% of patients suffering from hip implant loosening that was previously classified as aseptic. A key question arising from this finding was whether the infection initiated osteolysis or followed as a result of the immune response to the aseptic implant destabilization process, subsequently accelerating ossification. *E. cuniculi* could persist and replicate inside resting macrophages, where it can evade the immune response and be transported throughout the host [52]. Because aseptic implant loosening is associated with a response to implant biomaterials [53] or metal ions [54] by local immune activation – for example, of macrophages – it is more likely that dissemination of *E. cuniculi* to periprosthetic tissue followed an inflammatory response to debris. During latent infection in an immunocompetent host, persistent microsporidia could provide continuous immune stimulation, leading to chronic inflammation, progressive tissue destruction, and necrotic changes [55]. As opportunistic pathogens without organ specificity, *E. cuniculi* can readily spread throughout the body. This can occur by direct extension of spores into surrounding cells and by introduction into the vas-

cular system [56]. Finding *E. cuniculi* in periprosthetic tissue from 39% of revision hip arthroplasty patients shows that this fungus can occupy this unusual extraintestinal niche, and must therefore be considered as a contributing cause of periprosthetic osteolysis and implant destabilization after hip replacement. Increased presence of *E. cuniculi* spores in urine, not only of patients after hip joint replacement, might be considered as an indicator of chronic inflammatory process in the patient's body.

Generalized infections with *E. cuniculi* are not a commonly reported form of the disease. They were characterized in patients post kidney transplantation and patients with AIDS [49, 57, 58, 59, 60]. In some cases, this form of microsporidiosis may result in fatality of patients. Mertens et al. (1997) reported a case of a female AIDS patient, dying with widely disseminated *E. cuniculi* microsporidiosis. Indirect immunofluorescent antibody staining studies and molecular analyses identified the microsporidian as the dog strain of *E. cuniculi*. Autopsy revealed necrotizing microsporidiosis of the adrenal glands and kidneys, with lesser involvement of the brain, heart, trachea, urinary bladder, spleen, and lymph nodes. Cellular targets included macrophages, epithelium, endothelium, and cardiac myocytes. Spore detection was enhanced by Gram-staining, polarization, and fluorescence chitin stains. Central nervous system microglial nodules were present which contained microsporidia. This was the first demonstration of *Encephalitozoon* microsporidiosis of the brain, heart, and adrenal glands in a patient with AIDS.

Another curious case of human *E. cuniculi* infection was reported by Ditrich et al. (2011). A brain abscess caused by *E. cuniculi* genotype I occurred in a patient without major immunocompromise and with diabetes. The distinguishing clinical signs were hemiparesis and epilepsy. The microsporidium was observed in the abscess aspirate, and its specific DNA was also detected in stool and urine. The patient was successfully treated with albendazole and mebendazole.

The cases referred to above indicate a clearly high zoonotic potential of *E. cuniculi*, and infections caused by the fungi can widely vary in course. Patients who have severe cellular immunodeficiency appear to be at highest risk of developing the disease, but little is known about immunity to microsporidial infection. It is not understood whether microsporidial infection in these patients is primarily a reactivation of latent infection acquired before the state of suppressed immunity or whether microsporidial disease is caused by recently acquired infection.

3. *Encephalitozoon intestinalis*

E. intestinalis is the second most prevalent microsporidial species infecting humans. Infections in HIV-positive patients have been reported from the Americas [61, 62, 63], from Europe [13, 64, 65, 66, 67, 68, 69, 70, 71, 72], from Australia [62, 73, 74], and from Africa [74, 75, 76, 77]. Its occurrence has been primarily demonstrated in the organisms of farm animals (such as pigs,

cattle, and goats), companion animals (such as dogs), and wildlife [13, 76, 78].

The prevalence for *E. intestinalis* was 7.3% for 68 AIDS patients with diarrhea from the United States [79], 2% for 97 consecutive HIV-infected patients in Germany [69], 3% for 75 patients with chronic diarrhea from Zambia [77], and 0.9% for 320 patients with chronic diarrhea in Switzerland [18, 80].

E. intestinalis may also spread into biliary tract and into the gallbladder, causing cholangitis and cholecystitis. Systemic dissemination to kidneys and other sites without a luminal connection to the intestine may occur, but intestinal symptoms appear to predominate.

4. *Encephalitozoon hellem*

Birds are the primary reservoir for *E. hellem*. These microorganisms have been found in populations of pigeons, ducks, swans, geese, crows, cranes, puffins, and hummingbirds [81, 82, 83, 84, 85]. For reared birds, *E. hellem* was found in *Psittaciformes*, ostriches and Gouldian finches [1, 83, 86, 87, 88, 89, 90]. Infected animals are most often asymptomatic with periodic elimination of the spores in feces [81]. *E. hellem* mainly persists in the liver, the intestines, and the kidneys; however, it can be found in the eyes, the lungs, and the spleen of infected birds [33, 84, 87, 89, 90, 92].

E. hellem has so far been diagnosed only in low numbers of HIV-infected persons in a relatively few countries: Most cases were reported from the United States [27, 93, 94, 95, 96, 97, 98]. *E. hellem* was diagnosed in European patients from Italy [99], Switzerland [100, 101, 102], Germany [101], and the United Kingdom [103] and in one case in Africa (Tanzania) [100].

It is not clear whether epidemiological factors are responsible for a restricted distribution of this microsporidian or whether the relatively difficult identification of this species by immunological or molecular methods hampers its detection. *E. hellem* causes disseminated and ocular infections in HIV-positive patients, but asymptomatic infections of the respiratory tract have also been described [104]. *E. hellem* has so far been identified on two occasions in nonimmunosuppressed and HIV-seronegative patients, namely, from bronchoalveolar lavage of a patient with a simultaneous *Mycobacterium tuberculosis* coinfection [104] and in fecal samples from two diarrheic travelers returning from Singapore, who were coinfecting with *E. bieneusi* [105] as diagnosed by PCR and confirmed by sequencing [18].

5. Treatment of *Encephalitozoon* spp. infection in humans

In the treatment of human microsporidiosis, albendazole and fumagillin are known to have the highest clinical efficacy [61, 106]. Albendazole is the drug of choice for the treatment of intestinal, respiratory, and disseminated microsporidiosis caused by *Encephalitozoon* spp. [61]. Fumagillin is highly effective, but shows

toxicity and might cause thrombocytopenia, neutropenia, and hyperlipidemia when administered systemically in humans [107]. Also aseptic meningoencephalitis has been reported as a result of fumagillin treatment in a patient in whom albendazole treatment failed [108]. Moreover, both albendazole and fumagillin may not be fully effective and do not eradicate pathogens, especially in severely immunocompromised patients, which results in pathogen persistence and may lead to relapse of symptoms after the completion of treatment [61, 109, 110].

Since symptomatic microsporidial infection is related to the immune status of the host, restoration of the immune system may result in the resolution of microsporidiosis symptoms and elimination of the pathogen without the need for specific treatment. Such immune renewal might be achieved as a result of using highly active antiretroviral therapy (HAART) in AIDS patients [111] or dose reduction or temporary withdrawal of the immunosuppressant in the case of pharmacologically immunosuppressed patients [112, 113].

6. Conclusion

Microsporidia are identified as fungal organisms of almost every animal group, including numerous invertebrates and vertebrates. The sources of most microsporidia infecting humans and modes of transmission are still uncertain. Because microsporidial spores are released into the environment via stool, urine, and respiratory secretions, possible sources of infection may be persons or animals infected with microsporidia.

Current data suggest that microsporidia are important pathogens capable of causing opportunistic infections in severely immunodeficient patients. An increasing number of cases of human microsporidiosis will likely be reported as diagnostic

skills improve, and it would not be surprising if new microsporidial species were identified.

Research on these unique intracellular fungi may enhance our understanding of the evolutionary development of the host-parasite relationship, particularly of the mechanisms of the parasite's protection from the host's immune response, the mechanisms of host defense, and the pathogenesis of an over-reactive immune response by the host, which might itself cause disease. Improved diagnostic techniques will facilitate future studies on the incidence, risk factors, origins of infection, modes of transmission, clinical manifestations, pathogenesis, and treatment of this important emerging pathogen.

Authors' Contribution

A.W.: research concept and design, carrying out the experiments, acquisition of data, drafting the article or revising it critically for important intellectual content, writing the manuscript, literature review; **J.Z.:** research concept and design, acquisition of data, writing the manuscript; **O.T.:** carrying out the experiments, analysis and interpretation of data, designing the figures; **Ł.A.:** supervising the project, analysis and interpretation of data, final proofreading and approval of the version for publication.

ORCID

Anna Wilczyńska <https://orcid.org/0000-0002-1148-6515>

Jerzy Ziętek <https://orcid.org/0000-0002-2019-6640>

Łukasz Adaszek <https://orcid.org/0000-0003-0261-2695>

Conflict of Interest

The authors have no potential conflicts of interest to declare.

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