

Original papers

Occurrence of *Encephalitozoon intestinalis* in the Red ruffed lemur (*Varecia rubra*) and the Ring-tailed lemur (*Lemur catta*) housed in the Poznan Zoological Garden, Poland

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ABSTRACT. *Encephalitozoon intestinalis* is one of the most common microsporidial species found in humans worldwide but it has rarely been identified in animals. The presence of this pathogen has been detected in a few species of domestic, captive and wild mammals as well as in three species of birds. The aim of the present study was to examine fecal samples obtained from mammals housed in the Poznan Zoological Garden, Poland, for the presence of potentially human-infectious microsporidia. A total of 339 fresh fecal samples collected from 75 species of mammals belonging to 27 families and 8 orders were examined for the presence of microsporidian spores. Microsporidian spores were identified in 3 out of 339 (0.9%) examined fecal samples. All samples identified as positive by chromotrope 2R and calcofluor white M2R were also positive by the FISH assay. Using multiplex FISH in all 3 fecal samples, only spores of *E. intestinalis* were identified in 2 out of 14 Ring-tailed lemurs (*Lemur catta*) and in one out of 17 Red ruffed lemurs (*Varecia variegata rubra*). To our knowledge this is the first diagnosis of *E. intestinalis* in Ring-tailed and Red ruffed lemurs. It should be mentioned that both lemur species are listed by the IUCN Red List of Threatened Species. Although the lemurs were asymptotically infected, the possibility of widespread infection or death of these animals remains in the event of an elevated stress or a decrease in their immunological functions.

Key words: *Encephalitozoon intestinalis*, Zoo animals, diagnostics, FISH technique, microsporidia, asymptomatic encephalitozoonosis

Introduction

Microsporidia are obligate intracellular parasites in a wide range of invertebrate and vertebrate hosts that have recently been reclassified from protozoa to fungi [1]. So far, more than 1200 microsporidian species belonging to 143 genera have been described [2]. Only 14 species belonging to the *Enterocytozoon*, *Encephalitozoon*, *Pleistophora*, *Trachipleistophora*, *Nosema*, *Anncaliia* and *Vittaforma* genera are infectious for humans. Microsporidia are considered to be emerging pathogens and their routes and sources of transmission have not been well defined. Infections occur by ingestion of food or water contaminated

with microsporidian spores, or by inhalation of spores. Animal reservoirs of human-virulent microsporidia have recently been confirmed [3,4].

Encephalitozoon intestinalis was first identified in 1993 as an etiological agent of diarrhea and cholangiopathy in AIDS patients [5]. *E. intestinalis* mainly parasitizes the gastrointestinal and biliary tracts, though it may also cause disseminated infections. So far, at least 200 cases of *E. intestinalis* infections have been described in humans. However, many cases may be undiagnosed because the infections are often latent and difficult to diagnose. *E. intestinalis* has rarely been identified in animals. The presence of this pathogen has been detected in a few species of domestic [6–9], captive

[10], and wild mammals [11–13], as well as in three species of birds [7,14–16]. In animals, the infection due to *E. intestinalis* may be asymptomatic [9,16] or may be fatal without any previous clinical symptoms [10,11].

Since microsporidia are newly emerging pathogens affecting humans, and most of the emerging infectious diseases have a zoonotic origin, identification of animal reservoirs is epidemiologically important. Moreover, increased mortality of zoo animals due to microsporidial infections has been reported in North America, Australia and Europe [10,17–20].

The aim of the present study was to examine fecal samples obtained from mammals housed in the Poznan Zoological Garden, Poland, for the presence of potentially human-infectious microsporidia.

Materials and methods

A total of 339 fresh fecal samples collected from 75 species of mammals housed in the Poznan Zoological Garden, Poland, were examined for the presence of microsporidian spores. The animals represented to 27 families and 8 orders.

Fecal samples were collected by Zoo staff, placed in sterile plastic tubes, labeled, transported to the laboratory in a cooler, and stored at 4°C until they were analyzed. In the laboratory, two direct smears were prepared from all fecal samples and stained with chromotrope 2R and calcofluor white M2R [21,22]. The smears were microscopically screened for microsporidian spores using the 100x immersion oil objective.

Multiplex FISH assays for identification of *E. bienersi*, *E. intestinalis*, *E. hellem*, and *E. cuniculi* were performed as previously described [12,16]. The slides were examined with the Olympus BH2-RFL epifluorescence microscope using a 60x objective and a BP450-490 exciter filter. A sample was considered positive if a single microsporidian spore was detected. The FISH results were compared to the results obtained by conventional stain testing.

Results

Microsporidian spores were identified in 3 out of 339 (0.9%) fecal samples. All samples identified as positive by chromotrope 2R and calcofluor white M2R were also positive as determined by the FISH assay. Using multiplex FISH in all 3 fecal samples,

only spores of *E. intestinalis* were identified; the spores stained bright red. *E. intestinalis* spores were identified in 2 out of 14 Ring-tailed lemurs (*Lemur catta*) and in one out of 17 Red ruffed lemurs (*V. rubra*). None of the infected lemurs displayed any symptoms of infection.

Discussion

E. intestinalis has rarely been recognized in animals. As yet, this microsporidian species has been identified in fecal samples of domestic mammals such as pigs, goats, cattle, donkeys, cats and dogs, and in captive koalas, as well as in wild mammals such as red foxes, wild boars, the European brown hare, and mountain gorillas [6–8,10–13]. Moreover, *E. intestinalis* has been identified in a few species of birds such as pigeons, ostriches, and domestic geese [7,15,16].

The role of animal breeding within zoos in spreading microsporidia has not been widely studied, even though these animals are a potential source of zoonotic pathogens for zoo patrons as well as the animals' caretakers. In the present study spores of *E. intestinalis* were detected in two species of lemurs housed in the Poznan Zoological Garden, Poland. To our knowledge this is the first diagnosis of *E. intestinalis* in Ring-tailed and Red ruffed lemurs. Although the lemurs were asymptotically infected, the possibility of widespread infection or death in these animals remains, in the event of an elevated stress or a decrease in their immunological functions. Such an instance was observed in an Australian zoo, in which two young Koala bears died due to *E. intestinalis* infection without having any exhibited symptoms [10]. It should be mentioned that two of the lemur species in which microsporidia were found in the present study are listed by the IUCN Red List of Threatened Species. The Red ruffed lemur and Ring-tailed lemur are listed under endangered and vulnerable status species, respectively. It is unclear how these animals were infected. A potential source of the pathogen may be in fruits and vegetables, which comprise an essential part of the lemurs' diet. Recently, viable spores of *E. bienersi*, *E. intestinalis*, and *E. cuniculi* were found in food samples of plant origin, such as strawberries, raspberries, lettuce, sprouts, and other vegetables [23].

Diagnosing microsporidia in zoo animals has great significance since many animal species have

substantial economic and scientific value. Also, discovery of other animal-related reservoirs of microsporidian species is extremely important in order to minimize the risk of human infection. However, identification of microsporidian species is only possible by use of an electron microscope or molecular methods such as PCR or FISH. A fundamental limitation of these methods is the fact that they are only available in scientific laboratories. The electron microscope had a lower sensitivity than molecular techniques. On the other hand, the PCR technique can generate false-negative results due to the potential presence of polymerase inhibitors in the fecal samples of humans and animals, as well as environmental trials. The FISH method does not have these limitations, as evidenced by this and other studies [12,16,23–25]. The FISH technique has proven to be a very sensitive method. In all the fecal smears stained with chromotrope and calcofluor in which microsporidian spores were confirmed microscopically, the FISH technique not only corroborated the presence of microsporidian spores but also classified their species. Moreover, using the multiplex FISH technique significantly decreases the waiting time for results because it enables simultaneous identification of the four most commonly found microsporidian species. Since this method is not routinely used by veterinarians in zoological gardens, cooperation between zoos and research centers is imperative and will benefit the health of the animals.

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