Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 28, n. 1, p. 80-90, jan.-mar. 2019
Doi: https://doi.org/10.1590/S1984-296120180096

Original Article

# Occurrence of zoonotic *Enterocytozoon bieneusi* in cats in Brazil

Ocorrência de *Enterocytozoon bieneusi* zoonótico em gatos no Brasil

Jamille Batista Faria Prado[1]; Carlos Alberto do Nascimento Ramos[2]; Vagner Ricardo da Silva Fiuza[3]; Veronica Jorge Babo Terra[2]

[1] Programa de Pós-graduação em Ciências Veterinárias, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul – UFMS, Campo Grande, MS, Brasil

[2] Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul – UFMS, Campo Grande, MS, Brasil

[3] Instituto de Biociências, Universidade Federal de Mato Grosso do Sul – UFMS, Cidade Universitária - Pioneiros, Campo Grande, MS, Brasil

Received September 6, 2018
Accepted December 10, 2018

## Abstract

*Enterocytozoon bieneusi* is an opportunistic intestinal pathogen that infects humans and a wide variety of animals worldwide. Our aim in this study was to investigate the occurrence of *E. bieneusi* in a domestic cat population in Campo Grande, Mato Grosso do Sul, Brazil. Sixty fecal samples from diarrheic cats were subjected to polymerase chain reaction (PCR) and the amplicons were sequenced for identification. *E. bieneusi* was detected in two samples (3.3%), both identified as genotype D. This genotype has already been reported in animals and humans and is considered a zoonotic genotype. Our findings represent the first report of *E. bieneusi* in domestic cats in Brazil, reinforcing the importance of identifying this agent as a source of infection in animals and humans.

**Keywords:** Microsporidia, zoonosis, felines, diagnosis, PCR.

## Resumo

*Enterocytozoon bieneusi* é um patógeno intestinal oportunista que infecta humanos e uma variedade de animais em todo o mundo. O objetivo no presente estudo foi investigar a ocorrência de *E. bieneusi* em uma população de gatos domésticos em Campo Grande, Mato Grosso do Sul, Brasil. Sessenta amostras fecais de gatos diarréicos foram submetidas a reação em cadeia da polimerase (PCR) e os produtos de amplificação foram sequenciados para identificação molecular. *E. bieneusi* foi detectado em duas amostras (3,3%), ambos identificados como genótipo D. Esse genótipo tem sido relatado em animais e humanos e é considerado um genótipo zoonótico. Nossos resultados representam a primeira descrição de *E. bieneusi* em gatos domésticos no Brasil, reforçando a importância desse agente como fonte de infecção para animais e humanos.

**Palavras-chave:** Microsporídeo, zoonose, felinos, diagnóstico, PCR.

## Introduction

Microsporidia are obligate intracellular fungi. Currently between 1300 and 1500 different species have already been described, infecting a wide variety of invertebrate and vertebrate hosts, including humans (VÁVRA & LUKEŠ, 2013; SANTÍN, 2015). However, only 17 species are known to be pathogenic to humans, among which *Enterocytozoon bieneusi* is considered the most common disease-causing species (FAYER & SANTÍN, 2014; MATHIS et al., 2005). It is an opportunistic pathogen, which infects mainly immunocompromised individuals whose CD4+ cell counts are lower than 100 cels/mm$^3$ (ESPERN et al., 2007), causing clinical signs of chronic diarrhea associated with abdominal pain, fever and weight loss (AKINBO et al., 2012; BRASIL et al., 2000). Transmission occurs through the fecal-oral route by the accidental ingestion of spores eliminated within feces from infected animals or humans or by the ingestion of contaminated water and/or food (SANTÍN, 2015).

*E. bieneusi* was first identified in 1985 in enterocytes from an HIV positive human (DESPORTES et al., 1985). In animals, *E. bieneusi* was first reported in pig feces in 1996 (DEPLAZES et al., 1996), and since then it has been detected in the feces and intestinal

***Corresponding author:** Carlos Alberto do Nascimento Ramos. Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul – UFMS, Av. Senador Filinto Muller, 2443, CEP 79074-460, Campo Grande, MS, Brasil. e-mail: carlos.nascimento@ufms.br

tissue of 236 different animal species (MATHIS et al., 2005; SANTÍN & FAYER, 2011; WANG et al., 2018). Information about the occurrence of *E. bieneusi* in cats is scanty; a few studies have been conducted in Asian and European countries, and a single study in South America. Most of these studies have identified zoonotic *E. bieneusi* genotypes, which includes cats as dispersing agents and a potential source of infection in humans. Given the absence of studies on *E. bieneusi* in cats in Brazil, the purpose of our research was to investigate the occurrence of *E. bieneusi* in diarrheic domestic cats in the city of Campo Grande, state of Mato Grosso do Sul, mid west Brazil.

## Material and Methods

### *Samples*

This study was approved by the Ethics Committee on Animal Use of the Federal University of Mato Grosso do Sul (Protocol no. 787/2016).

Fecal samples from 60 diarrheic cats were carefully collected from the ground immediately after defecation, between September 2016 and May 2017. The fecal samples were collected in private residences and veterinary clinics. The cats ages varied from 45 days to 17 years old. All the samples (approximately 8 grams each) were placed in clean containers and immediately sent for processing at the laboratory of molecular biology at veterinary hospital facilities. Aliquots of the samples (approximately 1g) were transferred to 1.5 mL polypropylene tubes containing 500 µL of 0.9% sterile saline solution, and stored at -20 °C until DNA extraction. The classification of feces as diarrheic was based on a scoring system presented by Queen et al. (2012), in which score 1 was considered very firm, score 2 was considered well-formed, score 3 was considered soft-formed, and score 4 was considered watery. Only specimens with scores 3 or 4 were included in the diarrheic group.

### *DNA extraction*

Three hundred microliters of the fecal suspensions stored in microtubes were centrifuged (10,000 x *g* for 10 minutes). After discarding the supernatant, the pellet was suspended in 500 µL of 20% SDS (Sodium Dodecyl Sulfate), and 10 µL of Proteinase K (20 mg/mL) were added. The suspension was homogenized in a vortex mixer and incubated at 65 °C for 10 minutes. Then, 400 µL of chloroform was added and the suspension was vortexed again, after which 300 µL of protein precipitation solution (5M potassium acetate, 11% glacial acetic acid) was added. The microtubes were centrifuged (10,000 x *g* for 10 minutes) and the supernatant transferred to a new 1.5 mL microtube. One mL of ethanol was then added for DNA precipitation. After another centrifugation step (10,000 x *g* for 5 minutes), the supernatant was discarded and the pellet washed with 1 mL of 70% ethanol. The samples were centrifuged for 2 min (10,000 x *g*), and the pellets were allowed to dry at room temperature. Then, 100 µL of nuclease free water was added for DNA elution. An analysis of the material in an BioPhotometer Plus spectrophotometer (Eppendorf) indicated that all the samples showed a DNA concentration equal to or greater than 25 ng/µL and a ratio of 260/280nm equal to or greater than 1.75.

### *PCR and sequencing*

The molecular identification of *E. bieneusi* was performed using a nested protocol, as described by Buckholt et al. (2002). The primers for the first PCR were EBITS3 (5' GGT CAT AGG GAT GAA GAG 3') and EBITS4 (5' TCG AGT TCT TTC GCG CTC 3'). The second reaction was performed with the primers EBITS1 (5' GCT CTG AAT ATC TAT GGC T 3') and EBITS2.4 (5' ATC GCC GAC GGA TCC AAG TG 3'), amplifying a 392*bp* DNA fragment comprising part of the internal transcribed spacer (ITS) region of the *E. bieneusi* rRNA gene. Reactions were performed in a final volume of 25 µL containing 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl (pH 9.0), 0.2 mM dNTPs, 1 µM of each primer, and 2.5 U of Taq DNA polymerase (Ludwig Biotec). The conditions for the first reaction was 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30s, annealing at 57 °C for 30s, and extension at 72 °C for 40s, followed by a final extension at 72 °C for 10 min. The procedure for the second reaction was similar to the first, except the annealing temperature (55 °C), and the number of PCR cycles (30 cycles). *E. bieneusi* positive controls obtained from cattle from a previous study conducted in Brazil (FIUZA et al., 2016b), as well as negative controls (ultra-pure water), were included in all the reactions.

Amplification products were visualized in agarose gel (2%) stained with GelRed® (Biotium) under ultraviolet light after gel electrophoresis. Positive samples were purified using CleanSweep® PCR Purification (Applied Biosystems), following the manufacturer's protocol, and were sequenced in both directions by the Sanger method (SANGER et al., 1977). The resulting sequences were aligned with reference sequences downloaded from GenBank using the MEGA v. 7 program (KUMAR et al., 2016) for genotype identification.

## Results

Positive *E. bieneusi* PCR amplifications were observed in 2 (3.3%) of the 60 fecal samples. The samples came from a 3-month-old and a 5-year-old cat living in two different households. Both PCR products were successfully purified and sequenced, and presented 100% similarity with genotype D. The nucleotide sequences obtained in the present study were deposited in the GenBank database under accession nos. MH161409 and MH161410.

## Discussion

This is the first report of presence of *E. bieneusi* in domestic cats in Brazil. Indeed, *E. bieneusi* was previously identified through PCR in feces from cats in Asia: China (KARIM et al., 2014a; LI et al., 2015; XU et al., 2016b), Japan (ABE et al., 2009), Iran (JAMSHIDI et al., 2012) and Thailand (MORI et al., 2013); Europe: Germany (DENGJEL et al., 2001), Switzerland (MATHIS et al.,

1999), Portugal (LOBO et al., 2006) and Poland (PIEKARSKA et al., 2017); and in South America: Colombia (SANTÍN et al., 2006), with prevalence rates varying from 5 to 31.3% in studies involving 40 or more samples. The prevalence rate found in this study was 3.3%. In a study conducted in South America (SANTÍN et al., 2006), 46 cats from 15 days to 10 years of age were euthanized in Colombia and fecal samples were collected directly from the rectum and ileum; eight (17%) animals were considered *E. bieneusi* positive after molecular analysis.

*E. bieneusi* DNA has already been identified in fecal samples in Brazil collected from humans (FENG et al., 2011), cattle (FIUZA et al., 2016b), birds (CUNHA et al., 2016, 2017; LALLO et al., 2012), pigs (FIUZA et al., 2015) and sheep (FIUZA et al., 2016a). In addition, microsporidium was also detected in a sample of treated effluent water collected in the region of Campinas, São Paulo, demonstrating the significant possibility of dispersion of this agent even after sewage treatment (YAMASHIRO et al., 2017). However, epidemiologic factors and the occurrence of clinical disease in the country are still uncertain and need further clarification.

No clinical symptomatology has been associated with domestic animals in Brazil. In the state of Rio de Janeiro, Fiuza et al. (2016b) compared the feces consistency and body condition score of cattle of different age groups, but were unable to correlate the parameters with the occurrence of *E. bieneusi*.

In this study, all the fecal samples collected from cats were diarrheic. However, due to the low prevalence rate and the non-comprehensive diagnosis of other parasitic, bacterial and viral etiological agents wich are expected to cause diarrhea, we could not correlate the presence of *E. bieneusi* with this clinical manifestation. Similarly, Dengjel et al. (2001), who analyzed fecal samples from 60 diarrheic cats in Germany, found only three samples positive for *E. bieneusi*. Piekarska et al. (2017) analyzed fecal samples from 44 domestic cats (nine diarrheic) in Poland and found four *E. bieneusi* positive samples, only one of which was diarrheic. None of these studies was able to correlate the presence of *E. bieneusi* DNA with diarrhea in the cats.

*E. bieneusi* genotyping contributes as an essential information to a better knowledge on this microorganism, indicating both possible species-specific genotypes as well as those already found in more than one animal species. Genotypes found in humans and animals are considered zoonotic, even when clinical disease is not evident. The presence of DNA of this organism in feces suggests the presence of infection and the possibility of spore dissemination through feces.

Among more than 200 known *E. bieneusi* genotypes, 12-are considered specific to cats (Table 1), whereas two others have been reported in cats and other animals (Table 2). Hence, these genotypes are considered non-zoonotic. On the other hand, six genotypes that have been found in cats are considered zoonotic, since they have also been observed in humans (Table 3). Genotypes D and Type IV are of major importance and have been described in several countries in humans and in a wide variety of animal species (Table 3). In this study, both positive cats had the genotype D. This genotype was described in cats for the first time by Mori et al. (2013) in Thailand, where it was detected in 22 out of 25 *E. bieneusi* positive animals, demonstrating that domestic cats may play an important role in the propagation of zoonotic microsporidiosis caused by this genotype. Since that first description, genotype D has also been identified in cats by Karim et al. (2014a), Li et al. (2015) and Xu et al. (2016b), in researches carried out in China. Therefore, this is the first report of genotype D in cats outside the Asian continent. In Brazil, genotype D has already been found in fecal samples from humans (FENG et al., 2011), cattle (FIUZA et al., 2016b) and birds (CUNHA et al., 2016, 2017), suggesting the possible risk of zoonotic transmission in this country. In a study conducted in birds by Cunha et al. (2016) in the state of Minas Gerais, genotype D was the most prevalent, and was found in 58.3% of analyzed fecal samples from chickens purchased in public markets. In the other single research in cats in the Americas, Santín et al. (2006), using molecular methods, found 17% of 46 cats tested positive for *E. bieneusi* in Colombia, and also found four other genotypes (Type IV, WL11, Peru10 and D-like).

Our findings reveal the presence of *E. bieneusi* infection by a zoonotic genotype in domestic cats in Brazil. Cats, along with dogs, are the most common companion pets that live inside homes around the world, in very close contact with humans of all ages and health conditions. The detection of a zoonotic genotype

**Table 1.** *Enterocytozoon bieneusi* genotypes found only in cats.

| Genotype (Synonym) | Host | Geographic distribution (Reference) |
|---|---|---|
| EbfelA | Cat | Switzerland (Mathis et al., 1999) |
| L | Cat | Germany (Dengjel et al. 2001)<br>Portugal (Lobo et al., 2006) |
| PtEb III | Cat | Portugal (Lobo et al., 2006) |
| PtEb IV | Cat | Portugal (Lobo et al., 2006) |
| D-like | Cat | Colombia (Santín et al., 2006) |
| ETMK2 | Cat | Thailand (Mori et al., 2013) |
| ETMK3 | Cat | Thailand (Mori et al., 2013) |
| ETMK4 | Cat | Thailand (Mori et al., 2013) |
| CC1 | Cat | China (Karim et al., 2014a) |
| CC2 | Cat | China (Karim et al., 2014a) |
| CC3 | Cat | China (Karim et al., 2014a) |
| eb52 | Cat | Poland (Piekarska et al., 2017) |

**Table 2.** *Enterocytozoon bieneusi* genotypes found in cats and other hosts.

| Genotype (Synonym) | Host | Geographic distribution (Reference) |
|---|---|---|
| CC4 | Cat | China (Karim et al., 2014a) |
| | Cattle | China (Qi et al., 2017) |
| PtEb IX | Cat | China (Karim et al., 2014a) |
| | Dog | Poland (Piekarska et al., 2017) |
| | | Switzerland (Mathis et al., 1999) |
| | | Portugal (Lobo et al., 2006) |
| | | Japan (Abe et al., 2009) |
| | | Colombia (Santín et al., 2008) |
| | | United States of America (Feng et al., 2011) |
| | | China (Xu et al., 2016b) |

**Table 3.** *Enterocytozoon bieneusi* genotypes found in cats and humans (and some genotypes also in other hosts).

| Genotype (Synonym) | Host | Geographic distribution (Reference) |
|---|---|---|
| D (PigEBITS 9, WL8, Peru9, CEbC, PTEb VI) | Human | Cameroon (Breton et al., 2007) |
| | | Gabon (Breton et al., 2007) |
| | | Peru (Bern et al., 2005; Cama et al., 2007; Sulaiman et al., 2003a) |
| | | England (Sadler et al., 2002) |
| | | Niger, Vietnan (Espern et al., 2007) |
| | | Nigeria (Akinbo et al., 2012; Ayinmode et al., 2011; Maikai et al., 2012) |
| | | Malawi, Netherlands (ten Hove et al., 2009) |
| | | Iran (Agholi et al., 2013a; Agholi et al., 2013b) |
| | | Thailand (Leelayoova et al., 2006; Prasertbun et al., 2017; Saksirisampant et al., 2009) |
| | | Russia (Sokolova et al., 2011) |
| | | Congo (Wumba et al., 2012) |
| | | China (Wang et al., 2013a,b) |
| | | Portugal (Lobo et al., 2012) |
| | | Tunisia (Chabchoub et al., 2012) |
| | | Poland (Kicia et al., 2014) |
| | | India (Li et al., 2013) |
| | | Democratic Republic of São Tomé and Príncipe (Lobo et al., 2014) |
| | | Brazil (Feng et al., 2011) |
| | | Spain (Galván et al., 2011) |
| | Cat | China (Karim et al., 2014a; Li et al., 2015; Xu et al., 2016b) |
| | | Thailand (Mori et al., 2013) |
| | | Brazil (***this study)*** |
| | Wild boar | Czech Republic (Němejc et al., 2014) |
| | | Slovak Republic (Němejc et al., 2014) |
| | Pig | United States of America (Buckholt et al., 2002) |
| | | Japan (Abe and Kimata, 2010) |
| | | Thailand (Prasertbun et al., 2017) |
| | | China (Li et al., 2014a; Zhao et al., 2014b) |
| | | Czech Republic (Sak et al., 2008) |
| | Cattle | South Africa (Abu Samra et al., 2012) |
| | | China (Li et al., 2016a; Qi et al., 2017; Zhao et al., 2015c) |
| | | Argentina (Del Coco et al., 2014) |
| | | Brazil (Fiuza et al., 2016b) |
| | | Korea (Lee 2007, 2008) |
| | Sheep | China (Zhao et al., 2015b) |
| | Goat | China (Shi et al., 2016; Zhao et al., 2015b) |
| | Takin | China (Zhao et al., 2015a) |
| | Beaver | United States of America (Sulaiman et al., 2003b) |
| | Fox | United States of America (Sulaiman et al., 2003b) |
| | | Spain (Galván-Díaz et al., 2014) |
| | | China (Yang et al., 2015; Zhao et al., 2015d) |
| | Muskrat | United States of America (Sulaiman et al., 2003b) |
| | Northern raccoon | China (Li et al., 2016b) |
| | River otter | United States of America (Guo et al., 2014) |
| | Raccoon | United States of America (Sulaiman et al., 2003b) |
| | | China (Xu et al., 2016a; Yang et al., 2015; Zhao et al., 2015d) |
| | Falcon | Abu Dhabi (Muller et al., 2008) |
| | Rabbit | Spain (Galván-Díaz et al., 2014) |

**Table 3.** *Continued...*

| Genotype (Synonym) | Host | Geographic distribution (Reference) |
|---|---|---|
| | Horse | Czech Republic (Wagnerova et al., 2012) |
| | | Algeria (Laatamna et al., 2015) |
| | | China (Qi et al., 2016) |
| | | Colombia (Santín et al., 2010) |
| | African lion | China (Li et al., 2016b) |
| | Asian golden cat | China (Li et al., 2016b) |
| | Dog | Portugal (Lobo et al., 2006) |
| | | China (Karim et al., 2014a; Xu et al., 2016b) |
| | Pigeon | Iran (Pirestani et al., 2013) |
| | Mice | Czech Republic (Sak et al., 2011) |
| | | Germany (Sak et al., 2011) |
| | Birds | Brazil (Cunha et al., 2016, 2017) |
| | Northern white cheeked gibbon | China (Li et al., 2016b) |
| | Golden snub-nosed monkey | China (Li et al., 2016b) |
| | Olive baboon | China (Li et al., 2016b) |
| | Primate | China (Karim et al., 2015; Karim et al. 2014b,c) |
| | Baboon | Kenya (Li et al., 2011) |
| | Macaque | United States of America (Chalifoux et al., 2000) |
| | | China (Ye et al., 2014) |
| Type IV (K, Peru2, BEB5, CMITS1, BEB-var, PtEB III) | Human | Cameroon (Breton et al., 2007; Sarfati et al., 2006) |
| | | Gabon (Breton et al., 2007) |
| | | Peru (Bern et al., 2005; Cama et al., 2007; Sulaiman et al., 2003a) |
| | | England (Sadler et al., 2002) |
| | | Uganda (Tumwine et al., 2002) |
| | | Niger (Espern et al., 2007) |
| | | Iran (Agholi et al., 2013a) |
| | | China (Wang et al., 2013b) |
| | | Nigeria (Akinbo et al., 2012; Ayinmode et al., 2011; Maikai et al., 2012) |
| | | Portugal (Lobo et al., 2012) |
| | | France (Liguory et al., 1998, 2001) |
| | | Democratic Republic of São Tomé and Príncipe (Lobo et al., 2014) |
| | | Malawi (ten Hove et al., 2009) |
| | | Netherland (ten Hove et al., 2009) |
| | Cat | Germany (Dengjel et al., 2001) |
| | | Portugal (Lobo et al., 2006) |
| | | China (Li et al., 2015; Xu et al., 2016b) |
| | | Japan (Abe et al., 2009) |
| | | Colombia (Santín et al., 2006) |
| | Cattle | United States of America (Santín et al., 2012; Sulaiman et al., 2004) |
| | | Korea (Lee, 2008) |
| | Chipmunk | United States of America (Guo et al., 2014) |
| | Woodchuck | United States of America (Guo et al., 2014) |
| | Meadow vole | United States of America (Guo et al., 2014) |
| | Squirrel | United States of America (Guo et al., 2014) |
| | Black bear | United States of America (Guo et al., 2014) |
| | Primate | China (Karim et al. 2014b,c) |
| | Ostriches | Spain (Galván-Díaz et al., 2014) |
| | Birds | Brazil (Cunha et al., 2016) |
| | Snake | China (Karim et al., 2014d) |
| | Monkey | China (Karim et al., 2015) |
| | Rhesus monkeys | China (Ye et al., 2012) |
| | Dog | China (Karim et al., 2014a) |
| | | Colombia (Santín et al., 2008) |

**Table 3.** *Continued...*

| Genotype (Synonym) | Host | Geographic distribution (Reference) |
|---|---|---|
| I (BEB2, CEbE) | Human | China (Zhang et al., 2011) |
| | Cat | China (Karim et al., 2014a) |
| | Cattle | Germany (Dengjel et al., 2001; Rinder et al., 2000) |
| | | Czech Republic (Juránková et al., 2013) |
| | | Korea (Lee, 2007, 2008) |
| | | China (Jiang et al., 2015; Li et al., 2016a; Ma et al., 2015b; Qi et al., 2017; Zhang et al., 2011; Zhao et al., 2015c) |
| | | Argentina (Del Coco et al., 2014) |
| | | Brazil (Fiuza et al., 2016b) |
| | | South Africa (Abu Samra et al., 2012) |
| | | United States of America (Fayer et al. 2007, 2012; Santín et al., 2012; Santín and Fayer, 2009; Santín et al., 2005; Sulaiman et al., 2004) |
| | | Algeria (Baroudi et al., 2017) |
| | Primate | China (Karim et al., 2014b) |
| | White tailed deer | United States of America (Santín and Fayer, 2015) |
| | Yak | China (Ma et al., 2015a) |
| | Pig | Spain (Galván-Díaz et al., 2014) |
| BEB6 (SH5) | Human | China (Wang et al., 2013b) |
| | Cat | China (Karim et al., 2014a) |
| | Sheep | Brazil (Fiuza et al., 2016a) |
| | | China (Jiang et al., 2015; Li et al., 2014b; Shi et al., 2016; Ye et al., 2015; Zhao et al., 2015b) |
| | | Sweden (Stensvold et al., 2014) |
| | Cattle | United States of America (Fayer et al., 2007) |
| | Hog deer | China (Li et al., 2016b) |
| | Alpaca | China (Li et al., 2016b) |
| | Sika deer | China (Li et al., 2016b; Zhao et al., 2014a) |
| | Red deer | China (Li et al., 2016b) |
| | Primate | China (Karim et al., 2014B, 2015) |
| | Horse | China (Qi et al., 2016) |
| | Goat | Peru (Feng et al., 2011) |
| | | China (Shi et al., 2016; Ye et al., 2015; Zhao et al., 2015b) |
| Peru10 | Human | Peru (Bern et al., 2005; Cama et al., 2007; Sulaiman et al., 2003a) |
| | Cat | Colombia (Santín et al., 2006) |
| WL11 (Peru5) | Human | Peru (Bern et al., 2005; Cama et al., 2007; Sulaiman et al., 2003a) |
| | Cat | Colombia (Santín et al. 2006) |
| | Dog | Colombia (Santín et al. 2008) |
| | Fox | United States (Sulaiman et al. 2003b) |

emphasizes the risk of human infection, since cats can contribute to the direct and indirect transmission of this parasite through the contamination of water and food with feces containing *E. bieneusi* spores. This study imputes cats as potential dispersing agents of zoonotic genotype D in Brazil. However, further studies should be carried out to confirm this hypothesis.

Knowledge about and identification of possible pathogenic agents transmitted by these animals is of major public health importance, especially to immunocompromised patients. Thus, to gain a better understanding of the zoonotic transmission of *E. bieneusi* in Brazil, new epidemiological investigations on cats and other animals are needed, as well as simultaneous studies on animals and humans living together in the same house.

# References

Abe N, Kimata I, Iseki M. Molecular evidence of *Enterocytozoon bieneusi* in Japan. *J Vet Med Sci* 2009; 71(2): 217-219. http://dx.doi.org/10.1292/jvms.71.217. PMid:19262036.

Abe N, Kimata I. Molecular survey of *Enterocytozoon bieneusi* in a Japanese porcine population. *Vector Borne Zoonotic Dis* 2010; 10(4): 425-427.

Agholi M, Hatam GR, Motazedian MH. HIV/AIDS-associated opportunistic protozoal diarrhea. *AIDS Res Hum Retroviruses* 2013a; 29(1): 35-41. http://dx.doi.org/10.1089/aid.2012.0119. PMid:22873400.

Agholi M, Hatam GR, Motazedian MH. Microsporidia and coccidia as causes of persistence diarrhea among liver transplant children: incidence

rate and species/genotypes. *Pediatr Infect Dis J* 2013b; 32(2): 185-187. http://dx.doi.org/10.1097/INF.0b013e318273d95f. PMid:22982981.

Akinbo FO, Omoregie R, Xiao L, Dearen T, Leon ET, Okaka CE. Molecular epidemiologic characterization of *Enterocytozoon bieneusi* in HIV-infected persons in Benin City, Nigeria. *Am J Trop Med Hyg* 2012; 86(3): 441-445. http://dx.doi.org/10.4269/ajtmh.2012.11-0548. PMid:22403314.

Ayinmode AB, Ojuromi OT, Xiao L. Molecular Identification of *Enterocytozoon bieneusi* Isolates from Nigerian Children. *J Parasitol Res* 2011; 2011: 1-2. http://dx.doi.org/10.1155/2011/129542. PMid:22132304.

Baroudi D, Khelef D, Hakem A, Abdelaziz A, Chen X, Lysen C, et al. Molecular characterization of zoonotic pathogens *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in calves in Algeria. *Vet Parasitol Reg Stud Rep* 2017; 8: 66-69.

Bern C, Kawai V, Vargas D, Rabke-Verani J, Williamson J, Chavez-Valdez R, et al. The epidemiology of intestinal microsporidiosis in patients with HIV/AIDS in Lima, Peru. *J Infect Dis* 2005; 191(10): 1658-1664. http://dx.doi.org/10.1086/429674. PMid:15838792.

Brasil P, Lima DB, Paiva DD, Lobo MS, Sodré FC, Silva SP, et al. Clinical and diagnostic aspects of intestinal microsporidiosis in HIV-infected patients with chronic diarrhea in Rio de Janeiro, Brazil. *Rev Inst Med Trop São Paulo* 2000; 42(6): 299-304. http://dx.doi.org/10.1590/S0036-46652000000600001. PMid:11136515.

Breton J, Bart-Delabesse E, Biligui S, Carbone A, Seiller X, Okome-Nkoumou M, et al. New highly divergent rRNA sequence among biodiverse genotypes of *Enterocytozoon bieneusi* strains isolated from humans in Gabon and Cameroon. *J Clin Microbiol* 2007; 45(8): 2580-2589. http://dx.doi.org/10.1128/JCM.02554-06. PMid:17537939.

Buckholt MA, Lee JH, Tzipori S. Prevalence of *Enterocytozoon bieneusi* in swine: an 18-month survey at a slaughterhouse in Massachusetts. *Appl Environ Microbiol* 2002; 68(5): 2595-2599. http://dx.doi.org/10.1128/AEM.68.5.2595-2599.2002. PMid:11976142.

Cama VA, Pearson J, Cabrera L, Pacheco L, Gilman R, Meyer S, et al. Transmission of *Enterocytozoon bieneusi* between a Child and Guinea Pigs. *J Clin Microbiol* 2007; 45(8): 2708-2710. http://dx.doi.org/10.1128/JCM.00725-07. PMid:17537930.

Chabchoub N, Abdelmalek R, Breton J, Kanoun F, Thellier M, Bouratbine A, et al. Genotype identification of *Enterocytozoon bieneusi* isolates from stool samples of HIV-infected Tunisian patients. *Parasite* 2012; 19(2): 147-151. http://dx.doi.org/10.1051/parasite/2012192147. PMid:22550625.

Chalifoux LV, Carville A, Pauley D, Thompson B, Lackner AA, Mansfield KG. *Enterocytozoon bieneusi* as a cause of proliferative serositis in simian immunodeficiency virus-infected immunodeficient macaques (*Macaca mulatta*). *Arch Pathol Lab Med* 2000; 124(10): 1480-1484. PMid:11035580.

Cunha MJ, Cury MC, Santín M. Widespread presence of human-pathogenic *Enterocytozoon bieneusi* genotypes in chickens. *Vet Parasitol* 2016; 217: 108-112. http://dx.doi.org/10.1016/j.vetpar.2015.12.019. PMid:26827870.

Cunha MJR, Cury MC, Santín M. Molecular identification of *Enterocytozoon bieneusi, Cryptosporidium*, and *Giardia* in Brazilian captive birds. *Parasitol Res* 2017; 116(2): 487-493. http://dx.doi.org/10.1007/s00436-016-5309-6. PMid:27815734.

Del Coco VF, Córdoba MA, Bilbao G, Almeida Castro P, Basualdo JA, Santín M. First report of *Enterocytozoon bieneusi* from dairy cattle in Argentina. *Vet Parasitol* 2014; 199(1-2): 112-115. http://dx.doi.org/10.1016/j.vetpar.2013.09.024. PMid:24126086.

Dengjel B, Zahler M, Hermanns W, Heinritzi K, Spillmann T, Thomschke A, et al. Zoonotic potential of *Enterocytozoon bieneusi. J Clin Microbiol* 2001; 39(12): 4495-4499. http://dx.doi.org/10.1128/JCM.39.12.4495-4499.2001. PMid:11724868.

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(5): 93S. http://dx.doi.org/10.1111/j.1550-7408.1996.tb05018.x. PMid:8822883.

Desportes I, Le Charpentier Y, Galian A, Bernard F, Cochand-Priollet B, Lavergne A, et al. Occurrence of a new microsporidan: *Enterocytozoon bieneusi* n.g., n. sp., in the enterocytes of a human patient with AIDS. *J Protozool* 1985; 32(2): 250-254. http://dx.doi.org/10.1111/j.1550-7408.1985.tb03046.x. PMid:4009510.

Espern A, Morio F, Miegeville M, Illa H, Abdoulaye M, Meyssonnier V, et al. Molecular study of microsporidiosis due to *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* among human immunodeficiency virus-infected patients from two geographical areas: Niamey, Niger, and Hanoi, Vietnam. *J Clin Microbiol* 2007; 45(9): 2999-3002. http://dx.doi.org/10.1128/JCM.00684-07. PMid:17634305.

Fayer R, Santín M, Macarisin D. Detection of concurrent infection of dairy cattle with *Blastocystis, Cryptosporidium, Giardia*, and *Enterocytozoon* by molecular and microscopic methods. *Parasitol Res* 2012; 111(3): 1349-1355. http://dx.doi.org/10.1007/s00436-012-2971-1. PMid:22710524.

Fayer R, Santín M, Trout JM. *Enterocytozoon bieneusi* in mature dairy cattle on farms in the eastern United States. *Parasitol Res* 2007; 102(1): 15-20. http://dx.doi.org/10.1007/s00436-007-0746-x. PMid:17899197.

Fayer R, Santín M. Epidemiology of Microsporidia in human infections. In: Weiss LM, Becnel JJ. *Microsporidia: pathogens of opportunity*. 1st ed. Hoboken: John Wiley & Sons, Inc. 2014. p. 135-164. http://dx.doi.org/10.1002/9781118395264.ch3.

Feng Y, Li N, Dearen T, Lobo ML, Matos O, Cama V, et al. Development of a multilocus sequence typing tool for High-Resolution Genotyping of *Enterocytozoon bieneusi. Appl Environ Microbiol* 2011; 77(14): 4822-4828. http://dx.doi.org/10.1128/AEM.02803-10. PMid:21622791.

Fiuza VRS, Lopes CWG, Cosendey RIJ, Oliveira FCR, Fayer R, Santín M. Zoonotic *Enterocytozoon bieneusi* genotypes found in brazilian sheep. *Res Vet Sci* 2016a; 107: 196-201. http://dx.doi.org/10.1016/j.rvsc.2016.06.006. PMid:27473995.

Fiuza VRS, Lopes CW, Oliveira FC, Fayer R, Santin M. New findings of *Enterocytozoon bieneusi* in beef and dairy cattle in Brazil. *Vet Parasitol* 2016b; 216: 46-51. http://dx.doi.org/10.1016/j.vetpar.2015.12.008. PMid:26801594.

Fiuza VRS, Oliveira FCR, Fayer R, Santín M. First report of *Enterocytozoon bieneusi* in pigs in Brazil. *Parasitol Int* 2015; 64(4): 18-23. http://dx.doi.org/10.1016/j.parint.2015.01.002. PMid:25582928.

Galván AL, Sánchez AM, Valentín MA, Henriques-Gil N, Izquierdo F, Fenoy S, et al. First cases of microsporidiosis in transplant recipients in spain and review of the literature. *J Clin Microbiol* 2011; 49(4): 1301-1306. http://dx.doi.org/10.1128/JCM.01833-10. PMid:21325545.

Galván-Díaz AL, Magnet A, Fenoy S, Henriques-Gil N, Haro M, Gordo FP, et al. Microsporidia detection and genotyping study of human pathogenic *E. bieneusi* in animals from Spain. *PLoS One* 2014; 9(3): e92289. http://dx.doi.org/10.1371/journal.pone.0092289. PMid:24651457.

Guo Y, Alderisio KA, Yang W, Cama V, Feng Y, Xiao L. Host specificity and source of *Enterocytozoon bieneusi* genotypes in a drinking source

watershed. *Appl Environ Microbiol* 2014; 80(1): 218-225. http://dx.doi.org/10.1128/AEM.02997-13. PMid:24141128.

Jamshidi S, Tabrizi AS, Bahrami M, Momtaz H. Microsporidia in household dogs and cats in Iran; a zoonotic concern. *Vet Parasitol* 2012; 185(2-4): 121-123. http://dx.doi.org/10.1016/j.vetpar.2011.10.002. PMid:22035849.

Jiang Y, Tao W, Wan Q, Li Q, Yang Y, Lin Y, et al. Zoonotic and potentially host-adapted *Enterocytozoon bieneusi* genotypes in sheep and cattle in northeast china and an increasing concern about the Zoonotic importance of previously considered Ruminant-Adapted Genotypes. *Appl Environ Microbiol* 2015; 81(10): 3326-3335. http://dx.doi.org/10.1128/AEM.00328-15. PMid:25746997.

Juránková J, Kamler M, Kovarcik K, Koudela B. *Enterocytozoon bieneusi* in Bovine Viral Diarrhea Virus (BVDV) infected and noninfected cattle herds. *Res Vet Sci* 2013; 94(1): 100-104. http://dx.doi.org/10.1016/j.rvsc.2012.07.016. PMid:22858000.

Karim MR, Dong H, Li T, Yu F, Li D, Zhang L, et al. Predomination and new genotypes of *Enterocytozoon bieneusi* in captive nonhuman primates in zoos in China: high genetic diversity and zoonotic significance. *PLoS One* 2015; 10(2): e0117991. http://dx.doi.org/10.1371/journal.pone.0117991. PMid:25705879.

Karim MR, Dong H, Yu F, Jian F, Zhang L, Wang R, et al. Genetic diversity in *Enterocytozoon bieneusi* isolates from dogs and cats in China: host specificity and public health implications. *J Clin Microbiol* 2014a; 52(9): 3297-3302. http://dx.doi.org/10.1128/JCM.01352-14. PMid:24989604.

Karim MR, Wang R, Dong H, Zhang L, Li J, Zhang S, et al. Genetic polymorphism and zoonotic potential of *Enterocytozoon bieneusi* from nonhuman primates in China. *Appl Environ Microbiol* 2014b; 80(6): 1893-1898. http://dx.doi.org/10.1128/AEM.03845-13. PMid:24413605.

Karim MR, Wang R, He X, Zhang L, Li J, Rume FI, et al. Multilocus sequence typing of *Enterocytozoon bieneusi* in nonhuman primates in China. *Vet Parasitol* 2014c; 200(1-2): 13-23. http://dx.doi.org/10.1016/j.vetpar.2013.12.004. PMid:24388499.

Karim MR, Yu F, Li J, Li J, Zhang L, Wang R, et al. First molecular characterization of enteric protozoa and the human pathogenic microsporidian, *Enterocytozoon bieneusi*, in captive snakes in China. *Parasitol Res* 2014d; 113(8): 3041-3048. http://dx.doi.org/10.1007/s00436-014-3967-9. PMid:24906991.

Kicia M, Wesolowska M, Jakuszko K, Kopacz Z, Sak B, Kvetonova D, et al. Concurrent infection of the urinary tract with *Encephalitozoon cuniculi* and *Enterocytozoon bieneusi* in a renal transplant recipient. *J Clin Microbiol* 2014; 52(5): 1780-1782. http://dx.doi.org/10.1128/JCM.03328-13. PMid:24523472.

Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 2016; 33(7): 1870-1874. http://dx.doi.org/10.1093/molbev/msw054. PMid:27004904.

Laatamna AE, Wagnerova P, Sak B, Kvetonova D, Xiao L, Rost M, et al. Microsporidia and *Cryptosporidium* in horses and donkeys in Algeria: detection of a novel *Cryptosporidium hominis* subtype family (Ik) in a horse. *Vet Parasitol* 2015; 208(3-4): 135-142. http://dx.doi.org/10.1016/j.vetpar.2015.01.007. PMid:25638716.

Lallo MA, Calabria P, Milanelo L. *Encephalitozoon* and *Enterocytozoon (*microsporidia) spores in stool from pigeons and exotic birds: microsporidia spores in birds. *Vet Parasitol* 2012; 190(3-4): 418-422. http://dx.doi.org/10.1016/j.vetpar.2012.06.030. PMid:22853863.

Lee JH. Molecular detection of *Enterocytozoon bieneusi* and identification of a potentially human-pathogenic genotype in milk. *Appl Environ Microbiol* 2008; 74(5): 1664-1666. http://dx.doi.org/10.1128/AEM.02110-07. PMid:18192409.

Lee JH. Prevalence and molecular characteristics of *Enterocytozoon bieneusi* in cattle in Korea. *Parasitol Res* 2007; 101(2): 391-396.

Leelayoova S, Subrungruang I, Suputtamongkol Y, Worapong J, Petmitr PC, Mungthin M. Identification of genotypes of *Enterocytozoon bieneusi* from stool samples from human immunodeficiency virus-infected patients in Thailand. *J Clin Microbiol* 2006; 44(8): 3001-3004. http://dx.doi.org/10.1128/JCM.00945-06. PMid:16891527.

Li J, Luo N, Wang C, Qi M, Cao J, Cui Z, et al. Occurrence, molecular characterization and predominant genotypes of *Enterocytozoon bieneusi* in dairy cattle in Henan and Ningxia, China. *Parasit Vectors* 2016a; 9(1): 142. http://dx.doi.org/10.1186/s13071-016-1425-5. PMid:26968376.

Li W, Cama V, Akinbo FO, Ganguly S, Kiulia NM, Zhang X, et al. Multilocus sequence typing of *Enterocytozoon bieneusi*: Lack of geographic segregation and existence of genetically isolated sub-populations. *Infect Genet Evol* 2013; 14: 111-119. http://dx.doi.org/10.1016/j.meegid.2012.11.021. PMid:23262155.

Li W, Deng L, Yu X, Zhong Z, Wang Q, Liu X, et al. Multilocus genotypes and broad host-range of *Enterocytozoon bieneusi* in captive wildlife at zoological gardens in China. *Parasit Vectors* 2016b; 9(1): 395. http://dx.doi.org/10.1186/s13071-016-1668-1. PMid:27391225.

Li W, Diao R, Yang J, Xiao L, Lu Y, Li Y, et al. High diversity of human-pathogenic *Enterocytozoon bieneusi* genotypes in swine in northeast China. *Parasitol Res* 2014a; 113(3): 1147-1153. http://dx.doi.org/10.1007/s00436-014-3752-9. PMid:24442159.

Li W, Kiulia NM, Mwenda JM, Nyachieo A, Taylor MB, Zhang X, et al. *Cyclospora papionis, Cryptosporidium hominis*, and human-pathogenic *Enterocytozoon bieneusi* in captive baboons in Kenya. *J Clin Microbiol* 2011; 49(12): 4326-4329. http://dx.doi.org/10.1128/JCM.05051-11. PMid:21956988.

Li W, Li Y, Li W, Yang J, Song M, Diao R, et al. Genotypes of *Enterocytozoon bieneusi* in livestock in China: high prevalence and zoonotic potential. *PLoS One* 2014b; 9(5): e97623. http://dx.doi.org/10.1371/journal.pone.0097623. PMid:24845247.

Li W, Li Y, Song M, Lu Y, Yang J, Tao W, et al. Prevalence and genetic characteristics of *Cryptosporidium, Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. *Vet Parasitol* 2015; 208(3-4): 125-134. http://dx.doi.org/10.1016/j.vetpar.2015.01.014. PMid:25665462.

Liguory O, David F, Sarfati C, Derouin F, Molina JM. Determination of Types of *Enterocytozoon bieneusi* Strains Isolated from Patients with Intestinal Microsporidiosis. *J Clin Microbiol* 1998; 36(7): 1882-1885. PMid:9650929.

Liguory O, Sarfati C, Derouin F, Molina JM. Evidence of different *Enterocytozoon bieneusi* genotypes in patients with and without human immunodeficiency virus infection. *J Clin Microbiol* 2001; 39(7): 2672-2674. http://dx.doi.org/10.1128/JCM.39.7.2672-2674.2001. PMid:11427592.

Lobo ML, Augusto J, Antunes F, Ceita J, Xiao L, Codices V, et al. *Cryptosporidium* spp., *Giardia duodenalis, Enterocytozoon bieneusi* and Other Intestinal Parasites in Young Children in Lobata Province, Democratic Republic of São Tomé and Principe. *PLoS One* 2014; 9(5): e97708. http://dx.doi.org/10.1371/journal.pone.0097708. PMid:24846205.

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. *Int J Parasitol* 2012; 42(2): 197-205. http://dx.doi.org/10.1016/j.ijpara.2011.12.002. PMid:22265899.

Lobo ML, Xiao L, Cama V, Stevens T, Antunes F, Matos O. Genotypes of *Enterocytozoon bieneusi* in mammals in Portugal. *J Eukaryot Microbiol* 2006;53(s1 Suppl 1): S61-S64. http://dx.doi.org/10.1111/j.1550-7408.2006.00174.x. PMid:17169069.

Ma J, Cai J, Ma J, Feng Y, Xiao L. *Enterocytozoon bieneusi* genotypes in yaks (*Bos grunniens*) and their public health potential. *J Eukaryot Microbiol* 2015a; 62(1): 21-25. http://dx.doi.org/10.1111/jeu.12141. PMid:25040451.

Ma J, Li P, Zhao X, Xu H, Wu W, Wang Y, et al. Occurrence and molecular characterization of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in dairy cattle, beef cattle and water buffaloes in China. *Vet Parasitol* 2015b; 207(3-4): 220-227. http://dx.doi.org/10.1016/j.vetpar.2014.10.011. PMid:25541482.

Maikai BV, Umoh JU, Lawal IA, Kudi AC, Ejembi CL, Xiao L. Molecular characterizations of *Cryptosporidium, Giardia*, and *Enterocytozoon* in humans in Kaduna State, Nigeria. *Exp Parasitol* 2012; 131(4): 452-456. http://dx.doi.org/10.1016/j.exppara.2012.05.011. PMid:22664352.

Mathis A, Breitenmoser AC, Deplazes P. Detection of new *Enterocytozoon* genotypes in faecal samples of farm dogs and a cat. *Parasite* 1999; 6(2): 189-193. http://dx.doi.org/10.1051/parasite/1999062189. PMid:10416194.

Mathis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. *Clin Microbiol Rev* 2005; 18(3): 423-445. http://dx.doi.org/10.1128/CMR.18.3.423-445.2005. PMid:16020683.

Mori H, Mahittikorn A, Thammasonthijarern N, Chaisiri K, Rojekittikhun W, Sukthana Y. Presence of zoonotic *Enterocytozoon bieneusi* in cats in a temple in central Thailand. *Vet Parasitol* 2013; 197(3-4): 696-701. http://dx.doi.org/10.1016/j.vetpar.2013.07.025. PMid:23932454.

Müller MG, Kinne J, Schuster RK, Walochnik J. Outbreak of microsporidiosis caused by *Enterocytozoon bieneusi* in falcons. *Vet Parasitol* 2008; 152(1-2): 67-78. http://dx.doi.org/10.1016/j.vetpar.2007.11.019. PMid:18166273.

Němejc K, Sak B, Kvetonova D, Hanzal V, Janiszewski P, Forejtek P, et al. Prevalence and diversity of *Encephalitozoon* spp. and *Enterocytozoon bieneusi* in wild boars (*Sus scrofa*) in Central Europe. *Parasitol Res* 2014; 113(2): 761-767. http://dx.doi.org/10.1007/s00436-013-3707-6. PMid:24292543.

Piekarska J, Kicia M, Wesołowska M, Kopacz Ż, Gorczykowski M, Szczepankiewicz B, et al. Zoonotic microsporidia in dogs and cats in Poland. *Vet Parasitol* 2017; 246: 108-111. http://dx.doi.org/10.1016/j.vetpar.2017.09.011. PMid:28969771.

Pirestani M, Sadraei J, Forouzandeh M. Molecular characterization and genotyping of human related microsporidia in free-ranging and captive pigeons of Tehran, Iran. *Infect Genet Evol* 2013; 20: 495-499. http://dx.doi.org/10.1016/j.meegid.2013.10.007. PMid:24427811.

Prasertbun R, Mori H, Pintong AR, Sanyanusin S, Popruk S, Komalamisra C, et al. Zoonotic potential of *Enterocytozoon* genotypes in humans and pigs in Thailand. *Vet Parasitol* 2017; 233: 73-79. http://dx.doi.org/10.1016/j.vetpar.2016.12.002. PMid:28043391.

Qi M, Jing B, Jian F, Wang R, Zhang S, Wang H, et al. Dominance of *Enterocytozoon bieneusi* genotype J in dairy calves in Xinjiang, Northwest China. *Parasitol Int* 2017; 66(1): 960-963. http://dx.doi.org/10.1016/j.parint.2016.10.019. PMid:27794506.

Qi M, Wang R, Wang H, Jian F, Li J, Zhao J, et al. *Enterocytozoon bieneusi* Genotypes in grazing horses in China and their zoonotic transmission potential. *J Eukaryot Microbiol* 2016; 63(5): 591-597. http://dx.doi.org/10.1111/jeu.12308. PMid:26909747.

Queen EV, Marks SL, Farver TB. Prevalence of Selected bacterial and parasitic agents in feces from diarrheic and healthy control cats from Northern California. *J Vet Intern Med* 2012; 26(1): 54-60. http://dx.doi.org/10.1111/j.1939-1676.2011.00843.x. PMid:22182203.

Rinder H, Thomschke A, Dengjel B, Gothe R, Loscher T, Zahler M. Close genotypic relationship between *Enterocytozoon bieneusi* from humans and pigs and first detection in cattle. *J Parasitol* 2000; 86(1): 185-188. http://dx.doi.org/10.1645/0022-3395(2000)086[0185:CGRBEB]2.0.CO;2. PMid:10701590.

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(1): 39-42. http://dx.doi.org/10.1053/jinf.2001.0934. PMid:11972417.

Sak B, Kvac M, Hanzlikova D, Cama V. First report of *Enterocytozoon bieneusi* infection on a pig farm in the Czech Republic. *Vet Parasitol* 2008; 153(3-4): 220-224. http://dx.doi.org/10.1016/j.vetpar.2008.01.043. PMid:18342450.

Sak B, Kvac M, Kvetonova D, Albrecht T, Pialek J. The first report on natural *Enterocytozoon bieneusi* and *Encephalitozoon* spp. infections in wild East-European House Mice (*Mus musculus musculus*) and West-European House Mice (*M. m. domesticus*) in a hybrid zone across the Czech Republic-Germany border. *Vet Parasitol* 2011; 178(3-4): 246-250. http://dx.doi.org/10.1016/j.vetpar.2010.12.044. PMid:21269775.

Saksirisampant W, Prownebon J, Saksirisampant P, Mungthin M, Siripatanapipong S, Leelayoova S. Intestinal parasitic infections: prevalences in HIV/AIDS patients in a Thai AIDS-care centre. *Ann Trop Med Parasitol* 2009; 103(7): 573-581. http://dx.doi.org/10.1179/000349809X12502035776072. PMid:19825279.

Samra NA, Thompson PN, Jori F, Zhang H, Xiao L. *Enterocytozoon bieneusi* at the wildlife/livestock interface of the Kruger National Park, South Africa. *Vet Parasitol* 2012; 190(3-4): 587-590. http://dx.doi.org/10.1016/j.vetpar.2012.06.031. PMid:22824060.

Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977; 74(12): 5463-5467. http://dx.doi.org/10.1073/pnas.74.12.5463. PMid:271968.

Santín M, Cortes Vecino JA, Fayer R. *Enterocytozoon bieneusi* genotypes in dogs in Bogota, Colombia. *Am J Trop Med Hyg* 2008; 79(2): 215-217. http://dx.doi.org/10.4269/ajtmh.2008.79.215. PMid:18689627.

Santín M, Dargatz D, Fayer R. Prevalence and genotypes of *Enterocytozoon bieneusi* in weaned beef calves on cow-calf operations in the USA. *Parasitol Res* 2012; 110(5): 2033-2041. http://dx.doi.org/10.1007/s00436-011-2732-6. PMid:22146995.

Santín M, Fayer R. A longitudinal study of *Enterocytozoon bieneusi* in dairy cattle. *Parasitol Res* 2009; 105(1): 141-144. http://dx.doi.org/10.1007/s00436-009-1374-4. PMid:19259701.

Santín M, Fayer R. Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. *Res Vet Sci* 2011; 90(3): 363-371. http://dx.doi.org/10.1016/j.rvsc.2010.07.014. PMid:20699192.

Santín M, Fayer R. *Enterocytozoon bieneusi, Giardia*, and *Cryptosporidium* infecting white-tailed deer. *J Eukaryot Microbiol* 2015; 62(1): 34-43. http://dx.doi.org/10.1111/jeu.12155. PMid:25066778.

Santín M, Trout JM, Fayer R. *Enterocytozoon bieneusi* genotypes in dairy cattle in the eastern United States. *Parasitol Res* 2005; 97(6): 535-538. http://dx.doi.org/10.1007/s00436-005-1482-8. PMid:16167161.

Santín M, Trout JM, Vecino JAC, Dubey JP, Fayer R. *Cryptosporidium, Giardia* and *Enterocytozoon bieneusi* in cats from Bogota (Colombia) and genotyping of isolates. *Vet Parasitol* 2006; 141(3-4): 334-339. http://dx.doi.org/10.1016/j.vetpar.2006.06.004. PMid:16860480.

Santín M, Vecino JA, Fayer R. A zoonotic genotype of *Enterocytozoon bieneusi* in horses. *J Parasitol* 2010; 96(1): 157-161. http://dx.doi.org/10.1645/GE-2184.1. PMid:19799490.

Santín M. Enterocytozoon bieneusi. In: Xiao L, Ryan U, Feng Y. *Biology of foodborne parasites*. United States of America: CRC Press; 2015. p. 149-174.

Sarfati C, Ngole EM, Delaporte E, Moyou-Somo R, Molina J-M, Derouin F, et al. 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. http://dx.doi.org/10.4269/ajtmh.2006.74.162. PMid:16407362.

Shi K, Li M, Wang X, Li J, Karim MR, Wang R, et al. Molecular survey of *Enterocytozoon bieneusi* in sheep and goats in China. *Parasit Vectors* 2016; 9(1): 23. http://dx.doi.org/10.1186/s13071-016-1304-0. PMid:26782742.

Sokolova OI, Demyanov AV, Bowers LC, Didier ES, Yakovlev AV, Skarlato SO, et al. Emerging microsporidian infections in Russian HIV-infected patients. *J Clin Microbiol* 2011; 49(6): 2102-2108. http://dx.doi.org/10.1128/JCM.02624-10. PMid:21450962.

Stensvold CR, Beser J, Ljungström B, Troell K, Lebbad M. Low host-specific *Enterocytozoon bieneusi* genotype BEB6 is common in Swedish lambs. *Vet Parasitol* 2014; 205(1-2): 371-374. http://dx.doi.org/10.1016/j.vetpar.2014.06.010. PMid:24986434.

Sulaiman IM, Bern C, Gilman R, Cama V, Kawai V, Vargas D, et al. A molecular biologic study of *Enterocytozoon bieneusi* in HIV-infected patients in Lima, Peru. *J Eukaryot Microbiol* 2003a;50(s1 Suppl): 591-596. http://dx.doi.org/10.1111/j.1550-7408.2003.tb00642.x. PMid:14736175.

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* 2003b; 69(8): 4495-4501. http://dx.doi.org/10.1128/AEM.69.8.4495-4501.2003. PMid:12902234.

Sulaiman IM, Fayer R, Yang C, Santín M, Matos O, Xiao L. Molecular characterization of *Enterocytozoon bieneusi* in cattle indicates that only some isolates have zoonotic potential. *Parasitol Res* 2004; 92(4): 328-334. http://dx.doi.org/10.1007/s00436-003-1049-5. PMid:14727187.

ten Hove RJ, Van Lieshout L, Beadsworth MB, Perez MA, Spee K, Claas EC, et al. Characterization of genotypes of *Enterocytozoon bieneusi* in immunosuppressed and immunocompetent patient groups. *J Eukaryot Microbiol* 2009; 56(4): 388-393. http://dx.doi.org/10.1111/j.1550-7408.2009.00393.x. PMid:19602086.

Tumwine JK, Kekitiinwa A, Tzipori S, Buckholt MA, Akiyoshi DE, Nabukeera N. *Enterocytozoon bieneusi* among children with diarrhea attending Mulago Hospital in Uganda. *Am J Trop Med Hyg* 2002; 67(3): 299-303. http://dx.doi.org/10.4269/ajtmh.2002.67.299. PMid:12408671.

Vávra J, Lukeš J. Microsporidia and 'The Art of Living Together'. In: Rollinson D. *Advances in parasitology*. United States of America: Academic Press; 2013. p. 253-319.

Wagnerová P, Sak B, Kvetonova D, Bunatova Z, Civisova H, Marsalek M, et al. *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* in horses kept under different management systems in the Czech Republic. *Vet Parasitol* 2012; 190(3-4): 573-577. http://dx.doi.org/10.1016/j.vetpar.2012.07.013. PMid:22877825.

Wang L, Xiao L, Duan L, Ye J, Guo Y, Guo M, et al. Concurrent infections of *Giardia duodenalis, Enterocytozoon bieneusi*, and *Clostridium difficile* in children during a cryptosporidiosis outbreak in a pediatric hospital in China. *PLoS Negl Trop Dis* 2013a; 7(9): e2437. http://dx.doi.org/10.1371/journal.pntd.0002437. PMid:24069491.

Wang L, Zhang H, Zhao X, Zhang L, Zhang G, Guo M, et al. Zoonotic *Cryptosporidium* species and *Enterocytozoon bieneusi* genotypes in HIV-positive patients on antiretroviral therapy. *J Clin Microbiol* 2013b; 51(2): 557-563. http://dx.doi.org/10.1128/JCM.02758-12. PMid:23224097.

Wang SS, Wang RJ, Fan XC, Liu TL, Zhang LX, Zhao GH. Prevalence and genotypes of *Enterocytozoon bieneusi* in China. *Acta Trop* 2018; 183: 142-152. http://dx.doi.org/10.1016/j.actatropica.2018.04.017. PMid:29660311.

Wumba R, Jean M, Benjamin LM, Madone M, Fabien K, Josué Z, et al. *Enterocytozoon bieneusi* Identification Using Real-Time Polymerase Chain Reaction and Restriction Fragment Length Polymorphism in HIV-Infected Humans from Kinshasa Province of the Democratic Republic of Congo. *J Parasitol Res* 2012; 2012: 278028. http://dx.doi.org/10.1155/2012/278028. PMid:22811884.

Xu C, Ma X, Zhang H, Zhang XX, Zhao JP, Ba HX, et al. Prevalence, risk factors and molecular characterization of *Enterocytozoon bieneusi* in raccoon dogs (*Nyctereutes procyonoides*) in five provinces of Northern China. *Acta Trop* 2016a; 161: 68-72. http://dx.doi.org/10.1016/j.actatropica.2016.05.015. PMid:27260667.

Xu H, Jin Y, Wu W, Li P, Wang L, Li N, et al. Genotypes of *Cryptosporidium* spp., *Enterocytozoon bieneusi* and *Giardia duodenalis* in dogs and cats in Shanghai, China. *Parasit Vectors* 2016b; 9(1): 121. http://dx.doi.org/10.1186/s13071-016-1409-5. PMid:26932267.

Yamashiro S, Fiuza VRS, Teixeira ÂTLS, Branco N, Levy CE, Castro ICVS, et al. *Enterocytozoon bieneusi* detected by molecular methods in raw sewage and treated effluent from a combined system in Brazil. *Mem Inst Oswaldo Cruz* 2017; 112(6): 403-410. http://dx.doi.org/10.1590/0074-02760160435. PMid:28591400.

Yang Y, Lin Y, Li Q, Zhang S, Tao W, Wan Q, et al. Widespread presence of human-pathogenic *Enterocytozoon bieneusi* genotype D in farmed foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) in China: first identification and zoonotic concern. *Parasitol Res* 2015; 114(11): 4341-4348. http://dx.doi.org/10.1007/s00436-015-4714-6. PMid:26341801.

Ye J, Xiao L, Li J, Huang W, Amer SE, Guo Y, et al. Occurrence of human-pathogenic *Enterocytozoon bieneusi, Giardia duodenalis* and *Cryptosporidium* genotypes in laboratory macaques in Guangxi, China. *Parasitol Int* 2014; 63(1): 132-137. http://dx.doi.org/10.1016/j.parint.2013.10.007. PMid:24157444.

Ye J, Xiao L, Ma J, Guo M, Liu L, Feng Y. Anthroponotic enteric parasites in monkeys in public park, China. *Emerg Infect Dis* 2012; 18(10): 1640-1643. http://dx.doi.org/10.3201/eid1810.120653. PMid:23017776.

Ye J, Xiao L, Wang Y, Guo Y, Roellig DM, Feng Y. Dominance of *Giardia duodenalis* assemblage A and *Enterocytozoon bieneusi* genotype BEB6 in sheep in Inner Mongolia, China. *Vet Parasitol* 2015; 210(3-4): 235-239. http://dx.doi.org/10.1016/j.vetpar.2015.04.011. PMid:25944405.

Zhang X, Wang Z, Su Y, Liang X, Sun X, Peng S, et al. Identification and Genotyping of Enterocytozoon bieneusi in China. *J Clin Microbiol* 2011; 49(5): 2006-2008. http://dx.doi.org/10.1128/JCM.00372-11. PMid:21389159.

Zhao GH, Du SZ, Wang HB, Hu XF, Deng MJ, Yu SK, et al. First report of zoonotic *Cryptosporidium* spp., *Giardia intestinalis* and *Enterocytozoon bieneusi* in golden takins (*Budorcas taxicolor bedfordi*). *Infect Genet Evol* 2015a; 34: 394-401. http://dx.doi.org/10.1016/j.meegid.2015.07.016. PMid:26190449.

Zhao W, Zhang W, Wang R, Liu W, Liu A, Yang D, et al. *Enterocytozoon bieneusi* in sika deer (*Cervus nippon*) and red deer (*Cervus elaphus*): deer specificity and zoonotic potential of ITS genotypes. *Parasitol Res* 2014a; 113(11): 4243-4250. http://dx.doi.org/10.1007/s00436-014-4100-9. PMid:25185666.

Zhao W, Zhang W, Yang D, Zhang L, Wang R, Liu A. Prevalence of *Enterocytozoon bieneusi* and genetic diversity of ITS genotypes in sheep and goats in China. *Infect Genet Evol* 2015b; 32: 265-270. http://dx.doi.org/10.1016/j.meegid.2015.03.026. PMid:25818401.

Zhao W, Zhang W, Yang F, Cao J, Liu H, Yang D, et al. High prevalence of *Enterocytozoon bieneusi* in asymptomatic pigs and assessment of zoonotic risk at the genotype level. *Appl Environ Microbiol* 2014b; 80(12): 3699-3707. http://dx.doi.org/10.1128/AEM.00807-14. PMid:24727270.

Zhao W, Zhang W, Yang F, Zhang L, Wang R, Cao J, et al. *Enterocytozoon bieneusi* in dairy cattle in the Northeast of China: Genetic diversity of ITS Gene and Evaluation of Zoonotic Transmission Potential. *J Eukaryot Microbiol* 2015c; 62(4): 553-560. http://dx.doi.org/10.1111/jeu.12210. PMid:25712195.

Zhao W, Zhang W, Yang Z, Liu A, Zhang L, Yang F, et al. Genotyping of *Enterocytozoon bieneusi* in Farmed Blue Foxes (*Alopex lagopus*) and Raccoon Dogs (*Nyctereutes procyonoides*) in China. *PLoS One* 2015d; 10(11): e0142611. http://dx.doi.org/10.1371/journal.pone.0142611. PMid:26544711.