Veterinary Parasitology 217 (2016) 108–112

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Widespread presence of human-pathogenic *Enterocytozoon bieneusi* genotypes in chickens

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ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form
15 December 2015
Accepted 21 December 2015

Keywords:
Brazil
Birds
Enterocytozoon bieneusi
Genotypes
PCR

ABSTRACT

A total of 151 fecal specimens from chickens were randomly collected from local markets in Uberlândia and Belo Horizonte in the state of Minas Gerais, Brazil, to evaluate the presence of *Enterocytozoon bieneusi* by polymerase chain reaction (PCR). *Enterocytozoon bieneusi* was identified in 24 fecal samples (15.9%). This represents the first report of *E. bieneusi* in chickens in Brazil. All PCR-positive specimens were sequenced and 4 genotypes were identified, Peru 6, Peru 11, Type IV, and D. All four genotypes have previously been reported as human pathogens and are potentially zoonotic. Our results demonstrate that human-pathogenic *E. bieneusi* genotypes are present in chickens in Brazil, corroborating their potential role as a source of human infection and environmental contamination.

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1. Introduction

Microsporidia are a ubiquitous group of obligate intracellular parasites that infect all major animal groups, vertebrates and invertebrates, consisting of more than 1200 species. At least 17 are known to be pathogenic for human including among others *Enterocytozoon bieneusi*, *Encephalitozoon cuniculi*, *Encephalitozoon intestinalis* and *Encephalitozoon hellem* (Fayer and Santín, 2014). Among those pathogenic for humans, *E. bieneusi* is the most prevalent species in humans worldwide (Santín, 2015). It is an opportunistic parasite responsible of complications in immune deficient patients (Mathis et al., 2005). Gastrointestinal tract is the main localization with chronic diarrhea as the most frequent clinical manifestation of the infection.

E. bieneusi has also been found in a broad range of domestic and wild animals raising the question on the importance of animal reservoirs in the epidemiology of this parasite (Santín and Fayer, 2011). The identification of spores of *E. bieneusi* in water supplies suggests it can be a potential vehicle in the transmission of this parasite not only for humans but also for animals (Galván et al., 2013; Guo et al., 2014). Moreover, *E. bieneusi* has been detected in retail fresh food produce (raspberries, sprouts, and lettuce) (Jedrzejewski et al., 2007) and it has been found to be the cause of a large foodborne outbreak in Sweden (Decraene et al., 2012). Thus, transmission occurs mainly through fecal-oral routes, with sources including other infected humans or animals, contaminated water and food (Santín, 2015). However, not all *E. bieneusi* from animals have zoonotic potential; the use of nucleotide sequence analysis of the internal transcriber spacer (ITS) of the rRNA gene has revealed a considerable genetic diversity within isolates of human and animal origin with thus far more than 200 genotypes described. Some genotypes have been identified as host-adapted to humans or animals while others do not appear to have host specificity infecting both humans and animals. The later genotypes when found in human and animals and are thus considered zoonotic.

Information on birds is very scarce. The first case of *E. bieneusi* in birds, and also first in a non-mammalian host, was detected in 2 chickens (*Gallus gallus*) at a poultry abattoir in Germany (Reetz et al., 2002). Since then, there have been only two other reports in chickens from Peru (Feng et al., 2011), and China (Li et al., 2014). *E. bieneusi* has also been found in other bird species of the orders Columbiformes, Passeriformes, Psittaciformes, Struthioniformes, and Falconiformes suggesting that birds may play an important role in transmission of *this parasite* to humans (Haro et al., 2005,

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http://dx.doi.org/10.1016/j.vetpar.2015.12.019
0304-4017/Published by Elsevier B.V.

Table 1
Enterocytozoon bieneusi genotypes reported in birds. Bold denotes genotypes found in this study.

Genotype (synonyms)	Avian order (species)	Country	References
A*	Psittaciformes (*Agapornis roseicollis*, *Agapornis personata*, *Amazona leucocephala*, *Melopsittacus undulatus*, *Nymphicus hollandicus*, *Myiopsitta monachus*, *Alisterus scapularis*, *Polytelis swainsonii*, *Pyrrhura* sp., *Platycercus elegans*, *Platycercus eximius*) Passeriformes (*Serinus canaria*, *Temenuchus pagodarum*) Columbiformes (*Ocyphaps lophotes*)	Czech Republic	Kasicková et al. (2009)
Type IV*	Struthioniformes (*Struthio camelus*)	Spain	Galván-Díaz et al. (2014)
	Galliformes (*Gallus gallus*)	Brazil	(This study)
Peru6*	Psittaciformes (*Agapornis* sp.) Columbiformes (*Columba livia*)	Portugal	Lobo et al. (2006)
	Galliformes (*Gallus gallus*)	Brazil	(This study)
D* (CEbC, Peru 9, PigEBITS9, PtEbVI, WL8)	Falconiformes (*Falco* sp.)	Abu Dhabi	Müller et al. (2008)
	Columbiformes (*Columba livia*)	Iran	Pirestani et al. (2013)
	Galliformes (*Gallus gallus*)	Brazil	(This study)
Peru8*	Galliformes (*Gallus gallus*)	Peru	Feng et al. (2011)
EbpA* (F)	Psittaciformes (*Amazona aestiva*,*Melopsittacus undulates*) Passeriformes (*Sicalis flaveola*) Columbiformes (*Columba livia*)	Brazil	Lallo et al. (2012)
	Psittaciformes (*Agapornis fischeri*, *Agapornis nigrigenis*, *Agapornis cana* *Agapornis personata*, *Melopsittacus undulatus*, *Aratinga acuticaudata*, *Aratinga mitrata*, *Aratinga auricapilla*, *Barnardius zonarius*, *Cyanoramphus novaezelandiae*, *Nandayus nenday*, *Neophema splendida*, *Neophema pulchella*, *Polytelis alexandrae*, *Poicephalus senegalus*, *Psittacus erithacus*, *Pyrrhura* sp., *Platycercus elegans*, *Platycercus eximius*, *Platycercus caledonicus*) Columbiformes (*Geopelia cuneata*)	Czech Republic	Kasicková et al. (2009)
J*	Galliformes (*Gallus gallus*)	Germany	Reetz et al. (2002)
(BEB1, CEbB, PtEbX)	Columbiformes (*Columba livia*)	Iran	Pirestani et al. (2013)
PtEbII* (Peru6-var)	Psittaciformes (*Psittacus erithacus*) Columbiformes (*Columba livia*)	Portugal	Lobo et al. (2006)
Henan IV*	Galliformes (*Gallus gallus*)	China	Li et al. (2014)
M	Columbiformes (*Columba livia*)	Iran	Pirestani et al. (2013)
CC-1	Galliformes (*Gallus gallus*)	China	Li et al. (2014)
Col01[a]	Columbiformes (*Columba livia*)	Spain	Haro et al. (2005)
Col02[a]	Columbiformes (*Columba livia*)	Spain	Haro et al. (2005)
Col03[a]	Columbiformes (*Columba livia*)	Spain	Haro et al. (2006)
Col04[a]	Columbiformes (*Columba livia*)	Spain	Haro et al. (2006)
Col05[a]	Columbiformes	Spain	Haro et al. (2006)