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Prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China

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ABSTRACT

This study investigated 319 fecal specimens of cats ($n = 52$) and dogs ($n = 267$) from Heilongjiang province, China for the prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi*, and *Giardia duodenalis*. PCR and DNA sequence analysis of the small subunit rRNA gene identified *C. felis* and *C. parvum* in one cat each (3.8%) and *C. canis* and *C. ubiquitum* in 6 dogs (2.2%). Polymorphisms in the ribosomal internal transcribed spacer and phylogenetic analysis characterized zoonotic *E. bieneusi* genotypes D, EbpC, NED1, and NED2 and host-adapted ones NED3, NED4, and PtEb IX in 18 dogs (6.7%) and human-pathogenic genotypes D and IV in 3 cats (5.8%). Genotyping based on the hypermutation of *G. duodenalis* triosephosphate isomerase gene (TPI) facilitated identification of assemblage F in a cat (1.9%) and assemblages C and E in 12 dogs (4.5%). Subtypes of *G. duodenalis* isolates were determined by measuring the diversity of both TPI nucleotide and amino acid sequences. *C. canis*, *C. felis*, *C. parvum*, *E. bieneusi* genotypes D, EbpC, and IV, and *G. duodenalis* assemblage C identified herein have been documented in human infections in China. *C. canis*, *C. parvum*, *C. ubiquitum*, and *E. bieneusi* genotypes D, EbpC, and IV carried by cats or dogs also existed in wastewater in China. The finding suggested pet animals could be reservoirs for human cryptosporidiosis, microsporidiosis, and giardiasis and potential sources of water contamination in China.

1. Introduction

Cryptosporidiosis, microsporidiosis, and giardiasis represent significant contributions to the high zoonotic disease burden of diarrhea worldwide. *Cryptosporidium*, *Enterocytozoon bieneusi*, and *Giardia duodenalis* are the main causative agents of self-limiting disease in immunocompetent hosts and severe debilitating illness in immunocompromised individuals, especially AIDS patients (Feng and Xiao, 2011; Fletcher et al., 2012; Mathis et al., 2005; Santin and Fayer, 2011; Xiao, 2010). Although the three pathogens has been frequently detected in humans, animals, and water systems, little is known of the reservoirs and transmission routes, notably for *E. bieneusi* (Feng et al., 2009; Feng and Xiao, 2011; Feng et al., 2011; Fletcher et al., 2012; Li et al., 2012b; Liu et al., 2011; Mathis et al., 2005; Santin and Fayer, 2011; Xiao, 2010; Xiao and Fayer, 2008). Effective therapeutic treatments against the *Cryptosporidium* and *E. bieneusi* are still unavailable, emphasizing the

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necessity of discovering their genetic characteristics, elucidating the epidemiology, and planning and implementing prevention strategies (Anane and Attouchi, 2010; Feng and Xiao, 2011; Fletcher et al., 2012; Mathis et al., 2005; Santin and Fayer, 2011; Xiao, 2010).

Cryptosporidium is a genus of apicomplexan protozoans that colonize various vertebrate hosts and cause infectious diarrhea in humans. Thus far, over 20 *Cryptosporidium* species and more than 60 genotypes of uncertain species have been described. Although most of these *Cryptosporidium* species and genotypes are known to have some host specificity, identification of *C. hominis*, *C. parvum*, *C. canis*, *C. felis*, *C. ubiquitum*, *C. meleagridis*, *C. muris*, *C. andersoni*, *C. suis*, *C. scrofarum*, *C. cuniculus*, *C. fayeri*, and 4 genotypes including chipmunk genotype I and horse, skunk, and monkey genotypes in both humans and animals indicated that zoonotic transmission of cryptosporidiosis is a possibility (Fayer, 2010; Plutzer and Karanis, 2009; Slapeta, 2013; Xiao, 2010; Xiao and Fayer, 2008). In recent years, genotyping and subtyping of *Cryptosporidium* specimens from children, AIDS patients, and domestic animals have been widely used in the assessment and characterization of interspecies cryptosporidiosis transmission, and the zoonotic potential of *Cryptosporidium* from some animals has been well established (Slapeta, 2013; Xiao, 2010; Xiao and Fayer, 2008). However, very few studies have assessed the potential role of pets in zoonotic transmission of cryptosporidiosis in China.

A unicellular microsporidian parasite *E. bieneusi* affects a wide range of mammal hosts including humans around the world (Santin and Fayer, 2011), with over 200 genotypes distributed in several genetically isolated groups in phylogenetic analysis: zoonotic Group 1 and several host-adapted groups (Thellier and Breton, 2008). A variety of zoonotic genotypes (D, EbpC, IV, WL11, etc.) and some host-adapted ones (dominantly the outlier PtEb IX) contributed to *E. bieneusi* infections in cats and dogs (Abe et al., 2009; Dengjel et al., 2001; Karim et al., 2014; Lobo et al., 2006; Mathis et al., 1999; Mori et al., 2013; Santin et al., 2008; Santin et al., 2006; Zhang et al., 2011), the roles of pet animals in zoonotic transmission of microsporidiosis may be more common than currently believed.

A flagellated protozoan *G. duodenalis* is featured in high genetic diversity among isolates from various mammal hosts. Eight genetically distinct assemblages (A to H) have been defined for the parasite, with zoonotic assemblages A and B found both in humans and animals, host-adapted ones C and D primarily in dogs, E in ruminants, and F in cats (Ballweber et al., 2010; Feng and Xiao, 2011). Among sub-assemblages within assemblage A, AI has the capability to infect humans, cats, dogs, and ruminants; AII chiefly infects humans; and AIII infects wild mammals (Caccio et al., 2008; Feng and Xiao, 2011; Li et al., 2013). Cats and dogs were occasionally affected with assemblages B and E (Ballweber et al., 2010; Feng and Xiao, 2011). Sporadic infections of animal-derived assemblages C, D, E, and F were documented in individuals in developed and developing countries (Ballweber et al., 2010; Feng and Xiao, 2011; Liu et al., 2014).

There were very limited epidemiological data exhibiting the genetic characteristics of *Cryptosporidium*, *E. bieneusi*, and *G. duodenalis* in cats and dogs in China, notably for the former two pathogens (Jian et al., 2014; Karim et al., 2014; Li et al., 2012a; Li et al., 2013; Zheng et al., 2014). The three pathogens frequently appeared in the reports of human infections and water in China, but the likely sources are not clear (Feng et al., 2009; Feng et al., 2011; Li et al., 2012b; Liu et al., 2011; Wang et al., 2013a; Wang et al., 2013b; Wang et al., 2011). Frequent and close contact between humans and pet animals in China is of public health concern. The present work was undertaken to identify the species of *Cryptosporidium*, genotypes of *E. bieneusi*, and assemblages and subtypes of *G. duodenalis* in cats and dogs in China and evaluate the potential roles of pets in zoonotic transmission of human cryptosporidiosis, microsporidiosis, and giardiasis.

2. Materials and methods

2.1. Ethics statement

This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, China. Prior to experiment, the protocol of the current study was reviewed and approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University, under the approved protocol number SRM-08. In specimen collection, we obtained the permission from pet owners. No specific permits were required for the described field studies. And the locations where we sampled are not privately-owned or protected in any way. The field studies did not involve endangered or protected species.

2.2. Clinical specimens

A total of 52 fecal specimens were obtained from cats in a pet market in urban Harbin and grouped as follows: <3 months ($n = 6$), 3 to 12 months ($n = 30$), and > 12 months ($n = 16$). The number of male cats was 20, and females 32. Fecal specimen collection for dogs ($n = 267$) was processed from various sources: the pet market where cat specimens were collected ($n = 108$) and three pet hospitals ($n = 39$) in urban Harbin, stray ($n = 18$) in suburban Harbin, three pet hospitals ($n = 50$) in urban Daqing, and a pet hospital ($n = 52$) in urban Qiqihar. Likewise, dog specimens were divided into three age groups: <3 ($n = 34$), 3 to 12 ($n = 75$), and > 12 ($n = 158$). Male and female dogs had the specimen numbers of 133 and 134, respectively. The cities Daqing, Harbin, and Qiqihar are geographically linked and located at the southwestern end of Heilongjiang province, China. The stools of the market cats and dogs and the stray dogs were nondiarrheic at the time of sampling. Among the in-hospital dogs, 5 animals had diarrhea, while the others had nongastrointestinal illnesses. The sampling date for the market cats and dogs from Harbin was in June 2013, the in-hospital and stray dogs from Harbin during March to July 2013, and the in-hospital dogs from Daqing and Qiqihar in July 2014. The specimens were collected in 30 ml plastic containers and stored at −20 °C for DNA extraction. One specimen per animal was used in this study.