Letter to the Editor

Molecular identification of *Cryptosporidium* spp. from animal sources in China

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Introduction

Cryptosporidium spp. causes the diarrheal illness called cryptosporidiosis that may be fatal in the immunocompromised. More attention has been focused on the epidemiology and detection of *Cryptosporidium* in China including studies on infection in humans, animal populations and environmental water. *Cryptosporidium* has been an essential indicator in Standards for Drinking Water Quality (GB5749-2006) in China from 1 July 2008.

Domestic animals play an active role in family life, particularly dogs and cats involving continuous close contact with humans. In China, it was reported that the cities of Shanghai, Beijing, Guangzhou, Chongqing and Wuhan have been recognized as 'Pet City', and it was estimated that there are at least 150 million pet dogs around the country [1]. It is important to raise awareness that domestic animals may harbor zoonotic parasites such as *Cryptosporidium* asymptomatically. Infection of *Cryptosporidium* in dogs and cats has been reported in some countries, including the host-adapted and zoonotic *Cryptosporidium canis* infected in dogs from Japan [2] and Canada [3], *Cryptosporidium parvum* in dogs from Germany [4] and Costa Rica [5], and in cats from other countries [4, 5]. The host-adapted and zoonotic *Cryptosporidium felis* has been reported in cats from USA [6] as well as a report of a mixed infection in a cat involving *Cryptosporidium muris* and *Cryptosporidium felis* [7]. However, little relative information is known about the prevalence of *Cryptosporidium* in domestic animals in China. This study was aimed to estimate the prevalence of *Cryptosporidium* among the pet dogs and cats and other animals in a zoo of Shanghai, China.

The Study

Eighty-four fecal samples were collected from 19 caged dogs, 11 kennel cats from a pet hospital and 54 other animals (Table 1), as well as 8 wastewater samples from drainage channels from a zoo. Genomic DNA was extracted from feces using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, USA) following the manufacturer's instruction of Protocol, and DNA was stored at -20°C before it was used in PCR amplification reactions. Nested PCR was used to amplify an approximately 840 base pair (bp) long fragment of the SSU rRNA gene using two sets of oligonucleotide primers: F1: TTCTAGAGCTAATACATGCG and R1: CCCATTTCCTTCGAAACAGGA for primary PCR and F2: GGAAGGGTTGTATTTATTAGATAAAG and R2: CTCATAAGGTGCTGAAGGAGTAfor secondary PCR[8]. Amplification reactions were carried out in 25 µl volumes consisting of 12.5 µl Taq Green Master Mix (2X), 1 µl of each primer (10X), 9.5 µl nuclease-free water and 1 µl DNA. Reaction conditions were comprised of a hot start at 94°C for 1 min followed by 35 cycles at 94°C for 10 s, 55°C for 30 s and 72°C for 1 min and a final extension at 72°C for 10 min.

Table 1: The list of animals and *Cryptosporidium* spp. infection in this study

Genus	Species
Canis	Wolfhound*, Bernese Mountain dog*, Napoleon dog*, Rottweiler dog*, Boxer dog*, Alaska dog, Shiba, African hunting dog, Husky, LabradorRetriever (1), Miniature Pinscher, Great Pyrenees, Bangladesh dog, Akita, Japanese Spitz, Corgi, Chow, Maltese dog
Felis	Black Persian(1), Yellow Persian(1), Himalayan cat, Afghanistan's cat, Persian, White Persian(1), Short-haired cat, anonymous cat**
Others	Takin*, South China tiger (Qingfa), South China tiger (Xiaohu), South China tiger (Xiaoyong), South China tiger (Xiaoqing), South China tiger (Baobao), South China tiger (Xiaoying), Jaguar, Manchuriantiger, Bengal tiger, Black Panther, African lion, Blue fox, Leopard, Addax, Red deer(2), David'sdeer(1), Sika deer(1), Giraffe(1), Eland(3), Rhinoceros, Oryx(1), Yak (2), Tahr(1), Lynx, Zebra (3), Cheetah, Elephant(1), Panthera pardus, Panda, Wolf, Bharal, Black yak(1), Camel(1), Green Wolf, Gorals

*: *Cryptosporidium*-positive; **: a cat species recorded unclearly; 1, 2, 3 means 2, 3, 4 of the same kind of animal respectively;

Amplified regions were separated by 2% agarose gel electrophoresis and visualized following ethidium bromide staining. Amplified secondary PCR products were subjected to two directional sequencing with secondary primers by Invitrogen Ltd. (Shanghai). Sequences were blasted against sequences present in the NCBI database (http://blast.ncbi.nlm.nih.gov/) and multiple alignments were carried out using ClustalX 1.83. Phylogenetic relationship analyses were performed using MEGA 4.1 software.

A total of six *Cryptosporidium* positive samples with a infection rate of 7% (6/84) were detected from a Wolfhound, a Bernese Mountain dog, a Napoleon dog, a Rottweiler dog, a Boxer dog and a takin. No *Cryptosporidium* positive feces were detected from cats. There were some base substitutions found through multiplex alignments against each other and reference data. And the phylogenetic analysis turned out that the isolates from Wolfhound, Bernese Mountain dog, Napoleon dog, Rottweiler dog, and Boxer dog were belonged to *Cryptosporidium canis*, and the takin's isolate was *Cryptosporidium andersoni*. The nucleotide sequences in this study deposited in GenBank under accession numbers KF516539-KF516544.

Cryptosporidium canis has been found in human and considered as a zoonotic pathogen [9]. Xiao et al firstly reported the possible transmission of cryptosporidiosis between humans and dogs in a longitudinal cohort diarrhea study in Lima, Peru [10]. In Shanghai, infection with *C. canis* has also been reported in children from two pediatric hospitals [11]. Likewise, dogs can be the host of other types of *Cryptosporidium*, such as isolation of *C. muris* in a Texas canine population [12], although no positive was found in the present study, more attention should also be caused, and further works should be carried out to understand the real prevalence of this parasite or others infected in dogs.

Cryptosporidium andersoni is considered as a main type of *Cryptosporidium* in the bovine also has been isolated from humans. Furthermore, *C. andersoni* is recognized as one of the most common opportunistic and non-opportunistic pathogens HIV/AIDS patient with chronic diarrhea [13]. In China, *C. andersoni* was the dominant species in cattle [14]. And we have isolated this species from adult diarrhea patients from one Shanghai hospital (unpublished). It was also identified from diarrheal patients in others' study [15].

Fortunately, all the samples from cats and wastewater in the study were negative of *Cryptosporidium* spp., but the zoonotic species such as *C. parvum* and *C. felis* have been found in some studies [5,16], and in addition, more and more studies were involved in the water quality with this parasite including China, and different species and genotypes have been identified [17-19].

Consequently, the present work suggested that the infection of *Cryptosporidium* in pet dogs and other animals poses a significant potential public health threat and that surveillance practices must be established to prevent zoonotic disease of humans as well as pay more attention to the improvement of the management of animals.

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