Invited article

An investigation of parasitic infections and review of molecular characterization of the intestinal protozoa in nonhuman primates in China from 2009 to 2015

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A B S T R A C T

Parasites are a well-known threat to nonhuman primate (NHP) populations, and potentially cause zoonotic diseases in humans. In this study, the basic data was provided of the parasites in NHPs and the molecular characterization of the Enterocytozoon bieneusi, Giardia duodenalis, Cryptosporidium spp., and Entamoeba spp. were reviewed, which were found in these samples. A total of 3349 fecal samples were collected from 34 species reared at 17 districts in zoos, farms, free-range, or research laboratories, and examined microscopically. Eleven genera of intestinal parasites were detected: five genera of protozoans (Isospora spp., Entamoeba spp., Giardia spp., Cryptosporidium spp., and Cyclospora spp.) and six genera of helminths (Trichuris spp., Strongyloides spp., Ascaris spp., Physaloptera spp., Ankylostoma spp., and Enterobius spp.). The overall sample prevalence of parasitic infection was 54.1% (1811/3349). Entamoeba spp. was the most prevalent (36.4%, 1218/3349). The infection rate was the highest in free-range animals (73.0%, 670/918) (P < 0.01) and Guangxi Zhuang autonomous region (64.8%, 566/873). Mixed infections were mostly detected for Entamoeba spp., Trichuris spp., and Strongyloides spp. Molecular characterization was reviewed of Enterocytozoon bieneusi, Giardia duodenalis, Cryptosporidium spp., and Entamoeba spp., as these are zoonotic species or genotypes. This parasitological data for NHPs in China, provides important information for veterinarians and public health authorities for the elimination of such parasites and monitor the potential transmission of zoonotic infections from NHPs.

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1. Introduction

Nonhuman primates (NHPs), with their high level of genetic homology to humans, make them invaluable experimental models for biomedical research (Messoudi et al., 2011; Zhang et al., 2014). However, they are also an increasingly important source of emerging zoonotic diseases in humans, including human immunodeficiency virus (HIV), Ebola virus, malaria, etc (Poinar, 2009; Miller et al., 2013).

Several intestinal parasites occur in NHPs, causing asymptomatic or only mild disorders (Karim et al., 2014a; Kouassi et al., 2015; Li et al., 2015a). Potentially zoonotic protozoans (including Enterocytozoon bieneusi, Giardia duodenalis, Cryptosporidium spp., and Entamoeba spp.) could be maintained and transmitted with the attendant risk of human outbreaks originating in such animal reservoirs (Legesse and Erko, 2004; Ye et al., 2012). The health of NHPs is therefore important not only in terms of management objectives, but also concerning public health.

Compared with developed countries in America and Europe, China has relatively rich primate resources and is currently a leading producer and major supplier of NHPs to the international market (Zhang et al., 2014). NHPs are commonly maintained in zoos, natural reserves, and zoological gardens by different feeding habitats in China (Karim et al., 2014a). Therefore, it is important to understand the epidemiology of such intestinal parasites and their potential transmission from NHPs to humans.

The molecular characterization of NHP parasites is increasingly being studied (Berrilli et al., 2011; Íñiguez et al., 2012; Betson et al.,...
but there is a lack of comprehensive studies on the intestinal parasites in NHPs. Here, the prevalence of parasites in NHPs in China has been reported and the molecular characterization of the *Enterocytozoon bieneusi*, *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba* spp. found in these samples also had been reviewed.

### 2. Materials and methods

#### 2.1. Ethics statement

This study was conducted in accordance with the Chinese Laboratory Animal Administration Act (1988). The research protocol was reviewed and approved by the Research Ethics Committee of Henan Agricultural University. Appropriate permission was obtained from the director of animals and properties before the samples were collected. Veterinarians were notified of the parasitic infections identified in NHPs as soon as possible to expedite their management.

#### 2.2. Study area

A total of 3349 fresh fecal specimens were collected from 17 districts in two cities (Beijing and Shanghai), one autonomous region (Guangxi Zhuang autonomous region), and eight provinces (Hebei, Henan, Hubei, Hunan, Guangdong, Sichuan, Yunnan, and Shanxi) in China during the period between July 2009 to April 2015 (Fig. 1). This study included 34 NHP species (Table 1S). NHPs were grouped according to their feeding habits. 912 fecal specimens were subsequently collected from animals in zoos, 1402 from farms, 918 from free-range, and 117 from those in research laboratories (Table 1).

#### 2.3. Sampling

Fresh fecal samples from captive NHPs, which were kept in separate pens during the day, were collected in the early morning. The specimens from free-living animals were immediately collected from the ground after defecation.

Each specimen (about 10 g) was collected into a plastic container and labelled with the number, district, species, and clinical symptoms of the animal. Specimens were transported to the laboratory as soon as possible and stored in 2.5% (w/v) potassium dichromate solution at 4°C prior to microscopy. No animal exhibited any obvious clinical symptoms during the collection period.

#### 2.4. Microscopy

The fecal specimens were sieved through a sieve (7.62 cm diameter) with a pore size of 245 μm, transferred into a 50 ml centrifuge tube containing water, and precipitated by centrifugation at 5000 rpm for 10 min. A portion of each specimen was microscopically examined to detect protozoan and helminthic parasites with both Sheather’s sugar flotation technique and Lugol’s iodine staining (Huang et al., 2014). Wet smears were examined with a bright-field microscope at 100 × and 400 × magnification to determine the shape, size, and colour of the eggs/cysts.

#### 2.5. Review on molecular characterization of the intestinal protozoan

For *Giardia duodenalis*, a total of 1882 fecal specimens from NHPs were examined and characterized by ssrRNA (Appelbee et al., 2003), triosephosphate isomerase (*tpi*) (Sulaiman et al., 2003a), glutamate dehydrogenase (*gdh*) (Cacciò et al., 2008) and beta-giardin (*bg*) gene (Cacciò et al., 2002). 2660 specimens were...
identified for *Cryptosporidium* spp. by PCR amplification of the 18S rRNA (Xiao et al., 2001), 70 kDa heat shock protein (*hsp70*) (Xiao and Ryan, 2008) and genotyped by 60 kDa glycoprotein (*gp60*) gene (Alves et al., 2003). For *Enterocytozoon bieneusi*, there were a total of 1882 fecal specimens from NHPs that were screened and genotyped by SSU rRNA ITS gene (Sulaiman et al., 2003b); For *Entamoeba* spp., 531 specimens from 1059 *Entamoeba* spp. positive samples by microscopy, were randomly selected for PCR amplification based on SSU rRNA, using the specific primers of *E. histolytica* (Clark and Diamond, 1991), *E. dispar* (Clark and Diamond, 1991), *E. moskrovskii* (Ali et al., 2003), *E. nuttalli* (Verweij et al., 2001), *E. coli* (Tachibana et al., 2009) and *E. chattoni* (Tachibana et al., 2009) in order to identify the molecular characterization.

2.6. Statistical analysis

The statistical analysis was performed with SPSS software 19.0. The infection rates were compared with a χ² test, and differences were considered significant at $P < 0.01$.

3. Results

3.1. Occurrence of intestinal parasites

Eleven genera of intestinal parasites (five protozoan and six helminth genera) were found in the NHPs (Fig. 2). The overall sample prevalence of parasitic infection was 54.1% (1811/3349). *Entamoeba* spp. were the most frequently detected species, with an incidence of 36.4% (1218/3349), followed by *Trichuris* spp. (20.5%, 686/3349), *Strongyloides* spp. (6.2%, 206/3349), *Isospora* spp. (1.9%, 64/3349), *Giardia* spp. (1.3%, 43/3349), *Ascaris* spp. (1.0%, 32/3349), *Physaloptera* spp. (0.8%, 25/3349), *Cryptosporidium* spp. (0.5%, 18/3349), *Ancylostoma* spp. (0.5%, 16/3349), *Enterobius* spp. (0.4%, 12/3349), and *Cyclospora* spp. (0.2%, 7/3349) (Table 1).

3.2. Infection rate according to feeding habitats

The ratio of intestinal parasitic infections ranged from 46.0% to 73.0% among the four feeding habitats (zoos, farms, free-range, and research laboratories) (Table 1). The highest infection rate was found in those animals that were the free-range (73.0%, 670/918), followed by those in research laboratories (63.2%, 74/117), with lower infection rates at zoos (46.3%, 422/912) and farms (46.0%, 645/1402) ($p < 0.01$).

3.3. Geographic distribution of intestinal parasites

The sample prevalence of infection ranged from 32.6% to 64.8% among the 11 sampling locations. The Guangxi Zhuang autonomous region had the highest rate (64.8%, 566/873), and the lowest was found in Guangdong Province (32.6%, 107/328) (Table 2).

3.4. Mixed infections

The majority (74.3%, 1345/1811) of infected NHPs carried one parasitic species, 22.2% (402/1811) carried two parasitic species, and only 3.5% (64/1811) carried three or more parasite species (Table 2). The parasites most often involved in mixed infections were *Entamoeba* spp., *Trichuris* spp., and *Strongyloides* spp. (Table 1S).

3.5. Distribution patterns of infections among species

Six families, 20 genera, 34 species of NHPs, and 3349 individual specimens were detected, and the infections rates ranged from 0%
to 100% in different NHP species (Table 1S). Macaques monkey had the highest rate of parasitic infection with 80.1% (1908/2381). Interestingly, Ascaris spp. were only found in this species.

3.6. Molecular characterization of the intestinal protozoan

6.5% (122/1882) of specimens tested for Giardia duodenalis were positive by PCR analysis. The assemblages A (n = 4) and B (n = 118) were found, both which have zoonotic potential (Table 3). Assemblage A included subtypes A1, A2 and one novel subtype. Thirty-two assemblage B isolates with data at all three loci yielded 15 multi-locus genotypes (MLGs) (including 2 known and 13 new) (Karim et al., 2014a, 2015a). The occurrence of Giardia duodenalis assemblages in different species of nonhuman primate species are shown in Table 2S.

For Cryptosporidium spp., 0.7% (19/2660) were positive by PCR amplification (Karim et al., 2014a). 73.7% (14/19) of the positive specimens were found to be Cryptosporidium hominis, whilst 26.3% (5/19) were C. muris. The subtypes of the C. hominis were identified as IibA12G3 (7/14) and IiaA17 (1/14) by gp60 gene sequence analysis (Table 4). The occurrence of Cryptosporidium spp. and subtypes in nonhuman primate species based on PCR analysis are shown in Table 3S.

For Enterocytozoon bieneusi, there were 16.3% (306/1882)
positive specimens detected by PCR analysis. Altogether, 34 ITS genotypes were observed, including 16 known genotypes (Type IV, D, O, Henan V, Henan-IV, Peru8, PigEBITS5, PigEBITS7, EbpA, EbpC, EbpD, Peru11, BEB4, BEB6, I, and CS-1) and 18 new genotypes (CM1 to CM18) (Table 5). The new genotypes CM1 to CM3, CM6, CM8, CM10 to CM17 belong to the previously described group 1, which have zoonotic potential. Genotypes CM5, CM7, and CM9 clustered with group 2, whereas genotypes CM4 and CM18 formed new cluster (Karim et al., 2014b, 2015b). The occurrence of *Enterocytozoon bieneusi* and genotypes in different species of nonhuman primate species are shown in Table 4S.

For *Entamoeba* spp., the overall amplification efficiency was 87.19% (463) among the 531 positive specimens but only *Entamoeba dispar* (72.69%, 386/531) and *Entamoeba coli* (54.05%, 287/531) were amplified successfully. The mixed infections with *E. dispar* and *E. coli* were 27.1% (144/531) (Unpublished data).

### 4. Discussion

This study demonstrates a high sample prevalence (54.1%, 1811/3349) and diversity (five protozoan genera and six helminths genera) of intestinal parasites in NHPs in China. The prevalence varied with feeding habitats, NHP species, and geographic region. Similar infection ratio was found in pet macaques (59.1%, 52/88) in Indonesia (Jones-Engel et al., 2004), a zoo in Malaysia (54.5%, 54/99) (Lim et al., 2008), and pet monkeys in Cameroon (51.1%, 24/47) (Pourrut et al., 2011).

A diversity of intestinal parasites is frequently reported to infect NHPs (Jones-Engel et al., 2004; Legesse and Erko, 2004; Gillespie et al., 2005; Lim et al., 2008; Pourrut et al., 2011). Greater parasite species diversity was observed in Tai National Park, Côte d’Ivoire (with nine protozoans and 14 helminths in 3142 specimens) (Kouassi et al., 2015). Several studies had reported *Entamoeba* spp. as the most prevalent intestinal parasites in NHPs (Pourrut et al., 2011).
2011), whereas others reported that Strongyloides spp. were the most prevalent (Gillespie et al., 2005). All five genera of protozoans detected by microscopy, as well as Enterocytozoon bieneusi, are zoonotic (Mansfield and Gajadhar, 2004; Ye et al., 2012; Karim et al., 2014b; Plutzer and Karanis, 2016). Giardia duodenalis is a particularly zoonotic parasitic protozoan that infects a wide range of mammals, including NHPs (Feng and Xiao, 2011). Animals are infected when they ingest food or water contaminated with Giardia cysts (Graczyk et al., 2003). The assemblage B were the NHPs host-adapted, in 96.7% (118/122) of the positive isolates, which were zoonotic assemblage (Karim et al., 2015a).

The zoonotic Cryptosporidium spp. are usually associated with intestinal pathology, resulting in diarrhea in both humans and animals (Ryan and Hijjawi, 2015). They are transmitted via the fecal–oral route by either direct contact or the ingestion of contaminated food or water. The protozoan can disperse rapidly because they have a monoxenous life cycle, a low infective dose, and cysts (Graczyk et al., 2003). The assemblage B were the NHPs host-adapted, in 96.7% (118/122) of the positive isolates, which were zoonotic assemblage (Karim et al., 2015a).

The authors declare no conflicts of interest.
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