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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

Junqiang Li [a, b], Haiju Dong [a], Rongjun Wang [a, b], Fuchang Yu [a, b], Yayun Wu [a, b], Yankai Chang [a, b], Chenrong Wang [a, b], Meng Qi [a, b], Longxian Zhang [a, b, *]

[a] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, PR China
[b] International Joint Research Laboratory for Zoonotic Diseases of Henan, Zhengzhou 450002, PR China

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ABSTRACT

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* sp., *Cryptosporidium* spp., and *Cyclospora* spp.) and six genera of helminths (*Trichuris* spp., *Strongyloides* spp., *Ascaris* spp., *Physaloptera* spp., *Ancylostoma* 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.

1. Introduction

Nonhuman primates (NHPs), with their high level of genetic homology to humans, make them invaluable experimental models for biomedical research (Messaoudi 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; Iñiguez et al., 2012; Betson et al.,

* Corresponding author. College of Animal Science and Veterinary Medicine, Henan Agricultural University, 95 Wenhua Road, Zhengzhou 450002, PR China.
E-mail addresses: zhanglx8999@henau.edu.cn, zhanglx8999@gmail.com (L. Zhang).

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2014; Li et al., 2015a, 2015b), 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

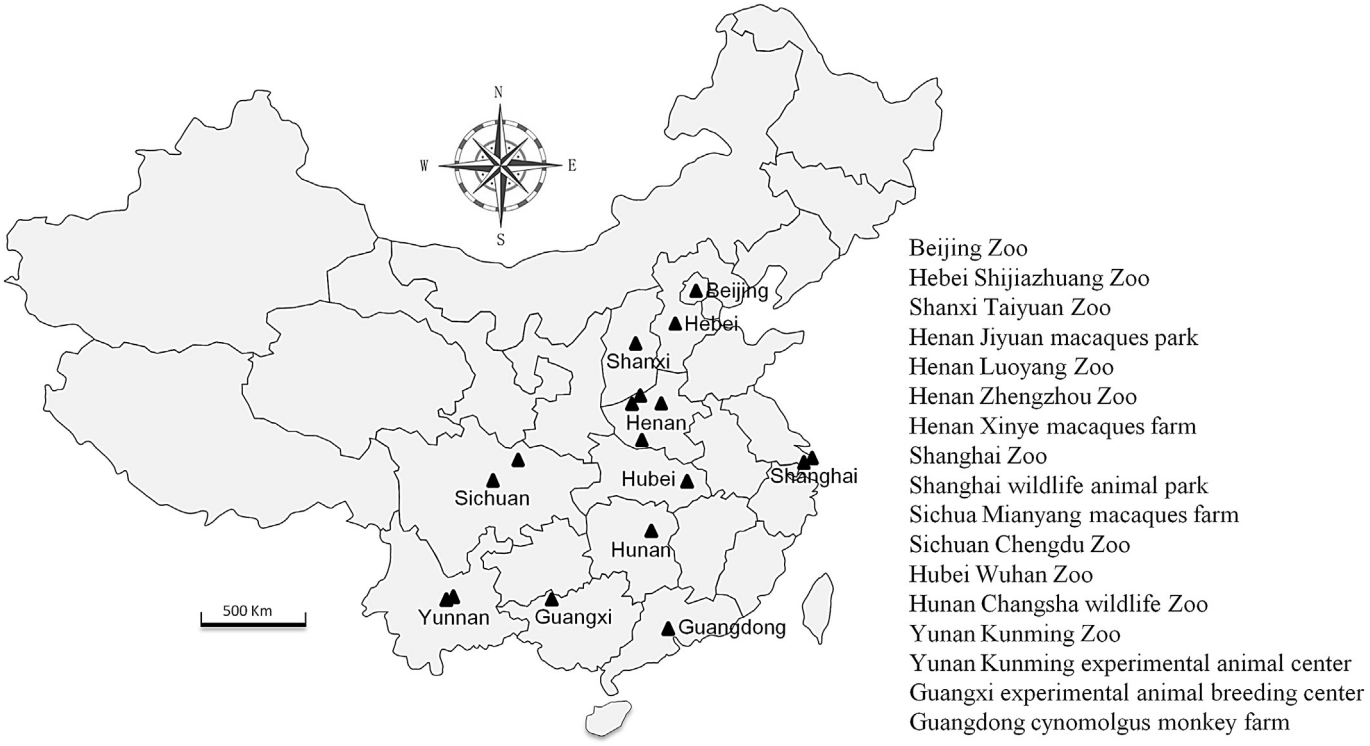

Fig. 1. Locations of the study area in China. Filled triangles indicate sampling sites.