Veterinary Parasitology 205 (2014) 518–522

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Natural infection of *Cryptosporidium muris* in ostriches (*Struthio camelus*)

Meng Qi[a,b], Lei Huang[a,b], Rongjun Wang[a,b], Lihua Xiao[c], Lina Xu[a,b], Junqiang Li[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
[c] Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

ARTICLE INFO

Article history:
Received 3 May 2014
Received in revised form 22 June 2014
Accepted 28 June 2014

Keywords:
Cryptosporidium muris
Ostrich
Cross-transmission
SSU rRNA
Actin
HSP70

ABSTRACT

A total of 303 fecal samples were collected from ostriches (*Struthio camelus*) and 31 samples (10.2%) were *Cryptosporidium*-positive upon microscopic analysis. The infection rate was 27.6% in ostriches aged 16–60 days, 1.2% in those aged 61–180 days, and 20.4% in those aged >10 years. The *Cryptosporidium*-positive isolates were genotyped with a restriction fragment length polymorphism analysis and DNA sequence analysis of the small subunit (SSU) rRNA gene. The 22 isolates from ostriches aged >10 years were identified as *Cryptosporidium muris*, whereas the nine isolates from ostriches <180 days were *Cryptosporidium baileyi*. Ten of the 22 *C. muris* isolates were analyzed based on the actin and HSP70 genes, and the results were identical to those observed for the SSU rRNA gene. Cross-transmission studies demonstrated that the *C. muris* isolate infected BALB/c mice and Mongolian gerbils, but did not infect chickens. *C. muris* isolated in this study appears to be host-adapted, consistent with a previous multilocus sequence typing analysis. Further studies are required to understand the prevalence and transmission of *Cryptosporidium* spp. in ostriches in different geographic areas.

1. Introduction

Cryptosporidium is an important zoonotic parasite throughout the world. It is one of the most common causes of diarrheal disease in humans and animals, and has significant public health implications. Twenty-seven valid *Cryptosporidium* species have been identified, and more than 70 host-adapted genotypes have been described (Qi et al., 2011; Liu et al., 2013; Kváč et al., 2014).

Cryptosporidium baileyi, *Cryptosporidium meleagridis*, and *Cryptosporidium galli* are the commonest *Cryptosporidium* species and have been identified in many avian hosts (Ng et al., 2006; Xiao and Fayer, 2008; Qi et al., 2011). A number of *Cryptosporidium* genotypes have also been detected, including avian genotypes (I–V), goose genotypes (I–IV), a black duck genotype, and a Eurasian woodcock genotype (Morgan et al., 2001; Jellison et al., 2004; Meireles et al., 2006; Ng et al., 2006; Xiao and Fayer, 2008; Abe and Makino, 2010). Cryptosporidiosis has been reported in ostriches and is implicated in phallus and cloacal prolapse, enteritis, and pancreatic necrosis (Bezuidenhout et al., 1993; Gajadhar, 1994; Penrith et al., 1994). *C. baileyi*, *C.*

* Corresponding author at: Corresponding author at: Henan Agricultural University, Veterinary Parasitology, College of Animal Science and Veterinary Medicine, No. 95 Wenhua Road, Zhengzhou 450002, Henan Province, PR China. Tel.: +86 371 63555689; fax: +86 371 63558180.
E-mail addresses: zhanglx8999@gmail.com, zhanglx8999@henau.edu.cn (L. Zhang).

http://dx.doi.org/10.1016/j.vetpar.2014.06.035
0304-4017/

meleagridis, and avian genotype II have been identified in ostriches in several countries, including Canada, Greece, Brazil, China, and Vietnam (Allwright and Wessels, 1993; Gajadhar, 1993; Sotiraki et al., 2001; Santos et al., 2005; Oliveira et al., 2008; Wang et al., 2011; Nguyen et al., 2013).

Cryptosporidium muris is usually a parasite of mammals, such as various rodent species, mountain goats, Bactrian camels, cynomolgus monkeys, cats, pigs, dogs, and humans (Dubey et al., 2002; Lupo et al., 2008; Xiao et al., 2004; Pavlasek and Ryan, 2007; Zintl et al., 2007). *C. muris* infection has also been reported in a tawny frogmouth, but whether the presence of fecal oocysts was attributable to mechanical transport (via the ingestion of an infected rodent) or an actual infection remains to be determined (Ng et al., 2006).

In China, the introduction and production of ostriches (*Struthio camelus*) commenced in 1992. Ostrich farms are mainly located in the Yellow River Basin (latitude ≈34°N) and are expanding into western regions. After 22 years of development, the breeding stock of ostriches has reached more than 30,000, and at present, China ranks first in Asia in the number of ostriches being reared. A previous study suggested that *C. baileyi* is the commonest *Cryptosporidium* species found in ostriches (Wang et al., 2011). In the present study, we characterized *C. muris* from ostriches with a DNA sequence analysis of the small subunit (SSU) ribosomal RNA (rRNA), actin, and 70-kDa heat shock protein (HSP70) genes, and characterized *C. baileyi* based on the SSU rRNA gene.

2. Materials and methods

2.1. Samples and microscopic examination

In total, 303 fecal samples were collected between August 2009 and March 2011 from an ostrich farm near the Yellow River in Zhengzhou, China. The farm was constructed in 2001 and 50 ostriches (15 males and 35 females) aged 1–2 years were introduced as the breeding birds from two local farms in Zhengzhou city.

All fecal samples were examined microscopically using both Sheather's sugar flotation and the modified acid-fast stain methods. The lengths and widths (to the nearest μm) of 76 oocysts were measured and the shape index (the ratio of length to width) of each oocyst was calculated. The *Cryptosporidium* oocysts were purified from the fecal samples by discontinuous sucrose gradient centrifugation (Wang et al., 2008) and stored in 2.5% potassium dichromate solution at 4 °C.

2.2. DNA extraction

After the *Cryptosporidium*-positive samples were stored in 2.5% potassium dichromate solution, they were washed three times with distilled water, and their genomic DNA was isolated using the MagExtractor *Genome* kit (Toyobo Co., Ltd., Osaka, Japan), as described previously (Wang et al., 2008).

2.3. Cryptosporidium genotyping

The primers and amplification conditions used in the nested PCR analysis of the partial 18S rRNA, actin, and HSP70 genes were as previously described (Xiao et al., 1999; Morgan et al., 2001; Sulaiman et al., 2002). The restriction fragment length polymorphisms (RFLPs) based on the SSU rRNA gene were analyzed with the restriction enzymes *Ssp*I and *Vsp*I (Xiao et al., 2001). The PCR products were sequenced on an ABI PRISM™ 3730 XL DNA Analyzer using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequence accuracy was confirmed by two-directional sequencing and by sequencing a second PCR product if necessary.

2.4. Sequence analysis

The sequences were aligned with the program ClustalX 1.83 (Thompson et al., 1997). Neighbor-joining trees were constructed with the program Phylip version 3.69, based on the evolutionary distances calculated with the Kimura two-parameter model.

Representative nucleotide sequences for the SSU rRNA, actin, and HSP70 genes have been deposited in GenBank under accession numbers GQ227706, KJ746834, and KJ746835, respectively.

2.5. Statistical analysis

The chi-square test was used to compare the *Cryptosporidium* infection rates, and differences were considered significant when $p < 0.01$ (Table 1).

2.6. Cross-transmission study

Sixteen 4-day-old Romance chickens, twelve 18-day-old BALB/c mice and twelve 20-day-old Mongolian gerbils were inoculated with *Cryptosporidium muris* oocysts. Before the inoculation, fecal samples from these animals were examined daily (3 days for chickens and 10 days for BALB/c mice and Mongolian gerbils) using the Sheather's sugar flotation technique. The *Cryptosporidium*-negative animals were used in the experimental infection. The doses used in the experimental infections are shown in Table 2.

3. Results

3.1. Prevalence of Cryptosporidium

Microscopic analysis showed that the average infection rate of *Cryptosporidium* spp. in the 303 samples was 10.2% (95% confidence interval [CI]: 10.2 ± 1.06%). The infection rate was 27.6% (95% CI: 27.6 ± 7.49%) (8/29) in ostriches aged 16–60 days, 1.2% (95% CI: 1.2 ± 1.67%) (1/83) in ostriches aged 61–180 days, and 20.4% (95% CI: 20.4 ± 2.65%) (22/108) in ostriches aged >10 years. No *Cryptosporidium* oocysts were detected in ostriches aged <15 days ($n = 20$), 181–360 days ($n = 34$), or 360–450 days ($n = 29$). The differences in the prevalence of *Cryptosporidium* spp. in the different age groups were significant ($\chi^2 = 38.42$, $p < 0.01$). *Cryptosporidium* infection