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PREVALENCE AND MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM SERPENTIS* IN CAPTIVE SNAKES IN CHINA

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KEY WORDS

Cryptosporidium serpentis
Snakes
China

ABSTRACT

Cryptosporidium species are the causative agent of cryptosporidiosis and common intracellular parasites that can infect a wide range of vertebrates, including snakes. In previous studies, *Cryptosporidium* species infections have been reported in snakes in Asia, Europe, and North America. However, limited information is available about the prevalence and molecular characterization of *Cryptosporidium* in captive snakes in China. Fecal specimens from 609 captive snakes were collected from Beijing (n = 227), Chengdu (n = 12), Dazhou (n = 359), and Ziyang (n = 11). The partial small-subunit (*SSU*) rRNA gene was amplified by nested polymerase chain reaction to determine the prevalence of *Cryptosporidium*, and a phylogenetic tree was constructed to assess evolutionary relationships and genetic characteristics. The overall prevalence of *Cryptosporidium* was 1.97% (12/609). BLAST and phylogenetic analysis of the small subunit ribosomal RNA (*SSU rRNA*) gene showed that the parasites belonged to *Cryptosporidium serpentis*. To the best of our knowledge, this is the first study to report the prevalence of *Cryptosporidium* in snakes of southwestern and northern China and provides preliminary data for the control and prevention of cryptosporidiosis in the investigated areas.

Cryptosporidium spp. are obligate intracellular protozoan parasites known to affect animals including humans, mammals, birds, fish, amphibians, and reptiles, and have been found in over 90 countries (Xiao et al., 2004a; Chen and Huang, 2012; Liu et al., 2015). *Cryptosporidium* species can be transmitted by direct contact with infected individuals or animals, as well as orally via contaminated food and water (Liu et al., 2014). Clinical symptoms vary depending on the age, species, and immune status of the host, and environmental conditions (Liu et al., 2014). Immunocompromised individuals aren't necessarily at higher risk of contracting an infection, but they are at higher risk of having a more severe disease progression and negative outcomes (Izadi et al., 2012; Zhang et al., 2018; Sannella et al., 2019).

Cryptosporidiosis is an important disease in reptiles, as it is highly contagious and associated with a high mortality rate. The first study on *Cryptosporidium* in snakes was conducted in 1977, wherein *Cryptosporidium* was detected in captive snakes with severe hypertrophic gastritis (Brownstein et al., 1977). Subsequently, infections have been reported in at least 57 reptilian species (Xiao et al., 2004b). *Cryptosporidium serpentis*, *Cryptosporidium parvum*, *Cryptosporidium parvum* mouse genotype, *Cryptosporidium muris*, *Cryptosporidium* sp., *Cryptosporidium tyzzeri*, *Cryptosporidium varanii*, and *Cryptosporidium andersoni* have been reported in various snakes worldwide (Plutzer and Karanis, 2007; Kuroki et al., 2008; Pedraza et al., 2009; Rinaldi et al., 2012; Yimming et al., 2016). However, 2 species are recognized as specific for reptiles: the gastric parasite *C. serpentis* and intestinal parasite *Cryptosporidium varanii* (syn. *Cryptosporidium saurophilum*; Fayer, 2010). *Cryptosporidium serpentis* has not been reported in humans; nevertheless, this species affects the health of snakes (Fayer, 2010). Furthermore, all *C. serpentis* infections occur in the stomach–abomasum of their respective hosts and cause a chronic, progressive disease that leads to death, thus eliminating valuable and biologically important ophidians (Graczyk et al., 1996). *Cryptosporidium varanii* is an intestinal parasite found primarily in lizards (Pavlasek and Ryan, 2008; Xiao et al., 2004b) and can cause anorexia, progressive weight loss, abdominal swelling, and high mortality, particularly in juvenile lizards (Koudela and Modrý, 1998; Dellarupe et al., 2016). As snake cryptosporidiosis results in the natural death of a snake or requires obligatory euthanasia to prevent spread of the pathogen, the economic loss can be immense.

There are approximately 2,800 different snake species reported around the world, out of which 200 species have been discovered

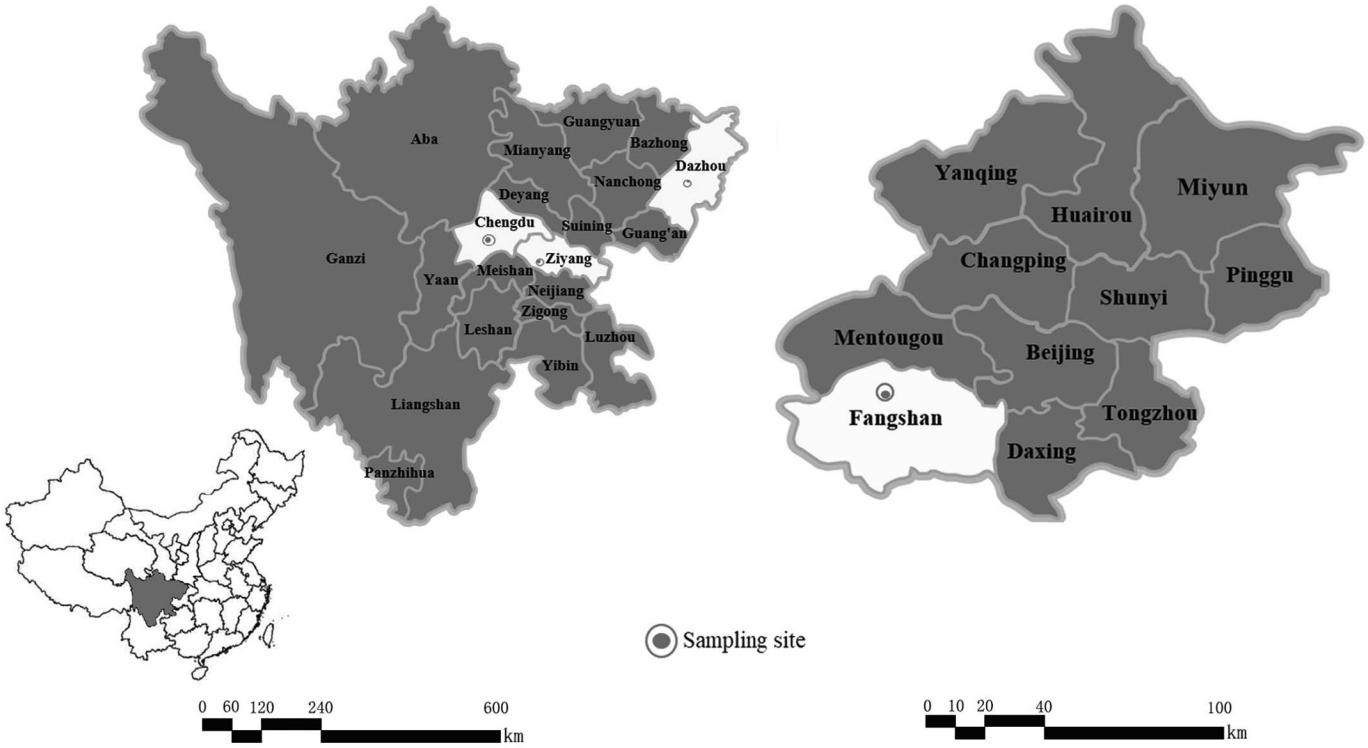

Figure 1. Sampling sites in Sichuan Province (left) and Beijing City (right), China.

in China. As global markets for live plants and animals have grown, the exotic pet trade is gaining attention over the past decade (Lockwood et al., 2019) and snakes have become increasingly popular pets (Pedraza-Díaz et al., 2009). Moreover, in China, snakes have long been considered a delicacy and are also utilized in traditional medicine (Zhou and Jiang, 2005). In recent years, the consumption of snake meat has become increasingly popular because of its taste and nutritive properties (Liu et al., 2020). Furthermore, swallowing raw gall bladder of snakes is a common practice in traditional Chinese medicine for its therapeutic effects (Sun and Li, 2004). Snakes with asymptomatic *Cryptosporidium* infections are difficult to diagnose (Xiao et al., 2019) and the aim of this study was to assess the current status of *Cryptosporidium* species prevalence in captive snakes in China using a molecular approach and to comprehend zoonotic implications of cryptosporidiosis.

MATERIALS AND METHODS

Ethical approval

This study was performed following the recommendations of the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, China. Before the initiation of the experiments, the protocol of the current study was reviewed and approved by the Institutional Animal Care and Use Committee of the Sichuan Agricultural University under permit. No animals were harmed during the sampling process. Permission was obtained from snake farm owners or shop managers before the collection of fecal specimens.

Sampling

A total of 609 samples were collected from the following 4 regions in the Sichuan province and Beijing City between March 2019 and June 2019 (Fig. 1): Chengdu (n = 12), Duzhou (n = 359), Ziyang (n = 6), and Beijing fangshan district (n = 227). The snakes in Dazhou and Ziyang are commercial snakes used for food, whereas those in Chengdu and Beijing are pet snakes. The fecal samples of the following snakes were sampled: black king snake, corn snake, Boulenger, Asian king snake, garter snake, king rat snake, sand boa, king snake, black rat snake, ball python, green snake, striped racer, Indo-Chinese rat snake, and cobra (Table I). Each snake was kept in a separate cage. Approximately 200 mg of fresh feces was collected from the bottom of each cage after defecation using sterile disposal latex gloves and immediately placed into individual disposable plastic bags. No obvious clinical signs were observed at the time of sampling, and the species and source were recorded at the same time. All fecal specimens were stored in 2.5% potassium dichromate solution at 4 C until further processing.

DNA extraction

The fecal specimens were washed 3 times in distilled water and centrifuged at 3,000 *g* for 10 min to remove the potassium dichromate. Genomic DNA was extracted from approximately 200 mg of each processed fecal specimen using an E.Z.N.A. Stool DNA kit (Omega Biotek Inc., Norcross, Georgia) according to the manufacturer's recommended instructions. The extracted DNA was stored at –20 C until molecular analysis was performed.

Table I. Prevalence and subtypes of *Cryptosporidium* spp. in different snakes in China.

Location	Host	Scientific name	No. of examined	No. of positive	Prevalence (%)	Species (n)
Beijing	Black king snake	*Lampropeltis getulus nigrita*	13	1	7.69	*Cryptosporidium serpentis* (1)
	Corn snake	*Elaphe guttata guttata*	22	1	4.55	*Cryptosporidium serpentis* (1)
	Macropisthodon rudis Boulenger	*Macropisthodon rudis*	18	0	0	
	Asian king snake	*Dinodon rufozonatum*	17	0	0	
	Garter snake	*Zaocys dhumnades*	10	0	0	
	King rat snake	*Elaphe carinata*	13	0	0	
	Sand boa	*Eryx*	16	0	0	
	King snake	*Lampropeltis getula californiae*	23	3	13.04	*Cryptosporidium serpentis* (3)
	Black rat snake	*Elaphe obsoleta obsolete*	36	2	5.56	*Cryptosporidium serpentis* (2)
	Ball python	*Python regius*	17	1	5.88	*Cryptosporidium serpentis* (1)
	Green snake	*Elaphe prasina*	2	0	0	
	Striped racer	*Elaphe taeniura*	18	1	5.56	*Cryptosporidium serpentis* (1)
	Indo-Chinese rat snake	*Ptyas korro*	22	1	4.55	*Cryptosporidium serpentis* (1)
Subtotal			227	10	4.41	*Cryptosporidium serpentis* (10)
Chengdu	Sand boa	*Eryx*	2	0	0	
	Black rat snake	*Elaphe obsoleta obsolete*	2	1	50	*Cryptosporidium serpentis* (1)
	Ball python	*Python regius*	2	0	0	
	Corn snake	*Elaphe guttata guttata*	2	1	50	*Cryptosporidium serpentis* (1)
	Black king snake	*Lampropeltis getulus nigrita*	2	0	0	
	King rat snake	*Elaphe carinata*	2	0	0	
Subtotal			12	2	16.67	*Cryptosporidium serpentis* (2)
Dazhou	Cobra	*Naja*	359	0	0	
Ziyang	Asian king snake	*Dinodon rufozonatum*	6	0	0	
	Striped racer	*Elaphe taeniura*	5	0	0	
Subtotal			11	0	0	
Total			609	12	1.97	*Cryptosporidium serpentis* (12)

Genotyping of *Cryptosporidium*

A nested polymerase chain reaction (PCR) targeting a ~830–base-pair (bp) fragment of the *SSU rRNA* sequence was performed to determine the *Cryptosporidium* species/genotype. The primers were F1 (5′-CCCATTTCCTTCGAAACAGGA-3′) and R1 (5′-TTCTAGAGCTAATACATGCG-3′) for the primary PCR, and F2 (5′-AAGGAGTAAGGAACAACCTCCA-3′) and R2 (5′-GGAAGGGTTGTATTATTAGATAAAG-3′) for the secondary PCR (Xiao et al., 1999). The annealing temperature was 55 and 58 C for the primary and secondary PCR, respectively. TaKaRa Taq DNA Polymerase (TaKaRa Bio Inc., Tokyo, Japan) was used for PCR amplification. Positive and negative controls were included in each amplification. Secondary PCR products were subjected to electrophoresis in a 1.5% agarose gel and visualized by staining with ethidium bromide.

Sequence analysis

All positive PCR products were directly sequenced on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster, California), using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Nucleotide sequences obtained in the present study were subjected to BLAST (http://www.ncbi.nlm.nih.gov/blast/), for analyzing the sequence similarity with reference sequences downloaded from the GenBank (http://www.ncbi.nlm.nih.gov). The sequences were aligned using Clustal X 2.0 (http://www.clustal.org/) to determine the *Cryptosporidium* species/genotypes. The nucleotide sequences generated in the present study have been deposited in GenBank under accession numbers MN474006, MN474009, MN474001, MN474011, MN545606, MN545609, MN545610, MN545612, MN545613, MN545618, MN545619, and MN545622 (Fig. 2).

Phylogenetic analysis

A neighbor-joining tree was constructed to assess the genetic relationship among the *Cryptosporidium* subtypes obtained in the present study and those identified in previous studies using the MEGA 7 software (http://www.megasoftware.net/). The sequences of the barcode region of *Cryptosporidium* were trimmed using trimAl (Capella-Gutiérrez et al., 2009). Evolutionary distances were calculated using the Kimura 2-parameter model. The reliability of the trees was assessed by bootstrap analysis with 1,000 replicates.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, Illinois). A chi-square test was used to compare the prevalence of *Cryptosporidium* in different species. Results with $P < 0.05$ were considered statistically significant.

RESULTS

The prevalence of *Cryptosporidium* in captive snakes

The overall prevalence of *Cryptosporidium* in captive snakes was 1.97% (12/609; 95% confidence interval [CI]: 1.1–3.4%). There were no significant difference in the prevalence of *Cryptosporidium* between the 4 locations tested, which ranged

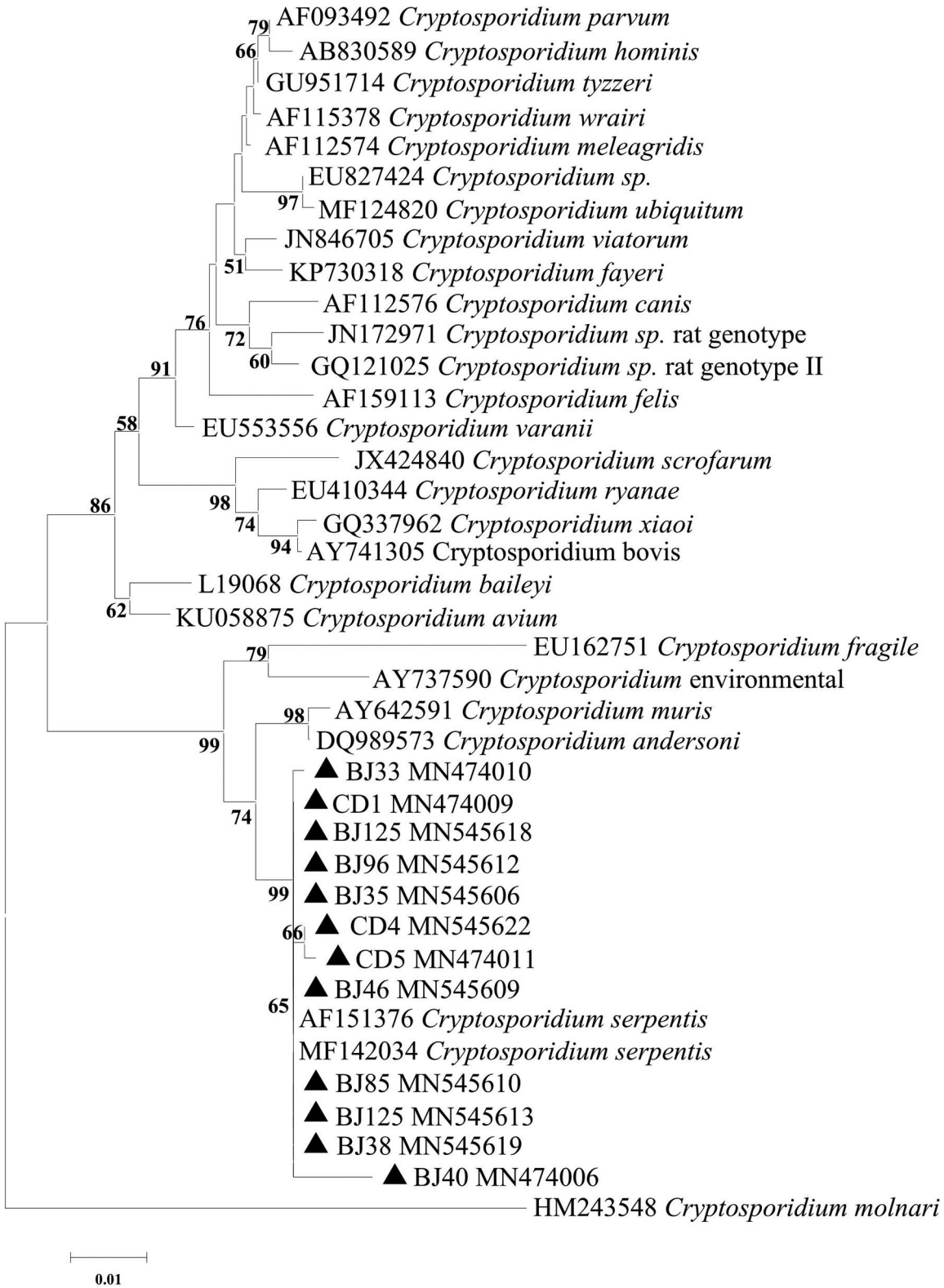

Figure 2. Phylogenetic relationships of *Cryptosporidium* snake isolates in this study to other known *Cryptosporidium* species inferred by neighbor-joining analysis of the *18S rRNA* gene. The neighbor-joining method was used to construct the trees from the Kimura-2-parameter model. Branch numbers represent percent bootstrapping values from 1,000 replicates, with values of more than 50% shown in the tree. *Cryptosporidium* genotypes found in this study are indicated in bold type. Genotypes in this study are marked with a ▲.

Table II. Prevalence and subtypes of *Cryptosporidium* spp. in different snakes in worldwide.

Location	Host	Scientific name	Technique	No. examined	No. positive	Prevalence (%)	Subtypes (n)	Reference
United States	Amazon tree boa	*Corallus hortulanus*	RFLP	123	48	39.02	*C. serpentis* (27)	Xiao et al. (2004b)
United States	Ball python	*Python regius*					*C. parvum* mouse	
United States	Black rat snake	*Elaphe obsoleta obsoleta*					genotype (12)	
United States	Boa constrictor	*Boa constrictor ortoni*					*C. muris* (2)	
United States	Boelen's python	*Morelia boeleni*					*C. saurophilum* (3)	
United States	Bornmueller's viper	*Vipera bornmuelleri*					*Cryptosporidium* sp. (1)	
United States	Bull snake	*Pituophis melanoleucuc sayi*					*C. serpentis* and *C. parvum*	
United States	California king snake	*Lampropeltis getulus californiae*					mouse genotype (2)	
							C. muris and *C. parvum*	
United States	Corn snake	*Elaphe guttata guttata*					mouse genotype (1)	
United States	Emerald tree boa	*Corallus caninus*						
United States	Fox snake	*Elaphe vulpina gloydi*						
United States	Louisiana pine snake	*Pituophis ruthveni*						
United States	Milk snake	*Lampropeltis triangulum*						
United States	Mountain viper	*Vipera wagneri*						
United States	Prairie king snake	*Lamproletis calligaster*						
Australia	Taipan	*Oxyuranus scutellatus*						
India	Indian rock python	*Python molurus*	Shaether's sugar floatation	1	1	100	ND	Akhila et al. (2018)
India	Reticulated python	*Reticulated python*	Shaether's sugar floatation	3	1	33.33	ND	Akhila et al. (2018)
India	Sand boa	*Eryx*	Shaether's sugar floatation	1	1	100	ND	Akhila et al. (2018)
India	Green anaconda	*Eunectes murinus*	Shaether's sugar floatation	7	3	42.86	ND	Akhila et al. (2018)
United States	Red corn snake	*Pantherophis guttatus*	Histologic sections of the stomach	1	1	100	ND	Bercier et al. (2017)
Italy	Boa constrictor	*Boa constrictor constrictor*	PCR	63	5	7.94	*C. parvum* (4), ND (1)	Díaz et al., (2013)
Italy	Epicrates cenchria	*Epicrates cenchria cenchria*	PCR	1	1	100	*C. serpentis* (1)	Díaz et al. (2013)
Italy	Florida king snake	*Lampropeltis floridiana*	PCR	1	1	100	*C. tyzzeri* (1)	Díaz et al. (2013)
Italy	Scrub python	*Morelia amethistina*	PCR	1	1	100	*C. tyzzeri* (1)	Díaz et al. (2013)
Italy	Carpet python	*Morelia spilota*	PCR	1	1	100	*C. muris* (1)	Díaz et al. (2013)
Italy	Chondropython viridis	*Morelia viridis*	PCR	1	1	100	ND (1)	Díaz et al. (2013)
Italy	Boa	*Python molurus molurus*	PCR	3	2	66.67	*C. parvum* (1), ND (1)	Díaz et al. (2013)
Italy	Ball python	*Python regius*	PCR	28	11	39.29	*C. tyzzeri* (7), *C. muris* (2), ND (2)	Díaz et al. (2013)
Italy	African rock python	*Python sebae*	PCR	1	1	100	*C. tyzzeri* (1)	Díaz et al. (2013)
Italy	Boa manditra	*Sanzinia madagascariensis*	PCR	1	1	100	*C. tyzzeri* (1)	Díaz et al. (2013)
Japan	Japanese grass snake		Nested PCR	223	57	25.6	ND	Kuroki et al. (2008)
Brazil	American spearhead	*Bothrops jararaca*	Indirect ELISA	126	86	68.25	ND	Paiva et al. (2013)
	Epicrates cenchria	*Epicrates c. cenchria*						
	Pantherophisobsoletus	*Pantherophis gutattus*						
	Ball python	*Python regius*						
Brazil	Green anaconda	*Eunectes murinus*	Semiquantitative evaluation	184	157	85.33	ND	Paiva et al. (2013)
	Pantherophisobsoletus	*Pantherophis gutattus*						
	Epicrates cenchria	*Epicrates c. cenchria*						
	Red-tailed boa	*Boa constrictor amarali*						
Spain	Boa constrictor	*Boa constrictor*	PCR	1	1	100	*C. varanii* (1)	Pedraza-Díaz et al. (2009)

Table II. Continued.

Location	Host	Scientific name	Technique	No. examined	No. positive	Prevalence (%)	Subtypes (n)	Reference
Spain	Kenyan or East African sand boa	*Erix colubrinus*	IF	1	1	100	ND	Pedraza-Díaz et al. (2009)
Spain	Corn snake	*Elaphe guttata guttata*	PCR	14	5	35.71	*C. serpentis* (5)	Pedraza-Díaz et al. (2009)
Spain	Milk snake, kingsnake	*Lampropeltis* spp.	PCR	36	19	52.78	*C. serpentis* (19)	Pedraza-Díaz et al. (2009)
Spain	Ball python	*Python regius*	PCR	11	5	45.45	*C. muris* (2), *Cryptosporidium mouse genotype* (1), *Cryptosporidium* sp. (*tortoise*) (1), *Cryptosporidium* sp. (1)	Pedraza-Díaz et al. (2009)
Japan	Corn snakes	*Elaphe guttata guttata*	Ziehl-Neelsen staining IFT PCR-RFLP	2	2	100	*C. saurophilum* (2)	Plutzer and Karanis (2007)
Austria	Corn snakes	*Pantherophis guttatus*	PCR	106	17	16.04	*Cryptosporidium varanii* (*saurophilum*) (17)	Richter et al. (2011)
Italy	Boa constrictor	*Boa constrictor constrictor*	IF PCR					Rinaldi et al. (2012)
Italy	Corn snake	*Elaphe guttata guttata*	IF PCR					
Italy	Indian rock python	*Python molurus*	IF PCR					
				125	6	4.8	*C. serpentis* (6)	
Brazil	Red-tailed boa	*Boa constrictor amarali*	PCR	7	4	57.14	*C. serpentis* (4)	Ruggiero et al. (2011)
Brazil	Vipera ruselli siamensis	*Bothropoides jararaca*	PCR	10	4	40	*C. serpentis* (4)	Ruggiero et al. (2011)
Brazil	South American rattlesnake	*Caudisona durissa*	PCR	8	3	37.5	*C. serpentis* (4)	Ruggiero et al., (2011)
Thailand	Boa constrictor	*Boa constrictor constrictor*	Nested PCR	17	5	29.41	*C. parvum* (4), *C. parvum mouse genotype* (1)	Yimming et al. (2016)
Thailand	Corn snake	*Elaphe guttata*	Nested PCR	76	19	25	*C. parvum* (10), *C. serpentis* (8), *C. saurophilum* (1)	Yimming et al. (2016)
Thailand	Milk snake	*Lampropeltis triangulum*	Nested PCR	7	2	28.57	*C. parvum* (2)	Yimming et al. (2016)
Thailand	Carpet python	*Morelia spilota*	Nested PCR	4	4	100	*C. parvum* (3), *C. andersoni* (1)	Yimming et al. (2016)
Thailand	Ball python	*Python regius*	Nested PCR	38	9	23.68	*C. parvum* (5) *C. muris* (3) *Cryptosporidium mouse genotype* (1)	Yimming et al. (2016)
China	Oriental rat snakes	*Ptyas mucosus*	Nested PCR	141	3	2.1	*C. serpentis* (3)	Karim et al. (2014)
China	Indian cobra	*Naja naja*	Nested PCR	99	0	0		Karim et al. (2014)
China		*Elaphe carinata*		12	3		*C. baileyi* (3)	Xiao et al. (2019)
		Naja atra		13	1		*C. baileyi* (1)	Xiao et al. (2019)
		Naja kaouthia		7	2		*C. baileyi* (2)	Xiao et al. (2019)
		Ptyas mucosa		12	6		*C. parvum* (2), *C. baileyi* (4)	Xiao et al. (2019)
		Deinagkistrodon acutus		6	1		*C. parvum* (1)	Xiao et al. (2019)
		Ptyas dhumnades		23	1		*C. serpentis* (1)	Xiao et al. (2019)
		Gloydius brevicaudus		12	1		*C. serpentis* (1)	Xiao et al. (2019)

* ND, no data; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; IF, immunoflourescence; IFT, indirect flourescent antibody staining technique..

from 0 to 16.67% ($\chi^2 = 2.473$, df = 3, $P > 0.05$) (Table I). The prevalence in different snake species ranged from 2.78% to 16.95% (Table I) and differed significantly between the 7 species ($\chi^2 = 9.699$, df = 4, $P < 0.05$).

Distribution of *Cryptosporidium* subtypes in captive snakes

Twelve samples were successfully sequenced, and all *SSU rRNA* PCR-positive samples were phylogenetically analyzed. According to the sequence data and genetic tree, the infection was caused by *C. serpentis* (Fig. 2; Table I). In the *SSU rRNA* gene, there were 1–5 nucleotide differences between the captive snake isolates and other known *C. serpentis* isolates. Nucleotide sequence comparison revealed genetic diversity between the 2 sequences from the 12 isolates. MN474011 contained 3 single-nucleotide polymorphisms (SNPs) within the 43-, 44-, and 83-bp regions of the *SSU rRNA* gene sequence of *Cryptosporidium serpentis* (addition: A, C, A), whereas isolate MN474006 contained 4 SNPs within the 41-bp (transversion: C/G), 61-bp (transversion: C/A), 108-bp (transition: C/T), and 132-bp (transversion: C/A) regions compared to the snake *Cryptosporidium serpentis* AF151376. The nucleotide sequences of MN474010, MN474009, MN545618, MN545612, MN545606, MN545622, MN545609, MN545610, MN545613, and MN545619, are the same as AF151376.

DISCUSSION

Cryptosporidium species have been reported in snakes from 9 different countries, including developed (USA, Australia, Italy, Japan, and Spain) and developing countries (India, Brazil, Thailand, and China; Table II; Brownstein et al., 1977; Xiao et al., 2004a; Chen and Qiu, 2012; Díaz et al., 2013; Yimming et al., 2016; Bercier et al., 2017; Akhila et al., 2018). In this study, the prevalence of *Cryptosporidium* was 1.97%, which is consistent with previous reports on the captive oriental rat snakes in Guangxi province in China (3/141, 2.1%; Graczyk et al., 1996; Zhou and Jiang, 2005; Karim et al., 2014; Bercier et al., 2017; Akhila et al., 2018; Xiao et al., 2019). However, the prevalence of *Cryptosporidium*-positive snakes in our study was lower than that of snakes in central China (Xiao et al., 2019). The 239 samples we collected in Beijing and Chengdu were sourced from pet snake shops. In our study, *Cryptosporidium* prevalence (5%) in pet snakes was similar to *Cryptosporidium* prevalence in Italian pet snakes (4.8%; Rinaldi et al., 2012). More importantly, *Cryptosporidium* was not detected in the commercial snake samples collected in Dazhou and Ziyang. Commercial snakes include cobras, Asian king snakes, and striped racers, which are used as a food source for consumers. The results demonstrate the occurrence of *Cryptosporidium* infection in pet snakes. However, the prevalence of *Cryptosporidium* infection in snakes from farms that provide snake meat was 0%. These results reveal that snakes bred under commercial conditions are relatively safe for consumption.

The prevalence in this study was lower than that of snakes in other countries where *Cryptosporidium* is found in snakes (Table II). This difference may be explained by several factors, such as the number of samples, geography of the source region, host health status, and raising and management systems. In our study, the relatively lower prevalence of *Cryptosporidium* may be because snakes were kept in separate cages and had a lower feeding density, which reduces the possibility of transmission between infected and healthy snakes. In addition, pet snake owners take precautionary measures to remove parasites, avoid food sourced from the wildlife by breeding their mice, and also breed the pet snakes themselves. Snake eggs are directly brought into the farms and raised. During the feeding process, farm owners break newborn chicken and pork meat into pieces for feeding. Great attention is paid to achieving isolation from the external environment and the prevention of diseases.

In our study, only *C. serpentis* was detected in snakes, and all *C. serpentis* isolates showed the *C. serpentis* genotype A *18S rDNA* sequence described by Xiao et al. (2004b). *Cryptosporidium serpentis* is an important parasite in snakes and is usually found in the gastric epithelium (Yimming et al., 2016). Moreover, *C. serpentis* was the only species identified in reptiles (Xiao et al., 2004b) until recently. Later studies have found that *C. serpentis* also occurs in cows and Alaskan caribou (Chen and Qiu, 2012; Paiva et al., 2013). Snakes infected with *C. serpentis* have increased mortality (Rinaldi et al., 2012; Paiva et al., 2013).

In conclusion, this is the first report of the prevalence of *Cryptosporidium* in snakes in southwest and north China. Moreover, this is the first time *C. serpentis* was detected in black king snakes, corn snakes, king snakes, ball python, striped racer, and Indo-Chinese rat snakes. Significantly, all of the above are common pet snakes in China and their proximity to humans can affect the health of handlers. Therefore, our findings contribute to the knowledge of *Cryptosporidium* distribution among snakes in China and provide baseline data for the implementation of effective measures and strategies to control and prevent snake and human infection with *Cryptosporidium*. However, there is a need to assess further the distribution of this group of parasites within the snake population as well as the potential risk that some of the *Cryptosporidium* species may pose to humans. Therefore, larger-scale surveys of snake cryptosporidiosis in various areas of China are needed to evaluate those in China and to assess the public health significance of the parasite better.

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