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Identification of zoonotic pathogens in zoo animals in the Republic of Korea

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ABSTRACT

Zoo animals may harbor various zoonotic pathogens and may pose a potential risk to humans through close contact. Nevertheless, epidemiological data on zoonotic diseases are scarce. Here, we conducted an epidemiological investigation and genetic characterization of *Coxiella burnetii*, *Cryptosporidium* species, *Enterocytozoon bieneusi*, and *Giardia duodenalis* in zoo animals in the Republic of Korea (ROK) and evaluated their zoonotic potential. A total of 261 fecal samples were collected from 27 animal species at one zoo between July 2022 and December 2023. Out of the four pathogens tested, one (0.4 %), twelve (4.5 %), and one (0.4 %) animals were found positive for *C. burnetii*, *E. bieneusi*, and *G. duodenalis*, respectively. *Cryptosporidium* species were not detected in zoo animals. Sequence analysis of *C. burnetii* based on the *IS1111* gene revealed that one sequence obtained from a black goat (*Capra hircus*) exhibited 100 % homology with those reported from a rodent in the ROK. A zoonotic assemblage B ($n = 1$) of *G. duodenalis* was identified in Patagonian mara (*Dolichotis patagonum*). Of the 12 *E. bieneusi*-positive samples, eleven novel genotypes (KRZB1−4 and KRZS2−8) were identified in four Asian black bears (*Ursus thibetanus ussuricus*) and eight squirrel monkeys (*Saimiri sciureus*). Phylogenetically, ten novel genotypes belonged to group 1 with zoonotic potential, and one novel genotype, KRZB4, was assigned to group 10. This study showed that *C. burnetii*, *E. bieneusi*, and *G. duodenalis* infections were identified in zoo animals in the ROK. Our findings highlight the need for ongoing monitoring and effective strategies to prevent and control these zoonotic pathogens.

1. Introduction

Zoos serve as popular urban recreational areas often featuring semi-forested or park-like surroundings. They create unique environments that gather diverse animal species for conservation, education, research, and exhibition purposes while also allowing the public to interact with wildlife prompting awareness of animal conservation and endangered species protection. Additionally, zoos facilitate frequent interactions between humans and animals. The semi-natural, fragmented environments within zoos are specifically designed to accommodate a variety of animals with differing habitat needs (Hrnkova et al., 2021). Due to spatial limitations, zoo animals often share small areas and frequently come into close contact with caretakers, visitors, and other wildlife (Kvapil et al., 2021). Recent studies have identified many zoonotic pathogens in zoo animals (Kvapil et al., 2021; Woolf et al., 2021; Zhang et al., 2021; Caballero-Gomez et al., 2022; Gottling et al., 2022; Hernandez-Colina et al., 2024; Moreira et al., 2024).

Coxiella burnetii is a globally prevalent zoonotic intracellular bacterium and the cause of Q fever in humans and coxiellosis in animals (Maurin and Raoult, 1999; Woldehiwet, 2004). This bacterium can persist in the environment for extended periods due to its highly infectious spore-like form (Marrie et al., 2015). Infection occurs mainly through inhaling contaminated aerosols or dust in nature or through direct contact with infected animal products (Angelakis and Raoult, 2010). Because *C. burnetii* can infect a wide range of hosts, its occurrence in wildlife near humans and livestock is virtually inevitable (Thomas et al., 2024). While coxiellosis is generally asymptomatic, it can lead to shedding of bacteria into the environment via feces or birth products, resulting in infection in various animal species from ticks to mammals and birds, with domestic ruminants being a significant source of human infections.

Cryptosporidiosis is caused by *Cryptosporidium* spp., protozoan

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parasites affecting the gastrointestinal tract of both humans and animals worldwide. These species are recognized as pathogens in livestock, poultry, companion animals, and wildlife, posing a threat to public health (Fayer, 2004). Transmission occurs via the fecal−oral route either through direct animal contact or ingestion of infective oocysts via contaminated water and food (Fayer, 2004; Xiao, 2010). Infections can range from asymptomatic to mild or severe watery diarrhea in host species (Monis and Thompson, 2003; Hunter et al., 2007; Ryan and Power, 2012; Xiao et al., 2012). Oocysts shed by infected animals remain viable for long periods, potentially transmitted to wildlife through infected food or water (Appelbee et al., 2005).

Microsporidia are obligate intracellular, spore-forming parasites that infect a broad spectrum of animals. Among these, *Enterocytozoon bieneusi* is the most commonly reported from livestock, companion animals, primates, wildlife, birds, and even wastewater (Guo et al., 2014; Stentiford et al., 2016). This species primarily transmits through the fecal-oral route, with contaminated water or food serving as potential infection sources (Mathis et al., 2005). Sequencing of the ribosomal internal transcribed spacer (ITS) region has shown that *E. bieneusi* genotypes cluster into at least 11 groups (Groups 1–11). Groups 1 and 2, found in multiple species, including humans, are considered zoonotic, while groups 3 to 11 appear to be more animal specific (Li et al., 2019).

Giardiasis is a zoonotic disease caused by *Giardia duodenalis*, reported in humans, companion animals, livestock, and wildlife (Thompson and Monis, 2012). *G. duodenalis* infections mainly spread through the fecal-oral route or through consuming food or water contaminated with cysts. Transmission can also occur through direct contact with infected animals (Adam, 2001). There are at least eight distinct assemblages of this species (A−H) (Cai et al., 2021), with assemblages A and B recognized as potentially zoonotic and found across a wide range of domestic and wild mammals, including humans. Conversely, assemblages C−H are more host-specific: C and D in canids, E in livestock, F in felids, G in rodents, and H in marine mammals (Monis and Thompson, 2003; Feng and Xiao, 2011).

To date, comprehensive research on zoo animals in the Republic of Korea (ROK) has been limited, with only a few studies conducted (Jung et al., 2007; Jang et al., 2008; Si et al., 2020; Hwang et al., 2021; Lee et al., 2024). Specifically, epidemiological data on zoonotic diseases in zoo animals remain scarce. Therefore, this study aimed to identify zoonotic pathogens in zoo animals in the ROK, characterize their genotypes, and assess their zoonotic potential. The insight gained will assist in developing control strategies to safeguard zoo animals.

2. Materials and methods

2.1. Sample collection

From July 2022 to December 2023, fecal samples were collected from 27 animal species at Cheongju Land in Cheongju, ROK. A total of 261 fecal samples were obtained from three time points: 79 samples between July and December 2022, 82 samples from January to June 2023, and 100 samples between July and December 2023. The species from which samples were collected were as follows: 130 fecal samples from 13 species of Carnivora, 41 samples from six species of Artiodactyla, 47 samples from three species of Primates, 25 samples from one species of Rodentia, four samples from one species of Lagomorpha, one sample from one species of Pilosa, six samples from one species of Marsupial, and three samples from one species of Perissodactyl. Detailed information regarding the animals and sample numbers is listed in Table 1. Upon collection, the feces were placed in sterile plastic tubes, immediately transported to the laboratory, and stored at −20 °C until DNA extraction. Health monitoring of zoo animals is performed annually by hematological examination. The results were within the normal range.

Table 1
Fecal samples collected from a Korean zoo between July 2022 and December 2023.

Order	Species	2022	2023		Total
		Jul.–Dec.	Jan.–Jun.	Jul.–Dec.	
Carnivora	*Ursus thibetanus ussuricus* (Asian black bear)	6	6	6	18
	Lutra lutra (Otter)	3	3	3	9
	Vulpes vulpes (Red fox)	0	4	4	8
	Panthera leo (Lion)	2	2	2	6
	Panthera tigris altaica (Tiger)	3	1	2	6
	Lynx lynx (Eurasian lynx)	5	5	6	16
	Suricata suricatta (Meerkat)	11	7	11	29
	Vulpes zerda (Fennec Fox)	4	3	3	10
	Nyctereutes procyonoides (Raccoon dog)	0	1	1	2
	Meles meles (Eurasian badger)	0	1	1	2
	Prionailurus bengalensis (Leopard cat)	3	3	5	11
	Nasua nasua (Coati)	1	0	0	1
	Canis lupus (Tibetan wolf)	6	0	6	12
Artiodactyla	*Ovis aries musimon* (Mouflon)	0	6	6	12
	Capra aegagrus hircus (Goat)	3	1	1	5
	Capra hircus (Black goat)	0	4	4	4
	Sus scrofa (Pig)	0	2	2	4
	Hemitragus jemlahicus (Himalayan Tahr)	5	5	5	15
	Naemorhedus caudatus (Long-tailed goral)	0	0	1	1
Primates	*Saimiri sciureus* (Squirrel monkey)	12	12	11	35
	Macaca fuscata (Japanese monkey)	3	3	3	9
	Macaca mulatta (Rhesus monkey)	1	1	1	3
Rodentia	*Dolichotis patagonum* (Mara)	8	10	7	25
Lagomorpha	*Oryctolagus cuniculus* (Rabbit)	0	0	4	4
Pilosa	*Choloepus didactylus* (Sloth)	1	0	0	1
Diprotodontia	*Macropus robustus* (Wallaroo)	2	2	2	6
Perissodactyla	*Equus caballus* (Horse)	0	0	3	3
Total		79	82	100	261

2.2. DNA extraction and polymerase chain reaction (PCR)

DNA was extracted from 200 mg of each fecal sample using an AccuPrep Stool DNA extraction kit (Bioneer, Daejeon, ROK), following the manufacturer's instructions, and was immediately frozen at −20 °C until use. *C. burnetii*, *Cryptosporidium* spp., *E. bieneusi*, and *G. duodenalis* were screened using specific primers with the nested PCR method under the following conditions: 93–95 °C for 5 min, followed by 30–35 cycles of 93–95 °C for 1 min, the appropriate annealing temperature for each pathogen, 72 °C for 1 min, and a final extension step at 72 °C for 7 min (Table 2). Distilled water was used as a negative control in all experiments. Amplified PCR products were separated by electrophoresis on 1.5 % agarose gels and visualized after staining with ethidium bromide.

2.3. Sequencing and phylogenetic analysis

For further DNA sequence analysis, the secondary PCR products of *C. burnetii*, *E. bieneusi*, and *G. duodenalis* were purified using the AccuPrep® PCR Purification Kit (Bioneer) and subsequently used for direct sequencing (Bioneer). All nucleotide sequences obtained from each

Table 2
The PCR primers for the four pathogens used in this study*IS1111*: *Insertion sequence 1111*; SSU rRNA: small subunit ribosomal RNA; ITS: internal transcribed spacer; *bg*: *β-giardin*.

Pathogen	Target gene	Sequence (5′–3′)	Amplicon (bp)	Annealing temp/time
Coxiella burnetii	*IS1111*	TATGTATCCACCGTAGCCAGTC CCCAACAACAACCTCCTTATTC	685	54 °C/30 s
		GAGCGAACCATTGGTATCG CTTTAACAGCGCTTGAACGT	202	
Cryptosporidium spp.	SSU rRNA	TTCTAGAGCTAATACATGCG CCCATTTCCTTCGAAACAGGA	1325	60 °C/1 min
		GGAAGGGTTGTATTTATTAGATAAAG AAGGAGTAAGGAACAACCTCCA	830	
Enterocytozoon bieneusi	ITS	GGTCATAGGGATGAAGAG TTCGAGTTCTTTCGCGCTC	410	55 °C/45 s
		GCTCTGAATATCTATGGCT ATCGCCGACGGATCCAAGTG	390	
Giardia duodenalis	*bg*	AAGCCCGACGACCTCACCCGCAGTGC GAGGCCGCCCTGGATCTTCGAGACGAC	753	65 °C/30 s
		GAACGAACGAGATCGAGGTCCG CTCGACGAGCTTCGTGTT	511	55 °C/30 s

pathogen were aligned using BioEdit software and then compared with reference sequences from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/BLAST). The percentage of nucleotide sequences similarities between our sample sequences and the reference sequences for each pathogen was assessed with Geneious Prime 2022.2 software (http://www.geneious.com). Phylogenetic analysis of each pathogen was performed using the maximum-likelihood method implemented in MEGA10, utilizing the best substitution model. Bootstrap values were calculated by analyzing 1000 replicates to evaluate the reliability of the clusters. The models used in this study were the Kimura 2-parameter model for *C. burnetii*, the Hasegawa-Kishino-Yano (HKY) + G model for *E. bieneusi*, and the Tamura-Nei (TN) 93 + G + I model for *G. duodenalis*. The nucleotide sequences obtained in this study were assigned the following accession numbers: PQ439336 for *C. burnetii*, PQ435993−PQ436004 for *E. bieneusi*, and PQ439337 for *G. duodenalis*, respectively.

3. Results

3.1. Prevalence of zoonotic pathogens

Among the 261 fecal samples collected from zoo animals, *E. bieneusi* (4.6 %, 12/261) was the most frequently detected pathogen, while *C. burnetii* (0.4 %, 1/261) and *G. duodenalis* (0.4 %, 1/261) were each found in only one sample (Table 3). There were no instances of co-infections with two or three pathogens in these animals. *Cryptosporidium* spp. was not identified in any of these samples (Table 3). Regarding the specific animals in which each pathogen was detected, *E. bieneusi* was found in four *Ursus thibetanus ussuricus* (Asian black bears) (22.2 %, 4/18) and in eight *Saimiri sciureus* (squirrel monkeys) (22.9 %, 8/35). *C. burnetii* was present in one *Capra hircus* (black goat) (12.5 %, 1/8), and *G. duodenalis* was identified in one *Dolichotis patagonum* (Patagonian mara) (4 %, 1/25) (Table 3).

3.2. Phylogenetic trees of pathogens detected in zoo animals

3.2.1. C. burnetii

One sequence obtained from a black goat was included in a phylogenetic analysis. Based on the *IS1111* gene, our sequence grouped with various hosts such as dogs, goats, sheep, cattle, rodents, humans, and wild animals reported in other countries, exhibiting 97.8−100 % identity (Fig. 1). The sequence from the black goat showed 100 % homology with those from rodents (OR284318), *Cerdocyon thous* (crab-eating fox, MK758120), *Nasua nasua* (South American coati, MK758117), and *Artibeus lituratus* (great fruit-eating bat, MH229950). Interestingly, the sequence from the black goat had 98.7 % identity with that of the Korean water deer. Additionally, our sequence shared 99.4 % identity with a virulent strain identified in pneumonia patient (JF970260).

3.2.2. E. bieneusi genotypes

To determine the genotypes of *E. bieneusi* detected in Asian black bears and squirrel monkeys, 12 ITS-positive samples were sequenced and used to construct the phylogenetic tree. Interestingly, 11 novel genotypes, KRZB1−4, and KRZS2−8, were identified in these animals (Table 4). Among them, 10 novel genotypes belonged to the zoonotic Group 1, whereas one novel genotype, KRZB4, was classified into Group 10 (Fig. 2). The phylogenetic analysis demonstrated that within Group 1, three new genotypes (KRZB1−3) detected in Asian black bears belonged to subgroup 1a (Fig. 2), displaying one to two nucleotide differences from one another. Notably, eight sequences obtained from squirrel monkeys exhibited greater diversity in genotypes, and seven novel genotypes (KRZS2−8) with two to four nucleotide differences were found. These genotypes were assigned to a new subgroup of Group 1 along with the ring-tailed lemur (KJ728809) reported in China (Fig. 2). The novel genotype KRZB4, identified in one Asian black bear, formed the same group as the Asian black bear (MK547516) in China (Fig. 2) and exhibited three nucleotide differences from genotype SCBB2.

3.2.3. G. duodenalis assemblage

Phylogenetic analysis based on the *bg* gene revealed that one sequence obtained from Patagonian mara was assigned to assemblage B, alongside those from calf, dog, gibbon, chinchilla, horse, human, and zoo animals, and diverged from assemblages A, C, D, E, and F (Fig. 3). By comparing sequences, the Patagonian mara sequence obtained in this study had 99.2 % identity with that from a Patagonian mara reported in China (PQ614260), showing four-nucleotide differences between the two sequences (Supplementary Fig. 1). Our sequence was found to be identical to that from chinchilla (KM977640) and bamboo rat (KY696832) in China, displaying 99.0−100 % identity with humans and other animals belonging to assemblage B (Fig. 3). Additionally, the Patagonian mara sequence exhibited 99.6 % and 99.8 % identity with those detected in beaver and coypus, respectively.

4. Discussion

Zoo animals can host potential zoonotic pathogens (Forsyth et al., 2012). The consequent diseases can affect not only animal populations but also humans (zookeepers and visitors) who interact closely with these species. This study investigated the prevalence of *C. burnetii*, *Cryptosporidium* spp., *E. bieneusi*, and *G. duodenalis* in various animal species at a Korean zoo. With the exception of *Cryptosporidium* spp.,

Table 3
The number of positive samples for each pathogen detected in this study.

Species	*C. burnetii*	*Cryptosporidium* spp.	*E. bieneusi*	*G. duodenalis*
Ursus thibetanus ussuricus (Asian black bear) (n = 18)	–	–	4	–
Lutra lutra (Otter) (n = 9)	–	–	–	–
Vulpes vulpes (Red fox) (n = 8)	–	–	–	–
Ovis aries musimon (Mouflon) (n = 12)	–	–	–	–
Capra aegagrus hircus (Goat) (n = 5)	–	–	–	–
Capra hircus (Black goat) (n = 8)	1	–	–	–
Sus scrofa (Pig) (n = 4)	–	–	–	–
Hemitragus jemlahicus (Himalayan Tahr) (n = 15)	–	–	–	–
Panthera leo (Lion) (n = 2)	–	–	–	–
Panthera tigris altaica (Tiger) (n = 3)	–	–	–	–
Lynx lynx (Eurasian lynx) (n = 16)	–	–	–	–
Macaca fuscata (Japanese monkey) (n = 9)	–	–	–	–
Macaca mulatta (Rhesus monkey) (n = 3)	–	–	–	–
Suricata suricatta (Meerkat) (n = 29)	–	–	–	–
Vulpes zerda (Fennec Fox) (n = 10)	–	–	–	–
Saimiri sciureus (Squirrel monkey) (n = 35)	–	–	8	–
Nyctereutes procyonoides (Raccoon dog) (n = 3)	–	–	–	–
Meles meles (Eurasian badger) (n = 2)	–	–	–	–
Dolichotis patagonum (Mara) (n = 25)	–	–	–	1
Macropus robustus (Wallaroo) (n = 6)	–	–	–	–
Prionailurus bengalensis (Leopard cat) (n = 11)	–	–	–	–
Nasua nasua (Coati) (n = 1)	–	–	–	–
Canis lupus (Tibetan wolf) (n = 12)	–	–	–	–
Choloepus didactylus (Sloth) (n = 1)	–	–	–	–
Oryctolagus cuniculus (Rabbit) (n = 4)	–	–	–	–
Equus caballus (Horse) (n = 3)	–	–	–	–
Naemorhedus caudatus (Long-tailed goral) (n = 1)	–	–	–	–
Total	1/261	0/261	1/261	12/261

"–": not detected.

C. burnetii, *E. bieneusi*, and *G. duodenalis* were detected. Notably, *E. bieneusi infection rates were high among zoo animals, while C. burnetii* and *G. duodenalis were each detected in only one animal. Nevertheless,* our results showed a low prevalence compared to previous studies (Li et al., 2015; Karim et al., 2021; Gottling et al., 2022; Zhao et al., 2025). These differences could be attributed to several factors, including the quality of sanitary control in the zoo, diversity of animal species, and environmental factors.

Coxiella burnetii infection is a concern for both public and veterinary health due to its rapid spread and highly infectious nature. Previous studies have indicated that *C. burnetii* infection occurs in numerous free-living and captive wildlife species globally, which are suspected of influencing the epidemiology of Q fever (Torina et al., 2007; Clemente et al., 2008; Gonzalez-Barrio and Ruiz-Fons, 2019; Zanatto et al., 2019; Celina and Cerny, 2022). In this research, *C. burnetii* DNA was found in a black goat, making the first identification in zoo animals in the ROK. Although the infection rate of *C. burnetii* (0.4 %) was low, it should not be dismissed due to the pathogen's high infectivity and potential for aerosol transmission. We previously identified *C. burnetii* in Korean water deer, rodents, horses, and domestic ruminants (Shin et al., 2020; Cho et al., 2021; Choi et al., 2024), with goats showing the highest rates. Additionally, *C. burnetii* is associated with reproductive problems in zoo animals (Clemente et al., 2008; Lloyd et al., 2010; Garcia et al., 2017). However, the black goat that tested positive for *C. burnetii* DNA appeared clinically healthy. Small ruminants are commonly used as contact animals in zoos. Given the absence of clear clinical symptoms for coxiellosis, contact with these animals poses a significant risk of pathogen transmission, particularly for visitors and zookeepers. Interestingly, the sequence obtained from the black goat matched 100 % with those from rodents identified recently in the ROK, while differing by four nucleotides from that of Korean water deer.

Recent studies indicate that wild rodents serve as a direct source of infections for both humans and animals (Reusken et al., 2011; Choi et al., 2024). Although the exact mode of infection for the black goat remains uncertain, the potential role of rodents in transmitting *C. burnetii* cannot be dismissed. Rodents may be more susceptible to *C. burnetii* infection because of their mobility and increased opportunities to come into contact with other animals, which could facilitate the spread of the infection. Similar to previous studies (Tozer et al., 2014; Mori et al., 2017; Abdel-Moein and Hamza, 2018), *C. burnetii* was detected in fecal samples. Since obtaining blood samples from zoo animals necessitates capture and restraint, potentially causing stress and injury, using fecal samples may be a more suitable approach in zoo environments. In general, bacterial numbers are highly variable, ranging from massive *C. burnetii* burdens in persistently infected tissue samples such as birth products or aborted materials to very low loads in environmental, milk, and blood samples (Mori et al., 2017). This suggests that feces, along with blood, urine, and vaginal swabs may also be suitable for screening *C. burnetii* in wildlife. Therefore, our results advocate for the need for epidemiological surveillance of *C. burnetii* infection and biosecurity measures to safeguard the health of

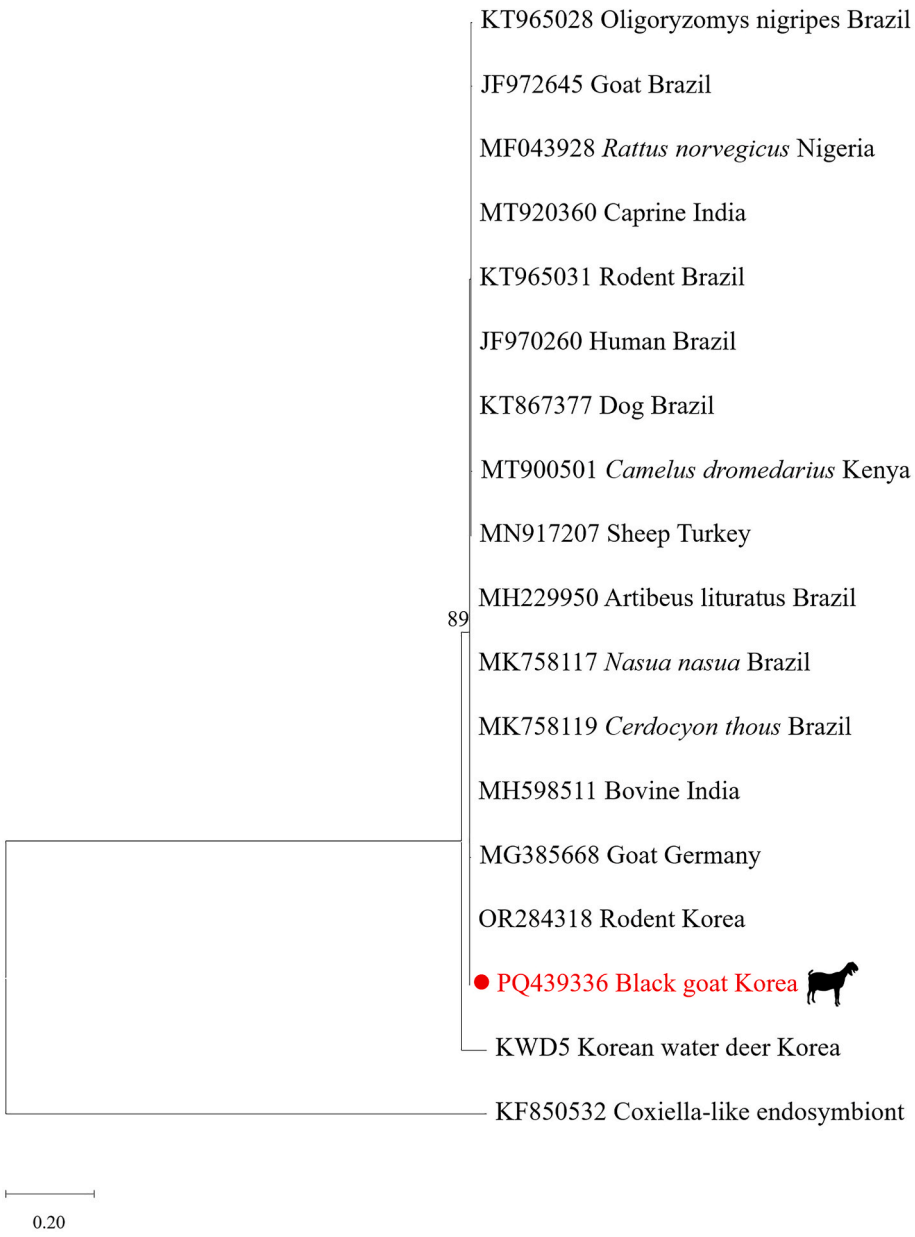

Fig. 1. Phylogenetic tree based on the *IS1111* gene of *Coxiella burnetii*. The evolutionary analysis was inferred using the Kimura 2-parameter model. Bootstrap values (1000 replicates) are indicated at each node. The scale bar implies nucleotide substitution per site. The sequence identified from the black goat is indicated by a red filled circle.

Table 4
Subtype distribution of *E. bieneusi* identified in zoo animals.

Group	subgroup	Genotype
Group 1	1a	KRZB1, KRZB2, KRZB3
	novel	KRZS2, KRZS3, KRZS4, KRZS5, KRZS6, KRZS7, KRZS8
Group 10		KRZB4

One sequence was identical to KRZS2.

zookeepers, visitors, and other animals in zoos in the ROK.

Enterocytozoon bieneusi is a known gastrointestinal pathogen affecting both humans and various animals. Several studies have documented the frequent detection of *E. bieneusi* in zoo animals (Sak et al., 2014; Li et al., 2015; Yu et al., 2017; Karim et al., 2020; Hwang et al., 2021; Koster et al., 2021; Zhang et al., 2021; Moreira et al., 2024). In this study, the infection rate of *E. bieneusi* was found to be 4.6 %, which is lower than the rates reported in China (13.5–20.8 %) (Zhang et al., 2021; An et al., 2024) and Bangladesh (16.5 %) (Karim et al., 2020), but higher than that in Portugal (1.57 %) (Moreira et al., 2024) and in previous findings from the ROK (Hwang et al., 2021). These variations in infection rates could be attributed to differences in environmental and management conditions across the zoos. Our results showed that *E. bieneusi* was primarily detected in squirrel monkeys and Asian black bears. However, we cannot currently ascertain the mode of transmission for this pathogen. It may involve the consumption of contaminated water and/or food, which are likely significant routes for the transmission of *E. bieneusi.* Microsporidiosis is regarded as a serious waterborne human disease, alongside cryptosporidiosis and giardiasis (Li et al., 2018). Given that *E. bieneusi*-positive animals in this study were housed together in the same barn, it is plausible that feces from infected animals contaminated the surrounding environment (including soil, water, and feeding troughs), potentially facilitating the spread of this pathogen.

As a parasite with zoonotic potential, *E. bieneusi* is suspected to be

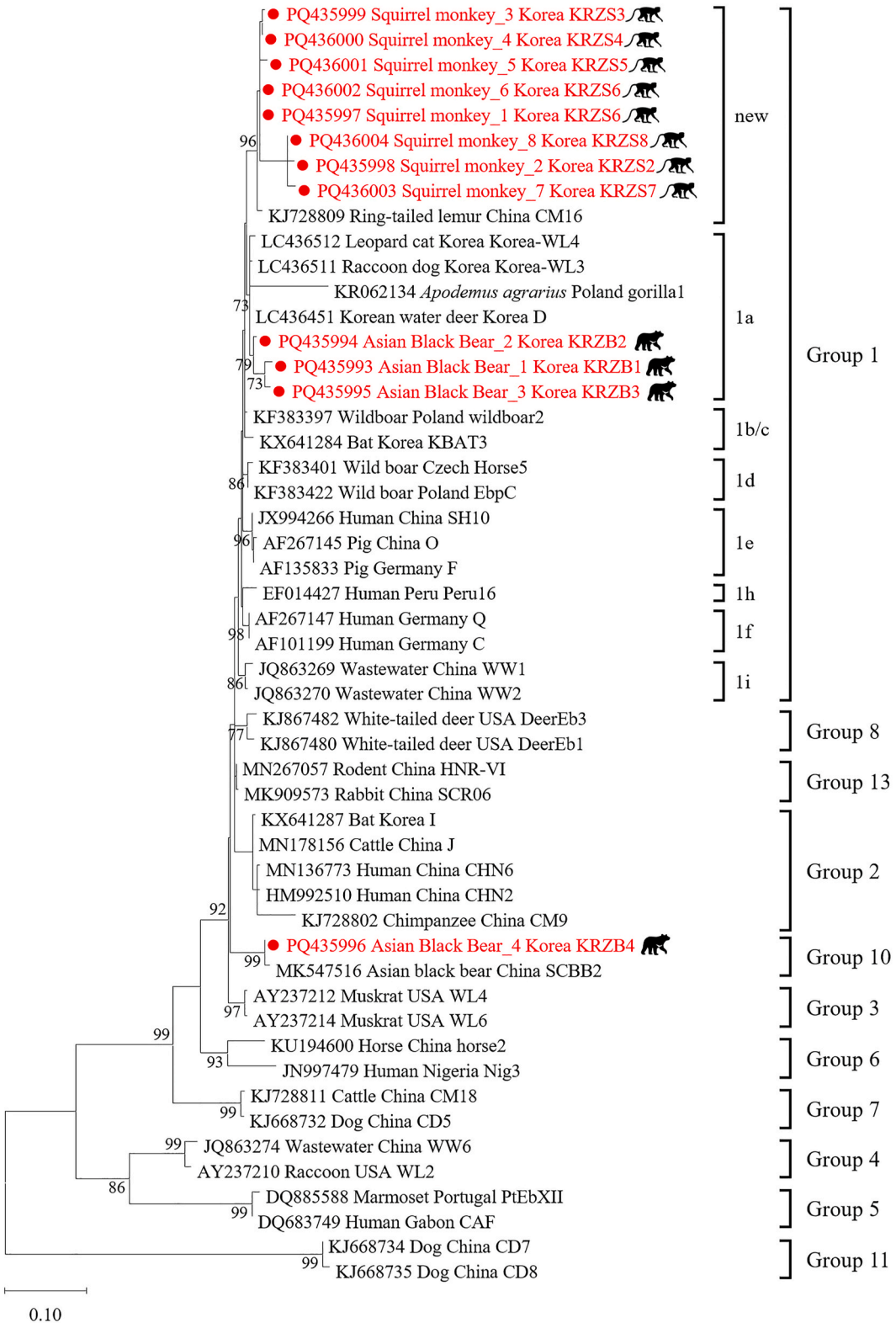

Fig. 2. Maximum-likelihood phylogenetic tree using the Hasegawa-Kishino-Yano (HKY) + G based on the ITS gene of *Enterocytozoon bieneusi*. Bootstrap values were calculated with 1000 replicates of the alignment. The scale bar represents the number of nucleotide substitutions per site. The sequences determined in this study are symbolized by red filled circles.

transmitted between animals and zookeepers (Karim et al., 2014; Yu et al., 2017). Prior studies have also detected *E. bieneusi* in non-human primates and carnivores (Sak et al., 2011; Karim et al., 2015; Yu et al., 2017, 2020; Li et al., 2018; Koster et al., 2021; Moreira et al., 2024), with various zoonotic genotypes identified from these animals (Karim et al., 2015; Yu et al., 2017; Zhong et al., 2017; Chen et al., 2020; Koster et al., 2021; Moreira et al., 2024). Remarkably, the genotypes shown in our study differ from those reported previously, suggesting that

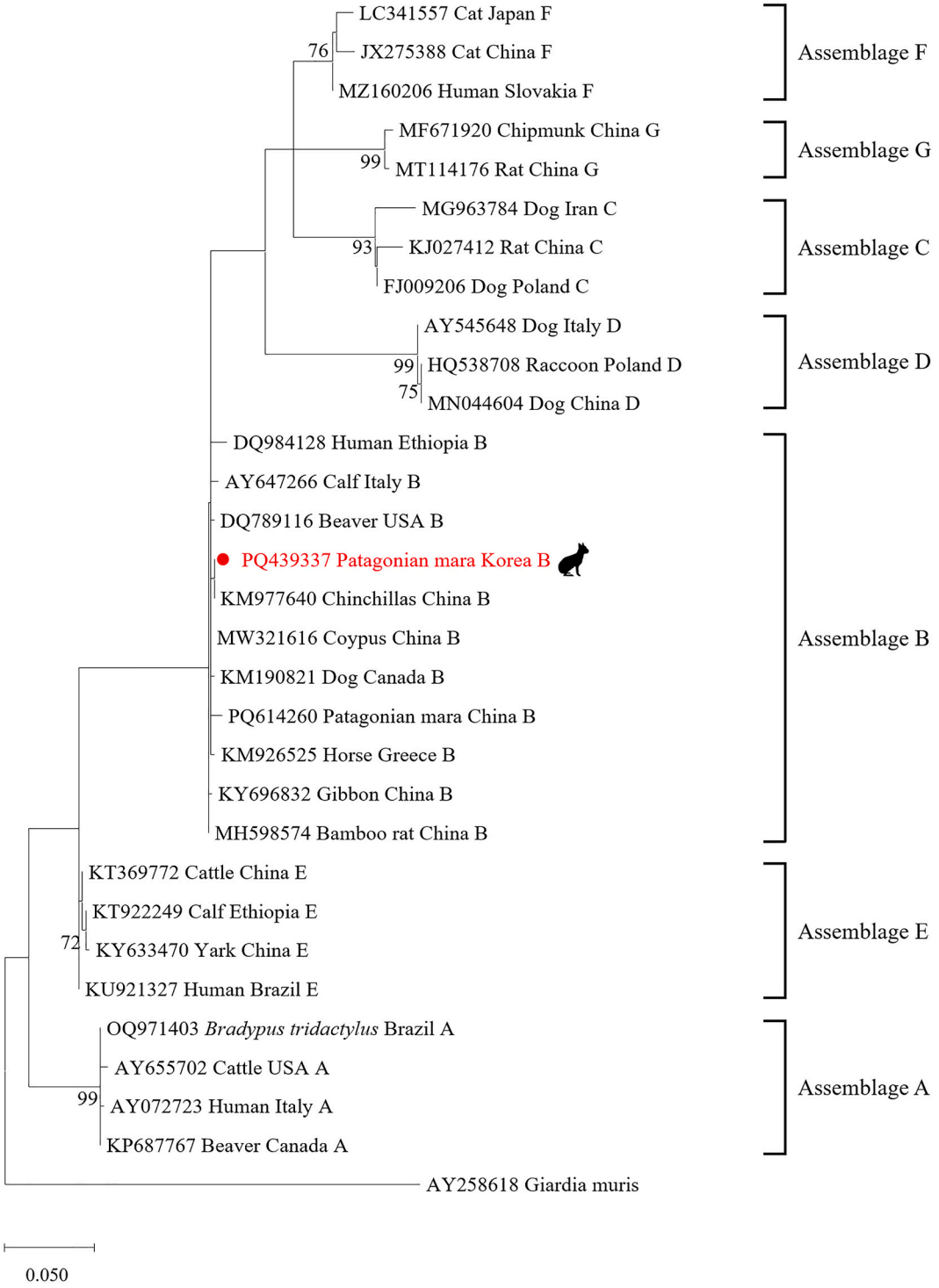

Fig. 3. Phylogenetic analysis based on *bg* of *Giardia duodenalis* using the Maximum-likelihood method with the Tamura-Nei (TN93) + G + I model. The numbers over the branches indicate bootstrap values as a percentage of 1000 replicates that support each phylogenetic branch. The scale bar represents the number of nucleotide substitutions per site. The sequence identified in Patagonian mara is shown by a red filled circle.

cross-species transmission of *E. bieneusi* may be occurring. Phylogenetic analysis revealed eight novel genotypes belonging to a new group, while three novel genotypes were assigned to group 1b. Besides KRZB4 classified into Group 10, ten novel genotypes fell into zoonotic Group 1, highlighting a potential public health threat. These findings suggest that squirrel monkeys and Asian black bears could also act as reservoir hosts for zoonotic *E. bieneusi* genotypes. Further investigation is needed to clarify the transmission routes of *E. bieneusi* in zoo animals.

Giardia duodenalis is a major pathogen associated with waterborne protozoan outbreaks of diarrhea worldwide. Waterborne transmission plays a crucial role in the epidemiology of this parasite, raising potential public health concerns (Baldursson and Karanis, 2011; Efstratiou et al., 2017). To date, *G. duodenalis* has been reported in various wildlife species (Karim et al., 2021; Kumari et al., 2021; Willenborg et al., 2022; Zou et al., 2022; An et al., 2024; Chen et al., 2024; Egan et al., 2024; Goncalves et al., 2024; Matas-Mendez et al., 2024), with assemblages A

and B predominantly found among them. In our study, *G. duodenalis* assemblage B was detected in a single Patagonian mara, which is unexpected given its previous identification in other rodent species, including beavers, muskrats, and chinchillas (Sulaiman et al., 2003; Levecke et al., 2011), indicating that *G. duodenalis* assemblage B appears to be susceptible to rodents. Moreover, according to recent results, *G. duodenalis* assemblage B was most frequently identified in several animal species, such as the families Lemuridae, Canidae, Viverridae, Rhinocerotidae, and Caviidae (Zhao et al., 2025). Given these findings, it appears that *G. duodenalis* assemblage B can infect a wide host range of mammals as well as humans (Cai et al., 2021).

In this study, the infection rate (0.4 %) of *G. duodenalis* was significantly lower compared to the results reported from other countries (Levecke et al., 2011; Karim et al., 2021; Willenborg et al., 2022; Chen et al., 2024; Goncalves et al., 2024; Matas-Mendez et al., 2024; Zhao et al., 2025). This can be explained by differences in environmental factors, detection methods, and sample number. To detect *G. duodenalis*, we used nested PCR based on the *bg* gene, one of the single-copy genes (*bg*, *tpi*, and *gdh*), which has considerably lower diagnostic sensitivity than those based on multiple-copy genes (e.g. SSU rRNA) (Zhang et al., 2020). It is speculated that the low infection rate may be due to the fact that multiple-copy genes were not used. A limitation of the present study is that that only one gene was used to detect this pathogen. Therefore, it may be necessary to utilize both genes to screen *G. duodenalis* infection.

At this point, the transmission route for *G. duodenalis* into Patagonian mara remains unclear; however, the possibility of waterborne infection cannot be discounted. Moreover, *G. duodenalis* has been detected in water sources, including surface water and wastewater, in other studies (Hatam-Nahavandi et al., 2017; Arussi et al., 2024). It is conceivable that cysts excreted from infected animals could inadvertently enter the water system, with cysts released through wastewater potentially contaminating surface water and leading to infection. Another possibility is that *G. duodenalis* may be propagated by rodents, which are known carriers and transmitters of enteric parasites like *Cryptosporidium* and *Giardia* (Hancke and Suarez, 2022; Li et al., 2023). Interestingly, the *G. duodenalis* assemblage B found in Patagonian mara showed 100 % homology with chinchillas and bamboo rats from China. This suggests that rodents could play a significant role in transmission. To date, there have been no reports of *G. duodenalis* in rodents within the ROK, indicating that further research is necessary to explore *G. duodenalis* by examining environmental sources.

Furthermore, a recent study reported a prevalence of *G. duodenalis* in a zoo in China, which was higher (3.82 %) than our results (Zhao et al., 2025). This discrepancy may have been influenced by environmental differences between the two zoos in addition to sample number. The zoo described in the previous study (Zhao et al., 2025) is located near a river, which may provide a more conducive environment for the survival of *G. duodenalis,* whereas this zoo (Cheongju Land Zoo) is located in a mountainous area with fewer natural water sources. Such environmental differences may contribute to the differing detection rates observed between the two studies. In the current study, the Patagonian mara in which *G. duodenalis* was detected, may have been attributed to the high population size in the group and frequent contact among animals, facilitating the transmission of intestinal parasites. It is important to note that various factors, such as environmental conditions or water resources, may influence *G. duodenalis* infection in wildlife beyond just host specificity. Therefore, these findings indicate that *G. duodenalis* contamination in water poses a potential risk to both human and wildlife health.

The transmission routes of these zoonotic pathogens remain undetermined; these pathogens can be transmitted not only from animals to humans but also from humans to animals. However, because the animals are confined in zoos, the likelihood of transmission to humans (keepers) is low. Most of all, the possibility cannot be excluded that these pathogens could be transmitted by zookeepers using contaminated hands to manage the enclosure environment or prepare food. The feed supplied to all animals is fresh twice a day, and the water is supplied from groundwater or surface water. It is speculated that the animals in this study were infected with these pathogens through contaminated water. Additionally, some animals may have already been infected before being brought to the zoo, and wild rodents and birds may have acted as infection source for these pathogens. Hence, additional research is necessary to elucidate *transmission pathways, and these findings underscore the importance of continual monitoring for zoonotic diseases and implementing* biosecurity practices.

One limitation of this study was that no microscopical analysis of feces was performed. Previous studies have also reported that PCR analysis is less sensitive if the number of oocysts is low or if oocysts are not completely broken (Fayer et al., 2007; Mueller-Doblies et al., 2008). Additionally, *Cryptosporidium* spp. was not detected; this might be attributed to the absence of *Cryptosporidium* spp. infection in zoo animals or the low sensitivity of PCR due to several inhibitors in the feces. Therefore, our results suggest that various methods should be performed to increase the accuracy of diagnosis.

5. Conclusion

This study demonstrated the detection of *C. burnetii*, several novel *E. bieneusi* genotypes, and *G. duodenalis* assemblage B in zoo animals, with *E. bieneusi* being the most frequently identified pathogen. Our results show that zoo animals carry a range of zoonotic pathogens. These findings imply that *E. bieneusi* and *G. duodenalis* could potentially cross-infect various host groups. The identification of zoonotic pathogens in zoo animals underscores the necessity for effective strategies to prevent and manage these pathogens. Consequently, future research is essential to clarify the transmission route of these zoonotic pathogens.

CRediT authorship contribution statement

Jinho Um: Writing – original draft, Visualization, Resources, Investigation, Formal analysis. **Jeongho Kim:** Resources, Conceptualization. **Seok-Jin Cho:** Investigation, Formal analysis. **Min-ho Park:** Investigation, Formal analysis. **Hyung-Chul Cho:** Formal analysis. **Yu-Jin Park:** Formal analysis. **Kyoung-Seong Choi:** Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethical approval

This study was exempt from ethical approval from the Institutional Animal Care and Use Committee (IACUC) at Kyungpook National University because the IACUC at this University only evaluates laboratory animals maintained within indoor facilities and not outdoor animals, and thus no ethics approval was required.

Data availability

All data generated and analyzed during this study were included in the article.

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Declaration of competing interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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References

Abdel-Moein, K.A., Hamza, D.A., 2018. Rat as an overlooked reservoir for *Coxiella burnetii*: a public health implication. Comp. Immunol. Microbiol. Infect. Dis. 61, 30–33. https://doi.org/10.1016/j.cimid.2018.11.002.

Adam, R.D., 2001. Biology of *Giardia lamblia*. Clin. Microbiol. Rev. 14, 447–475. https://doi.org/10.1128/CMR.14.3.447-475.2001.

An, D., Jiang, T., Zhang, C., Ma, L., Jia, T., Pei, Y., Zhu, Z., Liu, Q., Liu, J., 2024. Epidemiology and molecular characterization of zoonotic gastrointestinal protozoal infection in zoo animals in China. Animals (Basel) 14, 853. https://doi.org/10.3390/ani14060853.

Angelakis, E., Raoult, D., 2010. Q fever. Vet. Microbiol. 140, 297–309. https://doi.org/10.1016/j.vetmic.2009.07.016.

Appelbee, A.J., Thompson, R.C., Olson, M.E., 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife-current status and future needs. Trends Parasitol. 21, 370–376. https://doi.org/10.1016/j.pt.2005.06.004.

Arussi, D., Salant, H., Rojas, A., Dvir, E., 2024. Prevalence and molecular characterization of *Giardia duodenalis* in companion dogs, domestic livestock and wildlife in the Jordan Basin, Israel. Vet. Parasitol. Reg. Stud. Reports 52, 101042. https://doi.org/10.1016/j.vprsr.2024.101042.

Baldursson, S., Karanis, P., 2011. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010. Water Res. 45, 6603–6614. https://doi.org/10.1016/j.watres.2011.10.013.

Caballero-Gomez, J., Cano Terriza, D., Pujols, J., Martinez-Nevado, E., Carbonell, M.D., Guerra, R., Recuero, J., Soriano, P., Barbero, J., Garcia-Bocanegra, I., 2022. Monitoring of bluetongue virus in zoo animals in Spain, 2007-2019. Transbound. Emerg. Dis. 69, 1739–1747. https://doi.org/10.1111/tbed.14147.

Cai, W., Ryan, U., Xiao, L., Feng, Y., 2021. Zoonotic giardiasis: an update. Parasitol. Res. 120, 4199–4218. https://doi.org/10.1007/s00436-021-07325-2.

Celina, S.S., Cerny, J., 2022. *Coxiella burnetii* in ticks, livestock, pets and wildlife: a mini-review. Front. Vet. Sci. 9, 1068129. https://doi.org/10.3389/fvets.2022.1068129.

Chen, J., Zhou, L., Cao, W., Xu, J., Yu, K., Zhang, T., Wang, Y., Wang, J., Huang, H., Zhao, W., 2024. Prevalence and assemblage identified of *Giardia duodenalis* in zoo and farmed Asiatic black bears (*Ursus thibetanus*) from the Heilongjiang and Fujian Provinces of China. Parasite 31, 50. https://doi.org/10.1051/parasite/2024048.

Chen, L., Li, N., Guo, Y., Zhao, J., Feng, Y., Xiao, L., 2020. Multilocus sequence typing of *Enterocytozoon bieneusi* in crab-eating macaques (*Macaca fascicularis*) in Hainan, China. Parasites Vectors 13, 182. https://doi.org/10.1186/s13071-020-04046-w.

Cho, H.C., Hwang, S., Kim, E.M., Park, Y.J., Shin, S.U., Jang, D.H., Chae, J.S., Choi, K.S., 2021. Prevalence and molecular characterization of *Coxiella burnetii* in cattle, goats, and horses in the Republic of Korea. Vector Borne Zoonotic Dis. 21, 502–508. https://doi.org/10.1089/vbz.2020.2764.

Choi, K.S., Hwang, S., Kim, M.C., Cho, H.C., Park, Y.J., Ji, M.J., Han, S.W., Chae, J.S., 2024. Molecular surveillance of zoonotic pathogens from wild rodents in the Republic of Korea. PLoS Neglected Trop. Dis. 18, e0012306. https://doi.org/10.1371/journal.pntd.0012306.

Clemente, L., Fernandes, T.L., Barahona, M.J., Bernardino, R., Botelho, A., 2008. Confirmation by PCR of *Coxiella burnetii* infection in animals at a zoo in Lisbon, Portugal. Vet. Rec. 163, 221–222. https://doi.org/10.1136/vr.163.7.221.

Efstratiou, A., Ongerth, J.E., Karanis, P., 2017. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2011-2016. Water Res. 114, 14–22. https://doi.org/10.1016/j.watres.2017.01.036.

Egan, S., Barbosa, A.D., Feng, Y., Xiao, L., Ryan, U., 2024. Minimal zoonotic risk of cryptosporidiosis and giardiasis from frogs and reptiles. Eur. J. Protistol. 93, 126066. https://doi.org/10.1016/j.ejop.2024.126066.

Fayer, R., 2004. *Cryptosporidium*: a water-borne zoonotic parasite. Vet. Parasitol. 126, 37–56. https://doi.org/10.1016/j.vetpar.2004.09.004.

Fayer, R., Santin, M., Trout, J.M., 2007. Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. Vet. Parasitol. 145, 260–266. https://doi.org/10.1016/j.vetpar.2006.12.009.

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24, 110–140. https://doi.org/10.1128/CMR.00033-10.

Forsyth, M.B., Morris, A.J., Sinclair, D.A., Pritchard, C.P., 2012. Investigation of zoonotic infections among Auckland zoo staff: 1991-2010. Zoonoses Publ. Health 59, 561–567. https://doi.org/10.1111/j.1863-2378.2012.01496.x.

Garcia, E., Espeso, G., Fernandez, R., Gomez-Martin, A., Rodriguez-Linde, J.M., De la Fe, C., 2017. *Coxiella burnetii* detected in three species of endangered north African gazelles that recently aborted. Theriogenology 88, 131–133. https://doi.org/10.1016/j.theriogenology.2016.09.019.

Goncalves, G.H.P., Leal, D.A.G., Roratto, P.A., de Souza Junior, J.C., Souza, S.S., Peruchi, A.R., Nunes, A.J.D., da Silva Filho, H.H., Hirano, Z.M.B., Giongo, A., Greinert-Goulart, J.A., 2024. Diversity of gastrointestinal parasites and molecular characterization of *Giardia duodenalis* in free-living and captive howler monkeys (*Alouatta guariba clamitans*) in southern Brazil. Vet. Parasitol. Reg. Stud. Reports 52, 101048. https://doi.org/10.1016/j.vprsr.2024.101048.

Gonzalez-Barrio, D., Ruiz-Fons, F., 2019. *Coxiella burnetii* in wild mammals: a systematic review. Transbound. Emerg. Dis. 66, 662–671. https://doi.org/10.1111/tbed.13085.

Gottling, J., Heckel, J.O., Hotzel, H., Fruth, A., Pfeifer, Y., Henning, K., Kopp, P., Mertens-Scholz, K., Rietschel, W., Pfeffer, M., 2022. Zoonotic bacteria in clinically healthy goats in petting zoo settings of zoological gardens in Germany. Zoonoses Publ. Health 69, 333–343. https://doi.org/10.1111/zph.12922.

Guo, Y., Alderisio, K.A., Yang, W., Cama, V., Feng, Y., Xiao, L., 2014. Host specificity and source of *Enterocytozoon bieneusi* genotypes in a drinking source watershed. Appl. Environ. Microbiol. 80, 218–225. https://doi.org/10.1128/AEM.02997-13.

Hancke, D., Suarez, O.V., 2022. A review of the diversity of *Cryptosporidium* in *Rattus norvegicus, R. rattus* and *Mus musculus:* what we know and challenges for the future. Acta Trop. 226, 106244. https://doi.org/10.1016/j.actatropica.2021.106244.

Hatam-Nahavandi, K., Mohebali, M., Mahvi, A.H., Keshavarz, H., Mirjalali, H., Rezaei, S., Meamar, A.R., Rezaeian, M., 2017. Subtype analysis of *Giardia duodenalis* isolates from municipal and domestic raw wastewaters in Iran. Environ. Sci. Pollut. Res. Int. 24, 12740–12747. https://doi.org/10.1007/s11356-016-6316-y.

Hernandez-Colina, A., Seechurn, N., Costa, T., Lopez, J., Baylis, M., Hesson, J.C., 2024. Surveillance of *Culex* spp. vectors and zoonotic arboviruses at a zoo in the United Kingdom. Heliyon 10, e26477. https://doi.org/10.1016/j.heliyon.2024.e26477.

Hrnkova, J., Schneiderova, I., Golovchenko, M., Grubhoffer, L., Rudenko, N., Cerny, J., 2021. Role of zoo-housed animals in the ecology of ticks and tick-borne pathogens-a review. Pathogens 10, 210. https://doi.org/10.3390/pathogens10020210.

Hunter, P.R., Hadfield, S.J., Wilkinson, D., Lake, I.R., Harrison, F.C., Chalmers, R.M., 2007. Subtypes of *Cryptosporidium parvum* in humans and disease risk. Emerg. Infect. Dis. 13, 82–88. https://doi.org/10.3201/eid1301.060481.

Hwang, S., Kim, J., Park, Y.J., Jang, D.H., Shin, S.U., Cho, H.C., Choi, K.S., 2021. First report of *Enterocytozoon bieneusi* from an African lion (*Panthera leo*) in a zoo in the Republic of Korea. J. Zoo Wildl. Med. 52, 337–342. https://doi.org/10.1638/2020-0048.

Jang, Y.H., Lee, S.J., Lim, J.G., Lee, H.S., Kim, T.J., Park, J.H., Chung, B.H., Choe, N.H., 2008. The rate of *Salmonella* spp. infection in zoo animals at Seoul Grand Park, Korea. J. Vet. Sci. 9, 177–181. https://doi.org/10.4142/jvs.2008.9.2.177.

Jung, B.Y., Choi, J.S., Kim, K.T., Song, Y.K., Lee, S.H., Lee, K.W., Kim, J.Y., Moon, O.K., 2007. Seroprevalence of leptospirosis in Korean municipal zoo animals. J. Vet. Med. Sci. 69, 861–863. https://doi.org/10.1292/jvms.69.861.

Karim, M.R., Wang, R., Dong, H., Zhang, L., Li, J., Zhang, S., Rume, F.I., Qi, M., Jian, F., Sun, M., Yang, G., Zou, F., Ning, C., Xiao, L., 2014. Genetic polymorphism and zoonotic potential of *Enterocytozoon bieneusi* from nonhuman primates in China. Appl. Environ. Microbiol. 80, 1893–1898. https://doi.org/10.1128/AEM.03845-13.

Karim, M.R., Dong, H., Li, T., Yu, F., Li, D., Zhang, L., Li, J., Wang, R., Li, S., Li, X., Rume, F.I., Ning, C., 2015. Predomination and new genotypes of *Enterocytozoon bieneusi* in captive nonhuman primates in zoos in China: high genetic diversity and zoonotic significance. PLoS One 10, e0117991. https://doi.org/10.1371/journal.pone.0117991.

Karim, M.R., Rume, F.I., Rahman, A., Zhang, Z., Li, J., Zhang, L., 2020. Evidence for zoonotic potential of *Enterocytozoon bieneusi* in its first molecular characterization in captive mammals at Bangladesh National Zoo. J. Eukaryot. Microbiol. 67, 427–435. https://doi.org/10.1111/jeu.12792.

Karim, M.R., Li, J., Rume, F.I., Sumon, S.M.R., Selim, A.S.M., Hoda, N., Zhang, L., 2021. Occurrence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* among captive mammals in the Bangladesh National Zoo. Parasitol. Int. 84, 102414. https://doi.org/10.1016/j.parint.2021.102414.

Koster, P.C., Dashti, A., Bailo, B., Muadica, A.S., Maloney, J.G., Santin, M., Chicharro, C., Miguelanez, S., Nieto, F.J., Cano-Terriza, D., Garcia-Bocanegra, I., Guerra, R., Ponce-Gordo, F., Calero-Bernal, R., Gonzalez-Barrio, D., Carmena, D., 2021. Occurrence and genetic diversity of protist parasites in captive non-human primates, zookeepers, and free-living sympatric rats in the cordoba zoo conservation centre, southern Spain. Animals (Basel) 11, 700. https://doi.org/10.3390/ani11030700.

Kumari, P., Eo, K.Y., Lee, W.S., Kimura, J., Yamamoto, N., 2021. DNA-based detection of *Leptospira wolffii, Giardia intestinalis* and *Toxoplasma gondii* in environmental feces of wild animals in Korea. J. Vet. Med. Sci. 83, 850–854. https://doi.org/10.1292/jvms.20-0596.

Kvapil, P., Racnik, J., Kastelic, M., Markova, J., Murat, J.B., Kobedova, K., Pittermannova, P., Budikova, M., Sedlak, K., Bartova, E., 2021. Biosurveillance of selected pathogens with zoonotic potential in a zoo. Pathogens 10, 428. https://doi.org/10.3390/pathogens10040428.

Lee, H.Y., Kwon, Y., Lee, S.E., Kim, J., Choi, H., 2024. A *Mycobacterium bovis* outbreak among exhibition animals at a zoo in the Republic of Korea: the first contact investigation of zoonotic tuberculosis. Osong Public Health Res. Perspect. 15, 248–259. https://doi.org/10.24171/j.phrp.2023.0228.

Levecke, B., Meulemans, L., Dalemans, T., Casaert, S., Claerebout, E., Geurden, T., 2011. Mixed *Giardia duodenalis* assemblage A, B, C and E infections in pet chinchillas (*Chinchilla lanigera*) in Flanders (Belgium). Vet. Parasitol. 177, 166–170. https://doi.org/10.1016/j.vetpar.2010.11.027.

Li, J., Qi, M., Chang, Y., Wang, R., Li, T., Dong, H., Zhang, L., 2015. Molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in captive wildlife at Zhengzhou Zoo, China. J. Eukaryot. Microbiol. 62, 833–839. https://doi.org/10.1111/jeu.12269.

Li, J., Qin, H., Li, X., Zhang, L., 2023. Role of rodents in the zoonotic transmission of giardiasis. One Health 16, 100500. https://doi.org/10.1016/j.onehlt.2023.100500.

Li, W., Feng, Y., Zhang, L., Xiao, L., 2019. Potential impacts of host specificity on zoonotic or interspecies transmission of *Enterocytozoon bieneusi*. Infect. Genet. Evol. 75, 104033. https://doi.org/10.1016/j.meegid.2019.104033.

Li, W.C., Qin, J., Wang, K., Gu, Y.F., 2018. Genotypes of *Enterocytozoon bieneusi* in dogs and cats in eastern China. Iran. J. Parasitol. 13, 457–465.

Lloyd, C., Stidworthy, M.F., Ulrich, W., 2010. *Coxiella burnetii* abortion in captive dama gazelle (*Gazella dama*) in the United Arab Emirates. J. Zoo Wildl. Med. 41, 83–89. https://doi.org/10.1638/2009-0005.1.

Marrie, T.J., Minnick, M.F., Textoris, J., Capo, C., Mege, J.-L., 2015. Coxiella. In: Tang, Y. W., Liu, D., Schwartzman, J., Sussman, M., Poxton, I. (Eds.), Molecular Medical Microbiology. Elsevier, pp. 1941–1972. https://doi.org/10.1016/C2010-1-67744-9.

Matas-Mendez, P., Avalos, G., Caballero-Gomez, J., Dashti, A., Castro-Scholten, S., Jimenez-Martin, D., Gonzalez-Barrio, D., Munoz-de-Mier, G.J., Bailo, B., Cano-Terriza, D., Mateo, M., Najera, F., Xiao, L., Koster, P.C., Garcia-Bocanegra, I., Carmena, D., 2024. Detection and molecular diversity of *Cryptosporidium* spp. and *Giardia duodenalis* in the endangered Iberian lynx (*Lynx pardinus*), Spain. Animals (Basel) 14, 340. https://doi.org/10.3390/ani14020340.

Mathis, A., Weber, R., Deplazes, P., 2005. Zoonotic potential of the microsporidia. Clin. Microbiol. Rev. 18, 423–445. https://doi.org/10.1128/CMR.18.3.423-445.2005.

Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518–553. https://doi.org/10.1128/CMR.12.4.518.

Monis, P.T., Thompson, R.C., 2003. *Cryptosporidium* and *Giardia*-zoonoses: fact or fiction? Infect. Genet. Evol. 3, 233–244. https://doi.org/10.1016/j.meegid.2003.08.003.

Moreira, G., Cruz, A.V.S., Santos-Silva, S., Moreira, R.S.S., Mesquita, J.R., 2024. Detection of *Enterocytozoon bieneusi* in non-human primates in Portuguese Zoos. Animals (Basel) 14, 1874. https://doi.org/10.3390/ani14131874.

Mori, M., Mertens, K., Cutler, S.J., Santos, A.S., 2017. Critical aspects for detection of *Coxiella burnetii*. Vector Borne Zoonotic Dis. 17, 33–41. https://doi.org/10.1089/vbz.2016.1958.

Mueller-Doblies, D., Giles, M., Elwin, K., Smith, R.P., Clifton-Hadley, F.A., Chalmers, R. M., 2008. Distribution of *Cryptosporidium* species in sheep in the UK. Vet. Parasitol. 154, 214–219. https://doi.org/10.1016/j.vetpar.2008.03.021.

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A., Swart, A., 2011. *Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in The Netherlands; could *Rattus* spp. represent reservoirs for (re) introduction? Prev. Vet. Med. 101, 124–130. https://doi.org/10.1016/j.prevetmed.2011.05.003.

Ryan, U., Power, M., 2012. *Cryptosporidium* species in Australian wildlife and domestic animals. Parasitology 139, 1673–1688. https://doi.org/10.1017/S0031182012001151.

Sak, B., Kvac, M., Petrzelkova, K., Kvetonova, D., Pomajbikova, K., Mulama, M., Kiyang, J., Modry, D., 2011. Diversity of microsporidia (Fungi: microsporidia) among captive great apes in European zoos and African sanctuaries: evidence for zoonotic transmission? Folia Parasitol. (Praha) 58, 81–86. https://doi.org/10.14411/fp.2011.008.

Sak, B., Petrzelkova, K.J., Kvetonova, D., Mynarova, A., Pomajbikova, K., Modry, D., Cranfield, M.R., Mudakikwa, A., Kvac, M., 2014. Diversity of microsporidia, *Cryptosporidium* and *Giardia* in mountain gorillas (*Gorilla beringei beringei*) in volcanoes national park, Rwanda. PLoS One 9, e109751. https://doi.org/10.1371/journal.pone.0109751.

Shin, S.U., Park, Y.J., Ryu, J.H., Jang, D.H., Hwang, S., Cho, H.C., Park, J., Han, J.I., Choi, K.S., 2020. Identification of zoonotic tick-borne pathogens from Korean water deer (*Hydropotes inermis argyropus*). Vector Borne Zoonotic Dis. 20, 745–754. https://doi.org/10.1089/vbz.2019.2609.

Si, Y.J., Lee, Y.N., Cheon, S.H., Park, Y.R., Baek, Y.G., Kye, S.J., Lee, M.H., Lee, Y.J., 2020. Isolation and characterization of low pathogenic H7N7 avian influenza virus from a red-crowned crane in a zoo in South Korea. BMC Vet. Res. 16, 432. https://doi.org/10.1186/s12917-020-02645-4.

Stentiford, G.D., Becnel, J., Weiss, L.M., Keeling, P.J., Didier, E.S., Williams, B.P., Bjornson, S., Kent, M.L., Freeman, M.A., Brown, M.J.F., Troemel, E.R., Roesel, K., Sokolova, Y., Snowden, K.F., Solter, L., 2016. Microsporidia - emergent pathogens in the global food chain. Trends Parasitol. 32, 336–348. https://doi.org/10.1016/j.pt.2015.12.004.

Sulaiman, I.M., Fayer, R., Bern, C., Gilman, R.H., Trout, J.M., Schantz, P.M., Das, P., Lal, A.A., Xiao, L., 2003. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg. Infect. Dis. 9, 1444–1452. https://doi.org/10.3201/eid0911.030084.

Thomas, D.R., Garnish, S.E., Khoo, C.A., Padmanabhan, B., Scott, N.E., Newton, H.J., 2024. *Coxiella burnetii* protein CBU2016 supports CCV expansion. Pathog. Dis. 82, ftae018. https://doi.org/10.1093/femspd/ftae018.

Thompson, R.C., Monis, P., 2012. *Giardia*–from genome to proteome. Adv. Parasitol. 78, 57–95. https://doi.org/10.1016/B978-0-12-394303-3.00003-7.

Torina, A., Naranjo, V., Pennisi, M.G., Patania, T., Vitale, F., Laricchiuta, P., Alongi, A., Scimeca, S., Kocan, K.M., de la Fuente, J., 2007. Serologic and molecular characterization of tick-borne pathogens in lions (*Panthera leo*) from the Fasano Safari Park, Italy. J. Zoo Wildl. Med 38, 591–593. https://doi.org/10.1638/2007-0043R1.1.

Tozer, S.J., Lambert, S.B., Strong, C.L., Field, H.E., Sloots, T.P., Nissen, M.D., 2014. Potential animal and environmental sources of Q fever infection for humans in Queensland. Zoonoses Publ. Health 61, 105–112. https://doi.org/10.1111/zph.12051.

Willenborg, C.M., Cervena, B., Thompson, P., Rosario, E., Ruaux, C., Vogelnest, L., Slapeta, J., 2022. *Giardia duodenalis* in a clinically healthy population of captive zoo chimpanzees: rapid antigen testing, diagnostic real-time PCR and faecal microbiota profiling. Int. J. Parasitol. Parasites Wildl. 17, 308–318. https://doi.org/10.1016/j.ijppaw.2022.03.007.

Woldehiwet, Z., 2004. Q fever (coxiellosis): epidemiology and pathogenesis. Res. Vet. Sci. 77, 93–100. https://doi.org/10.1016/j.rvsc.2003.09.001.

Woolf, D., Sanchez, C., Gonzalez-Astudillo, V., Navarro, M., Tapia, C.C., Franco, M., Bustamante, J.A., Astudillo, M., Mukherjee, J., 2021. *Leptospira* species status of captive nonhuman primates and free-ranging rodents at the Barranquilla Zoo, Colombia, 2013. J. Zoo Wildl. Med. 51, 780–788. https://doi.org/10.1638/2019-0192.

Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: an update. Exp. Parasitol. 124, 80–89. https://doi.org/10.1016/j.exppara.2009.03.018.

Xiao, L., Ryan, U.M., Fayer, R., Bowman, D.D., Zhang, L., 2012. *Cryptosporidium tyzzeri* and *Cryptosporidium pestis*: which name is valid? Exp. Parasitol. 130, 308–309. https://doi.org/10.1016/j.exppara.2011.12.009.

Yu, F., Wu, Y., Li, T., Cao, J., Wang, J., Hu, S., Zhu, H., Zhang, S., Wang, R., Ning, C., Zhang, L., 2017. High prevalence of *Enterocytozoon bieneusi* zoonotic genotype D in captive golden snub-nosed monkey (*Rhinopithecus roxellanae*) in zoos in China. BMC Vet. Res. 13, 158. https://doi.org/10.1186/s12917-017-1084-6.

Yu, M., Liu, X., Xie, M., Li, D., Ni, Q., Zhang, M., Wu, J., Xu, H., Yao, Y., 2020. Epidemiological investigation and genotypes of *Enterocytozoon bieneusi* in 11 captive *Rhesus macaque* populations. Int. J. Parasitol. Parasites Wildl. 13, 191–195. https://doi.org/10.1016/j.ijppaw.2020.10.007.

Zanatto, D.C.S., Duarte, J.M.B., Labruna, M.B., Tasso, J.B., Calchi, A.C., Machado, R.Z., Andre, M.R., 2019. Evidence of exposure to *Coxiella burnetii* in neotropical free-living cervids in South America. Acta Trop. 197, 105037. https://doi.org/10.1016/j.actatropica.2019.05.028.

Zhang, K., Zheng, S., Wang, Y., Wang, K., Wang, Y., Gazizova, A., Han, K., Yu, F., Chen, Y., Zhang, L., 2021. Occurrence and molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis, Enterocytozoon bieneusi*, and *Blastocystis sp*. in captive wild animals in zoos in Henan, China. BMC Vet. Res. 17, 332. https://doi.org/10.1186/s12917-021-03035-0.

Zhang, X., Wang, L., Lan, X., Dan, J., Ren, Z., Cao, S., Shen, L., Deng, J., Zuo, Z., Yu, S., Wang, Y., Ma, X., Liu, H., Zhou, Z., Hu, Y., Fu, H., He, C., Geng, Y., Gu, X., Peng, G., Wang, Y., Zhong, Z., 2020. Occurrence and multilocus genotyping of *Giardia duodenalis* in captive non-human primates from 12 zoos in China. PLoS One 15, e0228673. https://doi.org/10.1371/journal.pone.0228673.

Zhao, Q., Pei, Z., He, Y., Jia, T., Zhang, Y., Zheng, M., Zhang, Z., Qi, M., 2025. Expansion of the known host range of *Giardia duodenalis* and *Cryptosporidium* spp. in captive wildlife at Beijing zoo. Parasite 32, 4. https://doi.org/10.1051/parasite/2024079.

Zhong, Z., Li, W., Deng, L., Song, Y., Wu, K., Tian, Y., Huang, X., Hu, Y., Fu, H., Geng, Y., Ren, Z., Peng, G., 2017. Multilocus genotyping of *Enterocytozoon bieneusi* derived from nonhuman primates in southwest China. PLoS One 12, e0176926. https://doi.org/10.1371/journal.pone.0176926.

Zou, Y., Li, X.D., Meng, Y.M., Wang, X.L., Wang, H.N., Zhu, X.Q., 2022. Prevalence and multilocus genotyping of *Giardia duodenalis* in zoo animals in three cities in China. Parasitol. Res. 121, 2359–2366. https://doi.org/10.1007/s00436-022-07565-w.