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RESEARCH

Besnoitia was detected in the fecal samples of a cattery in Shenyang, China

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Abstract

Besnoitia are a genus of cyst-forming protozoa, with cats serving as the definitive host for several species of *Besnoitia*. In order to gain insight into the epidemiology of *Besnoitia* infection in cats in catteries in Shenyang City, 58 fecal samples were collected from six catteries, and oocysts conforming morphologically to *Besnoitia* as well as *Besnoitia* ribosomal internal transcribed spacer 1 (ITS1) sequences were detected, confirming the presence of this parasite in a single cattery located in Shenyang, China. The ITS1 sequences were 100% identical to *Besnoitia acinonyx*. The findings of this study contribute to the existing epidemiological data on the disease, providing a valuable reference for the prevention and treatment of the disease.

Keywords *Besnoitia* · Cattery · Shenyang · *Besnoitia* acinonyx

Introduction

Besnoitia spp., classified within the phylum Apicomplexa and family Sarcocystidae, are cyst-forming protozoa closely related to *Toxoplasma gondii* and *Neospora caninum*. To date, ten species have been identified to date, including *B.akadoni, B.bennetti, B.besnoiti, B.caprae, B.darlingi, B.jellisoni, B.neotomofelis, B.oryctofelisi, B.tarandi* and *B.wallacei* (Table 1). While intermediate hosts (e.g., bovids, wild ruminants, equids, and goats) are well-characterized, definitive hosts for B. besnoiti, B. bennetti, B. caprae, and B. tarandi remain unknown. The pathogenicity of *Besnoitia* infections is primarily observed in natural intermediate hosts (Arya et al. 2005). In intermediate hosts, two asexual reproductive stages, namely tachyzoites and bradyzoites, lead to acute and chronic infections. Following invasion of the intermediate host, tachyzoites first proliferate rapidly in endothelial cells, forming tachyzoites and causing acute infection. To evade the host's immune response, tachyzoites then transform into bradyzoites, which form encapsulated cysts in subcutaneous tissues and mucous membranes, leading to chronic infection and the development of skin lesions and systemic clinical symptoms of varying degrees of severity, which show the characteristics of the disease (Oryan et al. 2014).

The are cats and cheetahs serve as definitive hosts for several *Besnoitia* species (Verma et al. 2017; Madder et al. 2019). Felids become infected after consuming prey (e.g., rodents) containing tissue-cyst bradyzoites. These bradyzoites undergo merogony and gametogony in epithelial intestinal cells and unsporulated oocysts are passed in the faeces (Dubey et al. 2002). After sporulation in a suitable environment, the oocysts are ingested by an intermediate host, where they develop into cysts in the tissues and organs and the life cycle is completed when the definitive host ingests these cysts in infected prey. As cats play a crucial role in the transmission of *Besnoitia* species, it is important to gain a deeper understanding of the infection status of *Besnoitia* in cats. Therefore, in this study, fecal samples from cats in several catteries in Shenyang City were collected and screened for *Besnoitia* species.

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Table 1 Species, natural hosts, and geographic distribution of *Besnoitia*

Species	Intermediate host	Definitive host	Geographical distribution
B.besnoiti	Cattle, antelope (*Aepyceros melampus*), impala, blue wildebeest (*Connochaetes taurinus*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*)	unknown	Africa, Asia, Europe and Venezuela
B.bennetti	Donkeys (*Equus africanus asinus*), horses (*E ferus caballus*) and zebras (*E zebra, E quagga, E grevyi*)	unknown	Africa, Asia, US and France
B.caprae	Goats (*Capra aegagrus hircus*)	unknown	Iran and Kenya
B.tarandi	Muskox (*Ovibos moschatus*), reindeer (*Rangifer tarandus*), mule deer (*Odocoileus hemionus*)	unknown	Canada, Finland, Russia and Sweden
B.akodoni	*Akodon motensis*	unknown	Brazil
B.jellisoni	*Dipodomys sppa, Peromyscus maniculatus*	unknown	US
B.darlingi	*Ameiva, Basiliscus, Didelphis marsupialis*	Cat (Felis catus)	US and Panama
B.neotomofelis	*Neotoma micropus*	Cat (*Felis catus*)	US
B.oryctofelisi	*Oryctolagus cuniculus*	Cat (*Felis catus*)	Argentina
B.wallacei	*Rattus exulans*	Cat (*Felis catus*)	Australia, Hawaii, Japan and Kenya
B.darlingi	*Acinonyx jubatus*	Acinonyx jubatus	Namibia

Research methodology

Samples collection

In December 2023, 58 fresh fecal samples were collected in batches from six catteries in Shenyang and subsequently transported to our laboratory for oocyst detection and polymerase chain reaction (PCR) analysis. These samples were obtained from the floors of the cat cages, which were routinely cleaned to uphold hygiene conditions.

PCR amplification

A pair of specific primers (Table 2) was designed based on the sequence of the internal transcribed spacer 1 (ITS1) of the 18S rRNA gene of *Besnoitia* (GenBank accession number: MW468050.1, positions 603 nt-946 nt). These primers were then sent to Sangon Biotech (Shanghai) Co., Ltd. for synthesis.

The DNA was extracted from fecal samples using the TIANamp Genomic DNA Extraction Kit, following the manufacture 's instructions precisely. The extracted DNA served as the template for PCR amplification with the designed primers. In the experiment setup, the reaction mixture comprised of 10 µL of 10× Ex Taq mix, 1 µL of each primer (10µM), 1 µL of DNA, and 7 µL of water. The PCR cycling conditions were as follows: an initial pre-denaturation step at 94 ℃ for 5 minutes, followed by 30 cycles of denaturation at 94 ℃ for 30 seconds, annealing at 68 ℃ for 30 seconds, and extension at 72 ℃ for 30 seconds, with a final extension setup at 72 ℃ for 2 minutes. Five µL of the PCR amplification product was subjected to electrophoresed on a 1.5% agarose gel. The electrophoresis results were visualized and documented using a gel imaging system (Wang et al.2015).

A blind testing approach was implemented during the PCR process. This invloved randomly shuffling samples from different sources and then transferring them to other personnel for analysis to minimize bias.

To validate the sequences of these amplified fragments, three samples (2, 10, 29) were randomly selected and sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing.

Microscopic examination of *Besnoitia* oocysts

Fecal samples were thoroughly mixed with 10-fold volume of saturated saline solution. The resulting mixture was then filtered through a 40- or 60- mesh sieve and allowed to stand undisturbed for 3–5 minutes. Subsequently, the upper layer of liquid was carefully aspirated using a fine rubber-tipped buret and transferred onto a microscope slide. Then the fecal suspension was examined under a microscope at

Table 2 Primer sequences used in the experiment

Primer name	Sequence (5' – 3')	length	Tm	locatin in MW468050.1	Amplification length(bp)
Besno F	GGATCATTCACACGTTGTCCT	21	58.30	603–623	344
Besno R	CATTGCGTTTCGCATTTCGC	20	59.98	927–946	

40× magnification to ascertain the presence of oocysts (Li et al.2019)

Clinical observations

To assess the pathogenicity and potential sources of *Besnoitia* infection, comprehensive information regarding the mental and physical status, as well as management practices, of cats in the PCR-positive cattery was collected.

Results and analysis

PCR and sequence BLAST

In the PCR assay of the first batch of 38 fecal samples target bands were successfully amplified in 33 fecal samples obtained from the same cattery, while five samples from another cattery yielded negative results (Fig. 1). In the second collection of fecal samples, no target bands were amplified, and all samples tested negative.

The PCR amplification products from samples 2, 10, and 29 were sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing. When the ITS1 sequences obtained from sequencing were compared using the Basic Local Alignment Search Tool (BLAST), it was founded that the ITS1 gene sequences of the three PCR amplification products were completely identical. The sequences *(Genbank accession number* PQ068757.1*)* exhibited 100% similarity with *Besnoitia-acinonyx (GenBank accession number MW468050.1),* a parasite previously identified in cheetah feces in Namibia. Additionally, the sequences showed over 90% similarity to those of *B.darlingi* and *B.oryctofelisi*, suggeting a high prevalence of *Besnoitia* infection in the cattery.

Oocyst detection

Each samples was analyzed individually. The upper layer of saturated saline was aspirated with a rubber-tipped burette and transferred onto a microscope slide. The slide was then examined under a microscope at 40× magnification to identify the oocysts. Through microscopic observation, among the 33 PCR-positive samples, no sample contained ovoid, coccidian-like structures measuring 10–14 × 10–13 µm in size, which is consistent with the literature description (Dubey et al.2003).

Clinical observations

The cattery accommodated 24 female cats and 37 kittens. All the felines were housed in cages and are routinely vaccinated. Thry are bred under the self-breeding and self-raising mode. The cats were treated annually with Drontal, Milbemycin Oxime, and Praziquantel tablets to combat intestinal parasites. they diet was formulated as a composite of commercially accessible cat food and freeze - dried feline meals, supplemented occasionally with chicken breasts. This information implies that *Besnoitia* may be present within the cattery environment, and it is improbable that the transmission of this organism occurs via food.

These cats demonstrated good mental states and were in good physical health, showing no signs of underweight condition. The feces matter was consistently observed to be of normal consistency and well-formed. Nevertheless, sporadic incidences of hairball and food regurgitation were noted. The findings from this cattery observations suggest that *Besnoitia* does not pose a substantial threat to cats, which is consistent with existing research (Dubey et al. 2002).

Discussion

In this study, *Besnoitia* infection was identified in domestic cats from a cattery in Shenyang through PCR-based fecal analysis. Comparaive analysis of the ITS sequence demonstrated 100% identity with *Besnoitia acinonyx*. Marking the first detection of this parasite in chinese cats and its first reported occurrence outside Africa.

While clinical cases of besnoitiosis have been documented in domestic animals across multiple Chinese

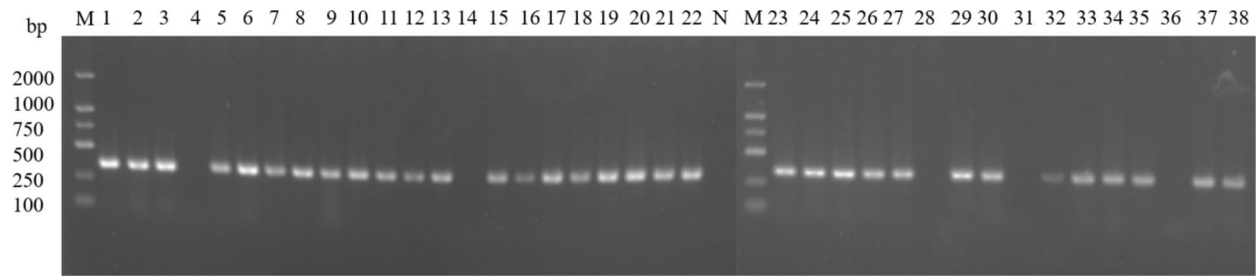

Fig. 1 Results of PCR amplification of *Besnoitia*. The blind method is employed during the PCR testing process. 33 positive samples were from the same cattery, while five samples from another cattery were negative.

provinces (Qiu et al.2017), prior reports of *Besnoitia* infections in feline definitive hosts remain absent. Our study findings confirmed the presence of *Besnoitia* in domestic cats using a molecular approach.

Although *Besnoitia* infects diverse hosts, clinical manifestations predominantly occur in intermediate hosts. To date, no cases have been reported in cats as definitive hosts. The absence of clinical signs in PCR-positive cats from the affected cattery aligned with the reported low pathogenicity of *Besnoitia* in definitive hosts. However, the limited detection of suspected oocyst (1/33 PCR-positive samples) suggests low parasitic burden or challenges in morphological identification, underscoring the necessity of molecular assay for accurate diagnosis.

The ITS1 sequence serves as the basis for identifying *Besnoitia* species (González-Barrio et al. 2021). The ITS1 sequences obtained in this study exhibited complete homology with *B.acinonyx* (MW468050.1), a species originally isolated from cheetah droppings in Namibia in 2021 (Schares et al. 2021). This genetic consistency highlights potential epidemiological links between geographically distinct populations.

Nevertheless, this study is constrained by its small sample size and localized sampling scope. Expanded surveillance across Shenyang, Liaoning Province, or other regions is imperative to elucidate the distribution and zoonotic implications of *Besnoitia* in China.

Conclusion

This study provides the pioneering evidence of the presence of *Besnoitia* in catteries within Liaoning Province, China. Furthermore, the strain has been precisely identified as *Besnoitia acinonyx*. These findings highlighted the imperative for in-depth investigations into *Besnoitia* infections, the characteristics of its strains, and their distribution pattens among domestic cats and livestock in Liaoning Province and across China. Conducting such research is indispensable for the effective prevention and control of *Besnoitiosis*.

Author contribution BaoShan LIU conceived the experiments. LiHui YU collected the fecal samples. TingTing Zhang conducted the data analysis and polished the manuscript. HaoYan YANG and Hao WANG performed the experiments. HaoYan YANG analyzed the data and drafted the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval Not applicable. No laboratory animals were involved.

Consent for publication All authors consent to publish the manuscript.

Competing interest The authors declare no competing interests.

Sequence information All sequences are publicly available. The found sequence has been deposited in the GenBank—sequence number PQ068757.1.

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