Theileria, Hepatozoon and Taenia infection in great gerbils (Rhombomys opimus) in northwestern China

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ABSTRACT

The great gerbil (Rhombomys opimus), widely distributed in Asia, is a natural reservoir for Yersinia pestis, Leishmania donovani and some species of helminths. In this study, 188 great gerbils were sampled in Alataw City and Manas County, northwestern China, and tested for the presence of Theileria, Hepatozoon and Taenia species by molecular methods. Theileria sp., named as "Candidatus Thelileria xinjiangensis", was detected in heart, liver, spleen, lung, and kidney of 6.9% rodents. Six genotypes of "Taenia sp. Rhombomys opimus", which were close to Taenia laticollis (87.3–94.0% identities), were detected in cyst liquid of 5.3% rodents. "Hepatozoon aegypti-lake" haemogregarines was detected in spleens of 1.6% rodents. To our best knowledge, Candidatus Thelileria xinjiangensis, Hepatozoon aegypti-lake and genotypes of "Taenia sp. Rhombomys opimus" were found for the first time in great gerbils. These results extend our knowledge on the diversity and pathogenesis of Thelileria, Hepatozoon and Taenia species.

1. Introduction

The great gerbil (Rhombomys opimus) is widely distributed in Asian countries, such as Afghanistan, China, Iran, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Tajikistan, Turkmenistan and Uzbekistan. To date, it is considered as a natural reservoir for Yersinia pestis, Taenia endophlora, Hymenolepis nana, Skrjabinotaenia lobate, Trichuris rhombo-midis, Trichuris muris, Syphacia muris and Dipetalonema vitaeae (Kamranrashani et al., 2013). However, little information is available on the susceptibility of great gerbils to other Taenia species and members of the genera Hepatozoon and Thelileria.

Thelileriosis is a hemolytic disease caused by protozoan parasites of the genus Thelileria, belonging to Piromplasidae together with Babesia and Cytotaxozoon (Mehlhorn and Schein, 1985; Kheirandish et al., 2020). Piromplasms may parasitize in the white and red blood cells of red fox, European badger, and wild cat, and can be transmitted by ticks to the predators. Considering rodents, Hepatozoon sp. in bank voles (Laakkonen et al., 2001), African jerboas (Jaculus jaculus), greater Egyptian jerboas (Jaculus orientalis) (Maia et al., 2014), cotton rats (Sigmodon hispidus), white-footed mice (Peromyscus leucopus) (Johnson et al., 2007) and wild mouse (Calomys callosus) (Wolf et al., 2016) were reported. Literature data also suggest that wild rodents (after acquiring the infection following ingestion of vectors) may contribute to the transmission of Hepatozoon spp. to predators (Moustafa et al., 2017).

Taeniosis (Cestoda: Cyclophyllidea) are tapeworm parasites of terrestrial mammals. Adult taenids occur in the small intestine of typically carnivorous definitive hosts, and their cystic larvae develop in tissues or body cavities of herbivorous or omnivorous intermediate hosts (Abuladze and Sludskia 1964). Wild mustelids and canids are definitive...
hosts of *Taenia* while rodents act as natural intermediate hosts (Deplazes et al., 2019). Within rodents, larval cestodes can cause serious pathological changes in viscera and tissues, and even death (Zhao et al., 2014), as exemplified by *Taenia hydatigena* in bull moose (*Alces alces*), *Taenia taeniaeformis* in house rat (*Rattus rattus*), and *Taenia mustelae* in plateau zokor (*Eospalax baileyi*).

Xinjiang Uygur Autonomous Region (XUAR), northwestern China, is adjacent to eight countries with 5600-km borderline (Wei et al., 2015). Previously, some emerging pathogens, such as Tacheng tick virus 1, Tacheng tick virus 2, Hantavirus and *Babesia occulta*, have been discovered in this region (Guo et al., 2016; Song et al., 2018; Liu et al., 2020). Here, we conducted an investigation on *Taenia*, *Hepatozoon* and *Theileria* in the great gerbils in XUAR (see Table 1).

### 2. Materials and methods

#### 2.1. Study area, sampling, blood collection and dissection

During 2019–2020, a total of 188 great gerbils were captured at 11 sampling sites in Alataw and Manas located in Gurbantungut Desert, XUAR (Fig. 1), where the predominant vegetation included clostridium (*Haloxylon ammodendron*) and saltcedar (*Tamarix sp.*) (Liu et al., 2012) while the climate was temperate continental climate with cool, dry winters, and hot, dry summers (Shu et al., 2020).

The great gerbils were captured in live traps (30 cm × 15 cm × 15 cm wire mesh), which were placed near the entrances of occupied burrows, baited with walnut, tomato or cucumber. Each survey site included 150 traps that were checked twice a day. Each trap was removed before nightfall and replaced on the survey site the following day (Kamranrashidi et al., 2013). All wild rodents were morphologically identified by an experienced zoologist. The rodents in Manas County were directly transported to our laboratory while the animals in Alataw City were transported to the Vector-borne Laboratory at Alataw Customs. Each animal was weighed, Zoletil 50 (Virbac, Paris, France) was used in anesthesia by intramuscular injection, and blood-taken from heart. The

![Fig. 1. Map showing the sampling sites in Alataw City (China-Kazakhstan border), Manas County (center of Gurbantungut Desert).](image-url)
Fig. 2. A-C Piroplasmic forms of Candidatus *Theileria xinjiangensis* in a microscopic field by Giemsa staining method [A single annular forms; B single circular form]. C The macroscopic appearance of splenomegaly in piroplasm-positive the great gerbil.

Fig. 3. A-C A Three kinds of cyst in thoracic cavity of great gerbils. The a-type cyst, belonging to *Taenia* sp. *Rhombomys opimus* A, contained 7 protoscolices. The b-type cyst, belonging to *Taenia* sp. *Rhombomys opimus* B, contained 13 protoscolices. The c-type cyst, belonging to *Taenia* sp. *Rhombomys opimus* C, contained 4 protoscolices. B A cyst attaching to the liver of a great gerbil in Manas County, XUAR. C Cysticercoids in cyst from the great gerbil in microscopic field by malachite green stain-method. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
heart, liver, spleen, lung and kidney of 188 wildlife carcasses were weighed, examined and stored at $-80^\circ C$.

2.2. Microscopic examination and quantitative inoculation

The weight ratio of each organ/corpse was recorded. Thin blood films were stained with Giemsa (Soulsby, 1982) and cysticercoids with malachite green stain-method. Briefly, the fixed smears were stained with 5% Giemsa diluted in phosphate buffer (pH 7.2). Microscopic examination was performed at 1000 × magnification (oil immersion) in 6 samples (Aziz and Al-Barwary, 2019).

To study the transmission of the cyst liquid from naturally infected great gerbils to laboratory mouse, four C57BL/6 and four Balb/c mice were intra-peritoneally injected with 0.2 mL fresh cyst liquid (~1000 cysticercoids), respectively. On the 90th day and 350th day after injection, two C57BL/6 and two Balb/c mice were dissected. The
morphological observation and molecular identification of cyst were carried out. All procedures performed in this study involving animals were in accordance with the ethical standards of Animal Ethics Committee of Shihezi University (Approval No. A2018-143-01).

2.3. DNA extraction and molecular identification

Each sample (~0.2 g) of the heart, liver, spleen, lung and kidney was used to extract genomic DNA using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China), following the manufacturer’s instructions. The DNA quantity and purity were assessed on a NanoDrop 2000 spectrophotometer analyzer (Thermo Fisher Scientific, USA). The concentration of DNA was at least 50 ng/μL for further detecting pathogens. A total of 940 organ samples were subjected to specific polymerase chain reaction (PCR) for Piroplasmida, Hepatozoon and Taenia. Two different (438-bp-long and 487-bp-long) fragments of the 18S ribosomal RNA (18S rRNA) gene of Piroplasmida were targeted with the primers BJ1/BN2 (Farkas et al., 2015) and PIRO-A/B (Sun et al., 2007), respectively. A longer, 1100 bp fragment of the 18S rRNA was used to detect Hepatozoon species. The genomic DNA was also extracted from cyst liquid, and two genetic markers (including a 450-bp-long fragment of the cytochrome c oxidase subunit I (cox1) gene (Liu et al., 2011) were used to identify members of the genus Taenia. The primers and PCR cycling conditions in this study are shown in Appendix Table 1. Each PCR assay included a negative control and a positive control (Yan et al., 2018; Song et al., 2018; Tian et al., 2011). The amplification products were cloned and sequenced. Sequences were manually edited, aligned with software BioEdit, and compared to the reference GenBank sequences (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic trees were constructed using the Maximum Likelihood or Neighbor Joining method with the MEGA 7.0 software (Kumar et al., 2016).

3. Results

Among the 188 the great gerbil samples analyzed, the hearts, livers, spleens, lungs and kidneys of 13 animals (6.9%, 13/188) were positive for both fragments of Piroplasmida 18S rRNA. Blood smear of PCR-positive animals showed an intraerythrocytic ring form piroplasm that measured about 2.98 × 2.24 μm (Fig. 2A and B). Splenomegaly was observed in the piroplasm-positive animals (Fig. 2C). The index of spleen/corpse ratio was significantly different (Fst = 20.015, P < 0.05) between piroplasm-positive and piroplasm-negative animals. BLASTn analysis showed that i) DNA sequences obtained in this study were 100%
identical to each other; and ii) Theileria sp., provisionally named here as “Candidatus Theileria xinjiangensis”, was closest related to Candidatus Theileria senegalensis from Nile rat (Arvicanthis niloticus) in Senegal (MK484070), with 97.4% sequence identity (Fig. 4).

Hepatozoon sp. were molecularly detected in three spleens (1.6%, 3/188). There was no obvious difference in the results of clinical pathological observation and spleen index analysis between PCR-positive and PCR-negative animals. BLAST and phylogenetic analysis showed that i) Hepatozoon species in this study, here designated as Hepatozoon ayorgbor-like, shared 98.2% (1020/1039) identity to Hepatozoon ayorgbor (EF157822) detected in ball pythons (Python regius) in Ghana, and ii) Hepatozoon ayorgbor-like from great gerbils was an ancestral species compared to Hepatozoon sp. (EF157822) detected in ball pythons (Python regius) in Ghana, and iii) Hepatozoon ayorgbor-like from great gerbils was molecularly detected in three spleens (1.6%, 3/188). There was no obvious difference in the results of clinical pathological observation and spleen index analysis between PCR-positive and PCR-negative animals. BLAST and phylogenetic analysis showed that the sequences for cysticercoid from thoracic cavity after 90 days and 350 days.

During the necropsy of great gerbils, large white and web-shaped cyst was found in the thoracic cavity (Fig. 3B). Many cysts with the diameter of 0.8 cm, attaching to the liver, was also found in the thoracic cavity of ten rodents (5.3%, 10/188), including field mouse (Apodemus flavicollis), African giant rat (Cricetomys gambianus), house mouse (Mus musculus), black rat (Rattus rattus), flying squirrel (Idiurus macrotis) and bushveld gerbil (Gerbilliscus leucogaster) (James Harris et al., 2017; Charles et al., 2012; Kamani et al., 2018; Killck-Kendrick, 2010; Moustafa et al., 2017). In the present study, Hepatozoon ayorgbor-like sequence was detected in three spleen samples of great gerbils in northwestern China, which shared 98.2% (1020/1039) identity to Hepatozoon ayorgbor in blood and liver of a ball python (Python regius).
Both inter- and intraspecific genetic diversities of Taenia species are known to exist in the same rodent species. For example, Taenia marsis, Taenia mustelae, Taenia polyacanthus were reported in bank voles (Myodes glareolus) (Lavikainen et al., 2008). At the same time, intraspecific genetic diversity of Taenia species, such as Taenia pisiformis (98.4%) and Taenia hydatigena (99.5%–99.7%), was also reported (Sarvi et al., 2020; Yang et al., 2013). Here “Taenia sp. Rhombomys opimus” A, B and C were found in great gerbils, which only shared 90.9%-97.6% identities based on cox1 analysis, and also showed three distinctive morphological characters based on the numbers of protoscolices. This finding suggests that “Taenia sp. Rhombomys opimus” has both inter- and intraspecific genetic diversity. The field investigation revealed that the cyst of “Taenia sp. Rhombomys opimus” is mostly present in the thoracic cavity. This finding suggests that “Taenia sp. Rhombomys opimus” has anatomical predilection site when infecting great gerbils, which is different from Taenia endothermicus in the great gerbil (Rhombomys opimus) and Lybian jird (Meriones lybicus) (Kamranrashani et al., 2013; Mowlavi et al., 2004). Previously, Taenia taeniaformis from house rat (Rattus norvegicus) was highly infective to SD, HA or AS rats but not infective to CBA or ICR mice, and Taenia crassiceps showed differences in susceptibility for the rat (Anderson and Griffin 1979; Azuma et al., 1995). Here a wild-type Taenia species, belonging to Taenia sp. Rhombomys opimus A, could naturally infected great gerbils, but didn’t infect C57BL/6 or Balb/c mice after being intraperitoneally inoculated with ~1000 cysticercoids. This survey findings demonstrate the transmission between genera of rats and mice has a biological barrier for “Taenia sp. Rhombomys opimus” cysticercoids.

Great gerbils located in the unique geographical environment between China and Kazakhstan serve as reservoirs for Hepatozoon ayorgbor-like genotype, “Taenia sp. Rhombomys opimus”, and Candidatus Theileria xinjiangensis. In the future, definitive hosts of the Hepatozoon ayorgbor-like genotype and “Taenia sp. Rhombomys opimus” should be identified among carnivores (eg. red foxes and wolves), and snakes including pythons. Previously, Hyalomma asiaticum and Dermacentor niveus were reported from great gerbils (Ye et al., 2006). Here Candidatus Theileria xinjiangensis was found in organs of great gerbils. Therefore, it will be important to extend the scope of these studies to larger sample size of great gerbils and their infesting ticks, in order to uncover their role in the life cycle of theileriae.

5. Conclusion

This study provided new data regarding Candidatus Theileria xinjiangensis, Hepatozoon ayorgbor-like genotype and “Taenia sp. Rhombomys opimus” which appear to be mostly associated with great gerbils. These results highlight the importance to continue research on vector-borne and zoonotic pathogens in wildlife in the region of Central Asia.

Declaration of competing interest

All authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.04.002.

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