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Clinical and epidemiological investigation of human infection with zoonotic parasite *Trypanosoma dionisii* in China

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SUMMARY

Background: Trypanosomiasis continues to pose a global threat to human health, with human infection mainly caused by *Trypanosoma brucei* and *Trypanosoma cruzi.*
Methods: We present a 30-year-old pregnant woman with persistent high fever from Shandong Province, China. High-throughput sequencing revealed the presence of *Trypanosoma dionisii* in blood. We conducted an analysis of the patient's clinical, epidemiological, and virological data.
Results: The patients exhibited fever, shortness of breath, chest tightness, accompanied by change in liver function and inflammatory response. She made a full recovery without any long-term effects. *T. dionisii* was detected in blood collected 23 days after onset of illness. The 18S rRNA gene sequence showed close similarity to *T. dionisii* found in bats from Japan, while the *gGAPDH* gene was closely related to *T. dionisii* from bats in Mengyin County, Shandong Province. Phylogenetic analysis demonstrated the current *T. dionisii* belongs to clade B within its species group. Positive anti-*Trypanosoma* IgG antibody was detected from the patient on Day 23, 66 and 122 after disease onset, as well as the cord blood and serum from the newborn. Retrospective screening of wild small mammals captured from Shandong Province revealed a prevalence rate of 0.54% (7/1304) for *T. dionisii*; specifically among 0.81% (5/620) of *Apodemus agrarius*, and 0.46% (2/438) of *Mus musculus.*
Conclusions: The confirmation of human infection with *T. dionisii* underscores its potential as a zoonotic pathogen, while the widespread presence of this parasite in rodent and bat species emphasizes the emerging threat it poses to human health.

Introduction

Trypanosoma, a genus of unicellular parasitic flagellate protozoa within the family *Trypanosomatidae* of order *Trypanosomatida,* is capable of infecting various vertebrates, including human beings. As one of the most significant parasitic diseases in the world,

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trypanosomiasis continue to pose threat to human and animal health globally, particularly in tropical regions of Africa, parts of Asia, and South America.[1] Taxonomically classified into ten clades,[2] two species cause human trypanosomiasis: human African trypanosomiasis (HAT), also known as sleeping sickness in Africa, caused by *T. brucei rhodesiense* and *T. brucei gambiense*,[3] and American trypanosomiasis (Chagas disease) in South America caused by *T. cruzi*.[4] Different transmission pattern has been revealed, HAT is mainly transmitted by tsetse flies, however, Chagas disease is mainly spread via insect vector, congenital transmission, blood transfusion, and organ transplantation.[4] The other species are largely known to be pathogenic to animals, such as *T. congolense* and *T. vivax* causing nagana in cattle in sub-Saharan Africa,[5] *T.equiperdum* causing dourine in equines[6] and *T. evansi* responsible for surra in camels, equids, cattle, and buffaloes worldwide,[7] which imposes a high economic burden.[1]

In China, human trypanosomiasis is considered as an imported disease. The first imported case of HAT with *T. brucei gambiense* infection in 2014 involved a male individual returning from West Africa.[8] Subsequently in 2017, two cases of HAT were confirmed, a 60-year-old man serving as a seaman and traveled between Libreville and Kango and a 41-year-old woman who traveled to Tanzania and Kenya.[9,10]To date, there is no evidence suggesting that *Trypanosoma* has established transmission cycle infecting humans within China, although positive serological results have been observed for *T. lewisi* infection in healthy people in Guangdong Province.[11]

In contrast, various animal pathogenic trypanosome species have been documented in China, including *T. lewisi*, *T. brucei*, *T. evansi*, *T. grosi*, and *T. dionisii*.[12–17] *T. lewisi* has been detected in various rodent species, such as *Rattus norvegicus*, *Rattus tanezumi*, *Rattus argentiventer*, and *Niviventer confucianus*[12,13]; *T. brucei* has been identified in bats and horses[14,18]; *T. evansi* was identified in buffaloes, horses, camels, and rats, while the presence of *T. grosi* was detected in *Apodemus agrarius*[16]; the most recent identification of *T. dionisii* in *Eptesicus serotinus* and *Myotis pequinius* in Shandong Province has further expanded its host range in China.[17] However, whether these trypanosome species could infect human being remains obscure, potentially unspecific clinical disease and low awareness in healthcare workers regarding neglected tropical diseases.

In this study, we report the first human case of *T. dionisii* infection in China, which was identified through Meta-genomics investigation; the epidemiological, clinical, and genetic characteristics associated with the case were identified as well.

Materials and methods

Epidemiological investigations and data collection

On 19 November 2023, a pregnant patient with recurrent fever of unknown reason was entered into the Qilu hospital, Jinan city of Shandong province, China. Following the identification of infection with *T. dionisii*, an epidemiological investigation was performed on the patient and her close-contact family members, to collect demographics information, exposure history prior to her illnesses, the presence of febrile disease resembling this patient during the last month. Paired serum and whole blood samples were collected from patients and the family members. Nasopharyngeal swab (NPS) was additionally collected from the patient on admission to the hospital; the umbilical cord blood and amniotic fluid were collected during her labor. Written informed consent was obtained from the patient, her family members after receiving approval from the Ethical Committee of Qilu Hospital of Shandong University (KYLL-202403–044). The procedures adhered to the Helsinki declaration of 1975, as revised in 1983.[19]

To determine the possible source of infection in the patient, we performed a retrospective detection on wild small animals collected from three locations in Shandong province: Linyi, Qingdao, and Yaitai from 2019 to 2023. Wild small mammals were captured with snap traps, and aseptic collection of tissues (heart, liver, spleen, lung, and kidney) were performed, stored at −80 ∘C for the current use. All tested wild animals were taxonomically identified to the species level by sequencing of mitochondrial cytochrome b (*mt-cyt b*) gene as previously described.[20]

Metagenomic next-generation sequencing

To identify the causative pathogen for the disease, metagenomic next-generation sequencing (NGS) was performed on whole blood obtained from both the patient and her newborn. In brief, DNA extraction was performed by using QIAamp DNA Micro Kit (Qiagen, Germany) following the manufacturer's instructions. Subsequently, a high-throughput sequencing library was constructed using the Qiagen library construction kit (QIAseq Ultralow Input Library Kit). Quality control of the library was performed by Qubit 3.0 Fluoremeter (Invitrogen, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, USA), followed by sequencing on the Illumina Nextseq 550 sequencing platform (Illumina, USA). Trimmomatic program (v0.39) was employed to filter low-quality raw reads for each library, and Botie2 (v2.4.5)[21] was used to remove the ribosomal (r) RNA reads while aligning them against the SILVA rRNA database. After data filtering, trimming, and error removal steps were completed, MEGAHIT (v1.2.9) with default parameter enabled de novo assembly of remaining high-quality reads,[22] which were then aligned with GenBank's nucleotide database available until 2023 July using Blastn (v2.15.0).[23] Pathogen-related contigs with e-values lower than $1e^{-5}$ were retained for further analysis, while potential host associations for these pathogen contigs were initially identified based on the taxonomic information obtained from the Blastn results and subsequently confirmed through phylogenetic relationships with pathogens having known host associations.

PCR amplification and sequencing

The *Trypanosoma*-specific DNA was tested on whole blood and serum samples of the patient, her close-contact family members, as well as mixed tissues (heart, liver, spleen, lung, and kidney) collected from wild small mammals (n = 1304). Briefly, DNA was extracted by using QIAamp DNA Micro Kit (Qiagen) following the manufacturer's instructions. Subsequentlye amplification of DNA was carried out targeting the 18S rDNA and the glycosomal glyceraldehyde phosphate dehydrogenase (*gGAPDH*) loci using previously described primers (Table S1).[24–26] The DreamTaq™ PCR Kit was utilized, and thermal cycling conditions consisted of an initial denaturation step at 95 °C for 5 min, followed by 40 cycles at 95 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s in a PCR System 9700 (Applied Biosystems, USA). The amplified products were analyzed by agarose gel electrophoresis, and bands of expected size were subjected to Sanger sequencing. All steps involving nucleic acid extraction and PCR testing were conducted in parallel with positive and negative controls.

Serological testing

The presence of anti-*Trypanosoma* IgG in serum specimens collected from the patient, newborn and the family members were tested using two methods: a rapid immunochromatographic assay by using the Chagas Detect Plus (CDP) (InBios, Seattle, WA), and a commercial ELISA IgG kit [NovaLisa® Chagas (*Trypanosoma cruzi*, NovaTec Immunodiagnostica GmbH; Germany]. All tests were performed following the manufacturer's instructions by technicians blinded to the participants's infection and clinical status.

Trypanosoma infection was confirmed based on consistent results obtained from both assays.

Tests for respiratory pathogens in the patient

The NPS sample was tested for the presence of nine respiratory pathogens, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Influenza, adenovirus, respiratory syncytial virus, parainfluenza virus, rhinovirus, human boca virus, coronavirus and human metapneumovirus by PCR or real-time RT-PCR as previously described[27–29] (Table S2).

Phylogenetic analysis

The nucleotide sequences of currently detected *T. dionisii* and representative species from genus *Trypanosoma* which were downloaded from GenBank were aligned using the default parameters in MAFFT (v7.505) (Table S3). Phylogenetic trees were established using the maximum-likelihood method with the best-fitting model determined by the ModelFinder program implemented in IQ-TREE (v2.1.4). Bootstrap values were calculated based on 1000 replicates.

Nucleotide sequence accession numbers

The sequences generated in this study were submitted to GenBank under the accession numbers PP564961, PP564968, PP555249, and PP563742-PP563748.

Results

Clinical features and laboratory abnormalities of the patient

The patient, a 30-year-old pregnant woman (34 1/7 weeks of gestation) living in Guangrao County, Dongying City of Shandong province (Fig. 1), presented with a febrile illness accompanied by chills, fatigue, and weakness on November 1st, 2023. She sought medical care at local clinics, where she received oral amoxicillin therapy without improvement. On the tenth day of illness, her highest temperature reached 39.5 °C. Subsequently on November 13, she was transferred to Dongying's People Hospital (Hospital A in Fig. 2) for further management. At the hospital, she underwent treatment with dexamethasone and antiviral therapy (Oseltamivir 75 mg twice daily), along with supportive care. Due to persistent high fever, the patient was subsequently transferred to Qilu Hospital (Hospital B in Fig. 2) on November 19, day 19 of her illness. Upon admission, she presented symptoms including fever, dyspnea, and chest tightness. Her physical examination showed no unremarkable findings, except for mild splenomegaly revealed by abdominal ultrasound. The electrocardiogram (ECG) and echocardiogram showed no abnormalities. Blood tests revealed a hemoglobin level of 101 g/L (normal range 115–150 g/L) and monocyte percentage of 12% (normal range 3–10%), as well as elevated level of lactate dehydrogenase (LDH, 254 U/L; normal range 120–230). An inflammatory response characterized by persistent elevated C-reactive protein (CRP, > 19.83 mg/L; particularly high at 71.3 mg/L on day-30 after disease), procalcitonin (> 0.108 ng/ML), and interleukin 6 (IL-6, > 8.79 pg/ML) were observed on day 21–32 after disease. Chest radiography showed no evidence of pneumonia; bacterial culture of blood was negative; test for T-SPOT.TB, antinuclear anti-bodies, lupus anticoagulant, etc., were all negative. NPS samples showed no positive results for any respiratory pathogens tested.

Considering the potential risks posed to the fetus by recurrent fever and the possibility of congenital transmission of *Trypanosoma*, a cesarean section was performed on November 29. Subsequently the high fever resolved one day after delivery. No abnormalities were observed for the newborn through physical examination, and scores of 10 on the Apgar scale at 1 min, 5 min, and 10 min post-delivery were obtained. She was treated with Benznidazole on November 4, day 34 of her illness. The patient made a full recovery without any clinical sequalae; no occurrences of cardiomyopathy, arrhythmias, megaviscera or other rare complications such as polyneuropathy or stroke were detected through medical examination. The patient was discharged on December 9th after a hospitalization duration of 27 days. Follow-up visit at Day 27 and Day 82 after discharge for both the patient and newborn revealed no abnormalities.

Identification of T. dionisii infection of the patients

By performing NGS on the blood sample collected on November 23 (day 23 of illness), a total of 256 reads were successfully aligned

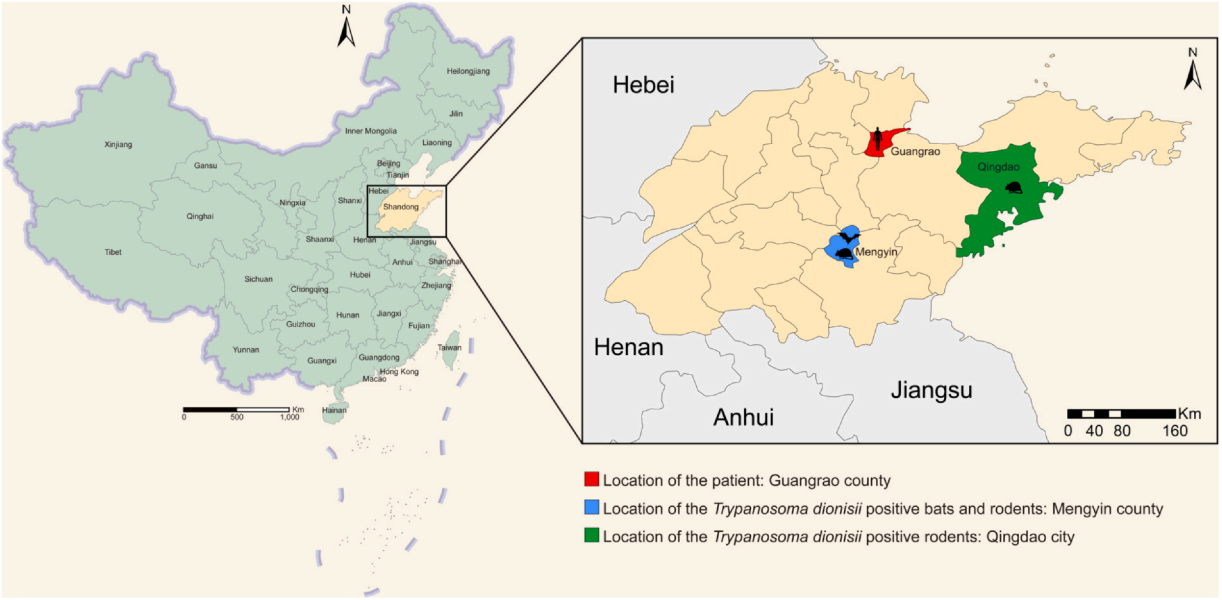

Fig. 1. Geographic distribution of *Trypanosoma dionisii* in Shandong province, China. The areas where surveillance of *T. dionisii* was carried out are shown in orange on the map. The location of the patient is highlighted in red. Blue color indicates the locations where *T. dionisii*-positive bats and rodents were captured in Mengyin county. Green color represents the location where *T. dionisii*-positive rodents captured in Qingdao. Maps were produced using ArcGIS Desktop 10.6.

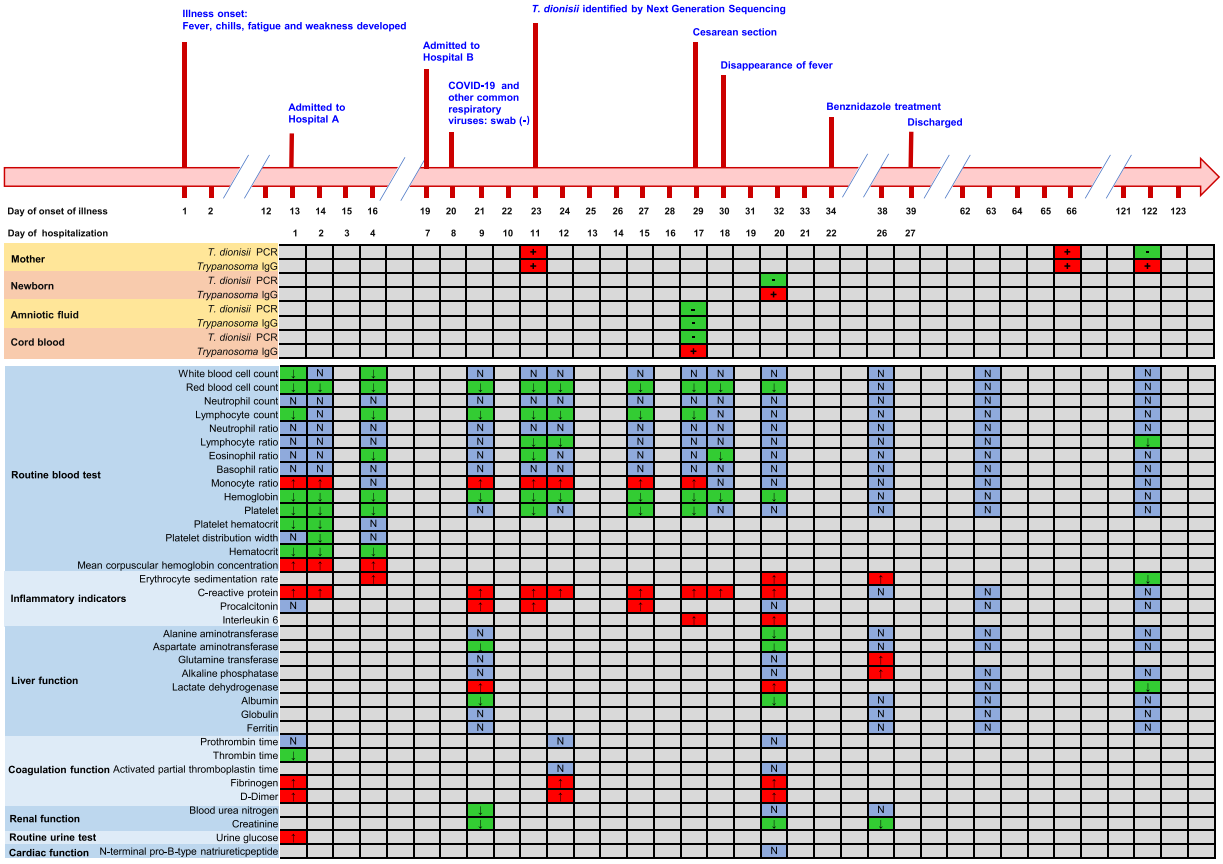

Fig. 2. Timeline of the clinical course of the patient and identification of *T. dionisii* infection.

to specific sequences of *T. dionisii* for the patient (Fig. 2), However, no *T. dionisii* specific sequence was detected for the newborn. PCR detection confirmed the presence of two genes for the *Trypanosoma spp.*, 18S rRNA gene and *gGAPDH* gene, in the blood sample of patients obtained on her 23 days of disease onset. The amplified sequence of the 18S rRNA gene spanned a length of 2106 bp and exhibited a remarkable similarity of 99.38% with *T. dionisii* isolated from bats in Japan (GenBank: LC326397) (Fig. 3A and Table S4). The amplified 799 bp within the *gGAPDH* gene exhibited a high similarity of 99.49% to *T. dionisii* isolated from bats in Mengyin County, Shandong Province, China (MH393931), located approximately 200 kilometers away from this patient' residence (Fig. 3B and Table S4).

The phylogenetic relationships of the currently determined *T. dionisii* to other *Trypanosoma* species at the partial 18S rRNA (2106 bp) and *gGAPDH* (799 bp) genes showed similar tree topologies, all clustering within the *T. dionisii* clade B (Fig. 3C). Based on the 18S rRNA gene, the current *T. dionisii* was most closely related to a *T. dionisii* isolated from Japan in 2016 (GenBank: LC326397) and phylogenetically grouped into the *T. dionisii* clade B group, along with other bat -originating *T. dionisii* isolates including TryCC 211(FJ001666) and TryCC 495 (FJ001667) from Brazil, x842 (FN599058) from United Kingdom, and SD109 (MH393947) collected in China in 2015 (Fig. 3C). Based on the *gGAPDH* gene, the curernt *T. dionisii* strain was most closely related to a bat-originating *T. dionisii* obtaimed from *Eptesicus serotinus*, collected from Mengyin County, Shandong Province, China in 2015 (GenBank: MH393931), and also phylogenetically grouped into the same *T. dionisii* clade B, along with other widely distributed *T. dionisii*, including TryCC 211 (GQ140362) from *Eptesicus brasiliensis* and TryCC 495 (GQ140363) from *Carollia perspicillata* collected in Brazil, x842 (FN599055) from *Nyctalus noctule* and gnash (FN599056) from *Ep. Serotinus* in United Kingdom, KTD (LC326399) from *Miniopterus fuliginosus* in Japan in 2016, and SD104 (MH393937) from *Myotis pequinius* and SD027 (MH393930) from *Ep. Serotinus* collected in China, all with bat origin (Fig. 3C).

The patient's sample collected on day 23 after disease, as well as on days 27 and 82 after discharge, yielded positive results for IgG antibodies agains *Trypanosoma* infection by both assays. No trypanosomes were found in the thin or thick peripheral-blood smears. Both the giemsa-stained thin and thick blood smears, as well as the malaria antigen test (immuno chromatographic test) showed negative results.

Epidemiological investigations

The patient is an employee of an insurance company who resides with her husband, daughter and mother. She recalled no blood transfusions or international travel in the past three years. There are no animals kept by her family, and she does not recall having any contact with birds, rodents, or other animals. She has no history of skin damage or tick bites; however, she did report a history of mosquito bites at the local hospital approximately two weeks prior to her current presentation. None of the three family members reported experiencing any illness similar to that of the patient during her illness period or during follow-up after the episode. PCR testing for *T. dionisii*, and serological testing yielded negative results for whole blood and serum samples collected from three family members. *T. dionisii* PCR detection were negative for amniotic fluid and cord blood collected during delivery, however, anti-*Trypanosoma* IgG antibody was positive in the cord blood. Positive IgG antibody was also identified from serum of newborn three days after birth (Fig. 2).

A total of 1304 wild small mammals belonging to 8 species from 6 genera were subjected to PCR analysis for the presence of *T. dionisii*

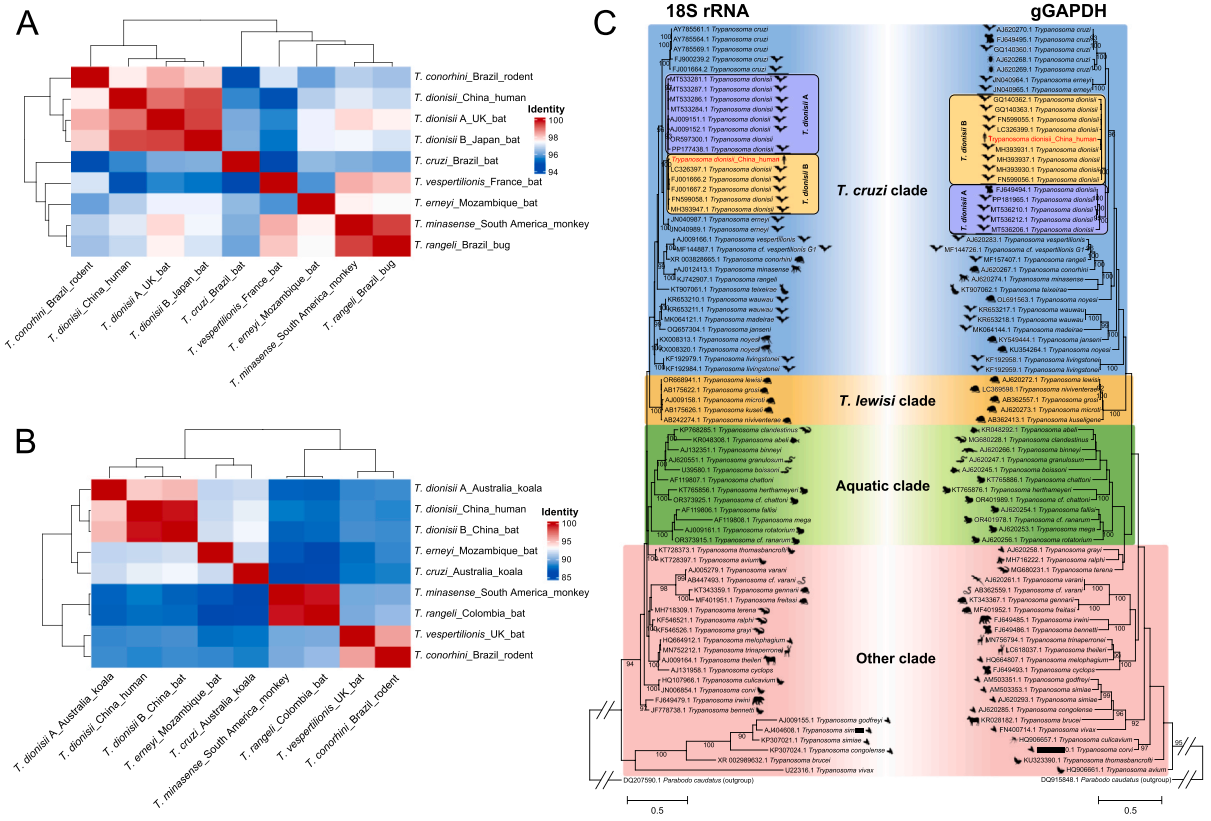

Fig. 3. Phylogenetic analysis of *Trypanosoma dionisii*. (A) The nucleotide similarity of 18S rRNA between the *T. dionisii* identified in this study and other related species in genus *Trypanosoma*. (B) The nucleotide similarity of *gGAPDH* gene between the *T. dionisii* identified in this study and other related species in genus *Trypanosoma*. Similarity values were shown as colored squares. The rows and columns were clustered based on Euclidean distance. The scale values range from 85 to 100. (C) The Maximum Likelihood phylogenetic tree constructed based on the 18S rRNA and *gGAPDH* sequences of the species in genus *Trypanosoma*. The best-fitting model was determined by the ModelFinder program implemented in IQ-TREE based on the Bayesian information criterion (BIC). Phylogenetic inference was performed using maximum likelihood (ML) method with 1000 bootstrap replicates. Branch lengths are indicated by the scale bar. Bootstrap values over 90% were shown in the nodes. *T. dionisii* detected in the patient in this study is labeled with red font. *Parabodo caudatus* was used as an outgroup.

(Table S5). Positive detection was obtained in two species, *Apodemus agrarius* (5/620, 0.81%) and *Mus musculus* (2/438, 0.46%), resulting an overall positive rate of 0.54% (7/1304). The rodents with positive detection was captured in Mengyin county, and Qingdao city. The remaining 6 species of wild small mammals tested negative for *T. dionisii*. A phylogenetic tree was constructed based on partial sequences of 18S rRNA (359-bp), demonstrating that the sequences from the two rodent species clustered together with those obtained from the current patients and bats collected in Mengyin county, while distant from other *T. dionisii* sequences obtained in Australia, United Kingdom, and Russia (Fig. 4). The nucleotide sequences of partial 18S rRNA from rodents showed a range of identity between 97.49–98.88% compared to those from the patient, and between 98.32–99.44% compared to those from bat-originating *T. dionisii* (MH393931) collected in Mengyin County, Shandong Province, China.

Discussion

Compared to the limited distribution of HAT and Chagas disease, animal trypanosomiasis is endemic in extensive tropical regions of Africa, parts of Asia, and South America.[30] A diverse range of domestic and wild vertebrate hosts (such as horses, deer, elephants, camelids, equines birds, reptile, amphibians and fishes) are known carrier or vectors for *Trypanosoma* transmission through various routes, such as blood-sucking arthropods (e.g., fly, tick, and flea), oral transmission, vertical transmission as well as through coitus.[31] Livestock worldwide suffer from trypanosomosis across a wide geographical area, which poses severe threats to livestock production, while hindering economic development and significantly impacting human health.[32]

Due to the broad host range of trypanosomes, various biological factors such as host spillover and close proximity between different animal species, along with abiotic factors like climate change, deforestation, and globalization, have contributed to the emergence of atypical human trypanosomosis (a-HT) caused by animal trypanosomes. While most cases of a-HT are transient, some require treatment and can be fatal.[33,34] In recent decades, there has been an increasing overlap between human settlements, grazing lands, and wildlife reserves leading to heightened risks of wildlife-livestock-human infections and exacerbating zoonotic transmission. An increasing number of a-HT cases that were previously limited to animals, such as *T. congolense*, *T. lewisi*, and *T. evansi*, have been reported,[35] indicating their potential for infecting humans under undetermined circumstances.

In 2021, there were reports of molecularly confirmed cases of *T. congolense* infection in 11 individuals from Southern Chad in Africa. Additionally, an infection caused by an unidentified *Trypanosoma* sp-125-H that phylogenetically clustered with a hyaena isolate was also reported in the same country.[36] The first documented case of a-HT caused by *T. lewisi* occurred in a 4-month-old infant from Malaysia in 1933.[37] A recent report from India in 2023 recorded an atypical neonatal infection of *T. lewisi* presenting with high fever, poor appetite, and lethargy for 3 days.[38] The first confirmation of human infection with *T. evansi* infection was identified in a herdsmen residing in Chandrapur, Maharashtra in 2004 who presented intermittent fever, chills and sweating.[39] A similar case involving *T. evansi* infection was reported in Vietnam in 2015.[40] The only documented

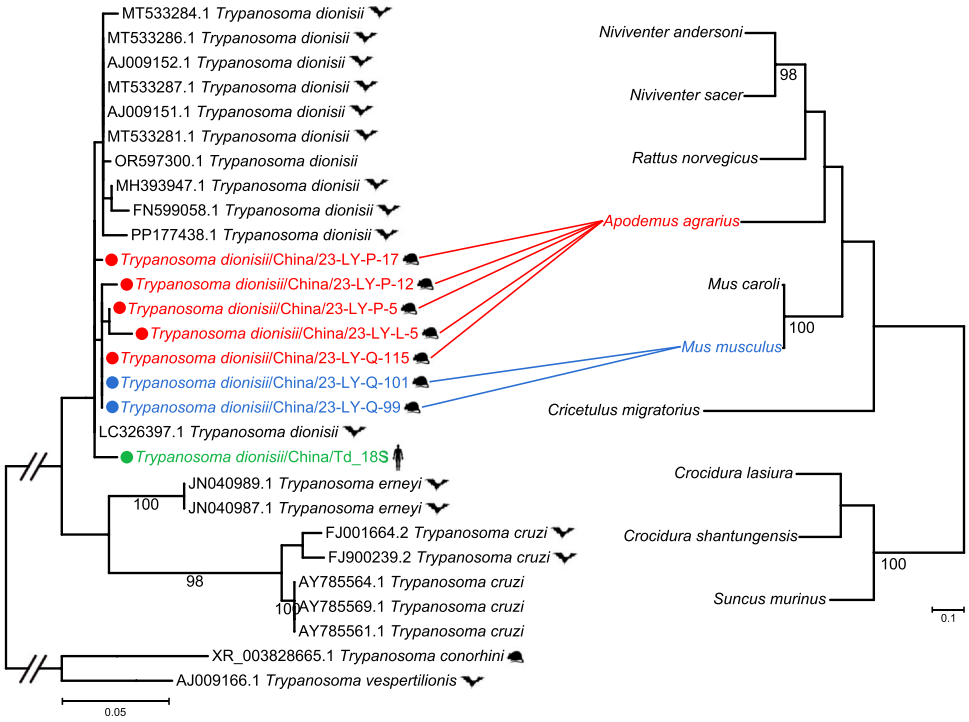

Fig. 4. Phylogenetic analysis of partial 18S rRNA of *Trypanosoma dionisii*. The Maximum Likelihood phylogenetic tree was constructed based on partial 18S rRNA of *T. dionisii* from seven *T. dionisii*-positive rodents. The Australia JPV sequences were labeled in orange. The current *T. dionisii* from two rodent species were labeled in red (*Apodemus agrarius*) and blue (*Mus musculus*). *T. dionisii* detected in the patient in this study is labeled with green font.The best-fitting model was determined by the ModelFinder program implemented in IQ-TREE based on the Bayesian information criterion (BIC). Phylogenetic inference was performed using maximum likelihood (ML) method with 1000 bootstrap replicates. Branch lengths are indicated by the scale bar. Bootstrap values over 90% were shown in the nodes.

case of human infection with *T. dionisii* involved the detection of the parasite in cardiac tissue from a child who succumbed to Chagas disease.[33]

The trypanosome *T. dionisii*, which is genetically related to *T. cruzi*, exhibits a global distribution and has been documented in bats across six continents, including North America, South America, Europe, Africa, Asia, and more recently Oceania.[41] While previously considered non-pathogenic, Maeda et al. reported the capacity of *T. dionisii* to invade and replicate within mammalian cells in vitro.[42] In China, only one study has identified the presence of *T. dionisii* in *Eptesicus serotinus* and *Myotis pequinius*[17]. Given its potential to infect mammalian species other than bats, understanding its pathogenesis to humans and the mode of transmission is imperative.

In this study, we present a comprehensive investigation into the first confirmed human infection of *T. dionisii* in a previously healthy progenant woman in China. The majority of pregnant women with Chagas disease are asymptomatic but may face an increased risk of preterm birth, low-birth weight and stillbirth.[43] In the current case, the patient exhibited symptomps such as febrile, chills, fatigue and weakness. Her illness resolved after anti-*Trypanosoma* therapy with no observed relapse during the three-month follow-up post-discharge. Unlike *T. cruzi*,[43] no congenital transmission was observed for this case presumably due to the implementation of cesarean section and avoidance of breastfeeding, which have been proven effective in interrupting *T. cruzi* transmission.[44,45]

The source of infection in the patient remained obscure, as no blood transfusions or organ transplant have been reported. Bats were previously considered to be the main hosts of *T. dionisii*, with a highly similar strain found in *Didelphis albiventris* (order Didelphimrphia)[46] and *carnivore Lycalopex gymnocercus* (order Carnivora).[47] However, considering the patient's limited exposure to bats, it is unlikely that they were the source of infeciton. To investigate the potential for *T. dionisii* to infect other mammals, we conducted a retrospective screening of mammal species in Shandong province and discovered its presence in at least two rodent species: *A. agrarius* and *M. musculus,* both abundant in peri-domestic habitats closely associated with humans and domestic animals. Furthermore, phylogenetic analyses of 18S rRNA and *gGAPDH* demonstrated a high similarity between the *T. dionisii* identified from the patient and those found in these two rodent species. The patient did not recall any animal exposure but remembered being bitten by mosquito before disease onset. Whether the patient was infected by arthropod vector remains unknown, although previous reported have mentioned infection or transmission of *T. dionisii* through bat bugs (family *Cimicidae*),[41] bat flies (family *Nycteribiidae*)[41]; gamasine mite Steatonyssus periblepharus,[48] there is still no direct evidence available regarding human infction via vector bites. The close phylogenetic relationship between *T. cruzi* and *T. dionisii* suggests that these parasites might employ similar strategies to complete their life cycles and share common vectors. Triatomines are natural vectors of *T. cruzi* and are mainly prevalent in the Americas. Two species of triatomine bugs, *Triatoma rubrofasciata* and *T. sinica* have been recorded elsewhere but not specifically within Shandong province.[49] Considering the diverse range of arthropods that feed on bats and rodents, there is a high likelihood of vector associated transmission for *T. dionisii*.

It is crucial to acknowledge the limitations of this study. With only one case available, our understanding of the clinical aspects related to *T. dionisii* infection remains limited. A full understanding of the clinical spectrum might be proposed based on a large-scale population surveillance or and active screening of pregnant women from endemic areas. Further investigation is required regarding similar transmission patterns for *T. dionisii* compared to its close relative, *T. cruzi*, particularly through Triatomine insect vectors, blood transfusion, organ transplantations, congenital infections and oral transmissions resulting from food contaminated with feces from insects or hosts.[43] Secondly, previous studies have demonstrated that closely related *Trypanosoma* species *T. dionisii* and *T. cruzi* share

common epimastigote epitopes, amastigote-specific epitopes, as well as trypomastigote epitopes.[50–52] Due to unavailability of antibody testing for *T. dionisii* in this study, serological tests for *T. cruzi* were employed to assess the presence of anti-*Trypanosoma* IgG antibodies. The positive serological findings presented herein suggest that cross-reaction with *T. cruzi* should be evaluated in the further study to improve serologic testing of *T. dionisii*.

In conclusion, this study presents the first documentation of human infection with *T. dionisii,* as well as its circulation in rodent species. These findings expand the range of host species for *T. dionisii*, and underscore its potential as a zoonotic pathogen.Given the wide distribution of the currently identified rodent species (*A. agrarius* and *M. musculus*) and bat species are widely distributed,[53] it is imperative to raise awareness regarding *Trypanosoma* as a potential differential diagnosis for patients presenting with fever of unknown cause.

Author contributions

SL, GW, QL, and WL designed the study. NX, XZ, HL, LZ, YH, MZ and GS did the experiments. NX, XZ, HL, YX, HLu, JZ, ZW, MC, YC, YZ, QW, YH, YL, ZZ, YG, CC, ML, CM, YW, and LF collected and analyzed the data. NX, XZ, HL, SL, GW, QL, and WL interpreted the data. NX, XZ, HL, SL, GW, QL, and WL wrote the paper.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106290.

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