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Prevalence of *Pentatrichomonas hominis* infection in wild rodents

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Abstract

Background *Pentatrichomonas hominis* is a zoonotic pathogen linked to gastrointestinal diseases in various animal species and humans. However, its prevalence of in wild rodents remains unexplored. Therefore, investigating the prevalence and molecular characteristics of *P. hominis* in wild rodents is essential.

Methods This study assessed the prevalence of P. hominis in wild rodents by analyzing 510 fecal samples using nested PCR. Statistical analysis of risk factors was performed with SAS (v9.0) and SPSS software.

Results The infection rates were 18.18% (16/88) in Yunnan, 0.94% (3/319) in Hunan, and 0% (0/103) in Guangxi. Among the species, *Rattus rattus sladeni* had the highest infection rate at 40% (2/5), followed by *R. flavipectus* at 23.08% (9/39), while *Niviventer lotipes* (0%, 0/23) and *R. losea* (0%, 0/41) showed no infection. Seasonally, the highest prevalence was observed in autumn (18.18%, 16/88) and the lowest in winter (0%, 0/103). Rodents from farmland had significantly higher infection rates than mountain areas (0%, 0/103) and lakeshores (0.32%, 1/312). Although female rodents had a higher infection rate (4.62%, 11/238) compared to males (2.94%, 8/272), the difference was not statistically significant. Phylogenetic analysis revealed that all *P. hominis* strains identified belonged to the zoonotic CC1 genotype.

Conclusions This study is the first to describe the distribution of *P. hominis* in wild rodents, paving the way for further epidemiological research on this parasite in wild animals. Such research is crucial for developing strategies to protect human health from zoonotic threats from wild rodents.

Keywords *Pentatrichomonas hominis*, Wild rodents, Prevalence, Genotype, China

Background

Pentatrichomonas hominis, formerly *Trichomonas hominis*, is a flagellated anaerobic protozoan classified as an opportunistic pathogen transmitted via the fecal-oral route [1]. It primarily resides in the large intestine of humans and other animals and was initially regarded as a commensal organism [2]. However, subsequent studies have confirmed its potential to cause gastrointestinal symptoms, including diarrhea, in humans, cats, dogs, and cattle [3–6]. Additionally, *P. hominis* has been implicated in respiratory infections, irritable bowel syndrome, rheumatoid arthritis, and systemic lupus erythematosus

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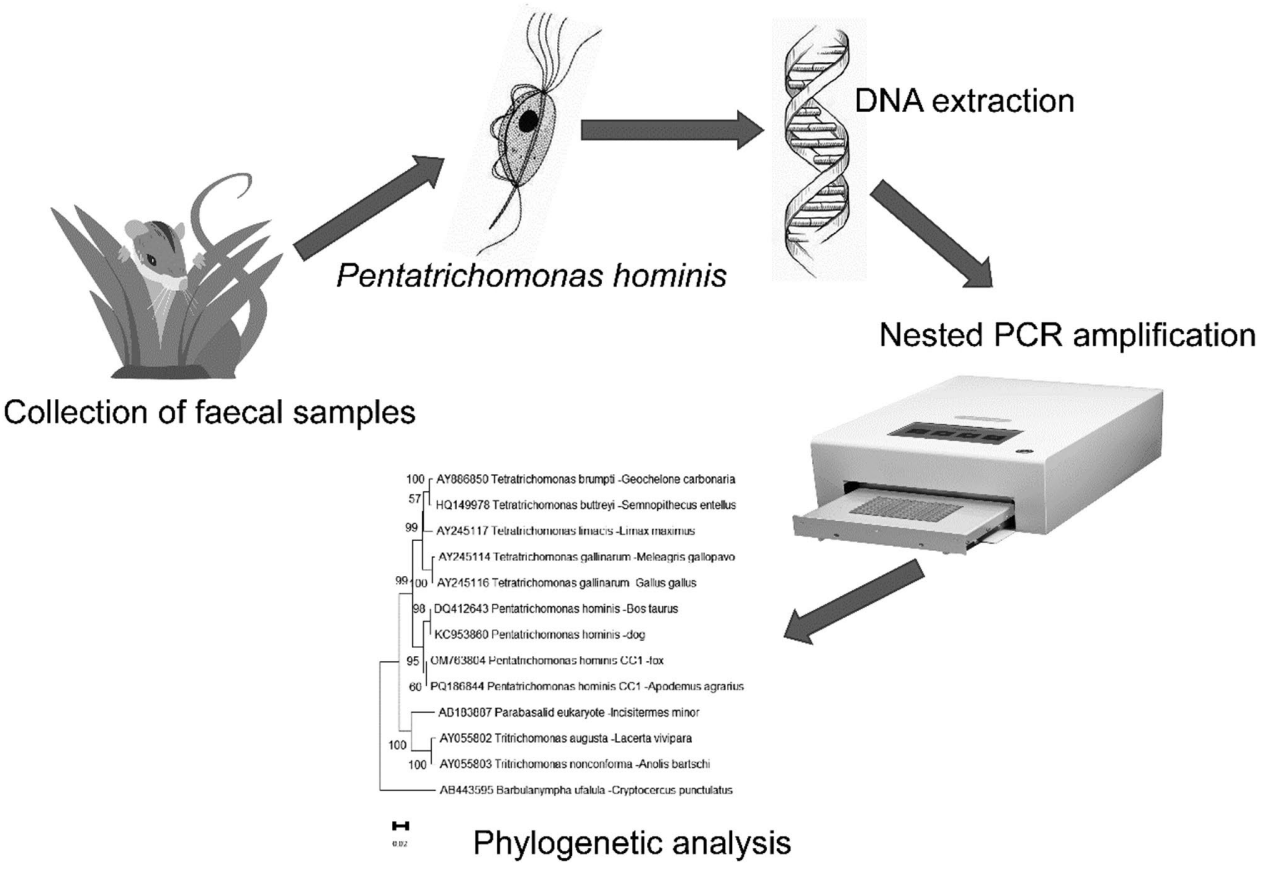
Graphical Abstract



in humans [7–10]. A recent study in China found that approximately 41.54% of patients with gastrointestinal cancer are infected with *P. hominis*, underscoring its potential threat to human health [11].

Currently, three genotypes of *P. hominis* have been identified: CC1, CC2, and CC3 [12]. CC1 is the most prevalent and has been detected in various animals, including humans, suggesting its zoonotic potential [2, 13]. In contrast, CC2 and CC3 have been found only in dogs in Northeast China, with no confirmed cases of zoonosis, indicating a need for further research.

Wild rodents, with their strong adaptability to different environments, are widespread in natural environments and closely interact with human populations. They have long been recognized as reservoirs for bacteria, viruses, and parasites, and can contaminate water and food sources by excreting pathogens [14–16]. Additionally, in some provinces of China, the consumption of field mice is a dietary habit, further heightening the public health risk. While the prevalence of *P. hominis* has been studied in various vertebrates, including farmed wild animals (e.g., foxes, raccoon dogs, sika deer, minks, and Siberian tigers), pigs, cattle, goats, dogs, cats, and non-human primates [17–21], its presence in wild rodents remains underexplored. Thus, this study aims to detect *P. hominis* in the fecal samples of wild rodents using nested polymerase chain reaction (PCR) to evaluate its prevalence in these populations. It is the first to focus on the prevalence of *P. hominis* in wild rodents, providing novel epidemiological data on this parasite in wild animals and offering a scientific basis for public health safety measures.

Materials and methods

Study design and sample collection

From August 2023 to May 2024, a total of 510 adult wild rodents were randomly captured for this study, representing the following species: *Bandicota indica* ($n = 39$), *Microtus fortis* ($n = 305$), *Rattus norvegicus* ($n = 31$), *Rattus rattus sladeni* ($n = 5$), *Rattus flavipectus* ($n = 39$), *Rattus losea* ($n = 41$), *Apodemus agrarius* ($n = 14$), *Niviventer lotipes* ($n = 23$), and *Mus musculus* ($n = 13$). The samples were collected from three regions in China: Hunan Province ($n = 319$), Guangxi Zhuang Autonomous Region ($n = 103$), and Yunnan Province ($n = 88$) (Fig. 1). Detailed information, including gender, sampling time, season, region, and species, was recorded for each rodent. Wild

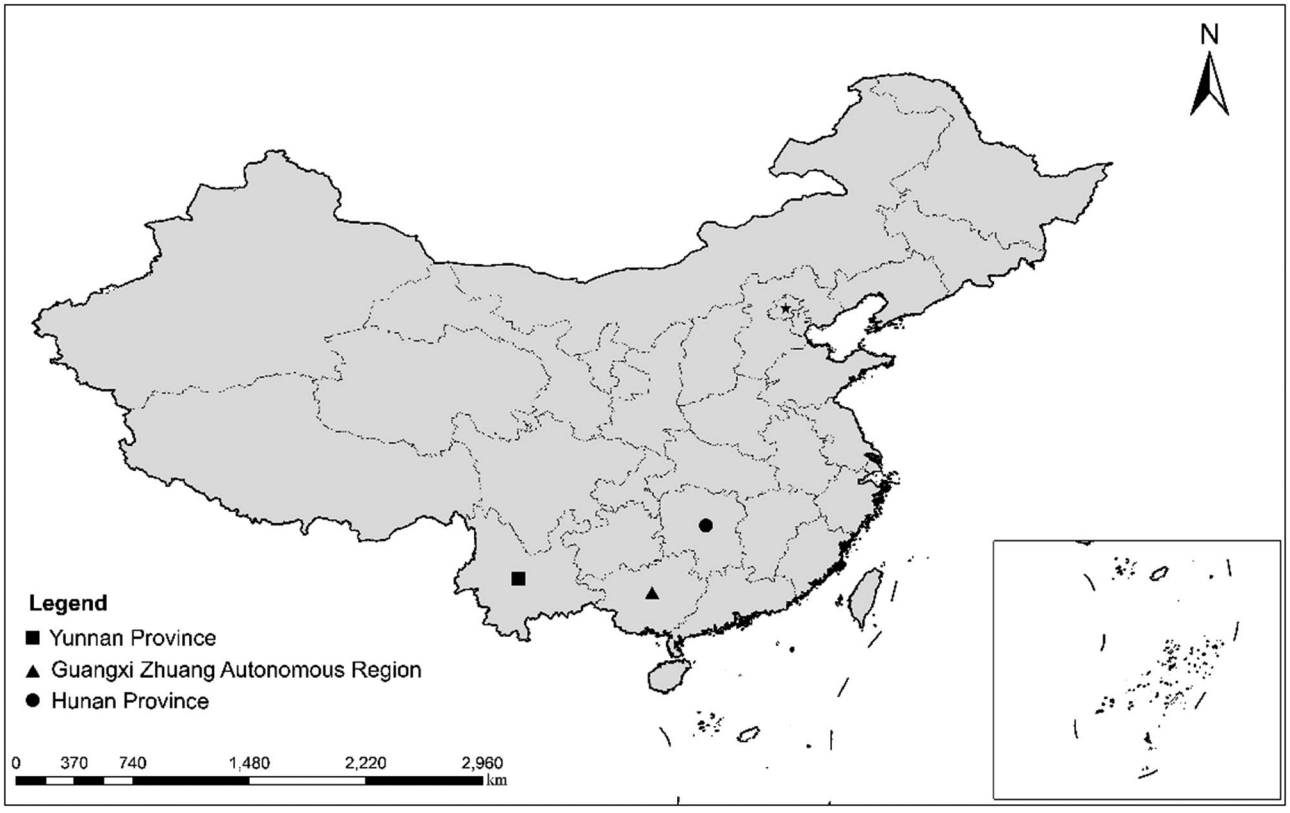


Fig. 1 Geographical locations of sample collection sites. The star marks the location of the capital of China

Table 1 Details of primers used in this study

Gene	Primer sequences	Target fragment size
18 S rRNA	F1:5′-ATGGCGAGTGGTGGAATA-3′	339 bp
	R1:5′-CCCAACTAGGCTAAGGATT-3′	
	F2:5′-TGTAAACGATGCCGACAGAG-3′	
	R2:5′-CAACACTGAAGCCAATGCGAGC-3′	

rodents were captured using mousetraps, and fecal samples were collected directly from the rectum. These samples were then stored on dry ice and promptly transported to the laboratory, where they were kept at -80 °C. All procedures for sample collection were approved by the Animal Ethics Committee of Qingdao Agricultural University (Approval No. QAU-AEW-20200701001) and adhered strictly to the relevant regulations.

DNA extraction

Genomic DNA was extracted from 200 mg of each fecal sample using the E.Z.N.A.® Stool DNA Kit (Omega Biotek, Inc., Norcross, GA, USA), following the manufacturer's instructions. Before extraction, the samples were homogenized using a vortex mixer (JOANLAB, Zhejiang, China). The extracted DNA was stored at -20 °C until further analysis.

PCR analysis

The presence of *P. hominis* was detected using nested PCR amplification targeting a 339 bp fragment of the 18 S rRNA gene (Table 1). The primers were designed based on studies by Crucitti et al. [22]. and Gookin et al. [23]. The PCR reactions were carried out on a Thermal Cycle gradient PCR instrument (Monad, Jiangsu, China). The first-round PCR conditions were: 95 °C for 5 min; followed by 33 cycles of 94 °C for 60 s, 59 °C for 60 s, and 72 °C for 60 s; and a final extension at 72 °C for 7 min. The second-round PCR used 4 μL of the first-round product with the following conditions: 95 °C for 5 min; 35 cycles of 94 °C for 60 s, 61 °C for 60 s, and 72 °C for 60 s; and a final extension at 72 °C for 7 min. Both positive and negative controls were included in each round of PCR. PCR products were analyzed using 1% agarose gel electrophoresis and all positive samples were sent to Anhui General Co., China, for sequencing.

Sequence alignment and phylogenetic analysis

The sequences obtained were aligned against reference sequences in the GenBank database using BLAST. To minimize redundancy and identify representative sequences, the data were compiled into a FASTA format file and clustered using the CD-HIT tool (v4.8.1) with a 99% similarity threshold. One representative sequence

was selected for phylogenetic analysis. The phylogenetic tree was constructed using MEGA11, employing the Kimura 2-parameter model, and the reliability of the clusters was evaluated with 1,000 bootstrap replicates.

Additionally, six sequences were aligned using MAFFT (v7.475), and phylogenetic trees were constructed using IQ-Tree (v2.1.2) with default parameters. Visualization was performed using iTOL (v7.0).

Statistical analysis

Statistical differences among species, regions, sexes, seasons, and environments were assessed using the chi-square test in the Statistical Analysis System (SAS, v9.0). Risk factors associated with the prevalence of *P. hominis* were also analyzed. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated using the Chi-square test in Statistical Product and Service Solutions (SPSS, IBM Corp., Armonk, NY, United States). Statistical significance was set at $P < 0.05$.

Accession numbers for nucleotide sequences

The representative sequence obtained in this study has been submitted to GenBank and assigned the following accession number: PQ186844.

Results

Out of 510 fecal samples, *P. hominis* was identified in 19 cases (3.73%). Yunnan Province had the highest infection rate at 18.18% (16/88), followed by Hunan Province at 0.94% (3/319), and no infections were detected in Guangxi Zhuang Autonomous Region (0%, 0/103; Table 2). Seasonal analysis revealed the highest prevalence in autumn (18.18%, 16/88), followed by summer (0.94%, 3/319), with no infections observed in winter (0%, 0/103). Infection rates varied by environment, with the highest in rodents from farmland (18.95%, 18/95), followed by those from lakeshores (0.32%, 1/312), and no infections in rodents from mountain areas (0%, 0/103). Significant differences were observed among species, with *R. rattus sladeni* (40%, 2/5) and *R. flavipectus* (23.08%, 9/39) showing the highest infection rates, while *R. losea* (0%, 0/41) and *Niviventer lotipes* (0%, 0/23) were uninfected. Gender did not significantly affect infection rates, with males (2.94%, 8/272) and females (4.62%, 11/238) showing no significant difference (Table 2).

Logistic regression analysis using forward stepwise selection in SAS revealed that region (χ^2=37.67, df=2, I^2=94.7%), season (χ^2=37.67, df=2, I^2=94.7%), and environment (χ^2=47.59, df=2, I^2=95.8%) significantly impacted the prevalence of *P. hominis* in wild rodents.

Molecular characterization of *P. hominis* was conducted through BLAST analysis of the 18 S rRNA

Table 2 Factors influencing the prevalence of *Pentatrichomonas hominis* among wild rodents, including environmental, ecological, and demographic variables

Variables	Categories	*/total	Positive rate	Heterogeneity	OR (95% CI)
			% (95% CI)	χ^2/df/I^2(%)/*P*	
	Hunan Province	3/319	0.94 (1.20–2.37)		Reference
Region	Guangxi Zhuang Autonomous Region	0/103	0.00 (-)		-
	Yuanan Procince	16/88	18.18 (10.74-27)	37.67/2/94.70/< 0.0001	23.41 (6.64–82.47)
Species	*Microtus fortis*	1/287	0.35 (0.00-1.49)	43.51/8/81.60/< 0.0001	Reference
	Bandicota indica	1/39	2.56 (0.00-10.68)		7.20 (0.46-112.85)
	Rattus norvegicus	3/41	7.32 (0.96–17.72)		21.00 (2.13-206.69)
	Rattus rattus sladeni	2/5	40.00 (1.85–86.22)		114.80 (8.89-1482.21)
	Rattus flavipectus	9/39	23.08 (11.00-37.76)		66.23 (8.17-537.02)
	Rattus losea	0/41	0.00 (-)		-
	Apodemus agrarius	2/22	9.10 (0.18–25.49)		26.09 (2.28-299.13)
	Niviventer lotipes	0/23	0.00 (-)		-
	Mus musculus	1/13	7.69(0.00-30.13)		22.08 (1.31-372.97)
Season	Winter	0/103	0.00 (-)	37.67/2/94.70/< 0.0001	Reference
	Summer	3/319	0.94(0.12–2.73)		0.44 (0.02–8.53)
	Autumn	16/88	18.18(10.74-27.00)		23.41 (6.64–82.47)
Gender	Male	8/272	2.94 (1.21–5.34)	0.98/1/0/0.3232	Reference
	Female	11/238	4.62 (2.27–7.71)		1.60 (0.63–4.04)
Environment	Lakeshore	1/312	0.32 (0.00-1.37)	47.59/2/95.80/< 0.0001	Reference
	Mountain	0/103	0.00 (-)		-
	Farmland	18/95	18.95 (11.63–27.51)		72.70 (9.56-553.04)
Total		19/510	3.73 (2.26–5.76)		

*:The number of positive samples out of the total number of samples tested

CI: Confidence intervval. *P*: p-value. *df*: dgree of freedom

sequences. The sequences (GenBank: PQ186844) showed 100% homology with the CC1 genotype (GenBank: KJ404269; Changchun; Canine). Phylogenetic analysis confirmed that all sequences obtained belonged to *P. hominis* (Fig. 2).

In the phylogenetic network, CC1 genotypes can be clearly distinguished from CC2 and CC3 genotypes (Fig. 3).

Discussion

Previous studies have demonstrated that *P. hominis* exhibits a high prevalence in various wild animals, including monkeys (46.67%), Siberian tigers (31.30%), sika deer (20%), and raccoon dogs (11.6%) suggesting its extensive transmission capability among wildlife [2, 14, 15, 24]. Additionally, *P. hominis* has been detected in a diverse range of species, such as boa *constrictors*, turkeys, bull, pigs, and goats, highlighting its strong host adaptability [17, 25–28].

Notably, many companion animals infected with *P. hominis* exhibit symptoms of diarrhea. For instance, three cases of *P. hominis* were identified among 14 diarrheic puppies in South Korea, and the parasite was found in all four kittens with diarrhea in the United States [29, 30]. Furthermore, *P. hominis* infections have been reported in humans, with two cases detected among 50 patients with

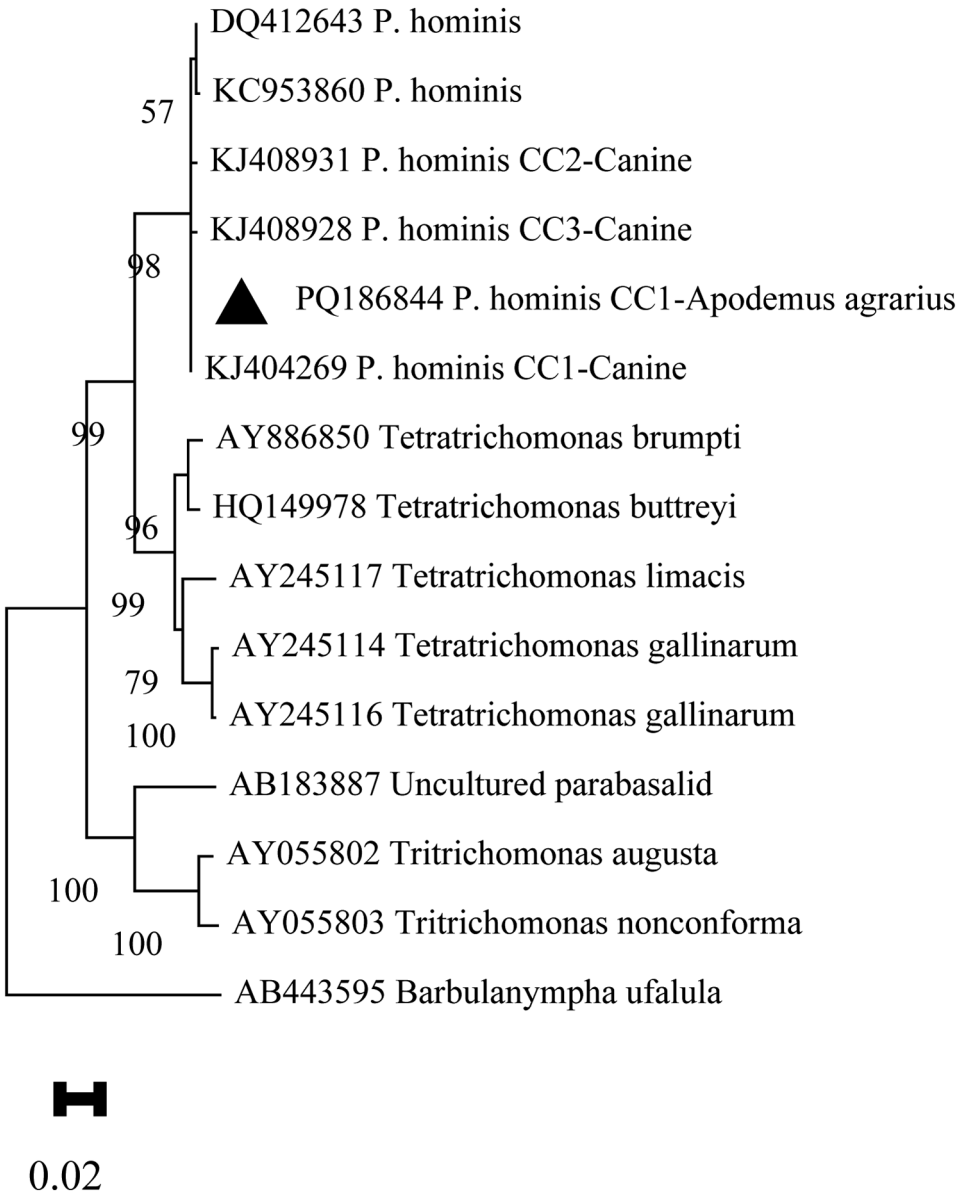


Fig. 2 Phylogenetic relationships of *Pentatrichomonas hominis* based on 18 S rRNA sequences. Phylogenetic trees were constructed using maximum likelihood analysis with genetic distances calculated by the Kimura 2-parameter model. Bootstrap values greater than 50% are indicated. The sequence of *P. hominis* isolated in this study is indicated with a triangle

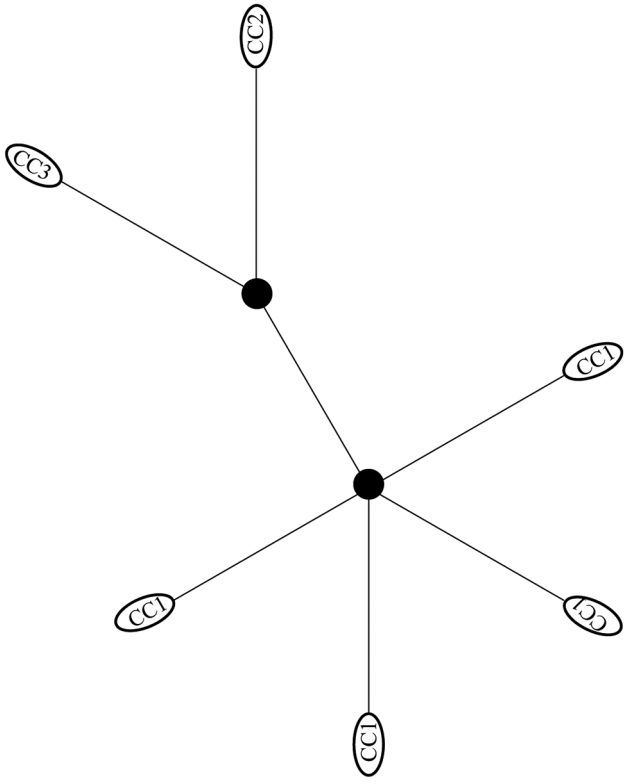


Fig. 3 The phylogenetic network of the genotypes of *P. hominis* in this study

diarrhea in China [2]. This underscores the potential for zoonotic transmission of *P. hominis* through contact with infected animals [31].

The study found an overall infection rate of 3.73% for *P. hominis* in wild rodents from southern China. This rate exceeds the prevalence of *Toxoplasma gondii* (3.2%) in the same host species from the same region [32]. Regionally, Yunnan Province exhibited the highest infection rate (18.18%), followed by Hunan Province (0.94%), with no infections detected in Guangxi Zhuang Autonomous Region (0%) These findings suggest that geographic location significantly influences parasite prevalence, which aligns with observations of *P. hominis* in Siberian tigers [15].

Significant differences in infection rates were also observed among rodent species. The highest infection rate was found in *R. rattus sladeni* (40%, 2/5), while no infections were detected in *R. flavipectus* (0%, 0/41) and *Niviventer lotipes* (0%, 0/23). This variation mirrors findings from studies on the prevalence of *P. hominis* in non-human primates in Brazil [33]. Seasonality also appeared to influence infection rates, with autumn showing a significantly higher prevalence (18.18%) compared to summer (0.94%) and winter (0%). It is hypothesized that the mild and humid conditions in autumn may facilitate the development and transmission of parasites, leading to increased infection rates [34].

The study also revealed that female rodents (4.62%, 11/238) had a higher infection rate than males (2.94%, 8/272), consistent with findings from Li et al. [2]. and similar research on Siberian tigers [15]. However, the lack of statistically significant differences between genders across studies suggests that *P. hominis* infection may be gender-neutral across various species and geographical distributions. Additionally, epidemiological data suggest that the infection rate of *P. hominis* is related to the age of the host [2, 15]. However, this study did not collect samples from juvenile wild rodents, resulting in insufficient data. Future research should expand the sampling range to address this gap.

Environmental factors were identified as significant risk factors for *P. hominis* infection. The highest prevalence was observed in farmland areas (18.95%, 18/95), compared to mountainous areas (0%, 0/103) and lakeshores (0.32%, 1/312). Given that *P. hominis* is transmitted via the fecal-oral route, the elevated infection rate in farmland areas may be attributed to the abundant food resources, which attract birds and other wild animals. This, in turn, increases the likelihood of rodents coming into contact with the feces of infected animals, thereby increasing the risk of infection. This scenario also increases the risk of transmission to humans and domestic animals, posing a threat to public health. Notably, the vertical transmission potential of *P. hominis* in wild rodents has not been studied. Future studies should focus on whether the parasite can be transmitted from mother to offspring and how long it survives in voles. This will help to understand the transmission dynamics of the disease in wild rodent populations and provide important scientific basis for the prevention and control of the disease.

The most common genotype of *P. hominis*, CC1, has been identified in a wide range of hosts, including raccoon dogs (GenBank: OM763803), foxes (GenBank: OM763804), dogs (GenBank: KJ408929), cats (GenBank: MG015711), monkeys (GenBank: KJ408932), and humans (GenBank: MK177542), indicating a lack of host specificity. Other genotypes, CC2 (GenBank: MJ40931) and CC3 (GenBank: KJ408928), have also been identified in dogs from the northeastern part of China [2]. In this study, genetic analysis of 18 S rRNA sequences confirmed that the *P. hominis* identified in wild rodents from Hunan and Yunnan Provinces belong to the CC1 genotype. The genetic similarity between the *P. hominis* sequences in this study and those from other hosts (e.g., dogs, foxes, cattle) [6] suggests that they belong to the same species, further confirming the zoonotic potential of *P. hominis*.

Conclusions

This study is the first to describe the distribution of *P. hominis* in wild rodents, paving the way for further epidemiological research on this parasite in wild animals. The findings confirm that wild rodents can serve as reservoirs for the zoonotic pathogen *P. hominis*, underscoring the need for expanded research to investigate the specific mechanisms of infection and transmission. Such research is crucial for developing strategies to protect human health from zoonotic threats from wild rodents.

Abbreviations

P. hominis	Pentatrichomonas hominis
PCR	Polymerase Chain Reaction
SAS	Statistical Analysis System
SPSS	Statistical Product and Service Solutions

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Not applicable.

Author contributions

Shuo Liu and Hai-Tao Wang: Methodology, Software, Writing-original draft. Qing-Yu Hou: Data curation, Methodology, Writing-review & editing. Si-Yuan Qin, Ya Qin, and He Ma: Data curation, Resources, Visualization, Writing-review & editing. Jing-Hao Li: Supervision, Writing-review & editing. Quan Zhao: Conceptualization, Resources, Supervision, Writing-review & editing. Hany M. Elsheikha, and Yan Tang: Conceptualization, Supervision, Writing-review & editing.

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None.

Data availability

The representative sequence obtained in this study has been submitted to GenBank and assigned the following accession number (GenBank: PQ186844).

Declarations

Ethics approval and consent to participate

All procedures in this study were approved by the Research Ethics Committee for the Care and Use of Laboratory Animals at Qingdao Agricultural University, China.

Consent for publication

All the authors have read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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