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RESEARCH

Prevalence of *Pentatrichomonas hominis* in foxes and raccoon dogs and changes in the gut microbiota of infected female foxes in the Hebei and Henan Provinces in China

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Abstract

Pentatrichomonas hominis (*P. hominis*) is a zoonotic parasite that affects a wide range of hosts, causing gastrointestinal diseases. The present study aimed to evaluate the prevalence of *P. hominis* among caged foxes and raccoon dogs and the effect of *P. hominis* on the gut microbiota in female foxes. A total of 893 fresh fecal samples were collected from the Hebei and Henan Provinces in China. *P. hominis* was screened based on 18S rRNA gene expression via nested PCR. The difference in the gut microbiota between nine *P. hominis*-positive and nine *P. hominis*-negative samples was investigated by 16S rRNA gene sequencing. The total prevalence of *P. hominis* infection in foxes and raccoon dogs was 31.7% (283/893). The prevalence rates of *P. hominis* infection were 28.2% (88/312) and 33.6% (195/581) in foxes and raccoon dogs, respectively. Phylogenetic analysis revealed that all *P. hominis* strains detected in foxes and raccoon dogs in the present study were the zoonotic genotype CC1. Moreover, compared with those in the *P. hominis*-negative group, the diversity of the gut microbiota in the *P. hominis*-positive group was lower, and the abundance of Firmicutes and the ratio of Firmicutes/Bacteroidetes (F/B) in the *P. hominis*-positive group were lower than those in the *P. hominis*-negative group. We speculate that these differences may be due to indigestion and diarrhea in infected female foxes. Overall, the present study evaluated the prevalence of *P. hominis* in foxes and raccoon dogs in the Henan and Hebei Provinces and revealed that *P. hominis* infection interrupted the diversity of the gut microbiota in female foxes.

Keywords *Pentatrichomonas hominis* · Gut microbiota · Foxes · Raccoon dogs

Introduction

Pentatrichomonas hominis (*P. hominis*) *is* an anaerobic flagellated protozoan of the Trichomonadidae family that inhabits the cecum or colon of its host (Delgado-Viscogliosi et al. 2000; Kim et al. 2010). *P. hominis* has a wide range of

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hosts and is transmitted through fecal-oral pathways, making it a potential pathogenic factor for zoonotic diseases (Zhang et al. 2019; Mahittikorn et al. 2021; Zhang et al. 2022b). The most common genotypes of *P. hominis* detected in dogs are CC1, CC2, and CC3, which have zoonotic potential (Li et al. 2016b). *P. hominis* was initially thought to be a symbiotic protozoan, but recent studies have shown that *P. hominis* mainly induces gastrointestinal diseases (Doğan and Tüzemen 2018; Zhang et al. 2019). *P. hominis* is also associated with human systemic lupus erythematosus, irritable bowel syndrome, rheumatoid arthritis, and gastrointestinal cancer (Compaoré et al. 2013; Doğan and Tüzemen 2018).

Microflora exist in various parts of the host, such as the skin and respiratory, intestinal, and urinary tracts (Dave et al. 2012). The gut microbiota forms a complex ecosystem within the host and plays important roles in the host's metabolism, immune response, and protection against pathogenic bacteria (Bäckhed et al. 2005; Mazmanian et al. 2008; Rossi et al. 2013; Sui et al. 2021). Studies have shown that gastrointestinal parasite infection initiates changes in the gut microbiota and affects the health of hosts (Berry et al. 2020; Fan et al. 2021; Zhang et al. 2022a). Harp et al. (1992) demonstrated that dysbiosis of the gut microbiota increased susceptibility to *Cryptosporidium parvum* infection in mice. Zhang et al. (2022b) analyzed the gut microbiota of patients with colorectal cancer who were infected with *P. hominis* and reported that infection with *P. hominis* increased the abundance of pathogenic bacteria, which might increase the risk of colorectal cancer. Studies have shown that *P. hominis* infection is often accompanied by diarrhea and emaciation (Li et al. 2018), and we hypothesize that disorders induced by *P. hominis* may be caused by dysbiosis of the gut microbiota. To exclude the influence of other factors (region, species, age, and sex), we noted that most female foxes had soft, watery stools; therefore, the gut microbiota of infected female foxes was evaluated in the present study.

It has been reported that the prevalence of *P. hominis* infection in wild animals such as silver foxes (45.0%) is very high (Li et al. 2017). Foxes and raccoon dogs are also hosts of *P. hominis* and can cause serious economic losses once infected with *P. hominis* (Li et al. 2017; Li et al. 2020). Microscopic examination was previously used for the detection of *P. hominis* (Bastos et al. 2018). With the development of molecular technology, polymerase chain reaction (PCR) is now considered to be the decisive method for detecting *P. hominis* (Kim et al. 2010; Liu et al. 2020). Foxes and raccoon dogs are widely farmed in the Hebei and Henan Provinces in China, but the prevalence of *P. hominis* infection in foxes and raccoon dogs and the effect of *P. hominis* infection on the gut microbiota of foxes in the Hebei and Henan Provinces are largely unknown. Therefore, the present study was conducted to evaluate the epidemiology of *P. hominis* in foxes and raccoon dogs in the Hebei and Henan Provinces based on the 18S rRNA gene locus and to evaluate the impact of *P. hominis* infection on the gut microbiota of female foxes.

Materials and methods

Ethics statement

This study was conducted according to the Chinese Laboratory Animal Administration Act of 1988. The research protocols were approved by the Ethics Review Committee of Henan Agricultural University (approval number: IRC-HENAU-20160225). The owners of the captive foxes and raccoon dogs gave permission to collect fecal samples, and no animals were injured during sample collection.

Sample collection

A total of 312 fecal samples from foxes and 581 fecal samples from raccoon dogs were collected from three different cities in the Henan and Hebei Provinces (Fig. 1) in China from June to December 2020. The foxes and raccoon dogs were kept in cages. Approximately 20 grams of fecal sample was collected from each animal from under the cages, and the samples were subsequently placed into clean plastic bags marked with sample information (including ID number, date, region, species, age, and sex). All the samples were stored at 4 °C until laboratory analysis.

DNA extraction and PCR amplification

Total genomic DNA was extracted from each fecal sample using an E.Z.N.A.™ Stool DNA Kit (Omega Biotek, Inc., Norcross, GA, USA) following the manufacturer's instructions and subsequently dissolved in 50 µl of elution buffer. The DNA was stored at −20 °C until PCR amplification.

P. hominis was determined by nested PCR based on the 18S rRNA gene. Primers were designed, and reaction procedures were conducted based on Li et al.'s report (Li et al. 2016a). The primer sequences were as follows: forward primer-1: 5′-ATGGCGAGTGGTGGAATA-3′ and reverse primer-1: 5′-CCCAACTAGGCTAAGGATT-3′, forward primer-2: 5′-TGTAAACGATGCCGACAGAG-3′ and reverse primer-2: 5′-CAACACTGAAGCCAATGC GAGC-3′. Amplification was performed in a final volume of 20 µl in an Applied Biosystems PCR thermal cycling apparatus (Thermo Fisher Scientific, Shanghai, China). The first-round PCR procedure was as follows: 95 °C for 5 min; 33 cycles of 94 °C for 60 s, 59 °C for 60 s, and 72 °C for 60 s; and 72 °C for 7 min. For the second round of PCR, 2 µl of the first-round amplicon was amplified with the following conditions: 95 °C for 5 min; 35 cycles of 94 °C for 60

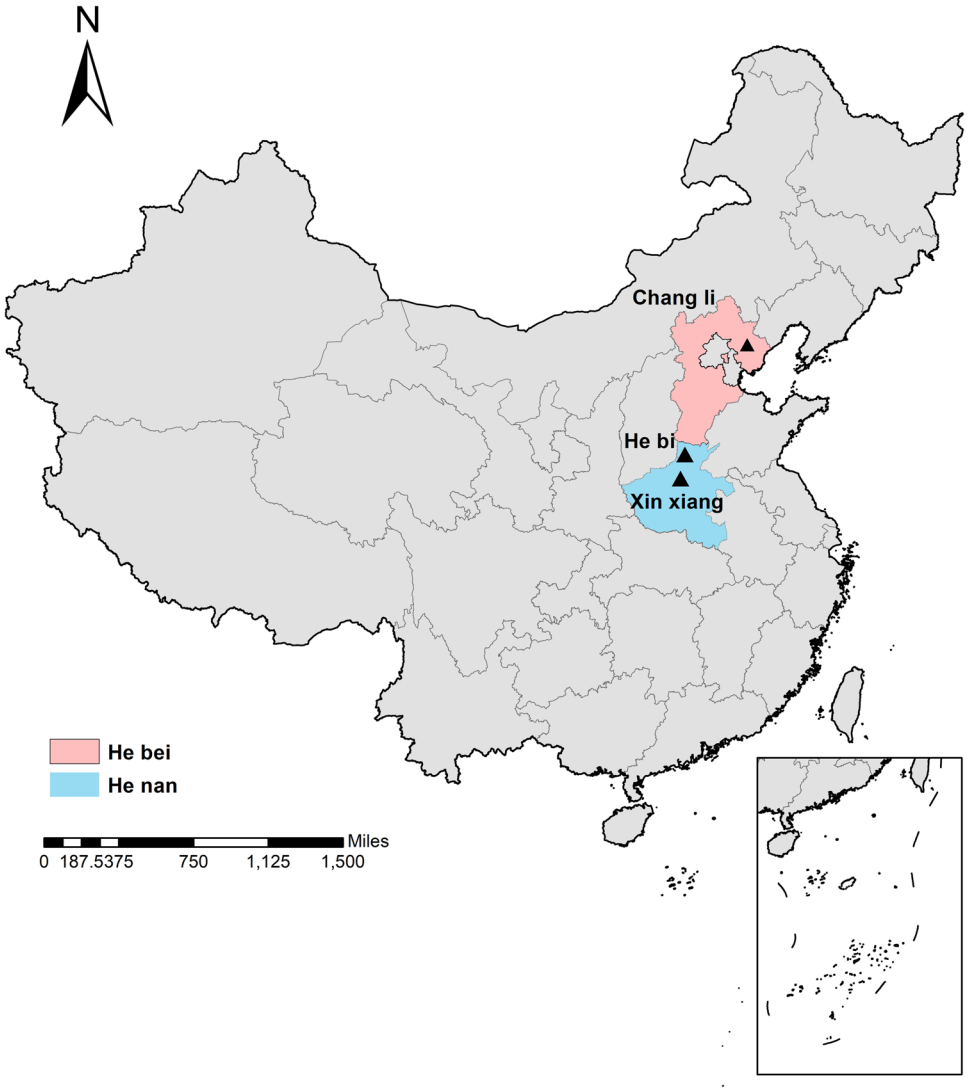

Fig. 1 Sampling locations in China. Triangles indicate the sites from which the samples were collected.

s, 61 °C for 60 s, and 72 °C for 60 s; and 72 °C for 7 min. The nested PCR results of the 18S rRNA target loci were detected via electrophoresis on a 1% agarose gel. Staining screening was performed with SYBR Green (TIANDZ, Inc., Beijing, China).

Sequence alignment and phylogenetic analysis

The *P. hominis*-positive PCR products of 18S rRNA were bidirectionally sequenced by SinoGenoMax (Beijing, China). To identify different genotypes, Chromas Pro 2.182 (Elegant Themes, USA) was used to assemble the sequences obtained in this study, which were then blasted against the reference sequence downloaded from GenBank (http://blast.ncbi.nlm.nih.gov). To better understand the taxonomic relationships among the *P. hominis* strains isolated in the present study, which were represented by reference sequences in GenBank, neighbor-joining trees were constructed with the Kimura 2-parameter model in MEGA (version 7.0.26), with 1000 bootstrap replicates. Representative 18S rRNA sequences obtained in this study have been deposited in GenBank under accession numbers OM763804 and OM763803.

16S rRNA sequencing

To exclude the influence of other factors (region, species, age, and sex), we noted that most female foxes had soft, watery stools; therefore, only nine *P. hominis*-positive samples (for which no other parasitic infection was detected except *P. hominis*) and nine *P. hominis*-negative (for which no parasitic infection was detected) samples from female foxes (female foxes >15 months old) were sent to Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) for gut bacterial analysis. PCR amplification of the V3–V4 region of the gut microbiota 16S rRNA gene from 18 fecal samples was performed using

the following primers: forward primer: 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer: 5′-GGTTACCTTGTTACGACTT-3′.

To understand the gut microbiota of female foxes, Illumina MiSeq sequencing of the 16S rRNA gene was completed by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The QIIME2-DADA2 pipeline and Vsearch software were used to process and analyze the sequencing data. The primer fragments and mismatched primer sequences were removed using the Cutadapt tool. Quality control, denoising, splicing, and chimera removal were performed by DADA2. Amplicon sequence variant (ASV) clustering was performed with 100% sequence similarity. The alpha diversity of the gut bacteria, particularly species richness and diversity, was evaluated by the Chao1 and Shannon indices, and diversity indices were calculated using Mothur (version 1.21.1) software. Beta diversity analysis was performed by calculating the unweighted UniFrac distance between each pair of samples. Principal coordinate analysis (PCOA) was also conducted based on the unweighted UniFrac distance to assess the similarity of the bacterial community structures. Taxon effect values between the two groups were estimated using linear discriminant analysis effect size (LEfSe) analysis.

Statistical analysis

SPSS 20.0 (IBM Corp., USA) was used to conduct the statistical analysis. The chi-square test was used to evaluate the significance of the differences in prevalence among different regions, species, ages, and sexes. The prevalence of *P. hominis* infection was calculated using the 95% confidence interval (CI). The difference in alpha diversity between groups was analyzed by Student's *t*-test using the Shannon and Chao1 indices. LEfSe analysis was used to identify differential gut microbiota between the two groups. Differences were considered to be statistically significant when $P < 0.05$.

Results

Prevalence of *P. hominis* in foxes and raccoon dogs

Fig. 2 shows the representative nested PCR results for the 18S rRNA target loci detected by 1% agarose gel electrophoresis. A total of 283 animals were infected with *P. hominis*. The total prevalence rate was 31.7% (283/893), and the prevalence rates of *P. hominis* infection were 28.2% (88/312) and 33.6% (195/581) in foxes and raccoon dogs, respectively (Table 1). The prevalence of *P. hominis* infection among foxes and raccoon dogs in different regions was significantly different ($\chi^2 = 86.8$, $df = 2$, $P < 0.01$). The highest prevalence of *P. hominis* infection was observed in Xinxiang city (43.1%, 233/540), followed by Changli city (18.7%, 36/193) and Hebi city (8.8%, 14/160) (Table 1). The prevalence of *P. hominis* infection in male foxes (37.6%, 32/85) was significantly greater ($\chi^2 = 5.1$, $df = 1$, $P = 0.02$) than that in female foxes (24.7%, 56/227) in the Henan and Hebei Provinces, but the difference in prevalence between the sexes of raccoon dogs was not statistically significant ($\chi^2 = 1.6$, $df = 1$, $P = 0.2$) (Table 1). The prevalence of *P. hominis* infection in raccoon dogs of different ages was significantly different ($\chi^2 = 62.7$, $df = 2$, $P<0.01$), with *P. hominis* infection occurring in raccoon dogs aged 6 to 15 months (61.3%, 49/80), followed by those aged younger than 2 months (48.5%, 65/134) and those aged older than 15 months (22.1%, 81/367); however, the difference in prevalence between ages of foxes was not statistically significant ($\chi^2 = 0.8$, $df = 2$, $P = 0.7$) (Table 1).

Genotypic and phylogenetic analyses of *P. hominis* in foxes and raccoon dogs

All the *P. hominis* strains isolated from foxes and raccoon dogs in the present study were identified as CC1 (Table 1). As shown in Fig. 3, the representative sequence registration numbers found in the present study are OM763804 and OM763803, which have more than 99% homology with the reference sequence (accession number: KJ408929) of *P.*

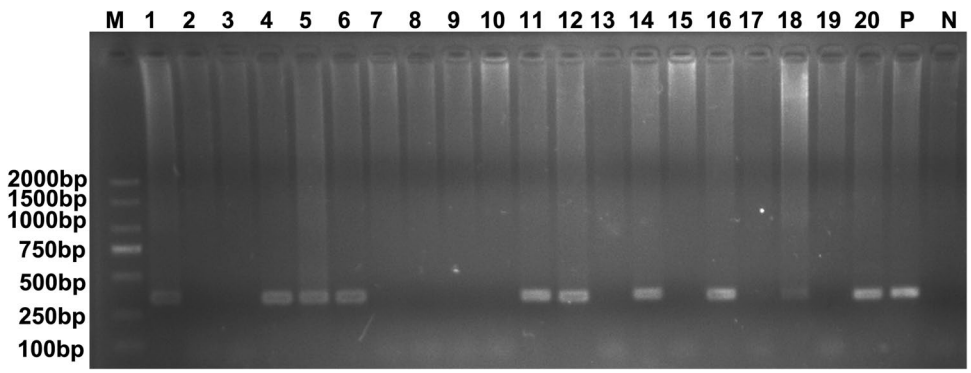

Fig. 2 Representative nested PCR results of 18S rRNA target loci detected by agarose gel electrophoresis. M: DL 2000 DNA Marker; 1–20: samples; P: positive control (positive control from Anhui Science and Technology University); N: negative control.

Table 1 Prevalence and genotypes of *P. hominis* in foxes and raccoon dogs in Xinxiang, Hebi, Henan, and Changli, Hebei.

Factor	Variable	No. of positive/no. of tested (%)	*P. hominis* genotype 18S rRNA	% (95% CI)	Statistical analysis
Region	Xinxiang	233/540	CC1	43.1 (39.0–47.3)	$\chi^2 = 86.8$, $df = 2$, $P < 0.01$*
	Hebi	14/160	CC1	8.8 (4.3–13.2)	
	Changli	36/193	CC1	18.7 (13.1–24.2)	
Species	Fox	88/312	CC1	28.2 (23.2–33.2)	$\chi^2 = 2.7$, $df = 1$, $P = 0.1$
	Raccoon dog	195/581	CC1	33.6 (29.7–37.4)	
Fox age	< 2 months	8/32	CC1	25.0 (9.1–40.9)	$\chi^2 = 0.8$, $df = 2$, $P = 0.7$
	6~15 months	10/44	CC1	22.7 (9.8–35.6)	
	≥ 15 months	70/244	CC1	28.7 (23.0–34.4)	
Raccoon dog age	< 2 months	65/134	CC1	48.5 (39.9–57.1)	$\chi^2 = 62.7$, $df = 2$, $P < 0.01$*
	6~15 months	49/80	CC1	61.3 (50.3–72.2)	
	≥ 15 months	81/367	CC1	22.1 (17.8–26.3)	
Fox sex	Male	32/85	CC1	37.6 (27.1–48.2)	$\chi^2 = 5.1$, $df = 1$, $P = 0.02$*
	Female	56/227	CC1	24.7 (19.0–30.3)	
Raccoon dog sex	Male	74/200	CC1	37.0 (30.3–43.8)	$\chi^2 = 1.6$, $df = 1$, $P = 0.2$
	Female	121/381	CC1	31.8 (27.1–36.5)	
	Total	283/893	CC1		

The chi-square test was used to evaluate the significance of the infection rates among different regions, species, ages, and sexes

*Statistically significant

hominis downloaded from GenBank (http://blast.ncbi.nlm.nih.gov/).

Effects of gut *P. hominis* infection on microbiota diversity and richness in female foxes

A total of 14,744 amplicon sequence variants (ASVs) were detected in the *P. hominis*-negative group, and 9785 ASVs were identified in the *P. hominis*-positive group. A total of 1144 ASVs were found in both groups (Fig. 4a). As shown in Fig. 4b, PCoA revealed a significant separation of the gut microbiota between the two groups (PCo1: 18.9%, PCo2: 13.0%, $P < 0.01$). As shown in Fig. 4c, d, the Chao1 ($P = 0.8$), Shannon ($P = 0.3$), and Simpson ($P = 0.3$) indices of the *P. hominis*-positive group were lower than those of the *P. hominis*-negative group.

Effect of gut *P. hominis* infection on the gut microbiota at the phylum level in female foxes

As depicted in Fig. 5a, at the phylum level, more than 90% of the gut microbiota in the two groups consisted of Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. Moreover, the relative abundances of Firmicutes ($P = 0.6$; Fig. 5b) and Proteobacteria ($P = 0.1$; Fig. 5e) in the *P. hominis*-positive group were relatively lower than those in the *P. hominis*-negative group. Similarly, the relative abundances of Bacteroidetes ($P = 0.1$; Fig. 5c) and Actinobacteria ($P = 0.2$; Fig. 5f) in the *P. hominis*-positive group were relatively greater than those in the *P. hominis*-negative group, and the F/B ratio ($P = 0.3$; Fig. 5d) in the *P. hominis*-positive group was relatively lower than that in the *P. hominis*-negative group.

Effect of gut *P. hominis* infection on the gut microbiota at the genus level in female foxes

As Fig. 6a shows, at the genus level, the gut microbiota of the *P. hominis*-positive group was mainly composed of *Streptococcus* spp., *Lactobacillus* spp., *Prevotella* spp., and *Bifidobacterium* spp., while that of the *P. hominis*-negative group was mainly composed of *Streptococcus* spp., *Clostridiaceae-clostridium* spp., *Blautia* spp., *Shigella* spp., *Kurthia* spp., and *Bacteroides* spp. Moreover, as shown in Fig. 6b–f, the relative abundances of *Streptococcus* spp. ($P = 0.1$), *Lactobacillus* spp. ($P = 0.1$), and *Prevotella* spp. ($P = 0.5$) and *Bifidobacterium* spp. ($P < 0.05$) in the *P. hominis*-positive group were greater than those in the *P. hominis*-negative group, while the abundance of *Clostridiaceae-closterium* spp. ($P < 0.01$) was significantly lower than that in the *P. hominis*-negative group.

Heterogeneity of the gut microbiota in the two groups

The heterogeneity of the gut microbiota between the *P. hominis*-positive and *P. hominis*-negative groups of female foxes was assessed by LEfSe analysis. As shown in Fig. 7,

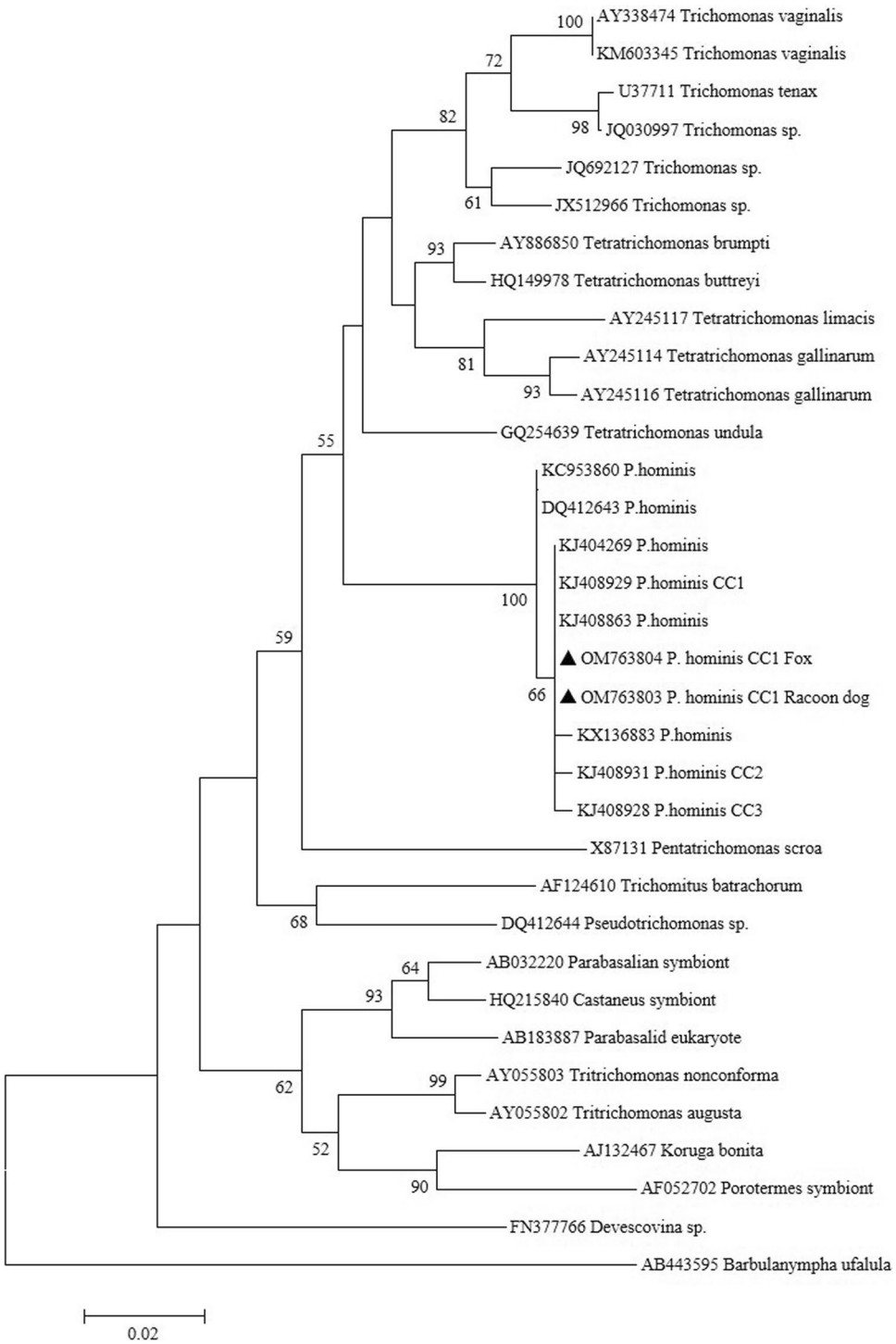

Fig. 3 Genotypic and phylogenetic analyses of *P. hominis* in foxes and raccoon dogs. Triangles indicate the *P. hominis* isolates from the present study. The data used for tree construction were obtained from the GenBank Data Libraries of the NCBI. The length of each branch is proportional to the amount of evolutionary distance between the different species. The scale bar indicates 0.02 substitutions (corrected) per base pair.

the classes Clostridia and Gammaproteobacteria, the orders Clostridiales and Bacillales, and the families Planococcaceae and Clostridiaceae (top 6 gut microbiota) were significantly more abundant in the *P. hominis*-negative group; and the families Lactobacillaceae, Prevotellaceae, Bifidobacteriaceae and Veillonellaceae, the order Bifidobacteriales, and the class Actinobacteria (top 6 gut microbiota) were significantly more abundant in the *P. hominis*-positive group.

Discussion

Trophozoites of *P. hominis*, which are anaerobic and flagellated protozoans, are opportunistic pathogens that mainly inhabit in large intestines of humans, cats, dogs, non-human primates, and pigs (Romatowski 2000; Gookin et al. 2005; Corbeil et al. 2008; Grellet et al. 2013). The zoonotic potential and pathogenicity of *P. hominis* have led to the increased

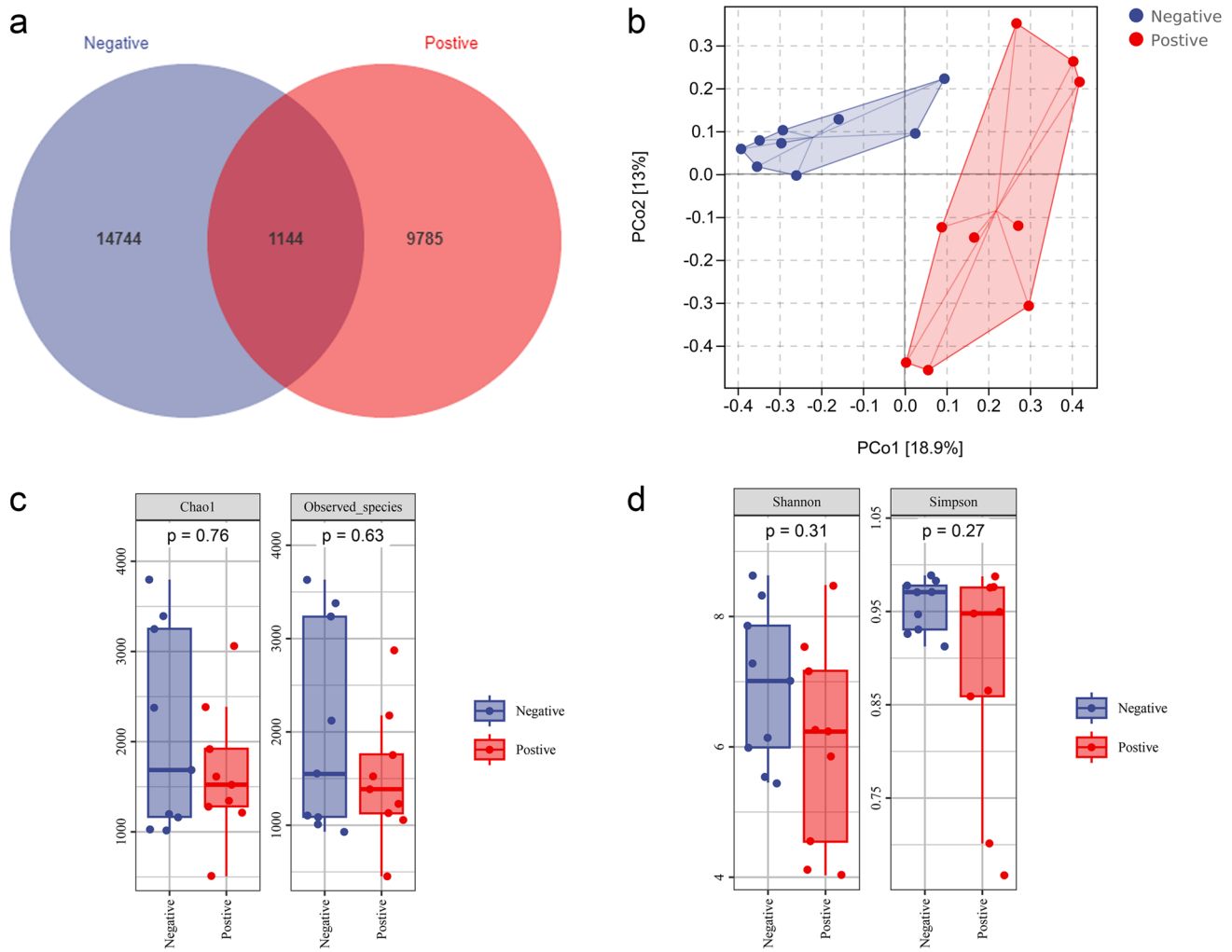

Fig. 4 Effects of gut *P. hominis* infection on the microbiota diversity and richness in female foxes. a Total amplicon sequence variants (ASVs) in different groups are shown as Venn diagrams. b Principal coordinate analysis (PCoA) by Bray−Curtis distance. c, d Species relative abundance and diversity determined by alpha diversity analysis.

awareness and detection of this parasite, especially in companion animals and farmed animals (Grellet et al. 2013; Li et al. 2015; Li et al. 2018; Li et al. 2020). Foxes and raccoon dogs are hosts of *P. hominis*, but there are few data on the prevalence of *P. hominis* in caged foxes and raccoon dogs in the Henan and Hebei Provinces in China (Li et al. 2017; Liu et al. 2020). Thus, 893 fecal samples were collected from caged foxes and raccoon dogs in the Henan and Hebei Provinces in the present study, after which the prevalence of *P. hominis* infection and the effect of *P. hominis* infection on the gut microbiota of female foxes were investigated.

At present, reports on the prevalence of *P. hominis* have mostly been concentrated in Asian countries such as China, Japan, and Thailand, though there have also been a few relevant reports in North America (USA), South America (Brazil), Europe (France, Poland), and Oceania (Australia) (Gookin et al. 2007; Dimasuay and Rivera 2013; Mahittikorn et al. 2021). Most of these reports focused on dogs, cats, pigs, cattle, and nonhuman primates, which are closely related to humans, while foxes and raccoon dogs were discussed relatively rarely (Gookin et al. 2007; Dimasuay and Rivera 2013; Grellet et al. 2013; Mahittikorn et al. 2021). In the present study, the prevalence rates of *P. hominis* infection in foxes and raccoon dogs in the Henan and Hebei Provinces were 28.2% and 33.6%, respectively. An epidemiological investigation of *P. hominis* infection in Jilin Province showed that the prevalence rates among wild silver fox, blue fox and raccoon dog infections were 43.3%, 45.0%, and 53.3%, respectively (Li et al. 2017), which are greater than the prevalence rates detected in the present study. The lower prevalence of *P. hominis* infection in the present study may be due to the intensive farming regime, which reduced the risk of *P. hominis* infection. Moreover, Zhang et al. (2022b) reported that the prevalence of *P. hominis* infection in Amur tigers

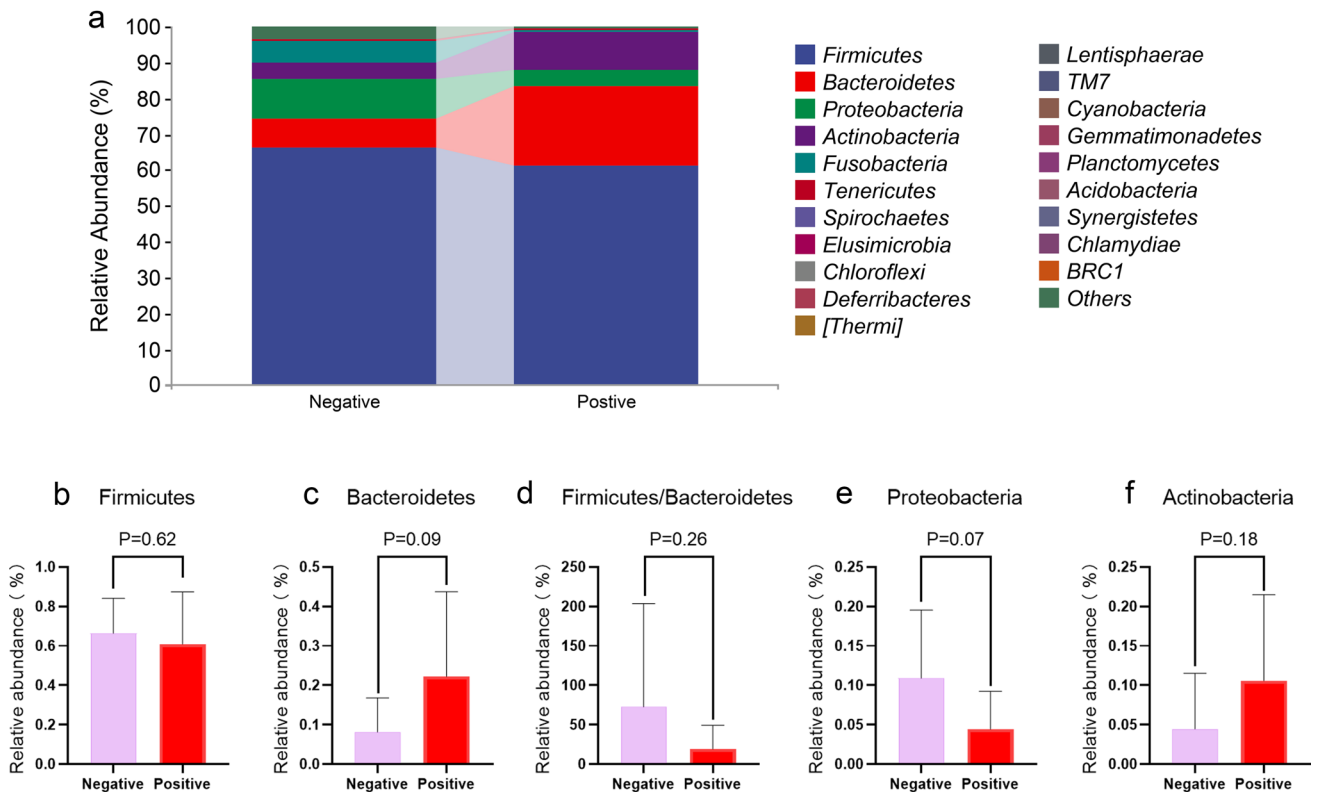

Fig. 5 Effect of gut *P. hominis* infection on the gut microbiota at the phylum level in female foxes. a Relative abundances of the 10 most abundant gut microbiota at the phylum level. b–f The relative abundances of Firmicutes and Bacteroidetes, the Firmicutes/Bacteroidetes (F/B) ratio, and the relative abundances of Actinobacteria and Proteobacteria in the different groups were analyzed by Student's t test.

in Northeast China was 31.3%. Li et al. (2018; 2020) found that the prevalence rates of *P. hominis* infection in goats and sheep in China were 0.3% and 0.0%, respectively, and that the total prevalence of *P. hominis* infection in cattle in Anhui Province in China was 8.1%. The above data indicated that the prevalence of *P. hominis* infection in carnivores (such as foxes and tigers) is greater than that in herbivores (such as cattle, sheep, and goats). Therefore, reducing meat supplementation during the feeding of foxes and raccoon dogs may reduce the risk of *P. hominis* infection.

Furthermore, the present study found that prevalence rates of *P. hominis* infection in foxes and raccoon dogs were significantly different in different regions, with the highest prevalence found in Xinxiang city (Henan province) followed by Changli city (Hebei province) and Hebi city (Henan Province), probably due to the different living conditions and feeding regimes, as reported by Li et al. (2017). In other protozoal infections, such as toxoplasmosis, female mice were more susceptible to *Toxoplasma gondii* infection than male mice were (Satoskar and Alexander 1995). Similarly, in the present study, the prevalence of *P. hominis* infection in male foxes was significantly lower than that in female foxes. In the present study, the prevalence rates of *P. hominis* in raccoon dogs older than 15 months and younger than 2 months were significantly lower than that in raccoon dogs aged 6 to 15 months, which indicated that the prevalence of *P. hominis* infection was related to the age of the host. However, Tolbert et al. (2012) found that *P. hominis* infection showed no age preference in dogs. Thus, the different incidences of *P. hominis* infection in foxes and raccoon dogs among individuals of different ages observed in the present study indicated that the effect of aging on susceptibility to *P. hominis* infection differed among the different species.

At present, among the CC1, CC2, and CC3 genotypes, CC1 is the most common *P. hominis* genotype detected in dogs and cats. Conversely, the CC2 and CC3 genotypes are rarely detected, and their host ranges are limited (Li et al. 2016a; Romatowski 2000). Previous data revealed that the CC1 genotype was the most common genotype of *P. hominis* detected in pet dogs in eastern China (Li et al. 2016a; Li et al. 2016b; Mahittikorn et al. 2021). Similarly, all 283 gene sequences obtained from the 18S rRNA gene of *P. hominis* from foxes and raccoon dogs were of the CC1 genotype (KJ408929), with no CC2 (KJ408931) or CC3 (KJ408928) detected in the present study. This finding indicated that the CC1 genotype is the most common genotype of *P. hominis* in foxes and raccoon dogs in the Henan and Hebei Provinces and has zoonotic potential (Li et al. 2017).

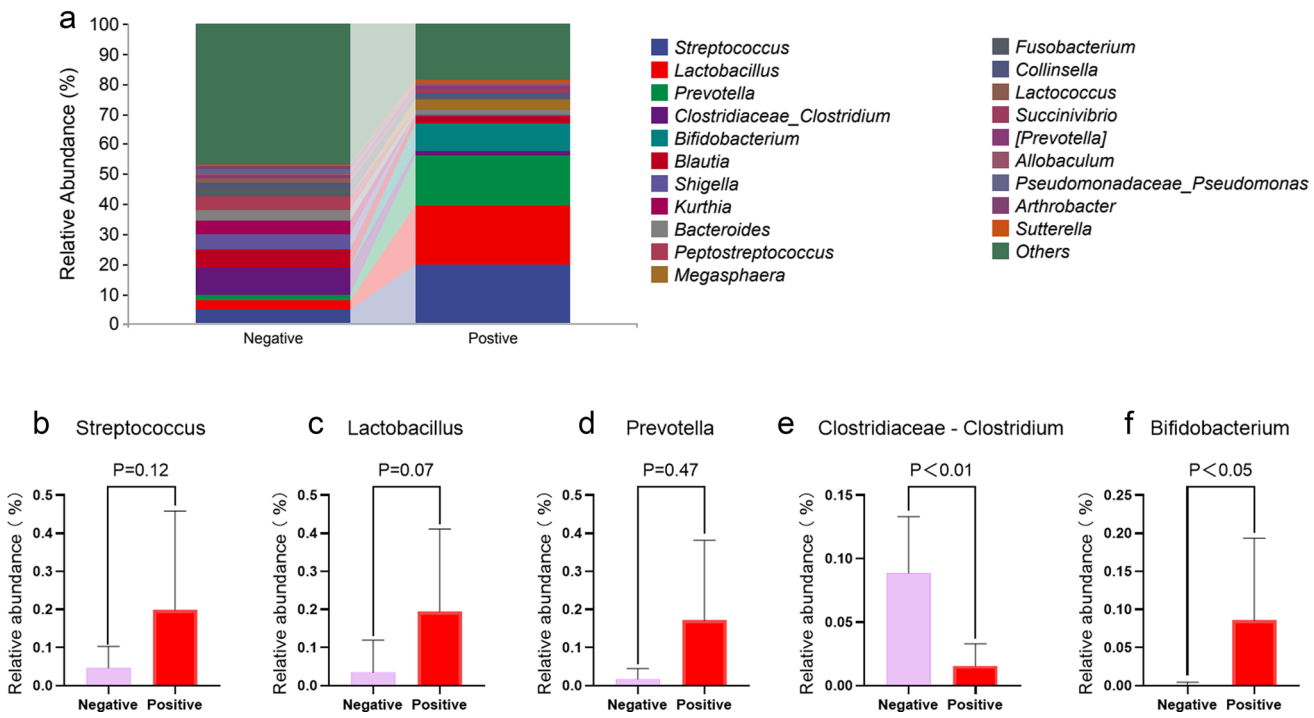

Fig. 6 Effect of gut *P. hominis* infection on the gut microbiota at the genus level in female foxes. a Relative abundances of the 20 most abundant gut microbiota at the genus level in the different groups. b–f The relative abundances of *Streptococcus* spp., *Lactobacillus* spp., *Prevotella* spp., *Clostridiaceae-Clostridium* spp., and *Bifidobacterium* spp. in the different groups were analyzed by Student's *t*-test.

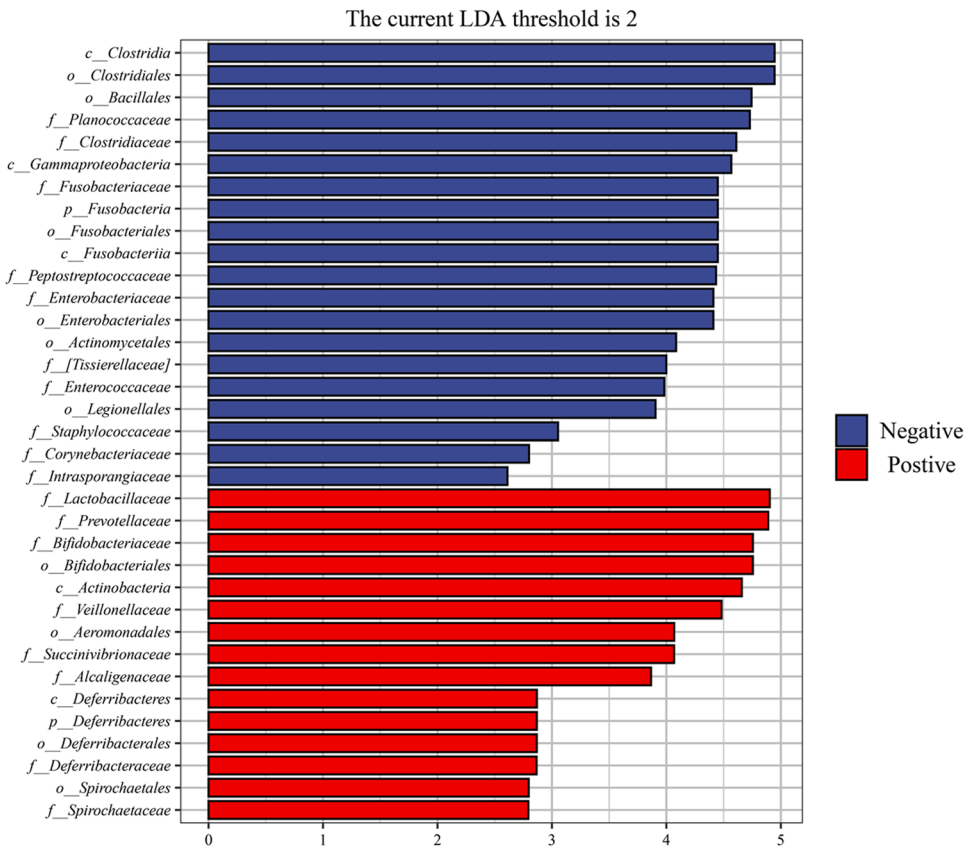

Fig. 7 Heterogeneity of the gut microbiota in the two groups. Differences in the gut microbiota between the two groups by LEfSe analysis. The highest levels of classification, species, and the length indicate the effect sizes associated with taxa. The LDA threshold was set at 2.

P. hominis can cause diarrhea and emaciation in the host, which we speculate may be caused by interference with the host's gut microbiota. To understand the pathogenicity of *P. hominis*, changes in the gut microbiota of female foxes were investigated. Many gastrointestinal parasites can cause host gut microbiota disorders, affecting the health of the host (Tolbert et al. 2012; Lynch and Pedersen 2016; Zhu et al. 2020). The present study revealed that Firmicutes and Bacteroidetes were the most abundant phyla in the female foxes, consistent with the findings of previous studies in which Firmicutes (73.4% in blue fox and 46.9% in raccoon dog) and Bacteroidetes (21.9% in blue fox and 44.3% in raccoon dog) were the most abundant phyla in the guts of healthy blue foxes and raccoon dogs (Liu et al. 2020; Wang et al. 2022). In the present study, the richness and diversity of the gut microbiota in foxes infected with *P. hominis* decreased, consistent with the findings of Sui et al. (2021; 2022), who reported that the diversity of the gut microbiota in foxes infected with *microsporidia* was lower than that in uninfected foxes. Thus, it can be concluded that *P. hominis* infection interrupted the diversity of the gut microbiota in female foxes.

Streptomyces and Bacteroides play important roles in host digestion (Berry 2016; Burgess et al. 2017; Liu et al. 2020; Wang et al. 2022). Firmicutes are the main microbes that digest fibers, producing volatile fatty acids and other byproducts (Berry 2016). In food, Bacteroides degrade proteins, carbohydrates, and compounds in plant cell walls (Huang and Zhang 2013). The Firmicutes⁄Bacteroidetes (F/B) ratio may be associated with fat accumulation in animals and humans (Turnbaugh et al. 2006; Bervoets et al. 2013). A higher F/B ratio might help hosts use nutrients more efficiently and generate more fat (Bervoets et al. 2013; Chavoya-Guardado et al. 2022). In the present study, analysis revealed that *P. hominis* infection decreased the F/B ratio in the gut of female foxes, which may be one of the reasons why female foxes infected with *P. hominis* became emaciated.

Lactic acid bacteria, including *Lactobacillus* spp. and *Bifidobacterium* spp., are probiotics that can inhibit pathogen invasion and enhance the immune response (Kim et al. 2019; Gómez-Gallego et al. 2021; Jang et al. 2021). However, at the genus level, *P. hominis* infection increased the abundance of "beneficial bacteria," including *Lactobacillus* spp., *Bifidobacterium* spp., and *Prevotella* spp., while decreasing the abundance of "potentially pathogenic bacteria," such as *Clostridiaceae-clostridium* spp. A previous study reported that *E. bieneusi* infection was associated with an increase in the proportion of "beneficial bacteria" and a decrease in "potentially pathogenic bacteria" (Sui et al. 2021). This phenomenon may be due to the pathogenic mechanism of *P. hominis* and the protective effect of the host microbiota against *P. hominis* infection; further studies are needed to determine the underlying mechanism involved.

In conclusion, the results of the present study indicated that the total prevalence of *P. hominis* infection in foxes and raccoon dogs was 31.7%, the prevalence of P. hominis infection in foxes was 28.2%, the prevalence of P. hominis infection in raccoon dogs was 33.6%, and the genotype was CC1, which is a risk factor for zoonosis. Moreover, *P. hominis* infection could cause a change in the gut microbiota in female foxes, the specific manifestations of which include gut microbiota diversity, the diversity of Firmicutes and the Firmicutes/Bacteroidetes (F/B) ratio. Moreover, the diversity of Actinobacteria increased, the diversity of *Streptococcus* spp., *Lactobacillus* spp., *Prevotella* spp., and *Bifidobacterium* spp. increased, and the diversity of *Clostridiaceae-Clostridium* spp. decreased, subsequently causing an imbalance in the gut microbiota of female foxes.

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Author contribution HJD designed the study and supplied the methodology. PTS conducted data analysis and article writing; YNG conducted material collection and testing; SJZ, LLL, FL, and TZ checked the manuscript for grammar and spelling; and HYD and HJD were responsible for supervision, writing review, and funding. All authors reviewed the manuscript.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication The authors declare that they know the content of this manuscript and agree to submit it to Parasitology Research.

Competing interests No potential conflict of interest was reported by the authors.

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