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Association between *Capillaria hepatica* infection-induced alterations in gut microbiota and estrogen expression in Brandt's voles (*Lasiopodomys brandtii*)

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Abstract

Background *Capillaria hepatica*, a zoonotic parasite, is present in the population of Brandt's voles (*Lasiopodomys brandtii*) and has been a central issue in ecological studies regarding its impact on host populations. Brandt's voles are known for their extremely high reproductive capacity, and the population explosion of Brandt's voles have occurred multiple times in the grasslands of Inner Mongolia over the past few decades. However, the mechanisms underlying the population dynamics of Brandt's voles, particularly in response to *C. hepatica* infection, remain poorly understood. Given the critical role of the gut microbiota in modulating hormones within the reproductive endocrine system, this study aims to explore how alterations in the gut microbiota influence the host's population dynamics in response to *C. hepatica* infection.

Methods Female Brandt's voles were inoculated with eggs of infected *C. hepatica*, and BALB/C mice were used as a control. At the end of the experimental period, cecal contents were collected for 16 S rRNA amplicon sequencing, and the expression levels of reproductive-related hormones were determined using enzyme-linked immunosorbent assay (ELISA).

Results *C. hepatica* infection leads to an increased diversity of gut microbiota in Brandt's voles, with significant changes in microbial composition. The relative abundance of *Muribaculaceae* and *Eubacteriaceae* increased significantly, while that of *Rikenellaceae* and *Lachnospiraceae* decreased significantly. The expression level of estradiol in the serum of infected Brandt's voles shows a slight decrease without statistical significance. However, the expression of equol is significantly higher in the infected group compared to the uninfected group, and the expression of enterolactone is significantly lower in the infected group than in the uninfected group.

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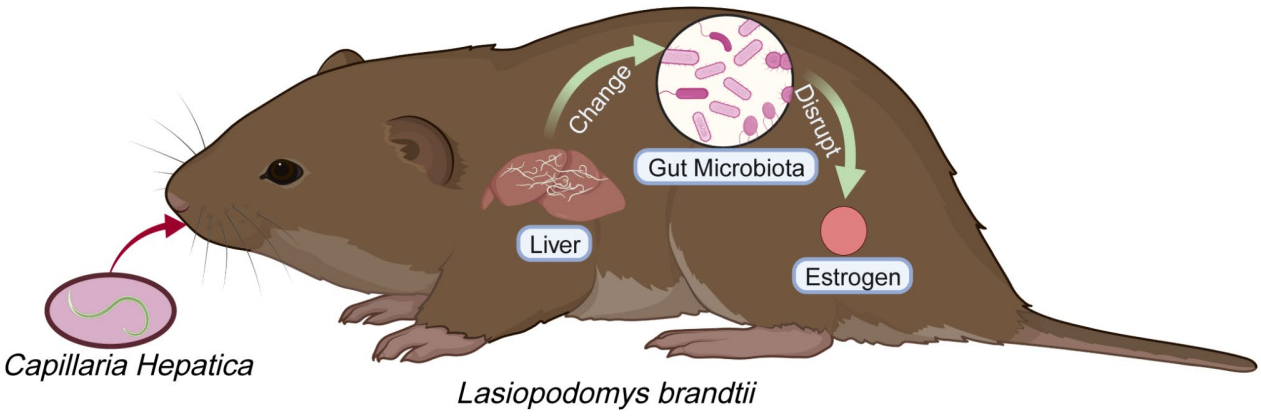
Conclusions This study demonstrates that infection with *C. hepatica* indirectly affect the abundance of specific gut microbiota in Brandt's voles, which are associated with reproductive hormones. This indirect effect on hormone expression can subsequently impact the reproductive function of the host. By investigating the changes in specific gut microbiota, this study sheds light on the mechanisms through which parasites regulate population fluctuations in Brandt's voles.

Keywords 16S rRNA amplicon sequencing, *Capillaria Hepatica*, Brandt's voles, Gut microbiota, Estrogen

Graphical abstract



Background

The Brandt's vole (*Lasiopodomys brandtii*) is a rodent belonging to the Hamster family Microtus subfamily, mainly distributed in the Northeast Asian steppe belt [1]. In China, Brandt's voles are found in the central part of Inner Mongolia, including the Xilingol grassland, the eastern Hulunbuir grassland, and the northern Zhangbei grassland in Hebei Province. Among these, the Xilingol and Hulunbuir grasslands are the main distribution areas [2]. Brandt's voles have a high reproductive capacity, and large-scale populations can cause damage to grassland environments. They compete with livestock for food resources and pose a serious threat to local animal husbandry, resulting in significant economic losses [3]. Over the past few decades, there have been multiple population explosions of Brandt's voles in the grasslands of Inner Mongolia, but the mechanisms underlying their population dynamics remain unclear [4].

Capillaria hepatica is a zoonotic linear parasite belonging to the Capillaridae family. It has a direct life cycle with no intermediate host. The parasite primarily resides in the host's liver, inevitably causing liver damage [5]. The adult worms live deep in the host liver and lay eggs in the surrounding tissues. These eggs become trapped in liver tissue and cannot be transmitted through the host's feces, but instead exit the body after the host dies and contaminate the soil [6]. In the past, the focus was mainly on the negative regulatory effects of pathogens on host population numbers [7]. Due to the detrimental effects of parasites on the host, such as nutrient uptake and pathological damage, more attention is usually paid to the study of diseases related to intestinal parasites, and less attention is paid to the interaction between parasite and the host gut microbiota [8].

The gut microbiota of the host is influenced by various factors, such as the host's genotype, dietary structure, and health status. External environmental disturbances also play a crucial role in shaping the structure and composition of the microbiota [9]. Over the course of evolutionary history, the interaction and mutual influence between the gut microbiota and its host have reached a state of balance. This maintenance of equilibrium is vital for the host in terms of metabolism, immunity, and nutrition [10, 11]. For example, in terms of metabolism, a classic example is the process of bile acid metabolism. Primary bile acids are synthesized from cholesterol in the liver, and it is only through the action of intestinal microbiota that primary bile acids can undergo 7α- deoxygenation to form secondary bile acids, which then participate in the enterohepatic circulation [12].

Recent studies have shown that the gut microbiota plays an important role in regulating sex hormones in the reproductive endocrine system. The gut microbiota can interact with estrogen, testosterone, insulin, and other hormones, influencing their metabolism and activity [13]. Firstly, the gut microbiota is involved in estrogen metabolism. Estrogen is a major regulator of the gut microbiota, and the gut microbiota gene pool involved in estrogen metabolism is referred to as the "estrobolome" [14]. Certain bacterial groups possess enzymes, such as β-glucuronidase and β-sulfatase, that can transform estrogen metabolites, thereby affecting their biological

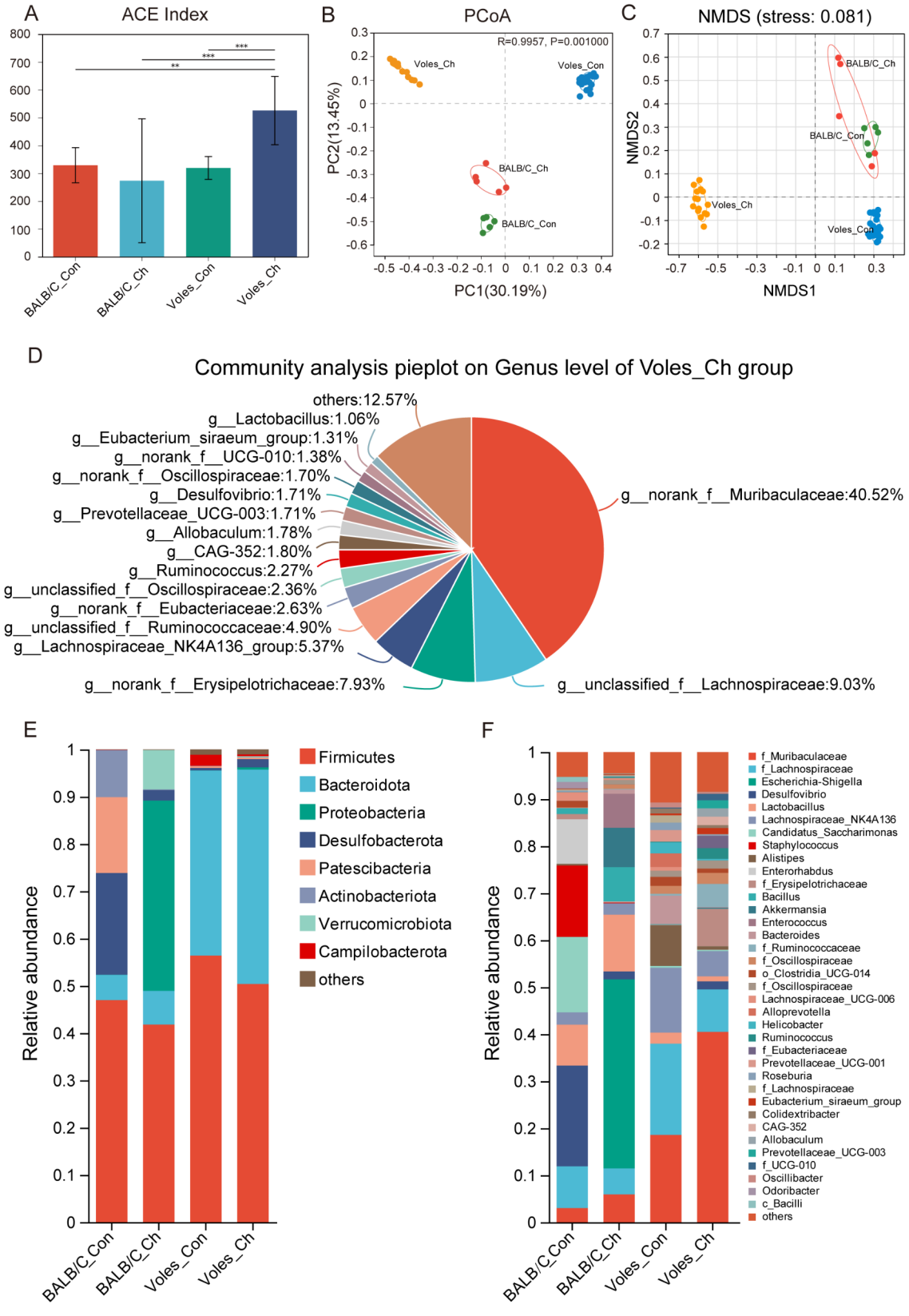


Fig. 1 (See legend on next page.)

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Fig. 1 Community diversity analysis. (**A**) Ace index was performed using the Kruskal-Wallis H test, marked with significance levels (* for $0.01 < P \leq 0.05$, ** for $0.001 < P \leq 0.01$, and *** for $P \leq 0.001$). The y-axis represents the average values of the index for each group. (**B**) PCoA on ASV level. The x-axis and y-axis represent the selected principal coordinate components, and the percentages indicate the contribution of each component to the compositional differences among samples. Points of different colors or shapes represent samples from different groups, and closer proximity between two sample points indicates a greater similarity in species composition. (**C**) NMDS analysis among all groups (Stress = 0.081). Each point on the graph represents a sample, and the distance between points represents the degree of difference. (**D**) Microbial community composition of infected Brandt's voles at the genus level. Different colors represent different species, and the size of each pie slice represents the percentage of that species. (**E**) Composition of gut microbiota of different groups at the phylum level. (**F**) Composition of gut microbiota of different groups at the genus level

activity [15]. Regulation of estrogen is crucial for female health, as the expression of estrogen receptor beta (ERβ) and serum concentrations of steroid hormones, especially estradiol, vary throughout an organism's lifespan. Previous studies have demonstrated that gut microbial lipids can activate estrogen signaling in both male and female mice [16]. Meanwhile, sex hormones can alter microbial diversity, with alpha diversity being negatively correlated with estradiol levels, although the mechanisms are still unclear [17]. For example, equol, a type of plant-derived isoflavone with estrogenic properties, binds to estrogen receptors and exhibits estrogenic effects [18]. The formation and clearance of these metabolites are essential for maintaining estrogen balance. Disruption of the gut microbiota may lead to disturbances in estrogen metabolism, subsequently affecting hormone levels and hormone-related physiological functions [19]. Secondly, the gut microbiota can influence the activity of sex hormones by modulating the immune system. The gut microbiota can regulate the function of immune cells by producing metabolites such as short-chain fatty acids (SCFAs) [18, 20, 21]. Additionally, the gut microbiota can affect the absorption and utilization of sex hormones by interacting with the intestinal mucosa. The gut microbiota can regulate intestinal barrier function, mucosal permeability, and intestinal motility, thereby influencing the absorption and utilization of sex hormones in the intestine [22].

In the wild environment, there are many uncertain factors that can potentially affect the gut microbiota of rodents, such as temperature, humidity, and other environmental factors. Additionally, rodents living in the wild are likely to carry both known and unknown pathogens [23], which can also disrupt the composition and structure of the rodent gut microbiota [24]. Although interactions between parasites and gut microbiota have been studied primarily in human, domestic animals and laboratory, research on the interaction between parasites and microbiota in wild populations is relatively limited [25, 26]. In order to further minimize the influence of other factors on experimental results, we conducted an indoor study on the effects of *C. hepatica* infection on the gut microbiota of Brandt's voles. Through indoor infection experiments, we aimed to reveal the association by which parasites regulate population fluctuations in field Brandt's vole populations from the perspective of the gut microbiota.

Results

The infection of *C. hepatica* affected the diversity and composition of gut microbiota

The interaction between the host and parasite may influence changes in the composition of the gut microbiota, and the genetic characteristics of the host itself may be one of the main influencing factors [27, 28]. Therefore, in our study, we simultaneously used BALB/C mice as a control host for comparative analysis. We referenced existing methods [29], employing necropsy to observe liver lesions to determine whether the mice were infected with the *C. hepatica*, and selected these confirmed infected mice for further experiments. To assess microbial diversity, we sequenced the V3-V4 regions of the 16 S rRNA gene in 46 Colorectal contents samples from 4 groups. ASV (Amplicon Sequence Variant) were taxonomically annotated, and the abundance information of each ASV annotation result in each sample was recorded [30]. A total of 8,941 ASVs were identified.

The results showed that after infection, the α-diversity of intestinal microbiota in Brandt's voles were significantly higher than that of other groups (Fig. 1A). Then, based on the Unweighted Unifrac and Weighted Unifrac distance information constructed based on ASV data, a principal-coordinate analysis (PCoA) (PC1 = 15.63%, PC2 = 14.46%, $P = 0.001$) (Fig. 1B) and Non-Metric Multi-Dimensional Scaling (NMDS) (Stress = 0.081) (Fig. 1C) based on the Bray-Curtis dissimilarity values were used to assess the differences in bacterial community structure between the groups. According to the taxonomic results, the dominant phyla in Brandt's voles-infected group were Firmicutes (50.41%) and Bacteroidota (45.34%). At the genus level, the dominant bacterial groups in the intestines of Brandt's voles-infected group were *Muribaculaceae* (40.52%), *Lachnospiraceae* (9.03%), and *Erysipelotrichaceae* (7.93%) (Fig. 1D).

For a more intuitive view of the relative abundance and proportions of gut microbiota in different groups at various taxonomic levels, we analyzed the composition of gut microbiota in these four groups at the phylum and genus levels. At the phylum level, the abundance of *Firmicutes* decreased after *C. hepatica* infection in both Brandt's voles and BALB/C mice. *Bacteroidota* had a

much higher relative abundance in Brandt's voles compared to BALB/C mice, and its relative abundance also increased after infection (Fig. 1E). However, *Proteobacteria* showed a significant increase in relative abundance after infection in BALB/C mice but not in Brandt's voles. At the genus level, both Brandt's voles and BALB/C mice showed an increase in the abundance of *Muribaculaceae* and a decrease in the abundance of *Lactobacillus* after *C. hepatica* infection. However, the abundance of *Escherichia-Shigella* increased significantly after infection only in BALB/C mice, not in Brandt's voles (Fig. 1F).

In summary, compared to BALB/C mice, Brandt's voles exhibited increased diversity of gut microbiota and significant changes in microbial composition after *C. hepatica* infection.

Specific gut microbiota may affect reproductive changes after infection with *C. hepatica* in Brandt's voles

In order to screen for gut microbiota that have a significant impact on host reproduction after infection with *C. hepatica* in Brandt's voles, we used LEfSe analysis based on the obtained community abundance data to identify significant differential species biomarkers between groups. We detected differentially abundant species between different groups using Kruskal-Wallis rank-sum test and assessed the magnitude of their effects using Linear Discriminant Analysis (LDA). The results showed that f_*Muribaculaceae*, f_*Erysipelotrichaceae*, o_*Oscillospirale*, f_*Ruminococcaceae*, f_*Eubacteriaceae* and g_*Ruminococcus* were significantly different in abundance in infected Brandt's voles (Fig. 2A) (Wilcoxon rank-sum test, $P < 0.01$, LDA score > 4). Furthermore, Wilcoxon rank-sum test was performed to analyze the differences between the infected and uninfected groups (Wilcoxon rank-sum test, $P < 0.05$) (Fig. S1A), revealing significant differences in the abundance of f_*Muribaculaceae*, f_*Erysipelotrichaceae*, f_*Ruminococcaceae*, f_*Eubacteriaceae*, g_*Ruminococcus*, and g_*Allobaculum*. Specifically, the relative abundance of f_*Muribaculaceae* ($P < 0.001$) (Fig. 2B) and f_*Eubacteriaceae* ($P < 0.001$) (Fig. 2C) significantly increased, while the abundance of *Rikenellaceae* ($P < 0.001$) (Fig. 2D) and *Lachnospiraceae* ($P < 0.001$) (Fig. 2E) significantly decreased. *Eubacteriaceae* can metabolize soy isoflavones (mainly genistin) to produce equol [31, 32], which is a phytoestrogen with estrogenic activity that can bind to estrogen receptors and exhibit estrogenic effects [18]. On the other hand, *Rikenellaceae* and *Lachnospiraceae* are involved in the generation of enterolactone [33], which may have positive feedback effects on female reproduction [34].

Meanwhile, One-way ANOVA was used to explore the differential bacterial communities among four groups (Fig. S1B). Compared to the other three groups, f_*Muribaculaceae* and *Ruminococcus* were highly enriched with significant differences in the infected Brandt's voles group (One-way ANOVA, $P \leq 0.001$). f__*Lachnospiraceae* showed significant enrichment in the uninfected Brandt's voles (One-way ANOVA, $P \leq 0.05$), while *Desulfovibrio* showed significant enrichment in the uninfected BALB/C mice. Additionally, differential analysis of the relative abundance of f_*Eubacteriaceae* (Fig. S2A), *Rikenellaceae* (Fig. S2B), and *Lachnospiraceae* (Fig. S2C) in the infected and uninfected Brandt's voles groups showed statistically significant differences (One-way ANOVA, $P \leq 0.001$).

Effects of infection with *C. hepatica* on the expression of reproductive hormones in Brandt's voles

From the above results suggest that the changes of gut microbiota related to endocrine hormones caused by *C. hepatica* infection may be associated the reproductive function of Brandt's voles. We first tested the expression levels of estradiol in the serum of the control group and the infection group of Brandt's voles. The results showed that although the expression level in the infection group decreased, there was no statistically significant difference (Fig. 3A) (one-way ANOVA, $P \leq 0.5$). Previous studies have shown a negative correlation between the level of estradiol and the reproductive ability of some animals [35, 36], while high concentrations of enterolactone were positively associated with improved reproductive success [37]. Rodents have the ability to produce equol. Then we tested the expression level of equol, and the results showed that the expression of equol in the infection group was significantly higher than that in the uninfected group (Fig. 3B) (one-way ANOVA, $P \leq 0.05$), while the expression of enterolactone was lower than that in the uninfected group ($P \leq 0.05$) (Fig. 3C) (one-way ANOVA, $P \leq 0.05$). These results indicate that *C. hepatica* infection may affect host reproduction by influencing the composition of the gut microbiota in Brandt's voles, thereby affecting the expression of hormones related to reproduction in Brandt's voles. The theory of the gut-liver axis provides a theoretical basis for understanding the interaction between the gut microbiota and liver diseases. The liver communicates with the intestine through the release of bile acids and many other bioactive substances into the biliary tract and systemic circulation. In the intestine, endogenous metabolites (such as bile acids and amino acids) and exogenous substances (from diet and environmental stress) produced by the host and gut microbiota are transported to the liver via the portal vein, thereby affecting liver function. Existing studies have shown that disruption of the gut-liver axis plays a crucial role in diseases such as alcoholic liver disease [38] and non-alcoholic liver disease [39].

Similar to other rodent species, at the phylum level, the highest abundance of bacteria in Brandt's voles is found

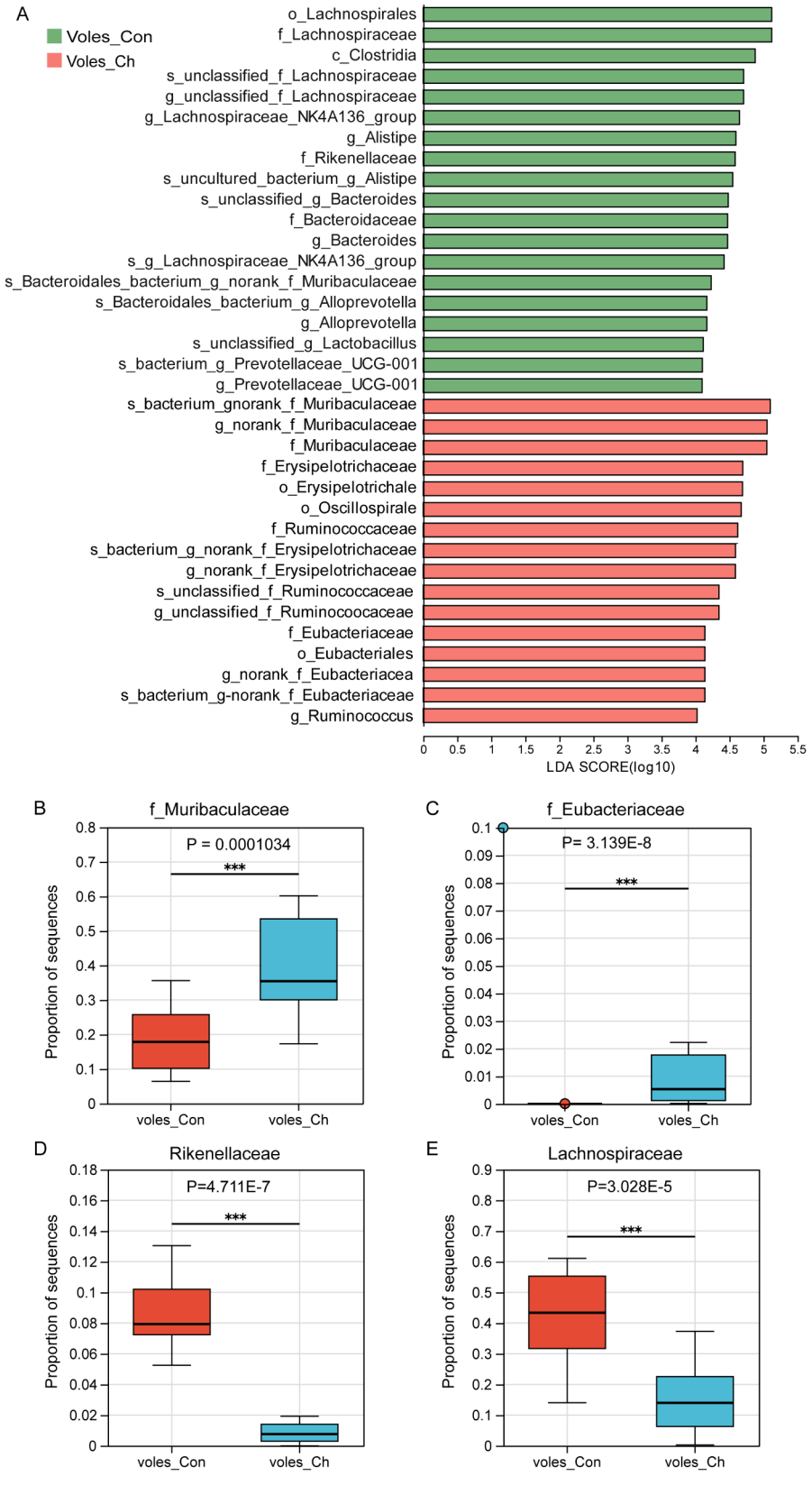

Fig. 2 Differential species abundance analysis. (**A**) Distribution of LDA values between uninfected and infected Brandt's voles. Bar chart displays microbial taxa with LDA scores greater than a set value (Wilcoxon rank-sum test, $P < 0.01$; LDA score > 4). Analysis of bacterial abundance differences of (**B**) Muribaculaceae (**C**) Eubacteriaceae (**D**) Rikenellaceae and (E) Lachnospiraceae were performed using the Wilcoxon rank-sum test. Significance levels are denoted as follows: (* for $0.01 < P \leq 0.05$, ** for $0.001 < P \leq 0.01$, and *** for $P \leq 0.001$)

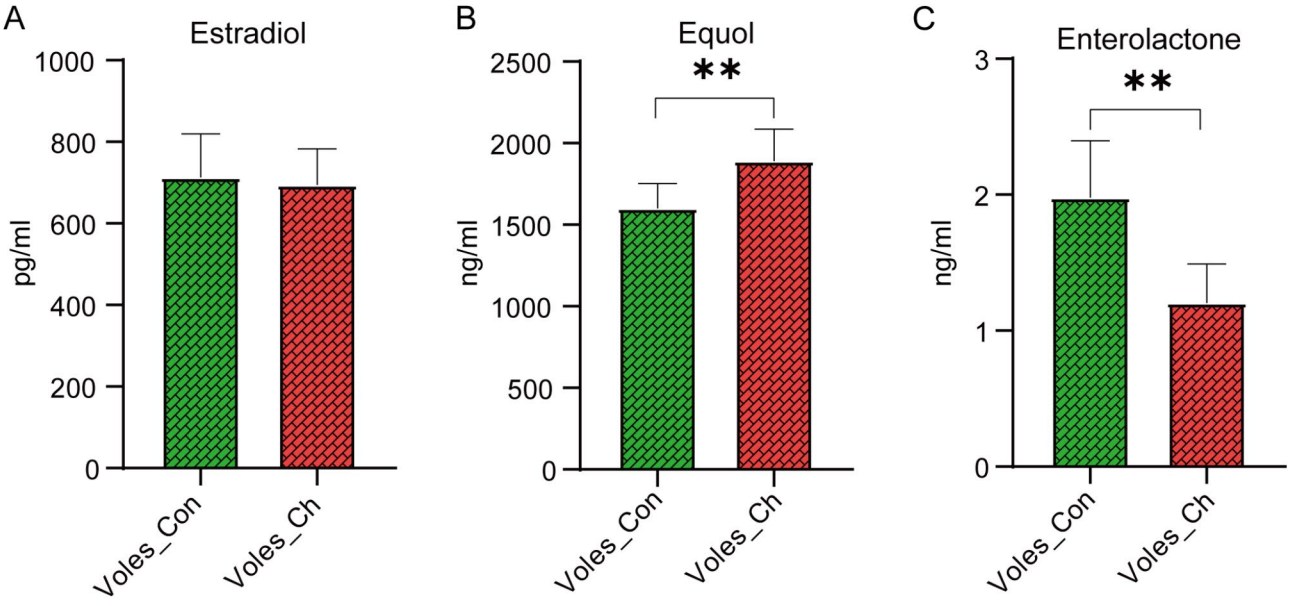

Fig. 3 Hormone level detection. (**A**) The concentration of estradiol. (**B**) The concentration of equol. (**C**) The concentration of enterolactone. Multiple comparisons were done by ordinary one-way ANOVA (ns: Not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, respectively)

in *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* [40, 41]. Among the highly abundant phyla, the abundance of *Firmicutes*, *Proteobacteria*, *Spirochaetes*, and *Cyanobacteria* significantly increases after *C. hepatica* infection, while *Bacteroidetes* and *Deferribacteres* decrease significantly. Studies have shown that dysbiosis of gut microbiota can lead to metabolic dysfunction, manifested by an increase in the ratio of *Firmicutes* to *Bacteroidetes* [42, 43]. *Bacteroidetes* is a crucial bacterial phylum in the gut, as it can degrade most carbohydrates as substrates [44], making it particularly important in herbivorous animals. The SCFAs released from the fermentation and breakdown of carbohydrates by *Bacteroidota* can be absorbed by the host and play important biological functions [45]. Brandt's voles feed on various plants, so a high abundance of *Bacteroidota* is beneficial for food digestion and absorption.

One notable feature of intestinal dysbiosis is a significant increase in the abundance of the phylum *Proteobacteria*. *Proteobacteria* are Gram-negative bacteria with lipopolysaccharides on their surface. Previous studies have found a positive correlation between the abundance of *Proteobacteria* and the occurrence of inflammation. For example, in patients with colitis and mouse models, there is a significant increase in the abundance of *Proteobacteria* [46]. Another study found that under chronic stress conditions, mice showed a significant increase in the abundance of *Proteobacteria* [47]. Furthermore, research has indicated that disrupted innate immune responses can promote the growth of *Proteobacteria*, which, in turn, can promote the progression of inflammation [48]. In this study, the increased abundance of *Proteobacteria* in the gut microbiota of infected Brandt's voles may be related to the host's immune response, and the high abundance of *Proteobacteria* may exacerbate intestinal dysfunction.

At the Family level, infection with *C. hepatica* also leads to significant changes in the gut microbiota. For example, *Ruminococcaceae* are symbiotic bacteria colonizing the cecum and colon that can produce SCFAs and play an important role in the degradation of various polysaccharides and fibers [49]. In Brandt's voles, we found a significant increase in the abundance of *Ruminococcaceae*, which may be a compensatory mechanism adopted by Brandt's voles to benefit themselves under infection status. *Erysipelotrichaceae* belong to the phylum Firmicutes and have potential roles in host diseases and physiology. For example, compared to healthy controls, patients with colorectal cancer, inflammatory bowel disease, and animal models of Crohn's disease showed significantly higher levels of *Erysipelotrichaceae* [50, 51]. The pathogenicity of *Erysipelotrichaceae* may be related to metabolic disorders in the host. For instance, the abundance of *Erysipelotrichaceae* is greatly increased in diet-induced obese animals and obese individuals [52, 53]. *C. hepatica* mainly parasitize the liver, inevitably causing liver damage. The liver plays an important role in host metabolism, immune responses, and other functions. After Brandt's voles were infected with *C. hepatica*, there was a significant increase in the abundance of *Erysipelotrichaceae*, which is likely related to the liver damage caused by *C. hepatica*.

The gut microbiota is referred to as a "virtual endocrine organ". Numerous studies have shown that the

composition of the gut microbiota is not only crucial for the host's metabolism and physical health but also regulates the hypothalamic-pituitary-adrenal axis [54, 55]. Although the exact signaling pathways between the gut microbiota and hormones have not been fully revealed, it is known that the microbial community can produce and secrete hormones, respond to host hormones, and regulate the expression levels of host hormones [54]. A recent study indicated that the reproductive success of Southern white rhinoceros (*Ceratotherium simum simum*) and Indian rhinoceros (*Rhinoceros unicornis*) may be influenced by certain gut microbiota in addition to the differences in estrogen receptor sensitivity to plant estrogens [35]. Research has shown that various microbial taxa are involved in the synthesis or transformation of sex hormones. Despite their low abundance, these taxa may have significant functionality [56]. For example, the *Coriobacteriaceae* can convert isoflavone genistein into equol [57],, and the level of equol has a negative correlation with the reproductive success of some animals [35, 36]. Consistent with this, the present study found a significant increase in equol concentration in the serum of Brandt's voles infected with *C. hepatica*. Therefore, the increased abundance of the *Eubacteriaceae*, which is potentially involved in the decrease of reproductive success in Brandt's voles, might be attributed to *C. hepatica* infection.

Conclusion

In summary, the infection of *C. hepatica* leads to the loss of homeostasis of gut microbiota, in which the abundance of disease-related intestinal microbes is increased, and the flora related to reproductive hormone metabolism is also altered, which may be one of the driving forces for the reduced fertility of Brandt's voles. These results reveal the potential role of gut microbiota in the regulation of the host population by the infection of *C. hepatica*, and provide important clues for further understanding the mechanism by which the parasite causes the fluctuation of the Brandt's voles population.

Methods

Sample collection and ethical statement

This study was conducted in accordance with the Guidelines for the Care and Use of Animals in Research published by the Institute of Zoology, Chinese Academy of Sciences. This study was reviewed and approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences (**2019FY100300-03**).

Experimental treatment of Brandt's voles and BALB/C mice

The experiments were conducted using 6-week-old female BALB/C mice purchased from SiPeiFu (Beijing) Biotechnology Co., Ltd. After one week of acclimation in the animal facility, the mice were randomly divided into a control group (BALB/C_Con) of 5 mice and an experimental group (BALB/C_Ch) of 5 mice. The experiments were conducted when the mice reached an average weight of approximately 30 g. The Brandt's voles used in this study were bred in our laboratory. Thirty-six female Brandt's voles with an average weight of approximately 30 g were selected, including 21 in the control group (Voles_Con) and 15 in the infection group (Voles_Ch). The temperature was controlled at around 23 °C, with a 12-hour light-dark cycle. All mice were fed under the same environmental conditions, and there was no contact between groups during the water and feeding process. The infection group was orally inoculated with approximately 500 *C. hepatica* eggs by gavage.

Culture of *C. hepatica* egg

The *C. hepatica* eggs used for infection were obtained by digesting the liver tissue of infected mice using the artificial digestion method. The liver tissue was washed with a suitable amount of physiological saline, centrifuged at 5000 rpm for 5 min, and the supernatant was discarded. The solution containing the *C. hepatica* eggs was slowly added to a culture dish containing DMEM medium supplemented with 0.5% serum and antibiotics (Penicillin 100U/ml and streptomycin 0.1 mg/ml). The culture dish was then placed in a 30 °C incubator. The medium was changed every 2 days, and microscopic observation was performed until the eggs developed to the infective stage.

Acquisition and preservation of sequencing samples

After 50 days of *C. hepatica* eggs infection treatment, samples were collected from the experimental and control animals. The BALB/C mice and Brandt's voles were euthanized using isoflurane, and cecal content samples were collected. The collected fecal content samples were immediately stored at -80 °C and sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. for sequencing.

Extraction, library construction and sequencing of fecal DNA

The Stool DNA was extracted according to the instructions of TIANamp Stool DNA Kit. After the purity and concentration of stool genomic DNA were determined, the stool was stored at -80℃. DNA concentration and purity was checked on 1% agarose gels. According to the concentration, DNA was diluted to 1 ug/μL using sterile water. 16 S rRNA genes of 16 S V3-V4 distinct regions were amplified used16S V4:515 F-806R specific primer with the barcode. All PCR reactions were carried out with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs; 0.2 μM of forward and reverse primers, and about 10 ng template DNA.

The PCR products were quantified using the QuantiFluor™ -ST Blue fluorescence quantification system (Promega) in reference to the initial quantitative results of electrophoresis, and then mixed proportively according to the sequencing volume requirements of each sample. Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. After the PE reads obtained by llumina sequencing were split, the double-ended Reads were quality-controlled and filtered according to the sequencing quality. Meanwhile, the double-ended Reads were spliced according to the overlap between them to obtain optimized data after quality control splicing. The optimized data is then processed using Sequence denoising methods (DADA2/Deblur, etc.) to obtain ASV (Amplicon Sequence Variant) representing sequence and abundance information.

Community diversity analysis

The richness and diversity of species in environmental communities can be obtained by evaluating Alpha diversity index, among which ace index can reflect Community richness. The degree of difference in species abundance distribution between samples can be quantitatively analyzed by the distance in statistics. The distance between two samples can be calculated by statistical algorithm to obtain the distance matrix, which can be used for further beta diversity analysis. PCoA is mapped based on the selected distance matrix, and the potential principal components affecting the differences in sample community composition are identified by dimensionality reduction.

Species difference analysis

One-way ANOVA was used to compare whether there were significant differences in the distribution of different species in 3 or more sample groups, and then post-hoc tests were carried out on different species to find out the sample groups with differences in multiple groups. FDR (false discovery rate) is used for multiple test correction, that is, multiple test correction of *P*-value. The Wilcoxon rank-sum test (also known as Mann-Whitney U test) was used to conduct non-parametric tests on two independent groups. At the same time, Linear discriminant analysis Effect Size (LEfSe) can be used to find the species characteristics that can best explain the difference between two or more groups of samples, and the degree of impact of these characteristics on the difference between groups [58]. Linear discriminant analysis (LDA) was used to assess the magnitude of the influence of the abundance of each component (species) on the differential effect.

ELISA detection

Fresh mouse heart blood was centrifuged in a centrifuge tube at 3000 rpm for 10 min to prepare serum. The estrogens, estradiol and equol were determined according to the Jianglai ELISA kit.

Abbreviations

C. hepatica	*Capillaria hepatica*
ELISA	Enzyme-linked immunosorbent assay
ASV	Amplicon Sequence Variant
PCoA	Principal co-ordinates analysis
FDR	False iscovery Rate
LEfSe	Linear discriminant analysis Effect Size
LDA	Linear discriminant analysis
SCFAs	short-chain fatty acids

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04524-2.

Supplementary Figure S1: Significance level analysis of species abundance differences. (**A**) Microbial taxa with significant abundance differences between control and infected groups in Brandt's voles. (**B**) Microbial taxa with significant abundance differences among all four groups.The x-axis represents the species names at different taxonomic levels, while the y-axis represents the percentage abundance of a particular species in each sample. Different colors represent different groups. One-way ANOVA was used to test the inter-group differences, and the corresponding *P*-values are shown on the right side. (* for $0.01 < P \leq 0.05$, ** for $0.001 < P \leq 0.01$, and *** for $P \leq 0.001$).

Supplementary Figure S2: Gut microbiota with significant abundance differences in all four groups. (**A**) Eubacteriaceae, (**B**) Rikenellaceae and (**C**) lachnospiraceae. The horizontal coordinate represents the name of the sample and the vertical coordinate represents the percentage of the abundance of A certain species in different samples. One-way ANOVA was used to test the inter-group differences. (* for $0.01 < P \leq 0.05$, ** for $0.001 < P \leq 0.01$, and *** for $P \leq 0.001$)

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Author contributions

H.H. and B.H. contributed to the conception of the study; B.H., K.Y. and S.F. contributed significantly to collect samples and perform the experiment; D.Z., N.Z. and G.L. contributed significantly to analysis and manuscript preparation; S.G., Y.X. and S.H. helped perform the analysis with constructive discussions; B.H. and D.Z. performed the data analyses and wrote the manuscript.

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Data availability

The data presented in the study are deposited in the **Genome Sequence Archive (GSA)**, accession number **CRA013885**. We encourage other researchers and colleagues in the academic community to utilize these resources and contact us through the provided contact information in the repository for further data discussion and collaboration opportunities. By sharing the data and related code extensively, we aim to promote openness and reproducibility in scientific research, as well as foster collaboration and advancement within the academic community.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the Guidelines for the Care and Use of Animals in Research published by the Institute of Zoology, Chinese Academy of Sciences. This study was reviewed and approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences (approval number: IOZ-15042). Animals were humanely euthanized to alleviate suffering.

Consent for publication
Not applicable.

Conflict of interest
All the authors have no conflict of interest to declare.

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