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Intestine microbiota and SCFAs response in naturally *Cryptosporidium*-infected plateau yaks

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Diarrhea is a severe bovine disease, globally prevalent in farm animals with a decrease in milk production and a low fertility rate. *Cryptosporidium* spp. are important zoonotic agents of bovine diarrhea. However, little is known about microbiota and short-chain fatty acids (SCFAs) changes in yaks infected with *Cryptosporidium* spp. Therefore, we performed 16S rRNA sequencing and detected the concentrations of SCFAs in *Cryptosporidium*-infected yaks. Results showed that over 80,000 raw and 70,000 filtered sequences were prevalent in yak samples. Shannon ($p<0.01$) and Simpson ($p<0.01$) were both significantly higher in *Cryptosporidium*-infected yaks. A total of 1072 amplicon sequence variants were shared in healthy and infected yaks. There were 11 phyla and 58 genera that differ significantly between the two yak groups. A total of 235 enzymes with a significant difference in abundance ($p<0.001$) were found between healthy and infected yaks. KEGG L3 analysis discovered that the abundance of 43 pathways was significantly higher, while 49 pathways were significantly lower in *Cryptosporidium*-infected yaks. The concentration of acetic acid ($p<0.05$), propionic acid ($p<0.05$), isobutyric acid ($p<0.05$), butyric acid ($p<0.05$), and isovaleric acid was noticeably lower in infected yaks, respectively. The findings of the study revealed that *Cryptosporidium* infection causes gut dysbiosis and results in a significant drop in the SCFAs concentrations in yaks with severe diarrhea, which may give new insights regarding the prevention and treatment of diarrhea in livestock.

KEYWORDS

***Cryptosporidium*, yaks, diarrhea, microbiota, SCFAs**

Introduction

The long-haired ruminant yak is a plateau bovine species living in the 3000-5000 m high-altitude regions and is mostly found on the Qinghai Tibet plateau (Li et al., 2022a). Diarrhea is a serious bovine problem detected globally in livestock farms associated with a decrease in fertility rate and milk production, especially neonatal diarrhea is usually found with high morbidity and mortality (Han et al., 2017; Li et al., 2019a; Lan et al., 2021)

Previously, studies revealed that diarrhea contributed to more than 50% of calf mortality in Canada (Smith et al., 2014), and affected 19% of the cattle population in the USA (Smulski et al., 2020), which indeed was the cause of huge economic detriment. Like other bovine animals, diarrhea has been commonly reported in yaks (Diao et al., 2020; Cui et al., 2022; Li et al., 2022a). There have been many biological factors which are associated for diarrhea and leading cause of death in calves (Kim et al., 2021). Many pathogens like bovine viral diarrhea virus, Noroviruses, *Escherichia coli*, *Salmonella* spp., and *Cryptosporidium* spp. have been commonly observed in infected cattle (Meganck et al., 2014; Cui et al., 2022). Among others, *Cryptosporidium* spp. are important zoonotic protozoa infecting various animal species (Li et al., 2019b; Kandeel et al., 2022), and are also generally recognized as the primary agent of cattle diarrhea (Li et al., 2019a; Li et al., 2019b). A previous study reported that the infection of *Cryptosporidium* spp. was an important issue in UK and Scotland (Smith et al., 2014). As yaks and cattle species are economically important for native herdsmen in China (Cheng et al., 2022), infectious diseases like those caused by *Cryptosporidium* spp. may not only affect animal health but are also potential threats leading to public health concerns.

Intestine microbiota is composed of millions of complex and diverse microorganisms, which contribute greatly to host health, nutrition absorption, host metabolism, and immunological development (Zeineldin et al., 2018). Previous studies demonstrated that this bacteria was related to various diseases like Type 2 diabetes (Martinez-Lopez et al., 2022), acute pancreatitis (Mei et al., 2022), obesity (Salazar et al., 2022), and diarrhea (Han et al., 2017; Zeineldin et al., 2018; Li et al., 2022b). Short-chain fatty acids are metabolic products of microbiota, which contribute to the cellular metabolism of the host (Bachem et al., 2019), regulating immune function and suppressing inflammatory reactions (Abdalkareem Jasim et al., 2022). In our previous study, we observed prominent changes in intestinal microbiota in a horse infected with *Cryptosporidium* spp. (Wang et al., 2022). However, scarce information is available about microbiota and SCFAs changes in plateau yaks infected with *Cryptosporidium* spp. Therefore, this study was conducted to explore intestinal microbiota and SCFAs response to natural *Cryptosporidium* infection in plateau yaks.

Materials and methods

Samples

Fecal samples (n=40) were collected from free-ranged yaks in Xining, Qinghai (North latitude 31°36′-39°19′, east longitude 89°35′-103°04′) and examined for *Cryptosporidium* spp. by employing nested PCR (Chen et al., 2022) and positive samples were saved for further analysis. In this study, all the *Cryptosporidium* spp. positive samples (n=4) with equal number of negative samples (n=4) were sequenced and divided into infected (INF) and healthy (H) groups, respectively.

DNA extraction and PCR amplification

The extraction of total genomic DNA was performed by utilizing a commercial TIANamp Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd, China) according to the product's specifications. Fecal DNA concentration, purification, and quality examination were performed through NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, USA) and 1.2% agarose gel electrophoresis, respectively. Then the hypervariable regions of bacterial 16S rRNA gene (V3-V4) were amplified using primers 338F and 806R as described in a previous study (Wang et al., 2019). All PCR products were individually subjected to agarose gel electrophoresis, gel extraction, and purification using the PureLink™ PCR Purification kit (Invitrogen™, USA). Finally, the purified DNA products were quantified by piloting QuantiFluor™-ST as guided by the instruction manual (Promega, USA).

Library construction, Illumina miSeq sequencing, and bioinformatics analysis

Library construction was carried out by employing commercial Hieff NGS® OnePot II DNA Library Prep Kit for Illumina® (Yeasen, China) according to the product's instructions, and sequenced through the Illumina NovaSeq platform (Illumina, San Diego, USA). Quality control of sequencing data was performed by employing QIIME2 (https://docs.qiime2.org/2019.1/) to generate amplicon sequence variant (ASV) (Callahan et al., 2016) and taxonomy table (Bokulich et al., 2018). Analysis of variance was performed using ANCOM (Analysis of Composition of Microbiomes), One-way ANOVA, Kruskal Wallis, LEfSe (LDA (Linear Discriminant Analysis) score >2), DEseq2 (p<0.05 and log2 (FoldChange) > 2), clustering heatmap (with Z-score > 0.5 or < -0.5) and evolutionary tree (p<0.05) methods to reveal differences in bacterial abundance among yak samples (Segata et al., 2011; Love et al., 2014; Mandal et al., 2015). Microbial alpha diversities analyses were performed through QIIME2 by calculating indices including observed OTUs, Chao1, Shannon, and Faith's. Microbial beta diversities of principal coordinate analysis (PCoA), nonmetric multidimensional scaling (NMDS) (Vazquez-Baeza et al., 2013), and partial least squares discriminant analysis (PLS-DA) were carried out to explore the structural variation of microbial communities across yak samples. The evolutionary relation tree was constructed by using ggtree in R package.

Function analysis

The potential KEGG Ortholog (KO) functional profiles of yak microbiota was predicted with PICRUSt (Langille et al., 2013) by annotating with MetaCyc and ENZYME database. One-way ANOVA was used to analyze the data, while Duncan test was used as *post-hoc* test to measure the individual differences in microbial function between the yak groups with a $p<0.05$ as statistically significant.

SCFAs detection

The concentrations of SCFAs in fecal samples were detected by employing GC-MS (Hsu et al., 2019; Zhang et al., 2019), and the differences between yak groups were explored *via* t-test.

Statistical analysis

The differences between different yak groups were calculated by the chi-square test piloting IBM SPSS Statistics (SPSS 22.0). *P* values < 0.05 were considered as statistically significant.

Results

Analysis of 16S rDNA sequencing data

In the current study, over 80,000 raw and 70,000 filtered sequences were obtained in yak samples. The non-chimeric sequences ranged from 62,133 to 73,453 in healthy yaks, and 68,173 to 74,350 in infected yaks (Table 1). There were a total of 1072 shared ASVs between the healthy (group H) and infected (group INF) groups. (Figure 1A). Alpha diversity index analysis showed that there was no significant difference in chao1, faith, and observed features between group H and INF, respectively. Shannon ($p<0.01$) and Simpson ($p<0.01$) were both significantly higher in group INF than in group H (Figure 1B).

Grouping of yak microbiota in different taxa

The sequence percentage in different taxa of group H and INF is shown in Figure 2A. At the phylum level, the dominant phyla were *Firmicutes* (69.61%), *Proteobacteria* (8.97%), and *Actinobacteria* (8.72%) in group H, while *Firmicutes* (56.38%) and *Bacteroidetes* (29.83%) were the main phyla in group INF (Figure 2B). At the class level, *Clostridia* (51.13%) and *Bacilli* (17.28%) were the primary classes in healthy yaks, while *Clostridia* (51.13%) and *Bacteroidia* (29.83%) were the major classes in infected yaks (Figure 2C). At the order level, *Clostridiales* (51.13%), *Lactobacillales* (8.20%), and *Bacillales* (8.10%) were the primary orders in healthy yaks, while *Clostridiales* (51.04%) and *Bacteroides* (29.83%) were the main orders in infected yaks (Figure 2D). At the family level, the main families were unclassified, *Ruminococcaceae* and *Lachnospiraceae* in groups H and INF (Figure 2E). At the genus level, unclassified (52.06%), *Pseudomonadaceae Pseudomonas* (6.13%), and *Lactobacillus* (6.00%) were the dominating genera in healthy yaks, while unclassified (69.25%), *Prevotellaceae Prevotella* (5.13%) and *Arthrobacter* (2.45%) were the main genera in infected yaks (Figure 2F). At the species level, the main bacteria in group H were unclassified (87.85%), *Veronii* (6.11%), and *Alactolyticus* (1.66%), while unclassified (95.15%), *Flavefaciens* (1.50%) and *Veronii* (1.12%) were the main bacteria in group INF (Figure 2G).

Shifts of yak microbiota infected by *Cryptosporidium*

To reveal the microbiota difference between healthy and infected yaks, beta diversity analysis was carried out through NMDS, PCoA, Qiime 2β, and PCA analysis. The results showed a huge difference in composition and structure between samples from group H and group INF animals (Figure 3). To explore the microbiota changes caused by *Cryptosporidium* in different taxa, a clustering heatmap (top 20 abundance) and evolutionary tree (top 50 abundance) with heat map analysis were plotted. The results revealed that at the order level, infected yaks showed an abundance of *Bacteroidia* and *Deltaproteobacteria*, while healthy animals

TABLE 1 The sequence data statistic analysis.

Samples	input	filtered	percentage of input passed filter	denoised	merged	percentage of input merged	non-chimeric	percentage of input non-chimeric
H1	93773	86437	92.18	82758	76547	81.63	73453	78.33
H2	88135	81010	91.92	77501	71312	80.91	67743	76.86
H3	91889	85557	93.11	82297	76216	82.94	72913	79.35
H4	80446	74466	92.57	71353	65482	81.4	62133	77.24
INF1	89759	83135	92.62	78940	72488	80.76	68173	75.95
INF2	92942	86291	92.84	82357	75217	80.93	71848	77.3
INF3	89810	83187	92.63	80203	75295	83.84	74350	82.79
INF4	88245	81820	92.72	78721	73786	83.61	71099	80.57

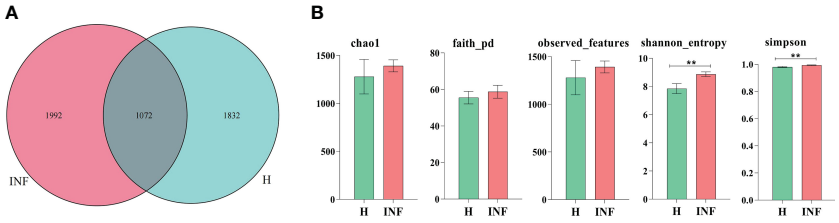

FIGURE 1
ASV venn map and Alpha diversity index analysis. **(A)** Venn map, **(B)** Alpha diversity index. ** refers to significance level, $p<0.05$.

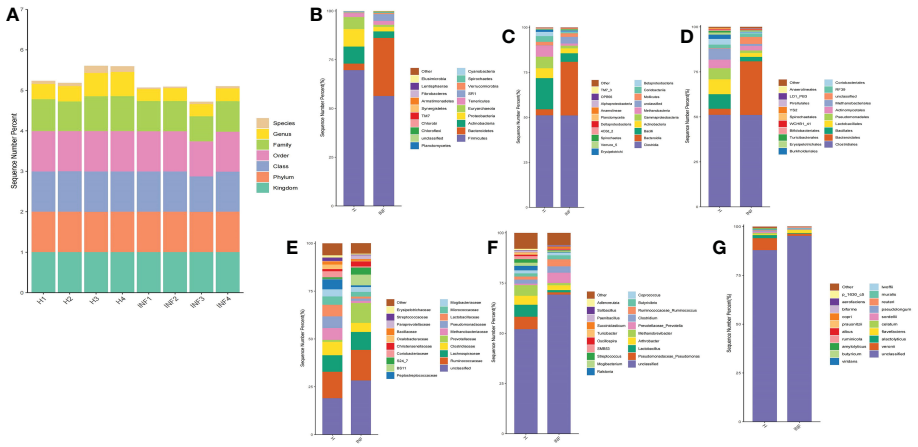

FIGURE 2
Statistical analysis of yak microbiota in different taxa. **(A)** Sequence percentages in different taxa, **(B)** Phylum, **(C)** Class, **(D)** Order, **(E)** Family, **(F)** Genus, **(G)** Species.

showed abundance of *Bacilli*, *Erysipelotrichi*, *Betaproteobacteria*, *Alphaproteobacteria*, and *Nitriliruptoria* as expressed in the clustering heatmap. The evolutionary tree also showed an obvious abundance difference in *Betaproteobacteria*, *Fibrobacteria*, SJA_176, 4C0d_2, *Nitriliruptoria*, *Clostridia*, and *Bacilli* between groups H and INF (Figure 4A). At the order level, the clustering heatmap revealed significant differences in the abundance of *Bacteroidales*, *Lactobacillales*, *Burkholderiales*, *Erysipelotrichales*, YS2, *Turicibacterales* and *Enterobacteriales* between healthy and infected animals. Evolutionary tree detected remarkable differences in the abundance of *Oceanospirillales*, *Burkholderiales*, *Enterobacteriales*, *Fibrobacterales*, *Turicibacterales*, RB046, YS2, *Nitriliruptorales*, *Clostridiales* and *Lactobacillales* between healthy and infected animals (Figure 4B). At the family level, there was a noteworthy difference of *Clostridiaceae*, *Prevotellaceae*, *Lactobacillaceae*, *Peptostreptococcaceae*, BS11, *Christensenellaceae*, *Oxalobacteraceae*, *Paraprevotellaceae*, *Streptococcaceae* and *Erysipelotrichaceae* between groups H and INF as revealed by the clustering heatmap. Evolutionary tree analysis showed a clear difference of *Halomonadaceae*, *Oxalobacteraceae*, *Enterobacteriaceae*, *Streptococcaceae*, *Peptostreptococcaceae*, *Turicibacteraceae*, *Dietziaceae*, *Sanguibacteraceae*, *Nitriliruptoraceae*, *Christensenellaceae*, *Clostridiaceae* and *Lactobacillaceae* between healthy and infected yaks (Figure 4C). At the genus level, interesting difference of *Lactobacillus*, *Prevotellaceae_Prevotella*, *Ralstonia*, *Streptococcus*, SMB53, *Turicibacter*, and *Adlercreutzia* was found between the two yak groups. Evolutionary tree analysis demonstrated that the abundance of *Halomonadaceae*, *Oxalobacteraceae*, *Streptococcaceae*, *Clostridiaceae*, *Turicibacteraceae*, *Planococcaceae*, *Erysipelotrichaceae*, *Sanguibacteraceae*, *Coriobacteriaceae*, *Paraprevotellaceae*, *Ruminococcaceae*, *Lachnospiraceae*, *Clostridiaceae*, *Lactobacillaceae* and *Lachnospiraceae* were significantly different between the two yak groups (Figure 4D). At the species level, the abundance of *alactolyticus*, *celatum*, *reuteri*, *butyricum*, *ruminicola*, *prausnitzii*, *biforme*, p_1630_c5, and *aerofaciens* were noticeably different in groups H and INF. Evolutionary tree analysis uncovered that the abundance of *alactolyticus*, *ruminis*, p_1630_c5, *biforme*, *umbonata*, *aerofaciens*, *prausnitzii*, *butyricum*, *celatum* and *reuteri* were significantly different between healthy and infected animals (Figure 4E).

To further uncover the marker bacteria between healthy and *Cryptosporidium*-infected yaks, we performed one-way ANOVA and Kruskal Wallis tests to determine the significance of the difference and depicted results by DESeq 2 volcano diagram and LEfSe chart, respectively. Results showed that at the phylum level,

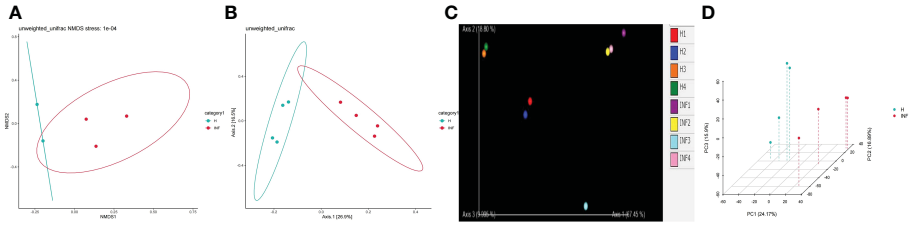

FIGURE 3
Beta diversity analysis between yak groups. **(A)** NMDS, **(B)** PCoA, **(C)** Qiime 2β, **(D)** PCA.

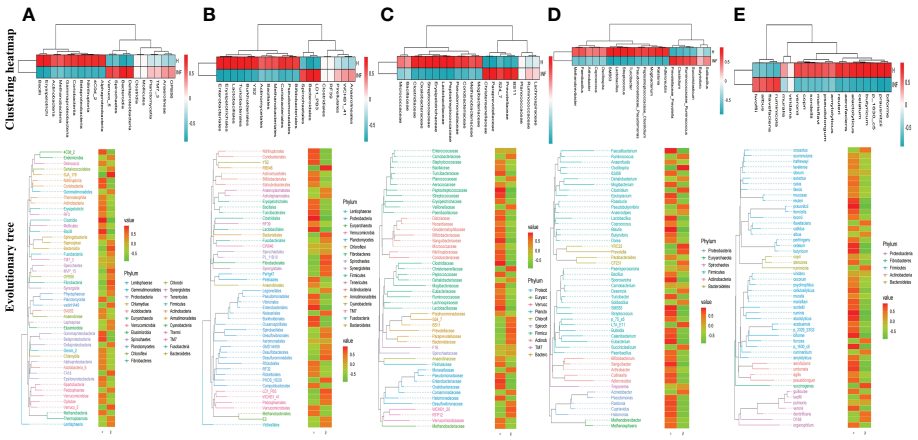

FIGURE 4
Clustering heatmap and evolutionary tree with heat map analysis of yak microbiota in different taxa. **(A)** Class, **(B)** Order, **(C)** Family, **(D)** Genus, **(E)** Species.

the abundance of SR1 ($p<0.0001$), *Bacteroidetes* ($p<0.0001$), *Armatimonadetes* ($p<0.0001$), *Fibrobacteres* ($p<0.01$), and *Synergistetes* ($p<0.01$) were visibly higher in infected yaks, while *Cyanobacteria* ($p<0.0001$), *Proteobacteria* ($p<0.0001$), *Armatimonadetes* ($p<0.0001$), *Euryarchaeota* ($p<0.0001$), *Actinobacteria* ($p<0.01$), *Firmicutes* ($p<0.01$), and *Elusimicrobia* ($p<0.05$) were significantly lower (Figure 5A). At the genus level, the abundance of YRC22 ($p<0.0001$), *Prevotellaceae_Prevotella* ($p<0.0001$), CF231 ($p<0.0001$), L7A_E11 ($p<0.0001$), BF311 ($p<0.0001$), *Desulfovibrio* ($p<0.0001$), *Succiniclasticum* ($p<0.0001$), *Desemzia* ($p<0.0001$), *Anaerovorax* ($p<0.0001$), *Pseudobutyrivibrio* ($p<0.0001$), *Acinetobacter* ($p<0.0001$), *Fibrobacter* ($p<0.0001$), *Ruminococcaceae_Ruminococcus* ($p<0.0001$), *Anaerorhabdus* ($p<0.0001$), *Treponema* ($p<0.0001$), *Selenomonas* ($p<0.001$), *Clostridium* ($p<0.001$), *Shuttleworthia* ($p<0.001$), *Dehalobacterium* ($p<0.001$), TG5 ($p<0.01$), unclassified ($p<0.01$), *Anaerostipes* ($p<0.01$), *Syntrophomonas* ($p<0.01$), *Brachymonas* ($p<0.01$), *Pyramidobacter* ($p<0.01$), SHD_231 ($p<0.05$), *Butyrivibrio* ($p<0.05$), *Desulfobulbus* ($p<0.05$), RFN20 ($p<0.05$), and *Anaerofustis* ($p<0.05$) were significantly higher in infected yaks, while *Turicibacter* ($p<0.0001$), *Lactobacillus* ($p<0.0001$), *Sporosarcina* ($p<0.0001$), *Ralstonia* ($p<0.0001$), *Akkermansia* ($p<0.001$), *Streptococcus* ($p<0.001$), *Methylobacterium* ($p<0.01$), *Adlercreutzia* ($p<0.01$), *Faecalibacterium* ($p<0.01$), *Roseburia* ($p<0.01$), *Paenibacillus* ($p<0.01$), *Methanosphaera* ($p<0.01$), *Pseudomonadaceae_Pseudomonas* ($p<0.01$), *Peptostreptococcaceae_Clostridium* ($p<0.01$), *Slackia* ($p<0.01$), *Cupriavidus* ($p<0.01$), *Halomonas* ($p<0.01$), *Gemmiger* ($p<0.01$), *Dietzia* ($p<0.01$), *Blautia* ($p<0.05$), *Agrobacterium* ($p<0.05$), *Nesterenkonia* ($p<0.05$), *Sanguibacter* ($p<0.05$), *Phascolarctobacterium* ($p<0.05$), *Actinomycetospora* ($p<0.05$), *Bifidobacterium* ($p<0.05$), SMB53 ($p<0.05$), and *Dorea* ($p<0.05$) were significantly lower in infected animals (Figure 5B).

Cryptosporidium infection potentially affected the microbiota function of yaks

The prediction of yaks' microbiota function was carried out by PICRUSt2, and the functional difference between yaks was explored by using one-way ANOVA and Duncan test through R language as previously reported (Zhai et al., 2020). A total of 235 enzymes with a significant difference in abundance ($p<0.001$) were found between healthy and infected yaks, with 119 higher and 116 lower abundance enzymes in INF yaks (Figure 6A). Only one different MetaCys pathway of pentose phosphate pathway (non-oxidative branch) was found between the two yak groups (Figure 6B). KEGG L1 analysis found that the abundance of genetic information

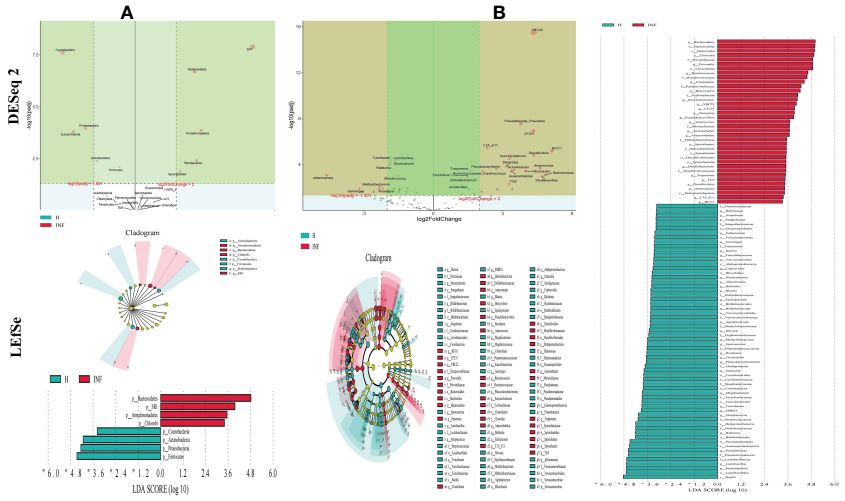

FIGURE 5
Cryptosporidium infection changes microbiota in different taxa through DESeq 2 volcano plot and LEFSe analysis. **(A)** Phylum, **(B)** Genus.

processing was prominently higher in infected yaks, while cellular processes and environmental information processing were significantly lower (Figure 7A). KEGG L2 analysis revealed that the abundance of biosynthesis of other secondary metabolites, glycan biosynthesis, and metabolism, metabolism of cofactors and vitamins, and nucleotide metabolism were remarkably higher in INF yaks, while amino acid metabolism, chemical structure transformation maps, lipid metabolism, metabolism of other amino acids, xenobiotics biodegradation, and metabolism were conspicuously lower (Figure 7B). KEGG L3 analysis discovered that the abundance of 43 pathways was significantly higher in INF yaks, while 49 pathways were significantly lower (Figure 7C).

Cryptosporidium infection decreased the concentration of SCFAs in yaks

The concentration of acetic acid ($p<0.05$), propionic acid ($p<0.05$), isobutyric acid ($p<0.05$), butyric acid ($p<0.05$) and isovaleric acid was significantly lower in infected yaks, respectively, while there was no significant difference of valeric acid and caproic acid between H and INF groups (Figure 8).

Discussion

Cattle diarrhea is still an important worldwide issue on farms, despite observing advanced preventive measures such as herd management, animal facilities and care, feeding and nutrition, and timely medication (Wei et al., 2021). The infectious *Cryptosporidium* was one of the main causative agents of diarrhea with limited available effective treatments (Li et al., 2019a). The harsh climatic conditions with heavy snowfall in the long frigid season (from October to May, with average temperature −15 to −5°C) didn't permit collection of many samples in the Plateau region. Also, very few positive samples (n=4) were observed out of total collected samples (n=40) in the present study. However, a prevalence as low as 1.3% of Cryptosporidium spp. positive samples has been reported in yaks in China region (Li et al., 2020). Moreover, despite the harsh climatic conditions and the low number of positive samples available for

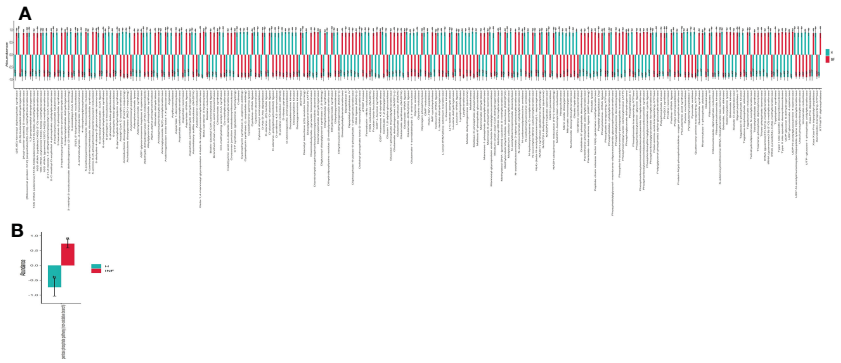

FIGURE 6
Cryptosporidium infection affected enzyme and MetaCys pathway abundance of yaks. **(A)** Enzyme ($p<0.001$), **(B)** MetaCys ($p<0.05$). "a, b" are showing significance relation.

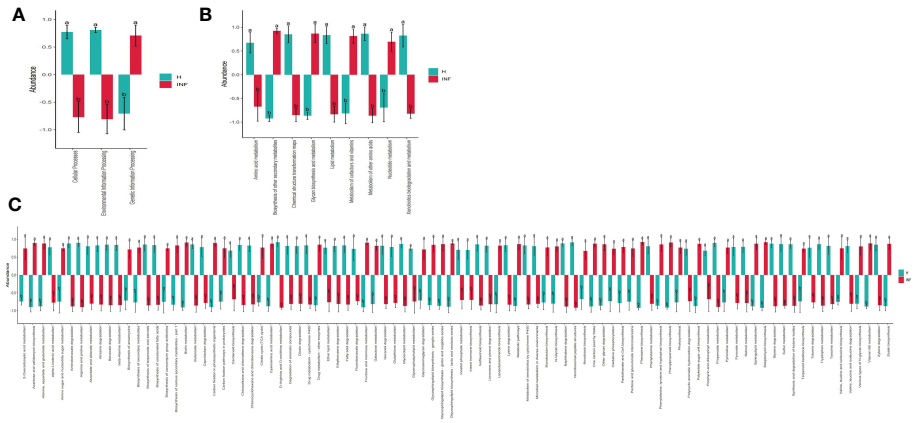

FIGURE 7
Cryptosporidium infection potentially affected the microbiota function of yaks. **(A)** KEGG L1 ($p<0.05$), **(B)** KEGG L2 ($p<0.05$), **(C)** KEGG L3 ($p<0.05$). "a, b" are showing significance relation.

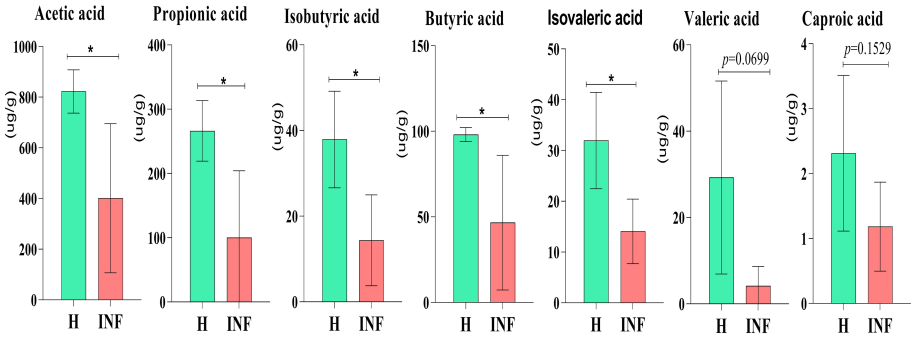

FIGURE 8
Concentration of SCFAs in yaks. Significance is presented as *$p < 0.05$; data are presented as the mean ± SEM (n = 4).

analysis, this number was above the minimum required for high throughput sequencing, and validation of changes of the microbiota (Ray et al., 2019). In the current study, we performed 16S rDNA sequencing of fecal samples collected from healthy and *Cryptosporidium*-infected yaks. Results showed that *Cryptosporidium* infection increased the alpha diversity index of Shannon ($p<0.01$) and Simpson ($p<0.01$) (Figure 1B), which demonstrated the increased microbiota complexity of infected animals. The current results are in line with our previous results found in *Cryptosporidium*-infected horses (Wang et al., 2022). Beta diversity analysis through NMDS, PCoA, Qiime 2β, and PCA analysis revealed microbiota differences between healthy and infected yaks (Figure 3), which were confirmed by comparing the dominating gut microbiota in different taxa (Figures 2, 4). Then we explored the significantly different bacteria between the H and INF groups through DESeq 2 volcano diagram and LEfSe chart analysis. The results showed that a total of 11 phyla and 58 genera were significantly different (Figure 5), which is in accordance with the previously reported results in a study conducted on infected people and horses (Chappell et al., 2016; Wang et al., 2022). The increased genera in yaks were in line with previous studies that found a higher abundance of *Desulfovibrio* and *Butyrivibrio* in colitis patients (Berry and Reinisch, 2013; Gryaznova et al., 2021), *Prevotellaceae_Prevotella* in diarrheic pigs (Yang et al., 2017), *Anaerovorax* in slow growth performers in nursery pigs (Zhai et al., 2020), *Succiniclasticum* in LPS induced dual-flow continuous culture system (Dai et al., 2019), *Pseudobutyrivibrio* in chronic kidney people (Wu et al., 2020), *Anaerorhabdus* in pulmonary fibrosis persons (Tong et al., 2019), *Selenomonas* in gastric cancer patients (Zhang et al., 2021), *Anaerostipes* in diabetic nephropathy patients (Du et al., 2021), *Pyramidobacter* in endoscopic sphincterotomy surgery gallstone patients (Shen et al., 2021), and *Anaerofustis* in Alzheimer people (Hou et al., 2021). The genus of *Acinetobacter* is an underrated food-borne pathogen (Amorim and Nascimento, 2017). A previous study found *Acinetobacter* in acute diarrhea of children (Polanco and Manzi, 2008). The genus of *Treponema* is the main pathogen in bovine dermatitis (Mamuad et al., 2020), *Clostridia* are clinical species and some of them may cause severe infections like colitis (Sanchez Ramos and Rodloff, 2018). Those increased genera may have contributed greatly to diarrhea caused by *Cryptosporidium*. The lower abundance of genera in yaks was in accordance with the results revealing *Turicibacter*

and *Lactobacillus* in *Salmonella*-infected pigs (Garrido et al., 2021), *Akkermansia* and *Roseburiain* in colitis in mouse (Bu et al., 2021; Li et al., 2021), *Adlercreutzia* in influenza virus-infected mouse (Lu et al., 2021), *Faecalibacterium* in pre-eclampsia people (Chen et al., 2020), *Methanosphaera* in sheep without treatment of anthelmintic (Moon et al., 2021), Slackia in Vogt-Koyanagi-Harada patients (Li et al., 2022a; Li et al., 2022b), *Gemmiger* in immune-mediated inflammatory people (Forbes et al., 2018), and *Dorea* in HIV patients (Xu et al., 2021). Those deficient genera in *Cryptosporidium*-infected animals may be the reason for diarrhea in yaks. The genus of *Cupriavidus* was related to mycotoxin biodegradation (AL-Nussairawi et al., 2020), and the dropped *Cupriavidus* in yaks may affect mycotoxin metabolism in yaks. The previous study uncovered probiotics of *Dietzia* as a new therapy for Crohn's disease (Click, 2015), and *Blautia*, *Phascolarctobacterium*, and *Bifidobacterium* are probiotic genera (Papizadeh et al., 2017; Chen et al., 2021; Liu et al., 2021), which demonstrated that *Cryptosporidium* led diarrhea may be due to the decrease of probiotics in the microbiota.

The shifted intestine microflora also changed their functions, as 235 significantly different enzymes were found between healthy and infected yaks ($p<0.001$) (Figure 6A). Only one obvious different MetaCys pathway of pentose phosphate pathway (non-oxidative branch) was found between the two yak groups (Figure 6B). Also, KEGG L3 analysis discovered that the abundance of 92 pathways was significantly different between healthy and infected animals (Figure 7C). Those results may infer that *Cryptosporidium* broke the balance of gut microbiota, which affected the microbiota function and caused diarrhea in yaks.

In the present study, significantly lower concentrations of SCFAs were found in *Cryptosporidium*-infected animals (Figure 8), consistent with yak diarrhea (Li et al., 2022a), LPS-induced piglets (Yang et al., 2021), and dextran sulfate sodium-induced colitis in mouse (Xu et al., 2020). SCFAs play very important roles in host physiology and energy homeostasis (Chambers et al., 2018). Among them, acetate and propionate can provide energy to peripheral tissues (den Besten et al., 2013). A previous study reported that acetate was responsible for maintaining intestine barrier integrity by inhibiting pathogens infection (Skonieczna-Żydecka et al., 2018). In a recent study, it was found that acetate could regulate IgA reactivity (Takeuchi et al., 2021), and propionate contributed to intestinal epithelial turnover and repair (Bilotta et al., 2021). Butyrate is highly related to intestine structure, energy providing to epithelial cells, and regulates immune function (Abdalkareem Jasim et al., 2022). Isobutyric acid and isovaleric acid may be related to mucosal and inflammation responses (Li et al., 2022a). Therefore, the decreased SCFAs in *Cryptosporidium*-infected yaks might have affected the intestinal barrier and immunity of the host (Aho et al., 2021), which potentially caused diarrhea in plateau yaks.

In conclusion, *Cryptosporidium* is an important zoonotic protozoon causing severe diarrhea in young animals; however, limited treatment measures are available. Here we reveal that *Cryptosporidium* infection causes dysbiosis and results in reduced SCFAs in yaks with severe diarrhea, which may give new insights regarding the prevention and treatment of diarrhea in livestock. The low sample size remains the limitation of our study.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA880359.

Ethics statement

The animal study was reviewed and approved by ethics committee of Nanjing Agricultural University.

Author contributions

KL and QW, research idea and methodology. HD, XC, XZ, and CZ, reagents, materials, and analysis tools. KL, writing-original draft and preparation. KM, MF-E-A, ZB, QW, JZ, SN, and KL, writing-review and editing. KL, JZ, and QW, visualization and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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