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RESEARCH ARTICLE

Association of *Giardia intestinalis* Infection with Intestinal Fungal Dysbiosis in Xizang Yaks

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ABSTRACT

Yak is an important bovine ruminant; however, little is known about fungal microbiota shifts in *Giardia (G.) intestinalis* infected yaks in Xizang. Therefore, we compared the fecal fungal microbiota of *G. intestinalis* positive (GD) and *G. intestinalis* negative (CG) yaks from Lhünzhub County, Xizang, China, using ITS amplicon sequencing. A total of 83 fecal samples were collected during 2026, including 53 samples from diarrheal yaks and 30 samples from clinically healthy yaks. Following parasite detection, six samples from each group (GD and CG) were selected for ITS-based molecular sequencing. A total of 890,652 filtered sequences were obtained, including 500,947 from the GD group and 389,705 from the CG group. Alpha-diversity analysis showed significantly higher ACE ($P<0.01$), Chao1 ($P<0.01$), observed species ($P<0.01$), Pielou evenness ($P<0.001$), Shannon ($P<0.001$), and Simpson ($P<0.0001$) indices in GD yaks than in CG yaks. Two phyla and 17 genera were identified as potential fungal biomarkers associated with *G. intestinalis* infection, including *Naganishia*, *Aspergillus*, *Cleistothelebolus*, *Xenodidymella*, *Piromyces*, *Urocystis*, *Fungi gen. incertae sedis*, *Plenodomus*, *Neoascochyta*, *Paraphaeosphaeria*, *Coniochaeta*, *Cystobasidium*, *Sporormiaceae gen. incertae sedis*, *Sclerostagonospora*, *Arthrinium*, *Neosetophoma*, and *Leucosporidium*. These findings provide preliminary insights into alterations in the fecal fungal microbiota associated with *G. intestinalis* infection in yaks on the Xizang Plateau.

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INTRODUCTION

The yak is a symbolic bovine breed that mainly lives on the Qinghai-Xizang Plateau and its adjacent areas, including Gansu, Sichuan, and Yunnan in China, with an average altitude over 3000 m (Li *et al*., 2014). This ruminant species is famous for its special ability to adjust to severe climatic conditions on the plateau, which include high altitude, hypoxia, gloomy and cold weather, and strong ultraviolet rays (Lu *et al*., 2025). Approximately 1800 million yaks are present in world, and 90% of them are distributed in Chinese plateau regions (Liu *et al*., 2023). Yaks are an important food resource for local herdsmen, which not only provides nutritious milk and meat products, but also valuable wool and leather, and useful fuel and means of transport (Lu *et al*., 2024; Xi *et al*., 2024). Therefore, efficient breeding of yaks is of great importance in plateau regions.

Cattle diarrhea is a commonly known digestive disease with typical symptoms of severe diarrhea, poor appetite, loss of appetite, and emaciation, with high morbidity and mortality, and is becoming a significant challenge for the cattle industry (Chen *et al*., 2025). Nowadays, diarrhea in yaks is reported in many regions, causing serious economic losses (Li *et al*., 2022; Li *et al*., 2026). There are many pathogens associated with cattle diarrhea, such as bacteria (*Escherichia coli*), viruses (bovine viral diarrhea, bovine rotavirus), and parasites (*Cryptosporidium*, *Giardia (G.) intestinalis*) (Li *et al*., 2022; Dong *et al*., 2023; Ji *et al*., 2025; Xie *et al*., 2025). The foodborne parasite *Giardia* is a serious protozoan causing millions of human infections every year, especially common in young children under five years (Ryan *et al*., 2019; Alawfi, 2024). Cattle are an important natural reservoir of *Giardia*, which can shed numerous cysts into the environment, and they transmit it to other animals or humans by ingesting contaminated food or water (Bulumulla *et al*., 2025). The global prevalence in cattle is 20-26% in calves, 17% in cattle, and 13% in

buffaloes (Taghipour *et al*., 2022). In yaks, the prevalence of *G. intestinalis* was 22.9% in Xizang and 10.4% in Qinghai (Jin *et al*., 2017; Ji *et al*., 2025).

Microorganisms like bacteria, viruses, fungi, and yeasts are present in the gut microbiota in trillions, positively associated with the host's countless functions, including intestinal homeostasis, immune modulation, and energy metabolism (Nathan *et al*., 2021; Xu *et al*., 2025). However, the intestinal microbiota structure is not invariable, which can be affected by various factors like gender, diet, disease and antibiotic utilization (Strasser *et al*., 2021; Zhu *et al*., 2024). Previously, dysbiosis was found in many gastrointestinal (GI) problems like diarrhea (Yu *et al*., 2025) Crohn's disease (Liu *et al*., 2024) and GI disorders (Saffouri *et al*., 2019). Intestinal parasites are also important inducing factors leading to the imbalance of microbiota, which has been reported in *Cryptosporidium* infected pigs (Chen *et al*., 2023), *Giardia* infected dogs (Boucard *et al*., 2021) and *Eimeria acervuline* infected chickens (Aida *et al*., 2023). However, we know little regarding microbiota changes in *G. intestinalis* infected plateau yaks. Therefore, the purpose of this study was to compare the intestinal microbiota of Xizang yaks that were positive and negative for *G. intestinalis*.

MATERIALS AND METHODS

Samples collection and parasites detection: A total of 83 fecal samples were taken from yaks in Lhünzhub County, Xizang, China, during 2026, including 53 samples from diarrheal yaks and 30 samples from clinically healthy yaks. The factors age, sex, diet, grazing management, antibiotic exposure, season and co-infections were not consistently recorded or available for all animals and therefore were not included as covariates in the fungal microbiota analysis. All samples were subjected to DNA extraction and amplification of 18S SSU rRNA gene for *G. intestinalis* detection (Ji *et al*., 2025; Liu *et al*., 2026). Multilocus markers, including glutamate dehydrogenase (*gdh*), beta-giardin (*bg*), and triosephosphate isomerase (*tpi*), were not analyzed because assemblage-level genotyping was beyond the scope of this study. Therefore, the present molecular analysis was limited to detection of *G. intestinalis* and did not determine its assemblage or subtype. Based on the parasite detection results, six samples from each group (GD and CG) were selected for subsequent ITS amplicon sequencing.

ITS amplicon sequencing of Xizang yaks: Based on the *G. intestinalis* PCR detection results, six positive (GD1-GD6) and six negative samples (CG1-CG6) were selected for subsequent ITS amplicon sequencing. The 12 samples were selected based on their infection status and adequate DNA quality and quantity for downstream amplification and sequencing, rather than parasite load or other biological characteristics. Due to the limited number of samples available for ITS sequencing, this analysis was designed as an exploratory comparison of fungal microbiota between *G. intestinalis* positive and negative yaks. For DNA extraction of those yak fecal samples, the Stool Genomic DNA Extraction Kit (Solarbio Life Sciences, Beijing, China) was used and quality of those extraction products was inspected via a spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, USA) and 1.2% agarose gel electrophoresis (Chen *et al*., 2023). Then, the fungi ITS region amplification of yaks was carried out using ITS1/ITS2 primers (5'-CTTGGTCATTTAGAGGAAGTAA-3', 5'-GCTGCGTTCTTCATCGATGC-3') (Wang *et al*., 2022) and further Illumina MiSeq platform at Bioyi Biotechnology Co., Ltd (Wuhan, China) used for paired-end 2 (250 bp) sequencing.

Fungi microbiota bioinformatics analysis of Xizang yaks: The fungi microbiota bioinformatics analysis of Xizang yaks in this study was performed mainly through QIIME2 (2023) and R packages (v4.5). The raw reads from Xizang yaks were demultiplexed via the tdemux plugin, and then filtered, denoised, merged, and chimera removed using the DADA2 plugin for quality control (Vogtmann *et al*., 2020). ASVs (Non-singleton amplicon sequence variants) were aligned with MAFFT (Katoh *et al*., 2019), which was then used for taxonomy assignment via the feature-classifier plugin against the UNITE database (https://unite.ut.ee/) (Bokulich *et al*., 2018). *G. intestinalis* positive and negative Xizang yaks' alpha diversity measures, including Chao1, Shannon, and Simpson, were computed using QIIME2 (Zhang *et al*., 2025). Similar to this, the beta diversity of Xizang yaks was assessed using Jaccard and Bray-Curtis metrics to investigate structural variation of fungal communities between *G. intestinalis* positive and negative animals. Principal coordinate analysis (Xiong *et al*., 2022), nonmetric multidimensional scaling analysis (Ma *et al*., 2023), arithmetic means hierarchical clustering analysis by unweighted pair-group method (Xi *et al*., 2023) and analysis of similarities (ANOSIM) (Xia *et al*., 2025). Linear discriminant analysis effect size and t-test methods were used to show the differentially abundant taxa between *G. intestinalis* positive and negative samples (Li *et al.,* 2024; Xu *et al*., 2025; Liu *et al*., 2026). Lastly, exploratory functional profiles were inferred using PICRUSt2 with reference to the MetaCyc and KEGG databases. Because PICRUSt2 was not specifically developed or validated for functional prediction from fungal ITS amplicon data, these results were considered preliminary and hypothesis-generating rather than direct measurements of fungal functional activity (Caspi *et al*., 2020; Kanehisa *et al*., 2023).

Statistical analysis: Statistical analyses were performed using SPSS 28.0. Normality was assessed by the Shapiro-Wilk test and alpha diversity was compared using the Wilcoxon rank-sum test. Beta diversity was assessed using Jaccard and Bray-Curtis distances with ANOSIM. Differential taxa were identified using LEfSe (LDA >2.0, $P<0.05$) and Wilcoxon rank-sum tests. Abundance data were normalized within LEfSe and the Benjamini-Hochberg method was used for multiple-comparison correction. A two-sided $P<0.05$ was considered significant.

RESULTS

Examination for *Giardia intestinalis* in Xizang Yaks: Among the diarrheic yaks, 11 samples (20.8%) were

found positive for *G. intestinalis*, while no positive samples were detected in healthy ruminants. Then, the 18S SSU rRNA genes of those positive samples were sequenced and six were successfully sequenced. The Xizang yak sample sequences were deposited into the NCBI database under accession numbers PZ544941-PZ544946 and their similarity to *G. intestinalis* reference genes (OQ376968.1, LC437354.1) was 99.66-99.62%.

Sequencing data and alpha diversity analysis of Xizang yaks: There were more than 85704 & 81954 raw sequences and 74604 & 56282 filtered sequences in all Xizang yak samples (Table 1), which were aligned to 1036 ASVs with 49 ASVs shared by *G. intestinalis* positive and negative animals (Fig. 1a). The Xizang yak's rarefaction curves peaked and remained horizontal, which showed that the current results are sufficient to present sample diversity (Fig. 1b). The rank abundance curves of *G. intestinalis* positive Xizang yaks were relatively steeper than those of *G. intestinalis* negative animals, which showed lower evenness in infected yaks (Fig. 1c). Group GD had considerably greater alpha diversity indices for ACE (P<0.01), Chao1 (P<0.01), Observed species (P<0.01), Pielou evenness (P<0.001), Shannon (P<0.001), and Simpson (P<0.0001) than group CG (Table 2, Fig. 1d).

Comparison of fungal microbiota of Xizang yaks: Ascomycota (71.74%), Basidiomycota (26.20%) and Neocallimastigomycota (1.33%) were the dominant phyla in group GD, while Ascomycota (56.97%), Basidiomycota (26.21%) and Fungi phylum Incertae sedis (12.24%) were primarily found in group CG (Fig 2a). Leotiomycetes (55.4%), Tremellomycetes (25.37%) and Dothideomycetes (9.38%) were the main classes in group GD, while Dothideomycetes (36.30%), Ustilaginomycetes (18.26%) and Fungi class Incertae sedis (12.78%) in group CG (Fig 2b). Thelebolales (55.16%), Filobasidiales (24.95%) and Pleosporales (8.97%) were the dominant orders in group GD, while in group CG Pleosporales (33.20%), Urocystidales (18.28%) and Fungi order Incertae sedis (12.56%) were the major orders (Fig 2c). At the family level, Thelebolaceae (55.25%), Filobasidiaceae (25.0%) and Sporormiaceae (7.91%) were the main families in group GD, while Urocystidaceae (18.48%), Sporormiaceae (7.91%) and Fungi family Incertae sedis (12.70%) were the primary families in group CG (Fig. 2d). *Naganishia* (50.93%), *Sporormiella* (10.79%), and *Preussia* (9.68%) were the main genera in group GD, while *Urocystis* (19.75%), Fungi genus *Incertae sedis* (13.44%), and *Preussia* (9.94%) were the dominating genus in group CG (Fig. 2e).

Table 1: Sequencing information of Xizang yaks

Samples	Input	Filtered	Denoised	Merged	Non-chimeric	Non-singleton
GD1	102481	92859	92583	90233	86960	86960
GD2	93790	82832	82698	80561	78542	78542
GD3	101644	89112	88999	87774	84559	84559
GD4	85704	74604	74485	73183	70530	70530
GD5	88962	80807	80726	80001	76473	76473
GD6	91489	80733	80530	79640	77161	77161
CG1	91572	65679	65445	64002	61941	61941
CG2	82174	58940	58631	57225	55137	55137
CG3	91235	65876	65546	63662	60017	60017
CG4	81954	56282	56020	54255	52221	52221
CG5	88444	74087	73622	71322	68233	68233
CG6	78555	68841	68547	66800	60924	60924

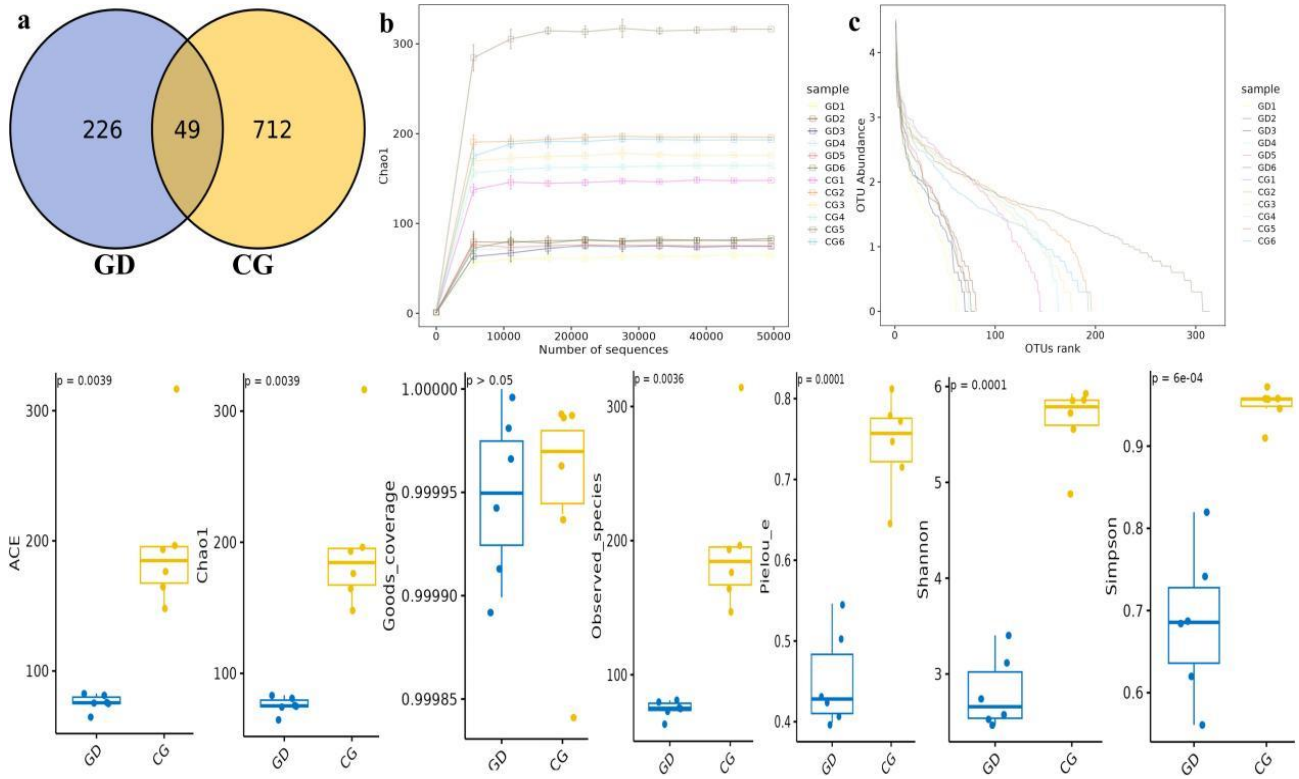

Fig. 1: Venn diagram and α-diversity analysis of *Giardia intestinalis* positive and negative yaks. (a) Venn diagram, (b) Rarefaction curve, (c) Rank abundance curve, (d) Indexes.

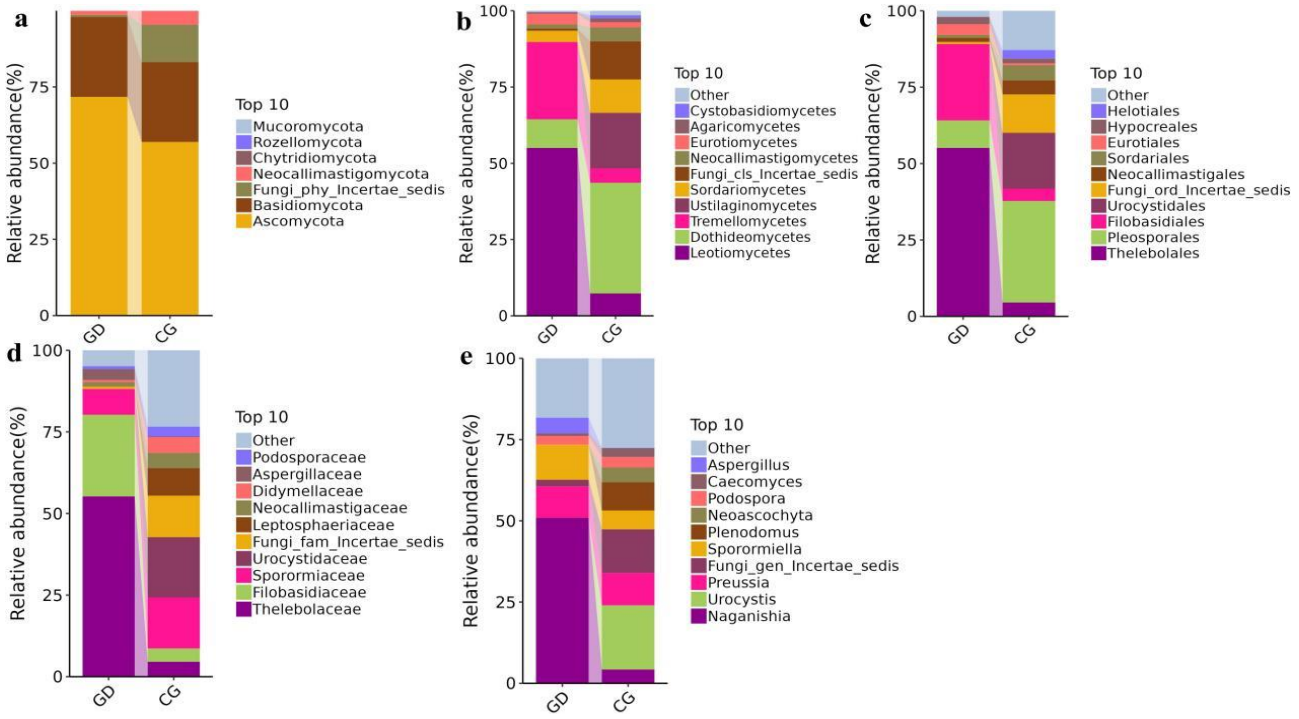

Fig. 2: Fungi microbiota comparing analysis of Xizang yaks in different taxa. (a) Phylum, (b) Class, (c) Order, (d) Family, (e) Genera.

Table 2: Alpha-diversity analysis of Xizang yaks in *Giardia intestinalis* positive and negative groups

Samples	Chao1	ACE	Good's Coverage	No of species Observed	Pielou evenness	Shannon	Simpson
GD1	64.5	64.5102556480381	0.999939527101937	63	0.422572260899602	2.52583269130316	0.687068240204953
GD2	81	81.2784218598884	0.999979842367312	81	0.406113476235019	2.57469852348007	0.560783002533134
GD3	74.5	75.4517066085694	0.99991936946925	73	0.398537237402906	2.4668755797047	0.619913202913416
GD4	75.5	75.729012345679	0.999959684734625	75	0.499999460453781	3.11440598451237	0.741421845132632
GD5	75	75	1	75	0.546315341319295	3.40289920891426	0.819696916540982
GD6	83.3333333333333	82.6496304308635	0.999899211836562	80	0.433672255760394	2.74164481766481	0.684210560853853
CG1	148	148.043599063942	0.999939527101937	147	0.813160714648991	5.85449070916572	0.972128366875185
CG2	196	196.323117925505	0.999979842367312	196	0.769427347083179	5.85896599416573	0.957800623545399
CG3	176	176.325121392245	0.999979842367312	176	0.744571666078342	5.55408142828624	0.956981891849076
CG4	164.5	164.59670781893	0.999959684734625	164	0.777793810597552	5.72265841034156	0.957636403587705
CG5	316.333333333333	316.588244995181	0.999838738938499	314	0.714397313974281	5.92565478344352	0.945644413400152
CG6	193	193.290575738375	0.999979842367312	193	0.642355159840699	4.87705395375798	0.909758539235549

Revealing fungi biomarkers between *Giardia* positive and negative yaks: Beta diversity ordination analyses (PCA, PCoA, NMDS and UPGMA) showed a visual separation of fungal communities between *G. intestinalis*-positive and negative yaks (Fig. 3a-d), with obviously difference via analysis of Anosim (P<0.01) and PERMANOVA (P<0.01) (Fig. 3e, 3f). However, quantitative statistical testing of the observed separation was not performed in the original analysis. The heatmap showed higher abundance of Ascomycota and Mucoromycota in group GD, while Basidiomycota, Rozellomycota, Fungi phylum Incertae sedis, Neocallimastigomycota and Chytridiomycota were higher in group CG (Fig. 4a). *Sporormiella*, *Panaeolus*, *Cyllamyces*, *Vishniacozyma*, *Sarocladium*, *Aspergillus,* and *Cleistothelebolus* were higher genera in group GD, while *Urocystis*, Fungi genus *Incertae sedis*, *Plenodomus*, *Neoascochyta*, *Caecomyces*, *Paraphaeosphaeria* and *Pilidium* were higher in group CG (Fig. 4b). LEfSe showed more significant value in group GD (*Naganishia* (P<0.01), *Aspergillus* (P<0.01), *Cleistothelebolus* (P<0.05), *Xenodidymella* (P<0.05) and *Piromyces* (P<0.01), while *Urocystis* (P<0.01), Fungi genus *Incertae sedis* (P<0.01), *Plenodomus* (P<0.01), *Neoascochyta* (P<0.01), *Paraphaeosphaeria* (P<0.01) and *Pilidium* (P<0.05) were observably higher in group CG (Fig. 5). T-test showed that Fungi phylum *Incertae sedis* (P<0.01) and Neocallimastigomycota (P<0.05) in group GD had lower values than group CG (Fig. 6a). At the genera level, the abundance of *Naganishia* (P<0.001), *Aspergillus* (P<0.05), *Cleistothelebolus* (P<0.05), *Xenodidymella* (P<0.05) and *Piromyces* (P<0.01) were noticeably higher in group GD, while *Urocystis* (P<0.01), Fungi genus *Incertae sedis* (P<0.01), *Plenodomus* (P<0.0001), *Neoascochyta* (P<0.05), *Paraphaeosphaeria* (P<0.05), *Coniochaeta* (P<0.05), *Cystobasidium* (P<0.05), *Sporormiaceae* genus *Incertae sedis* (P<0.05), *Sclerostagonospora* (P<0.05), *Arthrinium* (P<0.05), *Neosetophoma* (P<0.05) and *Leucosporidium* (P<0.05) were significantly higher in group CG (Fig. 6b).

Network analysis of yaks among several groups: Network analysis of Xizang yaks found that *Cladorrhinum* was positively associated with *Glutinomyces, Nectriella, Neonectria and Ascobolus. Phaeococcomyces* was positively related to *Nectriella, Glutinomyces* and *Calycina* (Fig. 7).

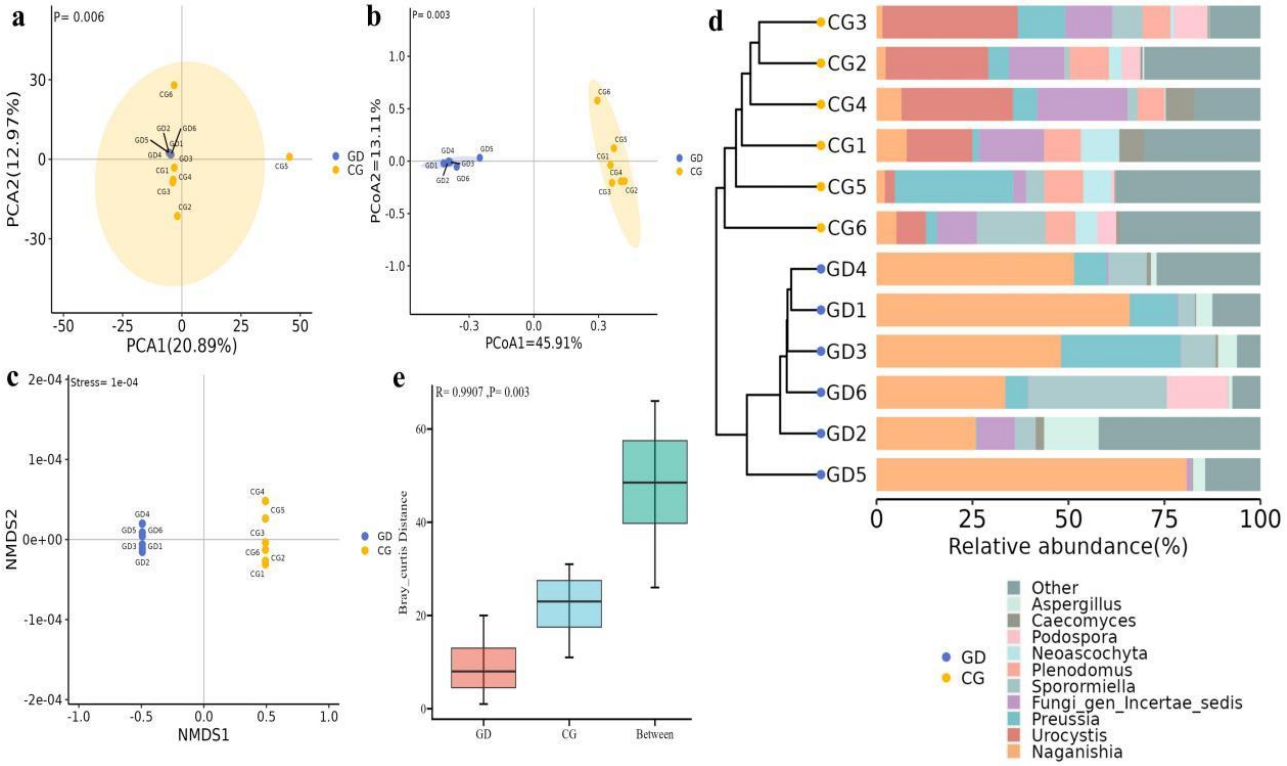

Fig. 3: Beta-diversity analysis of yaks in *Giardia intestinalis* positive and negative groups. (a) PCA, (b) PCoA, (c) NMDS, (d) UPGMA, (e) Anosim.

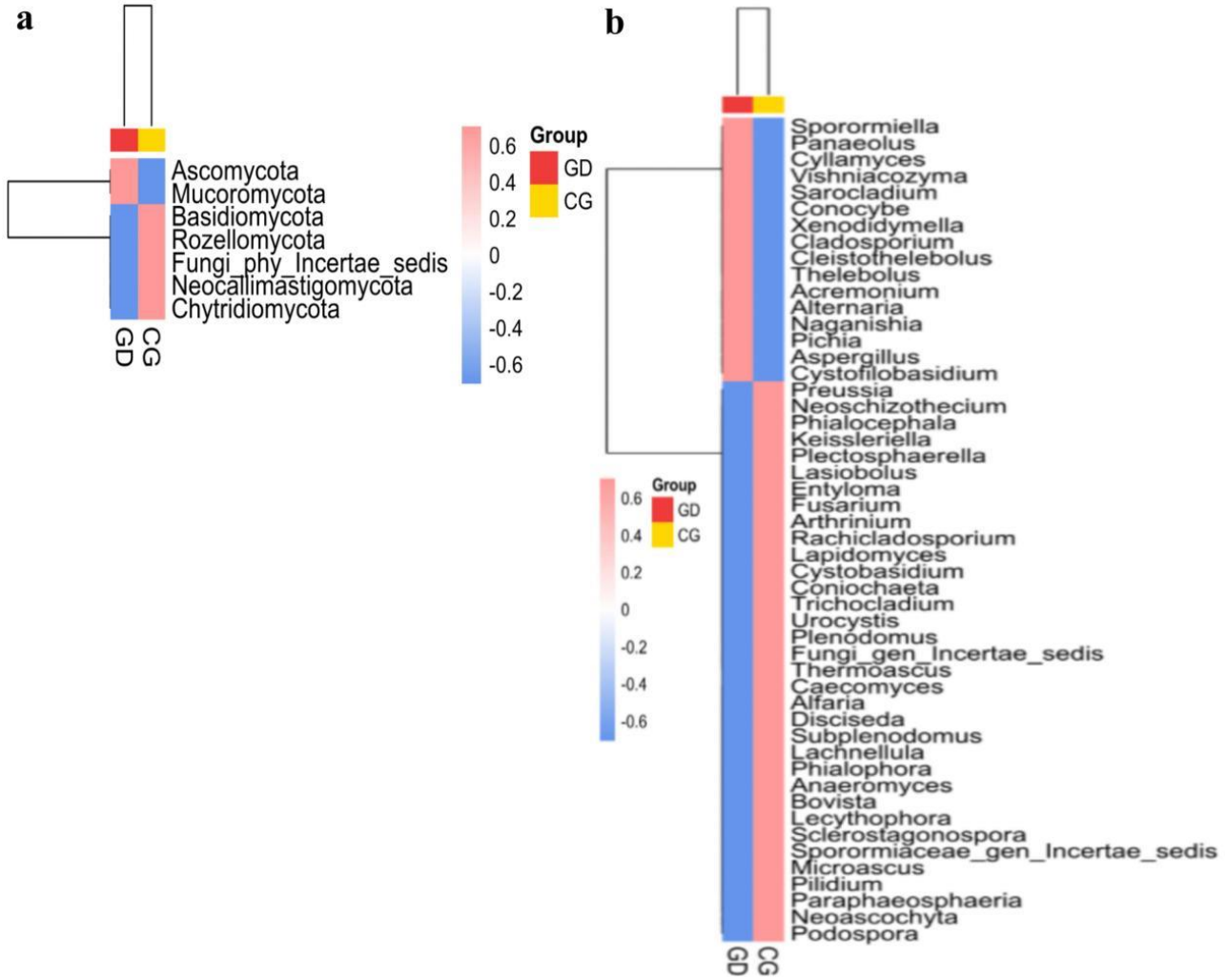

Fig. 4: Fungi microbiota comparison analysis of Xizang yaks in different taxa via heatmap. (a) Phylum, (b) Genera.

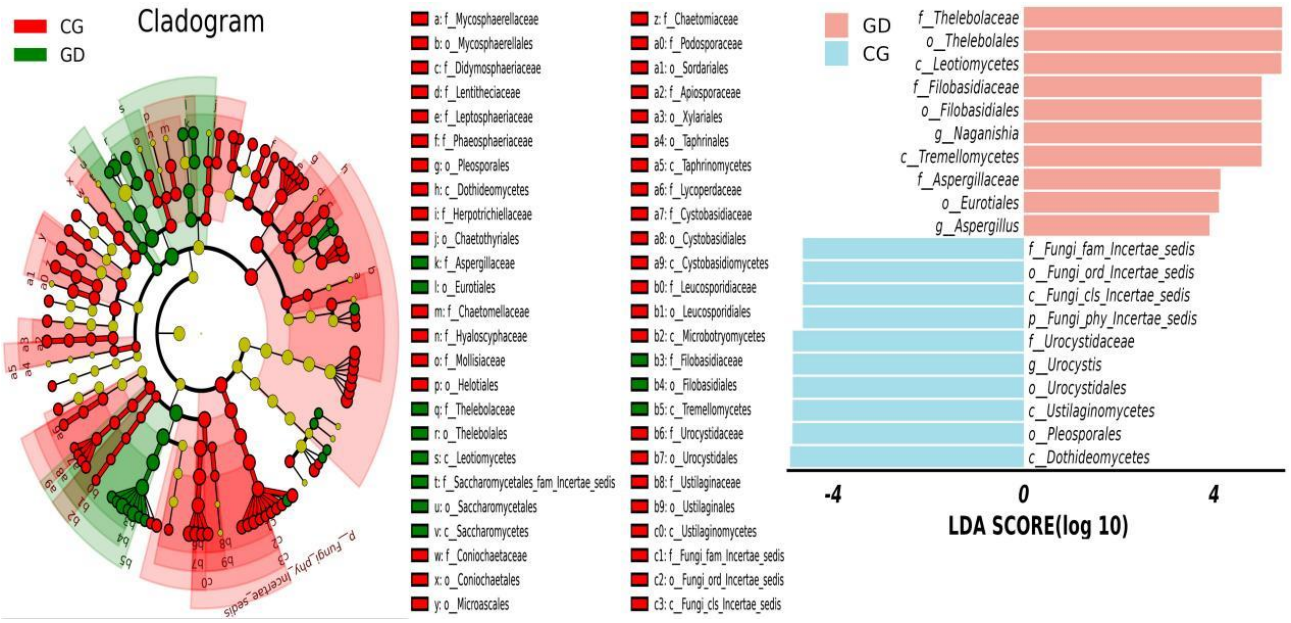

Fig. 5: Revealing fungi abundance difference between *Giardia intestinalis* positive and negative yaks via LEfSe. |LDA score| > 2.0, P<0.05.

DISCUSSION

The zoonotic protozoan parasite *G. intestinalis* is an important cause of severe diarrhea in people and food animals (Ryan *et al*., 2019). Xizang yaks are representative food ruminants and *G. intestinalis* infected individuals may transmit this causative agent to other animals or herd people by shedding infectious cysts to pastures and streams on the plateau (Abd El-Latif *et al*., 2020). The microbiota is widely recognized as the second genome, which plays vital roles in maintaining host health (Rowland *et al*., 2018). In this study, we performed ITS amplicon sequencing of positive and negative *G. intestinalis* from yaks for the first time and obtained 890 652 (GD=500 947, CG= 389 705) filtered sequences in Xizang yaks. Alpha diversity analysis discovered that *G. intestinalis* infection increased the richness and variety in yaks, with significant higher ACE, Chao1, observed species, Pielou evenness, Shannon and Simpson which was partly in agreement with *Cryptosporidium* infected yaks (Dong *et al*., 2023) and *G. intestinalis* infected dogs (Boucard *et al*., 2021).

The colonization of *G. intestinalis* can affect the intestinal flora via interactions with the intestinal epithelium, the intestinal environment, and immune responses of the host. GIA-induced changes in the gut barrier and mucus environment can alter nutrient availability and gut microbial communities, which can influence gut microbes (Fekete *et al*., 2024; Yao *et al*., 2024). The intestinal microbiome plays an important role in maintaining intestinal and immune homeostasis, while disturbances in microbial composition may contribute to intestinal inflammation and disease progression (Rychlik, 2025). Although further research is needed, experimental findings have shown that *Giardia* infection disrupts the intestinal balance of the microflora community, goblet cell responses, mucus barrier properties and alters mucin glycosylation, implying that there are cross-talks between parasite colonization, epithelial function, and the intestinal microflora community (Fekete *et al*., 2024).

Mucosal immunity may also be involved in these interactions. The gut epithelium and immune system are constantly communicating with gut microbiota to maintain their barrier integrity and immune homeostasis, and breakdown of this communication leads to microbial dysbiosis (Yao *et al*., 2024). Alterations in mucosal immunity, such as cytokine production and immune-cell activity, have been described in *Giardia* infection and may affect the intestinal microbial community (Xaplanteri *et al*., 2023; Sardinha-Silva *et al*., 2024). The exact link between these immune responses and the changes in the fungal communities, however, has not yet been fully understood.

Diarrhea can also induce alterations in the intestinal microbial communities through modifications of intestinal transit, luminal conditions, and nutrient availability. It may be associated with alterations in the abundance and distribution of enteric microorganisms; therefore, diarrheic status should be considered when interpreting microbiota differences between infected and control animals (Gurjar *et al*., 2024). Epithelial dysfunction, alterations in intestinal permeability, mucous disturbances, and altered intestinal transit have all been linked to *Giardia* infection, which may all impact the microbial ecosystem (Fekete *et al*., 2024). Fungal differences, however, were not a result of *Giardia* infection only, since controls without the presence of *Giardia* were not included in the present study but may have been due in part to diarrheic condition or other GI disturbances (Koyama *et al*., 2024).

The observed variation in types, abundance, and diversity of fungi may thus be a result of the interplay of changes associated with the parasite, host response, epithelial barrier, and (diarrhea-associated) ecological effects. However, these mechanisms are only suggested and not established cause-and-effect pathways, since this study did not directly measure epithelial barrier integrity, mucosal immune markers, intestinal metabolites, or fungal functional activity. New studies that focused specifically on fungi in individuals infected with *Giardia*

also suggest that the association between *Giardia* and the intestinal mycobiota is not yet well understood (Motta *et al*., 2025).

Alpha diversity analysis showed that *G. intestinalis* positive yaks exhibited higher fungal richness and diversity than negative yaks, which was also confirmed by taxon-level comparison analysis. The observed differences in fungal community composition were also accompanied by differences in predicted functional profiles; however, these predictions should be considered exploratory because fungal functional activity was not directly measured. Then, we further explored biomarkers between the two Xizang yak groups using LEfSe and a T-Test. The abundance of two phyla and seventeen genera was significantly different between group GD and CG. Among them, previous studies reported higher abundance of *Naganishia* in colorectal cancer mice (Wang *et al*., 2025), and *Cleistothelebolus* in *Cryptosporidium* infected yaks (Lu *et al*., 2023). The genus *Aspergillus* contains several pathogenic fungi producing mycotoxins threatening animal and human health (Xue *et al*., 2025) and *Xenodidymella* is also a pathogenic genus (Karimi *et al*., 2024). The greater abundance of these genera in *G. intestinalis* positive yaks may be associated with *G. intestinalis* infection and could potentially influence the host-microbiota interactions during infection. Previous studies found higher abundance of *Urocystis*, *Cystobasidium*, *Leucosporidium* and the fungal genus *Incertae sedi*s in healthy animals (Khadija *et al*., 2021; Cao *et al*., 2022; Lu *et al*., 2023; Zhong *et al*., 2026); the observed reduction in the abundance of these genera in infected Xizang yaks could potentially be associated with *G. intestinalis* infection; however, further studies are required to establish a causal relationship. *Arthrinium* has been reported to possess antagonistic or potential antifungal properties (Hong *et al*., 2015). Its higher abundance in healthy yaks may therefore be associated with a potentially protective role in maintaining fungal community balance; however, this association does not establish a direct antifungal effect in yaks. *Neosetophoma* can digest fiber and generate absorbable substances for ruminants (Wang *et al*., 2024); a higher abundance of this genus in *G. intestinalis* negative yaks may benefit forage digestion in ruminants. The fungal taxa identified in this study should be considered potential biomarkers associated with *G. intestinalis* infection rather than confirmed pathogenic or beneficial organisms. The gut mycobiome can influence intestinal homeostasis, immunity, and interactions with other microorganisms, but the biological functions of individual fungal taxa remain incompletely understood and may be context-dependent (Zhang *et al*., 2021; Zhang *et al*., 2022). The differential abundance of *Aspergillus*, *Naganishia* and *Cleistothelebolus* between groups represents an association with *G. intestinalis* infection status rather than evidence of pathogenic or protective effects. Further experimental studies are required to determine their biological roles. Functional and experimental studies are required to validate the roles of these taxa in *G. intestinalis*-associated fungal dysbiosis. The observed positive associations among fungal genera may indicate potential co-occurrence patterns within the yak intestinal mycobiota; however, correlation does not establish direct biological interactions. These associations may reflect shared ecological niches, similar responses to host conditions, or indirect microbial relationships. Therefore, the network results should be considered hypothesis-generating and require experimental validation (Zhang *et al*., 2022; Wang *et al*., 2025).

A major limitation of this study was the small sample size for ITS amplicon sequencing (six *G. intestinalis* positive and six negative yaks), which reduced statistical power and increased uncertainty in diversity and differential-abundance estimates; therefore, the findings should be considered exploratory and hypothesis-generating. The absence of systematic screening for other enteric pathogens and a diarrheic *G. intestinalis*-negative control group prevented differentiation of *Giardia* associated effects from those related to diarrhea or co-infections. In addition, incomplete information on age, sex, diet, grazing management, season, antimicrobial/antiparasitic exposure, and environmental factors limited adjustment for potential confounding. The lack of multiple comparison correction, quantitative ANOSIM statistics, and PERMANOVA further limited statistical support for beta-diversity and differential-abundance results. Sequencing-depth metrics, Good's coverage, rarefaction depth, negative extraction controls, and contamination assessment were also not documented. Finally, *G. intestinalis* detection relied solely on the 18S SSU rRNA marker, which has lower discriminatory resolution than Multilocus markers such as *gdh*, *bg*, and *tpi*; thus, assemblage identity and transmission relationships could not be determined. Future studies should include larger, well-controlled cohorts, diarrheic *Giardia* negative controls, systematic pathogen screening, comprehensive metadata, appropriate statistical corrections and sequencing quality controls and Multilocus genotyping to validate and clarify these findings.

Conclusions: In conclusion, *G. intestinalis* infection was associated with significant alterations in intestinal fungal diversity and community composition in Xizang yaks. Several fungal taxa, including *Naganishia*, *Aspergillus*, *Cleistothelebolus*, *Xenodidymella*, *Piromyces*, and *Urocystis*, were identified as potential biomarkers distinguishing infected from non-infected yaks. These findings provide preliminary evidence of an association between *G. intestinalis* infection and intestinal mycobiota dysbiosis and improve our understanding of host-parasite-microbiota interactions in plateau yaks. However, the cross-sectional design and lack of diarrheic *G. intestinalis* negative controls prevent causal inference and may introduce confounding by diarrhea. Future longitudinal and experimental studies are required to validate these fungal biomarkers and clarify the mechanisms underlying these microbial alterations.

Data availability statement: The *G. intestinalis* genes were added to the NCBI database under accession numbers PZ544941-PZ544946 and all accessible yak raw data were stored in the NCBI Sequence Read Archive under consent number PRJNA1479931.

Animal ethics approval: The Xizang Academy of Agricultural and Animal Husbandry Sciences Laboratory Animal Welfare and Ethics Committee approved every procedure used in this study.

Authors contribution: The research topic and technique were completed by BS. Solutions, reagents, lab supplies, and analytical instruments were prepared by BS, and Deji Q. The preparation, writing, and drafting were completed by BS. BS wrote, reviewed, and revised papers. Supervision and visualization were handled by BS. The final text was reviewed and approved by all authors.

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