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Minimal Alterations in Fecal Microbiota Composition Following Ivermectin Administration in Clinically Healthy Chinchillas

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ABSTRACT

The intestinal microbial community plays a vital role in maintaining host well-being, and growing attention has been drawn to the possible adverse impacts of pharmaceuticals on gut microbiota. The therapeutic application of ivermectin in chinchillas has not been previously examined. This investigation aimed to evaluate how ivermectin injections affect the fecal bacterial composition in chinchillas. A within-subject design was used, comparing microbial changes before and after treatment in 10 clinically normal animals over a 14-day period. Each chinchilla received the same ivermectin dose, and fecal samples collected pre- and post-treatment served as two experimental groups. Samples were taken on day 0 (prior to injection) and day 14 (following administration). Bacterial profiling was conducted using 16S rRNA gene sequencing. No abnormal clinical reactions were observed after subcutaneous ivermectin administration. Although the general abundance and diversity of gut bacteria remained largely unchanged, shifts in the prevalence of specific taxa were detected. In conclusion, ivermectin induced only minor modifications in fecal microbiota among healthy chinchillas, without noticeable short-term effects on their overall health.

Keywords: Chinchilla, Ivermectin, Fecal microbiota, 16S rRNA

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Introduction

Chinchillas are herbivorous mammals from the Chinchillidae family, native to the Andean region of South America. Although wild populations are nearly extinct, domesticated chinchillas, derived from Chinchilla lanigera, are widely kept both in fur production and as exotic pets [1, 2]. Parasitic organisms such as Syphacia obvelata, as well as ectoparasites including Ctenocephalides spp., Lagidiophthirus spp., and Atricholaelaps chinchillae, are occasionally found in these animals [3].

In recent years, research has increasingly focused on the relationship between pharmacological agents and intestinal microorganisms, which influence host metabolism and act as a defense barrier against pathogens [4, 5]. The gut microbial ecosystem contributes to the synthesis and degradation of compounds such as short-chain fatty acids, organic acids, conjugated linoleic acid, and phenolic molecules—key regulators of metabolic balance, immunity, and inflammation. Moreover, these microbial metabolites can influence the onset and development of various diseases [5–7]. Therefore, alterations in gut microbiota composition are closely linked to disease progression and immune homeostasis [4, 5, 8].

Ivermectin, a well-known antiparasitic drug, is commonly used for treating both internal and external parasitic infections in chinchillas, administered orally or via injection [3, 9, 10]. However, because ivermectin exhibits

certain antibacterial effects [11], it may disrupt microbial equilibrium in the intestine, potentially predisposing animals to disease [5, 6, 8]. Previous studies demonstrated that combined administration of fenbendazole and ivermectin altered gut microbial balance and metabolic pathways in Amur tigers [12], while treatment with tribendimidine plus ivermectin in hookworm-infected adolescents caused major shifts in gut bacterial composition [13].

To date, there have been no investigations on how ivermectin influences the fecal microbiota of chinchillas. The current study is the first to explore this subject by examining changes in bacterial communities after subcutaneous ivermectin administration, analyzed through 16S rRNA gene sequencing.

Materials and Methods

Animals

Ten adult chinchillas (five males and five females), aged between 1 and 2 years, were obtained from a commercial breeding facility in Beijing, China. None of the animals received medication prior to the experiment. They were individually housed in climate-controlled enclosures maintained at 21–23 °C and fed a commercial pellet diet (Mazuri® Chinchilla Diets, Land O'Lakes, Inc., Melrose, MN, USA) with fresh timothy hay and unlimited access to water. All subjects were verified to be clinically healthy through physical examination, normal food consumption, and consistent fecal output. Throughout the study, fecal pellets maintained normal shape and color. Microscopic analysis, including direct smears and zinc sulfate flotation tests of samples collected on days 0 and 14, confirmed the absence of gastrointestinal parasites. The protocol was reviewed and approved by the Animal Care and Use Committee of China Agricultural University, and informed consent was obtained from the breeder.

Study design and fecal sample collection

A within-subject pre–post experimental design was chosen instead of a crossover model, due to the lack of data regarding the washout period for ivermectin in chinchillas. All 10 animals received a subcutaneous injection of ivermectin (Ivomec®, Merial, Ingelheim am Rhein, Germany; 1.0% w/v sterile solution diluted tenfold in saline) at a dosage of 0.4 mg/kg on day 0.

Fresh fecal samples were collected at two time points—day 0 and day 14—forming the respective control and post-treatment groups. Each animal was placed in a clean cage during sample collection, and freshly excreted pellets were immediately transferred into sterile cryovials using sterile forceps. Samples were then frozen at −80 °C until subjected to microbial community analysis [14].

Extraction of DNA and sequencing of 16S rRNA

Genomic DNA from fecal specimens was isolated using a commercial extraction kit (PowerFecal® DNA Isolation Kit, MO BIO Laboratories, Inc., Carlsbad, USA) according to the manufacturer's protocol. To determine bacterial community composition, amplification targeted the V3–V4 hypervariable regions of the 16S rRNA gene using primers 338F (5′-ACTCTACGGAGCAGCA-3′) and 806R (5′-GACTACHVGGTWTCAT-3′). Amplicons were sequenced through the Illumina Novaseq6000 platform with paired-end 250 bp reads [15].

Statistical processing

Microbial diversity within the intestinal samples (α-diversity) was estimated using operational taxonomic unit (OTU) counts, the Chao1 and ACE richness estimators, and evenness indices. Sequences with at least 97% similarity were clustered into OTUs via USEARCH (v10.0), excluding those below 0.005% abundance. Community diversity and richness (Chao1, ACE, Shannon, and Simpson indices) were calculated in QIIME2 (v2021.4.0).

Differences in overall community structure between groups (β-diversity) were analyzed through the Bray–Curtis distance metric and illustrated using principal coordinate analysis (PCoA). Relative abundance variations were evaluated with the Wilcoxon rank-sum test, and the LEfSe (linear discriminant analysis effect size) method was used to pinpoint significant bacterial biomarkers. Statistical significance was assigned at $p < 0.05$.

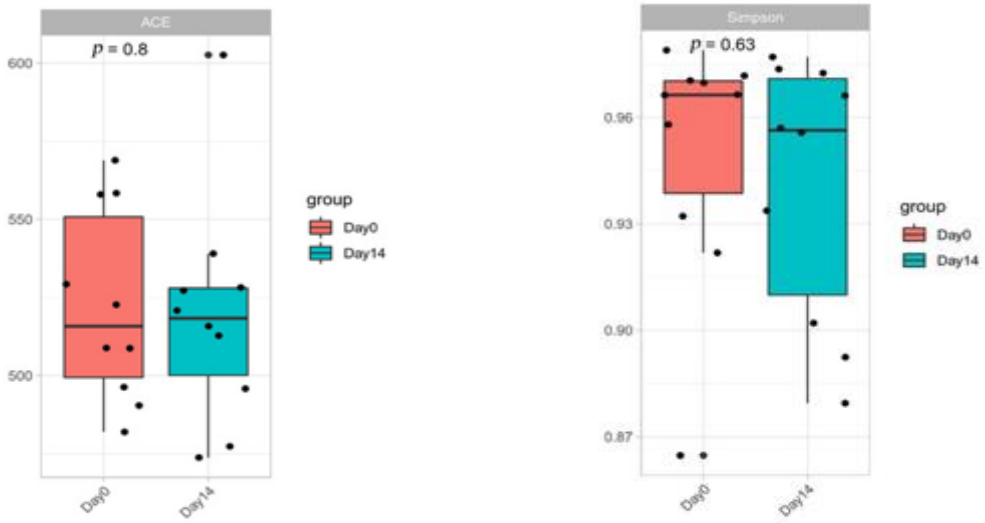
Results and Discussion

Clinical observations and parasitological tests

No adverse effects were observed in chinchillas following ivermectin administration. Fecal morphology remained constant—dry, firm, and oval. No diarrhea or abnormal behavior was recorded. Microscopic screening of fresh feces and zinc sulfate flotation tests before and after treatment revealed no parasitic organisms or ova.

Microbial diversity evaluation

OTU counts were 684 on day 0 and 662 on day 14, with 646 OTUs common to both. Indices measuring richness and diversity (Chao1, ACE, Shannon, Simpson) showed no statistical difference between sampling points: Chao1 ($p = 0.843$), ACE ($p = 0.823$), Shannon ($p = 0.464$), and Simpson ($p = 0.580$) **(Figure 1)**.

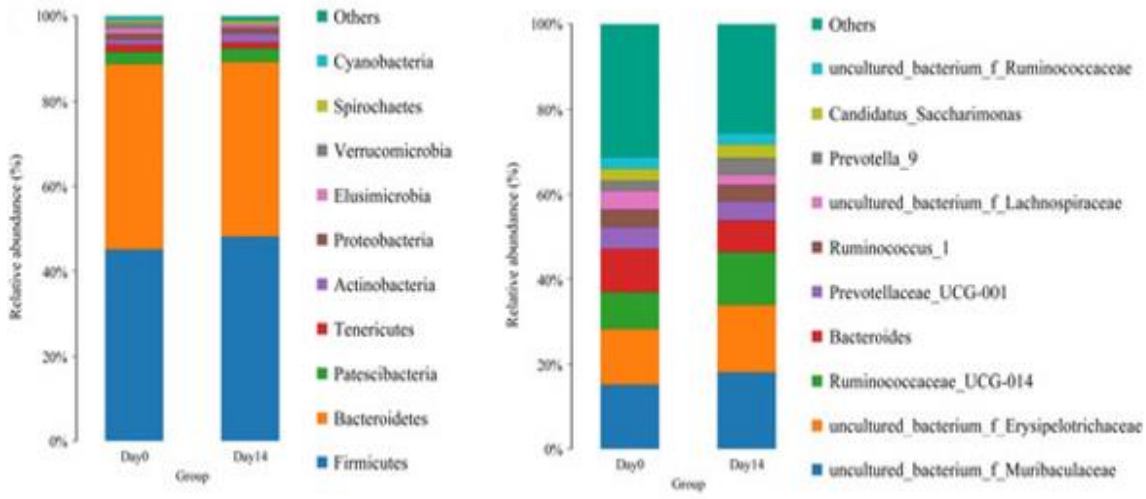


a) b)

Figure 1. ACE (left) and Simpson (right) indices comparing richness and diversity before and after treatment.

Taxonomic composition

At the phylum level, microbial patterns remained largely unchanged following ivermectin exposure. Both groups were dominated by Firmicutes, followed by Bacteroidetes, Patescibacteria, Tenericutes, Actinobacteria, Proteobacteria, Elusimicrobia, Verrucomicrobia, Spirochaetes, and Cyanobacteria **(Figure 2a)**. The combined proportion of Firmicutes and Bacteroidetes represented 88.76–89.22% of the microbiota. Cyanobacteria decreased from 0.73% (day 0) to 0.29% (day 14) with a significant difference ($p = 0.034$).



a) b)

Figure 2. (a) Phylum-level fecal bacterial distribution for day 0 and day 14. (b) Genus-level composition in both groups.

At the genus level, 180 OTUs were recognized. The dominant taxa were uncultured_bacterium_f_Muribaculaceae and uncultured_bacterium_f_Erysipelotrichaceae, followed by Ruminococcaceae_UCG-014, Bacteroides, Prevotellaceae_UCG-001, Ruminococcus_1, uncultured_bacterium_f_Lachnospiraceae, and Prevotella_9 **(Figure 2b)**. Abundances of Bacteroides (10.264%), Prevotellaceae_UCG-001 (5.156%), Ruminococcus_1 (4.177%), and uncultured_bacterium_f_Lachnospiraceae (4.435%) were slightly higher on day 0, but differences were not significant.

β-diversity based on Bray–Curtis metrics and PCoA visualization showed no evident separation between day 0 and day 14 groups at the phylum level.

LEfSe analysis identified taxa significantly differing between groups (LDA > 2, $p < 0.05$) **(Figure 3)**. The day 0 group was characterized by Rikenellaceae_RC9_gut_group, Lachnospiraceae_NK4A136_group, Methylobacterium, Angelakisella, and Oscillibacter. Conversely, Ruminococcaceae_UCG_013, Pediococcus, Eubacterium, Bacillus, and Catabacter were dominant in the day 14 samples.

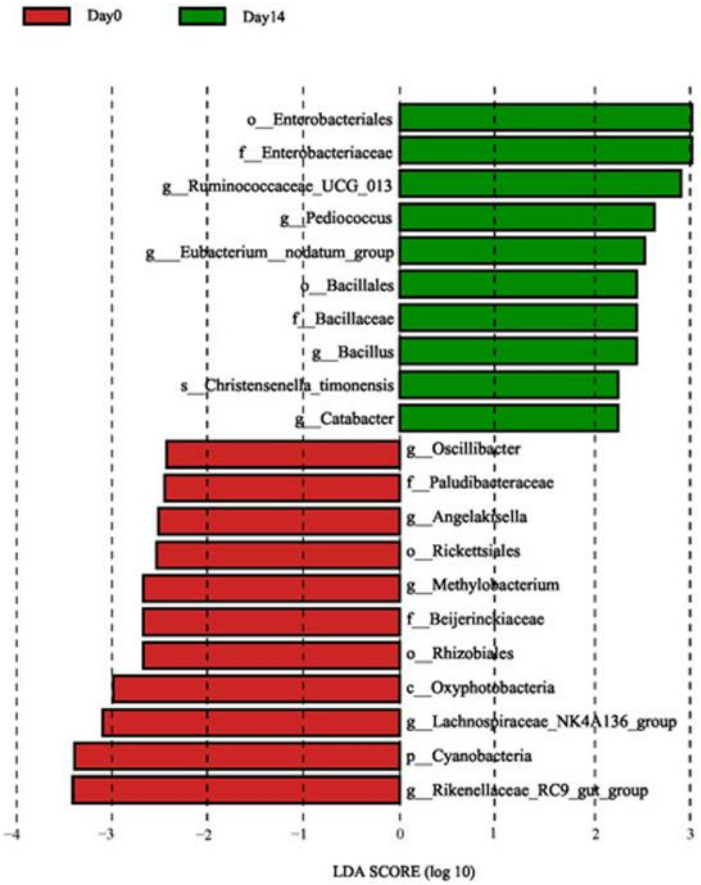


Figure 3. LEfSe analysis displaying significantly enriched bacterial taxa with LDA > 2 between groups.

To date, this appears to be the first investigation evaluating the influence of ivermectin administration on the fecal bacterial microbiota of chinchillas using 16S rRNA gene sequencing. In this pilot trial, only minor fluctuations in fecal microbial composition were detected before and after ivermectin injection, with no marked alterations in overall diversity or richness indices, and no apparent clinical abnormalities were observed.

Ivermectin treatment did not notably affect the predominant microbial taxa at either the phylum or genus level. Prior literature has indicated that Firmicutes, Bacteroidetes, Verrucomicrobia, Spirochaetes, and Proteobacteria represent the dominant fecal phyla in domestic herbivorous species, with a Firmicutes-to-Bacteroidetes ratio of less than 2 in hindgut fermenters [16–18]. Our data were consistent with these findings—Firmicutes and Bacteroidetes comprised the majority of the intestinal community, maintaining a ratio below 2. Although ivermectin exposure did not reshape the primary phyla, subtle variations in the relative abundance of certain microbial groups were detected between sampling periods.

At the phylum level, a significant post-treatment decline in Cyanobacteria was recorded. This phylum is ubiquitously present in environmental sources such as freshwater and marine ecosystems and has previously been

identified within the fecal microbiota of horses and volcano rabbits [19–21], both of which are hindgut fermenters. Although Cyanobacteria form only a minor fraction of intestinal flora, prior research has proposed a possible association between Cyanobacterial proliferation and neurotoxin production contributing to neurodegenerative conditions such as Equine Motor Neuron Disease [22]. Moreover, elevated intestinal Cyanobacteria levels have been linked with several disorders, including viral, metabolic, and gastrointestinal diseases, as well as neurodegeneration [19]. Despite being often connected with toxin formation, Cyanobacteria may also generate beneficial bioactive substances [23], and their reduction has been correlated with liver cirrhosis and obesity [19]. Thus, additional studies are needed to elucidate the biological role of Cyanobacteria in host health. This study newly identified Cyanobacteria as a notable constituent of the chinchilla gut microbiota, with ivermectin administration inducing a measurable decline. Considering its dual potential effects, further examination of Cyanobacteria's impact on chinchilla intestinal function is warranted.

At the genus level, uncultured_bacterium_f_Muribaculaceae emerged as the most prevalent genus, similar to observations in the ruminal microbiota of sheep [24], and was marginally more abundant on day 14 following ivermectin exposure. This genus is known for producing succinic acid—a key gluconeogenic precursor—and facilitating polysaccharide breakdown and metabolism [25]. In contrast, Prevotellaceae_UCG-001 was relatively higher on day 0. Given its role as a negative fermentation indicator [26], its reduction coupled with the increase in Muribaculaceae by day 14 implies no adverse fermentation outcome [24]. Bacteroides, involved in the degradation of plant polysaccharides in both terrestrial and marine herbivores [16], slightly decreased after treatment, suggesting ivermectin may modestly hinder fiber utilization.

LEfSe analysis revealed that several genera exhibited significant differences in abundance following ivermectin injection. Rikenellaceae_RC9_gut_group—part of the Rikenellaceae family known to produce hydrogen and modulate inflammatory cytokines—was enriched on day 0 [27]. This group also contributes to the decomposition of plant-derived polysaccharides and is positively linked with dietary fiber content [28]. Oscillibacter, another herbivore-associated genus implicated in polysaccharide metabolism [16], was also prevalent pre-treatment. Its metabolites, such as butyric and alpha-linolenic acids, have anti-inflammatory properties and support mucosal protection [29, 30]. Therefore, ivermectin's effect on these beneficial taxa may influence microbial equilibrium and fiber processing, potentially weakening normal intestinal defenses.

Ruminococcaceae_UCG_013, a Firmicutes genus within Ruminococcaceae, was strongly correlated with the day 14 samples. This genus synthesizes butyrate, promoting Treg cell differentiation and strengthening epithelial barrier integrity, contributing to anti-inflammatory and immunoprotective actions [31]. Additionally, Ruminococcaceae_UCG_013 degrades indigestible fibers, including cellulose and hemicellulose, playing an essential role in herbivore digestion [31, 32]. Other taxa significantly enriched at day 14 included Pediococcus, Eubacterium, and Bacillus. Pediococcus—a lactic acid bacterium—serves as a probiotic, producing pediocin, an antimicrobial peptide effective against E. coli, Salmonella enteritidis, Listeria monocytogenes, and Staphylococcus aureus [33]. Eubacterium, another butyrate producer, contributes to short-chain fatty acid (SCFA) generation, improving gut motility, immune modulation, and anti-inflammatory response [34, 35]. Bacillus, widely used as a probiotic, enhances host immunity and antibacterial resistance, with several commercial preparations available [36–38]. A Bacillus strain isolated from the donkey cecum demonstrated notable pathogen inhibition [38].

Gastrointestinal imbalance is a common issue in chinchillas, frequently related to microbial dysbiosis [39]. Syphacia obvelata, a parasitic nematode occasionally infecting chinchillas, is typically managed with ivermectin. However, due to ivermectin's antibacterial activity, it may inadvertently disturb intestinal microbial homeostasis and predispose animals to gastrointestinal disorders [8, 11]. Understanding this impact is therefore important for clinical application.

Our results indicate that a single therapeutic subcutaneous dose of ivermectin causes only minor changes in the fecal microbiota of healthy chinchillas, with no apparent short-term adverse effects. These findings may serve as a reference for the clinical management of helminth infections in this species. Nonetheless, given that this study employed a pre–post design without untreated controls, additional research incorporating control groups or longitudinal sampling is necessary to verify these observations.

Conclusion

In conclusion, administration of a single subcutaneous ivermectin injection exerted only a limited effect on the intestinal bacterial community of healthy chinchillas, with no significant short-term health disturbances. A notable decrease in Cyanobacteria was observed post-treatment at the phylum level. At the genus level, Rikenellaceae_RC9_gut_group, Lachnospiraceae_NK4A136_group, Methylobacterium, Angelakisella, and Oscillibacter predominated on day 0, whereas Ruminococcaceae_UCG_013, Pediococcus, Eubacterium, Bacillus, and Catabacter were associated with day 14. Future studies should further explore ivermectin's influence under parasitic infection or prolonged exposure conditions.

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