The impact of *Opisthorchis felineus* infection and praziquantel treatment on the intestinal microbiota in children

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**ARTICLE INFO**

**Keywords:**
- *Opisthorchis felineus*
- Parasite
- Gut microbiota
- 16S rRNA gene sequencing
- Children

**ABSTRACT**

The presence of some species of helminths is associated with changes in host microbiota composition and diversity, which varies widely depending on the infecting helminth species and other factors. We conducted a prospective case-control study to evaluate the gut microbiota in children with *Opisthorchis felineus* infection (*n* = 50) before and after anthelmintic treatment and in uninfected children (*n* = 49) in the endemic region. A total of 99 children and adolescents aged between 7 and 18 years were enrolled to the study. Helminth infection was assessed before and at 3 months after treatment with praziquantel. A complex examination for each participant was performed in the study, including an assessment of the clinical symptoms and an intestinal microbiota survey by 16S rRNA gene sequencing of stool samples. There was no change in alpha diversity between *O. felineus*-infected and control groups. We found significant changes in the abundances of bacterial taxa at different taxonomic levels between the infected and uninfected individuals. Enterobacteraeaceae family was more abundant in infected participants compared to uninfected children. On the genus level, *O. felineus*-infected participants' microbiota showed higher levels of *Lachnospira*, *Escherichia-Shigella*, *Bacteroides*, *Eubacterium eligens* group, *Ruminiclostridium 6*, *Barnesiella*, *Oscillibacter*, *Faecalitalea* and *Anaerosporobacter* and reduction of *Blautia*, *Lachnospiraceae FCS020* and *Eubacterium hallii* group in comparison with the uninfected individuals. Following praziquantel therapy, there were significant differences in abundances of some microorganisms, including an increase of *Faecalibacterium* and decrease of *Megasphaera*, *Roseburia*. Enterobacteraeaceae and *Escherichia* abundances were decreased up to the control group values. Our results highlight the importance of the host-parasite-microbiota interactions for the community health in the endemic regions.

1. **Introduction**

There is an increase in the prevalence of chronic, non-communicable diseases, including allergic and autoimmune diseases in developed countries (Burisch et al., 2014; Bai et al., 2019). The presence of some species of helminths is associated with changes in host microbiota composition and diversity, which varies widely depending on the infecting helminth species and other factors. We conducted a prospective case-control study to evaluate the gut microbiota in children with *Opisthorchis felineus* infection (*n* = 50) before and after anthelmintic treatment and in uninfected children (*n* = 49) in the endemic region. A total of 99 children and adolescents aged between 7 and 18 years were enrolled to the study. Helminth infection was assessed before and at 3 months after treatment with praziquantel. A complex examination for each participant was performed in the study, including an assessment of the clinical symptoms and an intestinal microbiota survey by 16S rRNA gene sequencing of stool samples. There was no change in alpha diversity between *O. felineus*-infected and control groups. We found significant changes in the abundances of bacterial taxa at different taxonomic levels between the infected and uninfected individuals. Enterobacteraeaceae family was more abundant in infected participants compared to uninfected children. On the genus level, *O. felineus*-infected participants' microbiota showed higher levels of *Lachnospira*, *Escherichia-Shigella*, *Bacteroides*, *Eubacterium eligens* group, *Ruminiclostridium 6*, *Barnesiella*, *Oscillibacter*, *Faecalitalea* and *Anaerosporobacter* and reduction of *Blautia*, *Lachnospiraceae FCS020* and *Eubacterium hallii* group in comparison with the uninfected individuals. Following praziquantel therapy, there were significant differences in abundances of some microorganisms, including an increase of *Faecalibacterium* and decrease of *Megasphaera*, *Roseburia*. Enterobacteraeaceae and *Escherichia* abundances were decreased up to the control group values. Our results highlight the importance of the host-parasite-microbiota interactions for the community health in the endemic regions.

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https://doi.org/10.1016/j.actatropica.2021.105835
Received 14 February 2020; Received in revised form 31 December 2020; Accepted 12 January 2021
Available online 21 January 2021
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Maizels, 2016; Zaiss et al., 2015). In this regard, it is important to study microbial composition affect the host immune response (Gause and modified the immune reactivity by increasing of the synthesis of sup-fibrosis and might increase the risk for cholangiocarcinoma (Gouveia and bile duct diseases, such as cholecystitis, cholangitis and periductal prevalence may exceed 50% in adults (Fedorova et al., 2018). The aim of this study was to identify potential interactions between infections on the development of chronic non-communicable diseases. The effect against allergies (Fedorova et al., 2017).

Chronic inflammation that promotes the fibrosis and malignancies posedly, liver flukes and helminth-modified microbiota together lead to chronic infection changed the composition of the gut and bile microbiome in experimental animals (Plieskatt et al., 2013). Supposedly, liver flukes and helminth-modified microbiota together lead to chronic inflammatory markers that promotes the fibrosis and malignancies (Chng et al., 2016; Sripa et al., 2012).

The liver fluke Opisthorchis felineus is endemic in Europe and in large parts of the Russian Federation, particularly in Siberia, where the prevalence may exceed 50% in adults (Fedorova et al., 2018). The infection occurs through the consumption of raw or undercooked cyprinoid fish. Chronic O. felineus infection may lead to several severe liver and bile duct diseases, such as cholecystitis, cholangitis and periductal fibrosis and might increase the risk for cholangiocarcinoma (Gouveia et al., 2017). Recent studies indicate that a chronic O. felineus infection modifies the immune reactivity by increasing of the synthesis of suppressors of the immune response IL10 and TGF-β and reducing the level of proinflammatory markers IL-4, IL-5, and might have a protective effect against allergies (Fedorova et al., 2017).

There is increasing evidence that helminth-induced changes in gut microbial composition affect the host immune response (Gause and Maizels, 2016; Zaiss et al., 2015). In this regard, it is important to study the mutual influence of the intestinal microbiota and helminth infections on the development of chronic non-communicable diseases. The aim of this study was to identify potential interactions between O. felineus infection, praziquantel treatment and intestinal microbiota.

2. Materials and methods

2.1. Ethics statement

Study procedures were followed in accordance with the ethical standards of the Helsinki Declaration of the World Medical Association. The study protocol was approved by the Ethical Committee of Siberian State Medical University (SibMed), Tomsk (N 4981, 24.10.2016). Written informed consents were obtained from adolescents 15 years and older, and parental consents were obtained for children under 15 years old.

2.2. Study design and sampling

A prospective case-control study was conducted in Tomsk Region (Western Siberia, Russian Federation) between November 1, 2016 and June 30, 2018. We recruited two groups of children aged 7-18 years consisting of subjects infected with the helminth O. felineus (n=50) and uninfected by O. felineus and other helminths (n=50) (Fig.S1 in the supplemental material). The recruitment of the infected patients was conducted at the Regional Children’s Hospital, uninfected controls were enrolled in the Center of Health for Children. Prior to the study, all subjects were asked to collect two stool samples collected on two consecutive days. Patients with O. felineus infection received standard treatment by praziquantel with a dose of 60 mg/kg body weight divided into three doses (at intervals of 4 hours) for one day. Follow-up period after the treatment was 3 months and included the assessment of clinical symptoms and efficacy of anthelmintic treatment via microscopy of stool samples.

Inclusion criteria for patients with O. felineus infection were participants who provided two stool samples for examination (microscopy) and at least one stool sample positive for the O. felineus infection. Inclusion criteria for uninfected controls and patients after treatment were: two stool sample negative for the O. felineus infection. Inclusion criteria for patients with O. felineus infection, patients after treatment and uninfected children were: anthelmintic and/or antimicrobial treatment 3 months prior to the study, probiotic treatment within the 4 weeks prior to the start of the study, were co-infection with other helminths/protozoa and/or had comorbidities, less than 5,000 reads per sample according to sequencing. In total, 50 infected and 49 uninfected children and adolescents were enrolled and completed assessments (two stool samples, completed examination, sequencing results); of those, 48 infected patients finished follow-up period after the treatment. Samples from two subjects, those collected after treatment, were taken out of the analysis.

2.2.1. Interviews and physical examination

Study participants were interviewed using a pre-tested questionnaire (Zvonareva et al., 2018). Data collected was about demographic status (age, gender), risk factors for O. felineus infection (habitual practices of raw fish consumption, personal hygiene) and comorbidities. Study participants passed clinical examination by a physician. We assessed complaints, skin, respiratory, cardiovascular, digestive and hepatobiliary systems, height and weight. Particular attention was paid to symptoms associated with O. felineus infection: pain in right subcostal area, nausea, skin rash before and after treatment. The biochemical tests of the blood serum samples were carried out usingDiaSys Diagnostic Systems GmbH kits (Table S1). The following indicators were measured: total protein, glucose, bilirubin total, the bound fraction bilirubin, enzymatic activity of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, and alkaline phosphatase. Ultrasonography of the liver and biliary ducts was performed by a mobile, high resolution ultrasound device (Shenzhen Mindray Bio-Medical Electronics, Co, Ltd).

2.2.2. Stool examination and sample storage

Prior to the study, all subjects were asked to collect two stool samples on two consecutive days. The participants were provided with sterile plastic containers to store the samples. The stool samples were transported in cooled containers (4°C) no more than one hour to the laboratory. We used PARASEP (DiaSys Ltd, UK) for the examination of the parasite eggs. The microscopy analysis for the presence of intestinal parasites/eggs was carried out in the diagnostic laboratory of SibMed by a certified specialist on the day of sampling. The number of eggs per gram (EPG) of feces was recorded for O. felineus and the infection intensity was calculated. The intensity O. felineus infection was grouped in low (0-999 EPG), moderate (1000-9999 EPG), and severe intensities (10,000 and above EPG) (Elkins et al., 1991). Three months post-treatment the microscopy were carried out using the same procedure as described above.

For the DNA extraction, there were stool samples collected from 50 uninfected children and from patients with O. felineus infection before (n=50) and 3 months after treatment (n=48). All post-treatment samples sequenced for this study were helmint-negative by microscopy: The collected stool samples were frozen and stored at -80°C at the “Biobank Sibmed” department until used for DNA extraction. DNA from all stool samples was isolated less than 2 months following collection.

2.3. DNA extraction and sequencing of 16S rRNA gene

The DNA samples were isolated from each sample (150 mg) by using
FastDNA SPIN Kit for Soil (MP Biomedicals, USA) and according to the manufacturer’s recommendations. The DNA samples were diluted 500 times. DNase and pyrogen free water (DES) from the FastDNA SPIN Kit for Soil was used for dilution of the purified DNA. Isolated DNA was stored at -80 °C. Blank extractions were included as negative controls to monitor for bacterial contamination. The V4 hyper-variable region of the 16S rRNA gene was amplified by PCR in 1 round using a modification of F515 and R806 barcoded primers (GTGBCAGCMGGCCGTTAA and GACTACNVGGGTATCTAATCC, respectively) with the double-index sample barcoding primer system described in (Fadrosh et al., 2014). Amplification was performed using C1000 Touch Thermal Cycler (Bio-Rad, USA) under the following cycling conditions: initial denaturation 60 s at 98 °C, followed by 30 cycles of 15 s at 98 °C, 15 s at 58 °C and 25 s at 72 °C, final extension of 5 min at 72 °C. Purification of the PCR products was performed using the Cleanup Mini kit for the extraction of DNA for reagent mixtures (Evrogen, Russia). The concentration of the obtained 16S rRNA gene libraries in the solution was measured with Qubit fluorometer (Invitrogen, USA) using Quant-IT dsDNA High-Sensitivity Assay Kit. The purified amplicons were mixed equally in accordance with the assessed concentrations. The quality of the library prepared for sequencing was assessed using the electrophoresis in 2% agarose gel. The final concentration of the pooled library prepared for sequencing on MiSeq platform was 12.5 pM (measured with Qubit fluorometer as described above for the individual libraries).

Sequencing of the pooled library was performed using MiSeq Reagent Kit v2 (2*250 cycles) on MiSeq sequencer (Illumina, USA) following the manufacturer’s instructions. Each sample was sequenced in duplicate to even the depth of coverage. In addition to patient samples, the sequencing of isolation, amplification and sequencing of control samples also was carried out.

2.4. Bioinformatic analysis

The pooled reads were demultiplexed using deML (Renaud et al., 2015). After the quality trimming, the concatenation of the paired reads was performed using SeqPrep tool. Prior the analysis, the replicates were concatenated into one FASTQ file. Sequence processing and diversity analyses were performed with QIIME 2 version 2019.1.0 (Bolyen et al., 2019).

After being imported into the QIIME 2 artifact, all sequences were quality-checked in sliding window via quality-filter q-score-joined script, filtered with a threshold of Phred score = 30 and then dereplicated. For chimeric reads identification and removing, a de novo approach was used with vsearch uchime-denovo script. Closed-reference clustering strategy was used for operational taxonomic units (OTUs) selection with 99% similarity SILVA 132 release (Yilmaz et al., 2014) as a reference database and q2-vsearch as picking algorithm. OTUs taxonomic classification was performed using q2-feature-classifier plugin with naïve Bayes classifier trained on 515F/806R region of 16S rRNA gene sequences from SILVA database. Technical control samples were manually checked and the reads identified as contaminants were subtracted from the microbiome samples. Samples containing less than 5000 reads were excluded from further analysis.

Prior to the taxonomic richness and α-diversity computation, the samples were rarefied to the depth of 5000 classified reads. Faith index, Shannon index, and the number of observed OTUs were calculated for each of the samples at the level of OTUs. The DECODE plugin of QIIME 2 with default parameters was used to calculate pairwise samples dissimilarity (β-diversity)(Martino et al., 2019). Beta-diversity was calculated for all samples and separately for the group of *O. felineus*-infected before and after treatment, and uninfected individuals.

2.5. Statistical analysis

Statistical analysis was conducted in R language, version 3.6.1 (R Core Team, 2019). Differences in measured clinical symptoms, biochemical and ultrasonography parameters of *O. felineus*-infected and uninfected individuals were assessed with Mann-Whitney-Wilcoxon test (for quantitative variables) and with chi-square test (for qualitative variables).

The three-groups (*O. felineus*-infected, after treatment, and uninfected) alpha-diversity comparison was implemented using a linear mixed model with *O. felineus* infection status as a fixed effect and patient’s identifier as a random effect (Bates et al., 2015). For post hoc analysis, the diffsmmeans function of lmerTest package was used (Kuznetsova et al., 2017). Associations between infection intensity and alpha-diversity indices were examined with negative binomial regression on *O. felineus* infected group (Venables and Ripley, 2002). The *O. felineus* EPG counts value was used as a dependent variable.

Beta-diversity analysis was performed for all samples and the groups of infected patients and uninfected controls. On whole sample, pairwise permutational analysis of variance (PERMANOVA) for distance matrices with 9999 permutations was performed on all samples to check the separation between *O. felineus*-infected, after treatment, and uninfected (R Core Team, 2019). For the *O. felineus*-infected and uninfected individuals, the impact of different clinical factors, including complaints, biochemistry, results of physical and ultrasound examination along with helminthic infection was tested in multivariate adonis model (Oksanen et al., 2019).

Prior to the statistical analysis of taxa abundance, OTU table was exported to R with import biom function, rarefied to the depth of 5000 reads per sample and aggregated on the levels of phyla, families, genera, and species with tax glom function (McMurdie and Holmes, 2013). All phylogenetic levels were inspected separately.

Analyses of taxa abundance were performed using a generalized linear mixed model with negative binomial distribution for taxa abundance (Brooks et al., 2017). Infection status was selected as a fixed effect and patient’s identifier as a random effect. All models were checked with II-type ANOVA (Therneau, n.d.); for the significant models, the estimated marginal means were calculated in each group, and Tukey post hoc analysis was performed (Lenth, 2019). Associations of infection intensity and microbial phylotypes abundance were identified similarly to α-diversity.

In case of multiple hypotheses testing, the p-values were adjusted with FDR method and referred as q-values. Visualization was performed with ggplot2 package (Wickham, 2006). Additional exploratory analysis of the microbiome data was performed using Kromics-Biota platform (Efimova et al., 2018).

3. Results

3.1. Characteristics of the study cohort

The study groups included 99 children (50 patients with *O. felineus* infection and 49 uninfected subjects). Two samples collected at post-treatment were excluded: one subject used antibiotics, another one refused to perform stool sample collection.

Participants’ age ranged from 7 to 18 years old, with a median age of 11 (8-14) for *O. felineus* infected patients and 10 (8-13) for the control group. Age and gender distribution were similar between the two groups. Table 1 summarizes the participants’ characteristics: clinical symptoms, biochemical tests, abdominal ultrasound. Among the most common complaint in *O. felineus* infected patients was pain in abdomen (q < 0.01), in particular, pain in the right subcostal area. According to the results of the biochemical test of the blood serum, the level of glucose and the activities of alkaline phosphatase, alanine aminotransferase, and gamma-glutamyltransferase were significantly higher in *O. felineus* infected participants than in uninfected ones. The levels of protein, total and conjugated bilirubin were statistically significantly lower in infected patients (Table 1). After anthelmintic treatment, the frequency of abdominal pain (94% vs 40%, q < 0.01) and other clinical symptoms

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3.2. Sequencing results

Infection intensity varied between 2 and 3800 eggs/gram, and the mean rate count was 363 ± 1004 EPG of feces, with 92.8% of participants having low intensity infection (2–500 EPG). There were no differences in α-diversity between *O. felineus*-infected, uninfected, and treated patients for any of these indices (Fig. 1A to C; Table S3; Fig. S2). However, we identified significant positive correlation between infection intensity pretreatment and richness and α-diversity for each of the 3 metrics (Fig. 1D to F). One patient with an outlying infection intensity of 3800 EPG of feces was excluded only from the EPG and bacterial abundance associations testing for the purpose to control false positives associations.

PERMANOVA applied to all samples showed separation between *O. felineus*-infected and uninfected microbiomes (R² = 0.053, p = 0.006), as well as between *O. felineus*-infected and treated groups (R² = 0.072, p = 0.002) (Fig. 2). There was no significant differences in gut microbiota between samples collected from infected children before and after anthelmintic treatment (R² = 0.018, p = 0.168). Significant associations were observed for *O. felineus* infection (R² = 0.065, p = 0.002), age (R² = 0.038, p = 0.017), and skin rash presence (R² = 0.033, p = 0.026) (Table S4).

3.3. Microbial diversity, helminthic infection and treatment

For the *O. felineus*-infected patients, negative binomial regression was performed to identify associations between the infection intensity and the levels of microbial taxa. On the level of phyla, positive associations were identified for Firmicutes and Tenericutes, and negative associations were identified for Verrucomicrobia and Proteobacteria (Fig. S3A). On the family level, Desulfovibrionaceae and Erysipelotrichaceae showed direct associations with infection intensity, and inverse correlations were found for Bifidobacteriaceae (Fig. S3B). Among the genera positively associated with the liver fluke infection, we identified Lachnospira, Escherichia-Shigella, Bacteroides, Eubacterium eligens group, Ruminiclostridium 6, Barnesiella, Oscillibacter, Faecalitalea, and Anaerospirobacter. Healthy volunteers showed higher levels of Blautia, Lachnospiraceae FCS020 group and Eubacterium hallii group in comparison with the infected individuals (Table 2). The observations were generally consistent with the results of a complementary pipeline available online as interactive reports at https://biota.knomics.ru/opisthorchis-and-gut-microbiome-2019.

3.4. Gut microbiota composition and *O. felineus* infection

On the level of phyla, patients with *O. felineus* infection had depletion in Firmicutes and increase of Proteobacteria abundance in gut microbiome compared to healthy controls (Table 2).

On the family level, *O. felineus* infection was linked to an increase of Enterobacteriaceae in the gut microbiota of children (Table 2).

Among the genera positively associated with the liver fluke infection, we identified Lachnospira, Escherichia-Shigella, Bacteroides, Eubacterium eligens group, Ruminiclostridium 6, Barnesiella, Oscillibacter, Faecalitalea, and Anaerospirobacter. Healthy volunteers showed higher levels of Blautia, Lachnospiraceae FCS020 group and Eubacterium hallii group in comparison with the infected individuals (Table 2). The observations were generally consistent with the results of a complementary pipeline available online as interactive reports at https://biota.knomics.ru/opisthorchis-and-gut-microbiome-2019.

3.5. Infection intensity and gut microbiota

For the *O. felineus*-infected patients, negative binomial regression was performed to identify associations between the infection intensity and the levels of microbial taxa. On the level of phyla, positive associations were identified for Firmicutes and Tenericutes, and negative associations were identified for Verrucomicrobia and Proteobacteria (Fig. S3A). On the family level, Desulfovibrionaceae and Erysipelotrichaceae showed direct associations with infection intensity, and inverse correlations were found for Bifidobacteriaceae (Fig. S3B). Among the genera positively associated with the liver fluke infection, we identified Catenobacterium, Desulfovibrio, Mitsuokella, Ruminococcaceae UCG-003, Enterorhabdus, Howardella, Olsenella, Moraya, Ripkenella RC9 gut group, Candidatus Soleaferrea, Senegalimassilia, Eubacterium coprostansogenes group, Erysipelatoclostridium, Anaerostipes, Bifidobacterium, Eubacterium hallii group, Ruminococcus gravis group, Gordonibacter and Akkermansia abundances demonstrated negative associations with the number of *O. felineus* EPG of faeces (Fig. S3C).

3.6. Liver fluke treatment affects gut microbiota structure

After anthelmintic treatment Firmicutes abundance was increased whereas Proteobacteria level was decreased to the uninfected participants’ proportions (Table 2). Enterobacteriaceae content also was decreased to the levels observed in uninfected children. Drug administration led to a decrease of Synergistetes and Atoptobacteria levels compared with *O. felineus*-infected and control groups (Table 2).

At the genus level, in gut microbiota of praziquantel-treated patients we identified the reduction of Lachnospira, Ruminiclostridium 6,
**Anaerosporobacter** and **Escherichia-Shigella** abundances along with increase in **Blautia, Eubacterium hallii** group and **Lachnospiraceae** FCS020 group levels up to the control group values (Table 2). Additionally, we observed the depletion of **Roseburia, Megasphaera** levels and expansion of **Faecalibacterium, Eisenbergiella, Sellimonas, Vartbaculum, Rikenellaceae** RC9 gut group, **Erysipelatoclostridium** in gut microbiota after treatment compared with **O. felineus**-infected and control groups. **Flavonifractor** and **Leuconostoc** had higher proportions in gut microbiota after treatment compared with controls (Table 2). In addition, praziquantel treatment results in reduction of **Ruminococcus gauvreauii** group, **Howardella** and **Slackia** abundances in gut microbiome compared with controls and, for **Lachnospiraceae** NK4A136 group and ND3007 group, **Tyzzerella** 4, **Prevotella** 2, in comparison with **O. felineus**-infected patients (Table 2). Praziquantel-treatment did not reduce levels of **Bacteroides**, **Oscillibacter, Faecalitalea, Eubacterium eligens** group and **Barnesiella** augmented in the gut microbiota of **O. felineus**-infected individuals (Table 2).

4. Discussion

In this study, we described for the first time the changes in the gut microbiota of **O. felineus**-infected children before and after anthelmintic treatment based on oral praziquantel application in an endemic rural area. Neither the presence of **O. felineus** infection nor the praziquantel treatment were associated with differences in the alpha-diversity, richness, or clinical symptoms compared with the control group. In other studies the analysis of the microbiota by high-throughput sequencing revealed that infection with the liver flukes **O. viverrini** and **C. sinensis** lead to increases in alpha-diversity in microbial communities in the colon (Plieskatt et al., 2013; Xu et al., 2018). Analysis of the composition of the bile microbiota in the presence of **O. felineus** infection was carried out in two studies (in experimental animals and patients with cholelithiasis) using the sequencing of the 16S rRNA gene (Saltykova et al., 2016, 2017). In an experimental study, the **O. felineus** infection was associated with increased bacterial diversity in the bile ducts as assessed by the Chao index (Saltykova et al., 2017). The study of patients with cholelithiasis showed that the presence of **O. felineus** infection did not...
lead to increased diversity of bile microbiota (Saltykova et al., 2016). However, we found significant positive associations between the O. felineus infection intensity and gut community structure in children. We did not detect increased alpha-diversity of the microbiota, probably due to the low intensity of invasion in the study group. Additionally, the O. felineus infection is endemic and hence may have already left its imprint on the gut microbiota composition of all individuals in the study region (Easton et al., 2019).

Many microbial taxa were differentially abundant between the infected and non-infected individuals. In the main, significant microbiota changes were observed within the Firmicutes phylum, including the Clostridiales order. This result was consistent with some previous studies of the gut microbiota in subjects infected by soil-transmitted helminths. (Cooper et al., 2013; Rosa et al., 2018).

Our data revealed that O. felineus infection is associated with an increase in the relative abundance of pro-inflammatory Proteobacteria, mainly Enterobacteriaceae, and a decrease in the relative abundance of commensal Firmicutes (Blautia, Eubacterium hallii group, Lachnospiraceae FCS020 group). At the same time, at the genus level we observed an increase in the abundance of some bacteria from the Firmicutes compared with the control group. In contrast, a positive correlation was identified for Firmicutes and a negative association was found for Proteobacteria with the infection intensity. The decrease in Firmicutes and an increase in Proteobacteria taxa is one of the signatures of the microbial dysbiosis in inflammatory bowel diseases (IBD) (Gophna et al., 2006). Enterobacteriaceae, including Escherichia, were also increased in O. felineus infected samples. This family is associated with the liver fluke O. viverrini infection in hamsters (Plieskatt et al., 2013).

Helminth-induced changes in gut microbial diversity can participate in the development of hepatobiliary diseases (Carpenter, 1998; Ithitae-trakool et al., 2016; Sanduzzi Zamparelli et al., 2017; Scholte et al., 2018). A number of studies demonstrated that patients with liver cirrhosis and primary biliary cholangitis showed a significant increase in the abundance of the Enterobacteriaceae family compared with the healthy group (Liu et al., 2012; Lu et al., 2011). There is growing evidence that the numbers of the Enterobacteriaceae are linked to IBD (Nishida et al., 2018; Swidinski et al., 2002; Tyakht et al., 2018; Varela et al., 2013). A number of studies have shown that patients with celiac disease, ulcerative colitis, and Crohn’s disease have an increased proportion of E. coli in the gut microbiota compared to healthy individuals (Collado et al., 2009; Mondot et al., 2011; Nadal et al., 2007; Swidinski et al., 2002).

Compared to healthy participants, O. felineus-infected patients manifested a significant decrease of Blautia, Eubacterium hallii group, and an increase of Lachnospira abundances, that may produce anti-inflammatory short-chain fatty acids (SCFAs) (Park et al., 2015, 2012). One of the potential biomarkers of a healthy gut often considered in microbiome surveys is the relative abundance of butyrate-producing microbes (Chang et al., 2014; Machiels et al., 2014). Bacteria that ferment dietary fibers to produce SCFAs are typically reduced in mucosa and feces of patients with IBD, as compared to healthy individuals (Kumari et al., 2013; Morgan et al., 2012; Takahashi et al., 2016). Infection with intestinal helminthes probably increases bacterially produced SCFAs concentrations, and, as a result, allergic airway inflammation is attenuated in the host (Zais et al., 2015). On the other hand, the ability of SCFAs to promote host regulatory T-cell responses may contribute to limited immuno-pathology and promotes chronic parasite infection (Arpaia et al., 2013; Furusawa et al., 2013). However, we did not directly measure intestinal SCFAs levels. More studies are needed to dissect this effect of O. felineus infection.

At the genus level, the abundance of Eubacterium eligens group was significant increased in the O. felineus-infected children. E. eligens is a pectin-degrading Firmicutes species that has the anti-inflammatory potential by promoting the production of IL-10 by epithelial cells (Chung et al., 2017). Two taxa belonging to Bacteroidetes were significantly increased in O. felineus infected patients. Consistent with previous reports, several Bacteroides species can produce enterotoxin previously associated with ulcerative colitis, stimulate interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) secretion in intestinal epithelial cells and intensify colitis symptoms in a murine model of ulcerative colitis (Ohkusa et al., 2009), while some strains of this genus have anti-inflammatory effects (Gauffin Cano et al., 2012; Hiipala et al., 2020; Ihekweazu et al., 2019). In previous studies, the lower abundance of Bacteroides was observed in the C. sinensis infected patients (Xu et al., 2018). Since changes were observed in populations of several bacteria with putative pro-/anti-inflammatory properties, whether the gut microbiota affected by the O. felineus infection could either contribute to or protect from inflammation is difficult to ascertain and, therefore, further analyses are required.

We also evaluated the association between intensity of helminth infection and gut microbiota composition. Bifidobacteriaceae, including genus Bifidobacterium, and Akkermansia were negatively correlated with the number of O. felineus EPG of feces. Bifidobacterium is an important probiotic bacterium, considered as potentially anti-inflammatory taxa (Khokhlova et al., 2012). As it shown in a previous study, the abundance of this genus also has a negative correlation with EPG of feces in C. sinensis-infected individuals (Xu et al., 2018). Akkermansia is a mucin-degrading bacteria, reduction in their abundance has been associated with inflammation and alterations of metabolic processes (Verhoog et al., 2019). On the family level, Desulfovibrionaceae and Erysipelotrichaceae showed direct associations with infection intensity. These taxa were implicated in the development of obesity-related metabolic disorders (Zhang et al., 2010). Apparently, the gut microbiome signature of O. felineus combines features known to be both directly and inversely associated with different disorders highlighting the complex nature of the helminth’s interactions with the host and commensal microbiome.

Our results show that deworming was not associated with changes in microbial alpha diversity, however, some significant differences were seen in the abundances of some microorganisms in the gut microbiota of the infected children before and after anthelmintic treatment. Specifically, the abundance of bacteria belonging to the Enterobacteriaceae family, Escherichia, Lachnospira, Anaerospirobacter, Ruminiclostridium 6, and Blautia genera (affected by O. felineus infection) after treatment resembled the levels typical for the control group. Conversely, the disease-specific shifts for some taxa persisted as long as 3 months after treatment (Barnesiellaceae, Bacteroides). At the phylum level, praziqantel treatment was associated with a decreased relative abundance of Synergistetes. This phylum is associated with periodontal diseases (Belibasakis et al., 2013; McCracken and Nathalia Garcia, 2020). In the other study, a higher proportion Synergistetes was evident in the group of participants infected with O. felineus in comparison with the...
uninfected participants (Saltykova et al., 2016). We found a significant decrease in the proportion of bacteria *Megasphaera*, *Roseburia*, and *Olsenella* in praziquantel-treated patients compared to infected and control participants. It has been reported that patients with cirrhosis and Hepatitis C virus infection have a higher level of *Megasphaera* compared to healthy controls (Chen et al., 2016; Lv et al., 2016). *Olsenella* also is positively associated with soil-transmitted helminths infections across Liberia and Indonesia (Rosa et al., 2018). We observed that abundance of *Olsenella* directly correlates with the *O. felineus* infection intensity. Species of *Roseburia* genus are known to be important butyrate-producing bacteria (Louis and Flint, 2009). The abundance of other butyrate-producing bacteria, namely *Fecalibacterium*, was increased in praziquantel-treated patients in comparison with infected or control participants.

The data presented here have some limitations. In our study, participants were examined one time posttreatment. The 3-month time point allowed us to examine the longer-term impact of helminth clearance. This study was not aimed to estimate the data regarding the effect of short term removal of helminths on microbiota and the impact of praziquantel treatment on uninfected children. The V4 region of 16S rRNA was analyzed. Although sequencing a longer region would result in a more precise taxonomic classification of each read, the V4 format allows a more accurate estimate of the gut archaea levels than V3-4 (due to variability in amplicon length for these prokaryotes). Moreover, V4 region is widely used in human gut microbiome surveys (Klimenko et al., 2018; McDonald et al., 2018; Wei et al., 2020). As each gene region produces a certain bias, an alternative would be to use a mix of primers covering multiple gene regions (Barb et al., 2016; Tremblay et al., 2015).

### Table 2

Significant differences in the abundances of gut bacteria (at several taxonomic levels) between comparison groups.

<table>
<thead>
<tr>
<th>Phylotype</th>
<th>Estimated marginal means</th>
<th>ANOVA p-value</th>
<th>Between group p-value</th>
<th>Uninfected vs O. felineus-infected</th>
<th>Uninfected vs after treatment</th>
<th>O. felineus-infected vs after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>uninfected (SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>71.64 (14.31)</td>
<td></td>
<td>2.58E-06</td>
<td>1.57E-05</td>
<td>0.520</td>
<td>0.001</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>3757.84 (118.74)</td>
<td></td>
<td>0.004</td>
<td>0.009</td>
<td>0.969</td>
<td>0.018</td>
</tr>
<tr>
<td>Synergistetes</td>
<td>0.37 (0.33)</td>
<td></td>
<td>0.014</td>
<td>0.916</td>
<td>0.039</td>
<td>0.012</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>31.63 (9.94)</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.616</td>
<td>4.80E-06</td>
</tr>
<tr>
<td>Atopobacteriaceae</td>
<td>12.44 (4.90)</td>
<td></td>
<td>0.57 (0.24)</td>
<td>1.23E-07</td>
<td>5.53E-06</td>
<td>5.36E-07</td>
</tr>
<tr>
<td>Escherichia-Shigella</td>
<td>27.71 (9.21)</td>
<td></td>
<td>6.00E-06</td>
<td>0.0004</td>
<td>0.002</td>
<td>0.662</td>
</tr>
<tr>
<td>Lachnospira</td>
<td>2.67 (0.78)</td>
<td></td>
<td>0.003</td>
<td>0.004</td>
<td>0.570</td>
<td>0.048</td>
</tr>
<tr>
<td>Anaeropasibacter</td>
<td>0.18 (0.10)</td>
<td></td>
<td>0.0002</td>
<td>0.004</td>
<td>0.187</td>
<td>0.001</td>
</tr>
<tr>
<td>Ruminococcaceae 6</td>
<td>1.72 (0.73)</td>
<td></td>
<td>0.006</td>
<td>0.002</td>
<td>0.999</td>
<td>0.001</td>
</tr>
<tr>
<td>Eubacterium eligens group</td>
<td>3.42 (1.13)</td>
<td></td>
<td>0.007</td>
<td>0.006</td>
<td>0.476</td>
<td>0.117</td>
</tr>
<tr>
<td>Faecalibacterium</td>
<td>0.02 (0.02)</td>
<td></td>
<td>0.005</td>
<td>0.030</td>
<td>0.004</td>
<td>0.531</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>1053 (24.28)</td>
<td></td>
<td>0.006</td>
<td>0.014</td>
<td>0.017</td>
<td>0.999</td>
</tr>
<tr>
<td>Eubacterium hallii group</td>
<td>182.82 (22.52)</td>
<td></td>
<td>0.010</td>
<td>0.026</td>
<td>0.011</td>
<td>0.937</td>
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<tr>
<td>Lachnospiraceae FC9020 group</td>
<td>13.90 (2.13)</td>
<td></td>
<td>0.007</td>
<td>0.005</td>
<td>0.296</td>
<td>0.229</td>
</tr>
<tr>
<td>Sellimonas</td>
<td>1.49 (0.76)</td>
<td></td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Eisenbergella</td>
<td>1.18 (0.58)</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.905</td>
<td>0.008</td>
</tr>
<tr>
<td>Faecalibacterium</td>
<td>0.90 (0.32)</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.0002</td>
<td>3.47E-05</td>
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<tr>
<td>Erysipelotrichaceae</td>
<td>1.13 (0.45)</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.998</td>
<td>0.033</td>
</tr>
<tr>
<td>Verrucomicrobiu</td>
<td>0.02 (0.02)</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.0004</td>
<td>0.0002</td>
</tr>
<tr>
<td>Megasphaera</td>
<td>16.96 (11.86)</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.0004</td>
<td>0.0002</td>
</tr>
<tr>
<td>Roseburia</td>
<td>54.37 (9.86)</td>
<td></td>
<td>0.003</td>
<td>0.018</td>
<td>0.001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Olsenella</td>
<td>3.51 (1.42)</td>
<td></td>
<td>0.003</td>
<td>0.0002</td>
<td>0.722</td>
<td>0.0002</td>
</tr>
<tr>
<td>Rikenellaceae RC9 gut group</td>
<td>2.31 (1.36)</td>
<td></td>
<td>0.001</td>
<td>0.006</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>0.12 (0.10)</td>
<td></td>
<td>0.003</td>
<td>0.003</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>Flavonifractor</td>
<td>0.36 (0.16)</td>
<td></td>
<td>0.003</td>
<td>0.003</td>
<td>0.170</td>
<td>0.170</td>
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<tr>
<td>Oscillobacter</td>
<td>0.99 (0.26)</td>
<td></td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.554</td>
</tr>
<tr>
<td>Howardella</td>
<td>3.39 (1.10)</td>
<td></td>
<td>0.006</td>
<td>0.008</td>
<td>0.738</td>
<td>0.738</td>
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<tr>
<td>Ruminococcus gauveraei group</td>
<td>45.35 (9.55)</td>
<td></td>
<td>0.007</td>
<td>0.008</td>
<td>0.0003</td>
<td>0.398</td>
</tr>
<tr>
<td>Oscilka</td>
<td>3.04 (0.87)</td>
<td></td>
<td>0.007</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Rikenellaceae ND5007 group</td>
<td>11.35 (2.13)</td>
<td></td>
<td>0.003</td>
<td>0.0004</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Prevotella 2</td>
<td>2.14 (1.28)</td>
<td></td>
<td>0.005</td>
<td>0.035</td>
<td>0.019</td>
<td>0.441</td>
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<tr>
<td>Tyzzerella 4</td>
<td>1.45 (0.74)</td>
<td></td>
<td>0.004</td>
<td>0.004</td>
<td>0.061</td>
<td>0.003</td>
</tr>
</tbody>
</table>

SE, Standard Error. P < 0.05 indicates significant difference.
5. Conclusion

We obtained new data regarding the impact of *O. felineus* infection and praziquantel treatment on the intestinal microbiome, and some of these effects were associated with the infection intensity. These results could be used to the prevention, and treatment of *O. felineus* infection, and the potential development of new strategies for the control of chronic non-communicable diseases. This requires further studies.

### Data availability

The data sets supporting the conclusions of this article are available in the European Nucleotide Archive (accession number PRJEB35995).

### Funding

This work was supported by the grant of the Russian Foundation for Basic Research (“Tools to diagnose carcinogenic liver fluke infection”, N19-51570004, to IS, VAP).

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgments

We acknowledge support and collaboration of the TOPIC (Tomsk OPistrictchoris Consortium), “www.topic-global.org”. We thank Mr. Jared Palazza, expert of Center for International Research and Development, Siberian State Medical University, for the efficient English editing.

### Supplementary materials


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