UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Posgrados

Gastrointestinal microbiota of children 6-8 years of age living in Cuenca, Guayllabamba and Uyumbicho (Ecuador)

Lucía Patricia Fiallos Cazar

Paúl Cárdenas, Ph.D.

Director de Trabajo de Titulación

Trabajo de titulación de posgrado presentado como requisito

para la obtención del título de Magister en Microbiología

Quito, 14 de mayo de 2019

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO DE POSGRADOS

HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

Gastrointestinal microbiota of children 6-8 years of age living in Cuenca, Guayllabamba and Uyumbicho (Ecuador)

Lucía Patricia Fiallos Cazar

	Firmas
Paúl Cárdenas, M.D., Ph.D. Director del Trabajo de Titulación	_____
Gabriel Trueba, Ph.D. Director de la Maestría de Mirobiología Miembro del Comité de Tesis	_____
Enrique Terán, Ph.D. Miembro del Comité de Tesis	_____
Hugo Burgos, Ph.D. Decano del Colegio de Posgrados	_____

Quito, 14 de mayo de 2019

© Derechos de Autor

Por medio del presente documento certifico que he leído todas las Políticas y Manuales de la Universidad San Francisco de Quito USFQ, incluyendo la Política de Propiedad Intelectual USFQ, y estoy de acuerdo con su contenido, por lo que los derechos de propiedad intelectual del presente trabajo quedan sujetos a lo dispuesto en esas Políticas.

Asimismo, autorizo a la USFQ para que realice la digitalización y publicación de este trabajo en el repositorio virtual, de conformidad a lo dispuesto en el Art. 144 de la Ley Orgánica de Educación Superior.

Firma del estudiante: _____

Nombre: Lucía Patricia Fiallos Cazar

Código de estudiante: 00109086

C. I.: 172091580-8

Lugar, Fecha Quito, 14 de mayo de 2019

DEDICATORIA

A mi padre Patricio Fiallos, por su apoyo, a mi madre Mariana Cazar por su amor y respaldo, a mi hermano Pedro Fiallos, a Boris Franco a mi hijo Ariel Franco, mi familia en general, a mis amigas y a mis compañeros del Instituto de Microbiología de la USFQ.

AGRADECIMIENTOS

Al Instituto de Microbiología de la Universidad San Francisco de Quito. A mi director de tesis Paúl Cárdenas, Ph.D. a Gabriel Trueba, Director de la maestría y Enrique Terán, Ph.D., miembro del tribunal y proyecto, por su constante ayuda. Al proyecto “Estudio del estado Inmunológico de niños en edad escolar y su relación con el microbioma intestinal y con el agua potable que consumen” y a los miembros del Centro de Biomedicina de la Universidad Central y a la Universidad de Cuenca. A María Daniela Garcés y María Belén Prado.

RESUMEN

La microbiota intestinal es una comunidad microbiana compleja compuesta de procariotas, eucariotas y arqueas que viven en simbiosis con su hospedador y están involucradas en el desarrollo saludable de los humanos. La microbiota intestinal aparece en el momento del nacimiento, pero se estabiliza después de los 3 años de edad, sin embargo, factores ambientales como la dieta, la ubicación geográfica, la presencia de parásitos, el estilo de vida y el consumo de antibióticos pueden afectarla y cambiarla. En este trabajo, comparamos la microbiota intestinal de niños de 6 a 8 años de edad de tres lugares diferentes (Cuenca, Guayllabamba y Uyumbicho) mediante la secuenciación del gen que codifica para el 16S ARNr obtenido de muestras de heces con tecnología MiSeq Illumina y analizamos los diferentes factores ambientales que podrían influir en la microbiota intestinal con el programa bioinformático Qiime2.org versión 2018.11. Los resultados encontrados mostraron que la ubicación geográfica y la presencia de *Entamoeba coli* y *Entamoeba histolytica* tienen un efecto en la beta diversidad de la composición de la microbiota, igualmente la distancia geográfica afectó la abundancia relativa de la microbiota pues se observó el incremento de géneros como *Prevotella* u órdenes como Clostridiales y frente a la infección con *Entamoeba* se observó el incremento de la abundancia relativa de la familia Ruminococcaceae, o el orden Bacteroidales en la microbiota intestinal de estos niños, sin embargo, no se observó ninguna diferencia en alfa diversidad.

***Palabras clave**: Microbiota intestinal, beta-diversidad, niños, Uyumbicho, Cuenca, Guayllabamba, Entamoeba histolytica, Entamoeba coli, ubicación geográfica.*

ABSTRACT

The intestinal microbiota is a complex microbial community composed of prokaryotes, eukaryotes and archaea that live in symbiosis with their host and are involved in the healthy development of humans. The intestinal microbiota appears at the time of birth, but stabilizes after 3 years of age, however, environmental factors such as diet, geographic location, presence of parasites, lifestyle and consumption of antibiotics can affect and change it. In this work, we compared the intestinal microbiota of children from 6 to 8 years of age from three different places (Cuenca, Guayllabamba and Uyumbicho) by sequencing the gene that codes for the 16S rRNA, obtained from stool samples, with MiSeq Illumina technology and analyzed the different environmental factors that could influence the intestinal microbiota with the bioinformatic program Qiime2.org version 2018.11. The results showed that the geographic location and the presence of *Entamoeba coli* and *Entamoeba histolytica* have an effect on the beta diversity of the composition of the microbiota, also the geographical distance affected the relative abundance of the microbiota because the increase of genera such as *Prevotella* or order as Clostridiales and in the same way in the infection with *Entamoeba* was observed the increase of the relative abundance of families like Ruminococcaceae, or order as Bacteroidales in the gut microbiota of these children. However, no difference in alpha diversity was observed.

Key words: *gut microbiota, beta-diversity, children, Uyumbicho, Cuenca, Guayllabamba, Entamoeba histolytica, Entamoeba coli, geographic location.*

CONTENTS

TABLES

FIGURES

INTRODUCTION

The gut microbiota

The gut microbiota is a complex and dynamic population of microorganisms formed by archaea, bacteria and eukarya. In healthy adults, it forms a symbiotic relationship that plays a role in human physiology (Bär, Phukan, Pinheiro and Simoes-Barbosa, 2015; Donaldson, Lee and Mazmanian, 2016; Morton, Lynch, Froment, Lafosse, Heyer, Przeworski, Blekhman and Ségurel, 2015; Burgess, Gilchrist, Lynn, and Petri, 2017; Thursby and Juge, 2017). There are approximately 100 trillion microorganisms within the gastro-intestinal tract of humans, which represent half the number of somatic cells within the body (Chong, Bloomfield and O´Sullivan, 2018; Wen and Duffy, 2017). Most of them are bacteria, but the gut can also harbor yeasts, single-cell eukaryotes, viruses and small parasitic worms (Rodríguez, Murphy, Stanton, Ross, Kober, Juge, Avershina, Rudi, Narbad, Jenmalm, Marchesi and Collado, 2015). The type, number and function of different bacteria who inhabit the intestine, vary according to its position in intestinal mucosa being in greater abundance and diversity found within the large bowel (Hillman, Lu, Yao and Nakatsu, 2017; Thursby and Juge, 2017). Bacterial gut microbiota contributes to nutrients absorption from food, and to respond to caloric deficit (since it intervenes in fermentation of undigested food components and fecal bulk) (Conlon and Bird, 2015; Gupta, Mohammed, Ghosh, Kanungo, Nair and Mande, 2011; Rinninella, Raoul, Cintoni, Franceschi, Miggiano, Gabarrini, Mele, 2019). It has been found that the composition of gut bacterial microbiota might be correlated to obesity (Sun, Ma, Ma, Zhang, Zhao and Nie, 2018), malnutrition (Gupta, et al., 2011), Crohn's disease., non-intestinal autoimmune diseases (type-1 diabetes, systemic lupus erythematosus, psoriasis, etc) (Opazo, Ortega-Rocha, Coronado-Arrázola, Bonifaz, Boudin, Neunlist, Bueno, Kalergis and Riedel, 2018). It also influences in intestinal mucus production,

adherence, liver function (Donaldson, Lee and Mazmanian, 2016) and even allergic diseases (Bunyavanich, Shen, Grishin, Wood, Burks, Dawson, Jones, Leung, Sampson, Sicherer, and Clemente, 2016).

Furthermore, the gut microbiota plays a key role in immunological and metabolic pathways (Marko and Pawliczak, 2017). For example, in the first months of life the gut microbiota induces the T cell response, and induces production of IL-10, whilst other cytokines that affect the Th1/Th2 balance and promote immunotolerance (Marko and Pawliczak, 2017; Francino, 2018). It is known that gut microbial composition depends principally of dietary habits and their interaction with the host systems (Sheflin, Melby, Carbonero and Weir, 2017). However, the association of microbiota with different diets in human populations, the effect of geographical location as well as the effect of eukaryotic parasites (protozoa and helminths) must be further studied.

Development of gut microbiota

Human intestinal microbiota is seeded before birth principally by the maternal microbiota (Wen and Duffy, 2017). For example, one-month-old babies who were delivered naturally had higher gut bacteria (colonized by maternal vaginal and fecal bacteria, including *Lactobacillus* and *Bifidobacterium* spp.) than those delivered by caesarean section (colonized with microbes associated with the skin and the hospital environment) (Francino, 2018; Laforest-Lapointe and Arrieta, 2017). Although perinatal factors such as mode of delivery, mode of infant feeding, use of special medicaments or antibiotic, diet and genetic can shape also the infant gut microbiota (Wen and Duffy, 2017). During lactation breast milk promotes the growth of *Lactobacillus* and *Bifidobacterium* in the infant gut, thanks to the presence of

oligosaccharides that serve as a substrate for these bacteria (Bardanzellu, Fanos, Strigini, Artini and Peroni, 2018). It has been reported that the major phyla found in the infant microbiota are Firmicutes, Bacteroidetes, Actinobacteria and Protecteobacteria, which help the immune system development (Conlon and Bird, 2015; Rutayisire, Huang, Liu and Tao, 2016). During weaning the relative abundances of Lachnospiraceae and Ruminococcaceae increases, but is important to mention that the microbiota composition is unique for each person (Leong, Haszard, Lawley, Otal, Taylor, Szymlek-Gay, Fleming, Daniels, Fangupo, Tannock and Heath, 2018).

In general, the establishing of human gut microbiota occurs by the end of the first 3-4 years old when infants acquire a microbiota profile converging towards the characteristic adult microbiota (Gilchrist, Petri, Schneider, Reichman, Jiang, Begum, Watanabe, Jansen, Elliot, Burgess, Ma, Alam, Kabir, Haque and Petri, 2016; Odamaki, Kato, Sugahara, Hashikura, Takahashi, Xiao, Abe and Osawa, 2016). The functional development of human microbiota includes the capacity of bacteria to produce vitamins and the increase of microorganism diversity that degrade fiber and other nutrients present in adult diets (Jandhyala, Talukdar, Subramanyam, Vuyyuru, Sasikala and Reddy, 2015; Thursby and Juge, 2017; Wang, Yao, Lv, Ling and L1, 2017). It is important to consider that the gut microbiota can be further altered as a result of bacterial infections, antibiotic treatments, lifestyles, surgeries, and diet changes (Lindsay, Oundo, Hossain, Antonio, Tamboura, Walker, Paulson, Paarkhill, Omore, Faruque, Das, Ikumapayi, Adeyemi, Sanogo, Saha, Sow, Farag, Nasrin, Li, Panchalingam, Levine, Kotloff, Magder, Hungerford, Sommerfelt, Pop, Nataro and Stine, 2015; Mueller, Bakacs, Combellick, Grigoryan and Dominguez-Bello, 2015).

Factors that model the gut microbiota

According to Lloyd et al. (2016), the microbiota is composed by a significant number of different bacteria nevertheless it has been identified a "core" set of bacterial taxa universally which proportions and compositions are susceptible to change for environmental factors like the family members and close relatives (Davenport, Sanders, Song, Amato, Clark and Knight, 2017), the geographical location (Laforest-Lapointe and Arrieta, 2017), dietary patterns and lifestyle in a specific area (Lloyd, Abu-Ali and Huttenhower, 2016).

The host genetics also contribute to shape the gut microbiota, but their interaction is difficult to elucidate (Rothschild, Weissbrod, Barkan, Kurilshikov, Korem, Zeevi, Costea, Godneva, Kalka, Bar, Shilo, Lador. Vila, Zmora, Pevsner-Fischer, Israeli, Kosower, Malka, Wolf, Avnit-Sagi, Lotan-Pompan, Weinberger, Halpern, Carmi, Fu, Wijmenga, Zhernakova, Elinav and Segal, 2018; Davenport,et al, 2017). A study of children showed that the degree of similarity in the gut bacterial community was higher in monozygotic twins compared with dizygotic twins and it was the lowest in the unrelated control group (Goodrich, Waters, Poole, Sutter, Koren, Blekhman, Beaumont, Van Treuren, Knight, Bell, Spector, Clark and Ley, 2014). This suggests that the possible impact of host genotype on microbiota is less pronounced due to the diet and other environmental and maternal factors on the structure of the infant gut microbiota (Rodríguez, et al., 2015).

Diet and lifestyle

The relative proportions of gut microbiota can vary between individuals or within an individual (Davenport, et al., 2017) according to life events such as puberty, ovarian cycle, pregnancy and aging (Jaŝarević, Morrison and Bale, 2016; Monda, Villano, Messina,

Valenzano, Esposito, Moscatelli, Viggiano, Cibelli, Chieffi, Monda, and Messina, 2017). However, diet is the most critical environmental factors shaping gut microbiota composition (Wen and Duffy, 2017) and in case of children, diet may have a particular influence on microbial diversity (De Filippo, Cavalieri, Di Paola, Ramazzotti, Poullet, Massart, Collini, Pieraccine and Lionetti, 2010; Turnbaugh, 2017). For instance, during childhood the major source of bacteria in the infant gut is obtained by the mother's milk, where the microbiota mainly includes *Staphylococcus*, *Streptococcus*, (Biagi, Quercia, Aceti, Beghetti, Rampelli, Turroni, Faldella, Candela, Brigidi and Corvaglia, 2017; Mueller, et la., 2015) *Serratia, Pseudomonas, Corynebacterium, Ralstonia, Propionibacterium, Sphingomonas,* and *Bradyrhizobiaceae* (Mueller, et al., 2015). The introduction of solid food in weaning is accompanied by an increase of gut microbiota diversity, specially the colonization of butyrate producers, like Prevotellaceae, *Bacteroides,* and certain *Clostridium* species (Gilchrist, et al., 2016; Rodríguez, et al., 2015).

Regarding to life style, the exercise may be an important influence in the enrichment of diversity and fitness improvement of commensal bacteria (Mach and Fuster-Botella, 2017). A recent study showed an increase in the diversity of gut microbial populations in response to exercise and diet, for example, people with obesity were found to have an increase of Firmicutes and a reduction of Bacteroidetes (Aydin, Nieuwdorp and Gerdes, 2018; Davis, 2016), which could potentially contribute to adiposity gain through greater energy harvest (Conlon and Bird, 2015; Monda, et al., 2017).

Geographic location

The geographical area of living jointly with diet also influence the composition of gut microbiota populations. It has been seen that the diversity and number of fecal microbes associated with metabolism of fiber in children from rural Africa is greater than children of Europe Union (De Filippo, et al., 2010). Other example was found in European children who consumed a calorie-dense, high-fat, low fiber diet resulting in a greater relative abundance of *Proteobacateria* in comparison to children from Burkina Faso who had a diet with low-fat and high-fiber (Shin, Whon and Bae, 2015). Thus, this study evidences that dietary preferences from different geographical regions contribute significantly to the gut microbiota although factors like infection diseases (including those causing diarrhea), poor sanitary conditions and poor personal hygiene (Burgess, et al., 2017; Lindsay, et al., 2015).

Gut microbiota and parasitic protozoans

The intestine is principally inhabited by commensal bacteria that influence overall the host metabolism, immune system, and interacts with other microorganisms like protozoan parasites (Burgess and petri, 2016; Hillman, et al., 2017). In the case of children in developing countries, are susceptible to parasitic infections because of the lack of health service and undernutrition, or limited access to clean drinking water allowing the prospering of intestinal parasites and modification of the gut microbiota (Mabbott, 2018; Toro-Londono, et al., 2019). Among the protozoan parasites that infect the intestine, *Entamoeba, Cryptosporidium* and *Giardia* are parasitic protozoans causing diarrhea (Mabbott, 2018; Osman, Safadi, Cian, Benamrouz, Nourrisson, Poirier, Pereira, Razakandrainibe, Pinon, Lambert, Wawrzyniak, Dabboussi, Delbac, Favennec, Hamze, Viscogliosi and Certad, 2016), and being amoebiasis one of the most common throughout the tropics (Berhe, Bugssa, Bayisa

and Alemu, 2018;). Although host genetics and variation in immune response contribute to protection to these parasites, the intestinal microbiota may have a significant influence over the disease progression (Bär, et al., 2015). For example, a study *in vitro* of the effects of *Lactobacillus acidophilus* strains and *Lactobacillus johnsonii* La1 (NCC533) on *Giardia duodenalis* strain WB trophozoites demonstrated that *L. johnsonii* La1 inhibited the proliferation of *Giardia trophozoites* and these results were confirmed with *in vivo* experiments with La1-treated gerbils which were protected against *Giardia* infection and mucosal damage leading to a resolution of infection (Berrilli, Cave, Cavallero and D´Amelio, 2012). In the same way another study showed, in a co-cultured of *Lactobacillus casei* or *Enterococcus faecium* (common human commensal bacteria) with *Entamoeba histolytica*, the reduction of parasite survival by 71%, and when both bacteria were combined the survival of parasite was reduced by 80% demonstrating that the gut microbiota can influence the progression of parasitic infection (Burgess, et al., 2017). Also, parasitic infections could alter the microbiota; *Entamoeba* feeds on bacteria and some *Entamoeba* trigger a strong inflammatory response upon invasion of the colonic mucosa (Huston and Petri, 1998; Loftus, Anderson, Davies, Alsmark, Samuelson, Amedeo, Roncaglia, Berriman, Hirt and Mann, 2005; Varet, Shaulov, Sismeiro, Trebicz-Gefen, Legendre, Coppée, Ankri, and Guillen, 2018)

It is important to mention that the characterization of microbiota is possible thanks to the development of next generation sequencing techniques and biological computational tools that allowed to perform multiplexing analyses that help to understand the interactions, mentioned before, between microbial communities, parasites and the host (De Filippo, et al., 2010 Cao, Fanning, Proos, Jordan, Srikumar; Toro-Londono, Bedoya-Urrego, García-Motoya, Galvan-Diaz and Alzate, 2019).

In this sense of research of child gut microbiota, the main objective of the current study is to analyze gut microbiome from 6 to 8 years old children from three different localities in Ecuador (Guayllabamba, Uyumbicho and Cuenca) using 16S rRNA gene sequencing, and the correlation with social demographic factors.

MATERIALS AND METHODS

Study design and participants

We conducted a cross-sectional study, to investigate gut microbiota profiles in children on ages ranging from six to eight years old, in the localities: Uyumbicho, Guayllabamba and Cuenca, Ecuador. From a total of 231 children recruited we collected 2 stool samples from each; one sample was used to extract DNA and perform microbiota analysis, whilst the other sample was used for parasitology determination. The participants were grouped according to the site of their residence: 1. Guayllabamba located in the canton Quito, province Pichincha, with a population of approximately 18,000 inhabitants. It is a rural area and its water network comes from Papallacta (Gobierno autonomo descentralizado Parroqui Rural, 2019); 2. Uyumbicho located in canton Mejía, province Pichincha, with a surface of 30 km^2 and a population of approximately 3,679 people. It is a rural area and its water network come from Cotopaxi (Gobierno autonomo Descentralizado Uyumbicho, 2019) and 3. Cuenca located in province of Azuay, with a population of approximately 580,000 people. It´s an urban area, with a surface of 15,730 hectares and its water network come from Tixán (Cuenca-Ecuador, 2019). Additionally, socio-economically information was collected using detailed questionnaires given to parents.

The project protocols and methods were approved by the Universidad San Francisco de Quito Research Ethics Committee 2017-152-M, and parents were asked for writing consent prior enrollment.

Samples

Fecal samples were collected between February, March and June 2018. Parents answered a questionnaire providing information about: antibiotics use during the last 15 days, illness, early diet, water consumption, and demographic data (age, sex, ethnic self-identity, etc.), by the time that samples were collected. The parents were instructed how to collect the fecal samples and immediately store them at -20° C. All samples were transported on ice to Instituto de Investigación en Biomedicina- Universidad Central del Ecuador- Calderón, where the parasitology analyzes were done and the microbiota samples was stored at -80° C, finally, the samples for microbiota analyses were transported on ice to Microbiology Institute - San Francisco University - Cumbayá and stored at -80° C until processing. Children included in this study had to regularly consume drinking water from their place of residence. Children who used antibiotics within the last 15 days preceding fecal sampling, didn´t filled questionnaires, or didn't give a stool sample for parasitology analyses were excluded. The final sample set analyzed were 105, in table 1 are summarized the samples collected and analyzed.

Laboratory Procedures:

DNA extraction

Microbial DNA was extracted using commercial FastDNA^{MT} SPIN Kit for Soil (MP Biomedical, Solon, OH, USA), according to the manufacturer´s instructions.

DNA quality and quantity

Concentration of extracted DNA (absorbance at 260 nm) and its purity (absorbance ratio 260/230 and 260/280) were measured using Nanovue plus (GE Healthcare, UK). All the DNA samples were lyophilized until analysis.

PCR amplification and sequencing

The V3-V4 region of the 16S rRNA gene was amplified using PCR with barcoded primers and sequencing was performed on the Illumina MiSeq platform. Sequences were generated using an Illumina MiSeq instrument (Bunyavanich, et al., 2016; Leong, et al., 2018). This procedure was realized in the University of North Carolina at Chapel Hill. A total of 41903835 quality reads were obtained.

Bioinformatics analysis

The sequences were analyzed using the third-party software Quantitative Insights into Microbial Ecology (QIIME2) 2018.11 (Bunyavanich, et al., 2016; Caporaso, et al., 2010), to perform 16S rRNA gene microbiome analysis from raw DNA sequencing data (Bolyen. et al., 2018). Within the 3 site residence groups for analysis, alpha- diversity (richness and evenness) were estimated using Faith's phylogenetic diversity (Bunyavanich, et al., 2016). Between the groups, beta-diversity was analyzed by: Bray-Curtis index, unweighted UniFrac, weighted UniFrac, and Jaccard distance, for visualized patterns between samples based on beta diversity distances we used principal coordinate analysis (PCoA), the statistic used to group significance for each metric was PERMANOVA (Bunyavanich, et al., 2016; Leong, et al., 2018). Significance of PERMANOVA tests was determined using 999 permutations with adjustment for multiple testing (Bunyavanich, *et al*., 2016). Taxonomy classifications were made by using Greengenes database (Almeida, Mitchell, Tarkowska and Fin, 2018), where the sequences only include 250 bases from the region of the 16S (Bolyen. et al., 2018). The variables analyzed in the tree groups were: residence site, province, date sampling, age, BMI, race, sex, stature, diarrhea, deworming, early nutrition, presence/absence of: *Ascaris lumbricoides*, *Entamoeba histolytica*, *Entamoeba coli*, *Giardia lamblia*, *Strongyloides stercoralis*, *Enteromonas intestinalis* and *Embadomonas intestinalis.*

The relative abundance was analyzed with statistic “Analysis of composition of microbiomes” (ANCOM), which is used for comparing the composition of microbiomes in two or more populations (Mandal, Van Treuren, White, Eggesbø, Knight & Peddada, 2015) and GNEISS. Both tests were run through the QIIME2 command line interface using default parameters. The test was out on the full OTU table and on OTU tables that were filtered to contain data from the residence site in order to concentrate on differences between the localities and the distinct social-economy variables (Leong, et al., 2018; Ma, et al., 2018). We focused in the variables: residence site, province, date of sampling, *Entamoeba histolytica* and *Entamoeba coli* for ANCOM, and for GNEISS analysis as were significant in the PERMANOVA analysis.

RESULTS

1. Children gut microbiota comparison between Cuenca, Guayllabamba and Uyumbicho

The relative abundances in proportion plots indicated the predominance of genera *Clostridium, Prevotella* and *Sporobacter* in positive subjects for *Entamoeba coli*, from Cuenca and Guayllabamba, as well as bacteria from the family Ruminococcaceae, order Bacteroidales and phylum Firmicutes, while the negative subjects did not exhibit an increase of bacterial abundance (figure 1)

2. Children gut microbiota comparison between Uyumbicho and Guayllabamba

We analyzed and compared the children gut microbiota composition between samples form Uyumbicho and Guayllabamba. In the comparison of the beta-diversity microbiota of children from the two localities, we found that only the variable *Entamoeba coli* would be modifying the gut microbiota composition. Unweighted UniFrac PCoA (variance= 23.70%) for *Entamoeba coli* showed a light clustering between positive subjects (figure 2), whilst weighted UniFrac PCoA analysis (variance= 40.51%) did not show any clustering between the samples. PERMANOVA analysis confirmed a significant difference in beta-diversity in microbiota composition (p=0.027), in children positive for *Entamoeba coli*, residents in Uyumbicho and Guayllabamba.

Relative abundance of gut microbiota between children positive and negative to *Entamoeba coli* in the two localities (Uyumbicho and Guayllabamba) indicated the predominance of genera *Clostridium, Prevotella, Bacteroides* family Ruminococcaceae, order

Bacteroidales and phylum Firmicutes in subject positive for *Entamoeba coli*, while the negative subject did not exhibit a significant increase in relative abundance (figure 3).

The alpha-diversity analysis of gut microbiota between children from Uyumbicho and Guayllabamba and in the other variables did not find any significance.

3. Children gut microbiota comparison between Uyumbicho and Cuenca

In the analysis of gut microbiota in children from Uyumbicho and Cuenca we found that some variables that were influencing the beta-diversity of microbiota: residence site, province, date sampling and *Entamoeba histolytica*. beta-diversity and PERMANOVA analyses between Uyumbicho and Cuenca like unweighted UniFrac PCoA (variance= 22.91%) and weighted UniFrac PCoA (variance= 40.45%) did not indicate differences between Uyumbicho and Cuenca. However, PERMANOVA analysis indicated that the structure of the infant gut microbiota is affected by this variable ($p = 0.034$) (table 2).

The relative abundances in proportion plots indicated the predominance of the genus *Prevotella* in children from Uyumbicho, while in children from Cuenca more abundant were *Butyrivibrio* and *Bacteroides*, the order Clostridiales and phylum Bacteroidetes (figure 4).

In the comparison of the beta-diversity between children gut microbiota of Uyumbicho and Cuenca we found that the variable “province” was influencing the gut microbiota composition. In the PERMANOVA analysis we encountered significant differences (p= 0.024) and the beta-diversity of gut microbiota was influenced by the location of children. However, unweighted UniFrac (variance= 23.91%) and weighted UniFrac PCoA (variance=

40.45%) did not show clustering. In the same way the analysis with the confounder *Entamoeba histolytica* the unweighted UniFrac PCoA (variance= 23.91%) and weighted UniFrac PCoA (variance= 40.45%) no obvious grouping was observed between the positive or negative samples. While in PERMANOVA analysis *Entamoeba histolytica* played an important role in modeling the gut microbiota in children because significant differences were found in beta-diversity of gut microbiota in children positive and negative to this parasite (p = 0.044), so it might mean that the beta-diversity of gut microbiota was influenced by the presence of *Entamoeba histolytica*

3.1. Gut microbiota comparison between negative/positive to *Entamoeba histolytica* from Uyumbicho and Cuenca.

Children positive to *Entamoeba histolytica* presented relative abundance for genera *Sporobacter* and *Prevotella* and families Ruminococcaceae and Veillonellaceae, and the orders Bacteroidales and Clostridiales (figure 5).

4. Comparison of gut microbiota between children from Pichincha and Azuay

The proportion plots indicated more relative abundance of genus *Prevotella* in children from Pichincha, while in children from Azuay the genera more abundant were *Butyrivibrio* and *Bacteroides*, the order Clostridiales and phylum Bacteroidetes (figure 6).

In alpha analysis we did not find any association or significance in the gut microbiota of children from Uyumbicho and Cuenca.

5. Gut microbiota comparison between Guayllabamba and Cuenca

In the analysis of beta-diversity of gut microbiota of children from Guayllabamba and Cuenca we found that only the confounder *Entamoeba coli* had a significance in the shaping of children microbiota composition

In the PCoA of unweighted UniFrac (variance= 22.85%) a tendency of clustering was observed between the positive samples for *Entamoeba coli* (figure 7), while in the weighted UniFrac (variance= 40.08%) any clustering was observed between the samples. The significances in PERMANOVA analysis indicated a strong correlation among the gut microbiota beta-diversity and the presence of the parasite in children from Guayllabamba and Cuenca, so *Entamoeba coli* would be the principal responsible of the possible differences between the gut microbiota of the two groups (p= 0.025).

The gut microbiota of children that were positive for *Entamoeba coli* presented a higher relative abundance for genera *Campylobacter, Prevotella* and *Desulfovibrio* and bacterial of families Lachnospiraceae and Ruminococcaceae, as well as class Clostridia and order Clostridiales (figure 1).

6. Other variables

In beta-diversity and α-diversity analysis we also evaluated the following variables: age, sex, weight, stature, body mass index, race, diarrhea, deworming, early nutrition (at first years of life), presence/absence of: *Ascaris lumbricoides, Giardia lamblia, Strongyloides stercoralis, Enteromonas intestinalis, Embadomonas intestinalis.* The results obtained were no

significant for beta-diversity and α-diversity. For this reason, we only presented the results of variables statistically significant.

DISCUSSION

In this cross-sectional study we compared children gut microbiota and different factors that could affect its composition. We concluded that geographical localization and the presence of *Entamoeba* spp. are two important factors that shape the gut microbiota in children from Cuenca, Guayllabamba and Uyumbicho.

There is a "core" of taxa prevalent among humans which varies according to the health of the individual (Lloyd-Price, et al., 2016). The gut microbiome starts developing at birth and its composition is shaped by multiple factors throughout life (Bär, et al., 2015). Environmental factors like diet and lifestyle predominantly shape gut microbiome composition; over 20% of beta-diversity variance is associated with them (Rothschild, et al., 2018).Evidence suggests also a link between gut microbiota composition and parasite colonization, since parasitic protozoa infect mucosal surfaces, which allows them to interact with local intestinal bacterial (Bär, et al., 2015; Chabé, Lokmer and Ségurel, 2017). Our results show that individuals who are infected with *Entamoeba coli* had a different gut microbiota composition. For example, children independent of their geographical location, who are positive for *Entamoeba coli,* had a predominance of the genera: *Clostridium*, *Prevotella* and *Sporobacter* (Ruminococcaceae family), as well as other species of the family Ruminococcaceae, order Bacteroidales and phylum Firmicutes. Comparatively, children from Uyumbicho and Guayllabamba infected with this parasite had increased relative abundance of genera *Bacteroides* (figure 3). On the other hand, children from Guayllabamba and Cuenca, positive for *Entamoeba coli,* presented predominantly *Campylobacter* sp., *Desulfovibrio* sp. and bacteria of the Lachnospiraceae and Clostridiaceae families. Regarding to *Entamoeba coli,* it is not considered an enteric pathogen (Hotez, 2000). Evidence suggests that parasites like *Entamoeba* spp.or other protozoa like *Blastocystis* spp. interact with the gut bacterial

community and the host modulating the host/gut/microbiota balance (Berrilli, et al., 2012; Lebba, Santangelo, Totino, Pantanella, Monsia, Di Cristanziano, Di Cave, Schippa, Berrilli and S´Alfonso, 2016). Burgess, et al. (2017) indicated that the taxa Prevotellaceae (order Bacteroidales) is an important predictor of *Entamoeba* spp. infection, a characteristic observed in our data where the relative abundance of genera *Prevotella* was higher in all individuals positive for infection with *Entamoeba coli*. Similarly, Lebba, et al. (2016) suggested that the increase of *Prevotella* sp. and *Bacteroides* sp. (phylum Bacteroidetes) could have a protective effect in the host, since these taxa are considered beneficial, and that the presence of *Entamoeba* spp. with other protozoa would induce an eubiotic condition (co-occurrence of potentially beneficial species together with a low percentage of potentially pathogenic species) in the intestine (Stensvold and Giezen, 2018).

Moreover, in the microbiota we can distinguish three bacterial patterns associated with the colonization of protozoa or other diseases, identifiable by the increase of *Bacteroides* sp., *Prevotella* sp., and Ruminococcaceae (Clostridiales) (Chabé, et al., 2017; Stensvold and Giezen, 2018; Toro-Londono, et al., 2019). Therefore, the data presented in this work on children from Cuenca, Guayllabamba and Uyumbicho, confirmed that infection with *Entamoeba coli* is associated with changes in the structure and composition of the gut microbiota in children, specifically by affecting the relative abundance of certain groups of bacteria such as *Prevotella* sp.. This alteration could happen because intestinal parasites constantly secrete molecules that change the intestinal environment, which could cause modification of the overall gut microbiota composition. In a similar way, different members of the microbiota could produce beneficial metabolites for the parasitic protozoa present (Berrilli, et al., 2012; Burgess, et al., 2017). Regarding *Bacteroides* genus, which relative abundance was increased in children from Uyumbicho and Guayllabamba positives to

infection with *Entamoeba coli*, is the most abundant genus in human gut microbiota and its species participate in carbohydrate metabolism, nevertheless according to (Verma, et al., 2012), abundance of *Bacteroides* sp. decreased in the gut microbiota in presence of illness (Verma, et al., 2012) but in our study, the genus *Bacteroides* increased in positive children to *Entamoeba coli*; this is agreement with other studies in which an enrichment of this genus has been observed in rotavirus infections (cause of diarrhea) (Sekirov, et al., 2010) or in diseases such as colorectal carcinoma (sometimes triggered by a infection by *H. pylori*) (Figura, Marano, Moretti and Ponsetto, 2016; Ishaq and Nunn, 2015), this is probably due to damage resulting from the alteration of host intestinal homeostasis, caused by the disease and not as a result of the interaction between the pathogen and the local microbiota (Sekirov, et al., 2010).

Bacteroidetes and Firmicutes are two of the most dominant phyla in human feces, (Dick, et al., 2009); in this study all of them were abundant in children infected with *Entamoeba coli.* In other studies, related to obesity or age, changes in the abundance of these bacteria have been observed, related to both their increase and reduction. These differences may be due to environmental factors, such as diet, physical activity and/or socioeconomic state (Koliada, et al., 2018). The same case would be happening with the family Lachnospiraceae and the genus *Clostridium* (phylum Firmicutes), which are susceptible to the effect of several environmental factors (Ravussin, et al., 2012), and probably in this case the environment factor that altered this composition was the presence of *Entamoeba coli.* With respect to the increase of *Desulfovibrio* sp. and genus *Campylobacter* , in children infected with *Entamoeba coli*, it has been observed that although *Desulfovibrio* sp. is part of "normal" microbiota (Dick, et al., 2005), some species are implicated in an intestinal damage by the hydrogen sulfide production, that would be contributing with chronic intestinal disorders (Verma, et al., 2012), instead, in general, *Campylobacter* sp. inhabits in the lower

gastrointestinal tract of healthy people (Burgess and Petri, 2016), and in the current study its abundance is higher in children infected with *Entamoeba coli*. Instead in the comparison of gut microbiota of children infected by *Entamoeba histolytica*, we observed an increase of genera *Sporobacter* sp. and *Prevotella* sp., families Ruminococcaceae and Veillonellaceae, and orders Bacteroidales and Clostridiales and the beta-diversity was statistically significant. With respect to *Entamoeba histolytica* has always been considered an intestinal pathogen that produces severe pediatric diarrhea and invasive amebiasis (Burgess and Petri, 2016; Verma, et al., 2012). It has previously been reported that *Entamoeba histolytica* is associated with increases in Clostridiaceae and Ruminococcaceae are increased within the gut microbiota composition (Morton, et al., 2015), as well as certain species of *Prevotella* sp. in infants from Bangladesh with asymptomatic diarrhea (Gilchrist, et al., 2016; Stensvold and Giezen, 2018) and in adults infected with *Entamoeba histolytica* from Cameroon (Burgess, et al., 2017; Morton, et al., 2015). In Indian patients this infection was related with the decreased prevalence of the genera *Clostridium*, *Bacteroides*, *Lactobacillus*, *Campylobacter* and *Eubacterium* (Chabé, et al., 2017; Verma, et al., 2012) which is consistent with our results. Regarding Veillonellaceae, Wang, et al (2017) indicated an increase of this family when there is dysbiosis in the gut microbiota, as a result of a disease. Altogether, these results suggest that this parasite is capable of modifying the intestinal microbiota composition, like we observed in our results.

In this work beta-diversity and gut microbiota composition were statistically different ($p=0.027$ and $p= 0.034$) when children infected and not infected with both *Entamoeba coli* and *Entamoeba histolytica* respectively were compared: other reports indicate that says that *Entamoeba* spp. infection is significantly correlated with microbiome diversity and composition, in fact in non-industrialized populations, the negative individuals for *Entamoeba*

spp. caused higher beta diversity, so the explanations could be related to a diet based on complex carbohydrates (Morton, et al., 2015; Rampelli, et al., 2015) or perhaps because of the infrequent or no use of antibiotics (Kim, Convington and Pamer, 2017), other explanation is that *Entamoeba* spp. preferentially feeding on certain bacteria, so this would promote the proliferation of other bacterial species, or induce a specific host immune response that could affect the survival of some microbes over others. (Berrilli, et al., 2012; Chabé, et al., 2017; Burgess and Petri, 2016; Morton, et al., 2015) Another possibility is that a specific gut microbiota predisposes a subject to *Entamoeba* spp. colonization (Burgess and Petri, 2016). However, both higher beta diversity and a different relative abundance in children infected with *Entamoeba* spp. were observed in our results.

The geographical location was another environmental factor that affected the beta-diversity in the gut microbiota in children of this study. We found a significant difference between gut microbiota in children from province locations Uyumbicho (Pichincha) and Cuenca (Azuay) (p= 0.034) and a different relative abundance in the comparison between the two locations and provinces, where we observed an increase in genus *Prevotella* sp. in children from Uyumbicho (Pichincha), while in children from Cuenca (Azuay) the more abundant genera were *Butyrivibrio* and *Bacteroides* , as well as the order Clostridiales and phylum Bacteroidetes (figures 4 and 6). Rodriguez et al. (2015) indicated that geographical location had an important impact on the microbiota, related to dietary patterns and lifestyle (city, town, country, etc.), whilst Chabé, et al. (2017) noted that the bacterial gut microbiome of industrialized populations had a marked decrease in bacterial diversity in comparison with the microbiome of non-industrialized as a result of lifestyle and hygiene differences. Furthermore, other studies (Fallani, et al., 2010) found that diet is the main influence on the infant gut microbiota, and this could affect the health of infants, as well as in later childhood

and adulthood. Additionally, they found a possible ‘‘geographic gradient’’ that impacts the gut microbiota composition between European infants, where those who came from northern areas had increased levels of *Bifidobacterium* sp., *Clostridium* sp. and *Atopobium* sp., while those who came from southern areas had increased abundance of *Eubacteria*, *Lactobacillus* sp., and *Bacteroides* sp.. Another study (De Filippo, et al., 2010) characterized and compared the gut microbiota of infants from Europe and Africa, and found that the microbiota of children from Burkina Faso (Africa), a rural population, had increased levels of the genera *Actinobacteria* sp. and *Bacteroides* sp., while in Florence (Italy), an urban area, the predominant bacteria were Firmicutes and Proteobacteria. These data could explain the results observed in the difference in microbiota composition of Uyumbicho and Cuenca because, firstly, the diet in the two locations is different, and secondly, that Uyumbicho is a rural area, located towards the northeast of the country with an undeveloped health and hygiene systems (Espín, 2014), while Cuenca is the urban capital of the Azuay province having a superior health system (Miller, n.d.). Even though these factors could affect the diversity and composition of the gut microbiota, however this effect was not observed in comparisons with Guayllabamba, which is also located in a rural area.

Diet is a factor that is also related to behaviors that may exist in different geographical areas, as well as to infection with parasitic protozoa, and is the predominant environmental factor that models the gut microbiota (Clarke, Murphy, Nilaweera, Ross, Shanahan, Toole and Cotter, 2012; Gupta, Paul and Dutta, 2017; Lloyd-Price, et al., 2016; Wen and Duffy, 2017). Diet could explain the presence of *Prevotella* sp. in the gut microbiota of children from Uyumbicho (Pichincha province), and of *Butyrivibrio* sp. in the gut microbiota of children from Cuenca (Azuay province), as both bacteria ferment xylane, carboxymethylcellulose and xylose to produce high levels of short-chain fatty acids (SCFAs). Physiologically, SCFAs

provide energy in the intestine and are rapidly and easily absorbed by the colon (De Filippo, et al., 2010). Nevertheless, it is important to make an analysis of the diet in these children to confirm this. In the case of *Prevotella* sp., it has been associated with rural populations and agrarian societies with a carbohydrate-based diet (Wu Chen, Hoffmann, Bittinger, Chen, Keilbaugh, Bewtra, Knights, Walters, Knights, Sinha, Gilroy, Gupta, Baldassano, Nessel, Li, Bushman and Lewis, 2011). On the other hand the genus *Bacteroides* sp., increased in the gut microbiota of children from Cuenca (Azuay), is usually related with western diets rich in animal protein and saturated fats (Conlon and Bird, 2015; Wu, et al., 2011), as well as *Prevotella* sp., the genus *Bacteroides* sp. also produces other short chain fatty acids (such as succinate and acetate) different from those produced by *Prevotella* sp. (Zhang, et al., 2015) and has a potentially anti-inflammatory role in the intestine (De Filippo, et al., 2010). Clostridiales and Bacteroidetes were increased in the children gut microbiota from Cuenca, both are related with a western diet (low in fiber, rich in animal protein, sugar, and milk consumption) (Clarke, et al., 2012). Additionally, an investigation by Gorvitovskaia, et al. (2016) showed that two biomarkers exist for diet and lifestyle: presence of either Bacteroidetes-Clostridiales or Prevotellaceae, with the presence of either group corresponding with a low abundance of the other. In conclusion, the presence of different bacteria with the same functionality in the two groups of children studied (children of Uyumbicho vs. children of Cuenca), indicates that, although the composition of the microbiota of the two groups is not the same, the functions that their members perform could be similar, or as Zhang, et al. (2015) defined it like a *"phylo-functional core of gut microbiota, is, the assemblage of bacterial genera with indispensable functions for human health, as the SCFA production"*.

CONCLUSIONS

- *Entamoeba* spp. altered the gut microbiota beta-diversity and relative abundances in children from the three localities analyzed (Cuenca, Guayllabamba and Uyumbicho) independently of geographic location.

- The genus *Prevotella* is one of the most abundant genera in children infected whit *Entamoeba*.

- Genera like *Campylobacter* and *Desulfovibrio* could help the establishment and progression of enteropathogenic organisms by changing the intestinal environment.

- The geographic location is another environmental factor that could modify the beta-diversity and gut microbiota composition in children from Uyumbicho (Pichincha province) compared to Cuenca (Azuay province), this indicated that the behaviors like, diet and life style (all factor related with a different location) shape the gut microbiota.

- The increased in relative abundance of *Prevotella* genus observed in the gut microbiota in children from Uyumbicho (rural area), agree with studies that showed that this genus are typical of gut microbiota in population of rural areas.

- We did not find differences in alpha diversity in gut microbiota of children from the three analyzed localities.

- Although geographical location influenced the beta diversity and composition of gut microbiota of children from Cuenca and Uyumbicho, in the results of the analysis of Guayllabamba we did not find that this factor was affecting the microbiota of these children.

- It is important to mention that in graphs (figures 1, 3, 4, 5, 6, 8) of relative abundance, although the error bars are large, the statistical significance is maintained.

- In this study we did not have information about diet and nutrition type of children so it is important to perform other studies that elucidate if this factor is influencing or modeling the microbiota of children from Cuenca, Guayllabamba and Uyumbicho.

- Another limitation of this study was that all participants had the same ethnical background.

REFERENCIAS

1. Almeida, A., Mitchell, A., Tarkowska, A. and Finn, R. (2018). Benchmarking taxonomic assignments based on 16S rRNA gene profiling of the microbiota from commonly sampled environments. *GigaScience*, 7, 1–10. doi: 10.1093/gigascience/giy054

2. Aydin, Ö., Nieuwdorp, M. and Gerdes, V. (2018). The Gut Microbiome as a Target for the Treatment of Type 2 Diabetes. *Current Diabetes Reports*, 18, 1-11 doi.org/10.1007/s11892-018-1020-6

3. Bär, A., Phukan, N., Pinheiro, J. and Simoes-Barbosa, A. (2015). The Interplay of Host Microbiota and Parasitic Protozoans at Mucosal Interfaces: Implications for theOutcomes of Infections and Diseases. *PLoS Neglected Tropical Diseases,* 9, 1-12. e0004176. doi:10.1371/journal.pntd.0004176.

4. Bardanzellu, F., Fanos, V., Strigini, F.A.L., Artini, P.G. and Peroni, D.G. (2018). Human breast milk: exploring the linking ring among emerging components. *Front. Pediatr,* 6, 1-9. doi: 10.3389/fped.2018.00215

5. Berhe, B., Bugssa, G., Bayisa, S. and Alemu, M. (2018). Foodborne intestinal protozoan infection and associated factors among patients with watery diarrhea in Northern Ethiopia; across-sectional study. *Journal of Health, Population and Nutrition*, 37, 2-7

6. Berrilli, F., Di Cave, D., Cavallero, S., and D´Amelio, S. (2012). Interactions between parasites and microbial communities in the human gut. *Frontiers in Cellular and Infection Microbiology*, 2, 1-6. doi: 10.3389/fcimb.2012.00141.

7. Biagi, E., Quercia, S., Aceti, A., Beghetti, I., Rampelli, S., Turroni, S., Faldella, G., Candela, M., Brigidi, P. and Corvaglia, L. (2017). The bacterial ecosystem of mother's milk and infant's mouth and gut. *Front. Microbiol*, 8, 1-9. .doi: 10.3389/fmicb.2017.01214.

8. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope E, Da Silva R, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley G, Janssen S, Jarmusch AK, Jiang L, Kaehler B, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MG, Lee J, Ley R, Liu Y, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton J, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson, II MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CH, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. (2018). QIIME 2:

Reproducible, interactive, scalable, and extensible microbiome data science. 6:e27295v2 https://doi.org/10.7287/peerj.preprints.27295v2.

9. Bunyavanich, S., Shen, N., Grishin, A., Wood, R., Burks, W., Dawson, P., Jones, S., Leung, D., Sampson, H., Sicherer, S. and Clemente, J. (2016). Early-life gut microbiome composition and milk allergy resolution. *American Academy of Allergy, Asthma & Immunology*, 138, 1122-1130. http://dx.doi.org/10.1016/j.jaci.2016.03.041.

10. Burgess, S. and Petri, W. (2016). The Intestinal Bacterial Microbiome and E. histolytica Infection. *Current Tropical Medicine Reports*, 3, 71–74, doi 10.1007/s40475-016-0083-1.

11. Burgess, SL., Gilchrist, C.A., Lynn, T.C., Petri., W.A., Jr. (2017). Parasitic protozoa and interactions with the host intestinal microbiota. *Infection and Immunity*, 85, 1-12. e00101-17. https://doi.org/10.1128/IAI.00101-17.

12. Cao, Y., Fanning, S., Proos, S., Jordan, K. and Srikumar, S. (2017). A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. *Front. Microbiol*, 8, 1-16 doi: 10.3389/fmicb.2017.01829.

13. Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Gonzalez, A., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koening, J. E., Ley, R. E., Lozupone, C.A., McDONALD, d., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R. (2010). QIIME allows

analysis of high-throughput community sequencing data. *Nature Methods*, 7, 335–336. doi:10.1038/nmeth.f.303.

14. Chabé, M., Lokmer, A. and Ségurel, L. (2017). Gut Protozoa: Friends or Foes of the Human Gut Microbiota?. *Trends in Parasitology*, 33, 925-934. http://dx.doi.org/10.1016/j.pt.2017.08.005.

15. Chong, C.Y.L., Bloomfield, F.H. and O'Sullivan, J.M. (2018). Factors Affecting Gastrointestinal Microbiome Development in Neonates. *Nutrients*, 10, 1-17. doi:10.3390/nu10030274.

16. Clarke, S. F., Murphy, E. F., Nilaweera, K., Ross, P. R. Shanahan, F., O´Toole, P. W. and Cotter, P. D. (2012). The gut microbiota and its relationship to diet and obesity. *Gut Microbes*, 3, 186-202. http://dx.doi.org/10.4161/gmic.20168.

17. Conlon, M. A. and Bird, A. R. (2015). The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. *Nutrients, 7*, 17-44. doi:10.3390/nu7010017.

18. Cuenca-Ecuador, F. municipal turismo para. (2019). Cuenca. Retrieved from http://www.cuencaecuador.com.ec/.

19. Davenport, E. R., Sanders, J. G., Song, S. J., Amato, K. R., Clark, A. G. and Knight, R. (2016). The human microbiome in evolution. *Bio Med Central*, 15, 1-12. doi 10.1186/s12915-017-0454-7.

20. Davis, C. D. (2016). The gut microbiome and its role in obesity. *Nutr Today*, 51, 167–174. doi:10.1097/NT.0000000000000167.

21. De Filippo, C., Cavalieri, D., Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G. and Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences*, 107, 14691-14696. doi/10.1073/pnas.1005963107.

22. Dick, L. K., Bernhard, A. E., Brodeur, T. J., Santo Domingo, J. W., Simpson, J. M., Walters, S. P. and Field, K. G. (2005). Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification. *Applied and Environmental Microbiology*, 71, 3184–3191. doi:10.1128/AEM.71.6.3184–3191.2005.

23. Donaldson, G., Lee, M. S. and Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*, 14, 20–32. doi:10.1038/nrmicro3552.

24. Dos Santos Pereira Indiani, C., Ferreira-Rizzardi, K., Midori-Castelo, P., Caldas-Ferraz, L., Darrieux, M. and Manzano-Parisotto, T. (2018). Childhood obesity and Firmicutes/Bacteroidetes ratio in the gut microbiota: A systematic review. *Childhood Obesity*, 8, 1-9. DOI: 10.1089/chi.2018.0040.

25. Espín, S. (2014). *Diagnóstico del potencial turístico de la parroquia de uyumbicho en el cantón mejía*. Dissertattion (Technician), Instituto Tecnológico Superior De Turismo Y Hotelería, Quito-Ecuador.

26. Fallani, M., Young, D., Scott, J., Norin, e., Amarri, S., Adam, R., Aguilera, M., Khanna, S., Gil, A., Edwards, C. A. and Doré, J. (2010). Intestinal Microbiota of 6-week-old Infants Across Europe: Geographic Influence Beyond Delivery Mode, Breast-feeding, and Antibiotics. *Journal of pediatric gastroenterology and nutrition*, 51, 77-84. doi: 10.1097/MPG.0b013e3181d1b11e.

27. Figura, M., Marano, L., Moretti, E. and Ponzetto, A. (2016). Helicobacter pylori infection and gastric carcinoma: Not all the strains and patients are alike. *World J Gastrointest Oncol*, 8, 40-54. Doi: 10.4251/wjgo.v8.i1.40.

28. Francino, M. P. (2018). Birth Mode-Related Differences in Gut Microbiota Colonization and Immune System Development. *Annals Nutrition & Metabolism*, 73, 12–16. doi: 10.1159/000490842.

29. Gilchrist, C. A., Petri, S. E., Schneider, B. N., Reichman, D. J., Jiang, N., Begum, S., Watanabe, K., Jansen, C. S., Elliot, K. P., Burgess, S. L., Ma, J. Z., Alam, M., Kabir, M., Haque, R. and Petri Jr, W. A. (2016). Role of the Gut Microbiota of Children in Diarrhea Due to the Protozoan Parasite *Entamoeba histolytica*. *The Journal of Infectious Diseases*, 213, 1579-1585. doi: 10.1093/infdis/jiv772.

30. Gobierno autonomo descentralizado Parroqui Rural, Guayabamba. (n.d.). Retrieved April 24, 2019, from http://gadguayllabamba.gob.ec/datos-generales/

31. Gobierno autonomo Descentralizado Uyumbicho. (n.d.). Retrieved April 24, 2019, from http://uyumbicho.gob.ec/pichincha/?p=110.

32. Goodrich, J. K., Waters, J. L., Poole, A. C. Sutter, J. L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J. T., Spector, T. D., Clark, A. G. and Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, 159, 789-799. doi:10.1016/j.cell.2014.09.053.

33. Gorvitovskaia, A., Holmes, S. P. and Huse, S. M. (2016). Interpreting *Prevotella* and *Bacteroides* as biomarkers of diet and lifestyle. *Microbiome*, 4, 1-12. doi 10.1186/s40168-016-0160-7.

34. Gupta, S. S., Mohammed, M. H., Ghosh, T. S., Kanungo, S., Nair, G. B. and Mande, S. S. (2011). Metagenome of the gut of a malnourished child. *Gut Pathogens*, 3, 1-9. doi: 10.1186/1757-4749-3-7.

35. Gupta, V. K., Paul, S. and Dutta, C. (2017). Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. *Frontiers in Microbiology*, 8:1-16. doi: 10.3389/fmicb.2017.01162.

36. Hillman, E.T., Lu, H., Yao, T. and Nakatsu, C.H. (2017). Microbial ecology along the gastrointestinal tract. *Microbes and Environments*, 4, 300-313. doi:10.1264/jsme2.ME17017.

37. Huston CD, Petri WA Jr. Host-pathogen interaction in amebiasis and progress in vaccine development. Eur J Clin Microbiol Infect Dis. 1998; 17(9):601–14. Epub 1998/12/01. PubMed PMID: 9832261.

38. Hotez, P. (2000). The Other Intestinal Protozoa: Enteric Infections Caused by Blastocystis hominis, *Entamoeba coli*, and Dientamoeba fragilis. *Seminars in Pediatric Infectious Diseases*, 3, 178-181. doi:10.1053/pi.2000.6228.

39. Ishaq, S. and Nunn, L. (2015). *Helicobacter pylori* and gastric cancer: a state of the art review. *Gastroenterology and Hepatology Bed Bench*, 8(Suppl.1), S6-S14. PMCID: PMC4495426.

40. Jandhyala, S., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M. and Reddy, D. N. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology*. 21, 8787-8803. doi: 10.3748/wjg.v21.i29.8787.

41. Jašarević, E., Morrison, K. E., Bale, T.L. (2016). Sex differences in the gut microbiome–brain axis across the lifespan. *Phil. Trans. R. Soc. B*, 371, 1-12. doi.org/10.1098/rstb.2015.0122.

42. Kedia S, Rampal R, Paul J, Ahuja V. Gut microbiome diversity in acute infective and chronic inflammatory gastrointestinal diseases in North India. J Gastroenterol. 2016; 51(7):660–71. Epub 2016/03/21. 10.1007/s00535-016-1193-1 [pii]. https://doi.org/10.1007/s00535-016-1193-1 PMID: 26994772.

43. Kim, S., Covington, A. and Pamer, E. G. (2017). The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. *Immunol Rev*, 279, 90–105. doi:10.1111/imr.12563.

44. Koliada, A., Syzenko, G., Moseiko, V., Budovska, L., Puchkov, K., Perederiy, V., Gavalko, Y., Dorofeyev, A., Romanenko, M., Tkach, S., Sineok, L., Lushchak, O. and Vaiserman, A. (2017). Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *Bio Med Central Microbiology*, 17, 1-6. doi 10.1186/s12866-017-1027-1.

45. Laforest-Lapointe, I. and Arrieta, M. C. (2017). Patterns of early-life gut microbial colonization during human immune development: An ecological perspective. *Front. Immunol,* 8, 1-13. doi: 10.3389/fimmu.2017.00788.

46. Lebba, V., Santangelo, F., TotinoV., Pantanella, F., Monsia, A., Di Cristanziano, V., Di Cave, D., Schippa, S., Berrilli, F. and D'Alfonso, R. (2016). Gut microbiota related to *Giardia duodenalis*, *Entamoeba* spp. and *Blastocystis hominis* infections in humans from Côte d'Ivoire. *J Infect Dev Ctries*, 10, 1035-1041. doi:10.3855/jidc.8179.

47. Leong, C., Haszard, J.J., Lawley, B., Otal, A., Taylor, R.W., Szymlek-Gay, E.A., Fleming, E.A., Daniels, L., Fangupo, L.J., Tannock, G.W., Heath, A-L.M. (2018). Mediation analysis as a means of identifying dietary components that differentially affect the fecal microbiota of infants weaned by modified baby-led and traditional approaches. *Appl Environ Microbiol*, 84, e00914-18. https://doi.org/10.1128/AEM.00914-18.

48. Lindsay, b., Oundo, J., Hossain, M.A., Antonio, M., Tamboura, B., Walker, A.W., Paulson, J.N., Parkhill, J., Omore, R., Faruque, A., Das, S.K., Ikumapayi, U.N., Adeyemi, M., Sanogo, D., Saha, D., Sow, S., Farag, T.H., Nasrin, D., Li, S., Panchalingam, S., Levine, M.M., Kotloff, K., Magder, L.S., Hungerford, L., Sommerfelt, H., Pop, M., Nataro, J.P., Stine, O. (2015). Microbiota That Affect Risk for Shigellosis in Children in Low-Income Countries. *Emerging Infectious Diseases*, 21, 242-250. DOI: http://dx.doi.org/10.3201/eid2102.140795.

49. Lloyd-Price, J., Abu-Ali, G. and Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, 8, 1-11. DOI 10.1186/s13073-016-0307-y

50. Loftus, B.; Anderson, I.; Davies, R.; Alsmark, U.C.M.; Samuelson, J.; Amedeo, P.; Roncaglia, P.; Berriman, M.; Hirt, R.P.; Mann, B.J.; et al. The genome of the protist parasite Entamoeba histolytica. Nature 2005, 433, 865–868.

51. Ma, B., McComb, E., Gajer, P., Yang, H., Humphrys, M., Okogbule-Wonodi, A.C., Fasano, A., Ravel, J. and Viscardi, R.M. (2018). Microbial biomarkers of intestinal

barrier maturation in preterm infants.*Front. Microbiol*, 9, 1-14.doi: 10.3389/fmicb.2018.02755.

52. Mabbott, N.A. (2018). The influence of parasite infections on host immunity to co-infection with other pathogens. *Front. Immunol*, 9, 1-14. doi: 10.3389/fimmu.2018.02579.

53. Mach, N. and Fuster-Botella, D. (2016). Endurance exercise and gut microbiota: A review. *Journal of Sport and Health Science*, 6, 179–197. http://dx.doi.org/10.1016/j.jshs.2016.05.001.

54. Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R. and Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health & Disease*, 26, 1-7. doi.org/10.3402/mehd.v26.27663.

55. Mariat, D., Firmesse, O., Levenez, F., Guimară es, V.D., Sokol, H., Doré, J., Corthier, G. Furet, J-P. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology*, 9, 1-6. doi:10.1186/1471-2180-9-123.

56. Marko, M. and Pawliczak, R. (2017). The role of microbiota in allergy development. *Alergologia Polska-Polish Journal of Allergology*, 4, 58-62. http://dx.doi.org/10.1016/j.alergo.2017.03.002.

57. Miller, P. (n.d.). Revista Cuenca Ilustra Ecuador. Retrieved from https://patomiller.wordpress.com/about/.

58. Monda, V., Villano, I., Messina, A., Valenzano, A., Esposito,T., Moscatelli, F., Viggiano, A., Cibelli, G., Chieffi, S., Monda, M and Messina, G. (2017). Exercise Modifies the Gut Microbiota with Positive Health Effects**.** *Oxidative Medicine and Cellular Longevity*, 2017, 1-8. http://dx.doi.org/10.1155/2017/3831972.

59. Morton, E.R., Lynch, J., Froment, A., Lafosse, S., Heyer, E., Przeworski, M., Blekhman, R. and Ségurel, L. (2015). Variation in rural african gut microbiota is strongly correlated with colonization by *Entamoeba* and subsistence. *PLoS Genet*, 11, 1-28. doi:10.1371/journal. pgen.1005658.

60. Mueller, N., Bakacs, E., Combellick, J., Grigoryan, Z. and Dominguez-Bello, M. (2015). The infant microbiome development: mom matters. *Trends in Molecular Medicine*, 21, 109-117. dx.doi.org/10.1016/j.molmed.2014.12.002.

61. Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J., Abe, F. and Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiology*, 16, 1-12. doi 10.1186/s12866-016-0708-5.

62. Opazo, M.C., Ortega-Rocha, E.M., Coronado-Arrázola, I., Bonifaz, L.C., Boudin, H., Neunlist, M., Bueno, S.M., Kalergis, A.M. and Riedel, C.A. (2018). Intestinal

microbiota influences non-intestinal related autoimmune diseases. *Front. Microbiol*, 9, 1-20. doi: 10.3389/fmicb.2018.00432.

63. Osman, M., El Safadi, D., Cian, A., Benamrouz, S., Nourrisson, C., Poirier, P., Preira, B., Razakandrainibe, R., Pinon, A., Lambert, C., Wawrzyniak, I., Dabboussi, F., Delbac, F., Favennec, L., Hamze, M., Viscogliosi, E. and Certad, G. (2016). Prevalence and risk factors for intestinal protozoan infections with *Cryptosporidium*, *Giardia*, *Blastocystis* and *Dientamoeba* among schoolchildren in tripoli, Lebanon. *PLoS Negl Trop Dis*, 10, 1-17. doi:10.1371/journal.pntd.0004496.

64. Padilla-Vaca F, Ankri S, Bracha R, Koole LA, Mirelman D. Down regulation of Entamoeba histolytica virulence by monoxenic cultivation with Escherichia coli O55 is related to a decrease in expression of the light (35-kilodalton) subunit of the Gal/GalNAc lectin. Infect Immun. 1999; 67(5):2096–102. Epub 1999/ 05/04. PubMed PMID: 10225860; PubMed Central PMCID: PMC115943.

65. Rampelli, S., Schnorr, S. L., Consolandi, C., Crittenden, A. N., Henry, A. G. and Candela, M. (2015). Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota. *Current Biology* 25, 1682–1693. doi.org/10.1016/j.cub.2015.04.055.

66. Ravussin, Y., Koren, O., Spor, A., LeDuc, C., Gutman, R., Stombaugh, J., Knight, R., Ley, R. E. and Leibel, R. L. (2012). Responses of Gut Microbiota to Diet Composition and Weight Loss in Lean and Obese Mice. *Obesity (Silver Spring)*, 20, 2-18. doi:10.1038/oby.2011.111.

67. Rinninella, e., Raoul, P., Cintoni, M., Franceschi, F., Donato Miggiano, G. A., Gasbarrini, A. and Mele, M.C. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms*, 7, 1-22. doi:10.3390.

68. Rodríguez, J.M., Murphy, K., Stanton, C., Ross, R. P., Kober, O.I., Juge, N., Avershina, E., Rudi, K., Narbad,. A., Jenmalm, M.C., Marchesi, J.R., and Collado, M.C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial Ecology in Health & Disease*, 26, 1-17. dx.doi.org/10.3402/mehd.v26.26050.

69. Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P.I., Godneva, A., Kalka, I. N., Bar, N., Shilo, S., Lador, D., Vila, A. V., Zmora, N., Pevsner-Fischer, M., Israeli, D., Kosower, N., Malka, G., Wolf, B. C., Avnit-Sagi, T., Lotan-Pompan, M., Weinberger, A., Halpern, Z., Carmi, S., Fu, J., Wijmenga, C., Zhernakova, A., Elinav, E. & Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature*, 555, 210-215. doi:10.1038/nature25973.

70. Rutayisire, E., Huang, K., Liu, Y. and Tao, F. (2016). The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterology*, 16, 1-12. doi 10.1186/s12876-016-0498-0.

71. Sekirov, I., Russell, S.L., Antunes, L.C.M. and Finlay, B.B. (2010). Gut microbiota in health and disease. *Physiol Rev*, 90, 859–904. doi:10.1152/physrev.00045.2009.

72. Shaulov Y, Shimokawa C, Trebicz-Geffen M, Nagaraja S, Methling K, Lalk M, et al. (2018) Escherichia coli mediated resistance of Entamoeba histolytica to oxidative stress is triggered by oxaloacetate. PLoS Pathog 14(10): e1007295. https://doi.org/10.1371/journal.ppat.1007295.

73. Sheflin, A. M., Melby, C.L., Carbonero, F. & Weir, T.L. (2017). Linking dietary patterns with gut microbial composition and function. *Gut Microbes*, 8, 113-129, doi:10.1080/19490976.2016.127080.

74. Shin, N.R., Whon, T. W. and Bae, J. W. (2015). Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology*, 33, 496-503. doi.org/10.1016/j.tibtech.2015.06.011.

75. Stensvold, C. and Giezen, M. (2018). Associations between Gut Microbiota and Common Luminal Intestinal Parasites. *Cell Press, Trends in Parasitology*, 34, 369-377, https://doi.org/10.1016/j.pt.2018.02.004.

76. Sun, L., Ma, L., Ma, Y., Zhang, F., Zhao, C. and Nie, Y. (2018). Insights into the role of gut microbiota in obesity: pathogenesis, mechanisms, and therapeutic perspectives. *Protein Cell*, 9, 397–403. https://doi.org/10.1007/s13238-018-0546-3.

77. Thursby, E. and Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, 474, 1823–1836. doi: 10.1042/BCJ20160510.

78. Toro-Londono, M.A., Bedoya-Urrego, K., Garcia-Montoya, G.M., Galvan-Diaz, A.L., Alzate, J.F. (2019). Intestinal parasitic infection alters bacterial gut microbiota in children. *PeerJ*, 7, 1-24. doi.org/10.7717/peerj.6200.

79. Turnbaugh, P. (2017). Microbes and diet-induced obesity: fast, cheap, and out of control. *Cell Host Microbe*, 21, 278–281. doi:10.1016/j.chom.2017.02.021.

80. Varet H, Shaulov Y, Sismeiro O, Trebicz-Geffen M, Legendre R, Coppee JY, et al. Enteric bacteria boost defences against oxidative stress in Entamoeba histolytica. Sci Rep. 2018; 8(1):9042. Epub 2018/06/15. https://doi.org/10.1038/s41598-018-27086-w PMID: 29899530.

81. Verma, A. K., Verma, R,m Ahuja, V. and Paul, J. (2012). Real-time analysis of gut flora in *Entamoeba histolytica* infected patients of Northern India. *BMC Microbiology*, 12, 1-11. doi: 10.1186/1471-2180-12-183.

82. Wang, B., Yao, M., Lv, L., Ling, Z. and Li, L. (2017). The human microbiota in health and disease. *Engineering*, 3, 71–82. doi.org/10.1016/J.ENG.2017.01.008.

83. Wen, L. and Duffy, A. (2017). Factors Influencing the Gut Microbiota, Inflammation, and Type 2 Diabetes. *The Journal of Nutrition*, 147(Suppl):1468S–75S. doi: https://doi.org/10.3945/jn.116.240754.

84. Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H. Bushman, F.D. and Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science,* 7, 105–108. doi:10.1126/science.1208344.

85. Zhang, J., Guo, Z., Xue, Z., Sun, Z., Zhang, M., Wang, L., Wang, G., Wang, F., Xu, J., Cao, H., Xu, H., Lv, Q., Zhong, Z., Chen, Y., Qimuge, S., Menghe, B., Zheng, Y., Zhao, L., Chen, W. and Zhang, H. (2015). A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *International Society for Microbial Ecology*, 9, 1979-1990. doi:10.1038/ismej.2015.11.

TABLES AND FIGURES

Table 1. Summarize of samples processing

	Group 1 Uyumbicho	Group 2 Guayllabamba	Group 3 Cuenca	Total samples
Samples collected	90	58	83	231
Samples that met the inclusion criteria	48	43	40	131
Samples that met the exclusion criteria	39	36	30	***105**

**105 were the final number of samples analyzed in this study*

Table 2 PERMANOVA of beta-diversity in gut microbiota between children from Uyumbicho and Cuenca

	PERMANOVA results
method name	PERMANOVA
test statistic name	pseudo-F
sample size	69
number of groups	2
test statistic	1.67134
p-value	0.035
number of permutations	999

Pairwise permanova results

		Sample size	Permutations	pseudo-F	p-value	q-value
Group 1	Group 2					
Cuenca	Uyumbicho	69	999	1.671344	0.034	0.034

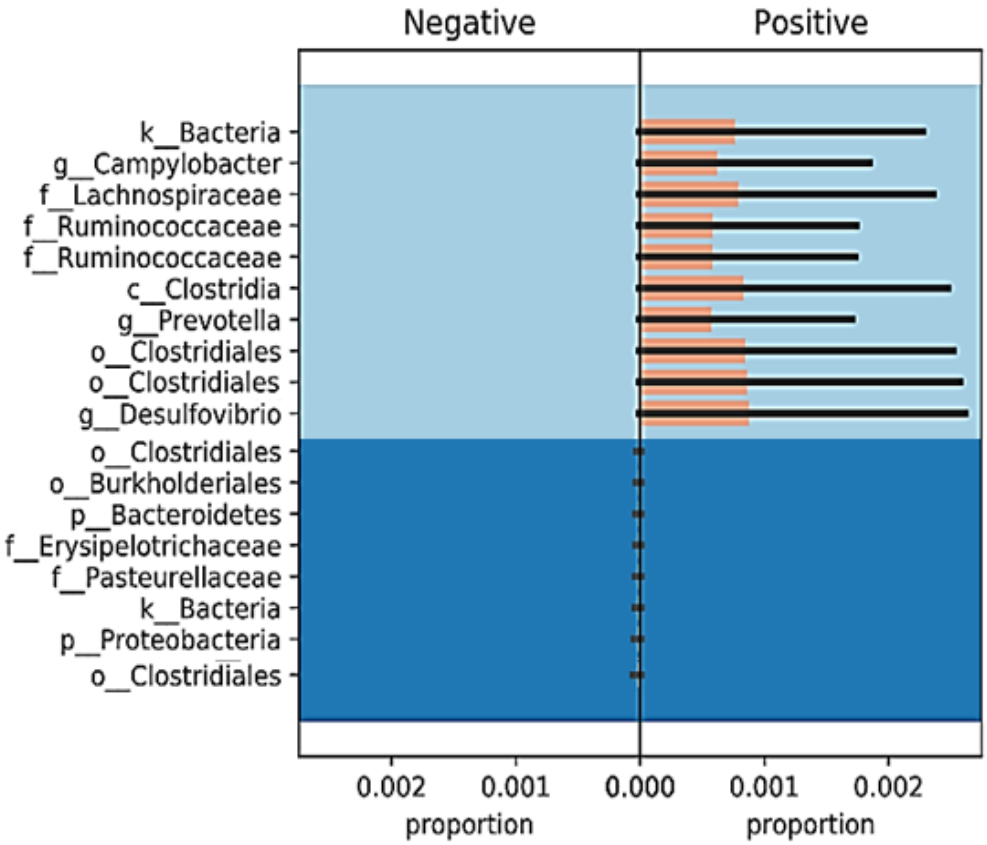

Figure 1. Proportion plots of differential abundance analysis using balances in gneiss according to the presence/absence of *Entamoeba coli* in children from Cuenca and Guayllabamba. In the right side the individuals positive for the parasite are observed and on the left side the negative individuals. The orange bars indicated the increased of the relative abundance of taxa in the positive individuals. The black bars represent the error bars (although the error bars are large, the statistical significance is maintained).

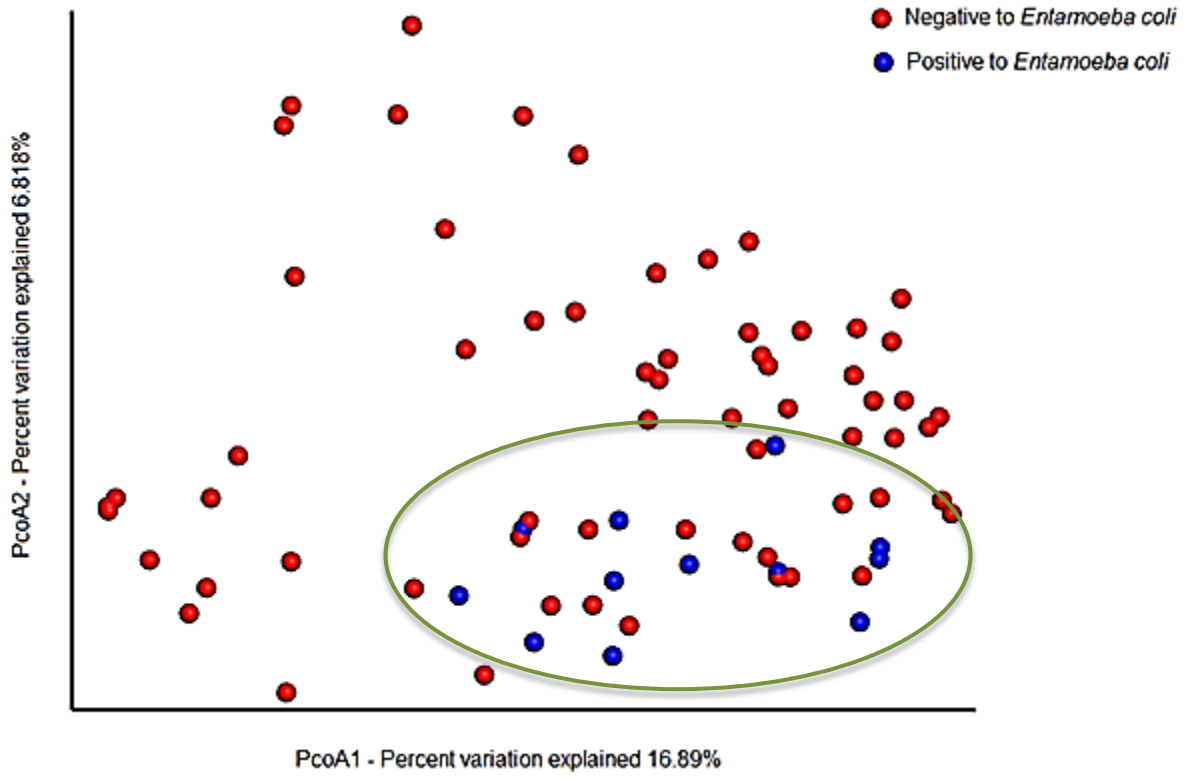

Figure 2. PCoA of unweighted UniFrac distances for all 105 fecal samples, indicating the effect of *Entamoeba coli*. The red dots correspond to negative for the parasite; the blue dots correspond to positive samples for the parasite. The analysis was done between children from Uyumbicho and Guayllabamba. The green circle shows a clustering between positive subjects.

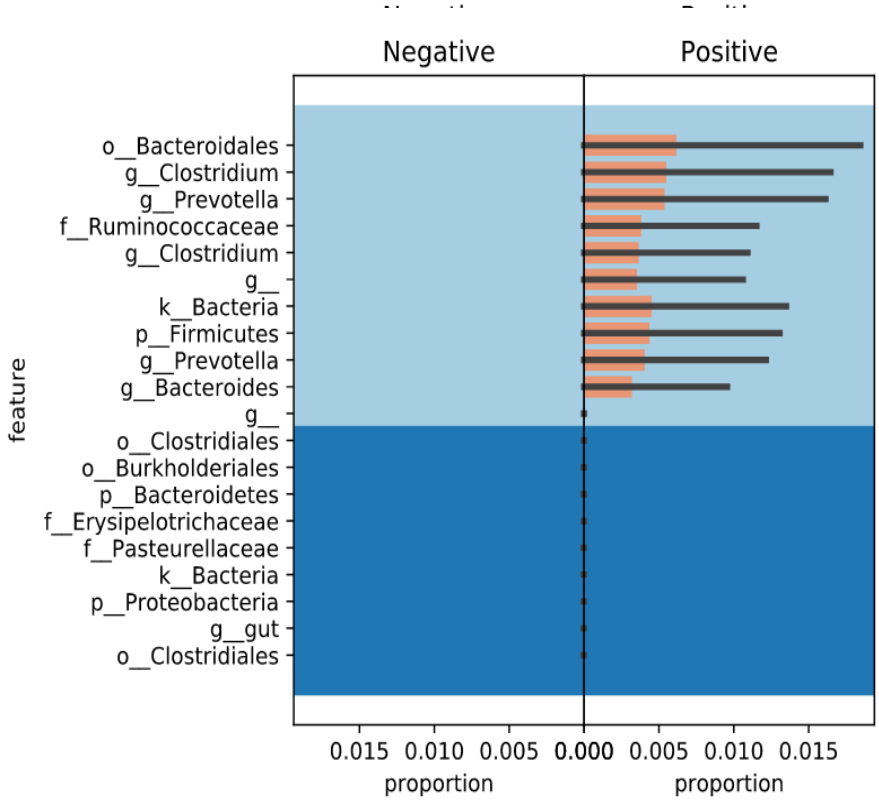

Figure 3. Proportion plots of differential abundance analysis using balances in GNEISS according to the presence/absence of *Entamoeba coli* in children from Uyumbicho and Guayllabamba. In the right side the individuals positive for the parasite are observed and on the left side the negative individuals. The orange bars indicated the increased of the relative abundance of taxa in the positive individuals. The black bars represent the error bars (although the error bars are large, the statistical significance is maintained).

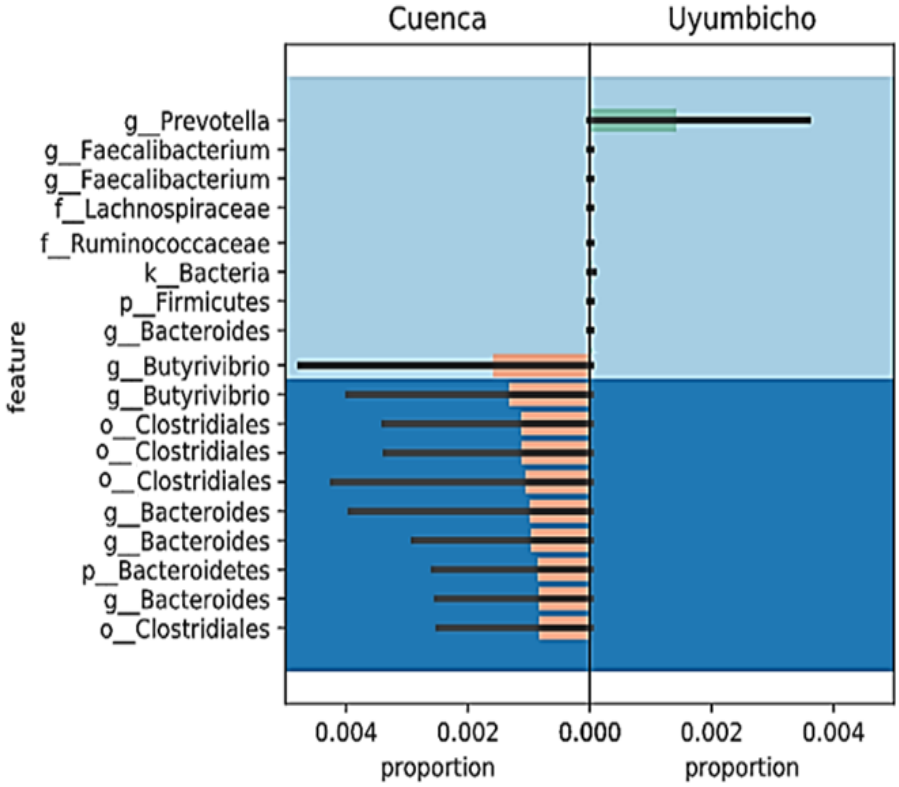

Figure 4 Proportion plots of differential abundance analysis using balances in gneiss according to the residence site of children from Uyumbicho and Cuenca, on the right side are observed children from Uyumbicho and on the left side the children from Cuenca. The green bar indicated the increased of the relative abundance of *Prevotella* in gut microbiota of children from Uyumbicho and the orange bars indicated the increased of the relative abundance of others taxa in gut microbiota of children from Cuenca. The black bars represent the error bars (although the error bars are large, the statistical significance is maintained).

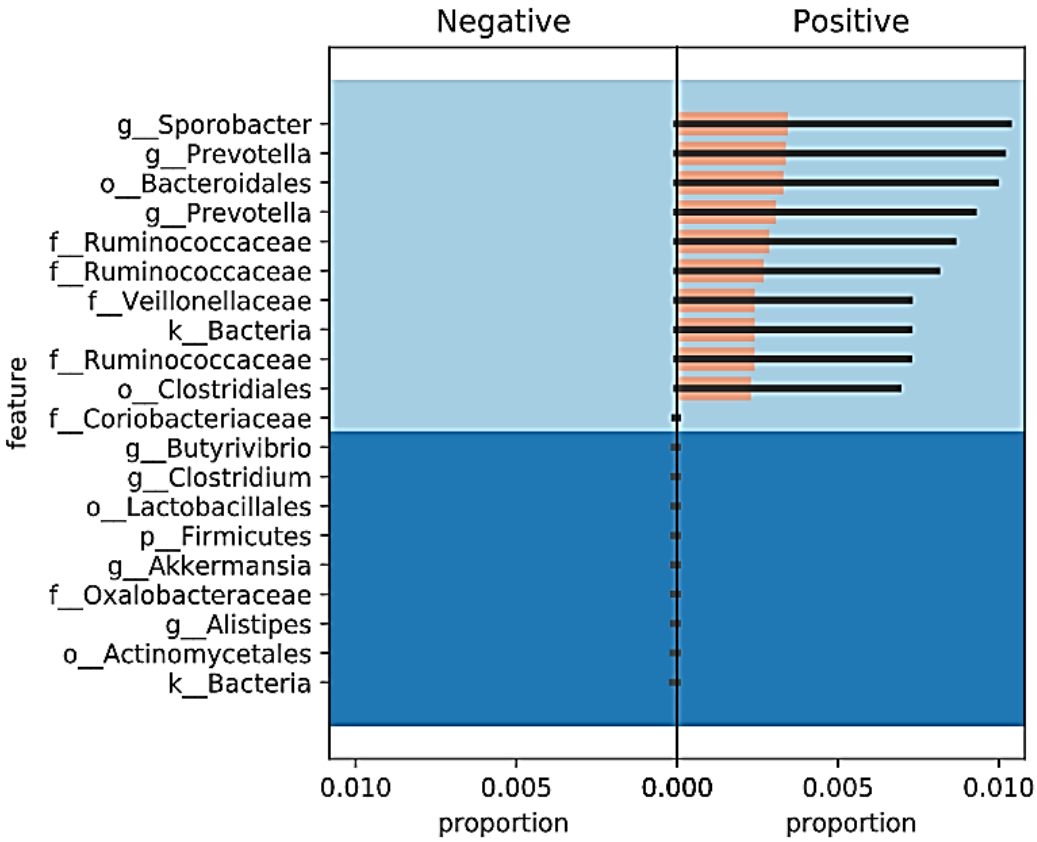

Figure 5. Proportion plots of differential abundance analysis using balances in GNEISS according to the children positive/negative to *Entamoeba histolytica* in children from Uyumbicho and Cuenca. In the right side the individuals positive for the parasite are observed and on the left side the negative individuals. The orange bars indicated the increased of the relative abundance of taxa in the positive individuals. The black bars represent the error bars (although the error bars are large, the statistical significance is maintained.

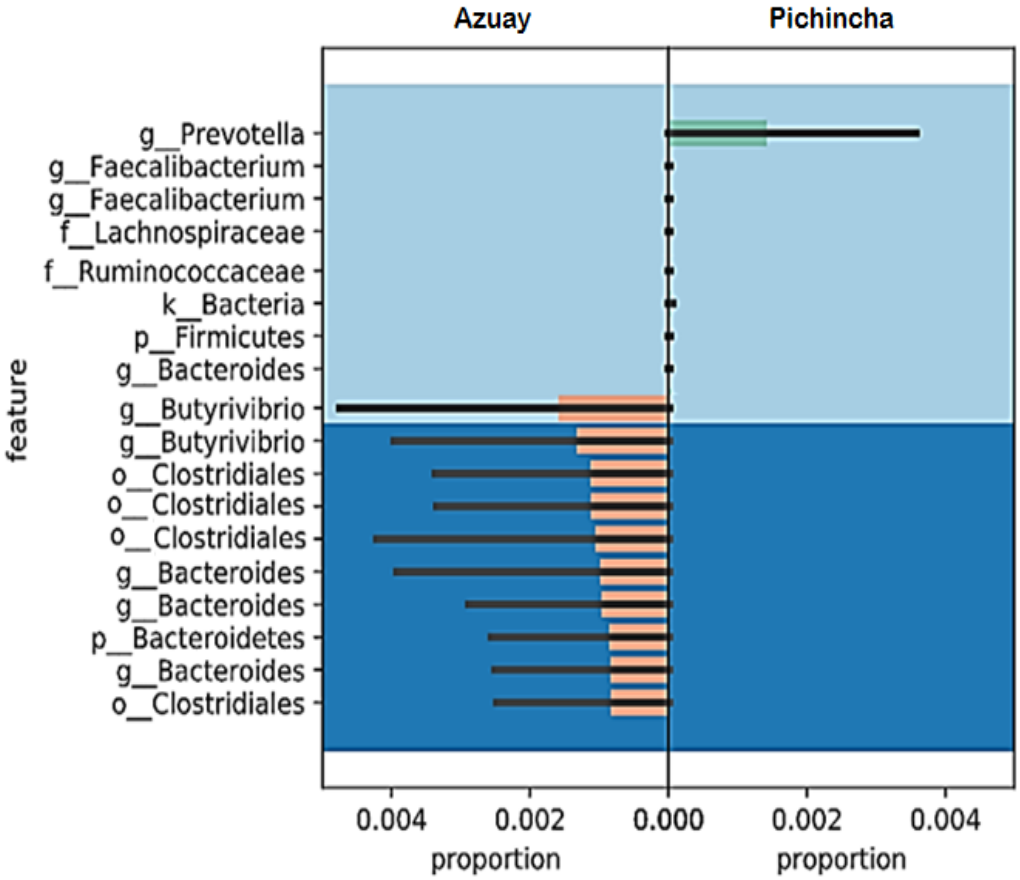

Figure 6 Proportion plots of differential abundance analysis using balances in GNEISS according to the province of residence of children from Pichincha and Azuay.In the right side are observed children from Pichincha and on the left side the children from Azuay. The green bar indicated the increased of the relative abundance of *Prevotella* in gut microbiota of children from Pichincha and the orange bars indicated the increased of the relative abundance of others taxa in gut microbiota of children from Azuay. The black bars represent the error bars (although the error bars are large, the statistical significance is maintained).

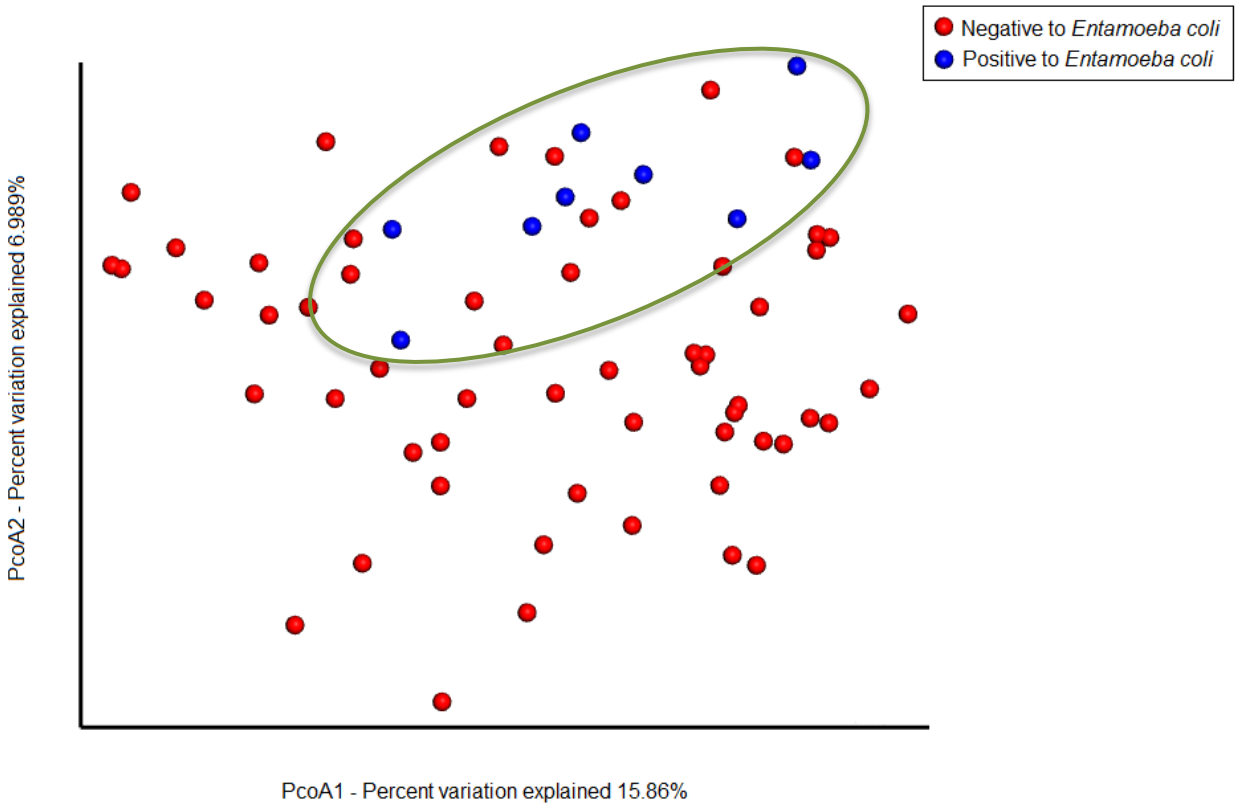

Figure 7 PCoA of unweighted UniFrac distances for all 105 fecal samples, indicating the effect of presence of *Entamoeba coli* in the composition of children gut microbiota from Guayllabamba and Cuenca. The red dots correspond to negative and the blue dots correspond to positive. The green circle shows a clustering between positive subjects.