Background

- **Dientamoeba fragilis** and Blastocystis have been described as non-pathogenic protozoa in human gut for decades.
- However, many case reports and other studies in early 2000s have indicated them as pathogens, and their eradication was suggested for effective treatment of patients.
- Recent screening studies using PCR indicated high prevalence rates for **Dientamoeba fragilis** (43% in Denmark) and Blastocystis (100% in Senegalese children) as “are they pathogens that require eradication or residents of a healthy gut?”
- Here, we present the outcomes of our assessments during the voluntary field studies in rural Nepal between 2013 and 2015 to contribute to this discussion.

**Materials and Methods**

- Stool samples of 203 children aged between 2 and 15 were collected during site visits between 2013 and 2015 (85 in 2013; 29 in 2014 and 89 in 2015).
- Parasitological examinations included routine O&P examination, which was done on site in 2015 and in the research laboratory of Acibadem University in Istanbul, where stool samples were transported at 4°C in two vials, with and without fixative solution.
- **Dientamoeba fragilis** was sought using Real-Time PCR, as described previously (Verweij et al., 2007).
- Prevalence of Blastocystis was assessed only by O&P examinations due to both budget and staff limits.
- Kinyoun acid fast staining was also applied to identify coccidian parasites in stool.

Results

- Routine O&P examinations showed Blastocystis was present in 58 (28.6%) of examined children.
- Adequate DNA was extracted from the stool samples of 153 children and **D. fragilis** DNA was identified in 52 (34.0%).
- Other intestinal parasites identified during O&P examination and Kinyoun-stained smears include Entamoeba histolytica/dispar (n=36), Giardia lamblia (n=34) Cryptosporidium spp (n=24), Cyclospora cayetanensis (n=14) and Hymenolepis nana (n=3).

Conclusions:

- Children in developing countries, such as Nepal are under the threat of parasitic diseases that directly disturb their physical and mental development.
- Children found to be positive for **D. fragilis** and Blastocystis were mostly non-symptomatic and healthy during the study.
- Unveiling the roles of **D. fragilis** and Blastocystis in human gut, identification of contributing factors associated with clinical disturbances in **D. fragilis** and Blastocystis positive individuals, and assessments of interactions of protozoa with other components of gut microbiota obviously deserves more attention.
- We plan to conduct PCR for Blastocystis to all DNA samples and include microbiota analyses to assess the relationships between the bacteria and eukaryotes in the gut.

Discussion

- High parasitic load in Nepal has been long described in all age groups, especially before 1990s when sanitation and hygiene were poorer (Estevé ez et al, 2016).
- Then, extensive nationwide measures were applied to improve the hygienic standards as well as the education and economic standards of the country.
- These measures were accompanied by widespread campaigns in primary schools such as “deworming programs” that aim to lower the prevalence of intestinal parasites, luncheon meals in schools and Vitamin A supplementation for school children (Khanal, 2002; Kuwar 2016).
- High rates of **D. fragilis** and Blastocystis in both sick and healthy children have been reported. In a previous study with asthmatic children, we found **D. fragilis** DNA in more than 76% of healthy children while it was lower in asthmatic children (52%). In addition to many recent articles, which all demonstrate the correlations between Blastocystis and **D. fragilis** and human health, it is noteworthy to assess their roles in our gut with further studies.