Prevalence of methanogens in fecal samples of dogs with chronic enteropathy

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Introduction

Methanogenic archaea are anaerobic microorganisms found in the mammalian intestinal tract and are responsible for methane production. In humans, a higher prevalence of methanogens was identified in fecal samples of patients with irritable bowel syndrome and lower prevalence in inflammatory bowel disease when compared to healthy controls. Limited data is available about the potential role of methanogenic archaea in the pathogenesis of intestinal disease of dogs

Objective

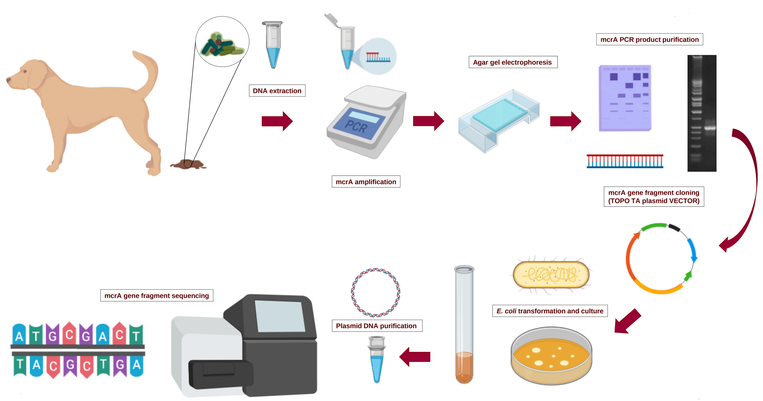
To evaluate the prevalence of methanogens in fecal samples from dogs with chronic enteropathy compared to healthy dogs

Materials and Methods

- Fecal samples were collected from 14 healthy dogs without gastrointestinal signs or antibiotic use in the last 6 months. 20 dogs with gastrointestinal signs for at least 3 weeks (vomiting, diarrhea, anorexia), defined as chronic enteropathy group, were included after systemic and parasitic diseases exclusion
- DNA extraction and PCR was performed targeting a ~460bp fragment of the mcrA gene (methyl-coenzyme M reductase alpha subunit) responsible for methanogenesis. Samples were run in duplicates and amplicons were visualized via agar gel electrophoresis. The amplicons were also sequenced by Sanger sequencing
- Fisher's exact test was used and significance was set at $p<0.05$

	HC	CE	p value
Sample size	14	20	N/A
Age: median-(range)	4(1-10)	5.1(1-11)	0.3711
Gender: (number F/M)	7/7	8/12	0.7282

Table 1. Gender and age information of dogs involved in the study. Mann-Whitney test was used and significance was set at $p<0.05$



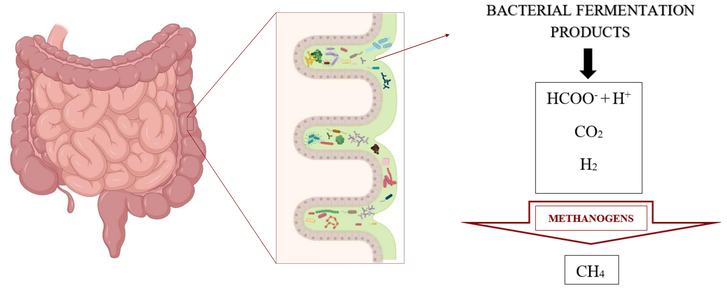


Figure 1. Methanogens are archaea that utilize bacterial fermentation products to produce methane. The methyl-coenzyme M reductase (MCR) catalyzes the reaction of methyl coenzyme M and coenzyme B to methane and the corresponding heterodisulfide CoM-S-S-CoB

Results

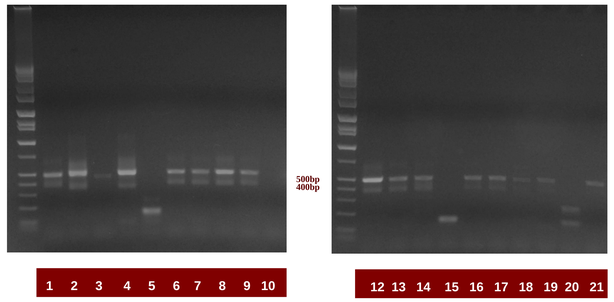


Figure 3. Agarose gel pictures of PCR amplification products obtained with mcrA primers specific for methanogens. Positive control fragment length: ~460bp

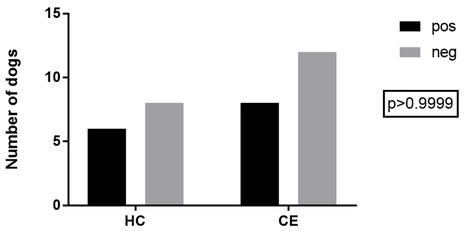


Figure 3. Bar diagram with the number of healthy dogs and dogs with chronic enteropathy that were positive/negative for methanogens in fecal samples

- 8/20 (40%) dogs with chronic enteropathy were positive for methanogens
- 6/14 (43%) healthy control dogs were positive for methanogens
- No significant difference was found in the prevalence of methanogens between both groups ($p=0.9999$)
- The mcrA sequences obtained were aligned using Blast and were identified as:
  - uncultured *Methanobrevibacter sp.* Isolate mcrA-II methyl-coenzyme M reductase (mcrA) partial gene (accession number: EU294497.1)
  - uncultured methanogenic archaeon partial mcrA gene for methyl-coenzyme M reductase alpha subunit (accession number: LT632515.1)

Discussion and Conclusion

- **This study did not identify a significant difference in the prevalence of methanogens in fecal samples of dogs with chronic enteropathy and healthy control dogs**
- **Further studies in a larger cohort of dogs are warranted to determine whether an increase in sample size would lead to different prevalence or abundance of methanogens**
- **Shotgun DNA sequencing to quantify and analyze at taxa level the total archaea present in fecal samples of the studied dogs is currently underway**

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Conflict of Interest

There are no conflicts of interest to disclose.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713714