Enterococcus faecium NCIMB 10415 may transiently colonise the gastrointestinal tract of healthy dogs but does not affect the faecal microbiota.

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Background

The principle behind the use of probiotic bacteria is that they mimic the actions of the commensal microbiota. Ideally a probiotic would survive transit through the gastrointestinal tract (GIT) in sufficient numbers to arrive at the site of action, where it would adhere to the intestinal mucosa and proliferate at this location. At this point it would effect it's function but it should probably only temporarily colonise the GIT.[1] The only EU-registered probiotic for dogs is *Enterococcus faecium* NCIMB 10415/DSM 10663 which is registered as a gut flora stabilizer and advertised to help repopulate the intestine with beneficial micro-organisms. However, the functional properties of this probiotic species in dogs are unknown. The aim of this study was to assess whether *Enterococcus faecium* NCIMB 10415 colonises and/or proliferates in the GIT and whether there is an effect on the faecal microbiota of healthy dogs following treatment with a commercial product containing these probiotic bacteria.

Methods

Twelve healthy dogs with normal faecal examinations and no history of gastrointestinal disease or antibiotic treatment in the last year were fed a commercial probiotic product containing 2 x 10^9 CFU of *E. faecium* NCIMB 10415 for 14 days. Five similarly healthy dogs, which were fed a placebo containing maltodextrin, acted as controls. Faecal samples were collected before, during and 4 days after feeding stopped and were stored at -80°C until faecal DNA extraction, using the MoBio PowerSoil® DNA Isolation Kit. Faecal samples were cultured using Slanetz and Bartley medium. A qPCR based on a unique plasmid of *E. faecium* NCIMB 10415[2] was developed to identify and quantify the probiotic bacteria, and one based on genomic DNA, to quantify all *E. faecium* strains. Faecal DNA was submitted to Mr DNA Molecular Research (Shallowater, Texas) for Illumina sequencing of the 16S rRNA gene. The QIIME v 1.8 open-source pipeline was used to analyse the sequence data and linear discriminant analysis effect size (LEfSe) (http://huttenhower.sph.harvard.edu/galaxy/) to compile the cladogram.

Results

The probiotic bacteria remained viable during transit through the GIT and could be isolated from dog faeces by culture. They were not detected by qPCR in the faeces of any dog prior to probiotic treatment. However, the probiotic bacteria were detected in the faeces of eight of the 12 dogs (up to 10^6 plasmids ng^{-1} faecal DNA) whilst taking the probiotic product but were only detected in small numbers (10^2 plasmids ng^{-1} faecal DNA) in the faeces of two dogs, four days after treatment stopped (Fig 1). They were not detected in the faeces of dogs that took the placebo. The qPCR to detect all *E. faecium* strains largely mirrored the results of the qPCR to detect the probiotic bacteria suggesting that the probiotic strain was the predominant strain of *Enterococcus faecium*.

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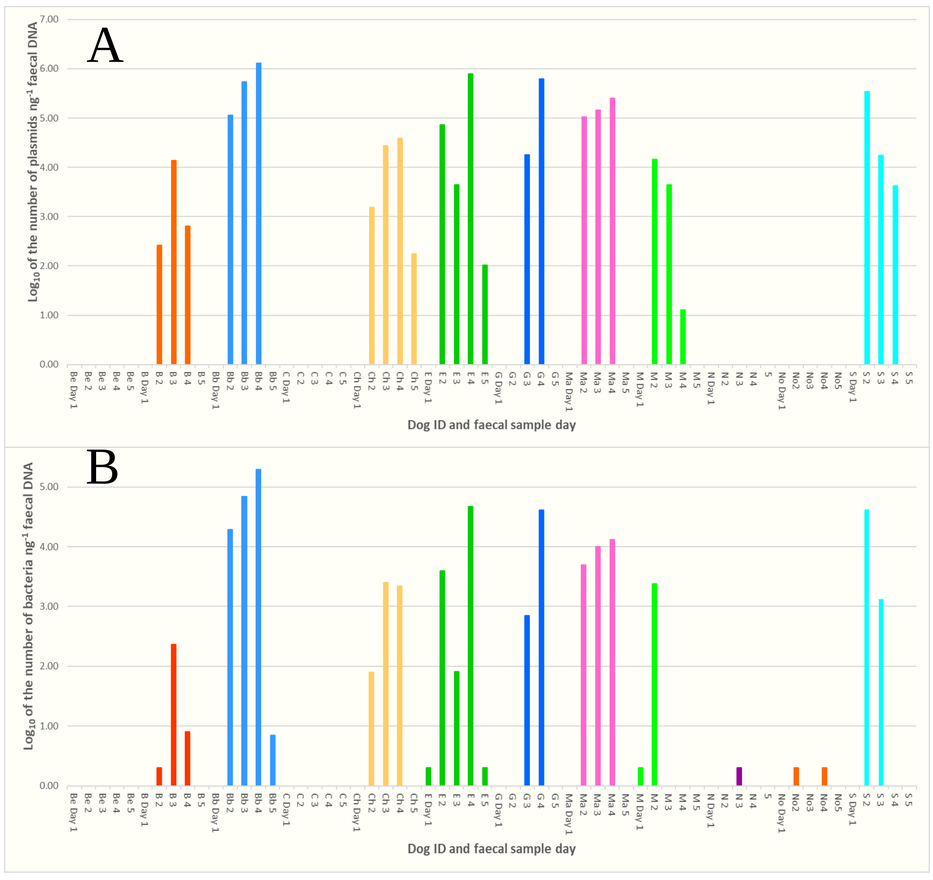

Fig. 1. ***The amounts of probiotic bacterial plasmids (A) and* E. faecium *(B) ng^{-1} of extracted faecal DNA excreted in healthy dog faeces.***

The results of qPCR to quantify the probiotic bacteria and all E. faecium *strains in the faeces of dogs that took the probiotic bacteria for 14 days. Each dog is represented by one colour and the sample days are given below*

Illumina sequencing returned 10 937 774 reads (mean 91 914 per sample; range 48 122 – 217 179) rarefied to 48 000 per sample for analysis (Goods coverage: 0.978). Alpha rarefaction analysis showed no effect of the probiotic bacteria on the numbers of observed species, nor the species richness or diversity of the faecal microbiota (Fig 2).

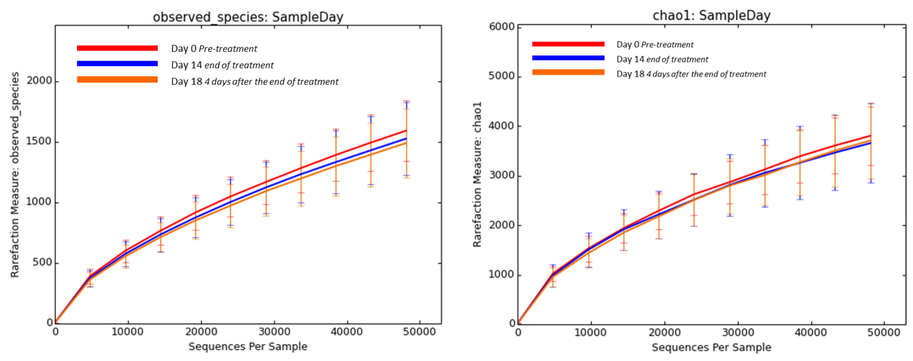

Fig. 2. ***Rarefaction analysis of the observed species and the species richness and diversity (using the Chao 1 index) in the faecal microbiota of 12 healthy dogs for each sample day.***

The vertical lines represent the means and the error bars, the standard deviations

An *Enterococcus* species was identified in the faecal microbiota of these dogs. The percentage of the microbiota comprised of this taxon was increased 14 days after treatment with the probiotic product but absent from most samples 4 days after treatment stopped, matching the qPCR findings (Fig 3).

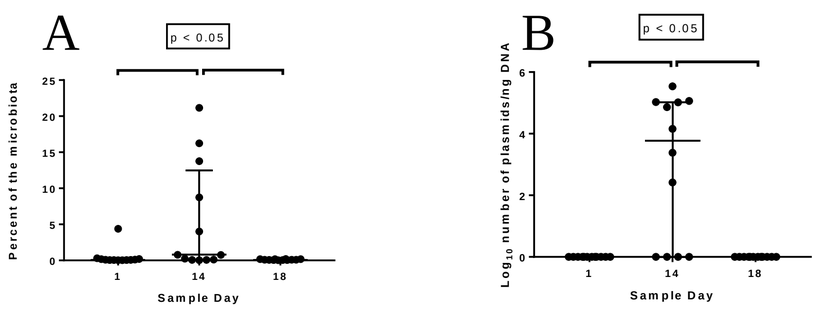

Fig. 3. ***The percentage of the microbiota comprised of an* Enterococcus *species (A) and the amount of the probiotic bacteria detected by qPCR (B) in healthy dog faeces.***

The results of illumina sequencing (A) and a qPCR to detect the probiotic bacteria (B) for samples taken before, at the end of, and 4 days after treatment had stopped.

Unifrac metric β-diversity-based principle coordinate analysis (PCoA) revealed that the faecal microbiotas of each dog were more similar to themselves than to other dogs and that the microbiota of dogs that lived together were more similar to each other than to other dogs ($p < 0.001$; $p < 0.01$ ANOSIM of unweighted (former) and weighted (latter) UniFrac distances). However, there was no effect of the probiotic bacteria on these faecal microbial communities over the treatment period (Fig 4) .

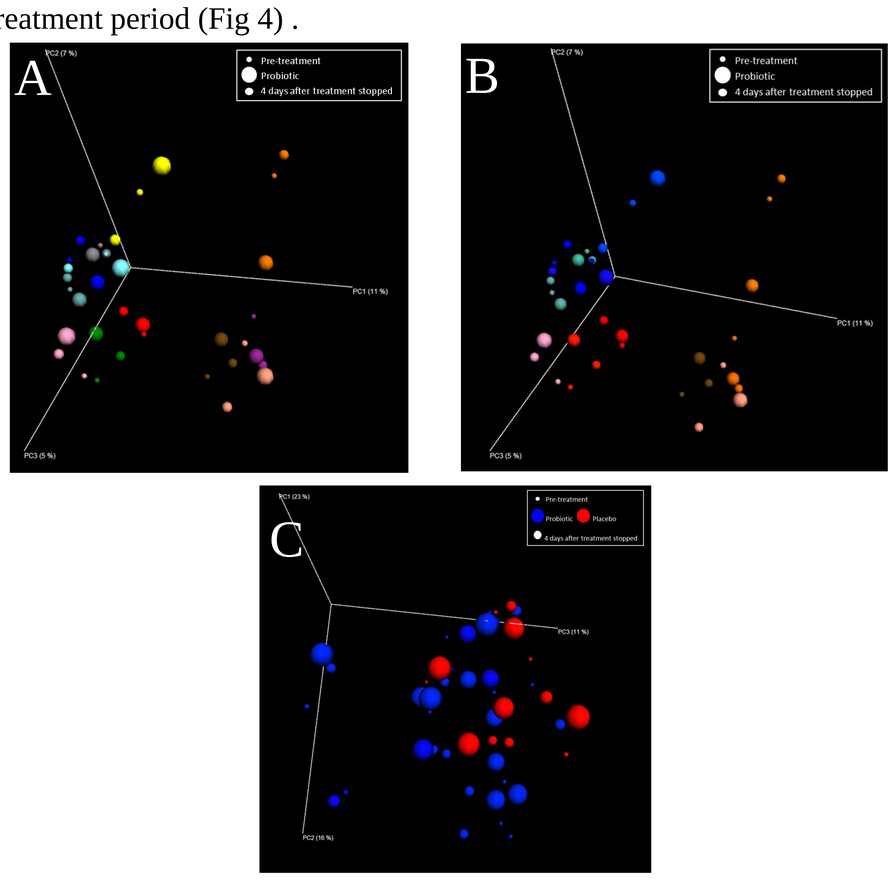

Fig. 4. ***Three dimensional PCoA of the faecal microbiota of healthy dogs that took the probiotic bacteria .***

A: each sphere represents the faecal microbiota of one sample, individually coloured for each dog and size-graded for sample day.
B: each sphere represents the faecal microbiota of one sample, individually coloured for dogs that live together and size-graded for sample day.
C: each sphere represents the faecal microbiota of one sample, coloured for probiotic or placebo treatment and size-graded for sample day.

A cladogram compiled from the data of all the faecal microbiota from each sample time showed that the only difference between all these faecal microbial communities was a significant increase in an *Enterococcus* sp. on day 14 at the end of treatment with the probiotic bacteria (Fig 5)

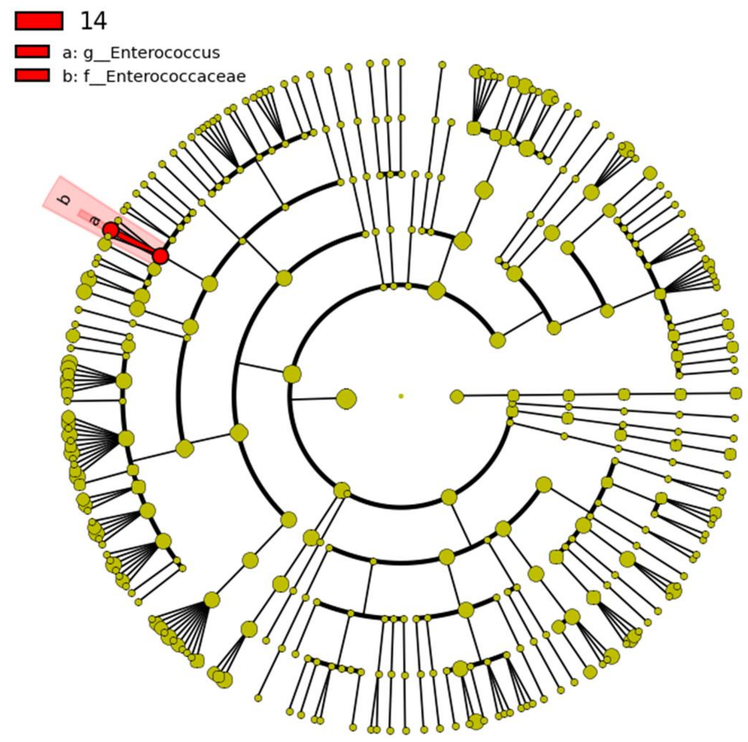

Fig. 5. ***LEfSe analysis of the microbiota of all the faecal samples.***

The samples were collected prior to and at the end of treatment (day 14), and 4 days after treatment stopped.

Discussion and Conclusions

- The probiotic species, *E. faecium* NCIMB 10415/DSM10663 survives transit through the GIT of the healthy dog.
- Thus, the bacterium could multiply in the GIT where the environment is favourable, may adhere and may temporarily colonise these regions.
- Probiotic bacteria were not detected in the faeces of all dogs suggesting that not all canine GITs provide favourable conditions.
- If colonisation occurred, it must have been transient because probiotic bacteria were not detected 4 days after treatment stopped.
- Regardless of whether the bacteria transiently colonise the GIT of the dog, they had no effect on the species richness and diversity of the faecal microbiota of these healthy dogs.
- There is a tendency for commercial packaging of probiotic products to be misleading and research is needed to prove the functions of each individual probiotic strain.

References

1. Thantsha M. S. *et al*. Probiotics – What They Are, Their Benefits and Challenges. 2012 In: New Advances in Basic and Clinical Gastroenterology Ed: T. Brzozowski. Pub: InTech. ISBN 978-953-51-0521-3
2. Vahjen W. *et al*. Effect of the Probiotic *Enterococcus faecium* NCIMB 10415 on Cell Numbers of Total *Enterococcus* spp., *E. faecium* and *E. faecalis* in the intestine of Piglets. 2007 Curr Issues Intest Microbiol 8 (1);1-7