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Characterisation of the Gut Microbial Community Structure of 15 Dogs with Chronic Anal Sac Disease and Effects of Glandex Chews® Supplement on Gut Microbiome Composition

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Abstract

Anal Sac Disease (ASD) is a common disease in dogs affecting the paired structures located on either side of the anus and is said to affect up to 12% of dogs [1,2]. This disease is primarily associated with the inability of the anal glands to empty and is said to be significantly affected by stool consistency among other factors. Composition of the gut microbiome can affect stool consistency [3] and inflammation hence may affect anal sac function and expression. The gut microbiome also contributes to host metabolism, protects against pathogens and 'educates' the immune system, hence affects directly and indirectly many physiologic functions of its host [4]. This study looked at the gut microbiome composition of dogs with documented chronic ASD without significant concurrent disease before and after 30day administration of Glandex supplement. We documented significant dysbiosis in these dogs which has improved somewhat with Glandex administration. Most significant changes documented were in gut microbial composition, most notably an improved Fusobacteria/Bacteroidetes (F/B) ratio.

Introduction

Anal sacs are pouch-like perianal structures of dogs and cats that have evolved for territorial marking and a defense against predators. These paired sacs (often incorrectly called Anal Glands) are located on either side of the anus between the internal and external sphincter muscles. They produce and can spontaneously release a watery to paste-like fluid that has a species-specific, unvaryingly disagreeable odor. This noxious material is formed by sebaceous glands, and in dogs, apocrine glands that line the sacs. Upon defecation, the pressure of the stool causes emptying of the contents through narrow ducts that open near the annus. Problems related to these structures are primarily associated with the inability to empty. Compression of the internal and external muscles of the anal sphincter and the concomitant mechanical pressure of stool during defecation effects a full or partial emptying of anal sacs. Anal Sac Disease (ASD) is very common presenting complaints at veterinary clinics. Affecting up to 12% of all canines [1]. Severity vary from mild irritation and itching; regional inflammation; infection; to cancer, presenting as excessive licking or biting at the rear end in an attempt to relieve discomfort, to considerable pain due to deep infection, abscess or tumor formation. It is suggested that a firmer stool can lead to reduction in the incidence of ASD as it exerts enough pressure on the anal glands to allow for natural gland expression. Stool consistency has also been associated with gut microbiota diversity and composition [3]. It is negatively correlated with species diversity, positively associated to the Bacteroidetes/Firmicutes ratio. The Bristol stool scale is a standardized method designed to classify the form of human faeces into seven categories. This scale was also used to score dog faeces in which the form of the stool depends on the time it spends in the colon [5].

The seven types of stool are:

1) Separate hard lumps, like nuts (hard to pass)
2) Sausage-shaped but lumpy
3) Like a sausage but with cracks on the surface
4) Like a sausage or snake, smooth and soft
5) Soft blobs with clear-cut edges
6) Fluffy pieces with ragged edges, a mushy stool
7) Watery, no solid pieces. Entirely liquid

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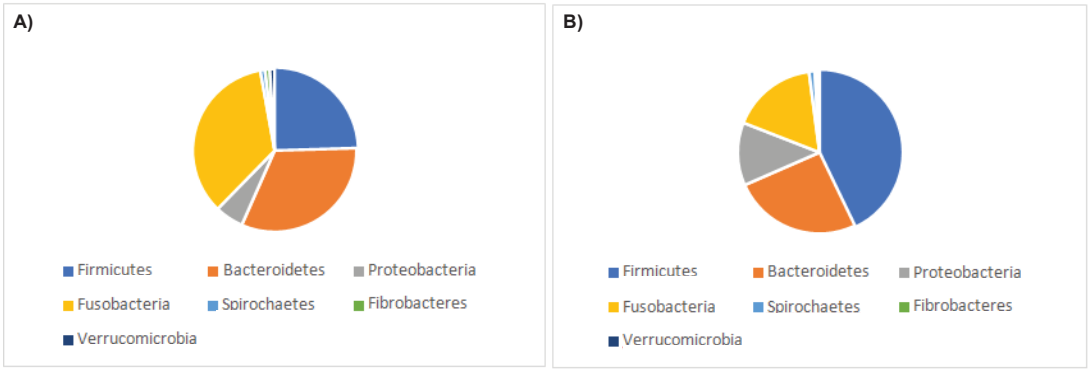

Figure 1 A: Healthy Dog (Phyla) B: Dogs with ASD (Phyla)

Types 1 and 2 indicate constipation. Types 3 and 4 are optimal, especially the latter, as these are the easiest to pass. Types 5–7 are associated with increasing tendency to diarrhoea or urgency5. Most bacterial analysis of the canine gastrointestinal tract fall into four main phyla: Firmicutes, Fusobacteria, Bacteroidetes and Proteobacteria 6. While there are variations in the microbiome composition along the GI tract, most clinical studies concentrate on faecal microbiota. Analyses of normal canine faecal microbiome indicated a predominance of these phyla in the below levels; Fusobacteria (24–40%), Bacteroidetes (32–34%), Firmicutes (15–28%), and Proteobacteria (5–6%) and Actinobacteria [6]. The two most important bacterial phyla in the gastrointestinal tract, Firmicutes and Bacteroidetes, have gained much attention in recent years. The Firmicutes/Bacteroidetes (F/B) ratiois widely accepted to have an important influence in maintaining normal intestinal homeostasis [7]. Increased or decreased F/B ratio is regarded as dysbiosis, whereby the former is usually observed with obesity, and the latter with inflammatory bowel disease (IBD). Age, diet, and many other environmental factors may play a significant role in the maintenance of a healthy microbiome, however, the alterations they cause pale in comparison with the alterations found in diseased animals. GI dysfunctions are the most obvious association with gut dysbiosis. In dogs, intestinal inflammation, whether chronic or acute, is associated with significant differences in the composition of the intestinal microbiota [6,8].

Materialsand Methods

This study was set out to look at the gut microbiome of 17 dogs with chronic ASD with 2 main objectives;

a) To explore specific changes in the gut microbiome of dogs with ASD when compared to healthy dogs and

b) To evaluate effects of feeding 30days of Glandex Chews® on the gut microbiome in these dogs.

To standardize the study and reduce as many variables as possible selected participant acted as its own control comparing before and after with minimal variations during the 30 day trial period each dog;

1) Had documented ASD for at least 12 months with repeated regular expression of the sacs by a veterinary surgeon (at least 4 times per annum)

2) Dogs were of both genders, various breeds, with ages between 2 and 7 years and body weights ranging 5. 1-21. 0 Kg

3) Had stable nutrition stable prior to and during the 30 day trial period. Owners were instructed to notify the investigators immediately if any significant change to the diet took place. Those dogs were excluded from the analysis.

4) We're not receiving any medication other than routine parasite control. Owners were instructed to notify the investigators immediately if any medication was administered. Those dogs were excluded from the analysis. 2 dogs that received antibiotic and anti-inflammatory medications were excluded from the trial analysis leaving only 15.

5) Participants had no documented concurrent disease

6) Participants were instructed to give the Glandex chews daily at the same time according to the manufacturer's dosing recommendation

First faecal samples were collected within 24 hours BEFORE feeding of Glandex Chews. And a second time within 24 hours AFTER the dog received the last daily Glandex Chew 30 days later (30 daily doses). Gut microbiome samples were submitted to Orivet Genetic Pet Care Laboratory and analysis was performed using an Illumina 16S metagenomics analysis at Orivet's research partner the University of Aberystwyth Institute of Biological, Environmental and Rural Sciences, center of excellence for genomic research, UK. Participants also filled a health questionnaire before and after the trial where Bristol stool scores were reported along with feedback on general wellbeing of the dogs and the owners' compliance and impressions of the efficacy of Glandex Chews.

Results

Pre-Glandex Treatment Analysis

Evaluation of the gut microbiome of participating dogs with documented ASD revealed low diversity scores with an average Shannon Index of 2. 81 (compared to average 3. 28 reported in the literature9). Participants had a Bristol Stool Score of 4. 53 (soft blobs with clear cut edges) which is slightly above normal (3-4) and perhaps suggesting slightly lacking in fibre. Participants showed significant dysbiosis compared to average documented healthy dog phyla population (Figure 1). Most significantly High level of Firmicutes (average 40. 9%), Low Levels of Bacteroidetes (24. 4%) with a F/B ratio of 1. 68. Average Fusobacteria levels were also low at 16. 2% and relatively high levels of Proteobacteria at 11. 9%.

Genus level analysis also documented significantly low levels of Fusobacterium, Prevotella, Blautia, Megamonas, Bifidobacterium and Streptococcus and high levels of Clostridium and Sutterella(Figure 2).

High level of Staphylococcus, common skin pathogenic bacteria in dogs was also noted at 24. 2% of top 30 most significant species.

The above confirmed significant dysbiosis of all 15 dogs

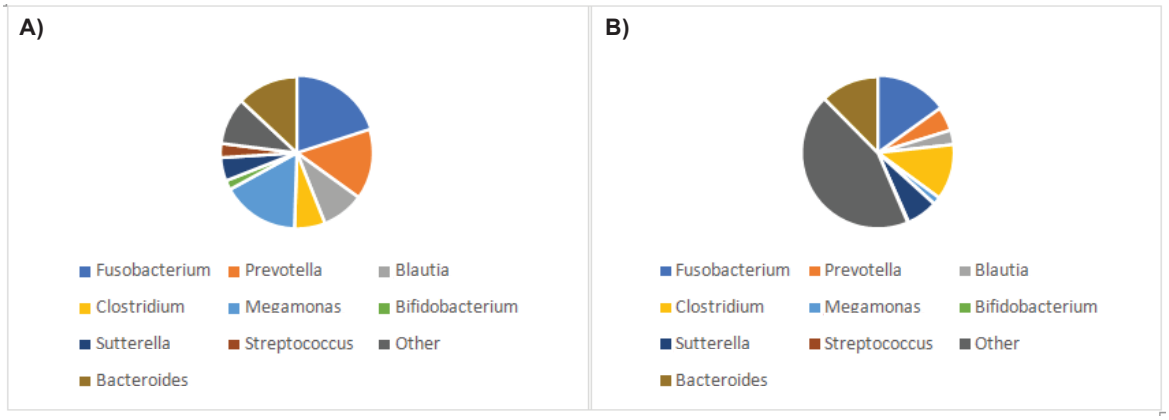

Figure 2A: Healthy Dog (Genus), B: Dogs with ASD (Genus)

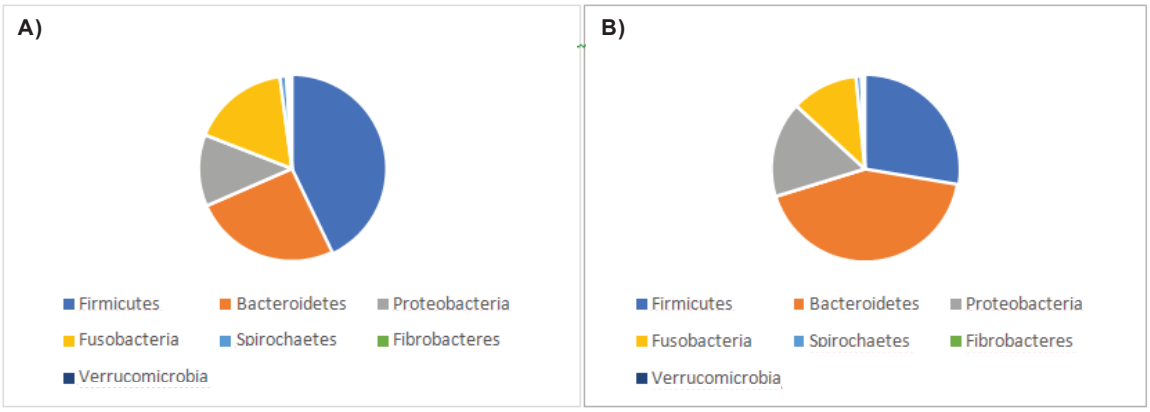

Figure 3A: Dogs with ASD (Phyla), **B:** Dogs with ASD Post Glandex (Phyla)

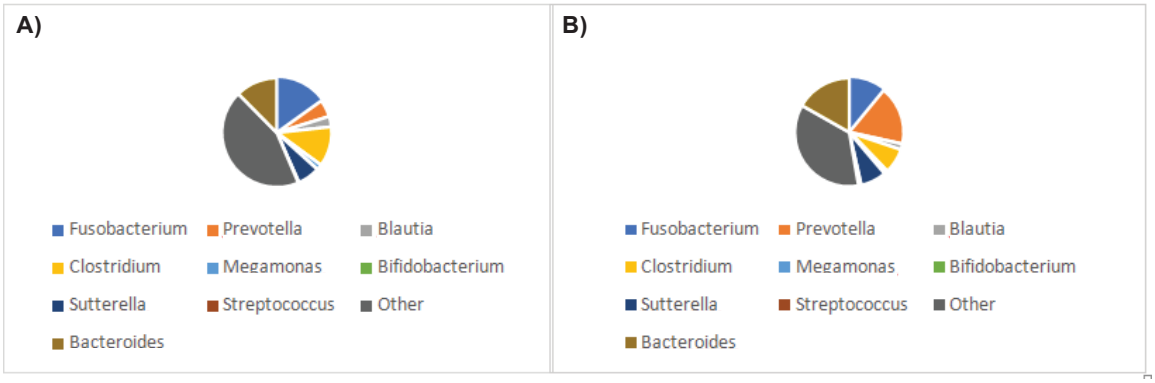

Figure 4A: Dod with ASD (genus), **B:** Dogs with ASD Post Glandex (genus)

with chronic anal sac disease. Post-Glandex Treatment Analysis (30 days administration of Glandex Chews). Average Shannon diversity index dropped slightly from 2. 81 to 2. 57. Owner reported Bristol stool score improved from an average of 4. 53 to 3. 63. Analysis of the gut microbiome was performed. On the Phylum level, most notable changes were significant reduction in Firmicutes (from 40. 9% to 26. 7%) and significant increase in Bacteroidetes (from 24. 3% to 41. 2%). With a F/B significantly reducing to 0. 64. Fusobacteria also reduced significantly from 16. 2% to 11. 2%), Figure 3 below.

Genus level analysis post Glandex Chews administration documented significantly changes most notably significant increase in Prevotella (from 5. 1% to 17. 6%) and reduction in Clostridium levels (from 11. 8% to 7. 6%) (Figure 4).

Staphylococcus levels also reduced significantly from 24. 2% to 6. 4% of top 30 most significant species.

Owners' Questionnaires

Owner compliance and impressions of the study were captured using a standard questionnaire. Owners confirmed no dietary changes or medication during the 30 days consumption of Glandex Chews. 2 dogs that received antibiotic and anti-inflammatory medications were excluded from the trial analysis. Over 90% compliance with all dogs receiving their daily dose (Figure 5) with no significant issues with palatability of the

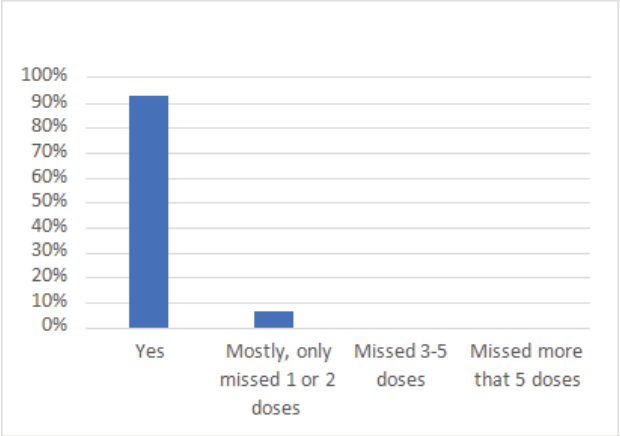

Figure 5: Did your pet receive Glandex Chews daily?

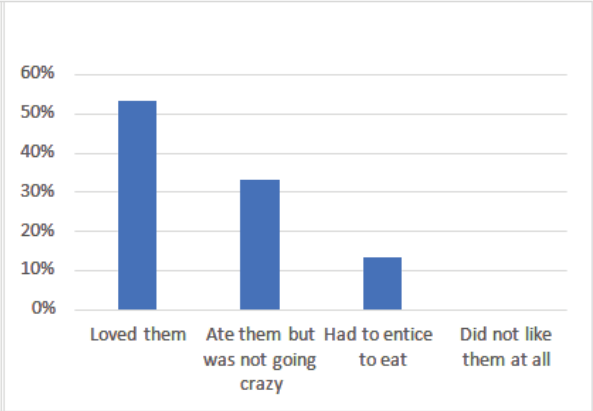

Figure 6: Palatability

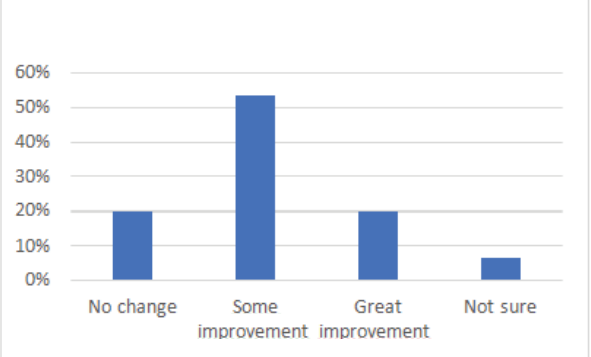

Figure 7: Change in AS symptoms

Glandex chews (Figure 6). 73% of pet owners reported "some improvement" or "great improvement" in their dog's anal sacculitis symptoms after 30 days. With 27% reporting "no change" or "not sure" if any notable change occurred (Figure 7).

Discussionand Conclusion

Gut microbiome analysis of healthy dogs with ASD revealed lower diversity compared to average documented healthy dogs. Shannon diversity score did not change significantly post 30 days of Glandex administration. Diversity scores have been suggested to be the single most important indicative measure of gut microbiome health. However other study utilizing mathematical modelling to investigate the ecological dynamics of microbial communities has shown that diversity and stability may not always be concomitant [9]. Specifically diversity comparisons between healthy and pathological states frequently yield contradictory results. There is a need to broaden our approach to the analysis of microbiome data if we are to better understand this complex ecological community and its role in animal health and disease. Gut microbiome analysis of healthy dogs with ASD revealed significant dysbiosis compared to average documented healthy dogs in both phyla and genus populations (figures 1 &2). One of the most significant changes is a change in F/B ratio of 1. 68 which is often seen in overweight dogs and is associated with changes in carbohydrate metabolism [7]. This change was also reported to be associated with human stool consistency scores [3] who may help explain effects on ASD. Firmicutes phyla is containing the classes Clostridia and Bacilli, are thought to more efficiently extract energy from food compared to other bacterial groups. In people, obesity has been found to correlate with an increased proportion of Firmicutes and a decreased proportion of Bacteroidetes [7]. It is not surprising that body conditions (overweight) will have an impact on the mechanics of anal sac expression as typically anal sacs position will be pushed further away from the annus making it harder to express. The documented improvement in F/B ratio with Glandex use may suggest affected dogs were overweight; however we did not record changes in body weight in this study. F/B ratio associated with stool consistency may suggest another mechanism by which ASD is affected. Indeed overall improvement noted in the gut microbiome composition may suggest combined effect on ASD. Affected dogs also had a documented low Fusobacteria and high Proteobacteria levels. These groups contain many pathogenic species, but they do have a role to play in the health of the biome, including the production of nitrogen (included in many hormones, amino acids & enzymes) and vitamins for use by the host.

Low levels of fusobacteria have been associated with poor nutrition. Most notable changes at the Genus level were increase in Prevotella (from 5. 1% to 17. 6%) and reduction in Clostridium levels (from 11. 8% to 7. 6%) (Figure 4). Prevotella are an important part of the normal healthy biome and contribute to the health of the dog by helping digestion and energy production. Low levels usually suggest a diet lacking in complex carbohydrates, such as the fibre found in Glandex, which may explain their level increase. Clostridium is very important members of the healthy biome and although some are pathogens, most are thought to play an important role in immune modulation and gut wall barrier integrity. High Clostridium is associated with high protein low carbohydrate diets. An interesting finding was high levels of Staphylococcus, a common skin pathogen noted in affected dogs which was significantly reduced post Glandex administration. This may be linked to increased licking of the perianal dermal area leading to colonization of the gut by these bacteria or may be associated with overall increased inflammation often seen in dogs with gut dysbiosis. Lastly some minor positive changes were noted in stool scores which may have had an impact on AS expression. Overall encouraging changes were seen in affected dogs following 30day use of Glandex administration but, dysbiosis still exist (all be in less severe). It is suggested the gut microbiome can take several months to re-balance and up to 12 months following prolonged antibiotic administration, hence it's entirely possible that 30 days may not be a long enough period to appreciate the full effect of Glandex administration on the gut microbiome. Further research is needed to document the long term change and the mechanism of this change in the composition of the gut microbiome community in dogs with ASD.

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