A comparison of culture vs 16S ribosomal RNA sequencing of chronic granulation tissue microbiota in cats and dogs.

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BACKGROUND

Chronically healing wounds affect many animals seen in general practice. Routinely, microbiological culturing is used to profile the bacterial species present in these wounds. Studies into the microbiota of these types of wounds in human medicine have shown that culture may be insufficient for detecting the extent of the bacterial species present. Culture-free 16S ribosomal RNA (rRNA) sequencing, being more sensitive than culture, may give a more accurate picture of the bacteria present. This study compares the results of culture and molecular testing in chronic wounds in animals.

METHOD

Samples from 10 chronic wounds (6 dogs and 4 cats) underwent culture and 16S rRNA sequencing. Genomic DNA was isolated and the 16S rRNA V3-V4 region was amplified by PCR. Subsequently, libraries of the genomic DNA were constructed for sequencing on the Illumina MiSeq platform. The resulting sequence reads were trimmed from primer sequences, paired-end reads were then joined and quality filtered. QIIME was used to cluster reads against the Greengenes database to provide taxonomic assignment, referred to as closed Operational Taxonomic Units (OTU) picking.

RESULTS

Culturing identified 6 genera across 5 samples; 5 samples were culture negative. In contrast, 98 unique genera were identified by 16S rRNA sequencing. *Campylobacter spp.* had the highest overall abundance however it was not detected by culturing in any of the samples, likely due to its growth requirements not being met using standard culturing procedures. Of the 6 genera identified by culturing, 3 could be classified to the genus level by 16S rRNA sequencing and 2 were assigned to family level. One case, which had a history of myiasis, showed a significantly different make-up of the microbiota detected by 16S rRNA sequencing.

CONCLUSIONS

The discordance between the culture and 16 rRNA sequencing was dramatic, with culture failing to identify the majority of the microbiota identified by sequencing. This suggests that culturing techniques may be inadvertently selecting for specific bacteria that are easily cultured rather than identifying the most abundant or clinically relevant bacteria. This may, in turn, be directing veterinarians towards the use of inappropriate antibiotics. It is, however, unclear as to whether some of the detected DNA may be from dead bacteria on the surface of the tissue rather than surviving within the wound. In depth analysis of OTU association with patient and clinical presentation characteristics is ongoing.

Canine raw meat diets and antimicrobial resistant *E. coli*: is there a link?

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Antimicrobial resistance is increasing amongst canine commensal and clinical bacteria. Risk factors for faecal carriage of AMR *Escherichia coli* have been reported, including the consumption of raw meat diets, however few studies have investigated dogs fed on such diets This study aimed to determine the prevalence of AMR (resistance to at least one antimicrobial), multidrug resistant (MDR; resistance to three or more antimicrobial classes) and third generation cephalosporin resistant (3GCR) in canine faecal *E. coli*. The University of Liverpool Ethics Committee approved this study in March 2015. Faecal samples (n = 190) were obtained between May and July 2015 from dogs eating raw-meat (n = 114), or cooked meat (n = 76) diets. Selective and enrichment culture were used to detect bacteria and biochemical testing and PCR assays for the *uid*A gene were used to confirm identification. *E. coli* were tested for antimicrobial resistance by disc diffusion (CLSI 2013) to a range of antimicrobials (amoxicillin, amoxicillin-clavulanate, gentamicin, ciprofloxacin, chloramphenicol, tetracycline and trimethoprim sulfamethoxazole) and isolates from 3GCR impregnated agar plates (1 µg/ml ceftazidime and 1 µg/ml cefotaxime) were additionally tested for cefpodoxime resistance. AMR was significantly more likely to be detected in raw-fed compared to cooked-meat-fed dogs: 54% of dogs (95% CI: 45 – 64) compared to 17% (95% CI: 9 – 26) of dogs ($P < 0.001$). Furthermore MDR was also more likely in raw-fed 25% (95% CI: 17 – 32) compared to 4% (95% CI: 0 – 8) of cooked-meat-fed dogs ($P < 0.001$). 3GCR *E. coli* was detected in 31% (95% CI: 22 – 39) of dogs that were raw-fed and only 4% (95% CI: 0 – 8) of dogs that were cooked-meat-fed dogs ($P < 0.001$). Raw-fed dogs may be a source of antimicrobial resistant *E. coli* for the household representing both a potential public health and animal welfare issue. Preventative measures need to be implemented to prevent dissemination of such bacteria.

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