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CASE REPORT

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Otic Microbiome Transplantation in a Dog with Recurrent Otitis Externa: Unraveling Allergic Secrets with Break Through Treatment Methodology

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ABSTRACT

Canine otitis externa (OE) is a common inflammatory disease that is frequently complicated by secondary bacterial and/or yeast infections. The otic microbial population is more complex than appreciated by cytological methods and aerobic culture alone. In the present case report a dog with recuurent OE and newly diagnosed otitis media was enrolled. Due to previous unsuccessful treatment trials with immunosupressive agents and antibacterial choices, with polypharmacy, we decided to perform manupilation of the microbiome in an attempt to investigate otic microecology. For this purpose first author gave his opinion for frontier knowledge and several years of experience for microbiome transplantation. Given literature data regarding ear canal microbiome among dogs with OE, is easily distinguishable to that of healthy ones, we decided to perform otic microbiome transplantation (omT). Microbiome samples were taken prior to and thereafter 10th days of omT, were collected and sent to MiDOG Limited Liability Company (LLC) service for next generation sequencing. Following omT, clinical signs were resolved and regressed, even starting on day 3 with satisfactory results (exudate was disappeared). Microbiome analytes showed *Porphyromonas cangingivalis*, *Peptoclostiridium* sp., *Pasteurella canis*, *Malassezia pachydermatis* and *Aspergillus* sp. with increased relative abundances, whereas no more pathogen were evident 10 days after omT. Pathogens were switched and substituted with new microorganisms with potential probiotic facilities. Observed species were increased following omT [prior to omT 47 vs. after omT as 98]. This case report for the first time, showed the efficacy of changing microechology of otic microechology by omT. This treatment modality would be developed by the resarcher group herein dedicated.

INTRODUCTION

Otitis externa (OE) and/or media, are unresolved inflammatory recation for external ear canal and middle ear cavity, respectively, among dogs (Doyle, 2004; de Bellis, 2015; Koch, 2017; Pye, 2018; Bajwa, 2019; Harvey, 2022). Altough veterinary surgeons cope with OE, otitis media keeps its position namely a disease condition that has been underrated in the vast majority veterinary practices (de Bellis, 2015). Given ear canal microbiome among dogs with OE, is easily distinguishable to that of healthy ones (Bradley et al., 2020; Kasai et al., 2020; Tang et al., 2020), we decided to perform otic microbiome transplantation (omT) in a dog with recurrent OE and newly diagnosed otitis media. Following first authors several years experience of both fecal and skin microbiota transplantation, he and his teammates decided to transplate otic microbiome in an unresolved case with several long duration of antibacterial trials and polypharmacy.

MATERIALS AND METHODS

Case history

A 3 years old dog was referred to several different veterinary private practices and finally the owner was instructed to a veterinary clinic in which an academician was in advisory board with a surgery certificate. However at that clinic 3 different antibacterial choices were prescribed, as was also the case in several clinics. Previously polypharmacy included antibiotics, antihistaminics and immunosupressive drugs. On referral there was bilateral exudate, rare head shaking and decerased hearing. There was no facial nerve paralysis nor Horner syndrome. Signs of OE were evident. Inflammation of the ear canal was strikingly evident. Ear canal was nearly closed, denoting terminal staging of inflammation. The owner also reported cutaneous adverse food reaction for several months, in which referred veterainary surgeons frequently offered and prescribed diets (none of them were low glycemic index, nor low carbohaydrates ratios).

Ethics statement

Both dogs participated at the present research study were referred to the University of Adnan Menderes, Faculty of Veterinary, Department of Internal Medicine. Both dogs were subjected to physical examination. Matched microbiome samples, 10 days apart prior to and thereafter omT, were collected and used solely for this case report with written owner consent. The present case report, as part of another research/clinical trial was approved by the local ethic committee of Aydın Adnan Menderes University-HADYEK on October 27, 2021 with no: 64583101/2019/022.

Microbiome analytes

A total of 2 seperate cotton swab samples were taken from middle ear, were analyzed. For sample collection, DNA extraction, library preparation, and sequencing brief explanation was follows. Samples were collected with swaps that were prepared and sent previously by MiDOG LLC service (including all neceesary instructions). Following unboxing, aseptical sterile, DNA free swab was handled and subjected gently to the middle ear of recipient. Briefly twisting off and twirling the swab 2-3-times over the ear charge and the swab tip was broken off into previously purchased sterile, sample administration material containing a DNA/RNA preservative, as was previously described (Tang et al., 2020), similarly to preior methodlogy (Ural et al., 2002; Ural et al., 2023) All samples were then shipped for processing to the MiDOG LLC testing center (Irvine, California). Genomic DNA available through ZymoBIOMICS™-96 DNA kit (Cat. No. D4304, Zymo Research Corp.) (Tang et al., 2020) along with 16S rDNA V1-V3 region was focused against bacterial analysis. Relative abundances regarding fungi were detected suggesting an equivalency of one 16S rDNA copy to one fungal ITS copy. Entire primer sequences were ready on the MiDOG LLC Service facilities. Methodology was previously determined (Tang et al., 2020).

Unenriched otic microbiome transplantation

This untold, entirely new technique was developed by the first author (K.U.) for several stages of scientific organization invoving consideration, planning, preparation and application. For every single moment first author tought like an engineer, who put measuring tape on his shoulder. Donor was a known owner kept dog without stress presentation, as was shown in table 1. Briefly omT was shown in figure 1. With every stages of it. Cotton swap was not subjected to any other condition, directly used fort his purpose. Material was collected from the donor and suddenly transferred to recepient.

Table 1. Stress interpretation, adopted from (Lloyd, 2017) for dogs enrolled herein for omT.

	donor	recipient
Clinical enterance	0	1
Following entrerance to practice room at faculty	0	0

Figure 1. Otic microbiome transplantation in a dog with recurrent otitis externa. A) preparation of cotton swap ready for use, b) cotton swap was directed to external ear canal and rotated for entire collection of presumably healthy microbiome, c)material was withdrawn from donor, d) recipient, namely a dog with recurrent otitis externa, was just prior to otic microbiome transplantation, e) cotton swap was directed to recipient's external ear canal and f) rotated for entire contact.

Assessing stress value for both donor and recipient dogs during omT

In an attempt to analyze the stress levels of both donor and recipeint dogs, assessment previously determined (Lloyd, 2017) was used by the reseracher team as follows.

This stress value was based on scoring from 0 [highest friendly attraction, outgoing and mindful/concerned of attention] to 5 [extreme presentation of stress; i.e., hiding, barking, howling etc].

RESULTS

Photographic evidence of proof was shown below at figure 2 also presenting both the donor and the recipient (before and after omT).

Figure 2. Showing the donor with a healthy ear canal (visible and easily seen/observed with an open tissue appearance), whereas c-d) presented the recipient with recurrent otitis externa that the ear canal is nearly be closed, just prior to terminal stage of allergy. At the bottom photograph recorded at 3rd day of omT. Ear canal was visible and quite opened in contrast to referral day. Inflammation was regressed.

Microbiome analytes

Table 2 and 3 presented all necessary data tabulated by MiDOG LLC service located in USA. Figure 3 depicted results of observed species belonging to microbiota members before and after omT. Following omT Alpha diversity was changed and new species were observed as follows (Table 3)

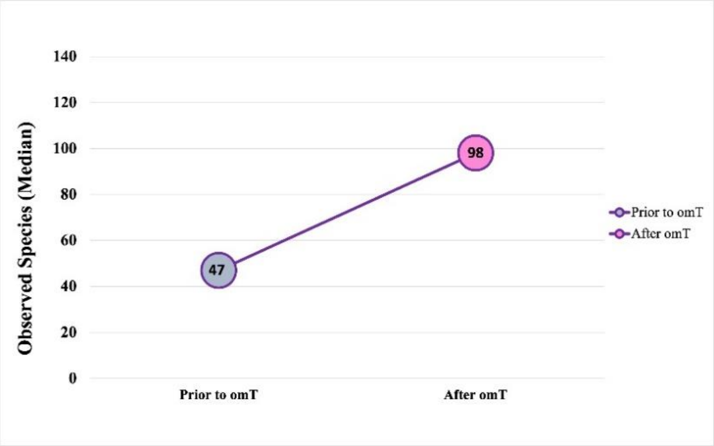

Figure 3. Depicting satisfactory results that was related to omT at ths present case. Observed species belonging to microbiota members were elevated from 47 to 98.

Table 2. *Malassezia pachydermatis*, *Aspergillus sp.*, *Acremonium hyalinulum* relative abundaces were detected as 0.2%, 0.1% and 0.0%, respectively.

	Percentage		**Cells per sample**		**Normal ranges**
Bacteria species detected with potential clinical relevant disease	**Prior to omT**	**After omT**			
Porphyromonas cangingivalis	25%	2.2%	15.000.000	1.400.000	0-46853
Peptoclostiridium sp.	8.9%	0	5.400.000	0	0-0
Pasteurella canis	1.0%	0%	600.000	0	0-1725
Corynebacterium mastitidis	0.8%	0%	480.000	0	0-770
Truperella bernardiae	0.7%	0%	450.000	0	0-0
Fungi species detected					
Malassezia pachydermatis	0.2%		17		0-22
Aspergillus sp.	0.1%		6		0-30
Acremonium hyalinulum	0.0%		2		0-0

Table 3. New fungal species that were absent prior to omT, which was existed following omT.

New bacterial species (selected ones) observed following omT	
Fusobacterium sp.	19%
(o) Fusobacteriales sp.	13%
Bacteroides plebeius	11.6%
f) Lachnospiraceae sp.	6.9%
Lachnoclostridium sp.	6.6%
Megamonas funiformis	5.2%
Streptococcus equinus-infantarius-luteiensis	4.8%
New fungal species (selected ones) observed following omT	
Saccharomyces cerevisiae	21.4%
Cladosporium sp.	14.3%
(c)Eurotiomycetes sp.	7.1%
Lophodermium sp.	7.1%
Stereum complicatum	7.1%
Penicillium sp.	7.1%
Malassezia restricta	4.8%
Candida sake	4.8%

DISCUSSION AND CONCLUSION

Clinical findings regarding otitis media among dogs enrolled were frequently accompany OE (shaking of head shaking, rubbing/scratching the infected ear, appearent exudate within the ear canal) (Doyle, 2004; Harvey, 2022; Koch, 2017, Pye, 2018). In the present case all aforementioned clinical signs were evident with recurrent presentation, unresponsive to several treatment trials of immunosuppressive and antibacterial choices. Polypharmacy was not accepted by us, and at initial referral with compaints of owner, whom was a teacher, we advised and told her that 'the past is no more concern'. Hence we informed the owner what we tought was 'treat to target' by omT. We, as reseracher group, were luckily in that stress values for both donör and recipient were acceptable, which prompted and motivated us for better application. To our knowledge, prior to transplantation procedure, veterinary surgeons should be aware of stress value for dogs.

In a prior study hypothesized that regarding canine OE; otic microbial echology is more compounded than detected by cytology and aerobic culturing. In that study 30 dogs with OE compared to 10 healthy ears; *Staphylococcus spp.*; *Pseudomonas aeruginosa* and *Malassezia spp*. were isolated with Staphylococcus as most abundant taxa (Bradley et al., 2020). Kasai et al. (2021) investigated alterations within the ear canal microbiota of dogs with OE. In that study ear swab samples were taken from 23 dogs with OE and to those 10 healthy controls. 16S rRNA gene sequenced using Illumina MiSeq and next generation sequencing revealed that otic microbiota in diseased ones exhibited significantly decreased alpha diversity. Elevated relative abundance of the genus Staphylococcus along with other relevant ones suggested that ear canal microbiota of dogs with OE was differing from healthy ones (Kasai et al., 2021). Relatively novel research identified microbiome of healthy/diseased canine ear samples. Out of 589 canine samples, 257 ear swab samples (128 healthy vs. 129 clinically affected) were subjected to next-generation sequencing. According to the results of that research overall loss of microbial diversity and altered relative/absolute abundances of bacteria and fungi were evident in diseased dogs. In comparison to healthy dogs, 78.3% of the diseased ear samples existed microbial overgrowth [i.e. 69.8% bacterial overgrowth, 16.3% fungal overgrowth, and 7.0% both with bacterial/fungal overgrowth]. Within the vast majority taxa enriched in diseased ears were *Staphylococcus pseudintermedius*, *Staphylococcus schleiferi*, *Malassezia pachydermatis* along with *Finegoldia magna*, *Peptostreptococcus canis*, and *Porphyromonas cangingivalis (Tang et al 2020)*. In the present article altough only 1 case was involved, same methodlogy was evident by MiDOG LLC testing center (Irvine, California), denoted as next-generation sequencing. In the present case, reported herein *Porphyromonas cangingivalis*, *Peptoclostiridium sp.*, *Pasteurella canis* were the most abundant bacterial species with relative abundaces of 25, 8.9 and 1%, respectively. On the other hand mycobiome revealed *Malassezia pachydermatis, Aspergillus sp., Acremonium hyalinulum with relative abundaces of* 0.2, 0.1 and 0.0%, respectively. Afterwards omT resulted with observed species increased from 47 to 98, as was shown in figure 3. Similar results were deemed available at our case report and research by Tang et al. (2020), in which following omT pathogen bacteria and fungal species were lost, indicating the efficacy of omT.

Regarding the significance and impact of this case report, for the first time omT was applied at veterinary field, alongwith comperative analyses of the otic microbiota, might provide practical strategy for battling OE and media and provide supportive data for the appropriate treatment of otitis among dogs

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: K.U., Design: K.U., Data Collection or Processing: K.U., H.E., S.E., C.B., Analysis or Interpretation: K.U., H.E., S.E., C.B., Literature Search: K.U., H.E., S.E., C.B., Writing: K.U.

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