Malassezia Otitis Externa in the Dog: The Effect of Heat-fixing Otic Exudate for Cytological Analysis

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Summary

This study was conducted on 32 dogs with *Malassezia* otitis externa in order to determine the effect of heat-fixing otic exudate on cytological analysis. *Malassezia* infection was confirmed by cytological examination of otic exudate. Otic discharge collected with cotton swabs was then rolled onto glass slides. One slide per dog was heat-fixed prior to staining; another slide was not heat-fixed. The number of yeast in 10 oil immersion fields (1000x) was counted for both slides from each dog. Statistical analysis revealed that heat-fixing did not systematically cause there to be increased or decreased numbers of *Malassezia* on cytology of otic exudate.

Introduction

Otitis externa is the most common condition of the canine external ear canal accounting for approximately 15% of all dogs presenting for veterinary care (Tater, et al., 2003). It is defined as inflammation of the ear canal with an underlying reason for the infection in essentially all cases (Rosser, 2004). In the early stages of this condition, inflammation of the external ear canal results in varying degrees of erythema of the pinnae, external auditory meatus, and lining of the external canal. This inflammation can result in clinical signs such as head shaking, ear scratching, ceruminous or purulent otic discharge,

excoriations, malodor, swelling and pain (Scott, et al., 2001; Girao, et al., 2004; Greene 2006). In chronic cases of otitis externa, proliferative changes can occur making treatment of clinical signs more difficult. The tympanum becomes susceptible to rupture and the development of concurrent otitis media is a potential sequela (Rosser, 2004). Fibrosis and mineralization of tissues surrounding the external ear canal can also occur and is most commonly seen in Cocker Spaniels with ceruminous or sebaceous gland hyperplasia (Greene 2006). In these cases, surgery may be the only option for treatment (Rosser, 2004).

Treatment of the clinical signs of otitis externa relies on cytological examination of otic exudate. Cytology is a mandatory test for every patient presenting with clinical signs of otitis externa. It is inexpensive, simple, and gives immediate results so that treatment can be initiated (Angus, 2004). It allows the investigator to identify and characterize microbial overgrowth or infection and is more sensitive in detecting *Malassezia* and bacteria than culture (Angus, 2004; Tater et al., 2003). Cytology strengthens interpretation of culture and susceptibility data, guides rationale for therapeutic decisions, and permits more accurate monitoring of response to treatment. In clinical practice, treatment of canine ear disease often targets the secondary causes of otitis externa, such as bacterial and yeast infections, and the primary and predisposing causes are neglected (Scott et al., 2001). It is essential that a systematic approach is taken to identify the underlying cause in order to prevent the recurrent nature of the disease. If this is not done successfully, bacteria and yeast become resistant to antimicrobial agents (Rosser, 2004).

Controversy exists over how to best prepare slides of otic exudate for cytological analysis. There is no definitive evidence in current literature that promotes heat-fixing otic exudate over not heat-fixing prior to staining. In clinical practice, it varies widely from clinician-to-clinician whether or not samples are heat-fixed. Many dermatologists have for years believed that heat-fixing is valuable in preventing loss of organisms, wax, and lipids in the staining process (Griffin, CE, 2001; Scott et al., 2001; Angus, 2004; Greene 2006). However, the authors of the *Atlas of Cytology of the Dog and Cat* are ambiguous as to whether or not to heat-fix. In fact, the authors state that slides of otic exudate may be "heat or alcohol fixed, or air dried" prior to staining with routine hematological stains (Baker, et al., 2000). The purpose of this study was to determine the necessity for heat-fixing otic exudate for routine cytological analysis.

Materials and Methods

Thirty-two dogs were included in this study. All dogs had clinical signs of otitis externa, including head shaking, ear scratching, otic discharge, excoriations, malodor, swelling and pain upon palpation of the auricular cartilage (Griffin, 1993; Scott et al., 2001). Diagnosis of *Malassezia* otitis externa was confirmed by counting greater than a median of 0.3 yeast per high power microscopic field (Tater, et al., 2003). *Malassezia pachydermatis* were chosen as the organism to study because they are (1) easy to identify and quantify, (2) prevalent among dogs with otitis externa, and (3) associated with a waxy and greasy otic discharge.

After the diagnosis was confirmed, one ear per dog was sampled using a non-sterile cotton-tipped applicator. The otic exudate was collected by inserting the cotton

swab into the external ear canal aiming for the junction between the vertical and horizontal ear canals where the cartilage bends at about a 45% angle (Angus, 2004). The exudate was rolled onto three glass slides. One slide per subject was heat-fixed by placing it over an open flame for 10 seconds and then stained with a modified Wright's stain (Diff-Quik®; Baxter Healthcare Co., McGraw Park, IL, USA), one slide per subject was not heat-fixed prior to staining with Diff-Quik®, and one slide per subject was kept unstained. Each stained slide was dipped in xylene and mounted with a cover slip using Permount® (Fisher Scientific, Pittsburgh, PA, USA). The stained slides were blinded by one of the investigators (DWS) prior to being read out by a different investigator (JSG).

The number of *Malassezia* yeast per oil immersion microscopic field (1000X) was counted in 10 fields for each of the paired samples from each dog. Ten fields were chosen based on evidence that 10 fields needed to be counted in order to get a representative count of the number of yeast on a slide due to the variability from field to field (Tater, et al., 2003).

Differences between fixed and unfixed samples within each dog were analyzed in order to describe changes within each dog attributable to the fixation process. The mean number of yeast was calculated for each of the paired slides for each dog (i.e. heat-fixed and unheat-fixed). As the results were not normally distributed, only non-parametric statistical tests were used. Wilcoxon's signed-ranks test evaluated whether the medians in the two paired sets of data for each dog were significantly different (Saunders-Dawson, 1990). Statistical significance was defined as $P \leq 0.05$. The data were analyzed using commercial software (Statistix 8.0, Copyright 2003; Analytical Software, Tallahassee, FL, USA).

Results

The study population included 23 purebreeds and 9 mixed-breeds (Table 1). No breed was over-represented. There were 17 spayed females, 5 intact females, 8 castrated males, and 2 intact. The dogs ranged in age from 9 months to 13.5 years (median age 6.5 years).

Table 1. Breeds of dogs represented in this study

Breed of dog*	No. of dogs in study
Basset Hound	1
Bichon Frise	1
Boston Terrier	1
Boxer	1
Bulldog	1
Cocker Spaniel	3
Collie	1
English Cocker Spaniel	1
German Shepherd Dog	1
Golden Retriever	5
Havanese	1
Labrador Retriever	2
Parson Russell Terrier (Jack Russell Terrier)	1
Pekingese	1
Poodle	1
Portuguese Water Dog	1
Mixed breed	9

*The American Kennel Club name for the dog breed was used for those breeds that are registered with the AKC.

The medians of the heat-fixed and unheat-fixed samples for each dog are presented in Table 2. Differences between the minimum, median and maximum yeast counts of fixed and unfixed samples were all close to zero meaning that heat-fixation did not systematically cause there to be either more or fewer yeast visible on fixed or unfixed slides (Table 2). The two-tailed *P* values for normal approximation of the minimums, medians, and maximums of the heat-fixed and unheat-fixed slides were $P = 0.20$, $P = 0.69$, and $P = 0.36$, respectively. There was no indication that heat-fixation increased or

decreased yeast counts when compared to samples that were not heat-fixed prior to staining.

Table 2. Medians of Heat-fixed and Unheat-fixed Samples

Case	Heat-fixed	Unheat-fixed	Case	Heat-fixed	Unheat-fixed
1	4.5	2.5	**17**	17	28.5
2	1.0	1.0	**18**	3.5	28.5
3	1.0	2.0	**19**	11	17.5
4	1.5	2.0	**20**	0	0
5	3.0	1.0	**21**	1.0	0
6	2.0	1.0	**22**	81	5.0
7	6.5	10.5	**23**	26	26
8	41.5	18.5	**24**	6.5	11.5
9	2.0	2.0	**25**	5.0	4.5
10	1.0	0	**26**	0.5	0
11	0.5	0	**27**	0.5	3.0
12	2.0	3.5	**28**	32.5	25.5
13	21	11	**29**	4.5	4.0
14	62.5	43.5	**30**	0	0
15	4.0	4.5	**31**	15.5	44.5
16	4.0	4.0	**32**	21.5	16

Discussion

Otitis externa is a multi-factorial disease that is initiated by many predisposing factors. One of the most common predisposing factors is conformational defects that alter the microclimate within the ear canal. This can lead to increased temperature and humidity within the canal. Conformational defects include stenotic canals, excessive hair in the canals, and pendulous pinnae. Excessive moisture due to excessive swimming or bathing can also lead to otitis externa. The excessive moisture causes maceration of the stratum corneum lining the external ear canal removing the normal protective barrier to secondary infection. Resident microflora of the external ear canal can then become opportunistic (Rosser, 2004).

Excessive cerumen production can also attribute to otitis externa. It provides a cutaneous microenvironment of increased moisture and surface lipids and a compromised barrier function that encourages the overgrowth of organisms, especially yeast (Crespo, et

al., 2002). Treatment effects that include trauma from cotton tipped applicators, irritating topicals, and super-infections resulting from altering the normal microflora predispose to otitis externa. Neoplasms, polyps and granulomas are examples of obstructive ear diseases that can initiate otitis (Rosser, 2004). Systemic diseases associated with immunosuppression, viral diseases, debilitation, and catabolic states can also predispose to otitis externa.

Primary causes of otitis externa are processes or factors that directly initiate inflammation of the external ear canal (Rosser, 2004). It is important to identify and treat these processes. These causes include hypersensitivity diseases, keratinization disorders, parasites, and foreign bodies. Hypersensitivity disorders include food hypersensitivity, atopic dermatitis, contact hypersensitivity, and adverse cutaneous drug reactions. Primary idiopathic seborrhea, hypothyroidism, sex hormone imbalances, and lipid-related conditions can all lead to keratinization disorders. The most notable parasite afflicting the external ear canal is the ear mite, *Otodectes cynotis.* However, other parasites include *Demodex, Sarcoptes,* and certain types of flies, fleas and chiggers. Foreign bodies include plant awns, hair, sand or dirt, and hardened medications or secretions (Scott, et al., 2001).

Secondary causes of otitis in the dog include topical reactions that require abnormal skin, treatment-derived foreign bodies, bacteria and yeast. Bacteria and yeast are normal commensal organisms of the external ear canal that become opportunistic as a result of alterations in the skin surface microclimate or host defense (Guillot, et al., 1999; Greene 2006). The most common bacteria associated with canine otitis externa is *Staphylococcus intermedius,* although *Proteus, Pseudomonas, Escherichia,* and *Klebsiella* species can all infect the canine ear. The most common yeast causing otitis

externa is *Malassezia pachydermatis.* It is the organism responsible for 57.3% of all infections (Cafarchia, et al., 2005). *Candida albicans* can also, though rarely, infect the ear.

Malassezia species in general are commensal organisms of human and animal skin that occasionally act as pathogens. There are 10 lipid-dependent species discovered to date and are named as follows: *M. dermatis, M. equi, M. furfur, M. globosa, M. japonica, M. nana, M. obtusa, M. restricta, M. sloofiae, and M. sympodialis* (Cafarchia, et al., 2005). These species have an absolute lipid requirement for growth. They are most commonly associated with dermatological conditions in humans such as Pityriasis versicolor, folliculitis, seborrheic dermatitis, and different forms of atopic dermatitis; however, occasionally systemic infections may occur (Cafarchia, et al., 2005; Crespo, et al., 2002).

Malassezia pachydermatis is the only non-lipid-dependent *Malassezia* species. It was first described in 1925 by Weidman in an Indian rhinoceros with exfoliative dermatitis and originally named *Pityrosporum pachydermatis* (Guillot, et al., 1999). It is round to oval in shape, 3 to 8 micrometers in diameter, and basophilic (Girao, et al., 2004). It undergoes monopolar, enteroblastic budding from one site on the cell wall forming a prominent bud scar or collar (Ginel, et al., 2002; Cafarchia, et al., 2005). This bud scar makes it easy to identify this yeast on routine cytology. The yeast can be found clustered on exfoliated keratinocytes or free in debris, and are said to resemble purple peanuts, footprints, or snowmen (Scott et al., 2001; Angus, 2004; Girao, et al., 2004). *Malassezia pachydermatis* are lipophilic yeasts that grow better when lipids are added to the medium but do not require them for growth (Guillot, et al., 1999). There are two

strains and 2 major phenotypes that exist; however, their clinical significance is unknown (Scott, et al., 2001).

Malassezia pachydermatis is most commonly associated with disease in animals; however, there have been reports of infection in low-birth-weight neonates who have received lipid-rich intravenous emulsions (Duarte, et al., 2002). Healthcare workers transmitted the yeast, acquired from pet dogs, to the neonates via contaminated hands from their dogs at home (Chang, et al., 1998; Crespo, et al., 2002). Breeds predisposed to infection by *Malassezia pachydermatis* include the Basset Hound, the American Cocker Spaniel, and the West Highland White Terrier (Dorogi, 2002). Dogs with pendulous ears and high levels of acids in their cerumen are also predisposed to infection (Masuda, et al., 2001). Adhesion to keratinocytes by *M. pachydermatis* is dose- and time-dependent and relies on lipid substances (Dorogi, 2002; Nardoni, et al., 2004; Girao, et al., 2004). Adhesion is essential before growth and pathogenicity of this yeast can occur (Guillot, et al., 1999; Girao, et al., 2004).

Otitis externa is one of the most common dermatological conditions in the dog and relies on cytology for proper diagnosis. *Malassezia pachydermatis* is the organism that attributes to over one half of all ear infections in dogs. Heat-fixing otic exudate prior to routine staining did not significantly increase *Malassezia pachydermatis* counts in dogs with clinical signs of otitis externa. Heat-fixing is an unneeded extra step that can be omitted from routine cytological analysis of *Malassezia* otitis.

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