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Short communication

Serum *Malassezia*-specific IgE in dogs with recurrent *Malassezia* otitis externa without concurrent skin disease

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ABSTRACT

Immediate-type hypersensitivity (ITH), mediated by IgE, to *Malassezia pachydermatis* is recognized in atopic dogs with recurrent yeast dermatitis and otitis externa (OE). *Malassezia*-associated OE commonly occurs in dogs without other signs of atopic dermatitis (AD). The aim of this study was to detect *Malassezia*-specific IgE in the sera of dogs with recurrent *Malassezia* OE without concurrent skin disease. Sera from healthy dogs were used for comparison. An FcεRIα-based ELISA was used to measure *Malassezia*-specific IgE. There was no significant difference between number of positive affected dogs (6/21, 29%) and number of positive unaffected dogs (15/86, 17%) ($P = 0.36$). There was also no significant difference in the concentrations of *Malassezia*-specific IgE between the two groups ($P = 0.97$). *Malassezia*-specific IgE did not distinguish between patient groups so, as with other canine allergens, serum IgE reactivity for *Malassezia* could not be used to differentiate between diseased and healthy patients. The presence of *Malassezia*-specific IgE in some of the affected dogs might indicate ITH to *Malassezia* in those dogs. Evaluation of ITH via intradermal test reactivity and response to allergen-specific immunotherapy might clarify the role of *Malassezia*-associated ITH in similarly affected dogs.

1. Introduction

IgE-mediated, or immediate-type hypersensitivity (ITH), to skin-associated microorganisms is recognized as an important factor in severity of atopic dermatitis (AD) in some human and canine patients (Santoro et al., 2015; Sonesson et al., 2013). Microorganism-associated hypersensitivity reactions are especially important in certain patient groups. One such group of people with AD, so-called "head and neck dermatitis" (HND), very frequently has elevated specific IgE for *Malassezia furfur*, a cutaneous commensal yeast of human beings (Darabi et al., 2009). These patients have an eczematous pruritic dermatitis affecting the scalp, face, and neck that responds to systemic antifungal therapy (Darabi et al., 2009). Multiple studies have reported a positive correlation between *Malassezia*-specific IgE levels and clinical severity of HND, with as many as 100% of affected patients having increased *Malassezia*-specific IgE (Devos and Van der Valk, 2000; Bayrou et al., 2005; Kim et al., 1999).

Recurrent dermatitis due to overgrowth of *Malassezia pachydermatis*, a commensal yeast organism of canine skin, is also a complicating factor for some dogs with AD. Immediate-type hypersensitivity to *Malassezia* allergens is increasingly recognized in some dogs with AD (Morris et al., 1998; Morris and DeBoer, 2003; Nuttall and Halliwell, 2001; Farver et al., 2005). Dogs affected with AD are also frequently affected with recurrent otitis externa (OE) (Favrot et al., 2010). Otitis externa is one of the most common reasons pet owners seek veterinary care for their dogs, accounting for 10–15% of diagnoses in primary care practices (Banfield, 2014; Veterinary, 2015; O'Neill et al., 2014). Overgrowth of *Malassezia* is also very often involved in canine OE. Reports of *Malassezia* in cases of canine OE range from 33 to 72% (Saridomichelakis et al., 2007; Campbell et al., 2010; Zur et al., 2011).

It is evident that *Malassezia* ITH contributes to the pathogenesis of certain skin disease syndromes in dogs and people. Given the high frequency of *Malassezia*-associated OE in dogs it is possible that ITH to *Malassezia* contributes to this disease too. It is not currently known if dogs with recurrent *Malassezia* OE in the absence of other skin disease have evidence of sensitization to *Malassezia pachydermatis*.

The purpose of this study was to determine how frequently *Malassezia*-specific IgE could be detected in the sera of dogs with recurrent *Malassezia* OE as their sole clinical problem. For compar-

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ison, the frequency of *Malassezia*-specific lgE detection in the sera of dogs with no history of OE or other skin disease was also evaluated. It is widely accepted that serum IgE tests for environmental allergens do not discriminate between dogs with AD and healthy dogs (Pucheu-Haston et al., 2015; Hensel et al., 2015) however this determination has not been made for *Malassezia*-specific IgE in dogs with recurrent *Malassezia* OE. The presence of *Malassezia*-specific lgE in serum from dogs with solely recurrent *Malassezia* OE might indicate further consideration of ITH to *Malassezia* in the pathogenesis of this common condition in dogs and point towards novel treatment options.

2. Materials and methods

2.1. Patient selection

Dogs with recurrent *Malassezia* OE were selected for inclusion. These dogs were recruited from the primary care patient population of a veterinary teaching hospital and surrounding referral primary care veterinary clinics. Candidate dogs were in good systemic health with no history of non-otic skin disease. Recurrent *Malassezia* OE was defined as three or more episodes in the 18 months prior to the time of serum collection. An episode of *Malassezia* OE was defined as clinical signs of otitis externa (ear pruritus, erythema, and exudate) combined with demonstration of yeast on ear swab cytology. No specific quantitative cytologic criteria for yeast organisms could be assessed due to variation in medical recordkeeping. Dogs with bacteria, or bacteria and yeast, on ear swab cytology were excluded. Dogs with systemic illness or generalized dermatologic disease were excluded. Serum samples collected from primary care patients as part of routine wellness visits were used for unaffected patient comparison. Medical records of unaffected dogs were screened to exclude those with any history of otic, dermatologic, or systemic illness. The protocol for animal use was approved by our institution's Animal Care and Use Committee.

2.2. Laboratory assay

Sera from all patients were analyzed for IgE against *Malassezia* by a commercial laboratory using an FcεRIα-based ELISA (Heska; Loveland, CO, USA), as described by Stedman et al. (2001) and Bevier et al. (1997).

2.3. Statistical analysis

Patient population characteristics were analyzed via an odds ratio calculator (MedCalc; medcalc.org/calc/odds_ratio.php, accessed May 12, 2015). Breed, age, and sex representation in each group was compared to the hospital's primary care service patient population for the same year.

Allergen-specific IgE was considered positive based on the commercial laboratory's threshold of 11 units or greater. Fisher's exact test was used to compare *proportion* of *Malassezia*-specific IgE positive to negative samples in both patient groups in a 2×2 contingency table. The Mann-Whitney *U* test was used to compare the *concentrations* of *Malassezia*-specific IgE between affected and unaffected dogs. Statistical analysis of data was performed with Prism 6 (GraphPad Software Inc.; La Jolla, CA, USA).

3. Results and discussion

It is commonly stated that OE can be the sole clinical sign of AD in dogs, and that underlying allergic disease must be considered for dogs with recurrent OE in the absence of other clinical signs, however there is little published evidence to support this recommendation (Harvey et al., 2001; Noxon, 2014; Paterson and Tobias, 2013; Scott, 1981). *Malassezia* OE specifically has been reported as more common than bacterial OE in dogs with AD (Favrot et al., 2010; Saridomichelakis et al., 2007; Zur et al., 2011). In Favrot's study (2010) that categorized the clinical criteria for diagnosis of AD in dogs, OE was reported as the initial clinical sign in 43% of dogs eventually diagnosed with AD based on the development of additional clinical signs. In the present study *Malassezia*-specific IgE was present in 29% of the dogs with recurrent *Malassezia* OE as a sole clinical problem (Table 1). *Malassezia*-specific IgE was also detected in 17% of the dogs with no history of skin or ear disease. There was no significant difference between the two groups, meaning that *Malassezia*-specific IgE tests would not differentiate between patient groups so could not be used to diagnose OE due to *Malassezia*. Diagnosis of *Malassezia* OE in dogs, however, is generally straightforward based on clinical and cytologic criteria. (Miller et al., 2013).

Table 1
Numbers of affected and unaffected dogs with positive or negative serum Malassezia-specific IgE (positive: 11 units or greater). There was no significant difference between dogs with recurrent otitis externa and unaffected dogs (Fisher's exact test, P = 0.36).

	IgE Positive	IgE Negative	Total
Recurrent Malassezia OE (otitis externa)	n = 6 (29%)	n = 15 (71%)	n = 21
No history of skin or otic disease	n = 15 (17%)	n = 71 (83%)	n = 86

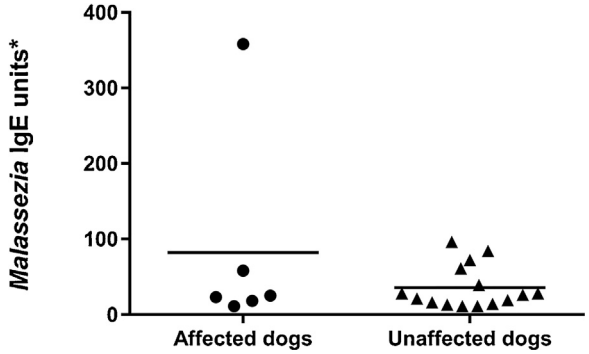

Fig. 1. Concentration of *Malassezia*-specific IgE in dogs affected and unaffected by recurrent *Malassezia* otitis externa. Each point represents one patient, bars indicate median values (Mann-Whitney U, $P = 0.97$). Negative values (<11 units) not represented. *Units expressed as HERBU (Heska IgE Receptor Binding Units).

It is well-recognized that serum IgE against environmental allergens does not always correlate with clinical signs and cannot be used to diagnose AD (Pucheu-Haston et al., 2015). In measuring allergen-specific IgE in retriever dogs, Lauber et al. (2012) demonstrated that healthy dogs can have as much, or more, environmental allergen-specific IgE as dogs with AD. The presence of allergen-specific IgE in healthy dogs does not however negate the potential importance of those tests for dogs diagnosed with AD, particularly for the formulation of allergen-specific immunotherapy (Olivry et al., 2015). Similarly, it appears that *Malassezia*-specific IgE also does not necessarily correlate with clinical signs of *Malassezia*-related skin or ear disease. Malassezia-specific IgE has previously been detected in the sera of healthy dogs at a level not different from that detected in the sera of atopic dogs affected with *Malassezia* dermatitis (Farver et al., 2005). Just as the presence of IgE against a variety of allergens in healthy dogs does not negate the value of IgE tests in atopic dogs, the lack of significant difference between the patient groups in the present study does not mean that *Malassezia*-specific IgE in affected dogs is unimportant. This IgE might suggest that ITH to *Malassezia* is present.

Fig. 1 illustrates the concentrations of *Malassezia*-specific IgE detected in sera of affected dogs compared to unaffected dogs.