Clinical features, cytology and bacterial culture results in dogs with and without cheilitis and comparison of three sampling techniques

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Abstract

Background - Cheilitis is a common presentation in dogs associated with a variety of skin diseases and often complicated by microbial infections.

Objectives - To describe and compare clinical features and cytology and bacterial culture results from the lower lip in dogs with cheilitis and in normal controls and to evaluate three cytology sampling techniques in their ability to differentiate between the groups.

Animals – Fifty-six dogs with cheilitis and 54 control dogs.

Methods – Anatomy and clinical signs of the lower lip were recorded. Cytology samples taken by tape strip, direct impression and swabs rolled over skin were scored semiquantitatively for microorganisms, inflammatory cells and keratinocytes. Cytology scores were correlated with semiquantitative bacterial culture scores.

Results – Pure breeds, frequency of lip folds and all cytology scores except keratinocytes were higher in dogs with cheilitis than in controls, but a substantial overlap was seen in all microorganisms between the groups. Hypersensitivity disorders were diagnosed in 40/56 cheilitis dogs. The tape strip technique yielded the greatest differences between groups. Bacterial growth was reported in 100% of cheilitis dogs and in 93% of the controls. Pathogens such as *Staphylococcus pseudintermedius*, ß-hemolytic streptococci and *Pseudomonas* spp. were most frequent and more common in cheilitis dogs. Cytology and bacterial culture were poorly correlated.

Conclusion – Cheilitis was associated with hypersensitivity as primary disorders and lip folds as predisposing factors. Culture findings showed similarities with skin elsewhere, except for higher rates of *Pseudomonas* spp.

Introduction

The lips surround the orifice of the mouth in humans and most mammals. In humans, they comprise four zones: the haired skin including the philtrum, the vermilion border, the vermilion (the red part of the lips) and the oral mucosa.[1-3] Cheilitis describes inflammation of any of these parts.[4,5] In the dog, the lips are less well defined than in humans and miss a distinctive vermillion. Adjacent to the oral mucosa is a small rim of smooth non-haired skin, approximately 0.5 to 1 cm in width, slightly broadening caudally towards the commissures and then a haired perioral part of the lips.[6] The cranial part of the lips with the philtrum and the more distant perioral skin is referred to as muzzle.[7,8] There may be a vertical and/ or horizontal lip fold, the upper canine tooth may overlap the lower lip, and excessive skin may droop in some breeds such as e.g. cocker spaniels, Saint Bernards and Bloodhounds.[9] If present, these features can trap air, moisture and food remnants on the lower lip, which may favor microbial colonization.

The term “cheilitis” is rarely used in the veterinary literature and remains poorly defined. In the most recent edition *Muller and Kirk’s Small Animal Dermatology,* cheilitis in the dog is cited twice, once to describe inflammatory changes of the lips in atopic dermatitis,[10] and in a potential case of mumps.[11] Other terms in the veterinary literature used to describe cutaneous changes in the lip region include “mucocutaneous” as in mucocutaneous pyoderma,[12,13] “perioral” as recently described for canine mucocutaneous lupus erythematosus,[14,15] and “muzzle” as in muzzle dermatitis,[7,8] however, distinctions between the terms remain unclear.

Changes associated with inflammation of the lip in the dog include swelling, erythema, alopecia, crusting, erosion, ulceration, hyper- or depigmentation.[7,9,12,14] Cheilitis can occur uni- or bilaterally and can be complicated by microbial infection.[9] The lip region can be the only area affected by skin disease (e.g. lip fold intertrigo) or be part of more widespread or generalized skin disease (e.g. atopic dermatitis, zinc deficiency syndrome, superficial necrolytic dermatitis).[15-19]

Culture-based studies concentrating on the microflora of the oral mucocutaneous region have sampled the buccal surface of the oral cavity and demonstrated the presence of *Staphylococcus pseudintermedius* and coagulase-negative staphylococci.[20-22] Only one study explicitly sampled the haired area of the lip, identifying large numbers of *Malassezia pachydermatis* by culture in healthy dogs of various breeds.[23]

Cytological examination is commonly used to determine type and quantity of microorganisms and inflammatory cells on skin.[24] Various sampling techniques are described including direct impression smears, acetate tape stripping and dry swabs rolled over an area. Sampling of the lip area can be challenging, for example if lesions are painful or if the patient is aggressive. A rapid sampling technique is desirable.

This study aimed to describe and compare clinical features and cytology and bacterial culture results from the lower lip in dogs with cheilitis and in normal controls and to evaluate three cytological sampling techniques in their ability to differentiate between the two groups.

Material and Methods

Animals

Dogs with cheilitis and control dogs were recruited at a private veterinary dermatology and ophthalmology referral practice and at one private dog day care facility in Germany between March and December 2014. All dogs were enrolled following their owner's written consent.

Clinical examination and inclusion criteria

Dogs were allocated to either group**,** based on history**,** physical and dermatological assessment. Cheilitis was defined as uni- or bilateral inflammation of the haired skin and/or the smooth non-haired rim of the lower lip as shown by one or more of the following clinical signs: erythema, alopecia, crusts, erosion, ulceration, hyperpigmentation and depigmentation. The severity was scored from 0 = absent, 1 = mild, 2 = moderate to 3 = severe, based on a scoring system established in the CADESI-4.[25] Scores ranging from 1-21 were possible for each side.

In the cheilitis group, there were no drug withdrawal requirements prior to enrolment but all topical or systemic antibacterial, antifungal, steroidal and non-steroidal anti-inflammatory treatments, as well as other prescribed medications prior to enrolment were recorded. Ecto- and endoparasite control was allowed. All dogs were further examined for signs of skin disease elsewhere. To identify underlying causes for cheilitis and other skin changes, a complete diagnostic work-up according to standard dermatological procedures were performed based on clinical signs and differential diagnoses.[26]

Control dogs were free of signs of cheilitis, had no history of skin disease reported by the owner and no signs of an inflammatory skin disease on any other part of the body on dermatological examination. Drug withdrawal periods in this group were six weeks for systemic antibacterial and antifungal agents, glucocorticoid and non-steroidal anti-inflammatory drugs, as well as any topical treatment on the lips or muzzle. Ecto- and endoparasite control treatment, as well as topical eye-drops were permitted. The latter were documented.

Anatomical characteristics of the lips on both sides were recorded for all dogs including presence or absence of a vertical and/or horizontal lip fold.

Sample collection

The lower lip was sampled on both sides of the mouth. In the cheilitis dogs, sample sites were chosen from lesional skin; in the controls, either the haired skin between the commissures and the canine tooth or, if present, the lip fold area was sampled. The following three sampling techniques were used to obtain specimen for cytological examination:

1. The tape strip technique involved pressing a 1x1cm area of an adhesive tape strip (tesa SE, Hamburg, Germany) against the skin for five seconds before removing it.[27]
2. The direct impression smear was obtained by pressing a glass slide (76x26mm, Engelbrecht Medizin und Labortechnik GmbH, Edermünde, Germany) twice onto the skin.
3. The rolled swab sample technique involved rubbing a cottonswab (Heinz Herenz Medizinal Bedarf GmbH; Hamburg, Ger-many) over the sample site for 5 s and then rolling the swabover a glass slide for staining. Twenty percent of dogs in each group were sampled in reverse order to assess whether the

order of sampling would influence the results.

For bacterial culture, a sterile swab (nerbe plus®, Winsen/Luhe, Germany) was rubbed against the sampling site for five seconds, placed in transport medium and submitted to an external veterinary diagnostic laboratory (synlab.vet GmbH, Hamburg, Germany) the same day. In dogs with cheilitis the side with higher clinical scores was sampled; in controls, only the left side was sampled.

Cytological Examination

Each specimen was stained with Hemacolor® Stain (In Vitro Diagnostic Medical Device, Merck KGaA, D-64271 Darmstadt, Germany) and analyzed microscopically (BX51; Olympus Imaging Europa, Hamburg, Germany) by the same investigator (MD) using ocular lenses of x10 magnification and Ultra-plan, Apochromat objectives with x4, x10, x40, x100 (oil immersion).

Slides were scanned using a low magnification (x100) for areas of interest (material of suitable and even density present). Subsequently, 10 high power fields (HPF) were examined at x400 magnification and cytological findings were scored semi-quantitatively. Using HPF oil immersion (x1000) and a quantitative scoring system as described by Udenberg[28] was found to be unsuitable in a small pilot study conducted by two of the authors (MD and ML, data not shown) because in contrast to other skin sites, samples from the lips revealed in many cases too many microbes to be counted. The following cytology findings were scored: coccoid and rod-shaped bacteria, *Malassezia* yeast, *Simonsiella* spp., inflammatory cells (differentiated into neutrophilic and eosinophilic granulocytes, macrophages, and nuclear streaming, defined as basophilic strands, variable in size, with at least one strand connected to a larger nuclear remnant,[28] cornified keratinocytes (including both pale staining flat and intensely stained elongated rolled-up squames) and nucleated keratinocytes (including squamous epithelial cells from mucous membranes). Semi-quantitative scores ranged from 0 to 4 as previously described: 0 = not seen; 1 = occasionally present but slide must be scanned carefully for detection; 2 = present in low numbers, but detectable rapidly without difficulties, 3 = present in larger numbers and detectable rapidly without any difficulties and 4 = abundant, as previously described.[29]

Samples from at least 20% of dogs from each group were evaluated in a blinded manner by a second investigator (ML) to assess interobserver reliability.

Bacterial Culture

Swabs were inoculated onto Columbia blood agar, Columbia blood agar with colistin-nalidixic acid (CNA) (for isolation and differentiation of gram-positive microorganisms) and MacConkey-agar (for isolation of *Escherichia coli*) and incubated at 37°C under aerobic conditions. Schaedler-agar and thioglycollate broth were used for incubation at 37°C under microaerophilic and anaerobic conditions to differentiate gram-negative and obligate anaerob rods, like *Bacillus* sp. and *Clostridium* spp. Bacteria were identified phenotypically by colony morphology, gram staining properties, and preliminary biochemical testing (catalase, cytochrome oxidase, indole). Haemolysing staphylococci were further tested for evidence of clumping factor and protein A (Pastorex™Staph-Plus, Bio-Rad) and speciated based on their biochemical properties (API ID 32 Staph, bioMérieux). Meticillin-resistant staphylococci were detected as growth on solid media containing 6µg/ml oxacillin (BD Oxacillin Screen

Agar; Becton Dickinson, Heidelberg, Germany) and mannitol (BD Mannitol Salt Agar, Becton Dickinson, Heidelberg, Germany). Beta-haemolysing streptococci were identified through catalase test and divided into serological groups according to Lancefield by the detection of group-specific antigens using latex-agglutination (Pastorex™Strep, Bio-Rad) and biochemical testing (API ID 32 Strep, bioMérieux). Gram-negative aerobic rods were identified by cytochrom oxidase reaction and API ID 32 E (bioMérieux). Anaerobic bacteria were classified due to gram staining and identified using API ID 32 A (bioMérieux).

After 24 and 48 hours, all media were examined visually for bacterial growth and semi-quantitatively scored as 4 = abundant; 3 = moderate; 2 = scattered; 1 = growth after enrichment or 0 = no growth. **All bacteria that could be identified using standard phenotypic and biochemical microbiology tests were reported, irrespective of their presumed pathogenic potential.**
Presumed pathogens were tested for their antibiotic susceptibility by agar dilution using ATB™VET (bioMérieux) according to the manufacturers instructions. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, according to proposed definitions;[30] the definitions for *S. pseudintermedius* were extrapolated from *S. aureus*.

Statistical Analysis

Analyses were performed using SigmaPlot software (SigmaPlot 11.0; Systat Software Inc., Erkrath, Germany). Comparison of mean cytology scores between groups for each sample technique was done by unpaired *t*-test with alpha 0.05. The no**n-**normally distributed data were evaluated by an analysis of variance after Kruskal-Wallace for each comparison. Tukey's tests were used for statistical comparison of the three different sampling techniques, in pairwise multiple comparison procedures (A vs B, A vs C, B vs C): the total of all cells and organisms was compared among the three sampling techniques (A, B, C). The possible influence of sampling order was analyzed by unpaired *t*-tests. Interobserver reliability was assessed by Cohen's kappa and Spearman-rank-coefficient test. Culture results and the presence of a lip fold were compared between cheilitis and control dogs, using a chi-square test. Cytology scores were correlated with semi-quantitative culture scores by Spearman-rank-coefficient. Statistical significance was defined as $P < 0.05$ in all cases.

Results

Animals

Fifty-six dogs with cheilitis and 54 control dogs were enrolled with age and gender evenly distributed in both groups (Table S1). There were more ($P = 0.008$) pure breeds in the cheilitis group ($n = 53$, 95%, 25 different breeds) than in the control group ($n = 35$, 64%, 27 breeds). The most common breeds with $n \geq 4$ were French bulldogs, German shepherd, golden retriever, Labrador retriever and West Highland white terriers in the cheilitis group, as well as Jack Russel terriers in the control group.

Pre-treatment

Thirty-seven (66%) cheilitis-group dogs had received medication prior to enrolment. Briefly, 13 dogs (23%) had been treated topically with antiseptic/-microbial and /or

antiinflammatory agents. Twenty-three dogs had received systemic medication (6 antimicrobials, 15 antiinflammatory drugs, 2 both) and nine of these in combination with antimicrobial shampoos.

Clinical findings

Erythema, alopecia and crusts were most commonly seen and 50% of the dogs had a score of 2 or 3 in erythema and alopecia (Table 1). Erosions and hyperpigmentation were present in about one third of the lips, whereas ulceration and depigmentation were rare. Fifty-two dogs (93%) had lesions on both sides of the lower lip and of those 77% were identical in clinical signs and scores. The mean clinical score was 5.8 on the left and 5.6 on the right side, ranging from 1 (erythema only) to 18.

A lip fold was more frequently present in dogs in the cheilitis group (*n* = 46; 82%) than in control dogs (*n* = 23, 43%)(*P* < 0.001). The lip fold was either horizontal (23 cheilitis 14 controls), vertical (14 cheilitis, 5 controls) or both (9 cheilitis, 4 controls).

Fourteen cheilitis-group dogs (25%) presented with skin lesions limited to the lower lips, five of them were diagnosed with mucocutaneous pyoderma[12,13] and nine with lip fold intertrigo.[19] In 42 dogs (75%), cheilitis was associated with other skin lesions. Forty of them (95%) were diagnosed with hypersensitivity skin disorders (canine atopic dermatitis (CAD) *sensu stricto* (*n* = 9), food-induced CAD (*n* = 6), adverse food reaction (*n* = 6) and flea-bite hypersensitivity (*n* = 5)). Thirteen dogs had an allergic phenotype according to published criteria of Favrot,[18] but a final diagnosis had not been achieved. One dog had adverse food reaction, flea allergy dermatitis and sebaceous adenitis concurrently. Two dogs with skin lesions beyond the lips had nonallergic diseases: sebaceous adenitis and idiopathic onychodystrophy.

Cytology findings

There was no difference (*P* = 0.175) in mean cytology scores recorded from the three sampling techniques when different sampling orders were used (i.e. tape strip, impression smear, swab or vice versa). Microorganisms were seen in the majority of dogs in both groups, whereas inflammatory cells were more frequently found in dogs with cheilitis (Tables 2 and 3). Long segmented filamentous bacteria were seen in six cheilitis and four healthy controls. These were not associated with high numbers of cocci or rods. Microorganism cytology scores did not differ between dogs with and without lip folds in the cheilitis group (Figure **1**). In the control group, these scores were higher if lip folds were present. The overall interobserver reliability was high with r_s = 0.81, (*P* < 0.001) and κ = 0.80.

Comparison of the three sampling techniques

Irrespective of the group, mean cytology scores for microorganism and inflammation categories were lower (*P* < 0.001) from swab samples (0.55 ± 0.97) than from impression smears (0.91 ± 1.21) and tape strip samples (0.90 ± 1.28), whereas the latter two showed no difference (*P* = 0.831). Scores for cocci, neutrophils and nuclear streaming were higher in dogs with cheilitis than in controls, using all sampling techniques (*P* < 0.001) (Table 3). The tape strip technique consistentely yielded higher scores for all microorganisms and for neutrophils in the cheilitis group than in the control group (Table 3).

Bacterial culture

Bacterial growth was reported from all swabs in cheilitis dogs (100%) and from 50 control dogs (93%) with a single bacterial species or group reported from 25% of swabs (12 from cheilitis group, 14 from controls). Bacteria typically considered as pathogens, like *S. pseudintermedius, Escherichia coli* and *Pseudomonas* spp. were more frequently isolated in cheilitis dogs, whereas swabs from control dogs more frequently yielded coagulase-negative staphylococci and alpha-haemolytic streptococci (Table 4). In addition, the pleomorph bacteria, *Acinetobacter* spp. and *Pasteurella* spp. were isolated from four cheilitis and three control dogs and from three cheilitis and two control dogs, respectively. Meticillin-resistant *S. pseudintermedius* was not isolated. Multidrug-resistance (resistance to at least three antimicrobial classes) was seen in eight of the 11 *Pseudomonas* isolates in cheilitis-group dogs and in one of four isolates from controls ($P = 0.174$).

There were no differences in bacterial species or bacterial groups reported from cultures of dogs with or without a lip fold within the control group (Table 4). Because most dogs with cheilitis had lip folds, differences in bacterial culture results could not be evaluated in this group.

Comparison of cytology and bacterial culture

Irrespective of the study group, bacterial culture results for Gram-positive cocci agreed with the detection of cocci in 88% of sames collected by the tape strip technique, 85% collected by impression smear and 57% collected by swab. Rods were identified more commonly by cytology than were Gram-negative bacilli with culture results. This difference was significant for the impression smear, where only 47% of rods seen with cytology were confirmed by culture ($P = 0.027$). Correlation of the semi-quantitative bacterial culture scores with the mean cytology scores was weak for both cocci and rods regardless of the sampling technique utilized. Although still weak (r = 0.38), the tape strip technique yielded the highest correlation ($r = 0.38$) (Table 5).

Discussion

Results from this study have provided data on possible contributing factors to cheilitis and have identified the tape strip technique as a reliable sampling method for this area. As expected, the clinical signs most commonly recorded in dogs with cheilitis were erythema, alopecia and crusts, similar to those seen in inflammatory diseases of the skin elsewhere.[31] Although lip folds were more frequently observed in dogs with cheilitis, they were still present in 43% of controls. By analogy with the classification system for the diagnosis of otitis externa[32] this indicates that lip folds may be predisposing factors in the development of cheilitis, but are unlikely to be a primary cause. Furthermore, as reported elsewhere,[18,33] hypersensitivity skin disorders may be a primary causes for cheilitis, as diagnosed in all but two of the 42 dogs with cheilitis in this study. A breed predisposition for cheilitis could not be assessed in this study due to differing source populations of participants and lack of comparison to the entire clinic population examined during the study period.

Cytological findings revealed few surprises. Dogs with cheilitis showed higher cytology scores for all potential pathogens compared with controls. Within the control group, microbe scores were higher in dogs with lip folds compared to those without, presumably due to increased moisture and temperature within the fold.[34] However, similar to results from ear canals of dogs with and without otitis,[35,36] we found a large overlap in microbe scores between groups. Absolute numbers for microorganisms on

cytology have been proposed to differentiate dogs with and without pyoderma.[28] Based on our findings of overlapping scores, such cut-off numbers cannot be recommended for lip cytology. Instead, a combination of clinical signs and cytological results should be considered. As expected, inflammatory cells were more frequent in cheilitis dogs and neutrophils dominated. More surprisingly, neutrophils were seen in 20% of control dogs. One study reported that occasional inflammatory cells can be detected histopathologically in healthy skin near mucosal sites as the canine nasal planum,[37] but their presence in surface cytology samples were unexpected. The majority of our control dogs from which neutrophils were observed had lip folds, and neutrophils might be a response to the higher amount of microbes in lip folds. The tape strip method produced the greatest yield for all cellular categories except eosinophils, macrophages and keratinocytes, in dogs with cheilitis. The method has previously been reported to provide higher yields for *Malassezia* yeast.[38] Factors that might influence tape strip yields include pressure used by the investigator and the size of sampled area, together with the ease and speed of use, especially in dogs that resent sampling of an area that can be painful.

Coagulase-negative staphylococci and alpha-haemolytic streptococci, which are members of the microbiota on healthy skin at other sites,[20,39] were more frequently isolated from the lips of healthy dogs. *Simonsiella* spp., considered part of the normal oral flora of dogs,[40,41] was significantly more common in cytologicalsamples from control dogs than from the cheilitis group. To the best of the authors' knowledge, studies on the role of *Simonsiella* spp. in disease have not been reported. It may be hypothesized that their growth or attachment is inhibited by inflammation or changes in the microbiota. The predominance of *S. pseudintermedius* from dogs with cheilitis is consistent with other types of skin infection, such as pyoderma and bacterial otitis.[42-44] The lack of isolation of meticillin-resistant *S. pseudintermedius* (MRSP) was not expected because this study was performed in a dermatology referral centre with apreviously reported MRSP prevalence of 27% amongst *S. pseudintermedius* from skin and ear canal infections. [54-57]

Isolation of *E.coli* in almost 20% of dogs with cheilitis was unexpected because *E.coli* is rarely reported from canine pyoderma and is not considered part of the normal oral flora.[45,46] This high prevalence may be due to anal licking because *E.coli* is a member of the faecal microflora[47] and anal pruritus has been associated with hypersensitivity disorders.[48] Similarly, *Pseudomonas* spp. are isolated infrequently from skin infections but were isolated from 11 dogs with cheilitis and four healthy controls.[49,50] *Pseudomonas* spp. are ubiquitous in the environment and typically associated with moist conditions. It is assumed that moist conditions on the lips can favour growth and adherence.[43,51,52,53]

The poor correlation between cytology and bacterial culture results has been reported for samples acquired from dogs with otitis externa and media.[58,59] However, in contrast to our study, culture from these ear sites was more efficient than cytological evaluation in detecting bacterial cocci and rods. On the lips a nonculturable oral microflora might have produced higher cytology scores and further studies should include molecular methods.

There are several limitations to this study. First, the order of cytological sampling was not randomized. However, a reversed order revealed no significant differences in

results. Secondly, indicators of dental/oral health, such as presence of plaque, tartar and gingivitis, were not assessed with validated scoring tools. It is possible that dental health and the oral microbiota could influence the microbiological findings on the lips. And finally, there could be breed-related effects that were not accounted for by the study design.

Dental health and oral microbiota could influence the microbiological findings on the lip. Therefore in a future study it would be worthwhile to collaborate with a veterinary dentist with respect to cheilitis. The sampling order was not randomized. Although a reversed order did not show a significant difference, a randomization could have revealed different results.

In summary, our results emphasize the importance of combining information from cytology and bacterial culture with clinical signs in dogs with cheilitis. Cheilitis was most often a bilateral problem, commonly found in purebred dogs and associated with hypersensitivity skin disorders. As expected, microbial and inflammatory cell parameters on cytology were higher in dogs with cheilitis, but the presence of a lip fold favored higher scores of microorganisms in both groups. Culture findings showed similarities with skin elsewhere, except for the predominance of *Pseudomonas* spp. in the cheilitis group and streptococci amongst controls. Tape stripping appears to be a reliable technique for cytological sampling of the lip and its routine use is also supported by the ease of administration at this body site.

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Tables and Figures

Table 1. Clinical signs, their frequency with the respective severity scores and the mean clinical score of 112 lower lips (56 dogs)

Clinical signs	Mean clinical scores	Number of lower lips *n* (%)			
		Score 0	Score 1	Score 2	Score 3
Erythema	1.66	13 (12%)	33 (29%)	45 (40%)	21 (19%)
Alopecia	1.43	25 (22%)	29 (26%)	43 (38%)	15 (13%)
Crusts	0.82	56 (50%)	21 (19%)	31 (28%)	3 (3%)
Erosion	0.55	75 (67%)	17 (15%)	15 (13%)	5 (4%)
Hyperpigmentation	0.44	86 (77%)	7 (6%)	15 (13%)	4 (4%)
Ulceration	0.15	102 (91%)	4 (4%)	5 (4%)	1 (1%)
Depigmentation	0.11	105 (94%)	2 (2%)	5 (4%)	0 (0%)

Table 2. Identification of cytological categories independent of the sample technique in dogs with and without cheilitis (*n* = number of dogs)

Category	Cheilitis group *n* (%)	Control group *n* (%)	*P* -value
Cocci	56 (100%)	54 (100%)	ND
Rods	50 (89%)	51 (94%)	ND
Malassezia spp.	37 (66%)	30 (56%)	0.2579
Simonsiella spp.	13 (23%)	28 (52%)	0.0012**
Neutrophils	48 (86%)	11 (20%)	0.0001***
Nuclear streaming	49 (88%)	16 (30%)	0.0001***
Eosinophils	2 (4%)	0 (0%)	ND
Macrophages	10 (18%)	2 (4%)	0.0169*
Keratinocytes	56 (100%)	54 (100%)	ND
Nucleated keratinocytes	47 (84%)	42 (78%)	ND

ND, not done.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3. Mean cytology scores of microorganism and inflammation categories in cheilitis and control dogs for the three sample techniques (swab, impression smear,

560 tape strip)

Category	Cheilitis group (n = 56)			Control group (n = 54)		
	Swab	Smear	Tape strip	Swab	Smear	Tape strip
Cocci	1.39***	2.23***	2.62***	0.79	1.59	1.70
Rods	1.06***	1.53	1.26*	0.51	1.25	0.94
Malassezia spp.	0.45	0.64	1.12*	0.41	0.42	0.69
Simonsiella spp.	0.03	0.06**	0.14*	0.02	0.23	0.31
Neutrophils	0.49***	1.37***	1.12***	0.00	0.09	0.41
Nuclear streaming	0.64***	1.39***	0.92***	0.05	0.12	0.06
Eosinophils	0.01	0.01	0.00	0.00	0.00	0.00
Macrophages	0.04	0.18***	0.03	0.02	0.01	0.00
Keratinocytes	2.24	2.64**	3.01	2.12	2.42	2.84
Nucleated keratinocytes	0.49***	1.12**	0.69***	0.19	0.78	0.33

561 Comparison within same technique between groups: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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563 **Table 4.** Frequency of isolated bacteria in dogs with cheilitis and control dogs (*n* =
564 number of dogs)

Shape	Bacterial family	Bacterial species or group	Cheilitis dogs (*n=56)*	Control dogs (*n*=54)	*P* - value
Cocci	*Staphylococcaceae*	Coagulase-negative staphylococci	3	10	0.031 *
		Staphylococcus pseudintermedius	45	26	0.02
	Staphylococcaceae	α-haemolytic streptococci	6	25	0.0001 ***
		β-haemolytic streptococci	19	12	0.172
	Micrococcaceae		0	1	ND
		Micrococcus sp.	0	2	ND
	Enterococcaceae	*Kokuria* sp.	3	0	ND
Rods	*Bacillaceae*	*Enterococcus* spp.	6	5	ND
	Pseudomonadaceae	*Bacillus* sp.	11	4	0.0612
	Corynebacteriaceae	*Pseudomonas* spp.	4	2	ND
	Clostridiaceae	*Corynebacterium* spp.	1	2	ND
	Enterobacteriaceae	*Clostridium* spp.	11	1	0.0025 **
		Escherichia coli	3	2	ND
		Enterobacter spp.	0	1	ND
		Serratia sp.	1	0	ND
		Klebsiella sp.	1	0	ND
		Pantoea sp.	1	0	ND
	Rhizobiaceae	*Proteus* sp.	0	1	ND
		Rhizobium sp.			

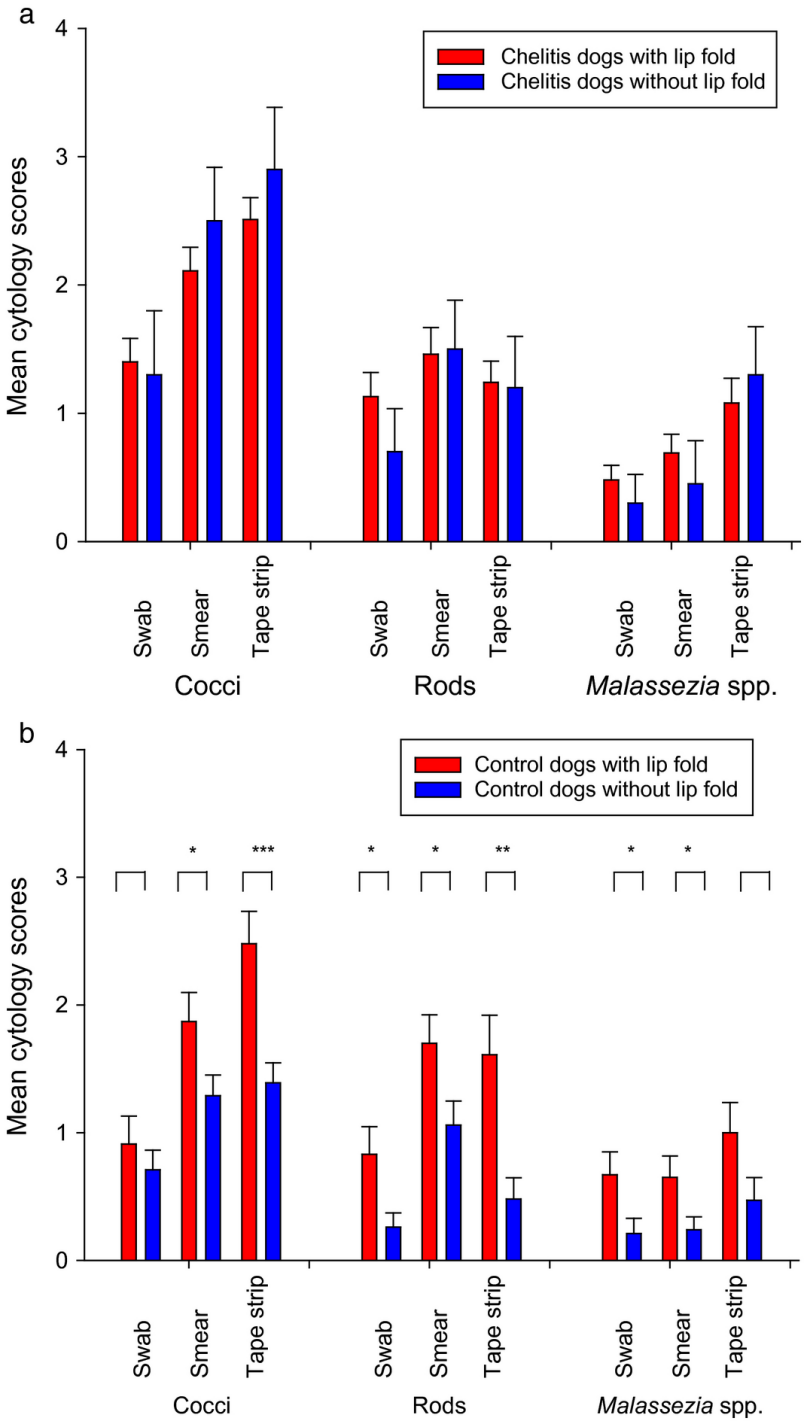
565 ND, not done.

566 Comparison between groups * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 5. Correlation between mean cytology and bacterial culture scores (0-4) of microorganisms for each sample technique; *r* = Spearman-rank-coefficient with corresponding *P*-value

Organisms	Sample technique	*r*	*P*-value
Cocci	Swabs	0.224	0.019
	Smear	0.239	0.012
	Tape strip	0.281	0.003
Rods	Swab	0.235	0.013
	Smear	0.244	0.010
	Tape strip	0.129	0.178
Cocci + Rods	Swab	0.280	<0.001
	Smear	0.313	<0.001
	Tape strip	0.384	<0.001

572 **Figure 1**. Mean cytology scores for microorganisms (cocci, rods, *Malassezia* spp.) in
573 (a) dogs with cheilitis and (b) control dogs, with or without a lip fold (*$P < 0.05$, **$P <$
574 0.01, ***$P < 0.001$). No significant differences were observed between samples from
575 cheilitis dogs.



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