

Evaluation of cytology collection techniques and prevalence of *Malassezia* yeast and bacteria in claw folds of normal and allergic dogs

Kimberly L. Lo and Wayne S. Rosenkrantz

Animal Dermatology Clinic, 2965 Edinger Avenue, Tustin, CA 92780, USA

Correspondence: Kimberly Lo, Animal Dermatology Clinic, 2965 Edinger Ave, Tustin, CA 92780, USA. E-mail: klo@adcmg.com

Background – Canine bacterial and *Malassezia* paronychia are common secondary complications of atopic dermatitis and adverse food reactions.

Hypothesis/Objectives – The aim of this study was to compare three different sampling methods for claw fold cytology and to evaluate the numbers of bacteria, *Malassezia* yeast and inflammatory cells.

Animals – Sixty client-owned dogs were classified into three groups: (A) normal dogs; (B) allergic dogs with no clinical evidence of claw disease (brown staining, erythema, swelling, crusts or exudates); and (C) allergic dogs with clinical paronychia.

Methods – A prospective, blinded, split-plot study design was used. Claw folds from each dog were sampled using either a toothpick, tape preparation or direct impression smear. Slides were evaluated by two investigators for inflammatory cells, nuclear streaming, debris, corneocytes, yeast, intracellular (IC) cocci, extracellular (EC) cocci, IC rods and EC rods. For each parameter, data were compared between groups and between methods. Inter-reader agreements were calculated.

Results – Group C had significantly higher values of EC cocci and corneocytes than Groups A or B. Although *Malassezia* organisms were more prevalent in allergic dogs than normal dogs, the counts were not significantly different. There were significantly higher numbers of *Malassezia* organisms ($P = 0.0016$) and EC cocci ($P = 0.0106$) retrieved from samples collected with a toothpick compared to other methods. Tape preparations were associated with significantly more debris and corneocytes (both $P < 0.0001$) and impression smears with significantly more nuclear streaming ($P = 0.0468$).

Conclusions and clinical importance – Sample collection using a toothpick optimizes the value of cytological results when sampling allergic dogs with clinical paronychia.

Introduction

Paronychia is defined as inflammation within the folds of tissue surrounding the claw and occurs secondary to many infectious and inflammatory diseases.¹ Of the infectious causes, bacteria are most often implicated and considered secondary to other causes or disease.² *Malassezia* paronychia is a common secondary complication of canine atopic dermatitis (CAD) and cutaneous adverse food reaction (CAFR).³ *Malassezia* paronychia is typically characterized by a dark, brown to red discolouration and a dry to moist waxy exudate affecting the proximal aspect of the claw base. Pruritus is a common feature.² Treat-

ment intervention for bacterial and yeast overgrowth or infection typically results in a reduction in the number of organisms, a resolution of infection and subsequent reduction in the level of pruritus.

There is a paucity of information available on bacterial populations of the canine claw fold. A study of dogs in which claw disease was the only clinical sign reported cytological evidence of bacteria in 11 of the 24 dogs (46%).⁴ Adverse food reactions were present in four dogs in that study; however, it is unclear whether any of these four were the same dogs that had bacteria identified. No previous study has specifically evaluated bacterial populations in the claw folds of normal or allergic dogs.

Studies designed to evaluate different cytological techniques for examination of the claw fold surface are also limited. Two techniques have been reported: direct impression smear with a glass slide and use of a cotton swab to retrieve exudate from the subungual fold.^{3–5} Anecdotal reports suggest that transparent acetate tape preparations and toothpick techniques are also used commonly in veterinary clinical practice. To the best of the authors' knowledge there have been no controlled studies to compare these collection techniques; further, expected counts of bacteria and *Malassezia* yeast from

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within the claw fold have not yet been established. Determination of the most effective technique for cytological sampling may enhance diagnostic accuracy and improve the management of paronychia secondary to allergic disease.

The aims of this study were to compare three different sampling methods for cytological evaluation of the skin surface of the subungual claw fold, and to quantify the number of *Malassezia* yeast, bacteria and inflammatory cells residing at this site.

Materials and methods

Study subjects and enrolment

This study was designed as a prospective, randomized, blinded, split-plot study. Sixty client-owned dogs were classified into three groups based on information obtained from physical examination, anamnesis and a questionnaire provided by the owners. Group A dogs had no history of skin or ear canal disease nor gastrointestinal disorders as defined by published criteria.^{6,7} Group B dogs had confirmed CAD and/or CAFR with no clinical evidence of claw disease (lack of brown staining, erythema, swelling, crusts, waxy debris and exudates). Group C dogs had confirmed CAD and/or CAFR with clinically apparent paronychia. The diagnosis of atopic dermatitis (AD) was confirmed by clinical signs which fulfilled at least six of Favrot's seven criteria.⁸ Atopic dogs were required to have a history of strictly seasonal pruritus or have completed an elimination diet trial using either a home-cooked or a commercial novel or hydrolysed protein diet for a minimum of 8 weeks to rule out CAFR. The diagnosis of CAFR was defined as continuous pruritus that decreased when an elimination diet was fed and then increased within 2 weeks after feeding the dog the original diet. A minimum pruritus visual analog score (PVAS)⁹ of 4/10, applied specifically as a measure of pedal pruritus, accompanied by brown staining, erythema, swelling, crusts, waxy debris or exudates affecting the claw fold was required for dogs to be included in Group C; see Figure 1.

Dogs were excluded from the study if they had been bathed or had any topical products applied within the 7 days prior to enrolment. The minimum drug withdrawal time prior to enrolment was as follows: 14 days for oral antibacterial agents and antifungals; 28 days for oral glucocorticoids; and 56 days for injectable glucocorticoids. All owners signed an informed consent form prior to enrolment in the study.



Figure 1. Paronychia. Group C dog with brown staining, erythema, swelling, crusts and waxy debris in the claw fold.

Sample collection and preparation

From each dog, samples were collected from adjacent areas of the most proximal aspect of each affected claw fold using three techniques (toothpick, tape preparation and impression smear). If no claws were clinically affected, samples were collected arbitrarily from the claw fold of the third digit of the left front and hind paws. One glass slide was used for each technique per animal, giving a total of 180 slides. The order of techniques was randomized to account for any variation in the same area being sampled multiple times.

For the toothpick technique the skin around the claw fold was everted and samples were obtained by inserting the sharp edge of a toothpick (Poly King Products; City of Industry, CA, USA) into the dorsal claw fold (Figure 2). The area was scraped gently three times. For the tape preparation method a piece of clear acetate tape (Scotch; St. Paul, MN, USA) was applied successively to the most proximal region of the claw fold three times, then placed sticky-side down on a glass slide. The impression smear technique involved applying pressure with a glass slide directly onto the claw fold and partially everting the fold with the slide to obtain material from the underlying fold.

With the exception of the tape preparations samples were placed on glass slides which were air-dried, heat-fixed and stained using Diff-Quik® (Medical Chemical Corporation; Torrance, CA, USA). Three drops of Sub-X® Mounting Medium (Surgipath Medical Industries, Inc.; Richmond, IL, USA) were applied to facilitate attachment of a coverslip. The tape preparations were not heat-fixed and 0.1–0.2 mL of the (basophilic) thiazine dye of Diff-Quik® Solution stain was injected under the tape. The slides were turned over and pressure applied to the back to allow excess dye to diffuse. All slides were stored in a slide box at room temperature (68–72°F) and coded randomly prior to evaluation by blinded investigators.

Cytological examination

The two investigators independently evaluated all slides at low power (4× and 10× magnification) and scanned for areas of cellular material in the following order of priority: the presence of inflammatory cells, nuclear streaming, debris and keratinocytes. Once a site was selected, three areas were examined at ×100 magnification using an oil immersion lens – the initial site and two immediately adjacent fields within a 360° radius. Fields with no detectable keratinocytes, inflammatory cells or nuclear streaming were omitted. This procedure was repeated in three different areas representative of the entire slide based on the above scanning criteria, for a total of nine oil immersion fields (OIFs) for each of the 180 slides. For each OIF; yeast, intracellular (IC) cocci, extracellular (EC) cocci, IC rods and EC rods were counted and recorded on a cytology data form. The



Figure 2. Toothpick sampling method.

counts were coded arbitrarily up to a prespecified maximum of 30, so that any field containing >30 organisms was counted as 30. Corneocytes were scored as 1 (rare 0–1/OIF), 2 (low 2–3/OIF), 3 (moderate 4–7/OIF) or 4 (high >7/OIF). For each OIF, polymorphonuclear leucocytes (PMNs), nuclear streaming and debris were scored as present (1) or absent (0). All scores were summed across the nine OIFs for each slide and inter-investigator agreement was assessed. The sums were then averaged over both investigators prior to analysis.

Statistical analyses

All analyses were performed using SAS v9.3 (SAS Institute; Cary, NC, USA). A nested analysis of variance model was used for analysis of all variables. The full model included three factors: group, dog and method. Group was a whole-subject factor although all three methods were repeated within each subject (split-plot factor). Dog was nested within group. If a significant interaction between group and method was found, then method differences were examined separately for each group. Multiple comparisons were adjusted for using Tukey's test and the significance threshold was set at 0.05. To assess inter-reader variability, pooled standard deviations were calculated on the paired readers values alternatively averaged and summed over nine fields for all variables. Pearson correlation coefficients were calculated for all variables between readers.

Results

Cytological examination

Yeast, EC cocci, EC rods, PMNs, nuclear streaming, corneocytes and debris were identified in all groups (Table 1). None of the slides contained IC rods. One of the slides (Group 1, toothpick) would not focus under oil immersion due to defective coverslip fixation. The coverslip was removed and both investigators scored and recorded data at the same time. Polymorphonuclear cells and/or nuclear streaming were identified on 13 of 180 slides (7.2%). Of these 13 slides, 11 had cocci, *Malassezia* or a combination of both present (five, one and five slides, respectively). Two of the 13 slides had PMNs and/or nuclear material without organisms.

Comparisons between groups

Least squares means and pooled standard errors by groups for each variable are provided in Table 1. Intracellular cocci were identified only in Group C. Group C dogs

Table 1. Least squares means and pooled standard errors by group. There were significantly more extracellular cocci and corneocytes for dogs in Group C (allergic dogs with clinical paronychia) compared to both Groups A and B (normal dogs and allergic dogs without clinical paronychia, respectively).

Variable [†]	A	B	C
Yeast	6.83 ± 6.27	11.9 ± 6.27	24.99 ± 6.27
EC cocci*	3.5 ± 7.4	0.82 ± 7.4	36.62 ± 7.4*
IC cocci	0 ± 0.33	0 ± 0.33	0.58 ± 0.33
EC rods	0.03 ± 2.14	0.06 ± 2.14	5.48 ± 2.14
IC rods	0 ± NC	0 ± NC	0 ± NC
PMNs	0.23 ± 0.11	0.03 ± 0.11	0.18 ± 0.11
Nuclear streaming	0.18 ± 0.17	0.1 ± 0.17	0.47 ± 0.17
Corneocytes*	24.33 ± 1	24.74 ± 1	30.55 ± 1*
Debris	3.46 ± 0.16	2.98 ± 0.16	3.13 ± 0.16

EC, extracellular; IC, intracellular; PMNs, polymorphonuclear leucocytes; NC, noncalculable because all values were 0.

* $P < 0.01$ (nested ANOVA).

[†]Summed over nine fields and averaged over both readers.

had significantly higher values of EC cocci and corneocytes than Groups A and B. Counts were not significantly different between Groups A and B. The mean pedal pruritus-specific PVAS scores were different ($P = < 0.0001$) between all groups: Group A ($0.1/10 \pm 0.07$); Group B ($3.7/10 \pm 0.5$); Group C ($7/10 \pm 0.34$).

Yeast organisms were present in higher numbers in allergic dogs than normal dogs, but this difference was not statistically significant. One normal dog had up to 30 yeast/OIF identified with the toothpick and impression smear methods, despite a lack of pruritus or clinical signs of paronychia. Neither investigator identified *Malassezia* on tape preparations from this dog.

Comparison of sampling methods

There were significantly higher numbers of *Malassezia* ($P = 0.0016$) and EC cocci ($P = 0.0106$) retrieved from samples collected with a toothpick compared to the tape preparation and direct impression smear methods. Increased amounts of cell debris and corneocytes were observed with the tape preparation method (both $P < 0.0001$), and increased amounts of nuclear streaming were observed with the impression smear method ($P = 0.0468$). In general, it was more challenging to identify organisms on tape preparation samples as a consequence of background debris (Tables 2 and 3, Figure 3a–c).

Table 2. Least squares means and pooled standard errors by methods. Method 1, toothpick; Method 2, tape preparation; Method 3, impression smear.

Variable [†]	1	2	3
Yeast*	27.13 ± 4.98	6.96 ± 4.98	9.64 ± 4.98
EC cocci*	22.62 ± 5.42	4.87 ± 5.42	13.46 ± 5.42
IC cocci	0 ± 0.33	0 ± 0.33	0.58 ± 0.33
EC rods	1.54 ± 1.66	1.73 ± 1.66	2.31 ± 1.66
IC rods	0 ± NC	0 ± NC	0 ± NC
PMNs	0.05 ± 0.11	0.15 ± 0.11	0.24 ± 0.11
Nuclear streaming*	0.2 ± 0.16	0.01 ± 0.16	0.54 ± 0.16
Corneocytes*	22.42 ± 0.87	35.03 ± 0.87	22.18 ± 0.87
Debris*	0.4 ± 0.15	8.66 ± 0.15	0.51 ± 0.15

EC, extracellular; IC, intracellular; PMNs, polymorphonuclear leucocytes; NC, noncalculable because all values were 0.

* $P < 0.05$ (nested ANOVA).

[†]Summed over nine fields and averaged over both readers.

Table 3. P -values between methods. Method 1, toothpick; Method 2, tape preparation; Method 3, impression smear.

Variable	1 versus 2	1 versus 3	2 versus 3
Yeast*	0.0026*	0.0107*	0.8933
EC cocci*	0.0073*	0.2551	0.2997
IC cocci	1.0000	0.4411	0.4411
EC rods	0.9951	0.9172	0.9511
IC rods	NC	NC	NC
PMNs	0.8034	0.4501	0.8319
Nuclear streaming*	0.6478	0.2557	0.0389*
Corneocytes*	<0.0001*	0.9748	<0.0001*
Debris*	<0.0001*	0.8553	<0.0001*

EC, extracellular; IC, intracellular; PMNs, polymorphonuclear leucocytes; NC, noncalculable because all values were 0.

* $P < 0.05$ (nested ANOVA).

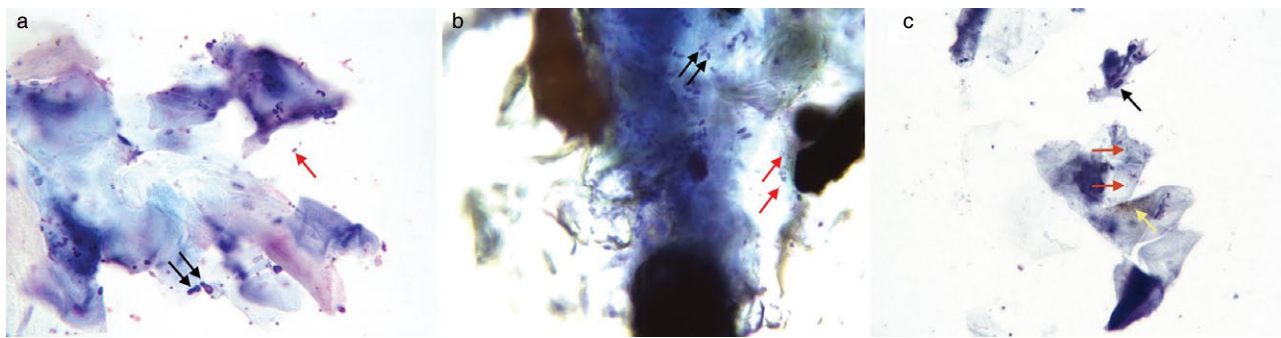


Figure 3. Comparison of three sampling methods for claw fold cytology for a subject in Group C (allergic dogs with clinical paronychia). Examples of *Malassezia* yeast are indicated with black arrows, cocci with red arrows and melanin with a yellow arrow. Diff-Quik® Solution stain, 100×. (a) Toothpick method showing > 30 *Malassezia* organisms and cocci. (b) Tape preparation showing numerous *Malassezia* organisms and cocci. Note the significant amount of debris and high number of corneocytes. (c) Impression smear showing scattered *Malassezia* organisms, cocci and melanin.

Table 4. Inter-reader variability

Variable	Pooled Standard Deviation (Sum)	Pooled Standard Deviation (Average)	<i>r</i>	<i>P</i> -value
Yeast	14.82	1.65	0.88	<0.0001
EC cocci	16.52	1.84	0.88	<0.0001
IC cocci	2.69	0.30	1.00	<0.0001
EC rods	6.93	0.77	0.80	<0.0001
IC rods	NC	NC	NC	NC
PMNs	0.91	0.10	0.46	<0.0001
Nuclear streaming	0.76	0.08	0.69	<0.0001
Corneocytes	5.68	0.63	0.76	<0.0001
Debris	1.47	0.16	0.88	<0.0001

EC, extracellular; IC, intracellular; PMNs, polymorphonuclear leucocytes; NC, noncalculable because all values were 0. Pearson's coefficient (*r*) interpretation: 0.00–0.25 little or no relationship, 0.25–0.50 fair, 0.50–0.75 moderate to good, >0.75 good to excellent.¹⁶

Inter-reader variability

Table 4 lists the pooled standard deviations, correlation coefficients (1.0 indicates perfect positive correlation, –1 indicates perfect negative correlation) and associated *P*-values. All variables that could be calculated were significantly correlated between readers (*P* < 0.0001). Correlation for IC rods could not be calculated, because all values were zero. The highest correlation was with IC cocci (*r* = 1), and the lowest was with PMNs (*r* = 0.46). Correlations for yeast, EC cocci, EC rods, nuclear streaming, corneocytes and debris ranged from 0.69 to 0.88 between readers.

Discussion

In this study, *Malassezia* organisms were commonly retrieved from the subungual claw fold region of normal healthy dogs. As previously reported, these organisms should be considered resident microflora of the normal canine claw fold. This study also suggests that high numbers of yeast can be detected on cytological evaluation of the skin surface of the canine claw fold from allergic dogs in both the absence and presence of concurrent signs of pruritus and paronychia. Whilst the number of *Malassezia* organisms retrieved was higher in allergic dogs with clinical signs compared to allergic dogs with no clinical signs

and normal healthy dogs, the counts were not significantly different between these three groups. This is an interesting observation and supports the hypothesis that a hypersensitivity reaction to *Malassezia* in allergic dogs may be more important as a contributory factor for pruritus than the actual number of yeast organisms present on the skin surface.^{2,10–12}

Significantly higher counts of EC cocci were found in claw folds of allergic dogs with clinical paronychia compared to normal and unaffected allergic dogs. Bacterial paronychia could be a key element of the higher pedal pruritus scores seen in this group and may have clinical implications for the successful reduction of pruritus in these cases.

When comparing the three sampling methods utilised for cytological evaluation of the claw fold region, our study suggests that the toothpick method is optimal for detecting yeast and coccoid bacteria. The toothpick provides a consistent, firm, pointed edge that is easily advanced into the claw fold. These findings contrast with those from a previous study which suggested that a tape preparation technique was more reproducible and accurate than other cytological methods for sampling yeast to confirm *Malassezia* dermatitis.¹³ This previous study did not, however, focus on the claw fold and the value of different collection techniques may vary with the location of sample collection. Another study evaluated a technique using the cut edge of a plastic cotton swab to gently scrape the claw fold of Devon Rex cats and cats of other breeds. Although the *Malassezia* yeast were of a different species in the cats, the number of organisms retrieved was comparable to the present study (8.63/OIF in Devon Rex cats and 0.59/OIF in other cats).⁵

Results presented here also suggest that the toothpick technique yields samples that are easier to interpret. Tape preparations were associated with more debris and corneocytes, and occasional hair or air bubbles were evident. Utilizing the tape preparation method for cytological collection of the claw fold could limit the ability to identify bacteria and yeast accurately, which can contribute to pedal pruritus in clinical patients. One limitation of this study was that samples were not evaluated until all claws had been sampled over an approximate 12-month period. Samples collected with tape preparations earlier in the study may have faded during storage, leading to an under-

estimate of the number of organisms. The investigators compared the number of *Malassezia* organisms identified on tape preparations during the first and last three-month periods of the study, and 9 of 12 dogs (75%) had significant numbers of organisms identified in samples stored between 9 and 12 months (data not shown). Future studies could investigate the effect of duration of tape preparation storage on the quantification of microorganisms.

Impression smears were frequently associated with nuclear streaming secondary to cell lysis. The pressure required to obtain a sample using this method often ruptures inflammatory cells, especially neutrophils. The nuclear material streaks the slide and is readily identified under low power magnification.¹⁴ Nuclear streaming is common, because neutrophils are fragile and vulnerable to trauma, and this feature has been identified as a reproducible indicator of these cells in cutaneous impression smears.¹⁵ Typically, the presence of PMNs or nuclear streaming with intracellular bacteria supports true infection, as opposed to overgrowth.¹⁴ In this particular study, the number of slides identified with PMNs or nuclear material was small (13 of 180), so statistical analysis was not performed. However, the majority of these slides (11 of the 13) contained identifiable bacteria and/or yeast. The small number of slides containing nuclear material made it difficult to draw any conclusions regarding the possible significance of neutrophils or nuclear material in the claw fold together with bacteria or *Malassezia* yeast.

When evaluating inter-reader variability, with the exception of PMNs, most correlations between readers showed good to excellent agreement ($r > 0.75$).¹⁶ The interpretation of nine fields was considered to be representative of the slide overall. Some of the variability can be explained by the fact that the two investigators likely did not evaluate the same nine OIFs on each slide. With the exception of one slide, all were evaluated independently to provide a realistic representation of clinical practice.

In summary, this study demonstrates that sample collection using the toothpick method optimizes the value of cytological evaluation of the subungual claw fold in cases of clinical paronychia. This determination is useful for clinicians in selecting the most effective method to evaluate bacterial and yeast infections of the canine claw fold.

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Résumé

Contexte – Une paronychie bactérienne ou à *Malassezia* est une complication fréquente de la dermatite atopique et des réactions alimentaires indésirables.

Hypothèses/Objectifs – Le but de cette étude est de comparer trois méthodes différentes de prélèvements de cytologie du bourrelet unguéal et d'évaluer le nombre de bactéries, *Malassezia* et cellules inflammatoires.

Sujets – Soixante chiens de propriétaires ont été classés en trois groupes : (A) chiens sains; (B) chiens allergiques sans signe de maladie des griffes (coloration brune, érythème, suintement, croûtes ou exsudat); et (C) chiens allergiques avec paronychie clinique.

Méthodes – Une étude pilote prospective en aveugle a été réalisée. Les bourrelets unguéaux de chaque chien ont été prélevés par impression directe, cellophane ou cure-dent. Les lames ont été évaluées par

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deux investigateurs pour les cellules inflammatoires, les filaments de chromatine, les débris, les cornéocytes, les levures, les coques intracellulaires (IC), extracellulaires (EC), les bâtonnets IC et les bâtonnets EC. Pour chaque paramètre, les données étaient comparées entre les groupes et entre les méthodes. Les concordances inter-observateurs ont été calculées.

Résultats – Le groupe C avait des valeurs significativement plus élevées pour les cocci EC et les cornéocytes que les groupes A ou B. Bien que les *Malassezia* soient plus prévalentes chez les chiens allergiques que chez les chiens sains, leurs dénombrements n'étaient pas significativement différents. Les nombres de *Malassezia* ($P = 0.0016$) et de EC cocci ($P = 0.0106$) étaient significativement plus élevés lorsque prélevés à l'aide de cure-dents comparés aux autres méthodes. Les tests par cellophane étaient associés à un plus grand nombre de débris et de cornéocytes ($P < 0.0001$) et les calques par impression présentaient davantage de filaments de chromatine ($P = 0.0468$).

Conclusions et importance clinique – La méthode de prélèvement à l'aide d'une cure dent optimise la valeur des résultats cytologiques pour des prélèvements de chiens allergiques avec paronychie.

Resumen

Introducción – la paroniquia bacteriana y por *Malassezia* en perros son complicaciones secundarias comunes de la dermatitis atópica y de las relaciones alimentarias adversas

Hipótesis/Objetivos – el propósito de este estudio fue comparar 3 métodos diferentes de toma de muestras para la citología del pliegue de la uña y evaluar el número de bacterias, levaduras *Malassezia* y células inflamatorias.

Animales – 60 perros de propietarios privados fueron clasificados en tres grupos: (A) perros normales; (D) perros alérgicos sin evidencia de enfermedad de las uñas (tinción marronácea, eritema, hinchazón, costras o exudados); y (C) perros alérgicos con paroniquia clínica.

Métodos – se utilizó un estudio prospectivo, ciego, de subdivisión de grupos (split-plot). Los pliegues de las uñas de cada perro fueron muestreados utilizando bien un palillo de dientes, una preparación con cinta adhesiva, o una impresión directa en el porta. Los portas fueron evaluados por dos investigadores buscando células inflamatorias, estrías nucleares artefactuales, detritus, corneocitos, levaduras, cocos intracelulares (IC), cocos extracelulares (EC), bacilos intracelulares (IC) y bacilos extracelulares (EC). Para cada parámetro los datos fueron comparados entre grupos y entre los métodos. Se calculó la similitud entre investigadores.

Resultados – el grupo C tuvo valores significativamente mayores de cocos extracelulares y corneocitos que los grupos A o B. Aunque los organismos de tipo *Malassezia* fueron más prevalentes en los perros alérgicos que en los perros normales, los conteos no fueron significativamente diferentes. Hubo un número significativamente mayor de organismos tipo *Malassezia* ($P = 0,0016$) y cocos extracelulares ($P = 0,0106$) en las muestras tomadas con un palillo comparadas con otros métodos. Las preparaciones con cinta adhesiva se asociaron con presencia de mayor detritus y corneocitos (ambos $P < 0,001$) y las preparaciones por impresión directa tenían mayor cantidad de estrías nucleares ($P = 0,0468$).

Conclusión e importancia clínica – la toma de muestras con un palillo optimiza el valor de los resultados citológicos cuando se toman muestras de perros alérgicos con paroniquia clínica.

Zusammenfassung

Hintergrund – Bakterielle Krallenbettinfektionen und Infektionen mit Hefepilzen sind häufige sekundäre Komplikationen bei atopischer Dermatitis und Futtermittelreaktionen.

Hypothese/Ziele – Das Ziel dieser Studie war ein Vergleich der drei unterschiedlichen Probeentnahmemethoden für die Zytologie des Krallenfalzes und eine Evaluierung der Bakterienzahlen, der *Malassezia* Hefen und der Entzündungszellen.

Tiere – Sechzig Hunde in Privatbesitz wurden in drei Gruppen eingeteilt: (A) normale Hunde; (B) allergische Hunde ohne klinische Evidenz einer Krallenerkrankung (Braunfärbung, Erythem, Schwellung, Krusten oder Exudat); und (C) allergische Hunde mit klinischer Paronychie.

Methoden – Eine prospektive, geblindete, Split-Plot Design Studie wurde verwendet. Von jedem Hund wurden vom Krallenfalz entweder mittels Zahnstocher, Klebestreifen oder direktem Abklatsch Proben genommen. Die Objektträger wurden von zwei UntersucherInnen evaluiert und das Auftreten von Entzündungszellen, Kernstreaming, Debris, Corneozyten, Hefen, intrazelluläre (IC) Kokken, extrazelluläre (EC) Kokken, IC Stäbchen und EC Stäbchen beurteilt. Für jeden Parameter wurden die Ergebnisse zwischen den Gruppen und zwischen den Methoden verglichen. Die Übereinstimmung zwischen den UntersucherInnen wurde kalkuliert.

Ergebnisse – Gruppe C wies signifikant höhere Werte an EC Kokken und Corneozyten auf als die Gruppen A oder B. Obwohl *Malassezia* Organismen bei allergischen Hunden häufiger auftraten als bei normalen Hunden, waren die Zahlen nicht signifikant unterschiedlich. Es bestanden signifikant höhere Zahlen an *Malassezia* Organismen ($P=0,0016$) und EC Kokken ($P=0,0106$) in Proben, die mittels Zahnstocher im Vergleich zu den anderen Methoden entnommen worden waren. Die Klebestreifenpräparate zeigten signifi-

kant mehr Debris und Corneozyten (beide $P < 0,0001$) und Abklatschpräparate weisen signifikant mehr Kernstreaming auf ($P = 0,0468$).

Schlussfolgerungen und klinische Bedeutung – Probenahme mittels Zahnstocher optimiert die Aussagekraft der zytologischen Ergebnisse bei Proben von allergischen Hunden mit klinischer Paronychie.

要約

背景 — イヌの細菌性およびMalassezia性爪周囲炎はアトピー性皮膚炎や食物有害反応で一般的にみられる二次的な合併症である。

仮説/目的 — この研究の目的は爪縁(claw fold)の細胞診の3つの異なるサンプリング方法を比較し、細菌、マラセチアならびに炎症細胞の数を評価することである。

供与動物 — 60頭の飼い犬を3つのグループに分類した:(A)正常犬、(B)爪疾患の臨床的な症状(茶色い着色、紅斑、腫脹、痂皮あるいは滲出物)のないアレルギー犬(C)臨床的に爪周囲炎のあるアレルギー犬。

方法 — 前向き、無作為、分割研究デザインを用いた。それぞれのイヌの爪縁(claw fold)を爪楊枝、テープ、あるいは直接押捺スミアのいずれかの方法でサンプリングした。スライドは2人の調査者が炎症細胞、核遊走、崩壊物、角質細胞、酵母、細胞内(IC)球菌、細胞外(EC)球菌、IC桿菌ならびにEC桿菌を評価した。それぞれのパラメータに対して、データをグループごとおよび方法ごとに比較した。評価者間の一致を計算した。

結果 — グループCはグループAあるいはBと比較し、有意に高いEC球菌値および角質細胞を示した。正常犬と比較し、アレルギーのイヌでマラセチア酵母菌の検出頻度は高かったが、数では有意差はみられなかった。爪楊枝を用いてサンプルを回収した方法は他の方法と比較し、マラセチア酵母菌の数($P = 0.0016$)およびEC球菌の数($P = 0.0106$)が有意に高かった。テープでのサンプリングは有意に多い崩壊物、および角質細胞と関連しており(both $P < 0.0001$)、押捺スミアは有意に多い核遊走と関連していた($P = 0.0468$)。

結論および臨床的な重要性 — 臨床的に爪周囲炎のあるアレルギー犬のサンプリング時には、爪楊枝を使用したサンプル収集が、細胞学的な結果の価値を最大限に得ることができる。

摘要

背景 — 犬細菌和马拉色菌性甲沟炎是异位性皮炎和食物副反应常见的继发疾病。

假设/目的 — 这项研究的目的是,比较三种不同的甲沟细胞学采样方法,并且评估细菌、马拉色菌和炎性细胞的数量。

动物 — 60只家养犬分为三组:(A) 正常犬; (B)过敏,但未表现爪部疾病的犬(褐变、红斑、肿胀、结痂或渗出); (C)临床表现为甲沟炎的过敏犬。

方法 — 使用前瞻性、双盲、裂区实验设计。分别使用牙签、胶带或直接压片三种方法,从每只犬的甲沟处取样。两位技术人员对玻片进行评估,包括炎性细胞、染色质、杂质、角质细胞、酵母菌、细胞内(IC)球菌,细胞外(EC)球菌、细胞内杆菌和细胞外杆菌。对于每项参数,在各组和各种方法间进行比较。计算不同读片者的一致性。

结果 — C组EC球菌和角质细胞的数量明显高于A组和B组。虽然过敏犬比正常犬的马拉色菌更常见,但是数量上并没有明显区别。牙签采样所取得的马拉色菌($P = 0.0016$)和EC球菌($P = 0.0106$)数量,显著高于其他采样方法。胶带样本的杂质和角质细胞(两者 $P < 0.0001$)明显较多,压片所取样本的染色质($P = 0.0468$)明显较多。

总结和临床意义 — 使用牙签法收集患甲沟炎的过敏犬的样本,其细胞学结果更有价值。