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Diversity of *Staphylococcus pseudintermedius* in carriage sites and skin lesions of dogs with superficial bacterial folliculitis: potential implications for diagnostic testing and therapy

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Background – *Staphylococcus pseudintermedius* is genotypically diverse within the canine population and multiple strains may colonize individual dogs at any given time. If multiple strains with distinct antimicrobial resistance profiles are present in superficial bacterial folliculitis (SBF), sampling a single skin lesion for culture and antimicrobial susceptibility testing (AST) might be inadequate to select effective therapy.

Hypothesis/Objectives – To investigate *S. pseudintermedius* diversity in carriage sites and lesions of dogs with SBF.

Animals – Fourteen dogs with SBF.

Methods – *Staphylococcus pseudintermedius* isolates obtained from perineum, gingiva and four to six skin lesions per dog were subjected to pulsed-field gel electrophoresis (PFGE) and AST to assess diversity between lesions. For two dogs, 14–16 isolates per lesion were included in the analysis to assess diversity within lesions.

Results – Analysis of one isolate per lesion revealed one to four strains displaying unique PFGE profiles, and up to three unique antimicrobial resistance (AMR) profiles for each dog. Multiple pustules from the same dog always harboured the same strain, whereas papules, crusts and collarettes did not. Up to four strains with distinct AMR profiles were isolated from the same lesion in two dogs. In 12 dogs, at least one carriage site strain also was represented in lesions.

Conclusions and clinical importance – Lesions of SBF may harbour multiple *S. pseudintermedius* strains with distinct antimicrobial resistance profiles. Pustules are the best target for bacterial culture. It remains unclear whether isolation of different strains from other lesion types is a consequence of contamination or co-infection by multiple strains.

Introduction

Skin infections represent the number one reason for antibiotic treatment of dogs and account for approximately 23–30% of all antimicrobials prescribed in small animal veterinary teaching hospitals.[1,2] Approximately 90% of these infections are caused by *Staphylococcus pseudintermedius*, an opportunistic pathogen residing on skin and mucosal sites of dogs.[3] Canine skin is particularly predisposed to superficial bacterial folliculitis (SBF), a common infection of the hair follicle.[4]

Staphylococcus pseudintermedius is genotypically very diverse in the canine population and multiple strains can colonize individual dogs at any given time.[5] Although clinical strains often derive from the commensal skin microbiome,[6,7] relatively little is known about strain diversity in canine skin lesions. In an earlier study conducted before the re-classification of the common canine pathogen from *S. intermedius* to *S. pseudintermedius*,[8] five of 11 dogs with deep pyoderma had distinct strains within the same lesion based on antimicrobial susceptibility testing (AST) and plasmid profiling of 10 isolates per lesion.[9] In a larger and more recent study, one *S. pseudintermedius* isolate from each of three pustular lesions in 40 dogs was analysed. Twenty six percent of the dogs had multiple strains as determined by pulsed field gel electrophoresis (PFGE).[6] In 95% of these dogs, PFGE profiles correlated with distinct AST profiles.[10] Information on strain diversity is limited for other lesions of SBF, such as papules, collarettes and crusts, which are more common than

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Abbreviations: AMR, antimicrobial resistance; AST, antimicrobial susceptibility testing; PFGE, pulsed-field gel electrophoresis; SBF, superficial bacterial folliculitis; TOF, time-of-flight.

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pustules and may be the only lesions available for bacterial culture in some dogs.[11]

Bacterial culture and AST are recommended for guiding antimicrobial treatment of canine skin infections, especially when cases do not respond to empiric treatment or when there is a prior history of multidrug-resistant isolates.[12] For this purpose, veterinarians tend to sample a single, representative skin lesion, even if more lesions are present. As part of the routine procedure for bacterial culture in veterinary diagnostic laboratories, a single colony representing the dominating growth on the agar plate is selected for identification and AST. It is unknown if these sampling and laboratory procedures enable detection of all strains involved in the infection. Failure to do so may potentially result in prescription of an ineffective antimicrobial and ultimately treatment failure. The need to address this issue was highlighted in a position paper by the ESCMID Study Group for Veterinary Microbiology (ESGVM) on diagnostic challenges in veterinary dermatology.[13]

The objective of the present study was to investigate strain diversity of *S. pseudintermedius* in carriage sites and skin lesions of dogs affected by SBF. The purpose was to provide informed background information for recommendations on sampling and bacterial culture of skin specimens from these dogs.

Materials and methods

Cases

Given informed owner consent, dogs with SBF were recruited at three companion animal clinics in Copenhagen, Denmark, between September 2013 and June 2014. Referral and first opinion cases presenting for a dermatological consultation were eligible for the study. The inclusion criteria were: (i) lesions consistent with SBF including a combination of at least two of the following lesion types: crusts, epidermal collarettes, papules and pustules; (ii) no systemic or topical antimicrobial treatment in the preceding 14 days; and (iii) cytological evidence of cocci and degenerate neutrophils in lesions.

Sample collection, cytology and bacterial culture

For cytology, an indirect impression smear on a glass slide was made from the content of one pustule per dog. In the absence of pustules, direct impression smears from underneath a crust or adhesive tape strip cytology from a collarette were used instead. Slides were stained with Hemacolor® staining kit (Merck; Kenilworth, NJ, USA) and evaluated microscopically.

For bacterial culture, two skin lesions of each type (when present) and the two most common *S. pseudintermedius* carrier sites, namely the superior gingival mucosa and the perineum,[14] were sampled using commercial swabs (Copan Venturi Transystem®, COPAN Diagnostics; Murrieta, CA, USA). Lesions of the same type were sampled as far apart as possible on dogs. Collarettes, crusts and pustules were sampled according to published guidelines,[12] whereas papules were swabbed after incision with the tip of a sterile needle.[11] Gingiva and perineum were sampled by rubbing a swab over the surface for approximately 3 s as described before.[14] Within 1 h after sampling, the swabs were streaked on 5% calf blood agar plates, followed by overnight incubation at 37°C. One colony with the typical morphology of *S. pseudintermedius* (medium-sized, raised and unpigmented, displaying large incomplete β - and small complete δ-haemolysis, either alone or in combination)[4] was subcultured from each sample and stored at −80°C. All remaining colonies resembling *S. pseudintermedius* were suspended in a mixture of 700 μL brain heart infusion broth and 300 μL 50% glycerol, and stored at −80°C.

Bacterial species identification and antimicrobial susceptibility testing

Bacterial isolates were identified to the species level by matrix-assisted laser desorption/ionization time-of-flight (Maldi-TOF) mass spectrometry (Vitek® MS RUO, bioMérieux; Craponne, France) using *Escherichia coli* ATCC 8739 as reference strain and the software Saramis™ 3.5 (bioMérieux) for spectra interpretation. Susceptibility to amikacin, ampicillin, cefazolin, chloramphenicol, clindamycin, enrofloxacin, erythromycin, gentamicin, oxacillin and trimethoprim/sulfamethoxazole was tested by broth microdilution using the COMPAN1F commercial panel (Thermo Fisher Scientific; Waltham, MA, USA) according to the Clinical and Laboratory Standards Institute guidelines (CLSI).[15] These drugs were included in the analysis as representatives of the principal antimicrobial classes used for treatment of SBF, plus the best indicator for prediction of meticillin resistance in *S. pseudintermedius* (oxacillin).[16] *Staphylococcus aureus* ATCC 29213 was used for quality control and isolates falling in the intermediate category were considered susceptible for ease of interpretation. The range of doxycycline concentrations in the test panel cannot predict susceptibility to doxycycline according to the current doxycycline clinical breakpoint for *S. pseudintermedius*.[17] Therefore, isolates were tested for susceptibility to doxycycline by agar dilution using Mueller–Hinton agar plates (Oxoid Ltd; Cheshire, UK) supplemented with 0.25 μg/mL doxycycline.

Pulsed-field gel electrophoresis

One *S. pseudintermedius* isolate per sample was subjected to PFGE using the Harmony protocol with modifications as described previously.[14] Multiple isolates from the same dog were included in the same gel. Band profiles were aligned according to an internal size standard in all gels (Low Range PFG Marker, New England BioLabs; Ipswich, MA, USA) using GelCompar II (Applied Maths; Sint-Martens-Latem, Belgium). Isolates displaying indistinguishable or closely related band patterns (up to three band differences) were assigned to the same PFGE type and considered to be the same strain.

Follow-up study on strain diversity within lesions

Based on PFGE analysis and AST of isolates in the primary study (see "Results") two dogs which carried distinct strains in different lesions were randomly selected to study diversity within lesions. The frozen mixture of colonies from each lesion was streaked on blood agar and – to select for strains with different antimicrobial resistance (AMR) phenotypes – on Mueller–Hinton agar plates (Oxoid Ltd) supplemented with one of seven different antimicrobial agents: (i) ampicillin (0.25 μg/mL), (ii) chloramphenicol (16 μg/mL), (iii) clindamycin (2 μg/mL), (iv) doxycycline (0.25 μg/mL), (v) enrofloxacin (2 μg/mL), (vi) gentamicin (8 μg/mL) and (vii) trimethoprim/sulfamethoxazole (2/38 μg/mL). Antimicrobial concentrations were selected based on the CLSI susceptible or intermediate breakpoints for staphylococci.[15] Ten colonies were selected from the blood agar plate and two colonies were selected from each Mueller–Hinton agar plate supplemented with antimicrobial when growth of presumptive *S. pseudintermedius* colonies was observed. The colonies were identified to the species level, tested for antimicrobial susceptibility and analysed by PFGE as described above.

Results

Fourteen dogs (A–N) representing three mixed breeds and 10 pure breeds (bearded collie, cocker spaniel, French bulldog, German shepherd dog, golden retriever, Havanese, miniature schnauzer, Welsh springer spaniel, West Highland white terrier and whippet) were enrolled in the study. Ages ranged from 4 months to 11 years (mean 4.8 years). Six dogs were male and eight were female. Based on clinical records,

13 dogs had previous episodes of SBF, whereas one dog presented with clinical signs of infection for the first time.

Staphylococcus pseudintermedius occurred in mixed cultures in 86% of carriage sites, 50% of collarettes, 33% of crusts, 22% of papules and 17% of pustules (Table 1). A single *S. pseudintermedius* colony was isolated from 28 carriage sites, 24 crusts, 18 papules, 12 collarettes and 12 pustules, leading to a total of 94 isolates. All isolates except one were typeable by PFGE, leading to identification of 28 unique strains (Table 2). A single strain was detected in five dogs, whereas two to four strains were detected in the remaining nine dogs. The same strain was isolated from all lesions in eight dogs, whereas the remaining six dogs had multiple strains across lesions. In most dogs (12 of 14), at least one strain from a carriage site also was isolated from one or more lesions. Multiple isolates representing the same strain generally had the same AMR profile, except three strains displaying variable susceptibility to ampicillin and doxycycline. In seven dogs, all isolates from lesions in each individual shared the same AMR profile. Either two (n = 6) or three (n = 1) unique AMR profiles were observed among isolates from lesions in the remaining seven dogs (Table 2).

In the follow-up study, 88 and 90 *S. pseudintermedius* isolates (14–16 per lesion) were obtained from dogs A and D, respectively. Isolate typing by PFGE confirmed the presence of the same two strains in Dog A that were detected in the primary study by analysis of one colony per sample. Strain 1 was recovered as the only strain in five lesions, whereas strain 2 was isolated from a single crust. The AST of the isolates belonging to this strain revealed three antimicrobial resistance profiles that were not detected in the primary study, two for strain 1 and one for strain 2 (Table 3). Some isolates belonging to strain 1, which was consistently resistant to trimethoprim/sulphamethoxazole, were additionally resistant to either ampicillin or doxycycline; three isolates of strain 2 displayed resistance to ampicillin in addition to chloramphenicol, clindamycin and erythromycin. Overall, each lesion in Dog A harboured isolates displaying either one or two distinct AMR profiles (Table 3). A higher genetic diversity was evident after PFGE analysis of the isolates from Dog D; five new strains (29–33) were found in addition to the two strains that were detected in the primary study. Three additional AMR profiles were observed. Each lesion in Dog D harboured isolates with one to four distinct AMR profiles (Table 3).

Discussion

Bacterial culture combined with AST is an important element of antimicrobial stewardship.[18] The current practice of culturing a single lesion and performing AST on one or few colonies could fail to detect multiple strains with distinct antimicrobial resistance profiles, and hence compromise the efficacy of antimicrobial treatment. The results of our study confirm this risk as indicated by the presence of genetically distinct strains in the lesions of six of 14 dogs. Up to four strains with three distinct antimicrobial resistance profiles were detected in the same dog (Dog F). This dog previously had received a wide range of topical and systemic antimicrobials including amoxicillin with and without clavulanic acid, doxycycline, framycetin, fusidic acid, gentamicin, gramicidin and metronidazole. This result highlights the need for future research investigating the relationship between strain diversity and antimicrobial exposure.

The degree of strain diversity correlated with the sample type in this study. Pustules, and to a lesser extent papules, were associated with less species and strain diversity than collarettes and crusts, as indicated by the relatively high frequency of *S. pseudintermedius* pure cultures (Table 1) and the detection of a single strain in pustules from the same dog (Table 2). The low species and strain diversity in pustules and papules can be attributed to the physical separation of their content from the environment and skin commensal microbiota, which reduces the risk of contamination before and during specimen collection. Samples collected from collarettes and crusts are more easily subject to contamination with bacteria from the environment and surrounding skin. These results support the current recommendations to use pustules as first-choice lesions for bacterial culture and diagnostic investigation of canine SBF.[11,12]

The data on treatment outcome were not consistently available for all dogs. One exception is Dog A, which carried a clindamycin-resistant strain in one of the six lesions tested and received systemic therapy with clindamycin 1 week after topical treatment with a shampoo containing chlorhexidine. Such treatment was effective despite the presence of the clindamycin-resistant strain, but it remains unknown whether cure was achieved because of clindamycin alone or its combination with chlorhexidine shampoo. Despite the presence of multiple strains and antimicrobial resistance phenotypes, dogs D and F were treated successfully using topical antimicrobial agents, namely chlorhexidine shampoo (both dogs) and fusidic acid ointment (Dog D). Topical agents are often favoured over systemic drugs for treating SBF due to their minimal

Table 1. Frequency of pure and mixed *Staphylococcus pseudintermedius* cultures from carriage sites and lesions of 14 dogs affected by superficial bacterial folliculitis

Sample site	*S. pseudintermedius* in pure culture	*S. pseudintermedius* in mixed culture
Carriage		
Gingiva	0	14
Perineum	3	11
Lesion		
Pustule	10	2
Papule	14	4
Crust	16	8
Collarette	6	6

Table 2. Distribution of *Staphylococcus pseudintermedius* strains in carriage sites and lesions of 14 dogs affected by superficial bacterial folliculitis

	Carriage site		Lesion							
Dog	Gingiva	Perineum	Papule 1	Papule 2	Pustule 1	Pustule 2	Crust 1	Crust 2	Collarette 1	Collarette 2
A	1 (SXT)	1 (SXT)	1 (SXT)	1 (SXT)	–	–	2 (CHL, DOX, ERY)	1 (SXT)	1 (SXT)	1 (SXT)
B	3	3	3	3	–	–	3	3	–	–
C	4	4	4	4	4	4	4	4	–	–
D	5	6 (CLI, DOX, ERY)	6 (CLI, DOX, ERY)	5	–	–	6 (CLI, DOX, ERY)	6 (CLI, DOX, ERY)	6 (CLI, DOX, ERY)	6 (CLI, DOX, ERY)
E	7 (DOX)	8 (DOX)	–	–	7	7	7	7		–
F	9 (DOX)	10 (CHL)	–	–	–	9 (DOX)	11	10 (CHL)	9 (DOX)	12 (DOX)
G	13	14	–	–	–	–	13	13	13	13
H	15 (DOX)	16	17	15 (DOX)	16	16	16	15 (DOX)	–	–
I	18 (DOX)	18 (DOX)	18 (AMP, DOX)	18 (DOX)	–	–	–	–	18 (DOX)	18 (DOX)
J	19	19	20	20	20	–	20	20	–	–
K	NT (DOX)	21 (DOX)	22	23	–	–	22	22 (DOX)	–	–
L	24	25	26 (DOX)	25	25	25	–	–	–	–
M	27	27	–	–	27	27	27	27	–	–
N	28	28	–	–	–	–	28	28	28	28

NT not typeable by PFGE, - no isolate; each strain was numbered according to its PFGE type (1–28). Resistance to ampicillin (AMP), clindamycin (CLI), chloramphenicol (CHL), doxycycline (DOX), erythromycin (ERY) and trimethoprim/sulphamethoxazole (SXT) is indicated in brackets. Strains were not resistant to any of the agents tested if no antimicrobial abbreviations are provided in brackets.

Table 3. Phenotypic and genotypic diversity of multiple *Staphylococcus pseudintermedius* isolates obtained from lesions of dogs A and D

Dog	Lesion	Number of isolates	Resistance profile	Strain number
A	Papule 1	11	SXT	1
		3	**AMP, SXT**	1
	Papule 2	14	SXT	1
	Crust 1	13	CHL, CLI, ERY	2
		3	**AMP, CHL, CLI, ERY**	2
	Crust 2	14	SXT	1
		2	**DOX, SXT**	1
	Collarette 1	14	SXT	1
	Collarette 2	13	SXT	1
		1	**AMP, SXT**	1
D	Papule 1	12	Fully sensitive	5
		1	**AMP, CLI, DOX, ERY**	6
		1	CLI, DOX, ERY	6
	Papule 2	12	Fully sensitive	5
		2	CLI, DOX, ERY	6
	Crust 1	8	CLI, DOX, ERY	6
		3	**CLI, ERY**	**29**
		2	**DOX**	**29, 30**
		2	Fully sensitive	**30**
		1	Fully sensitive	5
	Crust 2	14	CLI, ERY, DOX	6
	Collarette 1	13	CLI, ERY, DOX	6
		3	Fully sensitive	**30, 31, 32**
	Collarette 2	13	**CLI, ERY**	**33**
		3	CLI, ERY, DOX	6

AMP ampicillin, CHL chloramphenicol, CLI clindamycin, DOX doxycycline, ERY erythromycin, SXT trimethoprim/sulfamethoxazole.
Antimicrobial resistance profiles and strains that were not detected by the initial analysis of one isolate per lesion are highlighted in bold.

adverse effects and high efficacy, irrespective of antimicrobial resistance in clinical strains.[12]

Most dogs in this study (12 of 14) carried the strain associated with infection in the perineum and/or the gingiva (Table 2). Similar results were reported previously for 11 of 16 dogs,[7] and another study[6] found an even higher proportion of dogs (32 of 34) having identical strains of coagulase-positive staphylococci in carriage sites and lesions. Altogether, these studies confirm the view that dogs are generally auto-inoculated with *S. pseudintermedius* strains persisting on their body,[5] as seen for the majority of *S. aureus* infections in humans.[19]

The follow-up study revealed a high degree of genetic diversity among the isolates from the lesions in Dog D, from which seven genetically distinct strains were isolated (Tables 2 and 3). This level of strain diversity within lesions is higher than in a previous study of canine furunculosis, where a maximum of two AMR profiles were detected among 10 isolates per lesion.[9] The difference between studies may be partly attributed to methodological factors,

including that we plated samples on agar supplemented with antibiotics unlike the previous study. One limitation of our follow-up study on strain diversity within lesions is that only two dogs were investigated. Notably, these dogs were selected from those showing strain diversity across lesions in the primary study (Table 2) and therefore were not representative of the entire study population.

More research is needed to demonstrate whether co-infection by different strains with potentially different antimicrobial resistance profiles may occur in 'open' lesions such as collarettes and crusts. The occurrence of co-infection would pose a serious diagnostic challenge, as current methods used in diagnostic laboratories cannot reveal the entire spectrum of strain diversity. One possible solution could be the inclusion in primary culture of selective agar plates for detection of co-infecting strains of high clinical relevance (e.g. meticillin-resistant staphylococci).

In conclusion, lesions and carriage sites of dogs with SBF may contain a heterogeneous population of *S. pseudintermedius*. Our results confirm that the current recommendation to collect clinical specimens from pustules or papules (in absence of pustules) for bacterial culture is appropriate. Culture of samples from other lesions that are more exposed to contamination from the environment and skin commensal microbiota, such as collarettes and crusts, frequently leads to isolation of multiple bacterial species and *S. pseudintermedius* strains that may not necessarily be involved in the infection process. The biological and clinical significance of the isolation of different strains from these skin lesions remains unclear. Further research is warranted to clarify whether this observation is a consequence of contamination or co-infection by multiple strains. This information is important to understand the pathogenesis of SBF and to ensure effective antimicrobial treatment in clinical practice.

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Résumé

Contexte – *Staphylococcus pseudintermedius* est génotypiquement varié au sein de la population canine et des souches diverses peuvent coloniser chaque chien à un instant donné. Si des souches multiples avec des profils de résistance microbienne distincts sont présentes dans une folliculite bactérienne superficielle (SBF), prélever une seule lésion cutanée pour mise en culture et antibiogramme (AST) peut être insuffisant pour choisir le traitement approprié.

Hypothèses/Objectifs – Etudier la diversité de *Staphylococcus pseudintermedius* des sites de portage et des lésions des chiens atteints de SBF.

Sujets – Quatorze chiens avec SBF.
Méthodes – Les souches de *Staphylococcus pseudintermedius* obtenues à partir du périnée, des gencives et de quatre à six lésions par chien ont été analysés par PFGE (pulsed-field gel electrophoresis) et AST pour déterminer la diversité entre les lésions. Pour deux chiens, 14-16 souches par lésion ont été incluses dans l'analyse pour déterminer la diversité des lésions.
Résultats – Les analyses d'un prélèvement par lésion ont révélé une à quatre souches montrant des profils de PFGE uniques et jusqu'à trois profils de résistance antimicrobienne (AMR) unique pour chaque chien. Plusieurs pustules du même chien montraient toujours la même souche mais pas les papules, croûtes et collerettes. Jusqu'à quatre souches avec des profils d'AMR ont été isolés de la même lésion chez deux chiens. Chez 12 chiens au moins une souche de site de portage était représentée dans les lésions.
Conclusions et importance clinique – Les lésions de SBF peuvent présenter de multiples souches de *Staphylococcus pseudintermedius* avec des profils de résistance antimicrobienne différents. Les pustules sont les meilleures lésions à prélever pour culture bactérienne. Nous ne savons toujours pas si l'isolement de différentes souches des autres types de lésion est due à une contamination ou à une co-infection par des souches multiples.

Resumen
Introducción – *Staphylococcus pseudintermedius* es genotípicamente diverso dentro de la población canina y múltiples cepas pueden colonizar perros individuales en un momento dado. Si en la foliculitis bacteriana superficial (SBF) existen múltiples cepas con perfiles de resistencia antimicrobiana distintos, el muestreo de una sola lesión cutánea para cultivo y la prueba de susceptibilidad antimicrobiana (AST) podría ser inadecuado para seleccionar una terapia eficaz.
Hipótesis/Objetivos – Investigar la diversidad de *S. pseudintermedius* en sitios de colonización subclínica y lesiones de perros con SBF.
Animales – Catorce perros con SBF.
Métodos – aislados de *Staphylococcus pseudintermedius* obtenidos del perineo, la encía y cuatro a seis lesiones cutáneas por perro se sometieron a electroforesis en gel de campo pulsado (PFGE) y AST para evaluar la diferencia entre las lesiones. De dos perros se incluyeron 14-16 aislamientos por lesión en el análisis para evaluar la diversidad de cepas dentro de las lesiones.
Resultados – el análisis de un aislado por lesión reveló de una a cuatro cepas con perfiles únicos de PFGE y hasta tres perfiles únicos de resistencia antimicrobiana (AMR) para cada perro. Las pústulas múltiples del mismo perro siempre albergaban la misma cepa, mientras que las pápulas, las costras y los collaretes no. Se aislaron hasta cuatro cepas con distintos perfiles de AMR de la misma lesión en dos perros. En 12 perros, al menos una cepa del sitio de colonización subclínica también se representó en las lesiones.
Conclusiones e importancia clínica – las lesiones de SBF pueden albergar múltiples cepas de *S. pseudintermedius* con distintos perfiles de resistencia a los antimicrobianos. Las pústulas son el mejor objetivo para el cultivo bacteriano. No está claro si el aislamiento de diferentes cepas de otros tipos de lesiones es una consecuencia de contaminación o coinfección por múltiples cepas.

Zusammenfassung
Hintergrund – *Staphylococcus pseudintermedius* ist innerhalb der Hundepopulation ein genotypisch sehr unterschiedlicher Stamm und multiple Stämme können einzelne Hunde zum gegebenen Zeitpunkt besiedeln. Wenn viele Stämme mit deutlich unterschiedlichen antimikrobiellen Resistenzprofilen bei einer oberflächlichen Follikulitis (SBF)auftreten, kann die Probenahme von einer einzigen Hautveränderung für Kultur und Antibiogramm (AST) nicht ausreichend sein um eine wirksame Therapie auszusuchen.
Hypothese/Ziele – Eine Untersuchung der Diversität von *S. pseudintermedius* an typischen Trägerstellen und Hautveränderungen bei Hunden mit SBF.
Tiere – Vierzehn Hunde mit SBF.
Methoden – *Staphylococcus pseudintermedius* Isolate wurden vom Perineum, Gingiva und von vier bis sechs Hautveränderungen pro Hund entnommen und einer Pulsfeldgelelektrophorese (PFGE) und AST unterzogen, um die Verschiedenheit zwischen den Veränderungen zu untersuchen. Bei zwei Hunden wurden 14-16 Isolate pro Hautveränderung in die Analyse inkludiert, um Unterschiede zwischen den Veränderungen zu untersuchen.
Ergebnisse – Die Analyse eines einzelnen Isolates pro Hautveränderung zeigte, dass einer bis vier Stämme einzigartige PFGE Profile aufwiesen, und dass bis zu drei individuelle antimikrobielle Resistenzprofile (AMR) für jeden einzelnen Hund auftraten. Multiple Pusteln vom selben Hund zeigten immer denselben Stamm, während das bei Papeln, Krusten und Colaretten nicht der Fall war. Bis zu vier Stämme mit unterschiedlichen AMR Profilen wurden bei zwei Hunden von derselben Stelle isoliert. Bei 12 Hunden war ebenfalls zumindest ein Trägerstamm ebenfalls in den Hautveränderungen präsent.
Schlussfolgerungen und klinische Bedeutung – Hautveränderungen einer SBF können multiple *S. pseudintermedius* Stämme mit unterschiedlichen antimikrobiellen Resistenzprofilen aufweisen. Pusteln sind am besten für eine bakterielle Kultur. Es bleibt unklar, ob die Isolierung der verschiedenen Stämme aus

andersartigen Hautläsionen eine Folge von Kontamination oder eine Co-Infektion durch multiple Stämme darstellt.

要約

背景 – *Staphylococcus pseudintermedius*は犬集団の中でも遺伝的多様性をもち、いつでも複数の菌株が個々の犬に定着する可能性がある。表在性細菌性毛包炎(SBF)に異なる抗菌抵抗性プロファイルを有する複数の菌株が存在すると仮定すると、細菌培養検査および抗菌剤感受性試験(AST)の際、単一皮膚病変から採材することは効果的な治療法の選択に不適切な可能性がある。

仮説/目的 – 本研究の目的はSBFを有する犬のキャリッジ部位および病変における*S. pseudintermedius*の多様性を調査することである。

被験動物 – SBFを有する犬14頭。

方法 – 犬1頭あたり、会陰部、歯肉および4〜6つの皮膚病変から分離した*S. pseudintermedius*株を、パルスフィールドゲル電気泳動(PFGE)およびASTに供して病変の多様性を評価した。病変ごとの多様性を評価するため、2頭の犬において病変あたり分離株14〜16株を解析に含めた。

結果 – 病変あたりの分離株解析では、固有のPFGEプロファイルを示す1〜4株を明らかにし、各犬最大3つの固有の抗菌剤耐性(AMR)プロファイルを明らかにした。同一犬の複数の膿疱からは常に同一菌株が検出されたのに対し、丘疹、痂皮および表皮小環では同一菌株ではなかった。異なるAMRプロファイルを有する最大4つの分離株が、2頭の犬の同一病変から分離された。 12頭の犬において、少なくとも1つのキャリッジ部位の菌株もまた病変部に存在した。

結論と臨床的重要性 – SBFの病変には、異なる抗菌薬抵抗プロファイルを有する複数の*S. pseudintermedius*株が存在する可能性が考えられた。膿疱は細菌培養の最良の標的であった。他の皮疹から得られた異なる分離株が、汚染の結果であるかまたは複数の菌株による共感染であるかどうかは依然として不明である。

摘要

背景 — 犬群的假中间型葡萄球菌有多种基因型,在一定时间内,多种菌株也可以定植于犬个体。如果浅表细菌性毛囊炎(SBF)病例中存在明显耐药的多种菌株,那么对单个皮肤病变采样,并进行培养和药敏试验(AST),很可能无法选择有效的抗生素治疗。

假设/目的 — 调查SBF患犬正常和病变部位携带假中间型葡萄球菌的多样性。

动物 — 14只SBF患犬。

方法 — 从每只犬的会阴、牙龈和四到六个病变处采样,对获得的假中间型葡萄球菌菌株进行脉冲场凝胶电泳(PFGE)和AST,以评估不同病变的菌株多样性。其中两只犬,每个病变中发现14-16个菌株,对其分析以评估这些菌株的多样性。

结果 — 对每只犬的单个病变进行分析,一份分离样本可发现一到四个具有独特PFGE型的菌株,以及多达三种具有独特抗生素耐药型(AMR)的菌株。同一只犬的多个脓疱存在相同的菌株,而丘疹、结痂和表皮环的菌株并不一样。从其中两只犬的同一病变中,分离出多达4种不同AMR型的菌株。其余12只犬的病变中,至少存在一种正常携带的菌株。

结论和临床意义 — SBF病变可能含有多种假中间型葡萄球菌菌株,并具有不同耐药型。脓疱最适合采样并进行细菌培养。目前尚不清楚的是,来自其他病变类型的不同菌株,是污染菌还是合并感染的结果。

Resumo

Contexto – *Staphylococcus pseudintermedius* é genotípicamente diverso dentro da população canina e várias cepas podem colonizar o mesmo indivíduo em um dado momento. Caso múltiplas cepas com perfis de resistência a antimicrobianos distintos estejam presentes na foliculite bacteriana superficial (FBS), a coleta de apenas uma lesão de pele para cultura e antibiograma (ATB) pode não ser suficiente para selecionar uma terapia eficaz.

Hipótese/Objetivos – Investigar a diversidade de *S. pseudintermedius* em locais de carreamento e lesões de pele de cães com FBS.

Animais – Quatorze cães com FBS.

Métodos – *Staphylococcus pseudintermedius* isolados do períneo, gengiva e quatro a seis lesões de pele por cão foram submetidos à eletroforese em gel de campo pulsado (*pulsed-field gel electrophoresis,* PFGE) e ATB para avaliar a diversidade entre lesões. Quatorze a 16 isolados por lesão de dois cães foram incluídos na análise para avaliar a diversidade das lesões.

Resultados – A análise de um isolado por lesão revelou uma a quatro cepas apresentando perfis de PFGE distintos e até três perfis de resistência a antimicrobianos (RAM) para cada cão. Múltiplas pústulas do mesmo cão sempre continham a mesma cepa, enquanto pápulas, crostas e colarinhos epidérmicos não. Até quatro cepas com perfis RAM distintos foram isoladas da mesma lesão em dois cães. Em 12 cães, ao menos uma cepa oriunda de local de armazenamento estava presente também nas lesões.

Conclusões e importância clínica – As lesões se FBS podem apresentar múltiplas cepas de *Staphylococcus pseudintermedius* com distintos perfis de resistência a antimicrobianos. As pústulas são as melhores lesões para cultura bacteriana. Ainda não está esclarecido se o isolamento de diferentes cepas dos outros tipos lesionais é uma consequência de contaminação ou co-infecção por múltiplas cepas.