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Evaluation of weekly bathing in allergic dogs with methicillin-resistant *Staphylococcal* colonization

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Abstract

We evaluated the efficacy of weekly bathing in reducing methicillin-resistant *Staphylococcus* (MRS) colonization in canine allergic dermatitis in a pilot clinical trial. Six dogs with allergic dermatitis controlled by prescription medications were treated with weekly bathing for 1 month. The Canine Atopic Dermatitis Extent and Severity Index version 3 (CADESI-03) and pruritus scores and frequency of *mecA*-positive *Staphylococcus* spp. isolated from three body sites between weeks 0 and 4 were compared. There was no significant difference in CADESI-03 scores with bathing, whereas the pruritus scores were significantly reduced ($p < 0.05$). Furthermore, MRS frequency was decreased in four of the six dogs ($p < 0.05$). In conclusion, weekly bathing should be considered for reducing MRS colonization in canine allergic dermatitis.

Key Words: allergic dermatitis, bathing, *mecA*

Staphylococcus spp. are part of the commensal flora of canine skin. In dogs with allergic skin conditions, such as canine atopic dermatitis (CAD) and adverse food reactions (AFR), *Staphylococcus* spp. are common causative pathogens isolated from skin lesion^{6,7,13}. During antibiotic therapy, *Staphylococcus* spp. frequently

acquire genes that provide resistance to antimicrobial agents; one example is *mecA* found in methicillin-resistant *Staphylococcus* spp. (MRS)¹. These resistance genes in pathogenic *Staphylococcus* spp. constitute a risk for horizontal gene transfer to commensals and transients^{2,4}, which is particularly problematic for methicillin-

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resistant *Staphylococcus pseudintermedius* in dogs because of the risk of colonization or *mecA* gene transfer to owners^{3,6}. In this clinical pilot study, we evaluated the efficacy of weekly bathing in decreasing MRS colonization in the skin of allergic dogs.

Six dogs with chronic, recurrent, and pruritic skin symptoms that were diagnosed with CAD and/or concurrent AFR based on Favrot's criteria for canine atopic dermatitis, serum allergen-specific immunoglobulin E (IgE) test, and food elimination and provocation trials were enrolled (Table 1)^{5,9}. The allergic skin conditions of all dogs were under control by prescription medications and food avoidance. All six dogs were routinely bathed by their owners at a frequency of once a week or once every other week. All dogs had a history of superficial pyoderma due to *Staphylococcus* spp., in which *mecA*-positive *Staphylococcus* spp. was isolated from the skin surface. After informed consent was obtained, owners were instructed to declare any unusual events regarding their dogs such as the living environment and diet during the study period. The trial was conducted between September 2010 and December 2011. This trial was approved by the ethical committee for clinical trials at the Veterinary Teaching Hospital of Azabu University in Sagami-hara, Kanagawa, Japan.

The trial participants were bathed by veterinary technicians (VTs) once a week for 1 month. VTs photographed the dogs before their weekly baths. The Canine Atopic Dermatitis Extent and Severity Index version 3 (CADESI-03), which has a maximum score of 1,240, was used to evaluate skin symptoms⁸. Pruritus was assessed daily by the owners on a scale of 0-10 (0 = no pruritus; 10 = severe pruritus), and weekly average scores were calculated. To evaluate changes in clinical scores, the scores at the start (week 0) and end (week 4) of the trial were compared.

The swab samples were collected from the skin surface before the baths. Three swab samples were collected from three different affected body sites that showed signs such as erythema and

atopic dermatitis. At each site, an area of 5 cm² was swabbed and cultured for bacteria on blood agar. Five, morphologically similar colonies representing the majority from each agar plate were selected. The expression of *mecA* of gram-positive cocci was detected by polymerase chain reaction (PCR), as described by Sasaki *et al*¹². The bacterial species of *mecA*-positive gram-positive cocci were identified by sequencing 16S *rRNA* with species-specific primers using the Microseq 500 PCR kit (Applied Biosystems; Foster City, U.S.A.), according to the manufacturer's protocols¹². The frequencies of *mecA* in isolated *Staphylococcus* spp. from each body site at week 0 and 4 were compared. Changes in clinical scores were not available to the microbiologists until the end of the trial.

Changes in outcome measures between weeks 0 and 4 were compared using statistical analysis. Wilcoxon rank sum test was used for clinical scores, and Mann-Whitney *U* test was used for frequency of *mecA*-positive *Staphylococcus* colonization using the Microsoft Excel program with the Statcel software add-in (version 4; OMS Publishing, Japan). Data were presented as means \pm standard deviation, and *p* values of <0.05 were considered statistically significant for all analyses performed.

The changes in the frequency of *mecA*-positive *Staphylococcus* colonization, CADESI-03 and pruritus scores are presented in Fig. 1. Table 2 shows the PCR analysis for *mecA*. The frequency of *mecA*-positive *Staphylococcus* spp. isolated from all three body sites decreased in four dogs (cases 2, 4, 5, and 6). Specifically, the frequency at week 4 was significantly lower than that at week 0 (0.8 ± 0.4 versus 2.0 ± 0.9 , respectively; $p < 0.05$). At week 0, *mecA*-positive *Staphylococcus* spp. were isolated from 12 out of a total of 18 body sites (eleven *S. pseudintermedius* and one *S. aureus*). In contrast, *mecA*-positive *Staphylococcus* spp. were isolated from five body sites (four *S. pseudintermedius* and one *S. epidermis*) at the end of the trial at week 4. There was no significant difference in the CADESI-03

Table 1. Profile, skin symptoms, skin lesions, identified allergens, and prescribed medications and shampoos of six allergic dogs enrolled in the trial

Case No.	Breeds	Sex ¹⁾	Age (year)	1st onset (year)	Seasonality of symptoms	Major Skin symptoms	Major Skin lesions	Results of serum allergen specific Immunoglobulin E test	Results of food provocation test	Prescribed medications ²⁾	Prescribed shampoos ³⁾
1	Shiba Inu	SF	10	5	All year	Erythema, Scales, Lichenification, Hyperpigmentation, Alopecia	Muzzle, Periorcular, Feet, Axillae, Ventral abdomen, Prianal	<i>D. farinae</i> , <i>D. pteronyssinus</i>	Not determined	Clemastine fumarate, Glycyrrhizinate, Ointment	Chlorhexidine
2	Yorkshire Terrier	SF	6	1	All year	Erythema, Excoriation, Papule, Scales, Lichenification, Hyperpigmentation, Alopecia	Muzzle, Ear, Periorcular, Feet, Axillae, Dorsal trunk, Ventral abdomen	<i>D. farinae</i> , <i>D. pteronyssinus</i>	Chicken	Clemastine fumarate, Ointment	Chlorhexidine
3	Toy Manchester Terrier	SF	9	2	Autumn, Winter	Erythema, Scales, Alopecia	Axillae, Dorsal trunk, Ventral abdomen	<i>A. siro</i>	Not determined	Prednisolone, Ointment	Chlorhexidine
4	Shiba Inu	NM	10	1	All year	Scales, Lichenification, Hyperpigmentation, Alopecia	Muzzle, Periorcular, Feet, Axillae, Ventral abdomen	<i>D. pteronyssinus</i>	Chicken, Sardine, Sprout, Potato, Rice, Wheat	Prednisolone, Clemastine fumarate, Glycyrrhizinate, Ointment	Miconazole nitrate and Chlorhexidine
5	Brussels Griffon	NM	8	4	All year	Erythema, Excoriation, Papule, Scales, Lichenification, Hyperpigmentation, Alopecia, Fistula	Muzzle, Face, Dorsal trunk, Ventral abdomen, Interdigit	<i>A. siro</i>	Japanese sweets	Ointment	Chlorhexidine
6	Labrador Retriever	F	8	2	All year	Erythema, Papule, Lichenification, Hyperpigmentation, Alopecia, Fistula	Muzzle, Face, Ear, Axillae, Ventral abdomen, Interdigit	<i>A. fumigatus</i>	Chicken, Potato	Prednisolone, Thyroxin, Ointment	Chlorhexidine

1) F, female; SF, Spayed female; NM, Neutered male

2) Ointment, Hand-mixed topical corticosteroid (0.12% betamethasone valerate and 0.3% heparinoidm)

3) Chlorhexidine, Nolvasan, Fort dodge animal health, IA; Miconazole nitrate and Chlorhexidine, Malaseb, Fort dodge animal health, IA

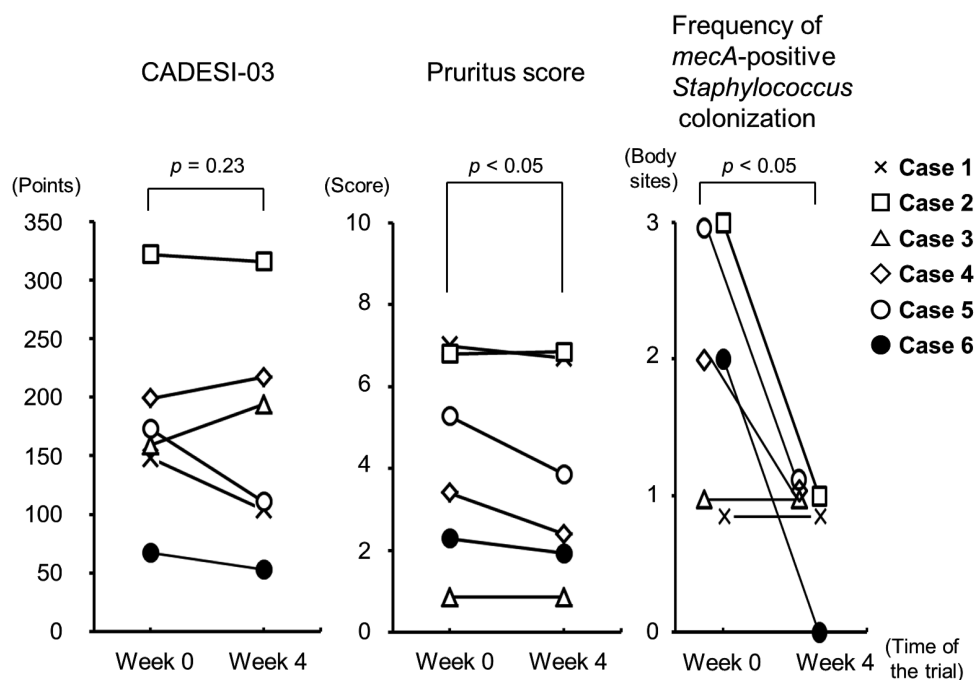


Fig. 1. Changes in the clinical scores and frequency of *mecA*-positive *Staphylococcus* colonization in six dogs enrolled in the trial.

score between week 0 (178 ± 83) and week 4 (166 ± 95); however, two of the six participant dogs (cases 1 and 5) exhibited decreases of $\geq 25\%$ in CADESI-03 scores compared with those at week 0. The pruritus score was significantly lower at week 4 than at week 0 (3.8 ± 2.5 versus 4.3 ± 2.5 , respectively; $p < 0.05$). Cases 4 and 5 showed decreases of $\geq 25\%$ in pruritus scores at week 4 compared with those at week 0.

In this trial, we were successful in maintaining allergic dermatitis under control using weekly bathing without antibiotics. Four weeks after the trial, the pruritus scores and MRS colonization on the skin surface decreased. Previous studies showed that transient infectious as well as resident non-infectious *Staphylococcus* spp. were present in the skin of atopic canines^{6,11,14}. While aggressive antibiotic therapy can be useful for the elimination of pathogenic *Staphylococcus* spp. from skin lesions, long term antibiotic use are likely to increase the risk for disruption of the skin and other organ-resident floras¹⁴. In addition, the risk of both pathogenic and non-pathogenic *Staphylococcus* spp. to acquire antibiotic resistance

genes are increased with this approach^{1-3,6,10,14}, as demonstrated by the colonization of healthy dogs by *mecA*-positive *S. pseudintermedius*¹⁰. In this study, *S. epidermis*, a typically non-pathogenic spp. that was isolated from case 3 at week 4 was found to have *mecA*. The roles of *S. epidermis* as a reservoir and transmitter of linezolid resistance genes were previously described². Restoration of commensal skin flora and prevention of drug resistance are considered as logical goals to control allergies in dogs¹⁴. Thus, weekly bathing following clinical cure with antibiotics should be useful in preventing the risk for development of multidrug resistance in not only skin microflora but also in other organs.

In the present study, we performed weekly bathing only for enrolled dogs that were being bathed two to four times a month by their owners before the trial. The environment and housekeeping conditions differed between the dogs. For example, the elderly owners may have had trouble in efficiently bathing their dogs, and one major reason for the success of this trial could be because of the experienced VTs who

Table 2. Summary of *Staphylococcus* spp. and their *mecA* status in six dogs enrolled in the trial

Case No.	Body sites where swab samples were collected	At week 0			At week 4		
		The results of the culture	The results of <i>mecA</i>	Isolated <i>Staphylococcus</i> spp. by PCR	The results of the culture	The results of <i>mecA</i>	Isolated <i>Staphylococcus</i> spp. by PCR
1	Right axillae	Positive	Positive	<i>S. pseudintermedius</i>	Positive	Positive	<i>S. pseudintermedius</i>
	Ventral abdomen	Negative			Negative		
	Right feet	Negative			Negative		
2	Right axillae	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
	Right ear	Positive	Positive	<i>S. pseudintermedius</i>	Positive	Positive	<i>S. pseudintermedius</i>
	Dorsal trunk	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
3	Left Axillae	Negative			Negative		
	Dorsal trunk	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
	Ventral abdomen	Negative			Positive	Positive	<i>S. epidermidis</i>
4	Muzzle	Positive	Positive	<i>S. pseudintermedius</i>	Positive	Positive	<i>S. pseudintermedius</i>
	Right Axillae	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
	Ventral abdomen	Negative			Negative		
5	Right Interdigit	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
	Dorsal trunk	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
	Ventral abdomen	Positive	Positive	<i>S. aureus</i>	Positive	Positive	<i>S. pseudintermedius</i>
6	Right Interdigit	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
	Muzzle	Positive	Positive	<i>S. pseudintermedius</i>	Negative		
	Right Axillae	Negative			Negative		

bathed the dogs in not a specific but a certain manner. Furthermore, worsening condition of the ventral abdominal lesions and increased CADESI-03 score were observed in case 3 at week 4. The ventral abdominal skin is comparatively thin in the breed phenotype of case 3. Therefore, it may be preferable to tailor the frequency of bathing in each dog based on breed characteristics to avoid excessive washing.

A limitation of the present trial was treatment necessity, and it was not possible to obtain permission from the owners for their animals to be bathed with only tap water as a control group participating in this trial. Further clinical trials with this control group are needed based on this pilot study. In conclusion, we propose weekly bathing as useful in controlling canine allergic dermatitis by preventing recurrent MRS skin infections.

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References

- 1) Beck, K. M., Waisglass, S. E., Dick, H. L. and Weese, J. S. 2012. Prevalence of meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) from skin and carriage sites of dogs after treatment of their meticillin-resistant or meticillin-sensitive staphylococcal pyoderma. *Vet. Dermatol.*, **23**: 369–375, e66–67.
- 2) Cafini, F., Nguyen le T. T., Higashide, M., Román, F., Prieto, J. and Morikawa, K. 2016. Horizontal gene transmission of the *cfr* gene to MRSA and *Enterococcus*: role of *Staphylococcus epidermidis* as a reservoir and alternative pathway for the spread of linezolid resistance. *J. Antimicrob. Chemother.*, **71**: 587–592.
- 3) Davis, J. A., Jackson, C. R., Fedorka-Cray, P.

- J., Barrett, J. B., Brousse, J. H., Gustafson, J. and Kucher, M. 2014. Carriage of methicillin-resistant staphylococci by healthy companion animals in the US. *Let. Appl. Microbiol.*, **59**: 1-8.
- 4) Hanssen, A. M., Kjeldsen, G., Sollid, J. U. 2004. Local variants of Staphylococcal cassette chromosome mec in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative Staphylococci: evidence of horizontal gene transfer? *Antimicrob. Agents Chemother.*, **48**: 285-296.
 - 5) Kawarai, S., Ishihara, J., Masuda, K., Yasuda, N., Ohmori, K., Sakaguchi, M., Asami, Y. and Tsujimoto, H. 2010. Clinical efficacy of a novel elimination diet composed of a mixture of amino acids and potatoes in dogs with non-seasonal pruritic dermatitis. *J. Vet. Med. Sci.*, **72**: 1413-1421.
 - 6) Lloyd, D. 2013. Chapter 4, Bacterial skin diseases. In: *Muller and Kirk's small animal dermatology*, 7 th ed., pp. 184-186, Miller, W. H., Jr, Griffin, C. E. and Campbell, K. L., eds., Elsevier Mosby, St. Louis, Missouri.
 - 7) McEwan, N. A., Mellor, D. and Kalna, G. 2006. Adherence by *Staphylococcus intermedius* to canine corneocytes: a preliminary study comparing noninflamed and inflamed atopic canine skin. *Vet. Dermatol.*, **17**: 151-154.
 - 8) Olivry, T., Marsella, R., Iwasaki, T. and Mueller, R. 2007. International task force on canine atopic dermatitis. Validation of CADESI-03, a severity scale for clinical trials enrolling dogs with atopic dermatitis. *Vet. Dermatol.*, **18**: 78-86.
 - 9) Olivry, T., DeBoer, D. J., Favrot, C., Jackson, H. A., Mueller, R. S., Nuttall, T. and Prélaud, P. 2010. International task force on canine atopic dermatitis. Treatment of canine atopic dermatitis: clinical practice guidelines from the international task force on canine atopic dermatitis. *Vet. Dermatol.*, **21**: 233-248.
 - 10) Priyantha, R., Gaunt, M. C. and Rubin, J. E. 2016. Antimicrobial susceptibility of *Staphylococcus pseudintermedius* colonizing healthy dogs in Saskatoon, Canada. *Can. Vet. J.*, **57**: 65-69.
 - 11) Rodrigues Hoffmann, A., Patterson, A. P., Diesel, A., Lawhon, S. D., Ly, H. J., Elkins Stephenson, C., Mansell, J., Steiner, J. M., Dowd, S. E., Olivry, T. and Suchodolski, J. S. 2014. The skin microbiome in healthy and allergic dogs. *PLoS. One*, **9**: e83197.
 - 12) Sasaki, T., Kikuchi, K., Tanaka, Y., Takahashi, N., Kamata, S. and Hiramatsu, K. 2007. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. *J. Clin. Microbiol.*, **45**: 1118-1125.
 - 13) Simou, C., Thoday, K. L., Forsythe, P. J. and Hill, P. B. 2005. Adherence of *Staphylococcus intermedius* to corneocytes of healthy and atopic dogs: effect of pyoderma, pruritus score, treatment and gender. *Vet. Dermatol.*, **16**: 385-391.
 - 14) Weese, J. S. 2013. The canine and feline skin microbiome in health and disease. *Vet. Dermatol.*, **24**: 137-145.