

Use of *Staphylococcus aureus* Phage Lysate Staphage Lysate (SPL)[®] for the Control of Recurrent Pyoderma Eczema in Dogs with Atopic Dermatitis

Suzana Evelyn Bahr Solomon¹, Marconi Rodrigues de Farias² & Claudia Turra Pimpão²

ABSTRACT

Background: Recurrent staphylococcal infections are frequent in dogs with atopic dermatitis (AD). Many factors seem to contribute to making bacterial pyoderma refractory to treatment. Short-term systemic antibiotic therapy is effective for the treatment of acute symptoms, and may, along with pulsatile therapy, contribute to the long-term control of the disease. However, microbial resistance has become a growing and alarming problem. The aim of this study was to evaluate whether the use of *Staphylococcus aureus* Phage Lysate Staphage Lysate (SPL)[®], can minimize the symptoms of recurrent pyoderma and increase the interval between acute atopic manifestations in dogs.

Materials, Methods & Results: Thirteen dogs with a history of Canine Atopic Dermatitis (CAD) and recurrent bacterial pyoderma received SPL at increasing intervals for 23 weeks. The contents of an intact pustule of each dog was collected and submitted to microbiological analysis. Systemic antibiotic therapy was established for the first 4-6 weeks of SPL protocol, based on the antibiotic sensitivity tests. The animals included in the study underwent a therapeutic protocol receiving shots of 0.5 mL of SPL subcutaneously (SC) twice a week for the first 12 weeks; 1.0 mL of SPL (SC) once a week for four weeks; 1.0 mL of SPL (SC) once every 15 days; 1.0 mL of SPL (SC) after a three-week interval from the last dose on week 20, until final observation at week 26, with no application. The animals underwent clinical examination every week and the evaluation of pruritus was used according Rybnicek *et al.* During the therapeutic protocol with SPL, a significant decline in the pruritus was observed in the treated dogs ($P < 0.05$). In week 1, the mean pruritus index was 7.33 on the Rybnicek scale; in weeks 12 and 23, the mean indices were 2.41 and 1.91. An effectiveness of 83.33% for the control of pruritus along with regression of the lesions was observed.

Discussion: Before treatment, the selected animals presented worsening of the pruritus during the pyoderma eczema episodes (pruritic), resulting in the emergence of a vicious cycle where the pruritus induced the appearance of new lesions, requiring the use of antibiotics for a long period. During the therapeutic protocol with SPL, a significant decline in the pruritus was observed in the treated dogs. The control of pruritus associated with pyoderma eczema of the dogs in this study before the vaccination protocol with SPL was satisfactory when they were subjected to antibiotic therapy; however, after suspending therapy, the bacterial infections recurred, on average, after 2-4 weeks. On the other hand, with the use of SPL, the animals were recurrence-free until the end of the experimental protocol. This was attributed to the antibiotic therapy administered at the beginning of the protocol, as this led to a regression of the bacterial pyoderma and involution of the lesions. However, after suspending antibiotics, it was observed that, by the end of the study, 83.33% of the dogs still had a low level of pruritus, few or no lesions, which were considered acceptable to most owners. At this moment none of these patients needed to be subjected to antibiotic treatment. The sums of the scores for the dogs on weeks 1, 12, and 23 were 53.33, 4.41, and 3.5, respectively, indicating significant improvements of the lesions, showing that the proposed protocol with SPL was able to prevent new episodes of pyoderma.

Keywords: atopic dermatitis, keratinocytes, pyoderma, bacterin.

INTRODUCTION

CAD is an inflammatory dermatopathy which occurs frequently in dogs. The epidermis tends to colonize and adhere to staphylococcal bacteria more frequently when compared to healthy dogs [7,17,29]. An antimicrobial immune response deficit has been reported, thus enabling bacterial proliferation [21] and recurrent pyoderma [7,22,28,29].

Staphylococcal infections correlate with the clinical signs of CAD. They play a role in the pathogenesis of this dermatopathy by perpetuating the cutaneous inflammatory response. The treatment of recurrent pyoderma seems to be associated with a partial or almost entire reduction of pruritus in these cases [5,26].

Acute episodes of bacterial pyoderma can be treated with topical or systemic antibiotic therapy [5]; however, when the symptoms recur or cannot be treated by other means, prophylactic antibiotics may represent an effective alternative [22], although this represents a risk for the development of a resistant infection [14].

The use of bacterins has been previously studied, mainly for the control of recurrent idiopathic superficial pyoderma [10]. SPL is a bacterin obtained from *Staphylococcus aureus*, which enhances the cellular and humoral responses against staphylococcal [6]. The aim of this study was to assess whether the use of SPL is effective in the control of recurrent staphylococcal infections in dogs with AD, as a means to reduce the frequency of pyoderma episodes, lessen the intensity of the lesions and pruritus, and consequently decrease the frequency at which antibiotics are used in these animals.

MATERIALS AND METHODS

Animals

A longitudinal, prospective, randomized study was conducted with 13 dogs, diagnosed with recurrent pyoderma secondary to AD at the Dermatology and Allergology Veterinary Service of the Veterinary Animal Hospital at PUCPR. The study dogs were all over one year of age, of both genders, pure or mixed breeds, with atopic dermatitis and a history of recurrent bacterial pyoderma, whose pruritus had improved by approximately 80% or more after an isolated antimicrobial therapeutic protocol, without the use of concomitant systemic or topical corticosteroid therapy. The average time to recurrence of pyoderma episodes

was 2-4 weeks, after suspension of antibiotic therapy for at least 21 days.

The diagnosis of AD was established by observing the presence of intense, chronic, primary pruritus responsive to corticosteroids in the distal portions of the limbs, abdomen, axillae, and ears, in which the clinical signs did not respond to the exclusion of allergens present in the saliva of arthropods or after dietary restriction with original protein for 6-10 weeks.

Culture and antibiotic sensitivity tests

For each dog, the contents of an intact pustule was collected with a sterile swab and subsequently submitted to microbiological analysis. The samples were inoculated in sheep blood agar (5%) and MacConkey agar and subjected to enzyme tests (catalase, coagulase, and oxidase), biochemical tests (mannitol fermentation), production of smears to investigate the staining characteristics, and isolation of the pathologic agent through inoculation in Brain Heart Infusion medium for susceptibility testing. After identifying the responsible agent, an antibiotic sensitivity test was produced to determine the sensitivity profile of the isolated agent against the following antimicrobials: amoxicillin and clavulanate, cefotaxime, cefovecin, clindamycin, marbofloxacin, ciprofloxacin, enrofloxacin, azithromycin, and oxacillin.

SPL protocol

The protocol was performed with *Staphylococcus aureus* Phage Lysate Staphage Lysate (SPL)[®]¹. The animals included in the study underwent the following therapeutic protocol: 1st phase (from week 1 to 12): 0.5 mL of SPL subcutaneously (SC) twice a week for the first 12 weeks; 2nd phase (from week 13 to 16): 1.0 mL of SPL (SC) once a week for 4 weeks; 3rd phase (weeks 18 and 20): 1.0 mL of SPL (SC) once every 15 days; 4th phase (week 23): 1.0 mL of SPL (SC) after a 3-week interval from the last dose on week 20; 5th phase (week 26): no application.

Systemic antibiotic therapy was established for the first 4-6 weeks of the SPL protocol, based on the antibiotic sensitivity tests.

Scores assessments

The animals underwent clinical examination every week and their owners were questioned regarding the pruritus and overall health of the pet. Subsequently, they were instructed to check a value

in the standardized pruritus score chart described by Rybnicek *et al.* [27].

Dermatological lesions (papules, pustules, crusts, erythema, and epidermal collarettes) were evaluated weekly, and a numerical lesion scale (modified from Carlotti *et al.* [3]) was used on weeks 1, 12, and 23, based on the dermatological evaluations. The total score of each examined week was obtained by adding up the individual scores of each lesion in each specific location (head, ears, chest, axillae, abdomen, perineum, limbs, and paws), ranging from 0-3, with 0 = no lesion; 1 = a few lesions; 2 = many separate lesions; 3 = many confluent lesions.

Statistical analysis

Fisher's exact test was used to verify the effectiveness of the treatment. The Kruskal–Wallis test was used for the statistical analysis of the nonparametric data, followed by Dunn's test for comparisons of the means. The significance level was set as 5% ($\alpha = 0.05$), with $P \leq 0.05$ considered statistically significant. All calculations were performed using the Statistical Software GraphPad Prism version 3.00 for Windows, (San Diego, CA, USA).

RESULTS

A total of 13 dogs with recurrent pyoderma secondary to atopic dermatitis were included in the experiment. Of these, there were 3 males and 10 females. The mean age was 5 years, and the breeds included were 3 Dachshunds, 2 Golden Retrievers, 2 Yorkshire terriers, 1 Poodle, 1 Maltese, 1 Lhasa Apso,

1 Shih Tzu, 1 English Bulldog, and 1 NBD. One dog (number 8) left the treatment before the end of protocol, all statistic data refers 12 dogs (except culture and antibiotic sensitivity tests).

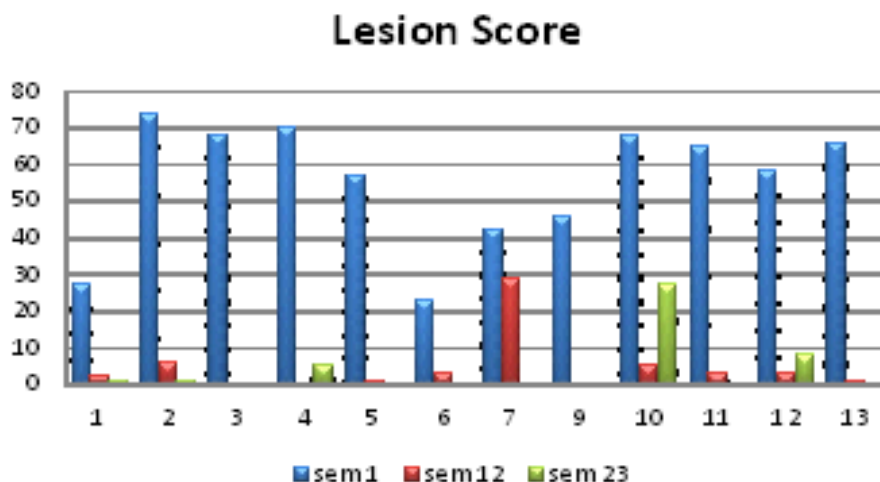
Among the bacteria isolated from the dogs, 5 of 13 dogs presented a positive culture for *Staphylococcus aureus*, and 8 of 13 dogs presented a positive culture for *Staphylococcus pseudintermedius*.

About the *S. aureus* strains, 20% were resistant to amoxicillin and clavulanate, 40 % to cefovecin, cefotaxime, ciprofloxacin, clindamycin, azithromycin, marbofloxacin and oxacillin and 60% to enrofloxacin. Two strains (40%) presented multi-resistant characteristics.

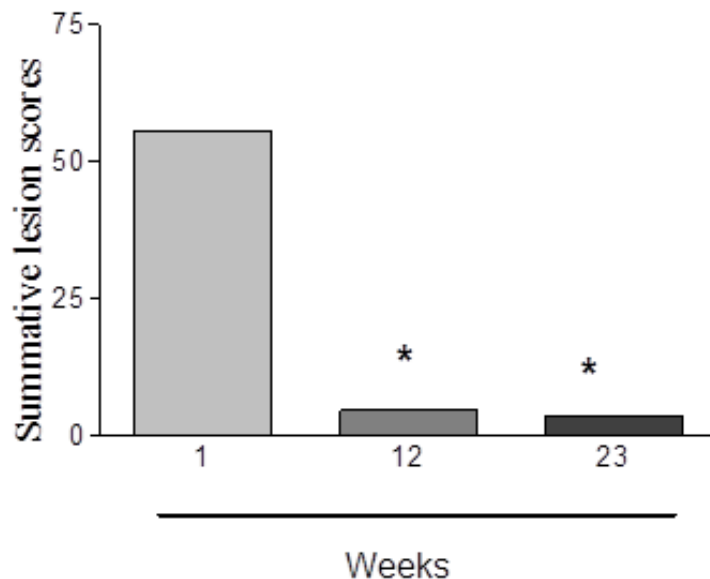
For *S. pseudintermedius* strains, 12,5 % were resistant to amoxicillin and clavulanate, 20% to clindamycin, azithromycin, marbofloxacin and oxacillin, 37,5% to cefovecin, cefotaxime, enrofloxacin and ciprofloxacin. Two strains (25%) presented multi-resistant characteristics.

The individual lesion scores obtained in the evaluations from weeks 1, 12, and 23 are shown in Graph 1. The sums of the scores for the dogs on weeks 1, 12, and 23 were 53.33, 4.41, and 3.5, respectively, indicating significant improvements of the lesions ($P < 0.001$), as shown in Graph 2.

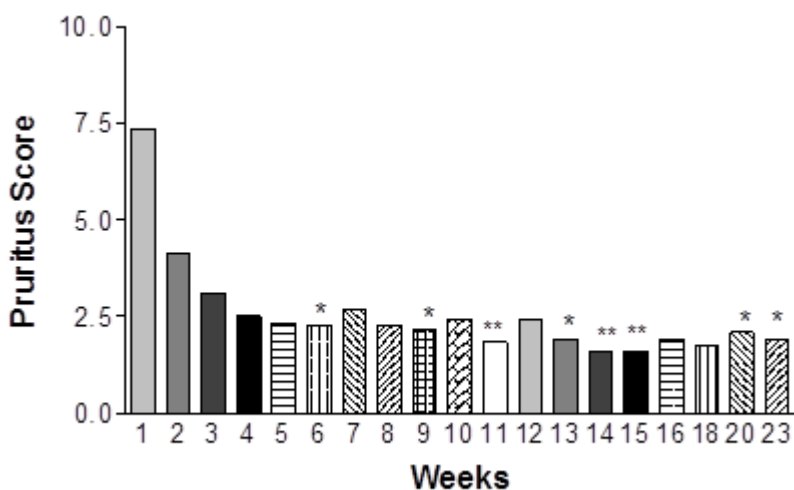
During the therapeutic protocol with SPL, a significant decline in the pruritus was observed in the treated dogs ($P < 0.05$). In week 1, the mean pruritus index was 7.33 on the Rybnicek scale [27]; in weeks 12 and 23, the mean indices were 2.41 and 1.91, respectively, as shown in Graph 3.



Graph 1. Individual lesion scores of the dogs on weeks 1, 12, and 23.



Graph 2. Summative lesion scores of the dogs on weeks 1, 12, and 23 (Dunn's test). * $P < 0.001$ compared to the first week.



Graph 3. Individual pruritus scores of the dogs during weeks 1 through 23 (Kruskal-Wallis). * $P < 0.05$ compared to the first week; ** $P < 0.001$ compared to the first week.

DISCUSSION

Fitzgerald [9] reported that *S. pseudintermedius* seem to show specificity for the corneocytes in dogs. In fact, several authors have described that this is the most isolated agent from the skin of dogs with bacterial pyoderma [1-4,29]. *S. aureus*, on the other hand, resides on the skin and mucous membranes of humans and has been commonly isolated from atopic patients [5,16,19], while its presence in dogs has been reported to occur mainly secondary to certain risk factors, such as chronic or recurrent infections

[14,20], as was the case in this study. However, in Brazil, Penna *et al.* [23] isolated *S. aureus* in only 12.5% of dogs with bacterial pyoderma, and in another similar study, the species was not isolated in any patient [24].

Resistance to enrofloxacin, a broad-spectrum veterinary antibiotic that is considered to be a second-line drug, coincides with the data obtained by Prescott *et al.* [25]. According to their study, the increased resistance to this and other antimicrobials can be attributed to their indiscriminate use in veterinary hospitals and private

clinics. Moreover, four dogs from this study (30.77%) showed multidrug resistance. According to Fitzgerald [9], multidrug resistance can be determined through the phenotypic data obtained from the antibiotic sensitivity tests, that is, when there is resistance to beta-lactam antibiotics along with resistance associated with the other three classes of antibiotics. In recent years, this phenomenon has been widely discussed and studied, not only due to its increasing prevalence in the veterinary field, but also due to the emerging growth of infections by multidrug-resistant strains of *S. aureus* and *S. pseudintermedius* [11].

Before treatment with SPL, the selected animals presented worsening of the pruritus during the pyoderma eczema episodes (pruritic), resulting in the emergence of a vicious cycle where the pruritus induced the appearance of new lesions. The improvement of the lesions in current study could be clearly correlated with control of pruritus. This findings are close to humans, which *S. aureus* colonization has a correlation with the severity of atopic dermatitis in chronic lesional patients and the treatment with antibiotics has a positive effect. Furthermore, trans-epidermal penetration of staphylococcal antigens was well demonstrated [5]. Several studies have demonstrated that *S. aureus* isolates from AD patients produce superantigens, the majority inducing production of superantigen-specific immunoglobulin E [7]. Staphylococcal activity could also cause enhance damage to the epidermal barrier. Ceramidase from *S. aureus* can cause hydrolysis of ceramides in atopic skin whilst IL-4 from T helper type 2 (TH2) cells has been shown to suppress ceramide production in epidermal sheets and induce synthesis of fibronectin, an adherence target of staphylococci [13]. It was also demonstrated that adherence of *S. pseudintermedius* to the corneocytes of atopic dogs is significantly greater than to those of healthy dogs [29]. According to Simou *et al.*, atopic dogs with a low degree of pruritus are similar to healthy dogs in terms of their staphylococcal adherence receptor expression, whereas more severe pruritus is associated with upregulation and results in an increase in adherence of *S. pseudintermedius* to canine corneocytes. Studies have been suggested that staphylococcal components could serve as allergens in dogs and stimulate an IgE response against these bacteria. Thus, atopic dogs are more likely to suffer flares when presented pyoderma [8].

The control of pruritus associated with pyoderma eczema of the dogs in this study before the vaccination protocol with SPL was satisfactory when they were subjected to antibiotic therapy; however, after suspending therapy, the bacterial infections recurred, on

average, after 2-4 weeks. On the other hand, with the use of SPL, the animals were recurrence-free until the end of the experimental protocol. Initially, at the beginning of the protocol, the improvement was attributed to the antibiotic therapy, as this led to a regression of the bacterial pyoderma and involution of the lesions. However, after suspending antibiotics, it was observed that, by the end of the study, 83.33% of the dogs still had a low level of pruritus, which was considered acceptable to most owners. Deboer *et al.* [5] subjected 13 dogs with recurrent idiopathic superficial pyoderma to therapy with SPL for 18 weeks and examined the lesions by the end of the experiment, after withdrawing treatment, as in this study. According to the author, the control of bacterial pyoderma was linked to a series of effects of SPL on the immune system, including increased cellular and humoral immunity, specifically against staphylococcus antigens by serum immunoglobulin E and G. In addition, SPL stimulates immunocompetent cells to produce tumor necrosis factor-alpha, interferon-gamma, and interleukin-1 and -2 cytokines, and increases bacterial adhesion, thereby enabling phagocytosis by macrophages and neutralization of toxic proteins [10,18], increasing the response by T-lymphocyte suppressors, and reducing bacterial hypersensitivity [12]. These effects are consistent with the findings regarding pruritus attenuation for the animals in this study. It is possible that these effects occur due to SPL promoting desensitization to bacterial antigens in allergic animals [18].

CONCLUSION

The use of SPL was able to significantly minimize pruritus and decrease cutaneous reactivity and the rate of recurrent tegumentary staphylococcal infections in dogs with atopic dermatitis. Thus, it appears to be a safe and effective option for the control of bacterial pyoderma secondary to atopic dermatitis in the long-term.

MANUFACTURERS

¹Delmont Laboratories Inc. Swarthmore, PA, USA.

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Ethical approval. This study was approved by the Ethics Committee on Animal Use for experimental protocols at the Pontifical Catholic University of Paraná, under registration number 461.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content of the paper.

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