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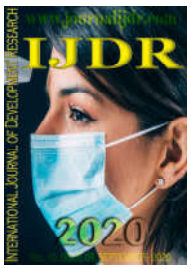

RESEARCH ARTICLE OPEN ACCESS

DEFENSIN GENE EXPRESSION LEVELS (CBD1 AND CBD103) IN SKIN SCRAPINGS OF THE POODLE DOGS WITH AND WITHOUT DERMATITIS

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ABSTRACT

Defensins represent an important group of antimicrobial peptides and are expressed in the skin and other tissues of various mammalian species. These peptides participate in innate immunity, aiding in the immune response. The objective of this work was to compare the levels of β-defensin gene expressions (cBD1 and cBD103) in skin scrapings of the Poodle dogs with and without dermatitis. Eight dogs with dermatitis and 13 healthy dogs from the Veterinary Hospital and privately owned had collected skin scrapings. RNA extraction was obtained by Trizol® reagent. mRNA expression levels were estimated by RT-PCR using the SYBR® Green One-step Kit on the CFX96 Touch ™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Data were compared by 5% Student's t-test. Relative cBD1 and cBD103 genes expressions were significantly higher in dogs with dermatitis ($P<0.05$). Dogs with dermatitis showed higher levels of defensin expression, suggesting a possible immune response in the skin of these animals.

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INTRODUCTION

Antimicrobial peptides (PAMs) are molecules with a variable number of amino acids that act to protect the host against various types of microorganisms, such as Gram-positive and Gram-negative bacteria, fungi, viruses and protozoa (HANCOCK, SCOTT, 2000; REDDY *et al*., 2004; MARR *et al*., 2006). The main mechanism of action of most PAMs is through rupture of bacterial membranes or by translocation of the peptide into the cell, acting on intracellular targets (HANCOCK, SAHL, 2006). Defensins are part of the innate immune system, being one of the main secreted PAMs in mammalian skin cells (HAZLETT, WU, 2011). The protein structure of these molecules is in β-leaf form, with three disulfide bonds and formed by six cysteine residues (YANG *et al*., 2002). Defensins are classified into three classes according to their structural characteristics: α-defensin, β-defensin and θ-defensin (YANG *et al*., 2002). α-defensins are made up of 29 to 35 amino acids, β-defensins have 38 to 42 amino acids and θ-defensins are the smallest defensins, with only 18 amino acids (YANG *et al*., 2002). α-defensins are secreted by neutrophils and gut cells, β-defensins secreted into the conjunctive membrane, skin, oral mucosa, urogenital and respiratory systems (YANG *et al*., 2002). While θ-defensins are found only in nonhuman primates (SELSTED, OUELLETTE, 2005). Bacterial resistance has motivated the development of new antibiotics, which has encouraged much research on new strategies to combat pathogenic microorganisms through the therapeutic potential of MAPs (HANEY *et al*., 2017). According to Afshar and Gallo (2013), several functions of MAPs such as antimicrobial activity and immune stimulators suggest that they may be promising therapeutic agents. Many defensins have now been studied on the skin of dogs with dermatological problems to determine if there is a stimulation in the production of these defensins during inflammatory and infectious processes (VAN DAMME *et al*., 2009; SANTORO *et al*., 2011; LANCTO *et al*., 2013). Atopic Dermatitis (AD) is an inflammatory skin disease, in which the main symptom is pruritus followed by erythema,

scaling and alopecia (HALLIWELL, 2006). The disease can affect several species of horses (WHITE, 2005), felines (MARSELLA, BENEDETTO, 2017) and dogs (BIZIKOVA *et al*., 2015), including humans (SILVESTRE-SALVADOR *et al*., 2017). The prevalence of AD can occur up to 15% in the general dog population (HILLIER, GRIFFIN, 2001). The expression of defensins has been widely investigated in dogs with atopic dermatitis compared to healthy dogs (VAN DAMME *et al*., 2009; LANCTO *et al*., 2013; SANTORO *et al*., 2013). However, the results of the studies are still divergent as to the defensing expression profiles. The use of a canine animal model allows a more efficient control of environmental and intrinsic variables (SANTORO *et al*., 2013). In addition, poodle dogs have a considerable predisposition to inflammatory skin processes such as sebaceous adenitis (PEDERSEN *et al*., 2012). In order to improvethe understanding, the effects of antimicrobial peptides on dogs' skin immunity, this study aimed to estimate the levels of β-defensin gene expression in skin scrapings of Poodle dogs with and without dermatitis.

MATERIALS AND METHODS

Ethical aspects: All owners signed a consent form authorizing the dogs' skin scraping. This study was developed following the welfare standards of the National Animal Experimentation Control Commission (CONCEA) and was approved by the Animal Ethics Commission from Federal Rural University of Amazon under 049/2015 protocol number.

Inclusion and exclusion standards: Dogs presenting skin lesions and clinical (pruritus and alopecia) characteristics of dermatitis were evaluated by anamnesis, physical examination and laboratory tests through skin scrapings, fungal culture and blood counts. Clinically healthy dogs with no history or presence of dermatological disease were included in the control group. The dogs used in this study were not on glucocorticoids and systemic antibiotics for at least one month. According to the owners only topical and systemic antiparasitics were used in some dogs with injured skin.

Dog samples: The dogs with injured skin were between 4 and 14 years old, four males and four females, uncastrated and with diet based on rations and natural food. Healthy dogs (normal skin) were aged from five months to eight years, 11 males and two females, uncastrated and on a diet based on rations and natural food.

Blood sampling and RNA extraction: The samples were collected at the veterinary hospital and some animal households. Skin scraping was performed using scalpel slides and added into 1.5 mL tubes containing RNAlater. Then, the samples were kept under low refrigeration at -80° C until RNA extraction. Total RNA extraction was performed using Trizol® reagent (Life Technologies Corporations, Carlsbad, CA, USA), adapted from the original method described by Chomczynski and Sacchi (1987), using a guanidine phenol and isothiocyanate solution employing 1 mL of Trizol for every 1 g of sample. Then, RNA concentrations were quantified using NanoDrop ND-1000 spectrophotometer (Agilent, Santa Clara, CA, USA). It was also possible to identify the purity of RNA by the A260/A280 nm absorbance ratio.

Primers and RT-PCR conditions: The primers used were cBD103 (Gene ID: 100170103), cBD1 (Gene ID: 611241) and GAPDH (Gene ID: 403755) as endogenous gene, all at 60 °C. The primers (Table 1) were developed with their gene sequences by the PRIMER3 program.

Table 1. Genes and primer sequences for RT-PCR

Genes	Primer sequences (5´-3´)	Sizes (base pairs)
cBD103	Forward:GCCCTTGCTGTTCTTGATGC Reverse: GCATTTTCGGCCAGTGGAAG	157
cBD1	Forward: TGCTGGCTTCCTTACGGG Reverse: TAACAGGTGCCATCGATCCT	118
GAPDH	Forward: GGGCCAAGAGGGTCATCATC Reverse: GATGCCGAAGTGGTCATGGA	167

cBD103= Canis Beta-Defensin 103; cBD1= Canis Beta-Defensin 1; GAPDH= Glyceraldehyde-3-Phosphate Dehydrogenase.

mRNA expression levels were estimated through triplicate using the SYBR® Green One-step RNA-to-Ct Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's recommendations at a final volume of 10 µl and analyzed on the CFX96 TouchTM. Real-Time Detection System (Bio-Rad, Hercules, CA, USA). The Ct values were obtained based on the threshold line that determines the end of the RT-PCR exponential curve and the expressions of each gene were determined by the $2^{-\Delta Ct}$ method, where the ΔCt corresponds to the difference between the Ct of the target gene and Ct of the endogenous gene.

Statistical analysis: Expression profiles were tested for normality by the Kolmogorov-Smirnov test. Then, Student's "t" test was applied to differentiate expression levels between animals with and without dermatitis through the SAS (University Edition) program. The significance level was $p < 0.05$.

RESULTS

The two evaluated defensin genes were expressed in the skins of all analyzed animals. The β-defensin1 gene (cBD1) has low expression (below 1.0) in animals with normal skin and moderate to high expression (from 1.0 to 1.2) in animals with lesioned skin. Therefore, this difference being very significant ($p < 0.05$) (Figure 1). The β-defensin 103 gene (cBD103) has already shown low expression in all animals (below 1.0). However, the expression of the same gene for animals with lesions were higher than animals without lesions ($p < 0.05$) (Figure 1).

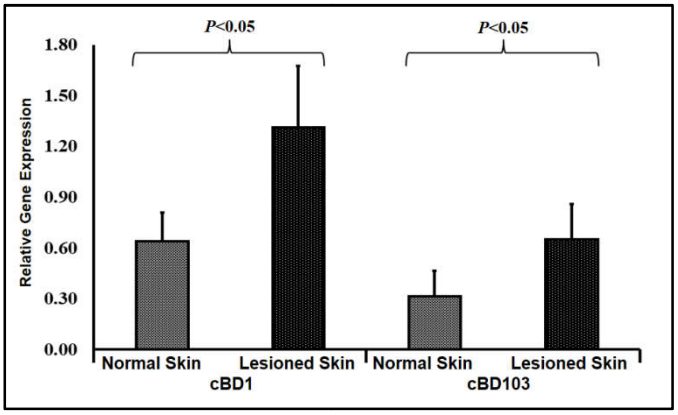

Figure 1. Profiles of the defensin gene expressions in dog skin of animals with or without lesions

DISCUSSION

β-defensins are considerably expressed in the skin of humans and various animal species such as dogs (HARDER *et al*., 2001; SANG *et al*., 2006; WINGATE *et al*., 2008). In this research we can observe the expression of cBD1 and cBD103 mRNA in the skin of dogs with atopic dermatitis and healthy dogs. Dogs with atopic dermatitis had higher levels of cBD1 and cBD103 defensin expression in the skin compared to healthy dogs. These results are in agreement with findings in the literature (VAN DAMME *et al*., 2009; SANTORO *et al*., 2011; SANTORO *et al*., 2013), which also evaluated mRNA expression of some defensins (cBD1, cBD2, cBD3 and cBD103) in healthy atopic skin of dogs. However, Mullin *et al*. (2013) who described mRNA expression for cBD1, cBD103 and TLR2 in infected and uninfected atopic skin samples, and also in healthy dog skin, but didn´t find no significant differences in the level of transcription of these defensins between healthy atopic dogs. CBD103 is the most widely expressed β-defensin in dog skin (WINGATE *et al*., 2008; LEONARD *et al*., 2012). However, in our study we observed a higher expression of cBD1 in dogs' skin compared to cBD103. Corroborating the results of van Damme *et al*. (2009), who reported a significant increase of more than ten fold in the expression of cBD1 detected in lesioned skin of atopic dogs compared to normal skin, while normal skin showed an increase of five fold compared to healthy skin. The interesting is that the animals analyzed by Damme *et al*. (2009) presented low cBD103 gene expression, contrary our findings, where the two defensins analyzed (cBD1 and cBD103) were most expressed on lesioned than health skin dogs. A research from Santoro *et al*. (2013) described a significant expression only to cBD103 on atopic skin than health skin of dogs. These differences may be some defensins are involved in determinate immunologic challenger as well as their expressions should vary depending of local and type of collecting, as related by Lancto *et al*. (2013) and Santoro (2018).

Some works relate that PAM expressions in dog and human skin presente divergent results about defensin expression. Some authors (GAMBICHLER *et al*., 2008; MULLIN *et al*., 2013; VAN DAMME *et al*., 2013; LEONARD *et al*., 2018) relate that may exist a tendency to reduce these PAMs on atopic skin, due to alteration on cutaneous barrier, what should increase the susceptibility to secondary bacterial infections. However, Lancto *et al*. (2013) did not observe significant differences for cBD1 and cBD103 expressions between animals with atopic dermatitis or other inflammatory conditions as sebaceous adenitis and suggest that reduction of defensin gene expressions is not a specific characteristic of atopic dermatitis. Some hypothesis from Santoro *et al*. (2013) based in previous studies (LAI, GALLO, 2009; AHRENS *et al*., 2011; SCHITTEK, 2011) to explain the alterations in the defensin production between atopic and health skin, they suggest that may occur fluency of immunologic environment local, cutaneous barrier alterations, degradations of proteins and presence of inflammatory cells. The cytokines may be involved with regulation of defensin expression on human and dog skins with atopic dermatitis (VAN HOWELL *et al*., 2006; DAMME *et al*., 2009). According to some works (MAEDA *et al*., 2002; NUTTALL *et al*., 2002), the level of TNF-α should be elevated on lesioned dog skin com atopic dermatitis, positively regulating the cBD1 expression. cBD1 was the defensin most expressed, but we did not analyze TNF-α expression to associate them. All animals in this research are Poodle breed, but others breeds as Golden retriever and Labrador present a polymorphism in cBD103 gene, that is responsible for hair dark coloration (CANDILLE *et al*., 2007; LEONARD *et al*., 2012). The role of defensin in atopic dermatitis and others dog skin infections is not yet fully understood, and need a deep analyze of these peptides, considering some variables as species, breed, sex, aging, alimentation, environment conditions, immunological state and recurrent infections. In conclusion, our study demonstrated differences on canine defensin gene expressions between atopic and health skin of dogs from Poodle breed, can suggest us that the lesioned skin could be producing a possible immunologic response through defensins. In other hands, there are several questions to understand about the skin immunology and defensin expressions and others skin inflammatory and infection processes.

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REFERENCES

Afshar, M, and Gallo, R.L., (2013). Innate immune defense system of the skin. Vet Dermatol. 24, pp.32-8.e8.0E9. doi:10.1111/j.1365-3164.2012.01082.x

Ahrens, K., Schunck, M., Podda, G.f., Meingassner, J., Stuetz, A., Schröder, J.M., Harder, J., and Proksch, E., (2011) Mechanical and metabolicinjury to the skin barrier leads to increased expression of murine β-defensin-1, -3, and -14. J Invest Dermatol. 131, pp. 443-52. doi:10.1038/jid.2010.289.

Bizikova, P., Santoro, D., Marsella, R., Nuttall, T., Eisenschenk, M.N., and Pucheu-Haston, C.M., (2015) Review: Clinicaland histological manifestations of can ineatopicdermatitis. Vet Dermatol. 26, pp. 79-e24. doi:10.1111/vde.12196

Candille, S.I., Kaelin, C.B., Cattanach, B.M., Yu, B., Thompson, D.A., Nix, M.A., Kerns, J.A., Schmutz, S.M., Millhauser, G.L., and Barsh, G.S., (2007) A -defensin mutation causes blackcoat color in domestic dogs. Science. 318, pp. 1418-1423. doi:10.1126/ science.1147880.

Gambichler, T., Skrygan, M., Tomi, N.S., Othlinghaus, N., Brockmeyer, N.H., Altmeyer, P., andKreuter,A., (2008) Differentialm RNA expression of antimicrobial peptides and proteins in atopicdermatitis as compared to psoriasisvulgar is and healthy skin. Int Arch Allergy Immunol. 147, pp. 17-24. doi:10.1159/000128582.

Halliwell, R., (2006) Revised nomenclature for veterinary allergy. Vet Immunol Immunopathol. 114, pp. 207-208. doi:10.1016/j.vetimm.2006.08.013.

Hancock, R.E., and Sahl, H.G., (2006). Antimicrobial and host-defense peptides as new anti-infective the rapeutic strategies. Nat Biotechnol. 24, pp. 1551-1557. doi:10.1038/nbt1267

Hancock, R.E., and Scott, M.G., (2009) The role of antimicrobial peptides in animal defenses. Proc Natl Acad Sci USA. 97, pp. 8856-8861. doi:10.1073/pnas.97.16. 8856.

Haney, E.F., Mansour, S.C., and Hancock, R.E., (2017) Antimicrobial Peptides: An Introduction. Methods Mol Biol. 1548, pp. 3-22. doi:10.1007/978-1-4939-6737-7_1

Harder, J., Bartels, J., Christophers, E., and Schroder, J.M., (2001) Isolation and characterization of human beta - defensin-3, a novel human inducible peptide antibiotic. J Biol Chem. 276, pp. 5707-5713. doi:10.1074/ jbc.M008557200.

Hazlett, L., and Wu, M., (2011) Defensins in innate immunity. Cell Tissue Res. 343, pp. 175-188. doi:10.1007/s00441-010-1022-4

Hillier, A., and Griffin, C.E., (2001) The ACVD task force on canine atopic dermatitis (I): incidence and prevalence. VetImmunol Immunopathol. 81, pp. 147-151. doi:10.1016/s0165-2427(01)00296-3.

Howell, M.D., Boguniewicz, M., Pastore, S., Novak, N., Bieber, T., Girolomoni, G., and Leung, D.Y., (2006) Mechanism of HBD-3 deficiency in atopic dermatitis. ClinImmunol. 121, pp. 332-338. doi:10.1016/ j.clim.2006.08.008.

Lai, Y., and Gallo, R.L., (2009). AMPed up immunity: howantimicrobialpeptideshavemultiple roles in immunedefense. TrendsImmunol. 30, pp. 131-141. doi:10.1016/j.it.2008.12.003.

Lancto, C.A., Torres, S.M., Hendrickson, J.A., Martins, K.V., and Rutherford, M.S., (2013) Altered expression of antimicrobial peptide genes in the skin of dogs with a topic dermatitis and other inflammatory skin conditions. Vet Dermatol. 24, pp. 414-e90. doi:10.1111/vde.12034.

Leonard, B.C., Marks, S.L., Outerbridge, C.A.,Affolter, V.K., Kananurak, A., Young, A., Moore, P.F., Bannasch, D.L., Bevins,C.L., (2012) Activity, expression and genetic variation of canine β-defensin 103: a multifunctional antimicrobial peptide in the skin of domesticdogs. J Innate Immun. 4, pp. 248-259. doi:10.1159/000334566..

Maeda, S., Fujiwara, S., Omori, K., Kawano, K., Kurata, K., Masuda, K., Ohno, K., andTsujimoto,H., (2002) Lesional expression of thymus and activation-regulated chemokine in canineatopicdermatitis. VetImmunol Immunopathol. 88, pp. 79-87. doi:10.1016/s0165-2427(02)00140-x.

Marr, A.K., Gooderham, W.J., andHancock, R.E., (2006) Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol. 6, pp. 468-472. doi:10.1016/j.coph.2006.04.006.

Marsella, R., and De Benedetto, A., (2017). Atopic Dermatitis in Animals and People: An Update and Comparative Review. VetSci. 4, pp. 37. doi:10.3390/vetsci4030037.

Mullin, J., Carter, S., Williams, N., Mcewan, N., and Nuttall,T., (2013) Transcription of caninetoll-like receptor 2, β-defensin 1 and β-defensin 103 in infected a topic skin, non-infectedatopic skin, healthy skin and the CPEK cellline. Vet Microbiol. 162, pp. 700-706. doi:10.1016/ j.vetmic.2012.09.017.

Nuttall, T.J., Knight, P.A., Mcaleese, S.M., Lamb, J.R., and Hill, P.B., (2002) T-helper 1, T-helper 2 and immunosuppressive cytokines in canine atopic dermatitis. VetImmunol Immunopathol. 87, pp. 379-384. doi:10.1016/s0165-2427(02)00076-4.

Pedersen, N.C., Liu, H., Mclaughlin, B.,andSacks,B.N., (2012)Geneticcharacterizationofhealthyandsebaceousadenitisaffected Standard Poodles fromthe United Statesandthe United Kingdom. TissueAntigens. 80, pp. 46-57. doi:10.1111/j.1399-0039.2012.01876.x.

Reddy, K.V., Yedery, R.D.,and Aranha, C., (2004) Antimicrobialpeptides: premisesandpromises. Int J AntimicrobAgents. 24, pp. 536-547. doi:10.1016/ j.ijantimicag.2004.09.005.

Sang, Y., Patil, A.A., Zhang, G., Ross, C.R.,andBlecha, F., (2006) Bioinformaticandexpressionanalysisof novel porcine beta-defensins. MammGenome. 17, pp. 332-339. doi:10.1007/s00335-005-0158-0.

Santoro, D., Bunick, D., Graves, T.K.,andSegre, M., (2013)Evaluationofcanineantimicrobialpeptides in infectedandnoninfectedchronicatopicskin. VetDermatol. 24, pp. 39-47.e10. doi:10.1111/j.1365-3164.2012.01091.x.

Santoro, D., Marsella, R., Bunick, D., Graves, T.K.,and Campbell, K.L., (2011) Expression anddistributionofcanineantimicrobialpeptides in theskinofhealthyandatopicbeagles. VetImmunolImmunopathol. 144, pp. 382-388. doi:10.1016/j.vetimm.2011.08.004.

Santoro, D., (2018) Evaluation of the secretion of antimicrobial peptides and antimicrobial effect of skin wash in atopic and healthy dogs: a preliminary study. VetDermatol. 29, pp. 402-e132. doi:10.1111/vde.12661

Schittek, B., (2011) The antimicrobialskinbarrier in patientswithatopicdermatitis. CurrProblDermatol. 41, pp. 54-67. doi:10.1159/000323296.

Selsted, M.E.,andOuellette, A.J., (2005)Mammaliandefensins in theantimicrobialimmune response. Nat Immunol. 6, pp. 551-557. doi:10.1038/ni1206.

Silvestre Salvador, J.F., Romero-Pérez, D.,and Encabo-Durán, B., (2017)AtopicDermatitis in Adults: A DiagnosticChallenge. J InvestigAllergolClinImmunol. 27, pp. 78-88. doi:10.18176/jiaci.0138.

Van Damme, C.M., Willemse, T., VanDijk, A., Haagsman, H.P.,andVeldhuizen, E.J., (2009)Alteredcutaneousexpressionof beta-defensins in dogswithatopicdermatitis. Mol Immunol. 46, pp. 2449-2455. doi:10.1016/j.molimm.2009.05.028.

White, S.D., (2005) Advances in equine atopic dermatitis, serologic and intradermal allergy testing. Clin Tech Equine Pract.4, pp. 311–313. Doi:10.1053/j.ctep. 2005.10.005.

Wingate, K.V., Torres, S.M., Silverstein, K.A., Hendrickson, J.A., and Rutherford, M.S., (2009) Expression of endogenous antimicrobial peptides in normal canineskin. Vet Dermatol. 20, pp. 19-26. doi:10.1111/j.1365-3164.2008.00707.x.

Yang, D., Biragyn,A., Kwak, L.W., and Oppenheim, J.J., (2002) Mammalian defensins in immunity: more than just microbicidal. Trends Immunol. 23, pp. 291-296. doi:10.1016/s1471-4906(02)02246-9.

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