

# Skin microbiome: eubiosis, dysbiosis and bacteriotherapy in veterinary dermatology – literature review

Microbioma cutáneo: eubiosis, disbiosis y bacterioterapia en dermatología veterinaria – revisión de la literatura

Larissa Botoni¹

1- DVM, Esp, MSc, PhD, DLACVD - Private Practice in Belo Horizonte-MG/Brazil

## **ABSTRACT**

Animals and humans' body surfaces, with its microenvironment particularities, are inhabited by unique microbial communities that play different roles like niche occupation, decomposing organic matter, producing nutrients, immune stimulation, among others. These communities are called the microbiome. As in any terrestrial biome, the microbiomes have a well-established structure, with resident and transitory members. Residents are those who constantly colonize a given habitat and transients are those who colonize temporarily. Both groups have potentially pathogenic microorganisms, called pathobionts, which only cause disease in the presence of predisposing factors. In order to perform its roles properly, it is important that the microbiome remains in balance, or eubiosis. The term dysbiosis is used to describe the disturbance of the balance of a microbiome, with overgrowth of pathobionts and/or mutualists. The aim of the present study was to review the main functions of the skin microbiome in health and disease and to discuss the new trend in dermatology research, the use of prebiotics, parabiotics and probiotics.

**Key words:** dermatology, microbiome, probiotics, postbiotics, parabiotics, prebiotics.

# RESUMEN

Las superficies corporales de animales y humanos, con sus particularidades microambientales, están habitadas por comunidades microbianas singulares que cumplen diferentes funciones como ocupación de nichos, descomposición de materia orgánica, producción de nutrientes, estimulación inmunológica, entre otras. Estas comunidades se denominan microbioma. Como en cualquier bioma terrestre, los microbiomas tienen una estructura bien establecida, con miembros residentes y transitorios. Los residentes son aquellos que constantemente colonizan un hábitat dado y los transeúntes son aquellos que colonizan temporalmente, Ambos grupos cuentan con microorganismos potencialmente patógenos, denominados patobiontes, que sólo provocan enfermedad en presencia de factores predisponentes. Para realizar sus funciones correctamente, es importante que el microbioma permanezca en equilibrio o eubiosis. El término disbiosis se utiliza para describir la alteración del equilibrio de un microbioma, con crecimiento excesivo de patobiontes y/o mutualistas. El objetivo del presente estudio fue revisar las principales funciones del microbioma cutáneo en salud y enfermedad y discutir la nueva tendencia en la investigación dermatológica, el uso de prebióticos, parabióticos y probióticos.

## PALABRAS CLAVE:

dermatología, microbioma, probióticos, postbióticos, parabióticos, prebióticos.

# INTRODUCTION

Planet Earth in all its extension is inhabited by a great diversity of living beings. Among them, microorganisms are the most abundant. All terrestrial surfaces, with their climatic and microclimatic particularities, are inhabited by unique microbial communities that play different roles like niche occupation, decomposing organic matter, producing nutrients, among others. Similarly, the body surfaces of humans and animals are also inhabited by complex and unique microbial communities called the microbiome. As in any terrestrial biome, the microbiomes that inhabit body parts have a well-established structure, with resident and transitory members. Residents are those who constantly colonize a given habitat and transients are those who colonize temporarily, without definitively establishing themselves. Both groups have potentially pathogenic microorganisms, called pathobionts, which only cause disease in the presence of predisposing factors. In order to perform properly its main functions, it is important that the balance between its members is maintained in the microbiome, so that beneficial species prevail over those with pathogenic potential. This microbiological balance is called eubiosis (Fig.1). The term dysbiosis is used to describe the disturbance of the balance of a microbiome. with overgrowth of pathobionts and/or commensals and alteration of the function of that community 1.

The microbiome lives in a close relationship with its host, Commonly, these microorganisms are called commensals, but the best symbiotic relationship to describe them would be mutualism<sup>2</sup>. In commensalism, the commensals benefit from the relationship without harming or helping the host. In mutualism, both hosts and microorganisms benefit from and contribute positively to each other's life. There is no relationship of indifference by the host. Some authors even describe the microbiome as an organ attached to the body. Some examples of the roles played by these microorganisms are the digestion of nutrients, modulation of the immune system, protection against colonization by pathogenic microorganisms, inhibition of pathobionts' overgrowth, skin and intestinal barrier function, among others. However, for the microbiome to function properly, it is imperative that

the community is in eubiosis, since dysbiosis favors the expression of the pathogenic potential of pathobionts with consequent inflammation, tissue injury and secondary infection. In addition, dysbiosis is not only harmful due to the damage generated by the dominant pathogen, but also due to the loss of symbiotic interactions with other beneficial microorganisms.<sup>1</sup>

There are several factors related to the emergence of dysbiosis in a microbiome, such as inflammatory processes, habits, diet, stress, drug use, host genetics. The intensity and duration of the dysbiotic stimulus will determine the ability to restore eubiosis.1 Whenever the microbiome suffers a stimulus for dysbiosis, as soon as it is removed, there is a trend is for the community to be restored and return to its original composition, However, there is a threshold to this resilience and resilience of the microbiome. Once the dysbiotic stimulus becomes persistent, these capacities are outdated, making it no longer possible to reverse dysbiosis 1,2. Then, community begins to present chronic dysbiosis, losing its ideal functioning hability. It is very import to understand and properly identify the primary causes of dysbiosis and correct treatment protocols to avoid this irreversible chronic changes.

The advances in microbial genomics research techniques allowed the study of microbial communities' structure, the interactions between its components, its relationship with various diseases, its roles in physiological processes and the use of the microbiome as treatment targets. The currently most studied forms of therapeutic manipulation of the microbiome are the use of prebiotics, probiotics, parabiotics and postbiotics. These treatments have aroused great interest in the scientific community, as they allow alternative treatments for infections without antimicrobial use, prevention of dysbiosis and immunemodulation in some inflammatory diseases like atopic dermatitis. Thus, the present study aims to discuss the key points regarding the skin microbiome in order to provide up-to-date information regarding its composition, microbiome in health and disease and bacteriotherapy.

Figure 1. Key concepts in microbiome and symbiotic relationshiops 1.2.

Concept	Definition
Eubiosis	Microbiological balance within the microbiome, with predominance of beneficial microorganisms.
Dysbiosis	The disturbance of the balance of a microbiome, with overgrowth of pathobionts and/or mutualistic organims and alteration of the function of the community.
Pathobionts	Mutualists with pathogenic potential when in a dysbiotic environment.
Microbiome	The mutualist community and its corresponding genome.

#### 1. Definition

Microorganisms are the smallest forms of life on the planet, and are also the most physiologically diverse and metabolically versatile organisms. The entire body surface of living beings is inhabited by a vast number of microorganisms, including bacteria, viruses, fungi, archaea and mites.3 This microbiota resides in the body of humans and animals as mutualists, without causing disturbances to the host. Thus, many body regions of these individuals, such as the skin, oral cavity, gastrointestinal and urogenital tracts, harbor complex microbial communities. When the genetic material of these communities is determined by genomic sequencing, the term microbiome becomes more appropriate, instead of microbiota.<sup>4-6</sup> Several studies in healthy humans and animals have shown that each body habitat has an unique microbiome, which can be compared to the fingerprint of humans <sup>6,7</sup>

#### 2. Microbial genomics

Since the last decades, new technologies involving the genomic identification of microorganisms have revolutionized knowledge about the microbiological world that lives around human beings, whether on terrestrial surfaces or in body areas of living organisms. These scientific tools allowed identification not dependent on pure culture, a great challenge for microbiologists in the past. In addition, they also provided the observation of the diversity and abundance of the microbiota that resides in different places and the dynamic interaction between

it and the environment.<sup>8</sup> The first complete genome sequencing took 13 years to complete, however, with the evolution of techniques, this process occurs in just a few days, currently <sup>8</sup>.

Culture-dependent methods are often used for the isolation and identification of microorganisms, even after the emergence of genomic sequencing methods. However, fastidious growth bacteria are difficult to isolate, and are often inhibited by other species. These factors make these methods limited, as they can underestimate the ecosystem's microbial diversity<sup>9</sup>. Culture-independent methods, which analyze DNA extracted directly from the community, allow the investigation of various aspects of the microbiome, such as taxonomic diversity and functional metagenomics.<sup>7</sup>

The term metagenomics is used to define the genomic methods not dependent on culture, which are used to study complex microbial communities within a given environment. The metagenomic sequencing is massively parallel and aims to determine the phylogenetic composition of the investigated microbiome, and the interaction between its members. Metagenomic studies can be performed in a specific way or by sequencing the complete genome of all microorganisms <sup>9</sup>.

The specific method is based on the sequencing of a marker gene capable of identifying the genome that contains it, without the need of sequencing it completely. The markers must be genes present in all members of the community and capable of differentiating genomes.

Therefore, it is necessary that the marker have regions of evolutionarily conserved DNA, so that it is possible to identify the community, but also, there must be adjacent hypervariable regions that allow, after sequencing, the differentiation of taxons based on the based pairs<sup>7</sup>. The 16S rRNA gene is the most used for bacterial microbiome studies, as it is common to all prokaryotic cells. For the classification of the sequences found, 16S rRNA libraries, such as the Ribosomal Database Project (RDP), GreenGenes and Silva, contain hundreds of thousands of cataloged sequences and are available online.<sup>79</sup>

The whole genome sequencing method, or whole-genome shotgun, sequences at the same time all existing DNA in the sample, allowing identification of the genes carried by the community. This genetic content can give an estimate of which proteins are being produced there. Thus, microorganisms are classified phylogenetically and according to the function performed in the environment. It is relatively simple and accessible to sequence solely the 16S rRNA of a community, which is characterized as a variety of sequences of this marker, and the number of times each of these sequences was identified, so that a taxonomic study of this microbiome can be carried out, <sup>79</sup>

A major challenge in the bioinformatic analysis of 16S rRNA is the definition of a standardized sequence for each community member, Although this marker is extremely conserved during evolutionary time, it has hypervariable regions (V1-V9). Thus, a small number of base pairs, in these regions vary within taxonomic groups, allowing their differentiation. After 16S rRNA sequencing, the sequences that show 97% of similarities are grouped in a taxon, or operational taxonomic unit (OUT) or phylotype. OTUs replace species in microbiome studies, as the classification of species by these markers is often not possible, and usually the phylotypes are classified until genera. The process of classifying sequences into OTUs is called binning, allowing community analysis. As OTUs or bins are grouped into phyla or other taxonomic units, the microbiome in question can be plotted in a histogram graph, demonstrating the taxonomic diversity and abundance of the community. 7

An important concept in microbiome studies is the population diversity, which is the number of each phylotype present in the sample, and their abundance. This is very relevant in human and animal health, as some diseases are being associated with low microbiome diversity, with the overgrowth of certain groups of microorganisms in the presence of predisposing factors.10 In the diversity calculation, to analyze the richness, number

of taxa, and the distribution, the proportion of each taxon, in a community, alpha-diversity is used, which is the evaluation of these variables within a single sample. Beta-diversity is used for diversity analysis when the objective is to compare different populations and determine their similarities. In conclusion, alpha-diversity is a statistical calculation within the same population and beta-diversity is a similarity score between two populations.<sup>7</sup>

In 2009, researchers recruited nine healthy people, of both genders, to collect samples of feces, oral cavity, nostrils, external auditory canal, scalp hair and different skin regions, with the aim of studying the bacterial microbiome of healthy humans using 16S rRNA sequencing, Among all samples collected from all individuals, the most frequent phyla were Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes. Each body region had a unique microbiome that reflected the conditions of the microenvironment. Comparing each habitat between people, there was important individual variation, however, they observed stability between samples collected at different times, demonstrating that the microbiome tends to remain stable under physiological conditions, in the same individual. <sup>12</sup>.

The Human Microbiome Project is a multicenter, international study that aims to characterize the human microbiota and microbiome. In 2012, researchers linked to the project, in the United States of America, recruited 242 adults of both genders, aged between 18 and 40 years, to characterize their bacterial microbiome, using massively parallel sequencing. Samples were collected from multiple body regions, including the skin, nostrils, oral cavity, oropharynx, vagina and feces, Samples were collected from the individuals again in 131 days, in an average of 219 days, after the first collection. This study corroborated with previous results that stated that each body region has a unique and specific microbiome. The oral cavity and feces showed the highest alpha-diversity, while the vagina showed the lowest, with a predominance of Lactobacillus. In terms of beta-diversity, the oral cavity presented the least and the skin the greatest measures, concluding that the first presented more similarity between individuals, unlike the skin. In samples from the gastrointestinal tract, an inverse relationship was observed between Bacteroidetes and Firmicutes, and in samples where the former was overrepresented, the latter was less concentrated. Considering the stability of the microbiome over time, in individuals who underwent second collections, it was observed that it remained stable in the same individual, but there was great interpersonal variation 10.

#### 3. Role of the microbiome in health and disease

The resident microbiota performs functions crucial to the host's health, such as resistance to pathogens, modulation of the immune system and nutritional support. 11. Body surfaces constitute stable, nutrient-rich ecosystems where microorganisms thrive. This microbiota constantly releases a complex mixture of metabolites, vitamins and nutrients that send signals to the immune system, shaping its responses. Therefore, a healthy animal must present two opposing reactions coexisting. The immune system must tolerate the microbiome and be able to mount a rapid response that efficiently eliminates pathogens. The selection of which response will be used for each situation will be defined according to how the antigen processing and signals sent by the microbiota occurs. If the balance between these pro-inflammatory and anti-inflammatory processes is disrupted, there will be a change in the level of immune system activation.<sup>12</sup>

The colonization of the newborn's skin, which is sterile in prenatal life, takes place from birth onwards. This initial microbiome shows little diversity and is closely related to the type of delivery through which the birth occurred. Neonates born by normal delivery present skin colonization more similar to that present in the mother's vaginal canal, with a predominance of Lactobacillus species, while those born by cesarean section will have their skin colonized first by contact with maternal skin and will then present a microbiome similar to this region, with a predominance of species of Staphylococcus. 7.9.13 This exposure to microorganisms in the early stages of life will determine how the immune system will develop, as germfree mammals have an underdeveloped mucosal immune system. The microbiota is able to stimulate enterocyte Toll-like receptors (TLR) and promote the development of the immune system.<sup>14</sup> Skin microbiota also contribute to this process during breastfeeding and contact with the mother.15

The intestinal microbiota has unique and essential functions in the body of healthy humans and animals. This microbiome has been associated with metabolic functions, inhibition of pathogen invasion, strengthening of the intestinal mucosa and modulation of the immune system. The gastrointestinal tract is a complex mixture of bacteria, archaea, fungi, and viruses. The most numerous are bacteria and it is estimated that trillions of individuals of various species are present. In mammals, there is a predominance of the Firmicutes and Bacteroidetes phyla, with a smaller fraction of Fusobacteria and Verrucomicrobia. Mammals have about 20,000 genes that code for proteins,

while their microbiota together have around 10 million. This diverse genome enhances the animal's metabolic function by increasing, for example, the ability to extract energy from plant structural carbohydrates and to absorb vitamins. A mouse with the conventional microbiota needs 30% fewer calories daily to maintain its body weight than germ-free mice.<sup>12</sup>

By occupying intestinal niches, commensal bacteria block subsequent colonization by pathogenic species. In addition, the intestinal microbiota modifies the local microenvironment by keeping pH and oxygen pressure low. In the intestinal mucosa, there is more activity of the immune system than in all lymphoid tissues combined.16 It is estimated that 80% of the activated B cells in the body are in the intestine and their function is to defend against possible microbial invasions. However, for the intestinal microbiota to colonize properly, there needs to be a balance between Th17 pro-inflammatory cells and Treg anti-inflammatory cells17. In a study that evaluated changes in the immune system of mice colonized by only one species of bacteria, it was demonstrated that many bacteria have different effects on immune function, Some species have similar, overlapping or even opposite functions. Some are potent stimulators of the Th17 response, while about a guarter of the bacteria studied stimulated Treg and others affected innate lymphoid cells and dendritic cells 1718.

In a study with seven Beagle dogs within ideal weight and seven obese, they observed that in lean dogs, the diversity of the microbiome was greater and there was a predominance of Firmicutes in intestinal samples, while in obese dogs the predominant phylum was Proteobacteria. This genus is composed of Gram-negative bacteria that have LPS in their membrane, a molecule capable of inducing chronic inflammation.<sup>19</sup>

In another study, with the aim of evaluating this relationship between LPS and obesity, researchers observed that mice fed a diet rich in lipids (72%) had a significant increase in the concentration of LPS in the plasma, to a lesser degree than what occurs in cases of sepsis, characterizing the state of metabolic endotoxemia. Subsequently, the same researchers performed a continuous infusion of LPS in animals fed a balanced diet, without increasing calorie intake. Thus, they observed that, after these four weeks, the animals that received LPS infusion obtained weight gain similar to those that received, for four weeks, a diet with 72% of lipids, Thus, it is suggested that the increase in LPS, causing metabolic endotoxemia, is related to obesity.<sup>20</sup>

Studies have also shown that the microbiome of the gastrointestinal tract controls a protein derived from the intestine that is very important in the metabolism of lipids in the host, Angiopoietin-like 4 (Angptl4). This protein regulates the oxidation of fatty acids in muscle and adipose tissue. Germ-free mice, when colonized with intestinal microbiota from obese rodents, showed suppression of Angptl4 production and more triglycerides were stored in the adipose tissue, leading to weight gain. In addition, an association has been made between the intestinal microbiome undergoing changes due to the consumption of a western diet, rich in red meat, lipids and few vegetables, and being able to produce carcinogenic substances and metabolize certain compounds, which contribute to the development of cancer.<sup>21</sup>

The microbiome is essential for the activation of the host's immune system and several autoimmune diseases are derived from acquired immunity imbalance, The mutualists that inhabit the entire body of humans and animals induce the differentiation of CD4+ T lymphocytes into four main types: Th1, Th2, Th17 and Treg. Each type has a specific immune function and secretes characteristic cytokines, Th1 cells are involved in the elimination of intracellular pathogens, Th2 control infection by parasites and Th17 has an important role in protecting against infections. Treg regulates the immune response. The resident microbiota modulates an appropriate balance between these four types of immune responses<sup>20</sup>. Intestinal T helper (Th) precursor cells can both differentiate into Treg and into Th17 depending on signals received from the microbiota, When the intestinal microbiome is eubiotic, Treg differentiation is favored while Th17 is suppressed and minimal inflammation occurs in the intestinal wall. In the absence of Treg, uncontrolled effector T cells respond to antigens and trigger inflammation,12

Regardless of the location, on the skin, gastrointestinal, respiratory, or genitourinary tract, the microbiome communicates directly and efficiently with the host's immune system. This communication is essential for the proper functioning of the innate and adaptive immunity, thus, dysbiosis has profound effects on immunity.<sup>22</sup> Dietary plant fibers contain complex carbohydrates that, when digested by Clostridia in the cecum and colon, generate metabolites such as acetate and butyrate that suppress macrophages and stimulate the production of Treg cells. Thus, consumption of a high-fiber diet plays a crucial role in controlling intestinal inflammation.<sup>12</sup> Furthermore, these clostridial colonies also increase the number of Treg cells in distant organs such as the spleen, lungs and skin, also

acting in inhibition of allergic responses. These stimulated T cells in the gut migrate to remote tissues and determine the systemic balance of T cells and their responses. <sup>23</sup>

The skin and the gastrointestinal tract have much in common and interact extensively. Both systems have dense vascularization, massive innervation and are intensely colonized by distinct microbial communities. The intestinal microbiome and its various metabolites can also induce skin changes, this is called the gut-skin axis. 12,24 The impact of the gut microbiome in the skin microbiome has been studied in both humans and dogs recently. 25,26 Leverett et al (2022) studied the impact of feeding fresh food instead of dry food in the composition of the skin microbiome of healthy dogs and observed an increase in alphadiversity in the fresh food group, showing this importance of the gut-skin axis. 26

#### 4. Bacterial skin microbiome

The skin is the most exposed organ of the entire body of humans and animals, responsible for providing the body with a barrier against the entry of possible harmful agents, such as pathogens and irritating substances, being able to act as a physical, chemical and immunological barrier, able to respond to the most diverse challenges. However, even with all their efficiency in exerting this protective barrier, various microbial communities live on the skin, which are tolerated by the cutaneous immune system. <sup>27</sup> Under physiological conditions, there is a balance between the host's immune system and the microbiome, which controls the proliferation of these microorganisms, however, when this balance is disturbed, dysbiosis may occur, causing disease. <sup>27–29</sup>

The cutaneous ecosystem is quite variable, being composed of several structures that differ from each other in terms of pH, humidity, temperature, lipids, antimicrobial peptides, among others. These different skin regions with different environmental conditions that lead to the formation of microbial niches. The hair follicles, sweat and sebaceous glands are regions that have a unique microenvironment, giving them a particular microbiome and differentiated from the horny layer of the epidermis, for example, even if the distance between the places is minimal <sup>5-29</sup>

Studies in humans show that four phyla of bacteria are more frequent in skin samples, Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes. The most identified genera are *Corynebacterium*, *Staphylococcus* and *Propionibacterium*, and the abundance of each genus is strongly dependent on the respective niche.

Staphylococcus and Propionibacterium predominate in regions with abundant sebaceous secretion on the face, while Corynebacterium predominates in the axilla and other more humid regions, but Staphylococcus may also be present. In dogs, the use of massively parallel sequencing for microbiome studies is not yet widely used, but the results obtained with healthy dogs showed data like those found in humans, with Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Cyanobacteria being the most abundant phyla in dog skin. In these animals, the topographic variation of the composition of the microbiome could also be observed.<sup>30-33</sup>

The skin microbiome can be altered by several factors, extrinsic or intrinsic. The extrinsic, or environmental, factors include humidity, environmental temperature, type of clothing, use of antibiotics and cosmetics, frequency of hygiene, among others. <sup>25</sup>The intrinsic ones are those related to the host and alter the composition of the microbiome. These factors are mainly anatomy, genetics, gender, age and immunity. <sup>9,25,34</sup> Most of the time, skin infections are the result of imbalances in the microbiota causing pathobionts' overgrowth under certain conditions. Skin dysbiosis changes the microbiome's ability to maintain the protective epidermal barrier function. <sup>9,35</sup>

Atopic dermatitis (AD) is a multifactorial skin disease, with a pathogenesis not completely understood, often associated with bacterial infections, mainly caused by the genus Staphylococcus, both in humans and in atopic dogs, 30,36. What makes these patients more prone to these infections are the skin changes associated with the disease. A few years ago, it was believed that AD was a disease caused by a genetic alteration of the immune system that generated an abnormal immune reaction, mediated by IgE-type antibodies, specific against certain innocuous allergens, this was the "outside-inside" theory. Currently, it is known that the dysfunction of the epidermal barrier facilitates and allows the entry of allergens and irritating substances, such as microorganisms, pollen, mites, acids, among others, increasing their contact and exposure to the immune cells of the epidermis. The latter completes the previous theory, from the outside to the inside, to form the "outside-inside-outside" theory. This new theory proposes that a primary defect of the epidermal barrier allows greater penetration of allergens, which will cause sensitization of the immune system and further damage to the epidermal barrier. Thus, it is currently believed that high levels of IgE are consequences of AD and not the cause. Furthermore, food allergens appear to play an important role in the pathogenesis of AD, contrary to what was previously thought, that AD and food allergy were completely and distinct diseases. 37.38

In a study carried out with atopic children, with the objective of evaluating the temporal variation of the microbiome throughout the evolution of the disease crises, the authors selected 12 atopic children of moderate to severe degree, and 11 healthy controls, and collected samples from the antecubital and popliteal flexures, sites known as atopic regions, their adjacent areas and the nostrils. The collections were performed before the crisis, at the time of the crisis and between 10 and 14 days after this period. By associating the severity of the disease with the Shannon alpha-diversity index, they found that these variables were inversely related, and the greater was the severity, the less diversity was observed. The reduction in diversity was closely linked to the atopic zones. In these places, there was a predominance of the genus Staphylococcus. In addition, patients who were not treated during the flares had lower Shannon diversity than the treated ones. The diversity of the adjacent regions and the nostrils remained quite stable, demonstrating that the alteration in the microbiome is related to the disease predilection sites.36 It has always been believed that S. epidermidis has some protective function against S. aureus infections due to its reduced abundance in crisis phases and an increase in stable phases<sup>39-41</sup>, Kong et al. (2012), observed that the abundance of S. epidermidis increased along with that of S. aureus during the flare, with a consecutive reduction after this period. This goes against what has been said so far, and these authors suggest that instead of inhibiting S. aureus, S. epidermidis has a symbiotic relationship with it.

The cutaneous microbiome of atopic and healthy dogs using non-culture-dependent methods has recently begun to be studied.<sup>26,30-33,42</sup> Hoffmann et al. (2014) performed the first study with these methods in dogs. These researchers evaluated the microbiome of 12 healthy and six allergic. The samples were collected from different skin regions, namely the nostrils, interdigital region, armpits, ear pinna, auditory canal, conjunctiva, lumbar region, perianal, inguinal and labial commissure. All atopic dogs included were in a stable phase of the disease, that is, outside the crisis. The researchers found a significantly lower alpha-diversity in the samples of allergic dogs when compared to healthy ones, even if they were out of the crisis period and with lesional skin. It is known that the alesional skin of atopic dogs presents a higher degree of inflammation and the defects in the epidermal barrier that are characteristic of the disease, this probably leads to

changes in the diversity of the skin microbiome of these animals.

It is known that, to control recurrent infections, it is essential that the inflammatory reaction of AD is well controlled and that the use of antimicrobials alone is not able to prevent the recurrence of infections, since the allergic disease is responsible for dysbiosis predisposing factors<sup>43</sup>. However, there are frequent cases in which, even if pruritus is being controlled satisfactorily, infections remain recurrent, making it necessary to use repeated antibiotic therapy. The extensive use of antimicrobials is associated with the emergence of resistant microorganisms, making infection control increasingly challenging.<sup>44</sup> Therefore, therapeutic alternatives for the control and prevention of dysbiosis have been researched. The use of probiotic therapy is a very current area of interest of the scientific community not only for the control of dysbiosis, but also for its immunomodulatory properties in various diseases such as atopic dermatitis, diabetes, obesity and inflammatory bowel disease. 45-54

#### 5. Bacteriotherapy

In the early 20th century, Ilya Ilyich Metchnikoff observed that regular consumption of lactobacilli from fermented dairy products such as yoghurts was associated with better general health and longevity in villages in Bulgaria. This observation that some bacteria have beneficial effects on the body led to the emergence of probiotics.<sup>52</sup>

Probiotics are live microorganisms that, when administered in adequate doses, are capable of producing beneficial effects to the host (FAO/WHO) and include both bacteria (mainly *Lactobacillus sp, Biphidobacterium sp and Bacillus sp)* and yeasts (mainly *Saccharomyces sp)*, <sup>51</sup> According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), the benefits of probiotics can be strain-specific (present only in some strains of a species), species-specific (present in all microorganisms of that species) or widespread (widely present in several species of probiotics). <sup>55,56</sup>

Most studies on the use of probiotics are related to their oral administration, but currently they have also focused on topical formulations containing probiotics for the treatment of various dermatopathies such as AD, psoriasis, acne, rosacea and anti-aging.<sup>45-51</sup> Contrary to popular belief, indications for both oral and topical supplementation with probiotics go far beyond restoring the microbiome through local colonization by mutualists. Probiotics have anti-inflammatory, immunomodulatory,

anti-proliferative, antioxidant and antimicrobial properties. <sup>57</sup> When administered orally, microorganisms act by interacting with the intestinal microbiome and immune system, which have important connections with the systemic immune system, being able to modulate inflammatory responses and also act beneficially in non-intestinal diseases. <sup>52,54</sup> The objective of applying these probiotic formulations through the skin is to directly stimulate the local microbiome and modulate the cutaneous immune system to intensify the response in inflammatory skin lesions and obtain more targeted effects on the skin. Studies in this regard are still early and recent, but they already show good results in the control of inflammatory dermatopathies in humans. <sup>45,47,50</sup>

In addition to the administration of live microorganisms, prebiotics are also used and, more recently, postbiotics and parabiotics have emerged, Prebiotics are nutritional ingredients not digestible by the host, mainly fibers, which are capable of stimulating the proliferation of beneficial microorganisms in the microbiome, providing an ideal microenvironment for the development of these microorganisms. Parabiotics are inactivated or lysed probiotic microorganisms in crude cell extracts, which, when administered in adequate amounts, produce effects in humans or animal consumers. Postbiotics are metabolites produced by probiotics or released after cell lysis that produce beneficial effects to the host. These substances are produced by microorganisms during the fermentation process. Parabiotics, prebiotics and postbiotics, similarly to probiotics, can be administered orally or topically.55.56

The use of parabiotics originated from questions about the feasibility and safety of administering live microorganisms. There are many factors involved in the activity of viable bacteria, such as adequate storage, their ability to colonize and their survival in the hostile environment of the host's skin or intestine. Studies show that the viability of probiotics is very dependent on the storage temperature, and at room temperature, the number of viable cells decrease considerably. 55.56 In addition, in any probiotic formulation, the proportion of live and dead bacteria can vary greatly, so the population of dead cells can be much greater than that of live cells. Thus, the beneficial effects attributed to viable cells may be overestimated, when in fact they are generated by the parabiotics, 56.58 Furthermore, one should also consider the possibility of horizontal transfer of resistance genes between pathobiont and probiotics, conferring them a pathogenic feature, no longer therapeutic potential, Thus,

the current trend on research is walking towards the supplementation with parabiotics and postbiotics, which have no storage limitations or safety issues. Although studies with the use of parabiotics and postbiotics are still in very initial, scientific evidence points to good efficacy and good prospects for future use.<sup>56,58</sup>

A common form of obtaining probiotics, prebiotics and postbiotics is through the fermentation of carbohydrates, such as milk and grains like oat, by certain strains of bacteria in order to promote colony growth and production of fermentation by-products, the prebiotics and postbiotics. Oats fermented by *Lactobacillus* strains have therapeutic use in topical formulations for dermatological use in humans and animals, due to their parabiotic, prebiotic and postbiotic properties.<sup>45,47-51,55,56,59,80</sup>

# Conclusions

Considering the data shown in the present review, the important role of the skin microbiome in the hosts' health by protecting against pathogenic microrganisms, acting as a part of the skin and intestinal barrier and in immunomodulation is highlighted. Therefore, it is mandatory that veterinarians consider this essential microbial community when establishing any treatment for their patients by the implementation of preventative measures for dysbiosis, adequate control of inflammatory diseases, causes of disruption of the eubiosis, and also the rational use of antimicrobials. Scientific evidence points to the use of formulations containing probiotics, parabiotics, postbiotics and prebiotics in maintaining the health of the microbiome and also as an important adjuvant in the treatment of inflammatory diseases such as canine atopic dermatitis. Therefore, these therapeutic modalities represent good future trends and should be used to improve patients' life quality and facilitate the control of challenging skin diseases.

# **REFERENCES**

- 1. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nat Rev Immunol [Internet]. 2017;17(4):219–32. Available from: http://dx.doi.org/10.1038/nri.2017.7
- 2. lebba V, Totino V, Gagliardi A, Santangelo F, Cacciotti F, Trancassini M, et al, Eubiosis and dysbiosis: The two sides of the microbiota. New Microbiologica. 2016;39(1):1–12.
- 3. Sabree ZL, Rondon MR, Handelsman J. Defining Statement. 2009;
- 4. Kil DY, Swanson KS. Companion animals symposium: Role of microbes in canine and feline health. J Anim Sci. 2011;89(5):1498–505.
- 5. Weese JS. The canine and feline skin microbiome in health and disease. Vet Dermatol, 2013;24:137-46.
- 6. Zapata HJ, Quagliarello VJ. The Microbiota and Microbiome in Aging: Potential Implications in Health and Age-Related Diseases. J Am Geriatr Soc. 2015;n/a-n/a.
- 7. Morgan XC, Huttenhower C, Chapter 12: Human Microbiome Analysis, PLoS Comput Biol, 2012;8(12).
- 8. Wizemann T, Olsen L a, Choffnes ER. The Science and Applications of Microbial Genomics:: Workshop Summary. 2013. 1–403 p.
- 9. Kong HH. Skin microbiome: Genomics-based insights into the diversity and role of skin microbes. Trends Mol Med IInternet], 2011;17(6):320–8. Available from: http://dx.doi.org/10.1016/j.molmed.2011.01.013
- 10. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–14.
- 11. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009;326(5960):1694–7.
- 12. Tizard IR, Jones SW. The Microbiota Regulates Immunity and Immunologic Diseases in Dogs and Cats. Veterinary Clinics of North America: Small Animal Practice [Internet]. 2017; Available from: http://linkinghub.elsevier.com/retrieve/pii/S0195561617301250
- 13. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):11971–5.
- 14. Gensollen T, Iyer SS, Kasper DL, Blumberg RS, How colonization by microbiota in early life shapes the immune system. 2016;539–45.
- 15. Brown RL, Clarke TB. The regulation of host defences to infection by the microbiota. Immunology. 2017;150(1):1-6.
- 16. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol. 2013;14(7):685–90.
- 17. Kamada N, Seo SU, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol, 2013;13(5):321–35.
- 18. Geva-Zatorsky N, Sefik E, Kua L, Pasman L, Tan TG, Ortiz-Lopez A, et al. Mining the Human Gut Microbiota for Immunomodulatory Organisms. Cell. 2017;168(5):928-943.e11.
- 19. Park HJ, Lee SE, Kim HB, Isaacson RE, Seo KW, Song KH. Association of Obesity with Serum Leptin, Adiponectin, and Serotonin and Gut Microflora in Beagle Dogs, J Vet Intern Med [Internet], 2015;29(1):43–50, Available from: http://doi.wiley.com/10.1111/jvim.12455
- 20. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Original Article. Diabetes. 2007;56(July):1761–72.
- 21. Muszer M, Noszczyńska M, Kasperkiewicz K, Skurnik M. Human Microbiome: When a Friend Becomes an Enemy. Arch Immunol Ther Exp (Warsz). 2015;
- 22. Tomkovich S, Jobin C. Microbiota and host immune responses: A love-hate relationship. Immunology. 2016;147(1):1–10.
- 23. Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, et al. The Key Role of Segmented Filamentous Bacteria in the Coordinated Maturation of Gut Helper T Cell Responses. Immunity. 2009;31(4):677–89.
- 24. Fang Z, Lu W, Zhao J, Zhang H, Qian L, Wang Q, et al. Probiotics modulate the gut microbiota composition and immune responses in patients with atopic dermatitis: a pilot study. Eur J Nutr [Internet]. 2020;59(5):2119–30. Available from: https://doi.org/10.1007/s00394-019-02061-x

- 25. Skowron K, Bauzakaszewska J, Kraszewska Z, Wiktorczykkapischke N, Grudlewskabuda K, Kwiecińskapiróg J, et al. Human skin microbiome: Impact of intrinsic and extrinsic factors on skin microbiota. Vol. 9, Microorganisms. MDPI AG; 2021. p. 1–20.
- 26. Leverett K, Manjarin R, Laird E, Valtierra D, Santiago-Rodriguez TM, Donadelli R, et al. Fresh Food Consumption Increases Microbiome Diversity and Promotes Changes in Bacteria Composition on the Skin of Pet Dogs Compared to Dry Foods, Animals, 2022 Aug 1;12(15).
- 27. Sanford J a., Gallo RL. Functions of the skin microbiota in health and disease. Semin Immunol (Internet). 2013;25(5):370-7. Available from: http://dx.doi.org/10.1016/j.smim.2013.09.005
- 28. Hoffmann AR, Patterson AP, Diesel A, Lawhon SD, Ly HJ, Steiner M, et al. The Skin Microbiome in Healthy and Allergic Dogs, 2014;9(1).
- 29. Schommer NN, Gallo RL. Structure and function of the human skin microbiome. Trends Microbiol [Internet]. 2013;21(12):660-8. Available from: http://dx.doi.org/10.1016/j.tim.2013.10.001
- 30. Hoffmann AR, Patterson AP, Diesel A, Lawhon SD, Ly HJ, Stephenson CE, et al. The skin microbiome in healthy and allergic dogs. PLoS One. 2014;9(1).
- 31. Rodriguez-Campos S, Rostaher A, Zwickl L, Fischer N, Brodard I, Vidal S, et al. Impact of the early-life skin microbiota on the development of canine atopic dermatitis in a high-risk breed birth cohort. Sci Rep. 2020 Dec 1;10(1).
- 32. Pierezan F, Olivry T, Paps JS, Lawhon SD, Wu J, Steiner JM, et al. The skin microbiome in allergen-induced canine atopic dermatitis. Vet Dermatol. 2016;
- 33. Bradley CW, Morris DO, Rankin SC, Cain CL, Misic AM, Houser T, et al. Longitudinal evaluation of the skin microbiome and association with microenvironment and treatment in canine atopic dermatitis. J Invest Dermatol. 2016;136(6):1182–90.
- 34. Lynde CW, Andriessen A, Bertucci V, McCuaig C, Skotnicki S, Weinstein M, et al. The skin microbiome in atopic dermatitis and its relationship to emollients, J Cutan Med Surg, 2016;20(1):21–8,
- 35. SanMiguel A, Grice E a. Interactions between host factors and the skin microbiome. Cellular and Molecular Life Sciences [Internet]. 2014;1499–515. Available from: http://link.springer.com/10.1007/s00018-014-1812-z
- 36. Kong HH, Oh J, Deming C, Conlan S, Grice E a., Beatson M a., et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res. 2012;22(5):850–9.
- 37. Le Roy L, Le Poder S, Desquilbet L, Perrot S, Cavana P, Marignac G. Canine Atopic Dermatitis Diagnostic Criteria: Evaluation of Four Sets of Published Criteria among Veterinary Students. J Vet Med Educ. 2015;42(1):79–84.
- 38. Santoro D, Marsella R, Pucheu-Haston CM, Eisenschenk MNC, Nuttall T, Bizikova P. Review: Pathogenesis of canine atopic dermatitis: skin barrier and host-micro-organism interaction, Vet Dermatol, 2015 Feb 13;n/a-n/a,
- 39. Christensen GJM, Brüggemann H. Bacterial skin commensals and their role as host guardians. Benef Microbes. 2014;5(2):201–15.
- 40. Gallo RL, Nakatsuji T, Microbial symbiosis with the innate immune defense system of the skin. J Invest Dermatol. 2011;131(10):1974-80.
- 41. Nakamizo S, Egawa G, Honda T, Nakajima S, Belkaid Y, Kabashima K. Commensal bacteria and cutaneous immunity. Semin Immunopathol. 2014;37(1):73–80.
- 42. Torres S, Clayton JB, Danzeisen JL, Ward T, Huang H, Knights D, et al. Diverse bacterial communities exist on canine skin and are impacted by cohabitation and time. PeerJ. 2017;5:e3075.
- 43. Tsakok T, Woolf R, Smith CH, Weidinger S, Flohr C. Atopic dermatitis: the skin barrier and beyond. Vol. 180, British Journal of Dermatology. Blackwell Publishing Ltd; 2019, p. 464–74.
- 44. Hillier A, Lloyd DH, Weese JS, Blondeau JM, Boothe D, Breitschwerdt E, et al. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). Vet Dermatol, 2014;25(3).
- 45. Lee GR, Maarouf M, Hendricks AJ, Lee DE, Shi VY. Topical probiotics: The unknowns behind their rising popularity. Dermatol Online J. 2019;25(5).
- 46. Taverniti V, Guglielmetti S. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: Proposal of paraprobiotic concept). Vol. 6, Genes and Nutrition. 2011. p. 261–74.

- 47. Gueniche A, Benyacoub J, Blum S, Breton L, Castiel I. Probiotics for Skin Benefits. In: Nutritional Cosmetics. 2009. p. 421–39.
- 48. Yu Y, Dunaway S, Champer J, Kim J, Alikhan A. Changing our microbiome: probiotics in dermatology. British Journal of Dermatology. 2020;182(1):39–46.
- 49. Lise M, Mayer I, Silveira M, Use of probiotics in atopic dermatitis, Rev Assoc Med Bras. 2018;64(11):997–1001.
- 50. Ouwehand AC, Bâtsman a., Salminen S. Probiotics for the skin: A new area of potential application? Lett Appl Microbiol. 2003;36:327–31.
- 51. Maguire M, Maguire G. The role of microbiota, and probiotics and prebiotics in skin health. Arch Dermatol Res. 2017 Aug 1;309(6):411–21.
- 52. Friedrich AD, Paz ML, Leoni J, Maglio DHG. Message in a bottle: Dialog between intestine and skin modulated by probiotics, Vol. 18, International Journal of Molecular Sciences. MDPI AG; 2017.
- 53. Pratap K, Taki AC, Johnston EB, Lopata AL, Kamath SD. A Comprehensive Review on Natural Bioactive Compounds and Probiotics as Potential Therapeutics in Food Allergy Treatment, Vol. 11, Frontiers in Immunology. Frontiers Media S.A.; 2020.
- 54. Fang Z, Lu W, Zhao J, Zhang H, Qian L, Wang Q, et al. Probiotics modulate the gut microbiota composition and immune responses in patients with atopic dermatitis: a pilot study. Eur J Nutr. 2020 Aug 1;59(5):2119–30.
- 55. P.F. Cuevas-González, A.M. Liceaga JEAT. Postbiotics and Paraprobiotics: From concepts to applications. Food Research International, 2020;
- 56. Barros CP, Guimarães JT, Esmerino EA, Duarte MCK, Silva MC, Silva R, et al. Paraprobiotics and postbiotics: concepts and potential applications in dairy products. Vol. 32, Current Opinion in Food Science. Elsevier Ltd; 2020. p. 1–8.
- 57. Huang R, Ning H, Shen M, Li J, Zhang J, Chen X, Probiotics for the treatment of atopic dermatitis in children: A systematic review and meta-analysis of randomized controlled trials, Front Cell Infect Microbiol, 2017;7(SEP):1–11,
- 58. Cuevas-González PF, Liceaga AM, Aguilar-Toalá JE. Postbiotics and paraprobiotics: From concepts to applications. Food Research International [Internet]. 2020;136:109502. Available from: https://doi.org/10.1016/j.foodres.2020.109502
- 59. Paetzold B, Willis JR, Pereira De Lima J, Knödlseder N, Brüggemann H, Quist SR, et al. Skin microbiome modulation induced by probiotic solutions. Microbiome. 2019;7(1):1–9.
- 60. Padeh Y, Expanding the Boundaries of Oral Probiotic Formulations to Target Atopic Dermatitis, 2019;5:10.



