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Ictiosis en un canino mestizo:
Reporte de un caso y revisión bibliográfica

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Prólogo

Apreciados colegas latinoamericanos:

Es un placer presentarles la novena edición de la revista de la Sociedad Latinoamericana de Dermatología Veterinaria. Nuestra revista se ha consolidado como una fuente de información de vanguardia para los profesionales de la veterinaria que nos dedicamos al estudio y tratamiento de esta hermosa especialidad.

En esta edición, se presenta un relato de caso de ictiosis canina y dos revisiones de literatura basadas en inmunoterapia alergeno específica y el microbioma cutáneo, con el fin de proporcionar a nuestros lectores una visión integral y actualizada de los avances en el diagnóstico, tratamiento y manejo de las enfermedades de la piel en pequeños animales.

Espero que los artículos seleccionados sean de utilidad, que contribuyan al desarrollo y mejora de la práctica clínica. Agradezco a todos los autores por sus valiosas contribuciones y a ustedes lectores, amigos y colegas, por su continuo apoyo a nuestra revista.

Quiero agradecer a la Dra. Wendy Roldán y a la comisión directiva de la SLDV por la confianza otorgada para esta nueva responsabilidad de ser editora jefe, la cual asumo con mucho respeto. Es un honor para mí estar con ustedes en este nuevo reto, para dar continuidad a este gran esfuerzo que han realizado desde hace varios años.

Con todo mi aprecio y deseando una lectura muy productiva,

Atentamente,

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Ictiosis en un canino mestizo: Reporte de un caso y revisión bibliográfica

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RESUMEN

La ictiosis es un trastorno congénito de baja prevalencia en caninos que se transmite de manera autosómica recesiva. Se caracteriza por la aparición de lesiones descamativas en todo el cuerpo, que suelen manifestarse a partir de las primeras semanas de edad. Las infecciones secundarias bacterianas y principalmente micóticas son de frecuente aparición agravando la signología cutánea. El estudio histopatológico de las lesiones revela hiperqueratosis ortoqueratótica laminar severa tanto epidérmica como folicular. El estudio genético permite determinar el gen mutado que da origen al trastorno de la queratinización que puede involucrar tanto a la queratina como a los lípidos o enzimas encargadas del proceso de cornificación. Debido a su carácter congénito y a la imposibilidad de revertir la mutación que promueve la alteración en el recambio epidérmico los tratamientos se centran principalmente en aliviar la sintomatología cutánea.

PALABRAS CLAVE: Ictiosis, Cornificación, Hiperqueratosis.

Ichthyosis in a mixed-breed dog: A case report and bibliographic review

SUMMARY

Ichthyosis is a congenital disorder of low prevalence in canines that is transmitted in an autosomal recessive manner. It is characterized by the appearance of scaly lesions all over the body, which usually manifest from the first weeks of age. Secondary bacterial and mainly fungal infections frequently appear, aggravating the skin signs. The histopathological study of the lesions revealed severe lamellar orthokeratotic hyperkeratosis, both epidermal and follicular. The genetic study makes it possible to determine the mutated gene that gives rise to the keratinization disorder that can involve both keratin and lipids or enzymes in charge of the cornification process. Due to its congenital nature and the impossibility of reversing the mutation that promotes the alteration in epidermal turnover, treatments are mainly focused on relieving skin symptoms.

Key words: Ichthyosis, Cornification, Hyperkeratosis.

INTRODUCCIÓN

La cornificación comprende el proceso por el cual los queratinocitos de la región basal de la epidermis se dividen, diferencian y migran hacia las capas más superficiales de la epidermis (1). En este proceso intervienen diferentes mecanismos a través de los cuales las células se queratinizan y finalmente se descaman (1,2,3). La correcta cornificación es indispensable para la formación de un estrato corneo eficaz en el control de las infecciones microbianas, en evitar la pérdida de agua transepidérmica, en proteger al individuo de las agresiones externas así como servir de barrera contra los antígenos del ambiente (1,2).

En la década de 1960, se definió a los trastornos de la cornificación como aquellos causados ya sea por un aumento en el recambio epidérmico o por una falta de descamación y consiguiente retención epidérmica (1). Dentro de los trastornos congénitos y/o hereditarios de la queratinización se incluyen, la ictiosis, la adenitis sebácea, la dermatosis psoriasiforme liquenoide del Springer spaniel inglés, la hiperqueratosis de las almohadillas plantares, la hiperqueratosis nasal, y la paraqueratosis folicular entre otras patologías de muy baja prevalencia (2).

El objetivo de este trabajo es presentar un caso de ictiosis en un cachorro mestizo y su evolución a lo largo de los años junto a una breve revisión de esta entidad y del proceso de cornificación normal.

RELATO DEL CASO

Se presenta a consulta en el servicio de dermatología del Hospital Escuela de la Facultad de Ciencias Veterinarias de la Universidad de Buenos Aires un canino, mestizo, macho de 2 meses de edad procedente de una camada de cachorros nacidos de una perra mestiza rescatada de la vía pública. Como datos de anamnesis, el paciente comía un balanceado premium, no estaba vacunado pero sí había recibido dos dosis de antiparasitario. Las lesiones dermatológicas comenzaron a partir de la quinta semana de edad. Ninguno de sus hermanos o la madre presentó lesiones. Al momento de la consulta, el paciente se encontraba alerta y clínicamente su estado general era bueno. A nivel dermatológico, se evidenciaba abundante cantidad de escamas laminares furfuráceas afectando el área de la cabeza, dorso, ventral del cuerpo, miembros y región perianal (Imagen 1). Dichas escamas estaban parcialmente adheridas al estrato córneo y a los pelos (Imagen 2). La piel del área ventral correspondiente a la región del pecho, del abdomen e inguinal era relativamente dura y áspera al tacto denotándose una marcada menor elasticidad (Imagen 3). En la cara externa de los pabellones auriculares se observaban escamas adheridas a los pelos, y en la cara interna eritema y secreción ceruminosa que se continuaba hacia el interior de los conductos auditivos (Imagen 4). A nivel podal, se encontraban placas descamativas en los espacios interdigitales y la piel de los pulpejos era dura al tacto (Imagen 5). El paciente manifestaba prurito moderado y presentaba un intenso olor rancio en la piel y en los conductos auditivos. Se procedió a realizar una serie de métodos complementarios que incluyeron análisis de sangre completo, estudio coproparasitológico seriado, raspaje cutáneo, tricograma, observación con lámpara de Wood y citología tanto de la piel como de los conductos auditivos. Tanto el raspaje como el tricograma fueron negativos y la observación con la lámpara de Wood no mostró refringencia. En la citología cutánea se identificaron abundantes células descamativas, neutrófilos, cocos libres y fagocitados en baja cantidad y abundante cantidad de estructuras de aspecto levaduriforme compatibles morfológicamente con *Malassezia sp.* La citología de los conductos auditivos reveló también elevada cantidad de estructuras de aspecto levaduriforme. El análisis de sangre no demostró ninguna alteración y el estudio coproparasitológico fue negativo. Se procedió a instaurar un tratamiento con un shampoo con azufre 2% y ácido salicílico 2% cada 48 horas, amoxicilina / ácido clavulánico 22 mg/kilo cada 12 horas por vía oral y gotas óticas con miconazol cada 12 horas. A los 7 días se observó una mejoría en el nivel de prurito y disminuyó significativamente el olor. La citología cutánea y ótica reveló moderada cantidad de levaduras. Se procedió a realizar una biopsia cutánea con un punch número 8 previa aplicación de lidocaína al 2% local. Las muestras fueron teñidas con la tinción rutinaria de hematoxilina eosina y con la tinción de ácido periódico de Schiff (PAS). El resultado de la histopatología informó hiperqueratosis ortoqueratótica laminar severa epidérmica y folicular (Imagen 6) junto a hipergranulosis del estrato granuloso (Imagen 7) y presencia de abundante cantidad de levaduras en estrato córneo (PAS) (Imagen 8). El resultado histopatológico sumado a la edad temprana de

presentación y la signología dermatológica condujo al diagnóstico de ictiosis. El paciente fue tratado con shampoo con sulfuro de selenio 2,5% dos veces por semana por su efecto queratolítico, queratoplástico y antifúngico y con aspersiones diarias de propilenglicol 50% por sus propiedades queratomoduladoras. Luego de 3 años de tratamiento el paciente continúa con buen estado clínico general aunque persisten lesiones dermatológicas (Imagen 9, 10, 11). Debido al carácter congénito de la enfermedad, las lesiones dermatológicas solo pueden manejarse parcialmente con terapia crónicas controlándose el desarrollo de infecciones bacterianas y micóticas secundarias las cuales suelen agravar el proceso de descamación y prurito. A causa de la proliferación de tejido queratinizado en las almohadillas podales (Imagen 12) que provocan incomodidad al caminar, deben de realizarse toilettes quirúrgicas debridantes de dichas lesiones cada 6 meses.

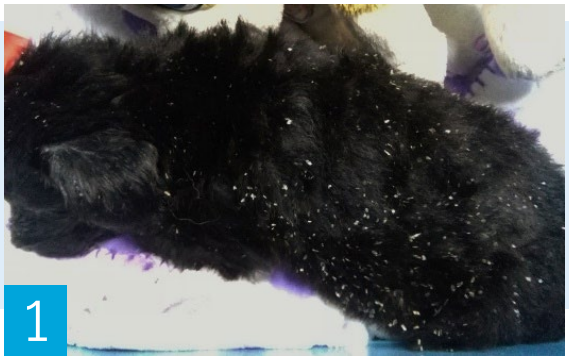

Imagen 1: Vista dorsal del paciente donde puede observarse abundantes escamas blanquecinas.

Imagen 2: Vista más cercana de las escamas parcialmente adheridas a los pelos.

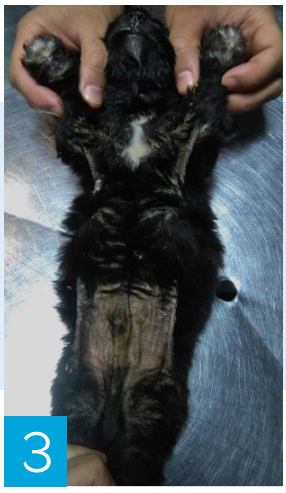

Imagen 3: Vista ventral del paciente donde se observa alopecia y puede apreciarse una alteración en el grosor de la piel la cual se haya además menos elástica y rugosa.

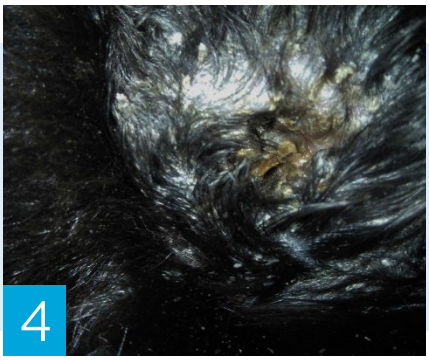

Imagen 4: Secreción ceruminosa abundante en conducto auditivo externo.

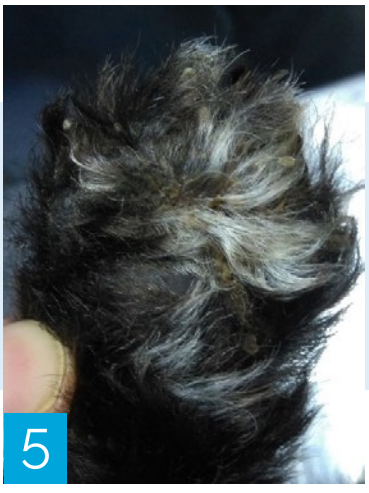

Imagen 5: Placas descamativas en espacios interdigitales.

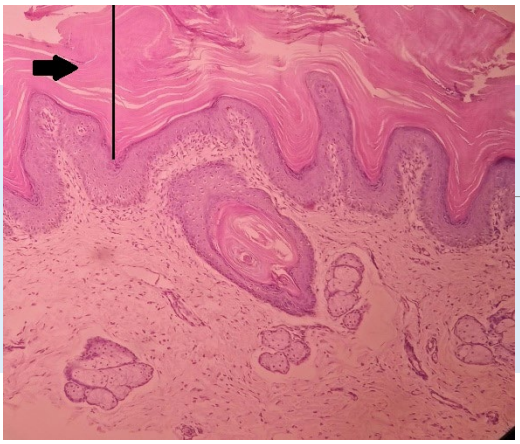

Imagen 6: Imagen histopatológica donde se aprecia hiperqueratosis ortoqueratótica laminar de la epidermis (Flecha negra). Tinción Hematoxilina-Eosina. Imagen a 10X.

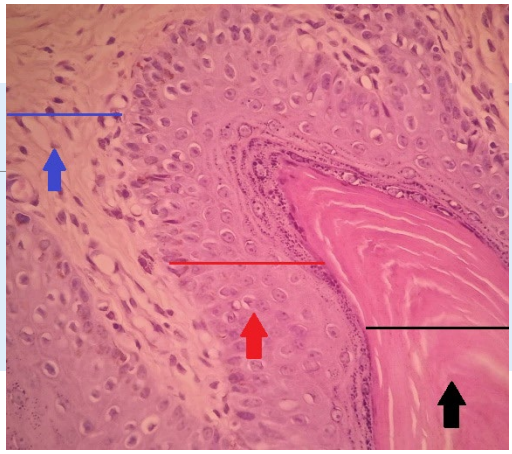

Imagen 7: Magnificación a 40X donde se observa con más detalle la epidermis y la hiperqueratosis ortoqueratótica laminar (Flecha azul: dermis. Flecha roja: queratinocitos nucleados de la epidermis. Flecha negra: hiperqueratosis ortoqueratótica laminar). Tinción de Hematoxilina-Eosina.

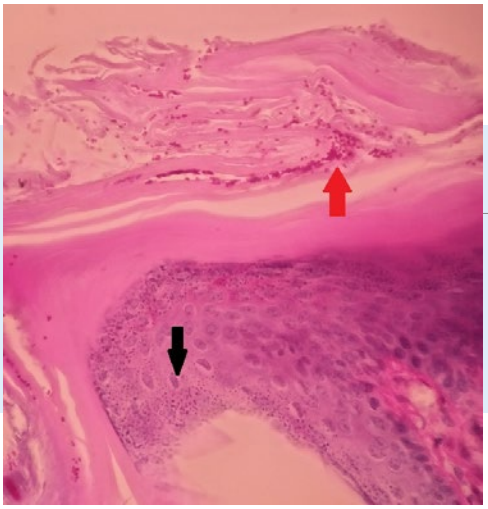

Imagen 8: Imagen a 40X donde se evidencia hipergranulosis (Flecha negra) y presencia de abundante cantidad de estructuras de aspecto levaduriforme compatibles morfológicamente con *Malassezia sp.*(Flecha roja) dispuestas en la capa córnea. Tinción PAS.

Imagen 9: Evolución del paciente a tres años del diagnóstico.

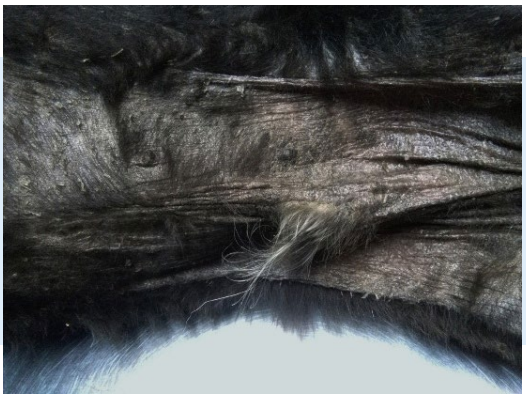

Imagen 10: Mantiene lesiones descamativas en área ventral donde la piel al tacto es más gruesa y áspera.

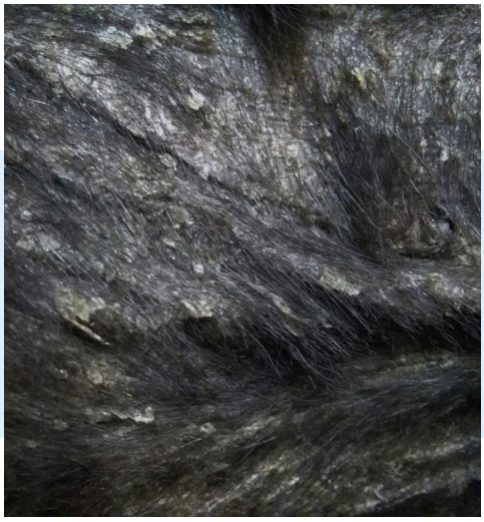

Imagen 11: Imagen a mayor aumento de piel del área ventral abdominal donde se evidencia la presencia de escamas adheridas al estrato córneo.

Imagen 12: Lesiones proliferativas de aspecto laminar en almohadillas podales.

DISCUSIÓN

La cornificación constituye un proceso complejo finamente regulado y orquestado. Durante este proceso, los filamentos de queratina citosólicos se agrupan y compactan para formar un core denso de proteína rodeados de una envoltura córnea y lipídica (1). Los gránulos de queratohialina evidenciables en el estrato granuloso contienen una proteína muy importante llamada profilagrina. La profilagrina es desfosforilada y luego fragmentada por la acción de proteasas séricas formando monómeros de filagrina (1). Esta última es la encargada de agrupar a los filamentos de queratina y conducir a un cambio conformacional celular. La membrana celular de las células epidérmicas en este punto es reemplazada por una envoltura córnea constituida por diferentes proteínas entre las que se incluyen a la involucrina, envoplaquina, periplaquina y loricrina (1). La enzima transglutaminasa interviene en la unión de dichas proteínas entre sí y con las colas de las ceramidas las cuales forman parte junto con colesterol y ácidos grasos libres de la envoltura lipídica. Esta capa se origina a partir de los cuerpos lamelares, vesículas pequeñas conteniendo diversos lípidos originados en la capa espinosa que son descargados al espacio intercelular del estrato corneo (3). La -O-acilceramida constituye un elemento clave en la formación de una membrana lamelar estable por lo que defectos en algunos genes encargados de su biosíntesis (PNPLA 1) (4) pueden conducir a graves alteraciones en la barrera epidérmica. Los filamentos de queratina están constituidos por dos tipos de fibrillas (Tipo I y II). Los defectos genéticos en algún tipo preciso de queratina se manifestarán predominantemente en el área de la epidermis donde predomine este tipo de queratina (1).

Los desórdenes de la cornificación se dividen en dos tipos a saber, primarios y secundarios (2). En los desórdenes de tipo primario, la descamación excesiva se debe a un defecto directo a causa de una mutación en alguno de los genes que codifican tanto a las proteínas estructurales involucradas en la formación del estrato córneo o enzimas indispensables para el transporte o formación de la capa lipídica (1). En cuanto a los desórdenes de tipo secundario, la causa de la excesiva descamación se asocia a otro desorden o condición que promueve generalmente una inflamación y consecuente alteración en el recambio celular epidérmico (2). Dentro de las posibles causas secundarias que promueven esta condición se incluyen dermatitis atópica canina, hipersensibilidad a la picadura de pulgas, sarna sarcóptica, dermatitis por *Malassezia sp.*, hipotiroidismo y linfoma epiteliotrópico (2). Según diversas investigaciones, se estima que cerca del 80% de los desórdenes de la cornificación se deben a procesos secundarios (3). Los desórdenes primarios de la cornificación se diagnostican a partir de una serie de datos que surgen tanto de la reseña, anamnesis, examen clínico general, examen dermatológico y la utilización de diversos métodos complementarios (2). La predisposición de ciertas razas, el inicio temprano de las lesiones, la presencia o ausencia de prurito, la realización de raspados y citologías cutáneas como de tricogramas y cultivos son orientadores. El diagnóstico definitivo se alcanza a partir de los resultados histopatológicos y en algunos casos la realización de estudios genéticos (3).

La ictiosis es un desorden primario de la cornificación de carácter congénito reportada en caninos, felinos, bovinos, porcinos, aves y humanos (2,5,6). Se caracteriza por la hiperqueratosis excesiva de todas las superficies de la piel incluyendo las almohadillas podales (5). Si bien tiene predilección por ciertas razas caninas, pueden surgir mutaciones espontáneas en otras razas o en animales mestizos (7). En medicina veterinaria, se divide en ictiosis epidermolítica y no epidermolítica a partir de los hallazgos microscópicos histopatológicos (5,6,8).

La ictiosis epidermolítica, se caracteriza a nivel histopatológico por la presencia de lisis de queratinocitos tanto en la capa espinosa como granular junto a hipergranulosis e hiperqueratosis (5,7). La visualización de estos hallazgos corresponde a alteraciones en la formación de queratina. Este tipo de defectos se han evidenciado en algunas razas de caninos como el Norfolk terrier, Rhodesian ridgeback y mestizo de Labrador (1,6). Se asocia a un defecto autosómico recesivo que causa una mutación en la queratina epidérmica (KRT10) (1,6). Los pacientes con dicha mutación presentan lesiones descamativas pigmentadas y alopecia multifocal (1,3).

La ictiosis no epidermolítica es una condición que se presume autosómica recesiva (1,5,6). En medicina humana, el termino ictiosis congénita autosómica recesiva (ARCI) hace referencia a un conjunto de condiciones causadas por una variedad de mutaciones que afectan tanto a proteínas como lípidos estructurales y que se desarrollan con diversos fenotipos (4,5). En medicina veterinaria no está caracterizado como en medicina humana pero pueden encontrarse diferencias en cuanto a razas (3,8). En los caninos, la ictiosis no epidermolítica ha sido reconocida en ciertas razas entre las que se incluyen Jack russell

terrier, Gran danés, Golden retriever, Bulldog americano, Cavallier king Charles spaniels (1,2,5), Chihuahua (9) y ha habido un reporte en un Boston terrier (7) y en un Ovejero Alemán (10). Estudios realizados en caninos Jack russel terriers han demostrado un recambio epidérmico acelerado de 3,6 días, lo cual es 6 veces más rápido que lo normal (2).

Dependiendo de la raza, los defectos genéticos autosómicos recesivos parecen enfocarse hacia diferentes blancos de acción (1,5). En la raza Golden retriever, el hallazgo de numerosos corneodesmosomas retenidos en epidermis, lo que sugeriría un retraso en su degradación, constituiría el principal defecto que conlleva al desarrollo de la signología clínica. Publicaciones más recientes han documentado una mutación en PNPLA1 (6,8,11,12). Este gen estaría involucrado en la organización y metabolismo de los lípidos en la capa lamelar, principalmente en la síntesis de -O-acilceramida (4). En esta raza la descamación no es tan intensa como en otras razas. Las escamas suelen ser grandes, adherentes de color blanco a gris las cuales se distribuyen principalmente a nivel del tronco. Puede acompañarse de una hiperpigmentación en el área ventral (axilas, tórax y región inguinal) (3,8,13). Ocasionalmente puede evidenciarse otitis ceruminosa y raramente hiperqueratosis de almohadillas podales (13). Si bien el diagnóstico se realiza generalmente antes del año de edad, también se han informado casos de pacientes con inicio de la enfermedad en la adultez (1,2).

En la raza Jack russell terrier, una mutación en el gen transglutaminasa 1 conllevaría al desarrollo de una hiperqueratosis marcada (1,2,3,6). En esta raza además se ha observado un descenso en los niveles de ácidos grasos libres y de acilceramidas junto a un incremento del nivel de ceramida III en el estrato córneo (1,3).

En lo que respecta a la raza Cavalier king Charles, se ha observado una hiperplasia epidérmica con un número alterado de gránulos de queratohialina los cuales además presentan atipia morfológica sumada a la presencia de alteraciones a nivel de los espacios entre los queratinocitos (3). En esta raza, la mutación afectaría al gen FAM83H aunque no se conoce a ciencia cierta la relación de este gen y el proceso de cornificación (1).

En la raza Gran danés, la mutación afecta al gen SLC27A4, el cual codifica para el transportador 4 de ácidos grasos (1,6). La afectación de dicho gen altera la normal organización de los lípidos en la capa lipídica (5). La histopatología suele ser similar a la de las otras razas descriptas pero llama la atención el acúmulo de un material eosinofílico a nivel folicular correspondiente a glicosaminoglicanos (5). En esta raza las lesiones pueden ser más severas que en las otras razas descriptas (1).

En la raza Bulldog americano, el defecto genético afecta al gen NIPAL-4 (1,6). Este gen también está relacionado con la correcta formación de la capa lipídica. Es notable en esta raza, que la afección por *Malassezia sp.* sea más grave y severa (1).

En la raza Ovejero Alemán, se ha descripto un caso en un canino donde se detectó una mutación en el gen ASPRV1 (6,10). Este gen codifica una proteasa símil retroviral involucrada en el proceso de conversión de profilagrina en filagrina (6,10).

En la raza chihuahua, una mutación en el gen SDR9C7 responsable de la formación de la envoltura lipídica del corneocito sería responsable del desarrollo de esta patología (9).

Si bien no es frecuente que esta entidad se manifieste en caninos mestizos, algunos trabajos publicados reportan la patología en este tipo de pacientes (12). Un caso reportado en 2016 de un canino mestizo de seis meses de edad cruza entre un Golden retriever y un caniche demostró por pruebas genéticas poseer una mutación homocigota en el gen PNPLA-1 (12). El paciente presentó lesiones cutáneas y resultados histopatológicos compatibles con ictiosis no epidermolítica (12). El tratamiento implementado con excelentes resultados consistió en aceites y ácidos grasos esenciales tópicos junto a baños humectantes y la administración de ácidos grasos esenciales por vía oral (12). Si bien en nuestro caso en particular no se realizaron pruebas genéticas, el inicio a temprana edad, el tipo de lesiones y la severidad de las mismas, junto al resultado histopatológico son pruebas suficientes para reportar el caso como un canino mestizo con ictiosis no epidermolítica.

En líneas generales, los pacientes comienzan a manifestar la signologia al momento del nacimiento o poco tiempo después (1,2) como en el caso de nuestro paciente el cual comenzó con los primeros signos dermatológicos a la quinta semana de edad. Los pacientes afectados por esta patología presentan una alteración epidérmica que se manifiesta por el desarrollo de lesiones características (2). Dichas lesiones incluyen la presencia de escamas adherentes sobre la superficie cutánea y adherida a los pelos (1,2,3). Es común observar dichas escamas, las cuales suelen ser gruesas y grandes ascendiendo por los tallos pilosos en forma de cilindros foliculares (2). Las escamas forman parches sobre la piel formando costras. La hiperpigmentación es notable en las áreas ventrales del cuerpo (1,2,3). El aspecto de la piel da la impresión de estar acartonada ya que el alto recambio epidérmico y la hiper-

queratosis promueve que la capa córnea de la epidermis se convierta en una estructura dura, seca y resquebradiza (1,2). Las áreas del puente nasal, trufa y almohadillas podales se engruesa a causa de la hiperqueratosis pudiendo desarrollarse en los márgenes de dichas estructuras áreas de sobrecrecimiento y proyecciones de tejido queratinizado. Las otitis ceruminosas acompañan estos cuadros observándose conductos y pabellones con hiperqueratosis y abundante secreción ceruminosa. Es frecuente el desarrollo de dermatitis por *Malassezia sp.* en forma secundaria al trastorno de la barrera cutánea por lo que estos cuadros suelen acompañarse de abundante grasitud, olor rancio y prurito (2). El dermograma incluye trufa, plano nasal, pabellones y conductos auriculares, dorsal de la cabeza, dorso del animal, axilas, piel del área ventral de cuerpo, inglés, almohadillas podales y espacios interdigitales (2). Las áreas con presencia de pliegues, espacios interdigitales y conductos auditivos suelen ser las áreas más afectadas por el desarrollo de *Malassezia sp.* secundaria (2). Nuestro paciente manifestó la presencia de abundante descamación generalizada, piel endurecida al tacto y de textura inelástica. Lesiones en almohadillas podales y espacios interdigitales junto a una otitis ceruminosa bilateral. Particularmente el paciente manifestó prurito intenso el cual aminoró al realizarse el tratamiento correspondiente para la dermatitis y otitis por *Malassezia sp.* que se desarrolló secundariamente al trastorno de la cornificación.

En lo que respecta al diagnóstico, la presentación de la signología desde el nacimiento o a las pocas semanas de edad es orientativo de esta entidad. Si en cambio las lesiones se desarrollan a edad adulta deberán considerarse todas aquellas causas de seborrea primaria o secundaria (2).

A nivel histopatológico las lesiones incluyen una epidermis de leve a moderadamente hiperplásica con hiperpigmentación variable (6). Es notoria la hiperqueratosis ortoqueratótica epidérmica y folicular junto a la presencia de tapones foliculares (6). Es característica de esta entidad la hipergranulosis de la capa granulosa epidérmica. El número de figuras mitóticas puede ser elevado (1,2,3). En el caso de nuestro paciente, las lesiones histopatológicas coincidieron con lo descripto en la literatura.

Los estudios genéticos son importantes al momento de definir la alteración genómica, sin embargo no son necesarios para confirmar la enfermedad (6,7).

Los diagnósticos diferenciales dependerán de la edad a la que se presenta por primera vez a consulta el paciente y de su historia clínica. La manifestación de lesiones a temprana edad reduce ampliamente el número de diferenciales (2,6). Tener presente que la signología puede agravarse por el desarrollo de infecciones secundarias bacterianas pero principalmente micóticas (2). En pacientes cachorros deberán considerarse como diferenciales procesos infecciosos como cheyletielosis, demodicosis, dermatofitosis y leishmaniosis, otras enfermedades primarias de la queratinización, dietas con déficit nutricional, parasitosis intestinales y alteraciones de la digestión que puedan afectar la absorción de nutrientes (2,3). En pacientes adultos tener presente también como posibles diferenciales además de los ya nombrados para los cachorros, adenitis sebácea, atopia, hipotiroidismo y linfoma cutáneo epiteliotrópico (3). En nuestro paciente, la realización de un examen clínico y dermatológico exhaustivo, junto a la realización de citologías cutáneas y óticas, raspajes, tricograma y observación con lámpara de Wood permitió descartar posibles agentes etiológicos infecciosos y parasitarios. La edad de presentación, la ausencia de signos digestivos, y los estudios de analítica sanguínea y coproparasitológico fueron de utilidad para descartar procesos que pudieran alterar el normal recambio epidérmico de origen metabólico y nutricional. Así mismo, el resultado histopatológico permitió finalmente confirmar la entidad presuntiva.

Con respecto al tratamiento, el carácter crónico e incurable de esta entidad dificulta su manejo. Las alteraciones en el recambio epidérmico conllevan al desarrollo concomitante de infecciones bacterianas y micóticas que empeoran el cuadro descamativo y agravan el prurito (2). Son esenciales los baños frecuentes con productos queratolíticos y queratoplásticos (2,3). La función de los productos con efecto queratolítico radica en favorecer la descamación al reducir la cohesión entre los queratinocitos y por ende reducir el grosor del estrato córneo (2). Los queratoplásticos en cambio promueven normalizar el recambio epidérmico. Si bien se desconoce el mecanismo exacto por el cual los agentes queratoplásticos actúan se presupone que su mecanismo estaría mediado por una acción directa o indirecta sobre el ADN celular disminuyendo el índice mitótico de los queratinocitos basales (2). Dentro de esta línea son útiles aquellos shampoos que contengan ácido salicílico 0,1-2% o peróxido de benzoilo 2,5-5%. También suelen ser muy útiles los shampoos con sulfuro de selenio 2,5% tanto por su efecto queratolítico-queratoplástico como también por su efecto antibacteriano y antifúngico (2). También son útiles las lociones emolientes como el ácido láctico en solución al 3 -12% y el propilenglicol al 50% (2,3). Un estudio realizado en caninos de raza Golden con ictiosis demostró eficacia en la reduc-

ción de descamación al utilizar baños y lociones conteniendo gluconolactonas (13). La administración de ácidos grasos esenciales orales y tópicos son reportados como efectivos cuando se utilizan a largo plazo (12). Los ácidos retinoicos son otra opción en el tratamiento de esta entidad (14). Intervienen en la diferenciación de los queratinocitos, modulación y maduración epidérmica lo que conduce a una reducción en el grosor de la epidermis. Dentro de estos la isotretinoina a una dosis de 1-2 mg/kilo cada 12 horas es reportado como efectivo a largo plazo (2,14). Debido a su carácter teratogénico no debe emplearse en hembras preñadas y deben realizarse periódicos controles de sangre (14). Las anormalidades de laboratorio incluyen hipertrigliceridemia, hipercolesterolemia, elevación de alanina aminotransferasa, aspartato aminotransferasa y fosfatasa alcalina (2). Dentro de los efectos colaterales, la queratoconjuntivitis seca es una de las complicaciones esperables (14). En nuestro caso en particular, la corta edad del paciente desalentó el uso de fármacos que pudieran a largo plazo propiciar el desarrollo de efectos colaterales por lo que se prefirió utilizar una terapia queratomoduladora tópica perdurable en el tiempo.

Conclusiones:

Los desórdenes de la cornificación debido a su carácter multimodal constituyen un desafío en la clínica dermatológica. En lo que respecta a aquellos de origen congénito, la ictiosis canina representa una condición de baja prevalencia. Si bien es más frecuente en determinados tipos de razas caninas también se reportan casos en mestizos. Este trabajo presenta un caso de ictiosis en un canino mestizo y su evolución a lo largo de los años. El inicio a temprana edad, la manifestación de lesiones descamativas severas con un dermograma generalizado junto al desarrollo de hiperqueratosis ortoqueratótica severa epidérmica y folicular fueron elementos claves al momento del diagnóstico. Si bien los estudios genéticos son imprescindibles para el reconocimiento de los genes mutados no son requisito sine qua non para el diagnóstico definitivo. El tratamiento es de por vida y consiste en la queratomodulación y el control de las infecciones secundarias concomitantes.

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ALLERGEN-SPECIFIC IMMUNOTHERAPY IN CANINE ATOPIC DERMATITIS

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ABSTRACT

Canine atopic dermatitis is a multifactorial disease with multiple options of treatments. Allergen-specific immunotherapy is the only therapy that is able to change the course of the disease. Many studies have been published using allergen immunotherapy in dogs with atopic dermatitis. However, many differences on the study methodology make difficult a meta-analysis. The main routes used in allergen immunotherapy are the subcutaneous route, sublingual/oral route and intralymphatic route. The immunological changes aimed with allergen immunotherapy include a shift from Th2 to Th1-biased responses, an increase of regulatory T cells, IL-10 and TFG- ß, besides IgE suppression and increase of IgG andibodies. No severe adverse effects are seen in dogs under allergen-specific immunotherapy.

Key words: allergy; dogs; immunology

RESUMEN

La dermatitis atópica canina es una enfermedad multifactorial con múltiples opciones de tratamientos. La inmunoterapia específica de alérgenos es la única terapia que puede cambiar el curso de la enfermedad. Se han publicado muchos estudios utilizando inmunoterapia con alérgenos en perros con dermatitis atópica. Sin embargo, muchas diferencias en la metodología del estudio dificultan un metanálisis. Las principales vías utilizadas en la inmunoterapia con alérgenos son la vía subcutánea, la vía sublingual/oral y la vía intralinfática. Los cambios inmunológicos buscados con la inmunoterapia con alérgenos incluyen un cambio de respuestas Th2 a Th1, un aumento de células T reguladoras, IL-10 y TFG-ß, además de supresión de IgE y aumento de anticuerpos IgG. No se observan efectos adversos graves en perros en inmunoterapia específica con alérgenos.

PALABRAS CLAVE: alergía; perros; imunología

INTRODUCTION

Canine atopic dermatitis is defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features and commonly associated with IgE antibodies to environmental allergens (1). Although it is a multifactorial disease with numerous treatment options, allergen-specific immunotherapy (ASIT) is the only therapy that can modify the course of the disease (2). ASIT is defined by the World Health Organization as the practice of a gradual and increasing administration of an allergen extract to an allergic subject to ameliorate the symptoms associated with subsequent exposure do the causative allergen (3). To this time, there are several studies using ASIT in the treatment of canine atopic dermatitis. However, a variety of open and uncontrolled studies with different protocols, methods and other variables make difficult to compare results and to state a standard regimen in dogs with atopic dermatitis. There are no differences between allergen tests used for screening allergen composition, with similarities observed whether used *in vivo* intradermal test or whether *in vitro* IgE serology (4). The objective of this article is to make a brief review on published literature regarding ASIT in atopic dogs. Although there are some few studies using food allergen-specific immunotherapy, this review is based on ASIT against environmental allergens.

LITERATURE REVIEW

Mechanism of action

Canine atopic dermatitis is characterized by an immunological dysfunction with up-regulation of Th2, Th1, Th17 and Th22 responses, and increasing expression of cytokines and chemokines, such IL-4, IL-5, IL-13, IL-31, IL-33, IL-22, IL-17, thymus and activation-regulated chemokine (TARC/CCL17), thymic stromal lymphopoietin (TSLP) among others (5,6,7).

The immunological changes observed in dogs under ASIT are synthetized in figure 1. The aim of allergen-specific immunotherapy is the induction of peripheral T cell tolerance, characterized by the production of regulatory T (Treg) cells and promote a shift from a Th2 to Th1-biased response (8). In dogs, this shift from Th2 to Th1 response was associated with an increasing level of interferon-gamma (IFN-**)**, whereas IL-4 level was not changed (9).

An increase of FoxP3+ Treg cells was associated to allergen immunotherapy in two studies (10,11). Treg cells can produce IL-10, which is associated to tolerance, by suppressing the expression of Th cells and activated monocytes and macrophages, and down regulates MHC class-II molecules and antigen-presenting cells capacity (8). Although one study find a significant increase in serum IL-10 in dogs receiving immunotherapy (10), other recent placebo-controlled studies failed to associate increasing levels of this cytokine with allergen immunotherapy (11,12). Transforming growth factor-beta (TGF-ß) is another tolerance-associated cytokine, which has been significantly increased in dogs after immunotherapy (11), although other study failed to do such association when comparing dogs receiving immunotherapy and placebo (12).

IgE suppression and an increasing concentration of IgG antibodies are associated with immunotherapy in dogs in many studies (10,13,14,15,16). IgG can act as a blocking antibody when competing with IgE for the same allergen (15).

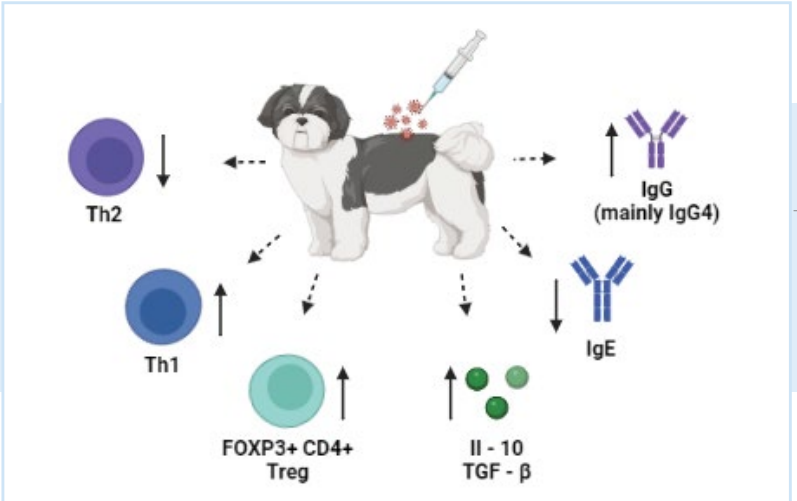

Figure 1. Expected immunological changes after allergen-specific immunotherapy in dogs.

Subcutaneous allergen-specific immunotherapy

The subcutaneous route is the standard route for injection of an allergen extract. Most of the studies of ASIT on atopic dogs use this route. Allergen extracts can be presented in aqueous solutions, which need more frequent injections, or with adjuvants, such aluminum hydroxide, which allow increasing the time between injections (17). Different protocols are available, depending of the manufacturer, but are generally divided into induction and maintenance phases. Maximal improvement can take one year after beginning and owners should be made aware (4). As ASIT is a long-term therapy with no immediate effects on clinical signs, owner compliance is essential for keeping the dog under therapy for at least 12 months and enhance the rates of success (18). Results of the success rate of subcutaneous ASIT from several published studies are disposed in box 1.

Box 1. Success rate of allergen immunotherapy in dogs with atopic dermatitis in several studies, using subcutaneous route. Adapted from Mueller, 2019 (4).

Study reference	Number of dogs	Success rate[a]
Scott et al., 1993 (19)	144	60%
Mueller and Bettenay, 1996 (20)	146	58%
Nuttall et al., 1998 (21)	186	22%
Zur et al., 2002 (22)	169	52%
Schnabl et al., 2006 (23)	117	64%
Plant and Neradilek, 2017 (24)	103	57%
Fennis et al., 2020 (25)	664	60%
Han et al., 2020 (26)	37	35%

a Percentage of "good to excellent" improvements.

Sublingual/oral immunotherapy

Sublingual immunotherapy relies on the fact that the patient needs to keep small amounts of allergens under the tongue for minutes before swallowing. This is feasible for humans, but not for dogs. In dogs, the extract is applied between the lips and gums once or twice a day. For this reason, oral immunotherapy is the best term for animals (4). This route induces a local immune response in the oral cavity to promote a tolerogenic environment, reducing Th2 polarization, through the allergen uptake by tolerogenic dendritic cells (27) Oral immunotherapy for the control of spontaneous canine atopic dermatitis has a success rate similar to subcutaneous ASIT (15), although a recent study demonstrated a lower success rate after 12 months when compared to subcutaneous and intralymphatic routes (28). The authors suspected that this lower response was related to inadequate contact time between the medication and oral mucosa (28).

Rush immunotherapy

With subcutaneous rush immunotherapy, the induction phase is given in 1 day, with allergens injected hourly and dogs monitored clinically in the hospital or veterinary clinic (4). A study showed 70% of good to excellent response in dogs with atopic dermatitis using aluminum-precipitated allergen extracts (29). It is recommended to give an antihistamine 1 to 2 hours before the injection of an allergen extract (4). It seems to be well tolerated in dogs, although it may increase pruritus (29,30).

Intralymphatic immunotherapy (ILIT)

Intralymphatic route is based on the injection of the extract directly in a lymph node, in order to reduce the time of the induction phase and enhance owner compliance (31). Previous studies showed that monthly injections of aluminum-precipitated allergen extract might reduce clinical scores in few months and provide long-

lasting effects in some dogs, with no major adverse effects (31,32). A recent study compared ILIT to subcutaneous and oral immunotherapies in dogs with atopic dermatitis (28). This study showed that dogs under intrapymphatic route had a higher return to normal rate compared to dogs under the other routes, in a 12-month follow-up (28). Finally, other study compared the efficacy of ASIT after an induction phase with ILIT and rush immunotherapy, with similar positive results on both alternatives (33).

Advances in allergen immunotherapy in dogs

Modern researches use new adjuvants, allergoids and recombinant allergens. These researches aim to develop products that have a shorter induction phase and a faster period for beneficial achievement.

The Allermune HDM (Nippon Zenyaky Kogyo – Zenoaq; Fukushima, Japan) is a subcutaneous immunotherapy with recombinant Der f 2 adjuvanted with the maltotriose polymer pullulan, already available on veterinary market. The first study used eight dogs experimentally sensitized to recombinant Der f 2, a low-weight *Dermatophagoides farinae*-derived allergen (34). In this study, six of eight dogs received Allermune immunotherapy under manufactures instructions, whereas two dogs received placebo. After 25 weeks, dogs receiving immunotherapy presented skin lesion scores significantly lower than dogs receiving placebo, with no adverse effects (34). Other study showed clinical benefits in the majority of the dogs on day 120 after beginning immunotherapy, with important reductions in the pruritus, skin lesions and the use of glucocorticoids (35). A third study showed the same beneficial responses in most of dogs as early as day 42 (36). It is interesting to note that on these two last cited studies, most of the dogs were sensitized to multiple allergens besides *D. farinae.*

Another novel allergen agents used in immunotherapy include glucotaraldehyde-polymerized allergoids conjugated with mannan (PM-allergoids). These allergoids target dendritic cells and enhance allergen uptake (37). A study used PM-allergoids from *D. farinae* for ASIT in *D. farinae*-sensitized atopic dogs, demonstrating reduction in pruritus and medications stores in a short period (few months), with no major adverse effects (37).

Adverse effects

ASIT is a very safe therapy. The most common adverse effect is an increased pruritus after allergen administration. Other very uncommon clinical signs may include localized injection reaction, anxiety, depression, hyperactivity, sleepiness, diarrhea, vomiting, urticarial, angioedema, weakness and anaphylaxis (4).

Conclusions

Allergen-specific immunotherapy is the only treatment for canine atopic dermatitis which can modify the course of the disease. It is a safe, medication-sparing therapy. The use of new adjuvants, allergoids and recombinant allergens seems to improve this kind of treatment, reducing time for clinical benefits and increasing owner compliance.

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

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

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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 [2] . 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.[1]

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 relationships [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.[4–6] 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 [6,7]

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.[8] 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 [8].

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[9]. 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.[7]

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 [9].

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[7]. 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.[7,9]

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. [7,9]

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.[7]

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. [11].

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.[12]

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 germ-free 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.[14] 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.[12]

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 quarter of the bacteria studied stimulated Treg and others affected innate lymphoid cells and dendritic cells 17[18].

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.[19]

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.[20]

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.[21]

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[20]. 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.[22] 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.[12] 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. [23]

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. [27] 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.[27–29]

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 [5,29]

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.[30–33]

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. [25]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. [9,25,34] 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.[9,35]

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[39–41], 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.[26,30–33,42] 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[43]. 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.[44] 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.[52]

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*).[51] 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).[55,56]

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.[45–51] 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. [57] 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[52,54] 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.[45,47,50]

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.[5,6,58]

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.[45,47–51,55,56,59,60]

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.

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