MYSTERY SLIDE SESSION

MYSTERY CASE #1:
Presenter: Judith Nimmo. ASAP Pathology, 53 Glenvale Cres, Victoria, Australia 3170.
A 4 year old male Maltese-cross dog had a > 2 year history of pruritic skin disease that had been treated with antibiotics, topical antifungal drugs, prednisolone and for the past 2 months with Atopica (cyclosporin A). The dog presented with a severe, progressive liquifactive cellulitis of the right foreleg. Despite aggressive antibiotic therapy the leg had to be amputated two weeks later.

MYSTERY CASE #2.
A common ringtail possum (Pseudocheirus peregrinus, Boddaert 1785) was trapped in the course of a surveillance project in south eastern Australia. The animal was lethargic and had scabbed and ulcerative lesions on face, feet and tail, with swelling of the nasal bridge, one hand, wrist and hock. The possum was euthanased and submitted for autopsy. The swollen hock yielded yellowish mucoid discharge; no bacteria were found on routine culture. Viscera were grossly unremarkable. The section provided is from the swollen hand, not at the site of ulceration.

MYSTERY CASE #3
Judith Nimmo. ASAP Pathology, 53 Glenvale Cres, Victoria, Australia 3170.
A 10 year old spayed female Labrador Retriever was presented with an ulcerative, lesion on nasal planum on the dorsal aspect of left naris that been present about a month. There had been no response to a week of antibiotics. There was no nasal discharge and the nasal mucous membranes look normal on scope. The nasal planum also appeared hyperplastic and dry.

MYSTERY CASE # 4:
Presenter: Emily Walder, Independent Slide Consultation Service, Venice, CA, USA
Jacob is a 4-year-old, neutered male Chihuahua with a 4 month history of a nonpruritic, alopecic patch on the top of the head. More recently, a similar lesion with mild erythema and scale developed near the base of the right pinna. DTM and skin scrapes were negative. There was no response to Revolution or ivermectin.

MYSTERY CASE #5,
Presenter: Emily Walder, Independent Slide Consultation Service, Venice, CA, USA
Sadie is a 7-year-old, neutered female Pomeranian with a history of moderate pruritus, partial to complete alopecia and scale on dorsal neck and trunk. She has polyuria/polydipsia and low T4. Low-dose dexamethasone suppression and ACTH stimulation tests were within normal limits

MYSTERY CASE #6.
Presenter: Emily Walder, Independent Slide Consultation Service, Venice, CA, USA and Sonya Bettenay Tierdermatologie Deisenhofen, Germany.
A 12 year-old MC Yorkshire terrier was seen for a progressive pawpad lesion of 5 months‘ duration, nonresponsive to antibiotics, starting as a small, superficial hole in the large RF pad and eventually involving the caudal haired skin junction. No other dermatologic or mucosal abnormalities were present.
INTRODUCTION
The goal of this interactive session is to interpret pathology reports, formulate a morphologic diagnosis and correlate both with clinical presentations.
In an interactive session we will establish morphologic diagnoses, differential diagnoses of each case, determine which clinical lesions would be expected and what techniques and procedures will assist to further evaluate the cases.

CASE 1: “Nantucket”, 10 year old Westfalian gelding
Histopathology report: Examined are sections of haired skin in which the epidermis is diffusely hyperplastic (acanthotic) and covered by a thick crust. The superficial, mid and deep dermal vessels are prominent and lined by plump endothelial cells. Many superficial dermal vessels contain homogeneous eosinophilic material and have indistinct brightly eosinophilic walls. Other vessels have pale vascular walls (hyalinization). Fragments of pyknotic nuclei are scattered within the perivascular space intermixed with few lymphocytes, plasma cells, neutrophils and red blood cells (micro-hemorrhage). The large crust is composed of brightly eosinophilic collagen embedded in degenerate inflammatory cells serum lakes, hemorrhage coagulative necrosis of epidermis. At the surface of the crust are numerous aggregates of bacterial colonies.

Questions for discussion:
Morphologic diagnosis?
What are the expected clinical lesions?
What additional information would you like to ask the referring clinician?
What are your differential diagnoses?

CASE 2: “Memphis Belle”, 8 year-old female spayed smooth coated collie
Histopathology report: Examined are four bisected punch biopsies of haired skin that extend to and include the subcutaneous adipose tissue. The epidermis is multifocally elevated in a plaquelike fashion. In the elevated areas, there is marked parakeratosis, which extends into the follicles. Multiple small, rather well defined crusts are embedded within this thick keratin layer. Some colonies of bacterial cocci are noted in the superficial crusts. Multifocally, small pustules are present within the hyperplastic epidermis. The superficial dermis is expanded by a continuous band of plasma cells with fewer lymphocytes. Large numbers of individual neutrophils transmigrate though the hyperplastic epidermis.

Questions for discussion:
Morphologic diagnosis?
What are your differential diagnoses?
What are the expected clinical lesions?
What additional information would you like to ask the referring clinician?

CASE 3: “Romy”, 10 year-old Thoroughbred gelding
Histopathology report: A1: Three replicate sections of a bisected punch biopsy are examined in which the surface is diffusely covered by a sero-cellular crust overlying intact epidermis. The dermis is infiltrated by a pleocellular inflammatory infiltrate. The crust is composed of necrotic epidermis, inflammatory cells and collagen bundles. The dermal infiltrate is composed of
histiocytes, fewer lymphocytes, plasma cells, rare granulocytes. Multifocally, there are multinucleate giant cells. Multifocal neutrophilic exocytosis is present.

B1: Two replicate sections of a bisected punch biopsy of haired skin are examined in which there are similar dermal lesions as described in A1. The inflammatory lesions are more severe, but there are no sero-cellular crusts.

Questions for discussion:
Morphologic diagnosis?
What are your differential diagnoses?
What are the expected clinical lesions?
What are your next steps to rule in/out differential diagnoses?

CASE 4: “Aiden” male castrated, 2 year-old Shepherd-mix
Histologic description: Examined are two bisected punch biopsies of haired skin, one of which includes a sinus hair (vibrissa). The dermis contains a superficial diffuse infiltrate composed of numerous histiocytes, plasma cells, lymphocytes and rare clusters of degenerate neutrophils. Some histiocytes contain melanin pigment. The epidermis is thickened to 9 layers and rare individual apoptotic keratinocytes are observed. There are multifocal intracorneal clefts that are filled with lakes of homogeneous eosinophilic material (serum). Multifocally, inflammatory cells are migrating into the epidermis resulting in obscuring of the dermo-epidermal junction.

Questions for discussion:
Morphologic diagnosis?
What are your differential diagnoses?
What are the clinical lesions observed?
Is there additional information you could get from the biopsy to confirm one of the diagnoses, rule out others?
What are your next steps to rule in/out differential diagnoses?

CASE 5: “Molly” female spayed, 10 year-old Labrador retriever
Histopathology report: A: Examined are duplicate sections of a bisected punch biopsy in which dermis and subcutis are largely effaced by nodular, coalescing cellular infiltrate composed of large round cells with round to oval or indented vesicular nuclei (histiocytes) and numerous smaller round cells with little cytoplasm (lymphocytes) and intermediate round cells. The latter population has round nuclei with stippled chromatin and a small to intermediate amount of pale cytoplasm. Anisocytosis and anisokaryosis of this latter cell population is moderate. Twenty mitotic figures are observed in ten 400x fields. Multifocally, there are areas of pale indistinct wispy collagen separating preexisting dermal collagen bundles and the nodular infiltrate is tightly surrounding dermal vessels. B. Examined are three bisected punch biopsies with lesions similar to A. In addition, the epidermis is regionally ulcerated and the associated superficial dermis is diffusely necrotic, with large numbers of neutrophils and abundant colonies of basophilic cocci.

Questions for discussion:
Morphologic diagnosis?
What are your differential diagnoses?
What are the expected clinical lesions deduced from the histopathology?
What are your next steps to rule in/rule out differential diagnoses?

CASE 6: adult female Ring-tailed Lemur
Histopathology report: A: Examined are multiple skin samples from “affected skin”, characterized by small hair follicles. Most follicles have an irregular outer root sheath and either lack hair bulbs (interpreted as telogen, resting phase) or have small remnants of hair bulbs. Many of these hair follicles lack hair shafts. There is no evidence of inflammation. The overlying epidermis is moderately acanthotic and there is compact to lamellar hyperkeratosis. B: Examined
are two sections of “non-affected” skin. The samples are within normal limits with many anagen hair follicles.

Questions for discussion:
Morphologic diagnosis?
Major clinical skin lesions deduced from the histopathology?
Differential diagnoses?
Do you have additional questions in regards to history?

CASE 7: “Salsa”, 4 year-old, male castrated, DMH, orange tabby cat
Histopathology report: A. Haired skin: Multiple sections of markedly acanthotic and hyperkeratotic haired skin are examined in which there is a similar process. There are multiple large subcorneal or intraepidermal accumulations of mostly viable neutrophils that are often associated with hair follicles extending into the infundibular region. The superficial dermis is expanded by edema, and expanded by a moderate to dense inflammatory cell infiltrate that is perivascular to diffuse. The inflammatory cell infiltrate consists of numerous neutrophils admixed with moderate numbers of mast cells, plasma cells, lymphocytes, and occasional Russell bodies. A few nodular foci of histiocytes and neutrophils are surrounding remnants of ruptured hair follicles, such as keratin squames and small clusters of disrupted glands. There is moderate to marked spongiosis of the epidermis in several areas. Within some of these intraepidermal pustules, there are individualized, rounded, brightly eosinophilic keratinocytes admixed with the inflammatory cells. In some sections there are copious amounts of parakeratotic keratosis, admixed with neutrophils, serum and granular, basophilic material forming a thick crust. There are occasional rounded, bright pink cells (keratinocytes) within the crusts. In less affected areas the subcorneal pustules coalesce to form a smaller crust. B. Crusts: These two slides consist of multiple sections of thick crusts that are composed of serum, keratin layers, degenerated neutrophils and occasional rafts of epithelial cells.

Questions for discussion:
Morphologic diagnosis?
Major clinical skin lesions deduced from the histopathology?
Differential diagnoses?
What are your next steps to rule in/rule out differential diagnoses?

CASES 8: “Micky”, 5 year-old, male neutered DSH cat
Histopathology report: Examined is a bisected punch biopsy of skin in which there is marked atrophy of the hair follicles, epidermal hyperplasia and a mild superficial perivascular dermal infiltrate. The hyperplastic epidermis and remaining superficial portion of the remaining follicular epithelia lack features of differentiation and are characterized by irregularly arranged keratinocytes with moderate to marked anisocytosis and anisokaryosis. Occasional karyomegaly is observed and numerous keratinocytes contain two to three nucleoli. There is mild multifocal micro-hemorrhage and thin wavy collagen bundles are seen within the superficial dermis. The mild perivascular dermal infiltrate is composed of mast cells and lymphocytes.

Questions for discussion:
Morphologic diagnosis?
What do you expect to see clinically?
What are your differential diagnoses?
Do you have specific questions in regards to history?

CASE 9: “Coco”, 5 year-old, mixed breed dog
Histopathology report: Three bisected punch biopsies of haired skin are examined in which there is a severe, inflammatory cellular infiltrate within the dermis, multifocal to coalescing transepidermal necrosis with ulceration. The inflammatory infiltrate is predominated by
eosinophils, which infiltrate and replace the follicular epithelia resulting in prominent eosinophilic cuffs surrounding free hair shafts (eosinophilic furunculosis). The inflammatory infiltrate coalesces to a diffuse dermal infiltrate in some areas. Admixed with the eosinophils are fewer neutrophils, macrophages, mast cells and occasional lymphocytes. The epidermis is multifocally eroded or ulcerated and there is marked protein rich dermal edema. The endothelial cells lining the small dermal vessels are plump and there are numerous eosinophils within the vascular Lumina. Inflammatory cells multifocally infiltrate, disrupt or efface hair follicles and adnexal structures in the most severely affected sections and extend into and replace regions of the epidermis. The infiltrate is composed of large numbers of neutrophils and eosinophils with fewer lymphocytes, plasma cells, macrophages, reactive fibroblasts, and occasional mast cells. Occasionally these cells transmigrate vessels walls, which are lined by plump reactive endothelium throughout the sections. Eroded and ulcerated surfaces are replaced by lakes of serum, blood, and non-degenerate neutrophils, and in these areas, there is severe edema in the dermis with marked acanthuses of the remaining epidermis and follicular epithelium.

Questions for discussion:
Morphologic diagnosis?
What do you expect to see clinically?
What are your differential diagnoses?
Do you have specific questions in regards to history?

CASE 10: “Diego”, 4 year-old, male Chihuahua-terrier mix
Histopathology report: A. Two bisected sections of haired skin are examined. One section is characterized by marked deposition of very fine fibrillar, to more homogeneous eosinophilic and partially basophilic material between the preexisting collagen bundles (interpreted as ischemic change of collagen). The follicles are atrophic and retracted. The epidermis is slightly acanthotic and occasional apoptotic basal calls can be seen. Small dermal vessels often have indistinct swollen vascular walls and endothelial cells are absent. B. Examined is a section of skin overlying a layer of cartilage. The latter is focally necrotic. There is a fine fibrillar matrix replacing normal superficial collagen. A mild mononuclear infiltrate is noted including some histiocytes that contain brown pigment (interpreted as pigmentary incontinence). Small linear epithelial structures represent remnants of atrophied hair follicles.

Questions for discussion:
Morphologic diagnosis?
Major clinical skin lesions?
What are your differential diagnoses?
Do you have additional questions in regards to history?
Autosomal recessive congenital ichthyosis in American bulldogs is associated with decreased expression of ICHTHYIN (NIPAL4).

MAULDIN EA1, WANG P1, EVANS E2, CANTNER CA3, FERRACONE JD1, CREDILLE KM4, Casal ML2

Department of Pathobiology1 and Section of Medical Genetics2, School of Veterinary Medicine, University of Pennsylvania, Pennsylvania, USA
3Brandywine Veterinary Hospital, Chadds Ford, PA
4Eli Lilly and Co., Indianapolis, IN

ABSTRACT: A minority of human patients with non-syndromic autosomal recessive congenital ichthyosis (ARCI) display mutations in ICHTHYIN (NIPAL4). This protein is thought to play a role in epidermal lipid metabolism, although the mechanism is unknown. A mild to moderate form of ICHTHYIN associated ARCI was identified in an extended pedigree of American bulldogs. The gross phenotype was evidenced by a disheveled pelage shortly after birth. All dogs had persistent generalized scaling, as well as adherent brown scale with erythema of the abdominal skin. Pedigree analysis was highly suggestive of an autosomal recessive mode of inheritance. Ultrastructurally, the epidermis showed abnormal lipid processing evidenced by discontinuous lipid bilayers in the stratum corneum, unprocessed lipid within corneocytes, and clear vacuoles within lamellar bodies. Linkage analysis revealed an association with NIPAL4, and an SINE insertion upstream of exon 1 in a highly conserved region was discovered and believed to be the cause of disease. Out of 545 DNA samples from American bulldogs, 32 dogs (17 females, 15 males,) were homozygous for the larger PCR fragment. All affected dogs were homozygous, with parents heterozygous for the insertion. Expression of NIPAL4, assessed by immunolabeling, showed an absence of ichthyin in the granular cell layer of the epidermis. This is the first description of a spontaneous autosomal recessive congenital ichthyosis associated with decreased expression of NIPAL4 in a nonhuman species.

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Canine Epidermal Neural Crest Stem Cells – Characterization, Isolation and Expansion
Barbara Gericota, Joseph S. Andersson, Gaela Mitchell, Dori L. Borjesson, Beverly K. Sturgess, Jan A. Nolta, Maya Sieber-Blum
(1) Stem Cell Program, Institute for Regenerative Cures, University of California Davis, USA
(2) School of Veterinary Medicine, University of California, Davis, USA
(3) Institute of Genetic Medicine, Newcastle University, UK

The embryonic neural crest has the ability to generate an astonishing variety of cell types and tissues in the adult vertebrate organism. The discovery of neural crest stem cells in an adult, readily accessible location opens a variety of opportunities for patient-specific therapies. We present, characterize, and provide protocols for the isolation of canine epidermal neural crest stem cells (cEPI-NCSC) - remnants of the embryonic neural crest in the adult hair follicle. Furthermore, we developed novel tools for research in canines. Similar to human and mouse EPINCSC, the neural crest origin of cEPI-NCSC is shown at the RNA and protein levels by expression of neural crest molecular signature and other neural crest-characteristic genes. In parallel to human EPI-NCSC, cEPI-NCSC also expressed pluripotency genes. We showed that cEPI-NCSC could generate all major neural crest derivatives. Multipotency and ability to self-renewal were demonstrated by in vitro clonal analyses, establishing cEPI-NCSC as multipotent somatic cells. A critical literature analysis on canine spinal cord injury (SCI) showed the need for novel treatments and suggested that cEPI-NCSC represent viable candidates for cell-based therapies in dogs with SCI. This concept is supported by the close ontological relationship between neural crest stem cells and spinal cord stem cells. Together, we provide the groundwork for the development of a novel cell-based therapy for a condition with poor prognosis and limited treatment options.

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Conflict of Interest
The authors indicate no potential conflicts of interest.
THE MILLIONS OF MICROORGANISMS INHABITING THE SKIN: THE CANINE SKIN MICROBIOME

Rodrigues Hoffmann A1, Patterson AP2, Diesel A2, Lawhon SD3, Ly HJ1, Stephenson C4, Mansell J1, Steiner JM4, Dowd SE5, Olivry T6, Suchodolski JS4

1Dermatopathology Specialty Service, Department of Veterinary Pathobiology; 2Clinical Dermatology Service, Department of Small Animal Clinical Sciences; 3Clinical Microbiology Laboratory, Department of Veterinary Pathobiology, 4Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX
5MR DNA Laboratory, Shallowater, Texas
6Department of Clinical Sciences, College of Veterinary Medicine, and Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC
arodrigues@cvm.tamu.edu

INTRODUCTION

Molecular-based sequencing studies targeting the bacterial 16S rRNA gene have revealed that the skin surface of humans is inhabited by a diverse and variable microbial population composed of commensal, symbiotic, and pathogenic bacteria, defined as the microbiome. Recent studies have shown that an imbalance in these microbial populations may result in damage to the skin, and development of skin lesions, although it is still unknown if an altered microbiome is the cause of skin lesions, or the result of an altered skin barrier.

The skin microbiome can be altered by host factors including presence of hair follicles, temperature, pH, moisture, environmental contact, and contact with mucous membranes. These different factors will influence the different skin microenvironments, which can be divided into dry, moist and sebaceous regions.

3-5,9 For instance, sebaceous areas are primarily colonized by Propionibacterium spp., whereas moist areas are more likely to be colonized by Staphylococcus spp. and Corynebacterium spp.

Age is also considered to be one of the host factors influencing the composition of the skin microbiome, with the proportions of Propionibacterium spp. increasing in the forehead with age, whereas Staphylococcus spp. and Streptococcus spp. proportions often decrease with age.1,10 These microbial populations can also be altered in disease states, which often result in lower diversity of these microbial populations.

The environmental factors have also been considered to be an important factor responsible for changes in the skin microbiome. A recent study demonstrated that individuals cohabiting with dogs often had a more diverse microbiome, and that family members that cohabited with dogs were more likely to have share their skin microbiome.

THE SKIN MICROBIOME IN HEALTHY DOGS

In a recent study,7 the sequence analysis revealed high individual variability between all samples collected from the different skin regions and between different dogs. Higher number of bacterial species (bacterial diversity) were observed on the haired skin (axilla, groin, periorcular, pinna, dorsal nose, interdigital, lumbar) when we evaluated the skin microbiome in different cutaneous and mucocutaneous regions in healthy dogs and in the axilla, groin, interdigital skin, and nostril of allergic dogs. A large scale DNA sequencing system (454 pyrosequencing) was used to sequence the bacterial 16S rRNA gene from skin swabs.

Compared to non-haired skin or mucocutaneous junctions (lips, nose, ear, and conjunctiva) (Figure 1). The nostril and conjunctiva were the skin regions with the lower diversity, whereas the axilla and dorsal nose had the higher diversity, with up to 486 bacterial genera being identified on the samples from the dorsal
nose from healthy dogs (average of 296 bacterial genera per dog).

Figure 1. Representative taxa at the genus level showing the higher diversity in the samples from haired skin (axilla), followed by mucocutaneous junction (lip comissure), with the nasal mucosa having the lower diversity. Each color shade represents a different genus.7

Some of the most abundant phyla identified across all samples included Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes (Figure 2). The bacteria in the genus *Ralstonia* (phylum Proteobacteria, family *Oxalobacteraceae*) were significantly more abundant in the samples from the healthy dogs. The family *Moraxellaceae* was the most abundant in the nostril.
Figure 2. Average of most common bacterial phyla and families in the different skin regions in the healthy dogs. The group “Other phyla” includes 12 phyla that each were present in only low abundance. *PLOS* one, 2014: e83197.

**THE SKIN MICROBIOME IN ALLERGIC DOGS**

Samples from the axilla, groin, interdigital skin and nose were collected from 6 dogs diagnosed with atopic dermatitis. None of the dogs had skin lesions at the time of collection or were being treated with antibiotics. The haired skin samples and nostril of dogs with allergic skin disease had lower number of observed bacterial species (median 125) and diversity when compared to the same skin sites of healthy dogs (median 239) (Figure 3).
Figure 3. Number of observed bacterial species in pooled samples from the nostril, interdigital skin, axilla and groin from healthy versus allergic dogs. The top curved line represents the healthy dogs and the lower line represents the allergic dogs. The samples from the healthy dogs had higher number of observed species when compare to allergic dogs. *PLOS one*, 2014: e83197.

Significant differences were observed at different phylogenic levels when comparing allergic versus healthy dogs (Figure 4). Some of the most common bacteria genera identified in the skin of allergic dogs included *Alicyclobacillus* spp., *Bacillus* spp., *Corynebacterium* spp. *Staphylococcus* spp., and *Sphingomonas* spp. One of the major differences was the abundance of *Ralstonia* spp. (Betaproteobacteria) in the skin of healthy dogs, whereas significantly lower proportions of this bacteria was observed in the allergic dogs. This was likely an environmental contaminant in the skin of healthy dogs.

Similar to what was observed in this study, lower bacterial diversity has been previously described in the skin of children with atopic dermatitis (AD). In AD children, reduction in microbial diversity, is followed by increased abundance of cutaneous *S. aureus* is described during skin flares. It is also described that increases in the abundance of *Staphylococcus* and reductions in microbial diversity precede an increase in the severity of AD. In AD children, antimicrobial or anti-inflammatory medications (hypochlorite baths) decreased, but did not eliminate, *S. aureus*. Interestingly, the changes in the microbial diversity during flares were reversed even before clinical improvement was seen. We have yet to evaluate the microbial diversity and composition of the skin following treatment with topical or systemic antimicrobial and anti-inflammatory drugs in dogs and other species.
Figure 4. Average of most common bacterial phyla in the axilla, groin, and nostril of allergic versus healthy dogs. The group “Other phyla” includes 12 phyla that each were present in only low abundance. On average, the nostril and groin of allergic dogs were colonized with higher proportions of bacteria in the family *Staphylococcaceae*, and lower proportions of bacteria in the family *Oxalobacteriaceae*. Higher proportions of bacteria in the family *Corynebacteriaceae* were also observed in the groin and interdigital region in allergic dogs.

FUTURE DIRECTIONS

Being able to examine the dynamics and how bacterial communities contribute to a health status or to the development of disease is one of the major advantages of microbial genomics. Examining these bacterial interactions could allow us to better understand how these communities contribute to health and disease, and perhaps identify successful treatment for skin diseases. A better understanding of the skin microbiome could allow us to develop new therapies that enhance beneficial microbes, and perhaps reduce the usage of systemic antibiotics. Our first study only included a small cohort of dogs, and in order for us to make any final conclusions about the microbes inhabiting the skin, additional studies are needed to evaluate a larger number of individuals, and it is necessary to develop serial studies to evaluate the skin microbiome shifts that occur during disease states in dogs and other animals.

LITERATURE CITED


