NAVDF

North American Veterinary Dermatology Forum

MAY 1-5

2018

MARRIOTT WAILEA BEACH RESORT & SPA

Maui, Hawaii

2018

31ST PROCEEDINGS OF

NAVDF

NAVDF

North American Veterinary
Dermatology Forum

NAVDF 2019

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TEXAS

April 10-13

HILTON AUSTIN

Changing the approach to the pruritic dog. Are you ready?

A dermatology portfolio of skin health solutions has transformed the approach to the pruritic dog. Zoetis treatments offer fast relief from pruritus, flea infestation and infection while still allowing you to diagnose and support long-term management. **Protect the bonds that matter—and let dogs and owners get back to enjoying life.**

Learn more at **www.ScienceOfStrongerBonds.com**

ZOETIS PETCARE

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ABSTRACTS

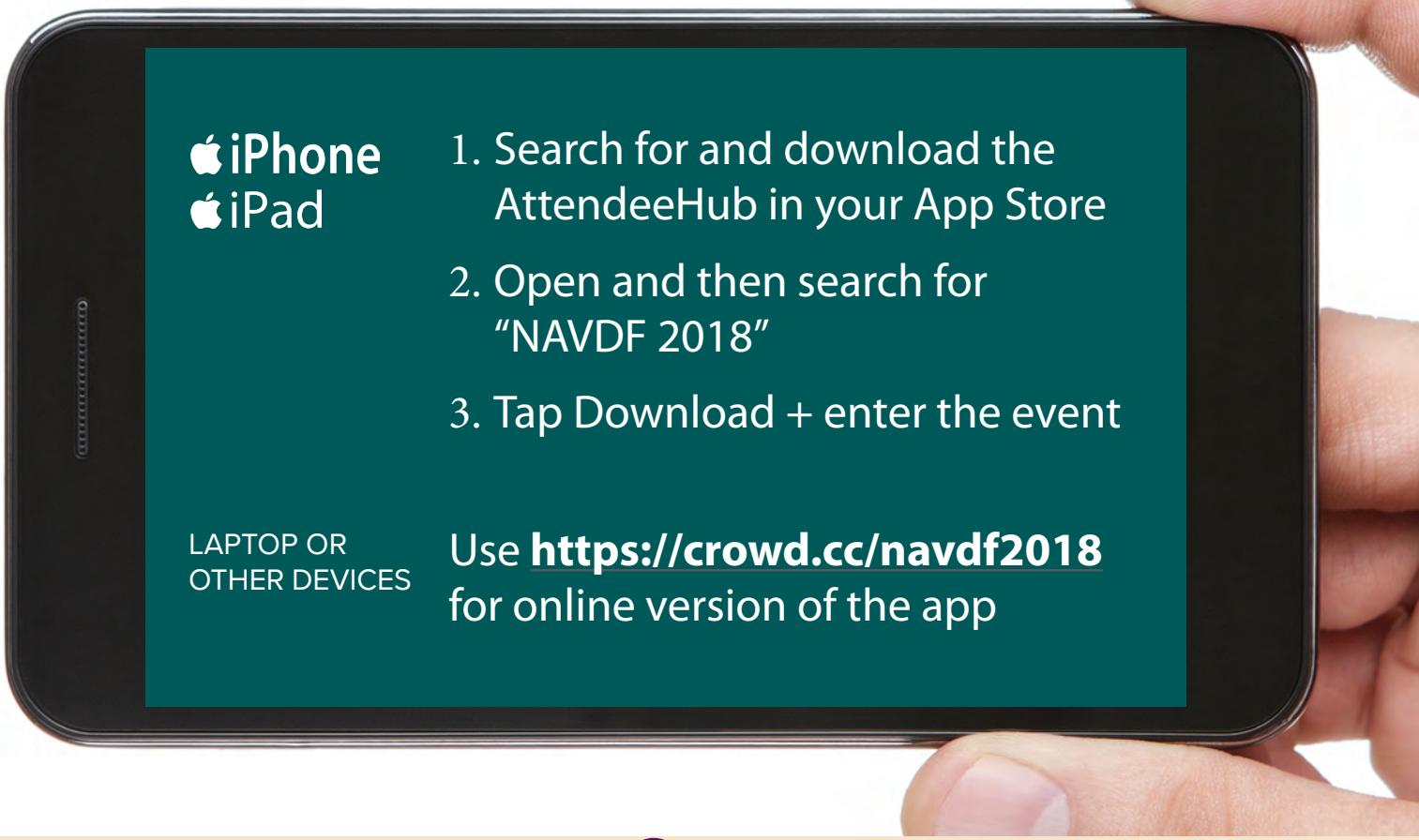
#detectDex

Dex is ready to explore Maui. While we'd love for him to take a few surf lessons, we want to make sure he's not crashing any luaus. Help us keep track of him during the conference.

If you spot him, make sure to snap a photo and share it on the app using your Instagram account. **Remember to tag the NAVDF (@navdf) and use the hashtag #detectDex.**

Once your photo is shared, return Dex to his dog house at NAVDF registration and claim your reward!

APP DOWNLOAD INSTRUCTIONS



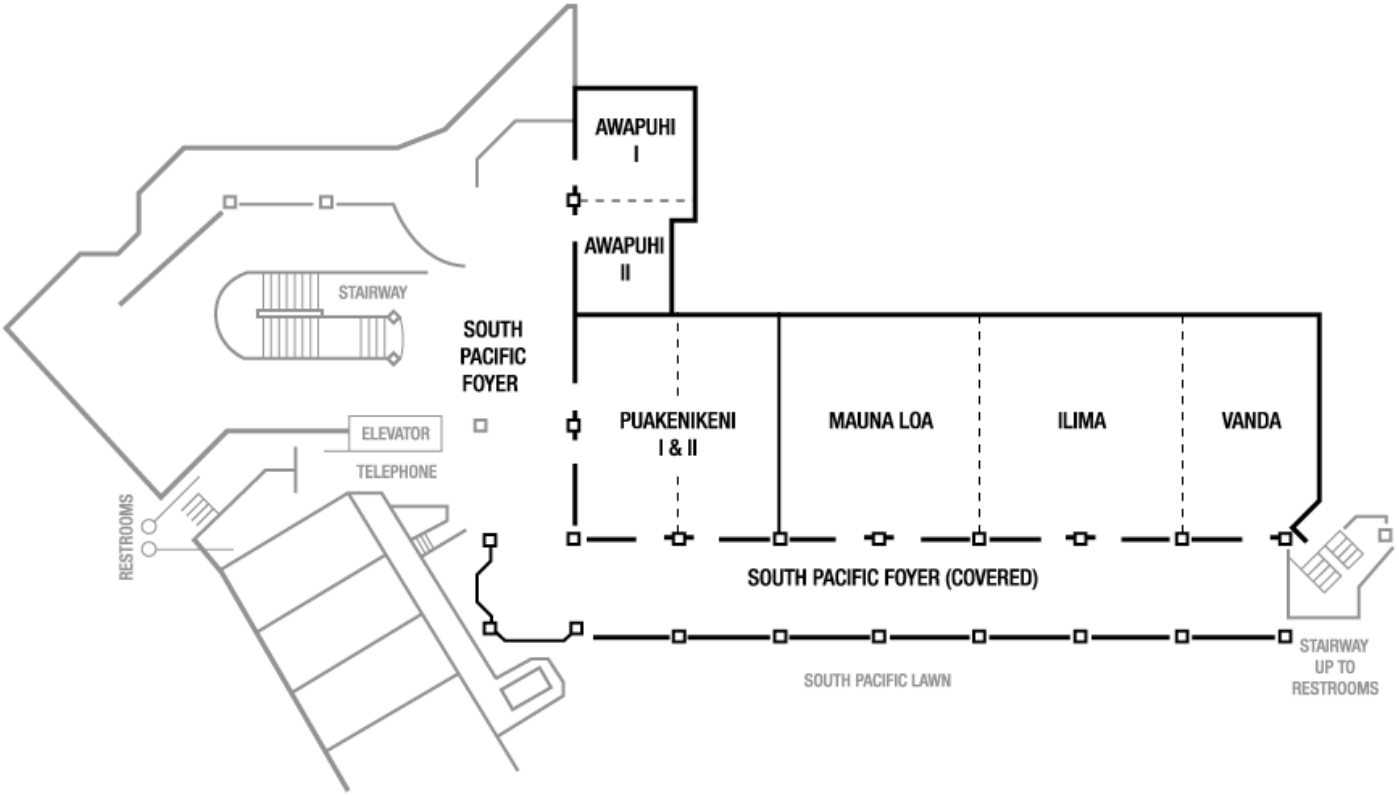
iPhone
iPad

1. Search for and download the AttendeeHub in your App Store
2. Open and then search for "NAVDF 2018"
3. Tap Download + enter the event

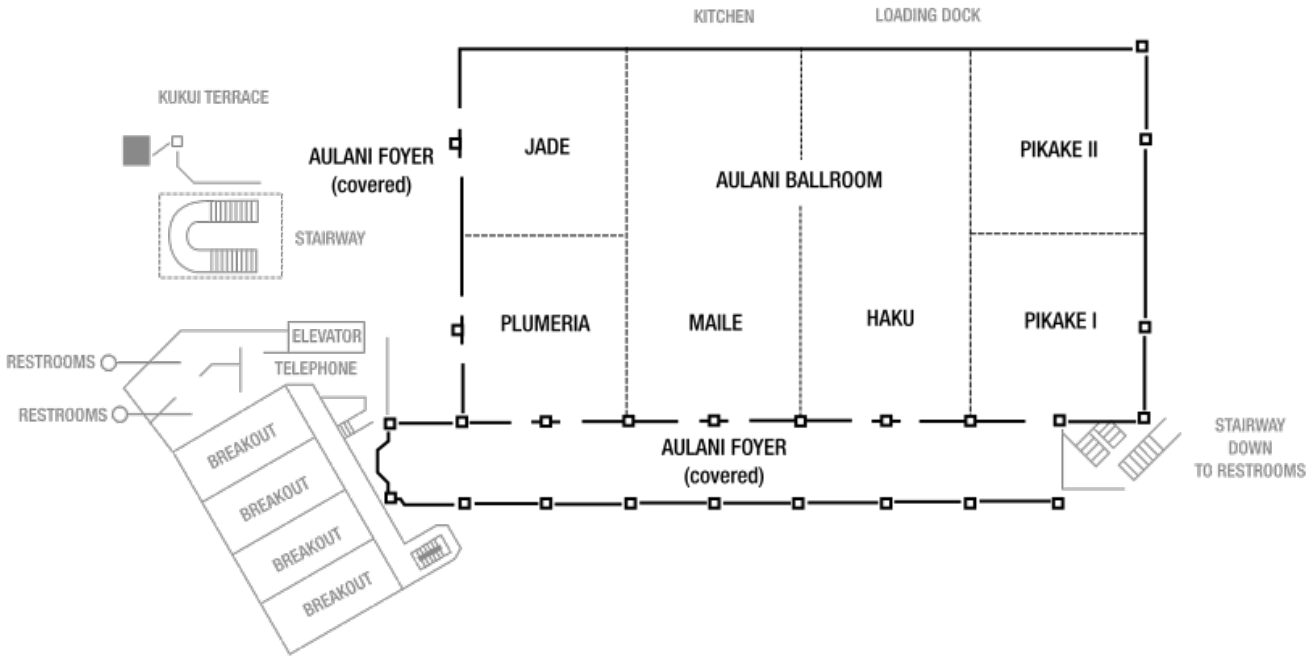
LAPTOP OR OTHER DEVICES

Use **https://crowd.cc/navdf2018** for online version of the app

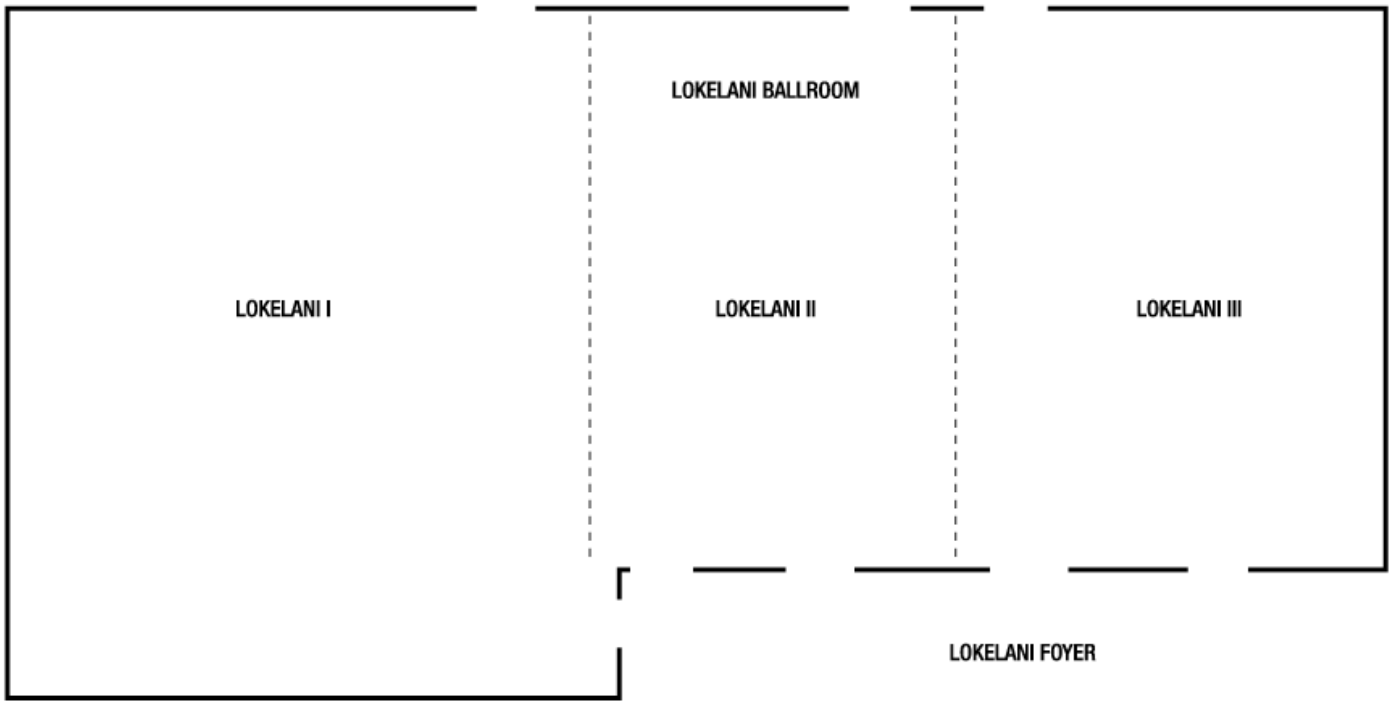
HOTEL MEETING SPACE



AULANI BALLROOM



HOTEL MEETING SPACE



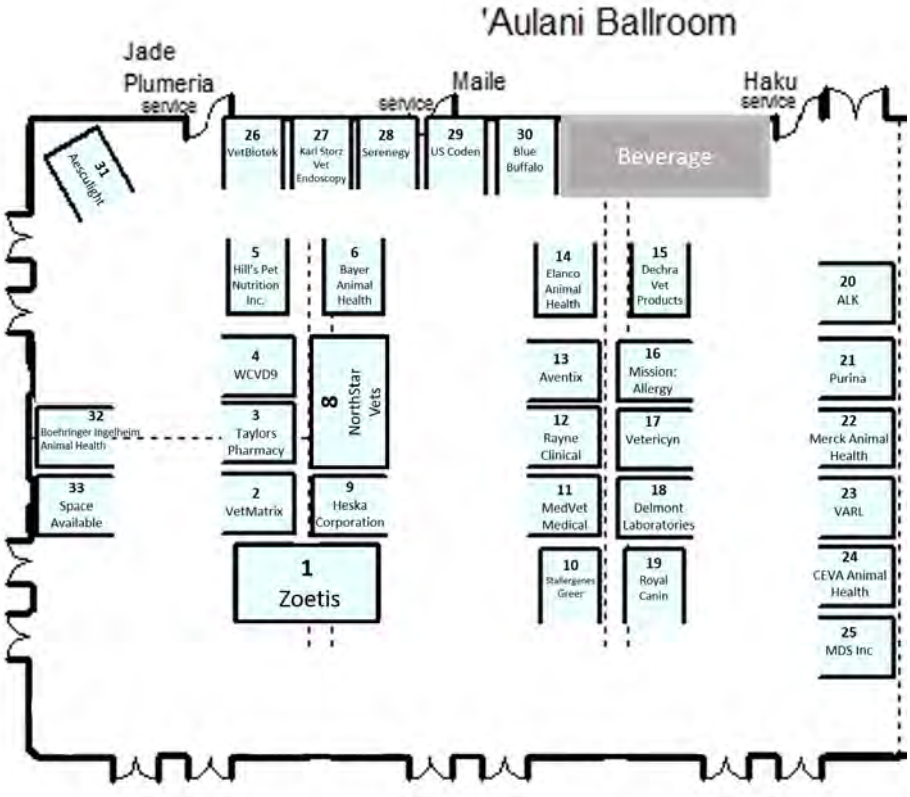
REGISTRATION & EXHIBIT HALL HOURS

REGISTRATION INFORMATION

Tuesday, May 1	5:00pm to 7:00pm
Wednesday, May 2	7:00am to 5:30pm
Thursday, May 3	7:00am to 5:30pm
Friday, May 4	7:00am to 12:30pm
Saturday, May 5	7:00am to 12:30pm

EXHIBIT HALL & POSTER HOURS

Wednesday, May 1	8:30am to 4:30pm
Thursday, May 2	8:30am to 4:30pm
Friday, May 3	8:30am to 11:30am



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IDEXX

ViN

When it comes to fast relief from allergic itch without steroid side effects,

IT WOULD BE A SHAME TO MAKE THEM WAIT

FIRST TIME, EVERY TIME —
Start and stay with APOQUEL (oclacitinib tablet) for relief of short- and long-term itch.

- Starts working in 4 hours[1]
- Controls itch within 24 hours without many of the side effects associated with steroids[2]
- Safe for long-term use[2]
- Does not interfere with diagnostic testing[3]

Learn more at APOQUEL.com

INDICATIONS
Control of pruritus associated with allergic dermatitis and control of atopic dermatitis in dogs at least 12 months of age.

apoquel® (oclacitinib tablet)

IMPORTANT SAFETY INFORMATION
Do not use APOQUEL in dogs less than 12 months of age or those with serious infections. APOQUEL may increase the chances of developing serious infections, and may cause existing parasitic skin infestations or pre-existing cancers to get worse. APOQUEL has not been tested in dogs receiving some medications including some commonly used to treat skin conditions such as corticosteroids and cyclosporine. Do not use in breeding, pregnant, or lactating dogs. Most common side effects are vomiting and diarrhea. APOQUEL has been used safely with many common medications including parasiticides, antibiotics and vaccines.

For more information, please see Brief Summary of full Prescribing Information on adjacent page.

References: 1. Gadeyne C, Little P, King VL, et al. Efficacy of oclacitinib (Apoquel®) compared with prednisolone for the control of pruritus and clinical signs associated with allergic dermatitis in client-owned dogs in Australia. *Vet Dermatol.* 2014;25(6):512-518. doi:10.1111/vde.12166. **2.** Cosgrove SB, Wren JA, Cleaver DM, et al. Efficacy and safety of oclacitinib for the control of pruritus and associated skin lesions in dogs with canine allergic dermatitis. *Vet Dermatol.* 2013;24(5):479-e114. doi:10.1111/vde.12047. **3.** Aleo MM, Galvan EA, Fleck JT, et al. Effects of oclacitinib and prednisolone on skin test sensitivity [abstract]. *Vet Dermatol.* 2013;24(3):297.

ZOETIS PETCARE

apoquel®
(oclacitinib tablet)

3.6 mg 5.4 mg 16 mg

Brief Summary of Prescribing Information

For oral use in dogs only

Caution: Federal (USA) Law restricts this drug to use by or on the order of a licensed veterinarian.

Indications: Control of pruritus associated with allergic dermatitis and control of atopic dermatitis in dogs at least 12 months of age.

Dosage and Administration: The dose of APOQUEL (oclacitinib maleate) tablets is 0.18 to 0.27 mg oclacitinib/lb (0.4 to 0.6 mg oclacitinib/kg) body weight, administered orally, twice daily for up to 14 days, and then administered once daily for maintenance therapy. APOQUEL may be administered with or without food.

Dosing Chart

Weight Range (in lb)		Weight Range (in Kg)		Number of Tablets to be Administered		
Low	High	Low	High	3.6 mg Tablets	5.4 mg Tablets	16 mg Tablets
6.6	9.9	3.0	4.4	**0.5**	-	-
10.0	14.9	4.5	5.9	-	**0.5**	-
15.0	19.9	6.0	8.9	**1**	-	-
20.0	29.9	9.0	13.4	-	**1**	-
30.0	44.9	13.5	19.9	-	-	**0.5**
45.0	59.9	20.0	26.9	-	**2**	-
60.0	89.9	27.0	39.9	-	-	**1**
90.0	129.9	40.0	54.9	-	-	**1.5**
130.0	175.9	55.0	80.0	-	-	**2**

Warnings:
APOQUEL is not for use in dogs less than 12 months of age (see **Animal Safety**).
APOQUEL is not for use in dogs with serious infections.
APOQUEL may increase susceptibility to infection, including demodicosis, and exacerbate neoplastic conditions (see **Adverse Reactions** and **Animal Safety**).

Human Warnings:
This product is not for human use. Keep this and all drugs out of reach of children. For use in dogs only. Wash hands immediately after handling the tablets. In case of accidental eye contact, flush immediately with water or saline for at least 15 minutes and then seek medical attention. In case of accidental ingestion, seek medical attention immediately.

Precautions:
APOQUEL is not for use in breeding dogs, or pregnant or lactating bitches.
The use of APOQUEL has not been evaluated in combination with glucocorticoids, cyclosporine, or other systemic immunosuppressive agents.
Dogs receiving APOQUEL should be monitored for the development of infections, including demodicosis, and neoplasia.

Adverse Reactions:
Control of Atopic Dermatitis
In a masked field study to assess the effectiveness and safety of oclacitinib for the control of atopic dermatitis in dogs, 152 dogs treated with APOQUEL and 147 dogs treated with placebo (vehicle control) were evaluated for safety. The majority of dogs in the placebo group withdrew from the 112-day study by Day 16. Adverse reactions reported (and percent of dogs affected) during Days 0-16 included diarrhea (4.6% APOQUEL, 3.4% placebo), vomiting (3.9% APOQUEL, 4.1% placebo), anorexia (2.6% APOQUEL, 0% placebo), new cutaneous or subcutaneous lump (2.6% APOQUEL, 2.7% placebo), and lethargy (2.0% APOQUEL, 1.4% placebo). In most cases, diarrhea, vomiting, anorexia, and lethargy spontaneously resolved with continued dosing. Dogs on APOQUEL had decreased leukocytes (neutrophil, eosinophil, and monocyte counts) and serum globulin, and increased cholesterol and lipase compared to the placebo group but group means remained within the normal range. Mean lymphocyte counts were transiently increased at Day 14 in the APOQUEL group.

Dogs that withdrew from the masked field study could enter an unmasked study where all dogs received APOQUEL. Between the masked and unmasked study, 283 dogs received at least one dose of APOQUEL. Of these 283 dogs, two dogs were withdrawn from study due to suspected treatment-related adverse reactions: one dog that had an intense flare-up of dermatitis and severe secondary pyoderma after 19 days of APOQUEL administration, and one dog that developed generalized demodicosis after 28 days of APOQUEL administration. Two other dogs on APOQUEL were withdrawn from study due to suspected or confirmed malignant neoplasia and subsequently euthanized, including one dog that developed signs associated with a heart base mass after 21 days of APOQUEL administration, and one dog that developed a Grade III mast cell tumor after 60 days of APOQUEL administration. One of the 147 dogs in the placebo group developed a Grade I mast cell tumor and was withdrawn from the masked study. Additional dogs receiving APOQUEL were hospitalized for diagnosis and treatment of pneumonia (one dog), transient bloody vomiting and stool (one dog), and cystitis with urolithiasis (one dog).

In the 283 dogs that received APOQUEL, the following additional clinical signs were reported after beginning APOQUEL (percentage of dogs with at least one report of the clinical sign as a non-pre-existing finding): pyoderma (12.0%), non-specified dermal lumps (12.0%), otitis (9.9%), vomiting (9.2%), diarrhea (6.0%), histiocytoma (3.9%), cystitis (3.5%), anorexia (3.2%), lethargy (2.8%), yeast skin infections (2.5%), pododermatitis (2.5%), lipoma (2.1%), polydipsia (1.4%), lymphadenopathy (1.1%), nausea (1.1%), increased appetite (1.1%), aggression (1.1%), and weight loss (0.7).

Control of Pruritus Associated with Allergic Dermatitis
In a masked field study to assess the effectiveness and safety of oclacitinib for the control of pruritus associated with allergic dermatitis in dogs, 216 dogs treated with APOQUEL and 220 dogs treated with placebo (vehicle control) were evaluated for safety. During the 30-day study, there were no fatalities and no adverse reactions requiring hospital care. Adverse reactions reported (and percent of dogs affected) during Days 0-7 included diarrhea (2.3% APOQUEL, 0.9% placebo), vomiting (2.3% APOQUEL, 1.8% placebo), lethargy (1.8% APOQUEL, 1.4% placebo), anorexia (1.4% APOQUEL, 0% placebo), and polydipsia (1.4% APOQUEL, 0% placebo). In most of these cases, signs spontaneously resolved with continued dosing. Five APOQUEL group dogs were withdrawn from study because of: darkening areas of skin and fur (1 dog); diarrhea (1 dog); fever, lethargy and cystitis (1 dog); an inflamed footpad and vomiting (1 dog); and diarrhea, vomiting, and lethargy (1 dog). Dogs in the APOQUEL group had a slight decrease in mean white blood cell counts (neutrophil, eosinophil, and monocyte counts) that remained within the normal reference range. Mean lymphocyte count for dogs in the APOQUEL group increased at Day 7, but returned to pretreatment levels by study end without a break in APOQUEL administration. Serum cholesterol increased in 25% of APOQUEL group dogs, but mean cholesterol remained within the reference range.

Continuation Field Study
After completing APOQUEL field studies, 239 dogs enrolled in an unmasked (no placebo control), continuation therapy study receiving APOQUEL for an unrestricted period of time. Mean time on this study was 372 days (range 1 to 610 days). Of these 239 dogs, one dog developed demodicosis following 273 days of APOQUEL administration. One dog developed dermal pigmented viral plaques following 266 days of APOQUEL administration. One dog developed a moderately severe bronchopneumonia after 272 days of APOQUEL administration; this infection resolved with antimicrobial treatment and temporary discontinuation of APOQUEL. One dog was euthanized after developing abdominal ascites and pleural effusion of unknown etiology after 450 days of APOQUEL administration. Six dogs were euthanized because of suspected malignant neoplasms: including thoracic metastatic, abdominal metastatic, splenic, frontal sinus, and intracranial neoplasms, and transitional cell carcinoma after 17, 120, 175, 49, 141, and 286 days of APOQUEL administration, respectively. Two dogs each developed a Grade II mast cell tumor after 52 and 91 days of APOQUEL administration, respectively. One dog developed low grade B-cell lymphoma after 392 days of APOQUEL administration. Two dogs each developed an apocrine gland adenocarcinoma (one dermal, one anal sac) after approximately 210 and 320 days of APOQUEL administration, respectively. One dog developed a low grade oral spindle cell sarcoma after 320 days of APOQUEL administration.

To report suspected adverse events, for technical assistance or to obtain a copy of the MSDS, contact Zoetis Inc. at 1-888-963-8471 or www.zoetis.com.

For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or online at http://www.fda.gov/AnimalVeterinary/SafetyHealth.

Storage Conditions:
APOQUEL should be stored at controlled room temperature between 20° to 25°C (68° to 77°F) with excursions between 15° to 40°C (59° to 104°F).

How Supplied:
APOQUEL tablets contain 3.6 mg, 5.4 mg, or 16 mg of oclacitinib as oclacitinib maleate per tablet. Each strength tablets are packaged in 20 and 100 count bottles. Each tablet is scored and marked with AQ and either an S, M, or L that correspond to the different tablet strengths on both sides.

NADA #141-345, Approved by FDA

Made in Italy

zoetis

Distributed by:
Zoetis Inc.
Kalamazoo, MI 49007

February 2013 428007800A&P

COMPLETE SCHEDULE

TUESDAY, MAY 1, 2018

7:30 AM – 6:30 PM	**ADVT VTS Board Examination**
7:30 am – 8:30 am	Set up
8:30 am –12:30 pm	4-hour Exam
12:30 pm – 6:30 pm	Grading
	● LOCATION: VANDA
8:00 AM – 4:30 PM	**ACVD Resident Forum**
7:30 – 8:30 am	Breakfast
8:00 – 9:30 am	Dr. Linda Frank – Hair Follicle
9:30 – 10:00 am	Break
10:00 – 11:30 pm	Dr. Robert Foster – Dermpath Basics & Pattern Recognition
11:30 – 12:15 pm	Dr. Dan Morris – Manuscript development and the publication process
12:15 – 1:00 pm	Lunch
1:00 – 2:30 pm	Dr. John Angus – Immune System Basics
2:30 – 3:00 pm	Break
3:00 – 4:30 pm	Dr. Stephen White – Small Mammal Dermatology
	Sponsored by Zoetis
	● LOCATION: LOKELANI 1
8:00 AM – 8:00 PM	Cyber Café
	Sponsored by Veterinary Information Network
	● LOCATION: AULANI FOYER
8:00 AM – 5:00 PM	ACVD Exam Committee Meeting
	● LOCATION: AWAPUHI 2
9:00 AM – 12:00 PM	NAVDF Organizing Committee Meeting
	● LOCATION: PUAKENIKENI 1
9:00 AM – 3:00 PM	WCVD9 – EOC Meeting
	● LOCATION: ILIMA
12:00 PM – 5:00 PM	AAVD Executive Board Meeting
	● LOCATION: PUAKENIKENI 1
12:00 PM – 5:00 PM	ACVD Executive Board Meeting
	● LOCATION: PUAKENIKENI 2

COMPLETE **SCHEDULE** CONT.

TUESDAY, MAY 1, 2018

1:00 PM – 5:00 PM	NAVDF Program Committee Meeting ● **LOCATION: BREAKOUT ROOM 4615**
2:00 PM – 5:00 PM	ACVD AOK Committee Meeting ● **LOCATION: AWAPUHI 1**
2:00 PM – 6:00 PM	Exhibitor & Poster Setup ● **LOCATION: AULANI BALLROOM**
5:00 PM – 7:00 PM	Registration Open ● **LOCATION: AULANI FOYER**
5:00 PM – 7:00 PM	Welcome Reception ● **LOCATION: PACIFIC TERRACE** ***Sponsored by Blue Buffalo Company***
7:30 PM	***Royal Canin*** – Young Vet Dinner *By invitation only.* ● **LOCATION: GANNON'S RESTAURANT**

COMPLETE **SCHEDULE** CONT.

WEDNESDAY, MAY 2, 2018

8:00 AM – 8:00 PM	Cyber Café ***Sponsored by Veterinary Information Network*** **● LOCATION: AULANI FOYER**		
7:00 AM - 5:30 PM	Registration Open **● LOCATION: AULANI FOYER**		
7:00 AM - 8:30 AM	Roundtable Breakfast Buffet (registration required) **● LOCATION: SOUTH PACIFIC FOYER**		
	ROUNDTABLE #1	**ROUNDTABLE #2**	**ROUNDTABLE #3**
7:30 AM – 8:45 AM	Cytopoint Anthea Schick, DVM **● LOCATION: AWAPUHI ROOM**	Dermatology in Academia Sandra Koch, DVM **● LOCATION: PUAKANIKENI 1**	Flea control hot button issues: resistance, repellents and systemics. Michael W. Dryden, DVM **● LOCATION: PUAKANIKENI 2**
7:30 AM – 8:45 AM	ADVT Business Meeting **● LOCATION: BREAKOUT ROOM 4615**		
8:30 AM – 4:30 PM	Exhibits & Posters **● LOCATION: AULANI BALLROOM**		
	SCIENTIFIC SESSION **● LOCATION: SOUTH PACIFIC BALLROOM**	**CONCURRENT SESSION** **● LOCATION: LOKELANI BALLROOM**	**ABSTRACT SESSION** **● LOCATION: PIKAKE**
9:00 AM – 10:00 AM	Epidermal Cornification and Disorders in Animals Keith Linder, DVM	Theory and Application of Modern Flea Control Michael Dryden, DVM, PhD	**Resident Abstracts** 9:00 AM | Dr. Karen Ho 9:15 AM | Dr. Karen Ho 9:30 AM | Dr. Curtis Plowgian 9:45 AM | Dr. Matt Levinson
10:00 AM – 11:00 AM			10:00 AM | Dr. Megan Boyd 10:15 AM | Dr. Nellie Choi 10:30 AM | Dr. Jay Korbelik 10:45 AM | Dr. Brooke Simon
11:00 AM – 11:30 AM	BREAK/ Visit Exhibits & Posters **● LOCATION: AULANI BALLROOM**		
11:30 AM – 12:30 PM	Erythema Multiforme and "Old Dog EM" Charles Bradley, VMD	Introduction to Hyperbaric Oxygen Therapy in Veterinary Dermatology John Angus, DVM, DACVD	**Resident Abstracts** 11:30 AM | Dr. Jacqueline Diamond 11:45 AM | Dr. Alicia Webb Milum 12:00 PM | Dr. Jennifer Thomas 12:15 PM | Dr. Austin Richman
12:30 PM – 2:00 PM	LUNCH on Your Own		
12:45 PM –1:45 PM	Sponsor Lunch Symposium: What's Dilution Got to Do with It? ADiscussion on Weight to Volume Dosing for Allergen Vials. *Pre-registration is required.* ***Sponsored by ALK*** **● LOCATION: PIKAKE 1**		

COMPLETE **SCHEDULE** CONT.

WEDNESDAY, MAY 2, 2018

	SCIENTIFIC SESSION	CONCURRENT SESSION	ABSTRACT SESSION
2:00 PM – 3:00 PM	Cutaneous Barrier Dysfunction and Ecosystem Part 1: Barrier Abnormalities and Diseases Koji Nishifuji, DVM, PhD	The Physics and Myths of Surgical Lasers Peter Vitruk, PhD	**Resident Abstracts** 2:00 PM | Dr. Clarissa Souza 2:15 PM | Dr. Meagan Painter 2:30 PM | Dr. Tyler Jordan 2:45 PM | Dr. Stephanie Abrams
3:00 PM – 4:00 PM	Cutaneous Barrier Dysfunction and Ecosystem Part 2: Cutaneous Ecosystem, Immunity and Diseases Koji Nishifuji, DVM, PhD	Compounded Veterinary Medications: Controversies and Guidelines Mark Papich, DVM	**Original Abstracts** 3:00 PM | Dr. Rosanna Marsella 3:15 PM | Dr. Rosanna Marsella 3:30 PM | Dr. Rosanna Marsella 3:45 PM | Dr. Rosanna Marsella
4:00 PM – 4:30 PM	BREAK/ Visit Exhibits & Posters ● **LOCATION: AULANI BALLROOM**		
4:30 PM – 5:30 PM	Mechanisms of Cell Death in The Epidermis Keith Linder, DVM	Analgesic Medications for Small Animals: What to use beyond NSAIDS? Mark Papich, DVM	**Original Abstracts** 4:30 PM | Dr. Caitlin Older 4:45 PM | Dr. Linda Frank 5:00 PM | Dr. Elodie Ollivier 5:15 PM | Dr. Elizabeth May
5:45 PM – 6:45 PM	AAVD – Business Meeting ● **LOCATION: SOUTH PACIFIC BALLROOM**		

COMPLETE **SCHEDULE** CONT.

THURSDAY, MAY 3, 2018

8:00 AM – 8:00 PM	Cyber Café ***Sponsored by Veterinary Information Network*** **● LOCATION: AULANI FOYER**		
7:00 AM - 5:30 PM	Registration Open **● LOCATION: AULANI FOYER**		
7:00 AM - 8:30 AM	Roundtable Breakfast Buffet (registration required) **● LOCATION: SOUTH PACIFIC FOYER**		
	ROUNDTABLE #4	**ROUNDTABLE #5**	**ROUNDTABLE #6**
7:30 AM – 8:45 AM	Advancements in Video Otoscopy Mike Canfield, DVM **● LOCATION: AWAPUHI ROOM**	Isoxazolines Paulo Gomes, DVM **● LOCATION: PUAKANIKENI 1**	Controversies in Drug Therapy Mark Papich, DVM **● LOCATION: PUAKANIKENI 2**
8:30 AM – 4:30 PM	Exhibits & Posters **● LOCATION: AULANI BALLROOM**		
	SCIENTIFIC SESSION	**CONCURRENT SESSION**	**ABSTRACT SESSION**
	● LOCATION: SOUTH PACIFIC BALLROOM	**● LOCATION: LOKELANI BALLROOM**	**● LOCATION: PIKAKE**
9:00 AM – 10:00 AM			9:00 am | Resident Research Awards Presentation
10:00 AM – 11:00 AM	Pemphigoid Diseases: From Pathophysiology to Novel Therapeutic Options Michael Kasperkiewicz, MD	Corticosteroids Mark Papich, DVM	**Original Abstracts** 9:15 AM | Dr. Rosanna Marsella 9:30 AM | Dr. Rosanna Marsella 9:45 AM | Dr. Rosanna Marsella 10:00 AM | Dr. Andrea Gonzalez 10:15 AM | Dr. Steve Dunham 10:30 AM | Dr. Andrea Myers 10:45 AM | Dr. Courtney Meason Smith
11:00 AM – 11:30 AM	BREAK/ Visit Exhibits & Posters **● LOCATION: AULANI BALLROOM**		
11:30 AM – 12:30 PM	Autoimmune Blistering Skin Diseases – Basement Membrane Autoimmunity Petra Bizikova, MVDr, PhD	Viral Skin Diseases Wayne Rosenkrantz, DVM, ACVD	**Resident Abstracts** 11:30 AM | Dr. Tara Denly/ Dr. FraneBanovic 11:45 AM | Dr. Kathy Tater 12:00 PM | Dr. Kathy Tater 12:15 PM | Dr. Andrea Wright
12:30 PM – 2:00 PM	LUNCH on Your Own		
12:45 PM –2:00 PM	ACVD Residency Mentors Meeting ***Sponsored by Stallergenes Greer*** **● LOCATION: PUAKENIKENI**		
2:00 PM – 3:00 PM **3:00 PM – 4:00 PM**	Pemphigus Diseases: From Pathophysiology to Novel Therapeutic Options Michael Kasperkiewicz, MD	Ecology and Control of Ticks Michael Dryden, DVM, PhD	2:30 pm – 4:00 pm WAVD Clinical Consensus Guideline
4:00 PM – 4:30 PM	BREAK/ Visit Exhibits & Posters **● LOCATION: AULANI BALLROOM**		

COMPLETE **SCHEDULE** CONT.

THURSDAY, MAY 3, 2018

4:30 PM – 5:30 PM	Desmosome Autoimmunity Petra Bizikova, MVDr, PhD		
5:45 PM – 7:15 PM	ACVD – Business Meeting ● **LOCATION: SOUTH PACIFIC BALLROOM**		
6:00 PM	ACVD Resident's Dinner ***Sponsored by Dechra*** ● **LOCATION: LUAU GARDENS, WAILEA BEACH RESORT**		
7:00 PM	ADVT VTS Dinner and Pinning Ceremony ***Sponsored by CEVA Animal Health*** ● **LOCATION: GRAND WAILEA**		
7:15 PM	ACVD – Diplomates Dinner ***Sponsored by Bayer*** ● **LOCATION: GRAND WAILEA**		

COMPLETE **SCHEDULE** CONT.

FRIDAY, MAY 4, 2018

8:00 AM – 8:00 PM	Cyber Café ***Sponsored by Veterinary Information Network*** ● **LOCATION: AULANI FOYER**		
7:00 AM - 12:30 PM	Registration Open ● **LOCATION: AULANI FOYER**		
7:00 AM - 8:30 AM	Roundtable Breakfast Buffet (registration required) ● **LOCATION: SOUTH PACIFIC FOYER**		
	ROUNDTABLE #7	**ROUNDTABLE #8**	**ROUNDTABLE #9**
7:30 AM – 8:45 AM	Feline Eosinophilic Diseases Michelle Woodward, DVM ● **LOCATION: AWAPUHI ROOM**	Treatment of Refractory Pruritus in Dogs and Cats Rendina McFadden, DVM ● **LOCATION: PUAKANIKENI 1**	Tech Roundtable - MDRO: Resistance in Companion Animals Amanda Friedeck, B.S., LVT ● **LOCATION: PUAKANIKENI 2**
8:30 AM – 11:30 AM	Exhibits & Posters ● **LOCATION: AULANI BALLROOM**		
	DERMATOPATHOLOGY SESSION (ISVD)	**CONCURRENT SESSION**	**ABSTRACT SESSION**
	● **LOCATION: SOUTH PACIFIC BALLROOM**	● **LOCATION: LOKELANI BALLROOM**	● **LOCATION: PIKAKE**
9:00 AM – 10:00 AM		Equine Dermatology - Correlation of Clinical Presentation with Histopathology Verena Affolter, PhD	9:00 am | ACVD/AAVD Research Grant Awards and Resident Externship Grant Awards
10:00 AM – 11:00 AM	The Hair Follicle: A Fascinating Miniorgan Monika Welle, Prof., Dr. med. vet	Update on Equine Dermatology Wayne Rosenkrantz, DVM, ACVD	**Clinical Abstracts** 9:15 AM | Dr. Elizabeth Martinez 9:30 AM | Dr. Christine Cain 9:45 AM | Dr. Ben Tham 10:00 AM | Dr. Liora Waldman 10:15 AM | Dr. Alena Ferrigno 10:30 AM | Dr. Larissa Botoni 10:45 AM | Dr. Andrea Wright
11:00 AM – 11:30 AM	BREAK/ Visit Exhibits & Posters ● **LOCATION: AULANI BALLROOM**		
11:30 AM – 12:30 PM	ISVD Mystery Slide Session ***Sponsored by IDEXX*** Dr. Janelle Novak Dr. Takafumi Osumi Dr. Juliana Werner Dr. Verena Affolter	Equine Panel Discussion Verena Affolter, Dr.med.vet, PHD Wayne Rosenkrantz, DVM, ACVD Stephen White, DVM Anthony Yu, DVM	
12:30 PM – 2:00 PM	EDUCATION DONE FOR THE DAY		
12:30 PM –2:00 PM	ACVD Resident Lunch ***Sponsored by Stallergenes Greer*** ● **LOCATION: PUAKENIKENI**		
12:45 PM – 1:45 PM	ACVD Website Committee Meeting ● **LOCATION: AWAPUHI 2**		
6:00 PM – 10:00 PM	Royal Canin Reception ● **LOCATION: GRAND WAILEA**		

COMPLETE **SCHEDULE** CONT.

SATURDAY, MAY 5, 2018

8:00 AM – 12:30 PM	Cyber Café ***Sponsored by Veterinary Information Network*** **LOCATION: AULANI FOYER**		
7:00 AM - 12:30 PM	Registration Open **LOCATION: AULANI FOYER**		
7:00 AM - 8:30 AM	Roundtable Breakfast Buffet (registration required) **LOCATION: SOUTH PACIFIC FOYER**		
	ROUNDTABLE #10	**ROUNDTABLE #11**	**ROUNDTABLE #12**
7:30 AM – 8:45 AM	Apoquel, beyond allergies Andrea Meyer, DVM **LOCATION: AWAPUHI ROOM**	Dermatology Photography David Duclos, DVM **LOCATION: PUAKANIKENI 1**	Resident Roundtable ***Sponsored by Royal Canin*** **LOCATION: PLUMERIA**
	DERMATOPATHOLOGY SESSION (ISVD)	**CONCURRENT SESSION**	**ADVT SESSION**
	LOCATION: SOUTH PACIFIC BALLROOM	**LOCATION: LOKELANI BALLROOM**	**LOCATION: PIKAKE**
9:00 AM – 10:00 AM	ISVD Clinicopathological Correlations Dr. Erica Noland / Dr. Annette Peterson Dr. Monika Welle / Dr. Mitchel Song Dr. Charles Bradley / Dr. Christine Cain Dr. Verena Affolter / Dr. Catherine Outerbridge Moderator: Dr. Derick Whitley	Video Otoscopy and Ct In the Diagnosis and Management of Canine and Feline Otic Disease Part 1 & Part 2 Mike Canfield, DVM, ACVD Rodney W. Rosychuk, DVM	What's all the fungus about? Fungal dermatoses in veterinary patients Amelia White, DVM, MS
10:00 AM – 11:00 AM			Who's behind Door 1? Making the diagnosis of fungal dermatosis Amelia White, DVM, MS
11:00 AM – 11:30 AM	BREAK		
11:30 AM – 12:30 PM	Case Discussion from The ISVD Listserv David Shearer, BvetMed, CertSAD, PhD	Selected Feline Dermatoses: Dermatoses Affecting Digits Petra Bizikova, MVDr, PhD	What's your diagnosis? A case-based approach to fungal dermatoses Amelia White, DVM, MS
12:30 PM – 2:00 PM	PROGRAM CONCLUDED		

ROUNDTABLE SCHEDULE

WEDNESDAY, MAY 2, 2018

1	**Cytopoint** | Anthea Schick, DVM	LOCATION: AWAPUHI ROOM
2	**Dermatology in Academia** | Sandra Koch, DVM	LOCATION: PUAKANIKENI 1
3	**Flea control hot button issues: resistance, repellents and systemics.** | Michael W. Dryden, DVM	LOCATION: PUAKANIKENI 2

THURSDAY, MAY 3, 2018

4	**Advancements in Video Otoscopy** | Mike Canfield, DVM	LOCATION: AWAPUHI ROOM
5	**Isoxazolines** | Paulo Gomes, DVM	LOCATION: PUAKANIKENI 1
6	**Controversies in Drug Therapy** | Mark Papich, DVM	LOCATION: PUAKANIKENI 2

FRIDAY, MAY 4, 2018

7	**Feline Eosinophilic Diseases** | Michelle Woodward, DVM	LOCATION: AWAPUHI ROOM
8	**Treatment of Refractory Pruritus in Dogs and Cats** | Rendina McFadden, DVM	LOCATION: PUAKANIKENI 1
9	**Tech Roundtable - MDRO: Resistance in Companion Animals** | Amanda Friedeck, B.S., LVT	LOCATION: PUAKANIKENI 2

SATURDAY, MAY 5, 2018

10	**Apoquel, beyond allergies** | Andrea Meyer, DVM	LOCATION: AWAPUHI ROOM
11	**Dermatology Photography** | David Duclos, DVM	LOCATION: PUAKANIKENI 1
12	**Resident Roundtable**	LOCATION: PLUMERIA

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References: 1. Data on file, Study Report No. C863R-US-12-018, Zoetis Inc. **2.** Gonzales AJ, Humphrey WR, Messamore JE, et al. Interleukin-31: its role in canine pruritus and naturally occurring canine atopic dermatitis. *Vet Dermatol*. 2013;24(1):48-53. doi:10.1111/j.1365-3164.2012.01098.x. **3.** Data on file, Study Report No. C362N-US-13-042, Zoetis Inc. **4.** Data on file, Study Report No. C961R-US-13-051, Zoetis Inc.

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ABSRACTS SCHEDULE

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Defining rifampin's minimum inhibitory concentration and killing properties against Staphylococcusintermedius group in the dog

K. HO*, A. CONLEY †, R. KENNIS*, T. HATHCOCK ‡, D. BOOTHE †, and A. WHITE*

**Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA*
†Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA
‡Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Abstract: Meticillin-resistant (MR) staphylococcal pyoderma in dogs has led to increased use of rifampin (RFP). Although RFP exhibits bactericidal activity against *Staphylococcus* spp. in humans this has not been studied in dogs. The study objectives were to determine the minimum inhibitory concentration (MIC) of canine *Staphylococcus intermedius* group (SIG) organisms and to determine killing properties of rifampin. One hundred canine SIG isolates, 50 meticillin-susceptible (MS) and 50 MR, were used to determine the MIC of RFP using the E-test. Two MS-SIG isolates with an MIC of 0.003 µg/ml and 0.008 µg/ml and two MR-SIG isolates with an MIC of 0.003 µg/ml and 0.012 µg/ml were used for the time-kill studies. Mueller Hinton Broth was supplemented with RFP at 0, 0.5, 1, 2, 4, 8, 16, and 32 times the MIC of the respective isolate for 0, 2, 4, 10, 16, and 24h. The number of viable colony forming units (CFU) was determined using the BacTiter-Glo™ Microbial Cell Viability Luciferase kit. The MIC50 was 0.004 µg/ml and the MIC90 was 0.008 µg/ml. Based on these methods RFP activity against all four isolates was consistent with time-dependent response since the magnitude of decrease in CFU was unchanged regardless of antimicrobial concentration. Two isolates (MS-SIG 0.003 and 0.008µg/ml) exhibited bacteriostatic activity while the other isolates (MR-SIG 0.003 and 0.012µg/ml) exhibited bactericidal activity. This study determined the MIC of RFP in canine SIG isolates, demonstrated that MS- and MR-SIG isolates are equally susceptible to RFP, and recommends dosing intervals designed for time-dependent efficacy.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Pharmacokinetics and pharmacodynamics of multiple oral dosing of rifampin in the dog

K. HO*, K. SMART*, C. CRUZ-ESPINDOLA†, D. BOOTHE †, and A. WHITE*

**Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA*
†Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Abstract: There is increased use of rifampin (RFP) in dogs; however, neither pharmacodynamics (PD) nor pharmacokinetics (PK) are available. This study aimed to determine the PK and PD of RFP in dogs. Ten healthy dogs were dosed orally with RFP at 5.9±1.1 mg/kg every 24 h for 13 days. On day 14, a trough plasma sample was collected; dogs received a single equivalent intravenous RFP dose and plasma was sampled intermittently for 48 h. Dogs were monitored throughout the 14 day period for adverse events, including physical examination and blood work. Nares and mouth cultures were collected on days 0 and 14. After oral dosing on day 7, C_{max} was 8.3±4.9 µg/ml. After intravenous dosing on day 14, C_{max} was 21.5+3.9 µg/ml and 3.7+0.7 µg/ml when adjusted for dose. AUC_{∞} was 57 ±12 hr (µg/ml), CLss was 0.019±0.004 L/kg/h, Vd was 0.28±0.9 L/kg and $t_{1/2}$ was 10.7±1.7 h. Based on a *Staphylococcus intermedius* group (SIG) MIC_{90} of 0.008 µg/ml for RFP, integration of PK and PD revealed a T>MIC of 48h, and an AUC:MIC_{90} of 7087±1.7. RFP C_{max} andC_{min} exceeded the MIC_{90} by more than 2500 and 125 fold, respectively. No significant adverse events occurred in any dog. RFP-resistant SIG was cultured from three dogs at study end but in no dogs at study start. This study provides supporting evidence for clinical trials evaluating oral RFP at 5 mg/kg every 24 h in the dog. Future studies are needed to support lowering the dose beyond what was tested herein.

Sources of funding: Self-funded

Conflict of Interest: None declared

Evaluation of breakpoints and the mutant selection window of moxifloxacin for canine isolates of *Staphylococcus pseudintermedius*

C. PLOWGIAN*, J. BLONDEAU†, W. ROSENKRANTZ‡, M. LEVINSON‡

** Animal Dermatology Clinic, Marietta, GA, USA*
†Royal University Hospital and University of Saskatchewan, Saskatoon, SK, Canada
‡Animal Dermatology Clinic, Tustin, CA, USA

Abstract: Moxifloxacin is a 4th generation fluoroquinolone that is approved for use in human medicine to treat a variety of infections. Pradofloxacin, its sister molecule, is used in veterinary medicine for similar infectious diseases. Veterinary laboratories currently include moxifloxacin in their culture and sensitivity profiles for staphylococcal infections, but use CLSI breakpoints for *Staphylococcus aureus*. Previous studies have shown S. aureus breakpoints can mischaracterize sensitivities of Staphylococcus pseudintermedius strains. No breakpoint data exist for moxifloxacin. The objective of this study is to establish minimum inhibitory concentrations (MIC) and mutant prevention concentrations (MPC) of moxifloxacin in canine isolates of *S. pseudintermedius*. The MIC and MPC of moxifloxacin and pradofloxacin were determined from 19 *S. pseudintermedius* isolates and a single isolate strain of *S. aureus*. The MIC50 values for moxifloxacin and pradofloxacin were both 0.031 µg/mL. The MIC90 for both drugs was 2 µg/mL. The MPC50 was 0.125 µg/mLand the MPC90 was 16 µg/mLfor both moxifloxacin and pradofloxacin. Using the CLSI breakpoint for *S. aureus* (S <0.5 µg/mL, R >2 µg/mL), 15/19 (78.9%) isolates tested were susceptible and 4/19 (21.1%) were resistant. The breakpoint values obtained were not markedly different from the CLSI breakpoints for *S. aureus*. The MIC, MPC, and mutant selection window (MSW) values for moxifloxacin were quite similar to that of pradofloxacin. The narrow MSW of both these drugs may reduce the risk of creating new antibiotic resistance. Pharmacokinetic and pharmacodynamic studies are needed before veterinary dosing recommendations for moxifloxacin can be calculated.

Sources of funding: ACVD Research Grant.

Conflict of Interest: None declared

The in-vitro antibacterial activity of an anthelmintic drug, oxyclozanide, againstcommon small animal veterinary bacterial pathogens

M. LEVINSON*, J. BLONDEAU†, W. ROSENKRANTZ*, C. PLOWGIAN‡

**Animal Dermatology Clinic, Tustin, CA, USA*
†Royal University Hospital and University of Saskatchewan, Saskatoon, SK, Canada
‡Animal Dermatology Clinic, Marietta, GA, USA

Abstract: With the increased prevalence of meticillin-resistant *Staphylococcus pseudintermedius* and other multi-drug resistant bacterial pathogens, treatments options are becoming limited. Oxyclozanide is in the anthelmintic drug class called salicylanilide which has been used mainly as a flukicide in ruminant veterinary medicine. It concentrates in the intestinal tract but has poor systemic gastrointestinal absorption. There is one report of a salicylanilide known as closantel that was used as a parenteral treatment for canine demodicosis with some success. Oxyclozanide also has *in-vitro* bactericidal activity against *Staphylococcus aureus* in humansbut its activity against common small animal bacterial pathogens such as *S. pseudintermedius* has yet to be studied. The aim of this study was to measure and establish the minimum inhibitory concentration (MIC) and mutant prevention concentration (MPC) of oxyclozanide against S. pseudintermedius. The MIC and MPC were determined from 17 *S. pseudintermedius* isolates as well as one isolate strain each of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis* with oxyclozanide using agar plate assays. The MIC of 16/17 and 1/17 isolate strains was 0.5µg/mL and 1 µg/mL, respectively. The MPC ranged between 16 and 32 µg/mL among all strains of *S. pseudintermedius.* No inhibition of growth was seen when testing bacterial isolate strains *E. coli, P. aeruginosa*, and *E. faecalis*. These results show that oxyclozanide has *in-vitro* antibacterial activity against *S. pseudintermedius*. Further studies are needed to evaluate the potential use of oxyclozanide as a topical or parenteral bactericidal agent.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Minimum inhibitory and bactericidal concentrations of antimicrobials found in ototopical formulations for resistant *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* with and without Tris-EDTA

M. BOYD*, D. SANTORO*, D. GRAM*, R. MARSELLA*

**College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: The usefulness of susceptibility testing when selecting topical antimicrobials for otitis externa has been questioned. Routinely, laboratories test minimum inhibitory concentration (MIC) up to the resistant clinical breakpoint. True MIC is therefore unknown. It is possible that such MIC may be achieved with high local concentrations of topical antimicrobials. Our primary aim was to measure the MIC and minimum bactericidal concentration (MBC) of enrofloxacin, gentamicin, marbofloxacin, neomycin, orbifloxacin, polymyxin B, and silver sulfadiazine (SSD) from concentrations of ototopical formulations (COF) until 1/59,000 dilutions. Our secondary aim was to evaluate the effect of Tris-EDTA on these values. Microbroth dilution was used to measure MIC and MBC of 10 resistant clinical isolates of *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa*. For all antimicrobials, COF were >26 times the MIC for Staphylococcus and>39 times for *Pseudomonas*. For *Staphylococcus*, all antimicrobial COFwere ≤ MBC. For *Pseudomonas*, Polymyxin B and SSD COF were >27 and all other antimicrobials were ≤ MBC. For *Staphylococcus,* Tris-EDTA significantly increased MIC for SSD and had no effect on other antimicrobials. For *Pseudomonas*, Tris-EDTA significantly reduced MIC for all antimicrobials except for SSD. There were no statistical differences with the addition of Tris-EDTA for MBC of either *Staphylococcus or Pseudomonas*. Ototopical formulations may provide antimicrobial concentrations above MIC when identified as resistant. Tris-EDTA is advantageous against *Pseudomonas* for all antimicrobials except SSD. Until the residual ear canal antimicrobial concentration is measured *in vivo*, the clinical application of these findings is unknown.

Sources of funding: University of Florida, Departmental start-up funds.

Conflict of Interest: None declared

Comparison of two ear cytology collection techniques in dogs with otitis externa

N. CHOI*, H.D. EDGINTON*, C.E. GRIFFIN†, J.C. ANGUS*

**Animal Dermatology Clinic, 150 N San Gabriel Blvd #400, Pasadena, CA 91107, USA*
†Animal Dermatology Clinic, 5610 Kearny Mesa Rd. Suite B-1, San Diego, CA 92111, USA

Abstract: Canine otitis externa is a common disease. Cytologic evaluation of otic exudate is a useful diagnostic test to direct and monitor treatment for otitis externa. The conventional method of collecting ear cytologic specimens utilizes a cotton tip swab inserted into the vertical ear canal. A proposed alternative method was to aspirate exudate from the deep horizontal canal using a rubber tube. The aim of this study was to compare cytological findings between these two collection techniques in 30 cases of canine otitis externa. This was a prospective, randomized, blinded comparison study. Ear canals from each dog were sampled using both methods. Ear cytologic preparations were stained with Diff-Quik® and blindly evaluated by two investigators for polymorphonuclear leukocytes (PMN), monocytes/lymphocytes, macrophages, yeast, intracellular (IC) cocci, extracellular (EC) cocci, IC rods and EC rods. A paired t-test was used to compare the two techniques. The inter-investigator reliability for PMN, EC rods and yeast was good, and for EC cocci was moderate. There were significantly higher numbers of PMNs obtained by the tube method (P =0.0024) than the cotton swab method. There were no statistically significant differences between cotton swab and rubber tube methods for monocytes/lymphocytes (P =0.7780), macrophages (P =0.1751), EC cocci (P =0.1262), EC rods (P =0.1162), yeast (P =0.5371), IC cocci (P =0.6606) or IC rods (P =0.6761). The technique was well tolerated. An alternative ear cytologic collection technique was identified which enables sampling of the deep horizontal canal.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Characterization of the otic bacterial microbiota in dogs with otitis externa compared to healthy individuals

J. KORBELIK*, A. SINGH†, J. ROUSSEAU‡ and J.S. WEESE§

** Department of Pathobiology, University of Guelph, Guelph, ON, Canada*
† Department of Clinical Studies, University of Guelph, Guelph, ON, Canada
‡Department of Pathobiology, University of Guelph, Guelph, ON, Canada
§Department of Pathobiology, University of Guelph, Guelph, ON, Canada

Abstract: Otitis externa is a common multifactorial disease with an incidence in dogs as high as 10-20%. The diversity of the cutaneous microbiota in dogs appears to decrease in diseased states. However, little is known about the microbiota of the canine ear and how it is altered by disease. The objective of this study was tocompare the otic bacterial microbiota in dogs with otitis externa vs. controls. Samples were collected from sixteen dogs with clinical and cytological evidence of otitis externa and nine clinically normal dogs. DNA from each sample was isolated and Illumina sequencing of V4 hypervariable region of the 16S rRNA gene amplicons was performed. Sequences were processed using the bioinformatics software MOTHUR. Bacteria from 25 different phyla were identified. Affected ears had significantly decreased Shannon's evenness and Simpson's diversity index ($P<0.001$) compared to healthy ears. Community structure and membership also differed between the two groups ($P<0.001$). According to Lefse analysis, 32 operational taxonomic units (OTUs) were differentially abundant ($P<0.05$). Five OTUs were over-represented in the affected ears, including *Staphylococcus*, *Parvimonas* and *Porphyromonas*. Twenty-seven OTUs were over-represented in healthy ears, including *Romboutsia, Megamonasand Faecali bacterium*. The otic microbiota is much more complex than has been identified with previous culture-based studies, and otitis externa is accompanied by broad and complex differences in the microbiota.

Sources of funding: Self-funded.

Conflict of Interest: None declared

A survey of antimicrobial use among dog breeders and prevalence of meticillin-resistant *Staphylococcus* spp. from the oral cavity and nares in individually owned breeding dogs

B. SIMON*, A. SCHICK*, T. LEWIS*

**Dermatology for Animals, 86 West Juniper Avenue, Gilbert, AZ 85233, USA*

Abstract: Meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) skin infections are an increasing concern in veterinary medicine. Previous reports have linked antimicrobial misuse in breeding kennels with increased risk of resistant staphylococcal strains. Additionally, there has been increased recognition of MRSP infections in juvenile dogs with no prior antibiotic exposure. The objectives of this study were to assess the prevalence of meticillin-resistant coagulase-positive staphylococci in breeding bitches and to survey antibiotic administration by their breeders. A total of 17 breeders participated, and 54 bitches of various ages and breeds were included. The bitches were kept in diverse housing environments throughout the Phoenix, AZ metropolitan region. Nasal and oral swabs were taken and submitted for aerobic bacterial culture. Susceptibility to oxacillin and the presence of the mecA gene were determined for each isolate. PBP2a latex agglutination testing revealed 0/54 samples were positive for meticillin-resistant *Staphylococcus aureus*, and 1/54 samples was positive for MRSP. These findings were interesting to note, as previous publications revealed that all isolated strains of S. pseudintermedius obtained from breeding bitches showed high percentages of resistance to various antimicrobials, yet were meticillin sensitive. Survey results revealed that 16/54 bitches received antibiotics within the year prior to sampling, including the bitch in which the MRSP positive sample was obtained. However due to the paucity of positive cultures, no statistically significant correlations could be made. Despite our findings, veterinarians and dog breeders should be cognizant of the risk for antimicrobial resistance with the overuse of antimicrobials.

Sources of funding: This study was funded by the American College of Veterinary Dermatology.

Conflict of Interest: None declared

Comparison of response to microneedling alone versus in conjunction with platelet rich plasma in the treatment of post-clipping alopecia

J. DIAMOND*, R. SCHICK*

**BluePearl – Georgia, Sandy Springs, GA, USA*

Abstract: Post-clipping alopecia often has a poor clinical response to therapy, and prolonged alopecia is a source of anxiety for some owners. In humans and dogs, superficial microtrauma using a microneedling device appears to induce mechanical stimulation of the hair follicle with resultant hair regrowth. Human studies suggest that application of platelet rich plasma (PRP) in conjunction with microneedling induces more rapid regrowth of better quality hair than microneedling alone. Four unrelated dogs diagnosed clinically and histologically with post-clipping alopecia for greater than 1 year were studied. The affected site was divided in half, with the first half treated with microneedling alone (MN) then the other half treated with PRP followed by microneedling (PRP+MN). Microneedling was accomplished under heavy sedation by rolling a new sterile microneedling device with moderate pressure in all directions until capillary bleeding is achieved. Hair regrowth was assessed by clinician and owner on a hair growth assessment scale (HGAS) (0: no improvement; 1: 1-25% improvement; 2: 26-50% improvement; 3: 51-75% improvement; 4: 76-100% improvement) at 1, 3, and 6 months. At 3 months, no dogs were scored 0, and 3/4 dogs had greater hair regrowth on the PRP+MN half. At 6 months, both PRP+MN and MN sites had a similar response, where 3/4 dogs scored 4, and one dog scored 2 on HGAS. Statistics were not performed due to small sample size. In dogs with post-clipping alopecia, PRP+MN appears to induce more rapid hair regrowth than MN.

Sources of funding: BluePearl Science.

Conflict of Interest: None declared

A cross-sectional study evaluating paw lesions, cytological findings, pruritic behaviors and gastrointestinal signs of show English bulldogs

A.N. WEBB MILUM*, C.E, GRIFFIN† and K. BLESSING†

** Animal Dermatology Clinic, Tustin, CA, USA*
† Animal Dermatology Clinic, San Diego, CA, USA

Abstract: English bulldogs (EBDs) are thought to be over-represented for pododermatitis and gastrointestinal disease. No study has looked at a population of breeding EBDs or reported pedal cytology findings. The study purposes were to compare dermatologic, pruritic and gastrointestinal signs and pedal cytology findings between healthy and non-healthy EBDs and also assess the frequency of dermatological abnormalities and cytological findings of lesional and non-lesional paw sites in non-healthy EBDs. Show bulldogs (n=34) received dermatologic examinations. Impression smear cytological samples were taken from the dorsal and palmar/plantar paw. Pruritic behaviors, gastrointestinal signs and antimicrobial and/or immunomodulatory drugs received within 12 months of show were also assessed. The dog owners completed questionnaires regarding history and assessed pruritus. No dogs met the standard criteria for healthy, thus comparison between healthy and non-healthy EBDs was not possible: 100% had an abnormal dermatologic exam with at least one site of erythema; 65% had recent drug history; 47% had history of dermatologic disease; 41% had history of other disease; 32% had history of pruritus and 26% had history of gastrointestinal disease. Compared to published normal values, EBD gastrointestinal signs differed in eructation, flatulence and regurgitation. Pedal erythema affected 94% of paws but did not correlate with pruritus or cytological findings. Sixty-one percent of paws had nuclear streaming or polymorphonuclear cells on cytology. White-colored pedal exudation correlated with inflammation and cocci, otherwise lesions did not correlate with cytology findings. No show EBDs met the criteria for healthy, and cytology was overall poor in distinguishing lesional from non-lesional skin.

Sources of funding: Self-funded.

Conflict of Interest: None declared

A pilot study describing histopathologic changes induced in the skin of domestic cats by the lone star tick, *Amblyomma americanum*

J.E. THOMAS*, J. MANSELL†, A. DIESEL*, A.P. PATTERSON*, and M.V. REICHARD‡

**Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA*
†Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA
‡Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA

Abstract: Tick bites and acarine hypersensitivity reactions cause skin damage and irritation to hosts. The tick-host interface has not been described in domestic cats. The purpose of the present study was to characterize the histopathologic changes induced by adult *Amblyomma americanum* bites on tick-naïve domestic cats (*Feliscatus*). Six purpose bred domestic cats (three males, three females) were used. Skin biopsies were collected from normal skin prior to tick infestation. Each cat was infested with 50 laboratory-reared, pathogen-free *A. americanum* adults. Subsequent biopsies were collected at 24 h, 48 h, and 96 h post-infestation at tick-bite sites. By 24 h post-infestation, mild, focal epidermal necrosis was observed, with a minimal increase in dermal inflammatory infiltrates. The tick feeding cavity extended through the epidermis into the dermis within 24 h of infestation. By 48 h post-infestation, inflammation extended into the dermis and subcutis; the predominant patterns included a moderate perivascular neutrophilic dermatitis and a moderate diffuse neutrophilic panniculitis. Coagulated collagen degeneration was observed in the dermis by 48 h. By 96 h post-infestation, a focally severe neutrophilic dermatitis and panniculitis was observed, with focal areas of necrosis and fibrin deposition extending through the subcutis into the muscle band of the deep subcutis. The histopathologic changes observed in this study incorporate specific responses previously described at the tick-host interface of dogs, cattle, rodents, and lagomorphs.

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Conflict of Interest: None declared

Trichographic features in normal black Doberman pinscher dogs

A.W. RICHMAN*, C.E. GRIFFIN†

**Animal Dermatology Clinic, 4834 Lincoln Blvd, Marina Del Rey, CA 90292, USA*
†Animal Dermatology Clinic, 5610 Kearny Mesa Rd. Suite B-1, San Diego, CA 92111, USA

Abstract: Doberman pinscher dogs (DP) can be affected by color dilution alopecia and follicular dysplasia. However, little research exists looking at normal canine hair shafts, nor is there any study with inter-observer agreement for canine trichographic features. Our objective was to characterize and establish trichographic features of healthy black DP hair shafts looking at variables that include; gross exam, primary versus secondary hairs, color changes, tip structure, curling, twisting, bending, cuticle changes (breaks, compression, overlapping), fractures and variations in hair diameter. The hair shafts from five body locations of 20 client owned healthy black DP of different ages and genders were quantitatively and systematically evaluated. To determine the reproducibility of these variables, the hair shafts were independently evaluated by two observers and inter-observer agreement was evaluated. Kappa statistics, positive and negative agreement were determined. Agreement between investigators ranged from 87% to 99% for each examined variable. Kappa statistics showed excellent agreement (0.81-1.00) to substantial agreement (0.61-0.80) for all variables besides cuticle compressions and variations in hair diameter which had moderate agreement (0.41-0.60). A previously undescribed observation of the "round hair tip" was made and it was more commonly found on the dorsal head. Color change to the proximal hair shaft was more common on the thighs and flanks. This study establishes normal trichographic findings in 500 hairs from 20 healthy black DP. It describes a systematic approach for evaluating hair shafts that can be applied in future studies for both normal and abnormal DP.

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Conflict of Interest: None declared

Evaluation of immunological parameters in pit bull dogs with juvenile onset generalized demodicosis and age-matched healthy pit bull dogs

C. P. SOUZA*, J. R. SCHISSLER*, E. T. CONTRERAS*, S. W. DOW*, L. S. HOPKINS*, J. W. COY*, R. A. W. ROSYCHUK*, J. R. HAWLEY*, M. R. LAPPIN*

**Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA*

Abstract: Juvenile-onset generalized demodicosis (JOGD) is thought to occur due to immunological abnormalities and is over represented in pit bull dogs. Flow cytometry and serological assays were performed in JOGD (n=12) and age-matched healthy pit bull dogs (n=12) to investigate for differences between groups. Flow cytometry measured B-cells expressing MHCII or surface bound IgG, CD4+ T-cells expressing MHCII, and CD8 T-cells expressing MHCII or CD11a. Surface expression was quantified by calculating the geometric mean fluorescence index. Wilcoxon rank-sum test was used to compare median results for Interleukins (IL)-2, IL-6, IL-7, IL-8, IL-10, IL-13, IL-18, FOXP3, MCP-1, GM-CSF, KC, IgE, IgA, IgG, IgM, CRP in the groups. IFNg, IP-10, IL-15, IL-31 and TNFa were also measured, however not enough dogs (<5) had values that were in range. Significance was defined as $P < 0.05$. IgG expression by B cells and T-cell activation markers (CD4+ T Cell MHC II and CD8+ T Cell MHC II) were not significantly different between the two groups of pit bull dogs ($P = 0.07$; $P = 0.45$;$P = 0.25$, respectively). Serum concentrations of the T-helper 1 cytokines IL-2 and IL-18, and MCP-1 were significantly higher ($P = 0.01$, $P = 0.01$, $P = 0.04$, respectively) in the JOGD group. Also, IgA median value was significantly higher ($P = 0.002$) in the JOGD group compared to healthy dogs. Results suggest an inflammatory/compensatory response by the dogs in the JOGD group, possibly induced by the abundant number of *Demodex* mites in their skin.

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Conflict of Interest: None declared

Use of the Health Belief Model to assess adherence to elimination diet trials in veterinary medicine

M. R. PAINTER*, T. TAPP† and J. PAINTER‡

**Department of Veterinary Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA* AnimalDermatologyClinic, Tustin, CA, USA*
†VeterinaryHealingArts, EastGreenwich, RI, USA
‡GeorgeMasonUniversity, Fairfax, VA, USA

Abstract: The current gold standard diagnostic test for cutaneous adverse food reaction (CAFR) in dogs is an elimination diet trial (EDT). The diagnostic value of this test relies on sustained adherence to strict feeding guidelines. The purpose of this study was to assess demographic, knowledge, and psychosocial factors associated with self-reported adherence to EDT recommendations. Owners of canine patients who were prescribed an EDT to diagnose CAFR in the past 5 years (2012 - 2017) were identified through a medical records review from a single veterinary dermatology specialty practice in East Greenwich, RI, USA. Potential participants (n=665) were contacted by email and invited to participate in an online survey. Survey items were guided by the Health Belief Model, a widely-used public health framework to assess how psychosocial attitudes impact health behaviors. Multivariablelogisticregre ssionwasconductedtoassessfactorsassociatedwithself-reportedadherenceto EDT recommendations. Of 192 survey participants, 77 (40.1%) reported perfectly strict EDT adherence. In multivariable analyses, participants with increased barriers (aOR=0.88, p=0.013) were significantly less likely to report strict EDT adherence. Participants with increased knowledge (aOR=1.30, p=0.049) or self-efficacy (confidence in their ability to follow strict adherence guidelines) (aOR=1.18, p=0.014) were significantly more likely to report strict EDT adherence. These findings indicate that the majority of EDTs prescribed are not followed strictly. Interventions which help clients reduce perceived barriers, increase self-efficacy and improve knowledge could improve adherence to EDT instructions and increase the utility of this diagnostic test.

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Conflict of Interest: None declared

Clinicopathologic findings and clinical outcomes in 49 cases of feline pemphigus foliaceus examined in northern California, USA (1987-2017)

T.J.M. JORDAN*, V.K. AFFOLTER†, C.A. OUTERBRIDGE‡, E.C. GOODALE‡, S.D. WHITE‡

** William R. Pritchard Veterinary Medical Teaching Hospital, University of California Davis, Davis, CA, USA*
† Department of Pathology, Microbiology & Immunology, School of Veterinary Medicine, University of California Davis, Davis, CA, USA
‡ Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, Davis, CA, USA

Abstract: The study objective was to describe the clinicopathologic features and treatment outcomes in cases of feline pemphigus foliaceus. Forty-nine cats met inclusion criteria requiring a histopathologic diagnosis and medical records available for review: 28 spayed (57%) and two intact (4%) females, and 19 castrated males (39%). The majority of cats (32/49 [65%]) were domestic short, medium or long hair. Median age at presentation was 6 years (range: 5 months – 15 years). Skin lesions included crusts (100%), alopecia (76%), erythema (73%), erosion/ulceration/excoriation (61%), and pustules (22%). Pruritus and pain were noted in 63% and 22% of cats, respectively. Affected sites included pinnae (92%), head/haired face (88%), nasal planum, philtrum, and alar folds (57%), ungual folds (47%), perineum (33%), and peri-areolar region (27%). Disease onset was temporally associated with recent vaccination in two cats, and pregnancy/parturition in two cats. When evaluated, anemia, leukocytosis, neutrophilia, and hyperglobulinemia were commonly noted. Histologic slides were available for review in 46 cats, and eight cats had suggestive vasculopathic changes. Complete remission was noted in 33 cats: 22 cats received corticosteroid monotherapy and 11 cats received corticosteroids with cyclosporine, chlorambucil, or gold salts. Twenty-six cats received oral antibiotics concurrently for superficial pyoderma. Relapse occurred in 19 cats, and was commonly associated with tapering/discontinuation of immunosuppressive medications. Complete discontinuation of immunosuppressive medications was not recorded in any cat. Spontaneous remission without treatment was noted in one cat with suspected metastatic thyroid carcinoma. Results indicated that cats with pemphigus foliaceus generally responded favourably to treatment and required long-term therapy.

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Conflict of Interest: None declared

Canine intradermal test threshold concentrations vary according to allergen extract and manufacturer

S. ABRAMS*, G. BROCK†,M. PALETTAS†, M. BOLNER‡, T. MOORE-SOWERS‡, G. PLUNKETT‡, L. COLE*, S. DIAZ* andG. LORCH*

**Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*
†Center for Biostatistics, Department of Biomedical Informatics, The Ohio State University, Columbus OH, 43210, USA
‡ ALK-Abelló, Round Rock, TX, USA

Abstract: Limited information is available on intradermal testing (IDT) threshold concentrations (TCs) for common allergens from different allergen extract suppliers.The objective of this study was to determine and compare the TCs for IDT allergenextractsin healthydogs from two allergen suppliers. We hypothesized that IDT TCs would vary by allergen extract and manufacturer. Allergen suppliers were ALK-Abelló and Stallergenes Greer. IDT was performed in 35 healthy non-allergic dogs. Eleven allergens from each company were diluted to six protein nitrogen unit concentrations. Reactions were scored subjectively(0-4+), and objectively as the average of the vertical and horizontal diameter of each reaction. Subjective and objective measurement concordance was determined for each company. Threshold concentrations were defined as the highest concentration of an allergen where ≤10%of dogs had a positive subjective reaction (≥2+) at 15 minutes. Using generalized estimating equations, the TCs of *Chenopodium album, Ulmus americana, Juglans nigra, Acer negundo, Juniperusvirginiana, Quercus alba, Sorghum halepense, Phleumpratense*, and *Dermatophagoides farinae* were determined for ALK-Abelló extracts. The TCs for *Ambrosia* spp., *Chenopodium album, Plantagolanceolata, Ulmusamericana, Juglans nigra, Acer negundo, Sorghum halepense* and *Phleum pratense* were identified for Greer extracts. Not all TCs could be determined for either company's extracts. Percent concordance ofthe objective measurement with the subjective score was 77.3% for ALK and 75% for Greer allergens. The TCs differed between allergens and manufacturers; therefore, not all allergens from different manufacturers are interchangeable nor should all allergens in an IDT panel be used at the same concentration.

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Conflict of Interest: None declared

Differences in tight junctions comparing canine epidermal keratinocytes and normal primary canine keratinocytes in culture

R. SANFORD*, K. AHRENS*, R. MARSELLA*

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Canine epidermal keratinocytes (CPEKs) are widely used as canine keratinocyte cell line. It is unknown if CPEKs are suitable for studies evaluating barrier function and tight junctions. A widely accepted method to evaluate skin barrier is the measurement of transepithelial electric resistance (TEER). Our study aimed to compare TEER and tight junction (TJ) protein expression in CPEKs and primary canine keratinocytes from normal dogs in culture until day 6. While TEER measurements in primary canine keratinocytes readings increased overtime to 2000 ohms/cm by day 6, CPEKs readings stayed around 100 to 150 ohms/cm. Students t-test of the TEER values showed significant difference between CPEKs and normal primary canine keratinocytes ($P = 0.0275$). TJ proteins (ZO1, claudin-1) were visualized by immunofluorescence. Slides were fixed on day 0 of confluence and stained with ZO1 and claudin-1 antibodies. Five images of each antibody were taken, randomized and evaluated blindly by three investigators for intensity, staining location, granularity, uniformity, and continuity. Cell size and variability of size were evaluated. Because we only had one slide of CPEKs cells per antibody, we tested our scores with a single sample z-score test. Size of CPEKs cells was significantly ($P < 0.0001$) smaller than primary normal keratinocytes and less variability of size was detected. For claudin-1, intensity of staining was greater in CPEKs ($P = 0.00362$) while granularity was less in CPEKs ($P < 0.0001$). No significant differences were seen for ZO1. In conclusion, CPEKs may not be representative of normal primary keratinocytes in terms of creating an effective skin barrier.

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Conflict of Interest: None declared

Transepithelial electrical resistance (TEER) of normal primary canine keratinocytes is significantly higher than atopic keratinocytes

K.AHRENS,* R. SANFORD,* R. MARSELLA*

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Skin barrier function is believed to play an important role in atopic dermatitis (AD). Debate still exists whether there may be some primary barrier dysfunction in canine AD or whether the impairment is purely secondary to inflammation. Transepithelial electrical resistance (TEER) is used extensively in human medicine as a measurement of skin barrier integrity and tight protein junction function without the influence of inflammation derived by leukocytes recruited in the skin. To the best of the authors' knowledge this parameter has not been assessed in canine AD. Thus the purpose of this study was to investigate TEER keratinocyte cell cultures from biopsies of normal and atopic dogs. Primary cell cultures from five normal and five atopic dogs grown on transwell inserts were measured up to 13 days after confluence using a TEER device that quantitatively measures the integrity of tight junctions and cell barriers in epithelial cell monolayers. Measurements were taken daily and ranged from 3 to 2359 ohms/cm. Normal cells were significantly higher than atopic cells with an average peak at day 6 of 1954ohms/cm. Atopic cells' highest levels were at day 13 of 520ohms/cm. Using 2-way repeated measures ANOVA there was a significant difference over time ($p<0.0001$) and between groups ($p<0.0001$). In conclusion, there is a significant difference in barrier function between normal and atopic keratinocytes. Future studies will need to address the cause of this intrinsic difference in behavior of atopic keratinocytes starting with expression of tight protein junction overtime in cell culture.

Sources of funding: Self-funded.

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The filaggrin story continued: investigation on filaggrin and filaggrin 2 in normal and atopic dogs

R. MARSELLA,* K. AHRENS,* R. SANFORD*

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Currently two proteins with filaggrin-like characteristics are described. We comparatively describe their distribution and morphology in normal and atopic canine keratinocytes cultures using fluorescently labeled antibodies to make tridimensional videos. At initial confluence, filaggrin-1 was nuclear and regularly organized in normal keratinocytes while it was both cytoplasmic, nuclear and irregularly structured in atopic keratinocytes. Four days later in normal keratinocytes distribution was primarily nuclear with some detection in the cytoplasm while in atopics it was all cytoplasmic. Filaggrin-2, in both normal and atopic keratinocytes, was more cytoplasmic and less abundant than filaggrin-1 and stained less in atopics. On day 4, in normal keratinocytes filaggrin-2 was detectable as discrete and well defined filaments while it looked decreased, patchy, and irregularly distributed in cytoplasm of atopic keratinocytes. Using QRT-PCR, higher gene expression for both filaggrins was found in atopics compared to normal. Next, biopsies from atopic beagles before (day 0) and after exposure to allergen (day 3 and 10) were stained by immunohistochemistry. Photographs were scored blindly for intensity, patchiness and distribution. No significant difference in intensity existed between filaggrin-1 and 2, overtime. Filaggrin-1 patchiness significantly increased at peak of clinical reactions (day 3). Both filaggrins were variably detected in both stratum granulosum and spinosum. Filaggrin-2 was also detected in stratum basale in 10% of slides. PCR showed increased gene expression for filaggrin-1 after allergen challenge (day 10). It is preliminarily concluded that altered organization of filaggrin filaments exists in atopic keratinocytes and that this may lead to increased production.

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Conflict of Interest: None declared

Investigation on the effects of current treatments for canine atopic dermatitis on skin barrier function in a colony of atopic beagles

R. MARSELLA,* K. AHRENS,* R. SANFORD,* TRUJILLO

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: We aimed to investigate the effect of currently approved treatments for canine atopic dermatitis on skin barrier function. Atopic beagles (n=19) were randomly divided into five groups and challenged with allergen (*Dermatophagoides farinae*, 12.5mg/dog/challenge) twice weekly for 4 weeks. Treatment groups included oclacitinib (n=4, oral, 0.5 mg/kg twice daily for 2 weeks, once daily for 2 weeks), cyclosporine (n=4, oral, 5 mg/kg once daily for 28 days), lokivetmab (n=4, one subcutaneous injection, 2mg/kg, on first day of challenge); prednisone (n=4, oral, 0.5 mg/kg twice daily for 2 weeks, 0.5 mg/kg once daily for 1 week, 0.5 mg/kg every other day for 1 week), andcontrol group (n=3, no treatment). Skin barrier function was assessed on days 0, 14 and 28on three sites (pinnae, axillae, inguinal area). Evaluations included dermatitis (CADESI-03), pruritus, transepidermal water loss (TEWL, closed chamber device) and hydration (corneometer). Significant decrease of hydration from baseline was found in control and prednisone groups in axillae on day 14 (P=0.004 andP=0.027, respectively). Lokivetmab significantly decreased hydration on day 28 compared to baseline (P=0.027) and with controls (P=0.023) in pinnae. Cyclosporine significantly increased TEWL from baseline to day 14 (P=0.029) and to day 28 (P=0.031) in axillae. Oclacitinib and lokivetmab did not significantly change TEWL overtime. Controls significantly increased TEWL in axillae on day 28 (P=0.0237). CADESI-03 correlated with TEWL (r=0.22; P=0.0043) and pruritus scores (r=0.22; P=0.0283). Hydration did not correlate with any parameters. In conclusion, none of the treatments improved skin barrier function parameters using technologies currently available.

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Conflict of Interest: None declared

Characterization of skin microbiota from superficial pyoderma forms in dogs

C. E. OLDER*, F. BANOVIC† and A. RODRIGUES HOFFMANN*

**Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA*
†Department of Small Animal Medicine & Surgery, College of Veterinary Medicine, University of Georgia, Athens, USA

Abstract: Canine superficial bacterial pyoderma is a common and recurrent disease characterized by three clinically andhistopathologically distinct forms: superficial bacterial folliculitis (SBF), bullous impetigo (BI) and exfoliative pyoderma-associatedepidermal collarettes (EC). Based on traditional culture sampling, *Staphylococcus pseudintermedius* is considered the major pathogen in canine superficial pyoderma. This study aimed to evaluate the skin microbiome and staphylococcal diversity of SBF- (12 lesions, eight dogs) and EC-associated (nine lesions, six dogs) superficial pyoderma phenotypes in dogs. Next-generation sequencing was performed on DNA extracted from superficial pyoderma lesions with primers targeting the V1-3 region of the bacterial 16s rRNA gene; sequences were processed in mothur using the RDP classifier and Silva reference alignment. *Staphylococcus* spp. accounted for 43% and 26% of the total sequences from SBF and EC samples, respectively. Staphylococcal sequences were further classified using a *Staphylococcus* spp. reference database as *S. pseudintermedius* (87%), *S. pasteuri* (3%), *S. felis* (3%) and *S. epidermidis* (2%). Relative abundance of *Staphylococcus* spp. was found to be significantly ($P < 0.01$) higher in the EC samples relative to the SBF samples, whereas *Corynebacterium* spp. abundancewas increased in SBF samples ($P < 0.05$). As expected, SBF and EC lesionscontained higher densities of *Staphylococcus* spp. measured by quantitative PCR than healthy canine skin (eight samples, four dogs) ($P < 0.01$ and $P < 0.01$, respectively). These results characterize differential contributions of microbial dysbiosis during SBF and EC pyoderma lesionformation; whether *Corynebacterium* spp. contributes to the SBF pathophysiology or merely reflects the abnormal environment needs to be further investigated.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Development of a vaccine to treat Staphylococcus pseudintermedius infections in dogs

M. ABOUELKHAIR*, L.A. FRANK†, A. SEWID*, D.A. BEMIS*, S.A. KANIA*

**Dept of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA*
†Dept of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

Abstract: There has been an increase in meticillin-resistant *Staphylococcus pseudintermedius* infections in dogs with most being multidrug- and extensively drug-resistant. Alternatives to antibiotics are needed to control staphylococcal infections. We found the bacteria inhibit phagocytosis, neutralize complement, bind immunoglobulin, induce B-cell apoptosis and secrete leukotoxins. The purpose of this study was to identify antigens of *S. pseudintermedius*, modify them, and test their antigenicity *in vivo* and *in vitro*. We developed a vaccine composed of antigens from key *S. pseudintermedius* defensive and offensive proteins secreted and/or exposed on the bacterial surface. Recombinant proteins attenuated by amino acid substitutions and antigenically very similar to the corresponding native proteins but without toxic and immune suppressive properties were produced. The proteins included coagulase, leukotoxin ED, protein A, 5'-nucleotidase, and exotoxin-15. Three injections were given subcutaneously to healthy dogs 1 week apart. Serum was collected before vaccination and weeks 1, 2, 3, and 5 post-vaccination. Complete blood count and chemistry panels were assessed at weeks 0 and 5 with no abnormalities noted. A good antibody response was obtained from individual proteins (100 µg/ injection), a protein mixture (20 µg of each protein in one vaccine) and a single chimeric protein with no adverse reactions. The antibodies neutralized the superantigenic effect of protein A (85% reduction in activity), overcame canine neutrophil migration inhibition by exotoxin-15 (55.5% improvement in neutrophil migration) and prevented coagulation of plasma by coagulase. These results show the identified proteins to be antigenic and non-toxic. The vaccine will be tested in a clinical trial.

Sources of funding: Self-funded.

Conflict of Interest: None declared

In vitro activity of phytosphingosine against Staphylococcus pseudintermedius: minimum inhibitory concentration and biofilm disruption after 10 min exposure

A. A. FERRAN*, E. OLLIVIER§, C. ZEMIRLINE§

**UMR 1331 Toxalim, INRA, ENVT, INP EIP, Toulouse, France*
§Ceva Santé Animale, Libourne, France

Abstract: *Staphylococcus pseudintermedius* is the most frequent pathogen isolated from skin and ear infections in dogs and cats. Extensive and repeated treatments with systemic antimicrobials contribute to the development of resistance. Topical therapy, including the use of antiseptics (e.g. chlorhexidine), is very important in antimicrobial stewardship. Investigations are necessary to better understand the effect of other topically applied ingredients. This study was performed to demonstrate the effectiveness of phytosphingosine against *Staphylococcus pseudintermedius*. First, the minimum inhibitory concentration (MIC) of phytosphingosine hydrochloride (HCl-PS) was determined for 10 strains of *Staphylococcus pseudintermedius.* Second, 7-hour old biofilms containing approximately 6 $\log_{10}$ *Staphylococcus pseudintermedius* in a 24-well plate were exposed to 0.1% (m/v) of HCl-PS, 0.3% (m/v) chlorhexidine digluconate or vehicle negative control. After 10 min at room temperature, the biofilms were rinsed twice and the bacteria in each well were counted before and after 15 min ultrasounds. For the 10 tested strains, the MIC of HCl-PS was below 0.003% (m/v), which is below its concentration in commercial products. After 10 min exposure to 0.1% (m/v) HCl-PS, biofilm embedded bacteria were not detected in six of six wells. Biofilm bacteria were also reduced by 0.3% chlorhexidine digluconate but completely undetected in only two of six wells. The vehicle had no effect on the bacteria. Under our *in vitro* conditions, HCl-PS was successful in reducing *Staphylococcus pseudintermedius* biofilm. These results suggest that topical products containing phytosphingosine could be effective in reducing *Staphylococcus pseudintermedius* biofilm on skin and ears in dogs and cats.

Sources of funding: Ceva.

Conflict of Interest: E. Ollivier and C. Zemirline are employees of Ceva.

Antibacterial effect of N-acetylcysteine in combination with antimicrobials on common canine otitis externa bacterial isolates

E.R. MAY*, B.E. RATLIFFE*, D.A. BEMIS†

**Department of Small Animal Clinical Sciences, University of Tennessee, Knoxville, TN, USA*
†Department of Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN, USA

Abstract: FDA approved canine otitis externa treatments are limited in variety and may contain ototoxic ingredients. Bacterial resistance is of continued concern, thus it would be ideal if non-ototoxic agents could be combined antibiotics for a synergistic effect, requiring lower concentrations of antibiotics to treat infections. Evidence of synergism and antagonism between N-acetylcysteine and various classes of antibiotics has been reported; our group was interested in further examining these interactions. The study aim was to determine if N-acetylcysteine, an otoprotective and antimicrobial compound, has synergistic activity when combined with enrofloxacin or gentamicin in vitro against bacterial isolates causing canine otitis externa. Twenty-two bacterial isolates from canine clinical cases were identified and tested: seven *Staphylococcus pseudintermedius*, 12 *Pseudomonas aeruginosa*, and three *Corynebacterium* spp. Each isolate was grown on blood agar for 24 h and transferred to Mueller-Hinton broth, resulting in a final concentration of 10^5 CFU/mL.Each well was inoculated with 50 µl of bacterial suspension. N-acetylcysteine was diluted in Mueller-Hinton broth to a starting concentration of 160 mg/ml, and both enrofloxacin and gentamicin were diluted to 64 µg/mL. Serial microdilution assays were performed in triplicate with negative controls for all isolates tested. For N-acetylcysteine, the minimum inhibitory concentration (MIC) values for all isolates tested ranged from 5-20 mg/mL, enrofloxacin MIC values ranged from 0.03->8 µg/ml and gentamicin MIC values ranged from <0.06->32 µg/mL. When combined, N-acetylcysteine with enrofloxacin and N-acetylcysteine with gentamicin resulted in both synergism and antagonism at the concentrations tested.

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Conflict of Interest: None declared

EPIDERMAL CORNIFICATION AND DISORDERS IN ANIMALS

LINDER KE, DVM, PhD, Dipl. ACVP

North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA

INTRODUCTION

The stratum corneum is the outer skin barrier to physical injury and fluid permeability and its functions are required to maintain life. Genetic alterations thataffect its many structural and biochemical components cause hereditary cornification defects, called ichthyoses. In these two lectures, a review of epidermal cornification will illustrate the specific location where each ofthe ichthyoses of animals disrupts cornification tocause disease. Only ichthyoses with known genetic causal variants (mutations) are covered.

CLINICAL FEATURES AND CLASSIFICATION

The clinical signs of ichthyoses are scaling, with or without hyperkeratosis,dry skin and variable erythroderma, which result from abnormal stratum corneum.Breed associations are important. Secondary infections are common. Ichthyoses sometimes mimic, and are thought often to potentiate, allergic disease. Ichthyoses are classified primarily based on clinical features and molecular geneticsand less now on histomorphological features. Non-syndromic ichthyoses affect only the skin, while syndromic variants affect skin and other organs. In humans, non-syndromic variants are sub-classified as common ichthyoses, autosomal recessive congenital ichthyoses (ARCI), keratinopathic ichthyoses (epidermolytic), and other forms of ichthyoses.[1] In animals, most ichthyoses are non-syndromic, have a lamellar pattern of cornification and are similar to ARCI in humans, while a few are keratopathic/epidermolytic and a few are other forms.

STRATUM CORNEUM

Cornification is the terminal differentiation step of the epidermis that results in formation of the stratum corneum. Composed of layers of dead corneocytes, sealed together by lipids, the stratum corneum continuously forms by cornification, matures biochemically and desquamates from the skin surface to maintain its composition and integrity. Four essential components of the stratum corneum are generated during cornification and include1) corneocytes 2) intercellular lipids 3) adhesion complexes and 4)a protease/protease-inhibitor network.

CORNEOCYTES

Corneocytes are tough, insoluble, dead cells that form the structural layers (20 to 50+) of the stratum corneum and are critical for support of intercellular lipids (see below) and to physically resist skin injury. Non-living corneocytes form through programed cell death of viable keratinocytes of the outerstratum granulosum (SG1) layer. In this process, key cellular changes include 1) cell flattening, 2) organelle loss, 3) cytoskeletal compaction, 4) cornified envelope formation and 5) structural protein cross-linking. Keratinocytes shrink and flatten dramatically, losing waterand cell volume (nearly 50-80%), into very thin disks withfaceted margins (about 1 um thick and 30 to 40 um in diameter). This shape change is facilitated by complete collapse and compaction of the keratin intermediate filament network by filaggrin, a filament aggregating protein that is released by caspase-14 and other enzymes. Intermediate filaments are rope-like, semi-elastic, heteropolymers of type-1 and type-2 keratin proteins, which are mostly KRT1, KRT2 and KRT10 in the stratum granulosum. Most organelles and non-keratin proteins are removed by autophagy, including the nucleus by nucleophagy, presumably because organelles impede cytoskeletal compaction.A cornified envelope forms by deposition of a dense layer of mixed proteins just below the plasma membrane and stabilizes the cell periphery. Cornified envelope proteins include envoplakin, periplakin, loricrin, involucrin, small proline rich proteins, and others. To strengthen the corneocyte, transglutaminases (TGMs 1, 3 and 5) covalentlycross-linkkeratin intermediate filaments, cornified envelopeproteins and adhesion complexes (corneodesmosomes). Key biochemical features of keratinocyte programed cell death are activation of caspase-14, autophagy and transglutaminase activity. Keratohyalin granules (KGs) store proteins needed for structural changes in cornification, including caspase-14, profilaggrin, loricrin, etc.

Ichthyoses in animals affecting corneocyte formation

Credille et al described a mutation affecting the *TGM1* gene for transglutaminase-1 in Jack Russell terriers with a recessively inherited cornification defect that resembles lamellar ichthyosis in humans.[2] Credille et alalso reported

a *KRT10* genemutation in Norfolk Terriers and confirmed an animal variant of epidermolytic hyperkeratosis.[3] Hypergranulosis, large KGs and eosinophilic keratin aggregates were observed. Unlike humans, inheritance is autosomal recessive.In Dogue de Bordeaux dogs with footpad hyperkeratosis, Plassais et al identified a mutation in *KRT16*, which resembles human palmarplantar non-epidermolytic keratoderma.[4] Hereditary footpad hyperkeratosis in the Kromfohrländer and Irish Terrier breeds was identified by Drogemuller et al to be associated with a mutation in *FAM83G*, but a molecular mechanism is unknown and is included here because of clinical similarities.[5] Gharahkhani et al found a possible association of a mutation of *ADAMTS17* for primary lens luxation in Miniature Bull terriers with abnormal footpad hyperkeratosis (data limited) - a possible syndromic form of palmar plantar keratoderma.[6] Forman et al identified a mutation in *FAM83H* (family with sequence similarity 83, member H)in Cavalier King Charles Spaniels with autosomal recessive,congenital keratoconjunctivitis sicca and ichthyosiform dermatosis (CKSID).[7] Keratinocyte hydropic degeneration and footpad ulcers suggest a keratinocyte cytoskeleton defectand a keratopathic ichthyosis. Ocular disease indicates a syndromic condition. In humans, *FAM83H* variants cause amelogenesis imperfecta. *FAM83H* functions as akeratin intermediate filament organizing protein (interacting with casein kinase-1alpha) and mutations disrupt thekeratin cytoskeleton and desmosome organization.[8] These findings support a role for *FAM83H* mediated disruption of intermediate filaments in dogs with CKSID. Hereditary nasal parakeratosis in Labrador retrievers is attributed to a mutation in *SUV39H2*,[9] which alters epidermal differentiation, interrupts corneocyte nucleophagy and induces parakeratosis.

INTERCELLULAR LIPIDS

Intercellular, non-polar lipids seal layers of corneoctyes in the stratum corneum and form the major permeability barrier of the skin to water and environmental molecules. The key components are the 1) lipid composition, 2) lipid envelope and 3) cornified lipid envelope. After hydrolytic modification, the intercellular lipid composition, by weight, is approximately 50% ceramides, 25% cholesterols and 10 to 15% fatty acids, although in nearly equimolar concentrations. The ratios are important for barrier function. Lamellar bodies are secretory vesicles that arise from the endoplasmic reticulum and deliver preformed stacks of lipids and proteins (proteases, inhibitors, etc.) to the external surface of the developing corneocyte.Lipid transporters load lamellar bodies with lipids, in the cytoplasm, such as ABCA14 for glucosylceramides. Lipid stacks unfurl and fuse to form a continuous layer of organized lipids called the lipid envelope that coatscorneocytes.The multilayered (stacked) structure of the lipid envelope is important for function and is altered by changing the lipid composition.The cornified lipid envelope replaces the corneocyte plasma membrane with long fatty acid chains of -(acylated)-OH-ceramides, derived predominately from linoleic acid, that are covalently anchored to involucrin of the cornified envelope by transglutaminases. The cornified lipid envelope seals the lipid envelop to the corneocyte surface.Linoleic acid is an essential fatty acid required forcornified lipid envelope formation and function.

Ichthyoses in animals affecting intercellular lipids

Several ichthyoses are described in animals that alter epidermal lipid metabolism and most are similar to ACRI in humans, affecting similar genes. Charlier et alreported a mutation in *ABCA12* in Italian Chianina cattle with severe hyperkeratosis and fissuring resembling harlequin ichthyosis in humans.[10] Grall et al reported a mutation in *PNPLA1* (patatin-like phospholipase domain-containing protein 1) in Golden Retriever dogs.[11] Disease can be noted at a young age or be so mild as to gounrecognized for years. Metzger et al identified a mutation in *FATP4 (SLC27A4)*, a fatty acid transporter protein, in Great Danes that present at a young age with thickened, folded and wrinkled skin, mimicking a mucinosis clinically.[12] Casal et al discovered a mutation in *NIPAL4* in American Bulldogs with generalized brown scale, erythroderma, secondary infections and variable pruritus.[13] *NIPAL4* codes a transmembrane protein (ichthyin) that likely is a Mg2+ transporter and impacts lipid metabolism.Transglutaminaseslink lipids to the cornified lipid envelope and mutations lead to bothkeratinocyte structural defects and altered lipid function (see above).

ADHESION COMPLEXES

Corneodesmosomes are the main, structural,cell adhesion junctions between corneocytes and form through modification of stratum granulosum desmosomes. Delivered by lamellar bodies, corneodesmosin binds extracellular cadherins, desmoglein-1 and desmocollin-1, of desmosomes and exhibits homo-oligomerization. Corneodesmosin stabilizes desmosomes and increases their flexibility and elasticity through glycine-loop properties.Internally,

periplakin binds the internal plaques of desmosomes and recruits envoplakin, thereby linking corneodesmosomes to the cornified envelope. Tight junctions form continuous, zipper-like, strings of cell-cell junctions that seal the periphery of keratinocytes in the stratum granulosum closely together, and create a selective, paracellular permeability barrier to electrolyte and solute movements in the upper epidermis. Tight junctions thus create an "internal skin barrier" below the stratum corneum and help to limit water loss through the epidermis. Tight junctions contain claudins, whichare differentially expressed in tissues and control selective permeability, including for Ca^{+2}, important for epidermal differentiation. Claudins 1, 4, 6, 7, 11, 12 and 18 are found in the epidermis. Tight junctions contribute to keratinocyte polarization (fence function) and to spatial coordination of corneodesmosome breakdown during desquamation.

Ichthyoses in animals affecting adhesion complexes
Ichthyoses affecting adhesion proteins are described in people but not yet in animals.

ENZYME NETWORK

A network of enzymes and enzyme inhibitors (protease, lipases, related inhibitors, etc.) orchestratecornification (see above), maturation and desquamation andrequire tight temporal and spatial control to maintain stratum corneum homeostasis. Importantly, biochemical maturation across the stratum corneum promotes deep corneocyte cohesion, but superficial corneocyte loss of cohesion and desquamation. There are several examples of biochemical changes occurring across the stratum corneum that impact how the enzyme network manages desquamation. Cholesterol sulfate is processed to free cholesterol by steroid sulfatase. Phospholipids are broken down to free fatty acids by phospholipases. Ceramides are broken down by ceramidase, sphingosine kinase and S1P lyase. Profilaggrin is processed to filaggrin and then to free amino acids (natural moisturizing factors) by caspase-14, matriptase, peptidylimidases, breomycin hydrolases, and others. As a consequence, the pH, calcium ion concentration and moisture drop,free amino acids increase, and conditions progressively favor activation of proteases involved in desquamation while causing inactivation of their inhibitors. The proteases kelikrien-5, 7 and 14 and others, progressively cleave corneodesmosin and then cleave desmoglein-1 and desmocollin-1 in corneodesomosomes. Their protease inhibitors, likelymphoepithelial Kazal type–related inhibitor-1, 2 and 3, and cystatin M/E become inhibited. Finally, desquamation occurs; corneodesmosome digestion and cellular delamination begin on the inner and outer faces of corneocytes and finish at the corneocyte periphery where tight junctions delay corneodesmosome cleavage.

Ichthyoses in animals affecting proteases or protease inhibitors
Naked foal syndrome in Akhal-Teke horses presents with mild ichthyosis and marked alopecia and horses die at a young age (syndromic features are yet uncharacterized). Bauer et al recently identified a mutation in *ST14* (suppressor of tumorigenicity 14 gene, Matriptase), a serine protease that is linked to filaggrin processing, corneodesmosome breakdown and terminal differentiation.[14] In a German Shepard dog with ichthyosis, Bauer et al also reported a de novo, dominant, gain of function, missense variant in *ASPRV1* (aspartic peptidase, retroviral-like 1, skin aspartic protease), a protease that processes profilaggrin to filaggrin.15

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Erythema Multiforme and "Old Dog Erythema Multiforme" Acute and Chronic Cytotoxic Dermatitis

Charles W. Bradley, VMD, Diplomate ACVP

University of Pennsylvania, School of Veterinary Medicine

INTRODUCTION

Erythema multiforme (EM)is anacquired cutaneous or mucocutaneous inflammatory disease that was first described in dogs in 1983(1)and in cats in 1984(2) with comparison to EM in people. As the name implies there can be a varied clinical presentation, frequently with maculopapular to vesiculobulous and ulcerative eruptions and crusts.Aspectrum of diseasehas been categorized as EM or highlighted as having histopathologic similarity.For example,studies demonstrate that EM, Stevens-Johnsons Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)have an overlapping spectrum of lymphocytic interface dermatitis and keratinocyte apoptosis, and are not histologically distinguishable. (3)With a histopathologic confirmation of a cytotoxic-interface dermatitis along the EM-TEN spectrum clinical features are more appropriate for differentiating EM, SJS and TEN. The reader is referred to these studies for information on differentiating these entities(3-5).There are other conditions that overlap with EM in both name and histomorphology for which continued investigation and refinement of the organizational (diagnostic and clinical) constructs are needed. These conditions can be grouped together as forms of interface or "cytotoxic" dermatitis.

The term cytotoxic dermatitis was first used by Murphy(6)to cover conditions with histopathologic evidence ofT-cell infiltration into the epidermis and keratinocyte death/apoptosis. This terminology has not been widely embraced by the human or veterinary literature, instead relying heavily on "interface dermatitis".At its most basic level cytotoxic dermatitis as a histopathologic reaction pattern can be divided into acute and chronic forms.

ACUTE CYTOTOXIC DERMATITIS

Acute cytotoxic dermatitis is manifest clinically as erythema, vesicles, erosions and ulcers. Histologically thedegree of satellitosis (i.e.cuffing of lymphocytes around apoptotic keratinocytes) is variable. Vacuolation and necrosis of the stratum basale is also identified in some circumstances, earning the designation of an interface dermatitis as seen in cutaneous lupus erythematosus and ischemic dermatopathy or dermatomyositis.Examples of acute cytotoxic dermatitis identified in veterinary medicine with a preponderance of suprabasilar involvement include erythema multiforme, SJS/TEN, graft-versus-host disease, and cytotoxic reactions to cutaneous viral infections.

Erythema multiforme spectrum: Little is known about the pathogenesis of EM in animals, but it is generally considered to be T-cell mediated hypersensitivity reaction. EM in companion animals differs from the human counterpart, with different triggers/associations, and clinical courses(4). Clinical lesions of EM in animals include erythematous macules, papules, bullae and erosions to ulcers that are symmetrical. The ventrum, mucocutaneous junctions, oral cavity, pinnae, and paw pads are common sites in dogs, with the trunk and mucocutaneous junctions being common foci in cats (Scott, Miller 1999). (7)Most cases could be classified as EM minor, with more severe and life-threatening manifestations classified as EM major, SJS or TEN. The disease course may be chronic or rarely recurrent.(7)At the more severe end of the spectrum (SJS/TEN) dogs may have systemic signs of fever, inappetence and lethargy with higher mortality. Cases often have concurrent mucosal involvement (oral, anal, or urogenital).(4, 5)

EM in people is thought to be a type IV hypersensitivity reaction, most often associated with viral infection (herpes simplex virus being predominant).Numerous putative causes have been associated with the EM-TEN spectrum in animals (Table 1). In animals, based on proposed theories, the EM spectrum may represent hypersensitivity reactions of types II, III or IV.Infectious associations are rarely identified, and most cases of EM remain idiopathic. While SJS/TEN are more frequently drug associated that is not necessarily the case for EM, where only 19% of cases were likely to be drug associated in one study.(3)In both EM and Graft-versus-host disease (below), there is a cytotoxic-interface dermatitis with upregulation of CD44, ICAM-1 (ligand of CD11a[LFA-1] of leukocytes; secondary to IFN-gamma production by T-cells) , and MHC class II adhesion molecules by keratinocytes.(8) CD44 is involved in T-cell activation and TNF-a release in NK cells. ICAM-1 and MHC class II are involved in the direct interaction between the keratinocyte

and lymphocytes. The intraepidermal and superficial dermal infiltrate consists of CD3+, CD8alpha, beta+positive lymphocytes.(8) Cases have also been shown with increased intraepidermal CD1+ Langerhans' cells, and increased numbers of dermal CD4+ Tcells and B cells.Direct T-cell mediated death or soluble ligands/factors (Fas ligand, granzymes, perforin and granulysin) may be responsible for keratinocyte death.(4, 9) Some cases may have more severe follicular involvement, and with the follicular involvement sebaceous gland loss is not uncommon. Cases in the veterinary literature with solely or at least initial mucosal involvement are rarely described(10).

From a histopathological perspective the EM cytotoxic reaction pattern is along a spectrum with SJS/TEN. A skin biopsy is needed for diagnosis, but disease categorization (e.g. TEN vs SJS) is best left to clinical assessment(4). According to Hinn et al cases clinically classified as EM are not frequently associated with causal drug exposure, whereas a positive drug score was more likely to be assigned to cases of SJS/TEN(3). Despite this, adverse drug reactions as a putative cause for EM remain pervasive. Some reported cases with a drug association may bebetter classified as SJS/TEN.

Graft Versus Host Disease (GVHD) is seen in dogs and cats as a complication of bone marrow transplantation, stem cell and rarely whole organ allografts. This may be secondary to the donor CD8+ and CD4+ T-cell recognition of and subsequent cytotoxicity of recipient epitopes (acute phase) or from the recipient T-cell recognition of the donated cells (chronic phase). The skin, liver and intestinal tract appear to be predilection sites for cytotoxic T-cell activity. In the skin the lesions overlap histologically with EM/TEN as a robust lichenoid interface cytotoxic dermatitis with lymphocyte satellitosis and keratinocyte apoptosis. In GVDH the degree of lymphocytic lichenoid inflammation in the superficial dermis tends to be more severe. Clinical lesions may include erythematous macules, multifocal alopecia, and ulcerative dermatitis with infrequent mucosal involvement.

Viral Induced Cytotoxic Dermatitis is rare in dogs and cats. Case reports of EM-like lesions secondary to canine parvoviral-2 infection indogs controversially places this virus as a putative cause for erythema multiforme(11, 12). Though the reaction pattern is similar it is best considered a cutaneous extension of a systemic infection by canine parvovirus with keratinocyte necrosis and cell-mediated immunity.EM in humans is typically caused by herpes simplex virus, and rarely by other viruses. The disease begins with the transport of HSV DNA fragments by circulating peripheral blood mononuclear CD34+ cells (Langerhans cell precursors) to keratinocytes, which leads to the recruitment of HSV-specific CD4+ TH1 cells. The inflammatory cascade is initiated by interferon-γ (IFN-γ), which is released from the CD4+ TH1 cells in response to viral antigens, and immune mediated epidermal damage subsequently begins. Active viral infection is not evident in the skin of herpesviral associated EM.Cytotoxic T-cells and apoptosis of infected keratinocytes can also be encountered at the base of regressing viral papillomas and is clinically and histopathologically distinct from EM.

CHRONIC CYTOTOXIC DERMATITIS

Murphy defined chronic cytotoxic dermatitis as being characterized by dyskeratosis, colloid body formation (keratinocyte mummification) and altered maturation of keratinocytes(6). Dyskeratosis in this context refers to theapoptotickeratinocytes.Several entities exist amongst dogs and cats that fall into this category of chronic or transepidermal cytotoxic dermatitis: hyperkeratotic/old-dog EM,canine proliferative, lymphocytic infundibular mural folliculitis and dermatitis (PLIMFD),and feline exfoliative dermatitis, and feline proliferative and necrotizing otitis externa.

Hyperkeratotic/Old-dog EM has been given honorable mention in main texts in veterinary dermatology with a one to two sentence description as a subset of erythema multiforme. (13, 14) There are two cases reported in the literature(4, 15). Cases are reportedlyas persistent, idiopathic and localized(13). Histologically there is cytotoxic dermatitis, often with targeting of the suprabasilar or upper half of the epidermis (sometimes called "superficial EM") with marked hyperkeratosis and parakeratosis. As the stratum basal is generally maintained there may be severe epidermal hyperplasia with rete ridge formation. Apoptotic keratinocytes may be retained in the stratum corneum and surface crust mimicking aged acantholytic cells. With severe parakeratosis and basal cell hyperplasia there may be histologic overlap with superficial necrolytic dermatitis SND. The degree of basal cell hyperplasia is frequently more severe in SND and careful histopathologic assessment and associated clinical features are required for differentiation.

In the reported cases there is a facial distribution. This condition should not be confused with human persistent EM for which hyperkeratosis is not a feature.

Feline exfoliative dermatitis has been attributed to a paraneoplastic syndrome (thymoma associated exfoliative dermatitis) and as an idiopathic condition when a thymoma cannot be identified (nonthymoma-associated exfoliative dermatitis)(16). There is significant histologic overlap with feline erythema multiforme histologically and clinically, and in cases where no thymoma is found it is not clear if these are distinct disease processes.Histopathologically feline exfoliative dermatitis tends to be more hyperkeratotic with less severe keratinocyte apoptosis than reported cases of feline EM and lymphocyte satellitosis may be equivocal.

Proliferative and necrotizing otitis externa has been reported in both kittens and in adult cats. The striking clinical presentation of crusted, proliferative plaques in the pinna and vertical canalis useful in making the diagnosis. Other locations including the face, and rarely the paw have been reported. Histologically there is cytotoxic dermatitis, frequently targeting acanthotic and hyperkeratotic (parakeratotic) follicular infundibulae. The infiltrating lymphocytes have been confirmed as CD3+ with associated caspase-3 positive apoptotic keratinocytes as reported in PLIMFD, below(17). Satellitosis is not as prominent as in reported feline EM and the intrafollicular epidermis is usually spared. Regions of apoptosis are localized to areas of hyperkeratosis. The cause is unknown and surveying for potential viral associations has been unrewarding to date.(18)

Proliferative, lymphocytic infundibular mural folliculitis and dermatitis (PLIMFD) has been reported as a rare condition in Labrador retrievers(19). The histologic features are nearly identical to those of PNOE, though the location may be more varied. This condition is distinguished from erythema multiforme by the latter lacking follicular distinction with parakeratotic hyperkeratosisand a lack of a papillated surface (due to infundibular involvement). Histopathologically, the interface dermatitis in the reported cases of PLIFMFD has a patchy distribution.

Summary

As a groupcytotoxic dermatitis appears to be an immune-mediated response to yet to be identified antigen(s) on keratinocytes. There is generally a response to immunosuppressive/immunomodulatory therapy and withdrawal of associated triggers (e.g. removal of thymoma in exfoliative dermatitis). Some cases (EM, feline exfoliative dermatosis) may be persistent and refractory to treatment, while others are self-limiting.

Pitfalls of histologic diagnoses: These conditions can have varied clinical and histopathologic presentations and multiple biopsy samples from a given case will be helpful in maximizing the potential for an accurate or helpful diagnosis. As mentioned above, though usually distinguishable, superficial necrolytic dermatitis can have some cross over with hyperkeratotic/old-dog EM. Some cases of cutaneous lupus (i.e. exfoliative cutaneous lupus erythematosus), with lichenoid interface dermatitis,may have suprabasilar keratinocyte involvement in addition to basal cell apoptosis and should not be confused with a chronic cytotoxic dermatitis. Occasionally early epitheliotropic T-cell lymphoma may overlap histologically and immunohistochemically with cytotoxic-interface dermatitis/EM. PARR/analysis for clonality may be useful in distinguishing these cases.(10)

Further investigation into the pathogenesis and underlying causes of acute and chronic cytotoxic dermatoses is needed. Clinical correlation with the histopathologic confirmation of an acute or chronic cytotoxic dermatitis is required as the biopsy is only a piece of the puzzle in classifying these conditions. Evaluation and scrutiny (drug-scoring) for underlying triggers is required.

Table 1: Etiologic Associations

EM (EM/SJS/TEN)[a]	Idiopathic, Drugs: Antibiotics: Amoxicillin, Amoxicillin-clavulanate, cefadroxil, cephalexin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, lincomycin, ometroprim-sulfadimethoxine, penicillin, tetracycline, trimethoprim-sulfas | Aurothioglucose, chlorpyrifos, diet (soy, beef-heartworm preventative chewable), diethylcarbamazine, D-limonene, ivermectin, levamisole, L-thyroxine, pentoxifylline, carprofen, griseofulvin, phenobarbital, moxidectin| Infections: anal sacculitis, feline herpesvirus, canine parvovirus, pseudomonal otitis externa, staphylococcal dermatitis|Neoplasia: thymoma, pheochromocytoma, CTCLb
GVDH	bone marrow, stem cell, organ allographs
Hyperkeratotic/OldDog/ Superficial EMc	leukemia, Pneumocystis; idiopathic
Feline Exfoliative Dermatitis	Thymoma; idiopathic
PNOE	Idiopathic
PLIMFD	Idiopathic; genetic (with breed association)?

[a]With scrutiny some cases may be reclassified amongst the EM/SJS/TEN spectrum and associations are listed for all entities.

[b]Early CTCL may mimic EM, and in some circumstances the two may not be able to be discerned leaving uncertaintyas to whether or not there is a neoplastic association with EM.

[c]Some cases may have associations reported as EM

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CUTANEOUS BARRIER DYSFUNCTION AND ECOSYSTEM PART 1: BARRIER ABNORMALITIES AND DISEASES

Koji Nishifuji, DVM, PhD, DiplAiCVD

Division of Animal Life Science, Tokyo University of Agriculture and Technology, Tokyo, Japan

INTRODUCTION

The epidermis resides in the outermost layer of the skin and play an important role in protect the mammalian body from penetration of external pathogens (inside-to-outside barrier) and loss of body fluids (inside-to-outside barrier). The structural integrity of the epidermis is therefore important in maintaining homeostasis of the mammalian body.

However, if the integrity is impaired by genetic alterations, environmental factors or the combination thereof, it allows colonization of microbes in the stratum corneum (SC), subsequent invasion of microbes or allergen and trans-epidermal loss of fluids. These conditions lead to bacterial skin infections and allergic skin diseases, which are developed primarily or secondary to genodermatoses. Also, inflammatory changes in the skin also disrupts the cutaneous barrier function. The aim of this lecture is to explain the main components associated with the cutaneous barrier and skin diseases associated with aberrant cutaneous barrier functions in humans and animals.

EPIDERMAL COMPONENTS CONTRIBUTE TO CUTANEOUS BARRIER FUNCTION

Corneocytes

Corneocytes are enucleated, terminally differentiated keratinocytes and the major cellular constituents in the SC. Corneocytes are differentiated from keratinocytes in the stratum granulosum (SG) through cornification process and cover the viable epidermis. In superficial SC, corneocytes shed from the SC surfaces through desquamation.

Corneodemosomes: Corneocytes are tightly linked to each other by corneodesmosomes with the extracellular components such as desmoglein (Dsg) 1, desmocollin (Dsc) 1 and corneodesmosins. The intercellular components of corneodesmosomes such as plakophilins, envoplakin and periplakin link the transmembrane constituents of corneodesmosomesand keratin filaments.[1]

Filaggrin and profilaggrin: In the cytoplasm of corneocytes, filaggrin (FLG) binds to keratin intermediate filaments to form keratin bundles, which provide mechanical strength.[2] The keratin bundles also collapse the corneocytes and result in their flattened shape. FLG is produced as the precursor protein profilaggrin, which contains 10-12 repeats of FLG monomers in humans[3] while it contains 4-6 repeats in dogs, in the SG and stored in keratohyalin granules.[4] The role of FLG in the SC barrier function has been well studied using FLG-deficient mice or flaky tail mice, the latter have spontaneous nonsense mutation in *FLG* gene.[5-7]

During cornification, profilaggrin is secreted to cytoplasm and cleaved to generate FLG monomers by proteases. Channel-activating serine protease (CAP) 1 and skin-specific retroviral-like aspartic protease (SASPase) play important roles in cleavage of profilaggrin to FLG monomers.[8, 9] Mice lacking those enzymes exhibited impaired cutaneous barrier function due to disturbed corneocyte morphogenesis and/or FLG processing.

In the superficial SC, FLG monomers dissociate from keratin filaments and subsequently degraded to urocanic acid (UCA) and pyrrolidine carboxylic acid (PCA) by caspase 14, calpain 1 and bleomycin hydrolase.1[0, 11] UCA decreases skin surface pH, and mice lacking FLG showed increase in the skin surface pH.[12] There is an *in vitro* evidence showing that UCA reduces the expression of co-stimulatory molecule on monocyte-derived dendritic cells and increases their ability to induce a regulatory T-cell phenotype in mixed lymphocyte reactions.[13] Indeed, epidermal Langerhans cells (LCs) expressing CD11c and CD83 increased in FLG-deficient mice,[13] suggesting that lack of UCA due to FLG-deficiency may be associated withimmune dysregulation in the skin. Meanwhile, PCA acts as a natural moisturizing factor, which may have water-holding capacity in the SC.[1]

FLG expression can be downregulated by immunological disturbances. Th2 cytokines such as IL-4, IL-13 and IL-31, as well as IL-33 that is stored in keratinocytes downregulate FLG expression.[1, 14, 15]

Cornified envelope: The cornified envelope (CE) resides beneath the plasma membrane of corneocytes and provides mechanical strength to the cell periphery. In early stage of CE formation, envoplakin, periplakin and involucrin accumulate beneath the plasma membrane and are crosslinked by transglutaminase (TG) 1 and TG5.[16] Subsequently, loricrin and small proline-rich (SPR) protein families are repeatedly crosslinked by TG3 to reinforce the CE. Loricrin and SPR protein crosslink onto the scaffold of involucrin by TG1 and TG5.[17] Among the CE constituents, involucrin acts as a scaffold of the CE, while loricrin forms the majority of the CE proteins.[1] The CE proteins are thought to have compensatory effects, because single knockout mice of genes encoding the CE proteins did not show obvious skin phenotypes.[18-20] In contrast, TG1-deficient mice showed neonatal death owing to increased trans-epidermal water loss and severe dehydration.[21] Similar to FLG, expressions of loriculin and involucrin can be downregulated by Th2 cytokines.[15]

Proteases: Kallikreins (KLKs) are chymotryptic serine proteases and play a role in desquamation of corneocytes in the upper SC. Keratinocyte-derived KLK5, KLK7 and KLK14 are involved in the proteolytic cascade associated with corneocyte desquamation, at least partly by disruption of corneodesmosomal adhesion.[22, 23] The enzymatic activity of KLKs are enhanced when skin surface pH is elevated.[22] The activated KLKs can induce expression of IL-1 cytokines in corneocytes and activate protease activated receptor type 2 (PAR2), thereby leading the expression of thymic stromal lymphopoietin (TSLP).[24, 25] Furthermore, hyperactivation of JAK1 tyrosine kinase led overexpression of serine proteases including KLKs and resulted in progressive pruritic dermatitis with impaired barrier function and subsequent Th2 response.[26] Interestingly, topical application of emollients suppressed the development of dermatitis in JAK1 hyperactive mice, suggesting that topical application of emollients when infants or puppies is important for prevention of AD development. The enzymatic activities of KLKs are inhibited by lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI) expressed in granular cells.[27]

Extracellular lipids

The extracellular lipids embed corneocytes and act as the "mortar" of the SC barrier. The extracellular lipids in the SC are the mixture of ceramides, cholesterol and free fatty acidsgenerated in granular cells. Ceramides are the major constituents of the SC extracellular lipids both in humans and dogs.[28, 29]

Precursor lipids of ceramides are produced in granular cells and packed into lamellar granules. It has been reported that two transmembrane lipid transporters, ATP-binding cassette subfamily A member 12 (ABCA12) and transmembraneprotein 79/mattrin (Tmem/Matt) play important roles in transportation of lamellar granule contents. ABCA12-deficient mice exhibited severe fatal skin barrier defects with accumulation of intracellular lipids in keratinocytes,[30] while Tmem-deficient mice exhibited spontaneous dermatitis with defective skin barrier[31] Glucosylceramide and sphingomyelin are ceramide precursors recognized in lamellar granules.[2]

During cornification, the contents of lamellar granules are secreted to the SC-SG boundary. Glucosylceramide and sphingomyelin are metabolized into free extractable ceramides by β-glucocerebrosidase and sphingomyelinase, respectively.[2] β-glucocerebrosidasedeficiency in mice resulted in lipid barrier defects in the SC.[32] It has been reported that sustained SC chymotryptic enzyme activity by prolonged increase in skin pH leads to degradation of those ceramide-producing enzymes,[33] suggesting that the ceramide-producing enzymes are potential substrates for KLKs. Free extractable ceramides in human and canine SC can be divided into 11 groups according to their sphingoid and fatty acid structures.[34] Among the ceramide groups, esterified ω-hydroxyceramides are converted into non-esterified ω-hydroxyceramides by 12R-lipoxygenase (12R-LOX), epidermal lipoxygenase-3 (eLOX3) and hydrolase. The ω-hydroxyceramides crosslink onto scaffold of involucrin by TG1, and form protein-bound ceramides that anchor the extracellular lipid lamellae consists of free extractable ceramides to the CE.[35, 36] In addition to suspected primary role of ceramides in the SC barrier function, ceramide production can be downregulated by Th2 cytokines,[1] Moreover, interferon-γ appeared to have the adverse effect on the SC structure and function, possibly by decreasing ceramides long-chain fatty acids.[37, 38] These findings suggest that the skin inflammation leads to decrease of the SC ceramides.

Tight junction

Tight junction (TJ) is an occlusive junction expressed in various epithelial tissues. It has been reported that functional TJs are recognized in superficial layer of the SG.[39] TJs consist of the extracellular proteins occludin family and claudins,

as well as cytosolic scaffold proteins such as zonulae occludens (ZOs).[1] Functional TJs act as zippers those tie plasma membranes of adjacent keratinocytes and hamper the penetration of large molecules or the external pathogens through the structure.

CORNEOCYTE ABNORMALITIES AND DISEASES

Filaggrin and diseases

In humans, loss-of-function mutations in *FLG* gene is associated with ichthyosis vulgaris and atopic dermatitis (AD).[40, 41] *FLG* gene mutation is considered as the most significant risk factor of human AD, although significant proportion of human patients with AD do not have such mutations. It has also been reported that IL-1 cytokine levels in the SC increased in human AD patients with loss-of-function mutations in *FLG* gene.[42]

Although canine *FLG* gene was completely cloned, *FLG* gene mutations in canine genodermatosis or the other skin diseases have not been reported. Previous studies demonstrated a possible association of altered *FLG* transcription or protein expression in canine AD. Quantitative real-time PCR analysis revealed that *FLG* gene transcription in clinically non-lesional skin decreased in the subgroup of West Highland White terriers with AD.[43] Immunostaining analysis revealed the missing C-terminal *FLG* expression in the small subgroup of dogs with spontaneous AD.[44] In contrast, another study reported that *FLG* gene transcription appeared to increase in the skin of dogs with spontaneous and experimental AD as determined by quantitative real-time PCR analysis.[45, 46] Immunostaining intensities of *FLG*-metabolizing enzymes such as calpain 1, caspase 14 and matriptase increased in the skin obtained from an experimental canine model of AD,[47] suggesting abnormal catabolism of *FLG* in canine atopic skin. To date, there is no consensus among researchers regarding abnormal *FLG* expression in CAD. Recently, heterozygous variant in the *ASPRV1* gene encoding SASPase, which leads to altered *FLG* expression, was reported in a German Shepherd dog with ichthyosis.[48] Moreover, a single point mutation in the suppressor of tumorigenicity 14 gene (ST14), which encodes matriptase causes naked foal syndrome in Akhal-Teke horses. Horses with this disease are born hairless and often die within days after birth, possible due to cutaneous barrier defect.[49]

Proteases

Enhanced KLKs activity may lead to profound skin barrier defect due to excess shedding of corneocytes. Netherton syndrome is a genetic human skin diseasewith abnormal cornification and severe allergic manifestations such as dermatitis, rhinoconjunctivitis, asthma and a high titer of serum IgE. It has been reported that this syndrome is caused by loss-of-function mutations in *SPINK5* gene that encodes LEKTI, an inhibitor of KLKs.[50] In addition, the 4bp insertion polymorphism of KLK7 gene resulted in gain-of-function mutation in KLKs and associated with human AD in the UK cohorts.[51] Involvement of KLKs in skin diseases has not been reported in veterinary literatures.

Corneodesmosomes

In human medicine, homozygous mutations in *DSG1* gene resulted in lack of membrane expression of Dsg1 in patients with severe dermatitis, multiple allergies and metabolic wasting (SAM syndrome).[52] In addition, corneodesmosin-deficiency causes peeling skin syndrome, a recessive genodermatosis in humans characterized by lifelong widespread, reddish peeling of the skin with pruritus.[53]

In veterinary medicine, there is an immunofluorescence evidence showing that fluorescent intensities of corneodesmosin and claudin-1reduced in experimental AD dogs with house dust mite sensitization.[54] Genodermatoses caused by mutation in genes encoding corneodesmosomal proteins have not been reported in veterinary literatures.

Cornified envelope

TG1-deficiency causes severe autosomal recessive congenital ichthyosis in humans (ARCI1 orichthyosiform erythroderma). To date, broader spectrum on *TG1* mutations have been reported in human patients with ARCI.[55] Also, homozygous missense mutation in *TG5* has been reported to cause peeling skin syndrome in humans.[56]

In veterinary literatures, *LINE-1* insertion in *TG1* gene has been reported to cause lamellar ichthyosis in Jack Russell terrier dog.[57] In addition, it was reported that transcription of involucrin gene was upregulated in dogs with spontaneous AD, although its clinical and biological significance remains to be further elucidated.[45]

CUTANEOUS LIPID ABNORMALITIES AND DISEASES

In human medicine, mutations in genes encoding lipid transporters or lipid metabolism enzymes have been reported to cause some form of ichthyosis. Loss-of-function mutations in *ABCA12* gene causes disrupted skin lipid barrier in human harlequin ichthyosis.[58] It has also been reported that some spectrum of *ACBA12* gene mutations are associated with ARCI.[59] Mutation in *SLC27A4* gene encoding fatty acid transport protein 4, which enhances the uptake exogenous, long chain fatty acids into keratinocytes, causes ichthyosis prematurity syndrome in humans.[60] Nonsense mutations in *ALOX12B* and *ALOXE3* genes, which encode 12R-LOX and eLOX3, respectively, cause milder form of congenital ichthyosis (ARCI2 and ARCI3) in humans.[61] Deficiency in β-glucocerebrosidase and steroid sulfatase, the latter degrading cholesterol sulfate to cholesterols in keratinocytes, cause type II Gaucher disease and recessive X-linked ichthyosis, respectively.[55, 62] Human ARCI with CERS3 gene mutations encoding ceramide synthase 3 exhibits disturbed sphingolipid profile with reduced skin-specific long-chain ceramides.[63, 64] Recently, missense mutation of the *TMEM79* gene has significant association with human AD.[31]

Accumulating evidences indicate that mutations in lipid transporters or lipid metabolism enzymes are also recognized in genodermatoses recognized in domestic and companion animals. A single point mutation in *ABCA12* gene causes ichthyosis fetalis in Chianina cattles.[65] PNPLA1 mutation was recognized in golden retrievers and its cross breed with ARCI.[66, 67] It has been reported that PNPLA1-deficiency in mice leads to defect in omega-O-acylceramide synthesis,[68] indicating that the gene is involved in ceramide metabolisms in the skin. A splice acceptor site mutation in *SLC27A4* gene and deficiency of NIPAL4 have been shown to cause ARCI in Great Danes and American bulldogs, respectively.[69-71] NIPAL4, also referred to as ichthyin localizes to keratins and desmosomes in the epidermis and is considered to play a role in the epidermal lipid metabolism.[72]

Decrease of the SC ceramides and alteration of ceramide profiles have been reported in medical and veterinary literatures. It has been reported that esterified or non-esterified ω-hydroxyceramides in the SC decreased in human and canine AD,[28, 73-75] suggesting that reduction of free extractable and protein-bound ceramide classes with long carbon chain is associated with this disease. In addition, non-hydroxy acylceramides have also shown to be decreased in the SC of dogs with spontaneous AD.[36, 76]

Authentic or artificial ceramide-containing cleansers and moisturizers have been widely used to restore ceramide-associated cutaneous barrier function in human and canine AD.[77-80] A systemic review to evaluate the clinical effectiveness of moisturizers in human AD: it appeared that human AD patients treated with steroids with concomitant use of ceramide-containing cleanser and moisturizer increased the chance of disease clearance and significantly improved disease severity, although the efficacy of ceramide-containing products appeared to be less documented rather than urea.[77] Our preliminary study showed that a shampoo containing artificial ceramide-like ingredient rapidly restored the impaired barrier function by cleansing than control shampoo (Yoon JS and Nishifuji K: manuscript in preparation).

TIGHT JUNCTION ABNORMALITIES AND DISEASES

In human AD patients, reduced expressions of claudin-1, claudin-23 and ZO-1 have been reported.[81, 82] It was also reported that claudin-1 polymorphism was associated with susceptibility to AD in humans.[82] Immunofluorescence studies revealed that fluorescent intensities of claudin-1 and ZO-1 were reduced in dogs with experimental AD.[54]

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TANEOUS BARRIER DYSFUNCTION AND ECOSYSTEM PART 2: CUTANEOUS ECOSYSTEM, IMMUNITYAND DISEASES

Koji Nishifuji, DVM, PhD, DiplAiCVD

Division of Animal Life Science, Tokyo University of Agriculture and Technology, Tokyo, Japan

INTRODUCTION

As the interface with the external environment, the stratum corneum (SC) is always exposed to commensal and environmental microorganisms.[1] Recent advances in sequencing analysis such as bacterial 16S ribosomal RNA (rRNA) gene sequencing as well as fungal 18S rRNA of ITS sequencing revealed that cutaneous microbiota is more diverse thanseen in bacterial cultures.[2] It is also revealed that resident microbials play an integral part in the function of the skin, by interacting with innate immunity and the cutaneous barrier function. Accumulating evidences further suggest that cutaneous dysbiosis drives or aggravatesthe inflammatory reaction in some skin diseases such as atopic dermatitis (AD). The aim of this lecture is to explain the current knowledge of the cutaneous ecosystem and its relationship in innate immunity and allergic skin diseases.

SKIN BACTERIAL MICROBIOME AND DISEASES

Bacterial microbiota in normal skin

Bacterial microbiota in mammalian skin mainly consists of four phyla: *Firmicutes, Actinobacteria, Proteobacteria* and *Bacteroidetes*.[3] Most of bacteria belong to *Firmicutes* are gram-positive strains: *Staphylococcaceae* belong to this phylum and are well known bacterial habitants in the skin.[4] *Actinobacteria* are also a phylum of gram-positive bacteria: *Corynebacteriaceae* and *Propionibacteriaceae* belong to this phylum and are also known as commensal bacteria in the skin.[4] *Proteobacteria* is a major phylum of gram-negative bacteria: *Pasteurellaceae* including *Haemophilus* sp. belong to this phylum.[5] *Bacteroidetes* are also known to be frequently isolated in feline oral cavity.[2]

The diversity of bacterial microbiota in the skin is influenced by topographical areas, environment and disease status. In humans, topographical areas can be classified into three areas based on the cutaneous environment and bacterial microbiota: (i) sebaceous areas wherein *Propionibacteriaceae* and *Staphylococcaceae* predominate, (ii) moist areas wherein *Corynebacteriaceae* predominates and (iii) dry areas that have higher bacterial diversity than seen in other areas.[6] In contrast, bacterial microbiota in canine and feline skin is more diverse than see in human skin, and highly valuable across individuals and body sites.[7, 8] A recent study demonstrated that the structure of bacterial community varied among season, and *Propionibacterium acnes*, *Haemophilus* sp., *Corynebacterium* sp. and *S. epidermidis* were key members of the bacterial community in dog skin.[9] Feline skin appeared to have more abundant *Bacteroidetes*, suggesting that grooming behavior of cats affects to the cutaneous flora.[8]

Possible roles of commensal bacteria in the cutaneous immunity

Among the commensal bacteria from mammalian skin, possible roles of *Staphylococcaceae, Corynebacteriaceae* and *Propionibacteriacea*e in the cutaneous immunity have been reported:

Staphylococcus epidermidis: Accumulating evidences showed that *S. epidermidis* provides a negative effect on proliferation of *S. aureus* in human AD. Serine proteases produced by *S. epidermidis* has a potential to inhibit and destroy *S. aureus* biofilm.[10, 11] There is an *in vitro* evidence showing that small molecules produced in *S. epidermis* activated Toll-like receptor (TLR) 2 signaling and enhanced human β-defencin (hBD) 2 production in cultured human keratinocytes.[12] In addition, a study using experimental mice showed that *S. epidermidis* enhanced production of IL-17A and IFN-γ by skin γδT cells,[13] which promote protective immunity not only against *S. aureus* but other pathogens such as *Leishmania* sp.[14] IL-17A is also known to promote recruitment of neutrophils to the skin and to enhance the production of antimicrobial peptides at the local sites.[15] Furthermore, *S. epidermidis* promotes the activity of regulatory T cells and suppresses the proliferation of $CD4^+$ lymphocytes.[16] Hence, *S. epidermidis*, a major commensal organism of the humanand canine has a protective role in the skin, by direct inhibition of pathogens orby activating innate and adopted immune functions.

S. aureus: Besides beneficial effects of *S. epidermidis* on cutaneous immunity, *S. aureus* is considered to enhance inflammatory reactions. Previous studies demonstrated that S. aureus colonization in the SC was recognized in mice with aberrant cutaneous barrier function including filaggrin (FLG)-deficient flaky tail mice.[17] Percutaneous entry of *S. aureus* in flaky tail mice directly correlated with increased expression of IL-4, IL-13, IL-22, thymic stromal lymphopoietin (TSLP) and other cytokines associated with AD, and with decreased expression of an antimicrobial peptide cathelicidin. In addition, *S. aureus* colonization was also recognized in mice lacking a disintegrin and metalloproteinase 17 (ADAM17) that regulate epidermal growth factor receptor ligand-dependent terminal keratinocyte differentiation.[18] The ADAM17-deficient mice showed the cutaneous bacterial dysbiosis and spontaneous dermatitis associated with *S. aureus* colonization. It has been reported that mouse lacking ADAM17 in keratinocytes showed skin barrier defect with disintegrity of cornified envelopes.[19] It has been reported that δ-toxin produced in *S. aureus* induced mast cell degranulation, which resulted in releasing of histamine and Th2 cytokines IL-4 and IL-13.[20] Conversely, production of IL-17A, but not that of IL-4, in CD4+ cells from lymph nodes increased in ADAM17 deficient-mice inoculated by *S. aureus.*[18] Additional information showed that Langerhans cells (LCs) played an important role in Th17 and γδTh17 responses driven by percutaneous inoculation of *S. aureus*.[18]

S. pseudintermedius: Despite the frequent isolation of *S. pseudintermedius* from the skin of dogs with AD and secondary superficial pyoderma, the role of this strain in the innate immunity has been insufficiently investigated. However, increased serum IgE against *S. pseudintermedius* in dogs with spontaneous AD suggests the role of this strain in adoptive immune reaction of the canine allergic skin disease.[21]

Corynebacterium sp.: It has been reported that *C. bovis* enhanced production of IFN-γ, IL-4 and IL-17A in $CD4^+$ cells from lymph nodes of ADAM17 deficient-mice,[18] suggesting the combinational effect of this bacterial strain with *S. aureus* in development of AD.

P. acnes: The bacterial strains arewell known as the causative microorganism of acne vulgaris in humans. Previous studies indicated that *P. acnes* inhibited growth of *S. aureus* by fermentation of glycerol into variety of fatty acids that decrease pH.[22] It has also been reported that *P. acnes* induced production of hBD2 andproinflammatory cytokines/ chemokines from cultured human sebocytes.[23]

Bacterial dysbiosis and allergic skin diseases

Skin colonization by *Staphylococcaceae* has been reported in human, canine and feline allergic skin diseases.In 2012, Kong et al first reported that increased *S. aureus* in the skin lesions was associated with disease flares in human AD.[24] The same group also reported that commensal *S. epidermidis* also increased during disease flares,[24] suggesting antagonistic mechanisms of this strain towards pathogenic *S. aureus*. Of particular interest is that skin colonization by *S. aureus* was recognized in vast majority of human patients with AD, whereas the colonization was recognized in only a minority of healthy people.[24, 25] Moreover, increased proportions of *Streptococcus, Propionibacterium* and *Corynebacterium* species were observed following therapy of human AD.[24]

In veterinary literatures, it has been reported that bacterial diversity in axilla and pinnal skin decreased, while the proportion of *Staphylococcaceae*, particularly *S. pseudintermedius*, as well as that of *Corynebacteriaceae* increased in dogs with spontaneous AD compared to healthy dogs.[26] It was also reported that dysbiosis and *Staphylococcaceae* proportions restored following therapy.[26] Moreover, bacterial diversity was negatively correlated with transepidermal water loss, a quantitative parameter of inside-to-outside skin barrier function.[26] Skin colonization by *Staphylococcaceae* and *Corynebacteriaceae* was also observed in experimental atopic dogs subjected to percutaneous sensitization by house dust mites. In the experimental dogs, increased *Corynebacteriaceae* proportion was recognized shortly after the development of the skin lesions, while increased *Staphylococcaceae* proportion continuedafter the skin lesions remitted.[27]

Increased proportion of *Staphylococcaceae* in the skin was also recognized in cats with allergic skin diseases, although bacterial diversities did not differ among healthy and allergic cats.[8]

SKIN FUNGAL MICROBIOME AND DISEASES

Fungal flora in normal skin

Recent metagenomics analyses have shown that some sites of the skin have relatively high proportions of fungal sequences. *Malassezia* sp. are the most frequently identified commensal fungi in human skin.[28] In humans, *M. restricta* predominates on head and face, whereas *M. globosa* predominates on the trunk.[29] The skin in other sides have more diverse *Malassezia* proportions including *M. sympodialis*.[29] Human feet have diverse fungal proportions including *Aspergillus, Cryptococcus, Rhodotorula* and *Epicoccum*.[29]

In dogs, skin mycobiota (fungal microbiota) was shown to be more diverse than seen in human skin[2, 30] *Alternaria, Cladosporium* and *Epcoccum* were predominant fungal genera at any body site.[30] Surprisingly, *Malassezia* sp., which are frequently isolated by traditional culture technique and considered as pathogens in some skin diseases, did not predominate skin mycobiota in dogs. *Malassezia* proportions were higher in nostril, conjunctiva and ear canal than the other body sites.[30] It has been shown that the most abundant fungal sequences from feline skin were *Cladosporium* and *Alternaria*.[31]

Possible roles of *Malassezia* in the cutaneous immunity

The current knowledge of host-*Malassezia* interactions are largely depend on the results of *in vitro* studies. Therefore, the exact pathophysiology of *Malassezia*-associated skin diseases in humans and animals is largely unknown. The roles of other fungi listed above in skin homeostasis and diseases are poorly understood.

Malassezia sp. resides in the SC and/or hair follicles can interact primarily with viable keratinocytes, LCs or dendritic cells (DCs), This interaction may be directly through membrane bound pattern recognition receptors (PRRs), or indirectly through soluble metabolites secreted by the yeasts.[32] The sensitization of the host cells by *Malassezia* sp. may be accelerated by disruption of the cutaneous barrier function and mechanical trauma. Moreover, it has been shown that immunogenic proteins secreted from *Malassezia* sp. triggered DCs and enhance cytokine production.[33]

Among PRRs, two Fcγ-like receptorsDectin-2 and Mincle, a β-glucan receptor Dectin-1, Langerinand TLR2are known to sense *Malassezia* sp. through ligands expressed on fungal cell wall.[34-41] Mincle and Dectin-1 and -2 are expressed by antigen presenting cells, whereas Langerin is expressed exclusively by Langerhans cells. The sensing of *Malassezia* sp. by host cells induces proinflammatory response characterized by production of cytokines/chemokines or antimicrobial peptides.[38-41] It has been reported that lipid components of *M. restricta* and *M. globose* induceed production of TSLP, which is strongly associated in skin inflammation, from keratinocytes.42

Besides innate immunity against *Malassesia* sp. adoptive immune response characterized by increased serum anti-MalasseziaIgEin human and canine AD has been reported.43-48 The titer of anti-*Malassezia* IgE positively correlated with the severity of human AD.[44-46] Also, *Malassezia*-reactive skin homing T cells in human patients of AD have been reported.[49] Those T cells comprise not only Th1 and Th2 lymphocytes but Th17 cells and IL-22 secreting cells.

Fungal dysbiosis and allergic skin diseases

In human AD, it appeared that *Malassezia*species predominated in the samples obtained from atopic and non-atopic individuals, whereas the diversity of non-*Malassezia* yeast mycobiotawas higher in ADpatients than seen in healthy individuals.[50]

The mycobiota of allergic dogs was significantly less rich than that of healthy dog skin.[30] It has been shown that the skin of allergic cat had significantly greater amounts of *Agariomycetes* and *Sordariocycetes*, and significantly less Epicoccus compared to healthy cat skin.[31]

Although changes in fungal diversity and fungal proportion in human, canine and feline allergic skin diseases have been reported, the exact roles of dysbiosis in pathophysiology of the disease are currently unknown and have yet to be elucidated.

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MECHANISMS OF CELL DEATH IN THE EPIDERMIS

Linder KEDVM, PhD, Dipl. ACVP

North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA

INTRODUCTION, MORPHOLOGY AND EARLY INTERPRETATION

Our early understanding of mammalian cell death was driven in large part by its morphology, which was classified in the early 1970s and then refined to include apoptosis, necrosis and autophagic cell death.[1] *Apoptosis* is characterized by cells that round, shrink, form plasma membrane protrusions and blebs, and ultimately fragment into membrane bound apoptotic bodies. The nucleus fragments and chromatin condenses and moves peripherally. Ultrastructurally, cytoplasmic organelles and the plasma membrane remain intact until late. *Necrosis* is recognized by cell swelling, disruption of plasma membrane integrity, nuclear condensation, and organelle swelling and rupture. *Autophagic cell death* manifests as variably swollen and clear cells with autophagic vacuolization of the cytoplasm, best seen utrastructurally. Classically, vacuoles have double-membranes and contain degenerating organelles. A simple operational dichotomy of uncontrolled passive necrosis and cell-controlled active apoptosis dominated to explain most cell death in relation to interpreted causes, mechanisms and significance to the host.[1] In this scheme, necrosis is caused by cell injury that irreversibly unbalances homeostatic cell mechanisms, causes cell swelling and rupture, and induces violent, pro-inflammatory injury to the host. In contrast, apoptosis is caused by specific molecular signals that trigger preprogramed enzymatic machinery to control orderly dismantling of the cell into apoptotic bodies for removal by quiet phagocytosis. Being non-inflammatory, apoptotic bodies allow for orderly remodeling of tissues and management of diseased cells. This scheme has now changed greatly. Necrosis is often cell-controlled, now called regulated cell necrosis, and apoptosis is not always quiet. Indeed, the known molecular pathways leading to cell death are increasingly more numerous, and are complex and interconnected.

TYPES OF CELL DEATH

Cell death is now divided into two main types, either cell uncontrolled, called accidental cell death, and cell controlled, called regulated cell death, according to the Nomenclature Committee on Cell Death (NCCD).2 Accidental cell death occurs when cells die abruptly and uncontrollably from severe external chemical or mechanical injury, and it is insensitive to pharmacologic or genetic intervention to manipulate its outcome. The morphology is chiefly that of necrosis. Regulated cell death occurs when cells contribute biochemical events that control the process, and its morphology, and is modifiable pharmacologically or genetically. Regulated cell death includes apoptosis, necrosis and autophagy. Autophagic cell death (including the autosis form) can be an independent death modality but mostly autophagy enhances or inhibits cell death by other mechanisms in a context dependent manner. Regulated cell death can be programmed genetically (programmed cell death (PCD)), or be induced by cell injury. Programmed cell death controls tissue remodeling in development, organ homeostasis and tissue differentiation. Cornification is an example of the latter. With injury-induced regulated cell death, injury triggers preformed cellular machinery to carry out distinct biochemical subroutines that lead to controlled cell death, and thus the type and morphology (apoptosis, necrosis, autophagy) of cell death that occurs.

MECHANISMS OF REGULATED CELL DEATH

Over a dozen mechanisms of regulated cell death are recognized, but only a limited number of cell death morphologies are known to occur. Therefore, the NCCD recommended that the unique molecular features of effector phases of each cell death type be used for their identification.[2] Yet naming discrete mechanisms masks the complexity. Redundant initiation mechanisms lead to interconnected signally networks that result in common or unique execution mechanisms. Ultimately, the mode by which cells die depends on the cell type, cell status, stimulus and dose.

APOPTOSIS

Apoptosis is triggered through the extrinsic and intrinsic cell signaling pathways that lead to caspase-mediated cell death and result in cellular contents being isolated in membrane-bound apoptotic bodies.[3] Mostly phagocytes clear apoptotic bodies via recruitment (find me) signals, receptor binding (eat me) signals, such as phosphatidylserine exposure, phagocytosis and digestion. Free apoptotic bodies can undergo secondary necrosis (rupture) and release

damage-associated molecular patterns (DAMPs) extracellularly. The intrinsic pathway of apoptosis is initiated by cellular stressors, such as DNA damage, oxidative stress, ER stress, cytosolic calcium overload, growth factor withdrawal, etc., that lead to mitochondrial outer-membrane permeabilization (MOMP). The MOMP pore releases cytochrome c to the cytoplasm to bind APAF1 and initiate the apoptosome, leading to activation of the initiator caspase-9 and then the effector caspases, caspase-3 and caspase-7. The extrinsic pathway is initiated by a subset of the TNF receptors called death receptors (DR, i.e. TNFR1) and by stress signals that are classically FasL, TNF and TNF-related apoptosis-inducing ligand (TRAIL). Death receptors recruit and activate caspase-8, via the death-inducing signaling complex (DISC), which in turn cleaves caspases-3 and -7 to carry out apoptosis. In some cell types, caspase-8 can induce intrinsic apoptosis, which is key because not all cells readily trigger extrinsic apoptosis by DR activation.

REGULATED NECROSIS

Necroptosis is the best characterized form of regulated necrosis, but proposed types also include pyroptosis, NETosis, parthanatos, entosis, ferroptosis, oxytosis and others.[3] Morphologically, cell swelling and loss of membrane integrity occur, i.e. necrosis. Autoschizis shares morphologic features of necrosis and apoptosis.

Necroptosis

Necroptosis is form of regulated cell death that is triggered by TNF, FasL, Trail, and certain TLRs, RIG-1-like receptors, genotoxic stress, UV injury, and others.[3] TNF triggered necroptosis is the best studied. TNF activation of TNFR1 can initiate NF-☐B activation and inflammatory cytokine/chemokine production, apoptosis, or necroptosis. Apoptosis (extrinsic pathway) appears to be the preferred cell death outcome but, if blocked by caspase-8 inhibition (viral inhibitors), then necroptosis ensues through necrosome formation, a complex of RIP kinases (RIPK1, RIPK3) and mixed lineage kinase domain-like (MLKL). Activated MLKLoligomerizes and forms pores in the plasma membrane to cause cell swelling, rupture and death, i.e. necrosis.

Pyroptosis, NETosis, and Parthanatos

Pyroptosis is an inflammatory form of regulated necrosis that amplifies immunity during infection – it was discovered in macrophages, but is now known in other cells, including skincells.[3] Post inflammasome activation by pathogen signaling, pyroptosis occurs from caspase-1 or caspase-11/4/5 dependent release of the amino-terminus fragment of gasdermin D, which oligomerizes to form the pyroptotic pores in the cell membrane. Pore formation causes cell swelling and rupture (pyroptosis) and release of proinflammatory IL-1☐ and IL-18. Neutrophils undergo NETosis, a regulated process that extrudes neutrophil extracellular traps (NETs) - a meshwork of chromatin, histones and antimicrobial molecules, which traps and kills extracellular bacteria. NETosis is activated by microbial and sterile mediators as well as by specific receptor activation (complement, antibodies, cytokines and TLRs). Elastase and myeloperoxidase enter the nucleus and cause histone degradation and chromatin condensation and initiate NET formation.[3] Parthanatos is regulated cell death from over activation of Poly(ADP-ribose) poly¬merase (PARP) proteins that transfer ADP-ribose groups from NAD to target proteins, a process called poly(ADP-ribosyl)ation (PARylation).[3] There are many cellular targets. Reactive oxygen species, UV DNA damage, alkylating agents, calcium flux, and others activate PARPs, which are then thought to deplete cellular NAD+ and ATP to cause regulated necrosis.

CORNIFICATION

Cornification is specialized form of PCD-mediated tissue differentiation that continually remodels the viable epidermis into the stratum corneum.[2] Dead corneocytes, sealed together by lipids, are continuously supplied by keratinocyte cell death and lost by desquamation. According to the Galluzzi,[2] the key biochemical features that identify this cell death biochemical subroutine are transglutaminases (TGM1, 3 and 5) and caspase-14 activity. However, these are not essential and clearly many differentiation specific molecules are expressed in cornification. Furthermore, cornification cell death is synchronized with autophagy that removes nearly all organelles, including the nucleus.

CELL DEATH IN CONTEXT

Solar injury: UVB damage induces pyrimidine dimers and oxidative DNA injury and causes keratinocyte apoptosis (sunburn cells) and regulated-necrosis, and injury to multiple dermal cells. Intrinsic and extrinsic apoptotic mechanisms involve TNF-☐, TRAIL, p53, and others. **Hypoxia(ischemia):** Hypoxia mediates apoptosis through intrinsic mechanisms, can trigger regulated necrosis and induces autophagy. Oxidative injury and DNA damage contribute,

while context dependent, complex interactions of hypoxia inducible factor with p53 alter apoptosis outcomes. **T-cells and NK cell mediated cell death - viruses, cancer, and aberrant responses:** Cytotoxic T-lymphocytes (CTLs) and natural killer (NK) cells mediate cell death of virus-infected cells and cancer cells. When misdirected, these mechanisms contribute to epidermal injury in autoimmune disease (i.e. lupus), graft vs host dz., erythema multiforme (EM) and drug reactions (fixed-type, toxic epidermal necrolysis (TEN), etc.). Both CTLs and NK cells kill directly by cytotoxic granule release (perforin, granzyme B, granulysin), which trigger intrinsic apoptosis, and by expression of DR ligands (TRAIL and FasL), which trigger extrinsic apoptosis.[4] Importantly, cell target recognition differs between CTL and NK cells, being antigen based and non-antigen cell-stress based, respectively, which allows for detection and cell killing of viral infected and tumor transformed cells. Altered viral (herpesvirus) and drug antigen presentation contribute to EM and drug reactions (TEN, others), respectively. In cutaneous and systemic lupus, apoptosis appears increased and apoptotic body clearance delayed.[5] Secondary apoptotic body necrosis releases DAMPs with self-antigens, including nucleic acids. The plasmacytoid dendritic cell driven interferon response, key for CTL and NK cell viral responses, is activated, promotes the cytotoxic response and more apoptosis. Solar exposure exacerbates lupus lesions by causing apoptosis and releasing cytokines and DAMPs. **Pemphigus vulgaris (PV):** The role of apoptosis (esp. apoptolysis) in PV skin lesion development remains controversial but it has been proposed as an initiating mechanism of acantholysis. **Toxic epidermal necrolysis:** Kim et al. identified significantly up-regulated RIPK3 levels, reactive oxygen species, and activation of MLKL and necroptotic cell death of keratinocytes in toxic epidermal necrolysis.[6] Possible mediators include TNF, FasL, granulysin and annexin 1A from cytotoxic T-cells, NK cells and monocytes (annexin). In this disease, cell death occurs initially by CTL and NK cell mediated keratinocyte apoptosis and then quickly transitions to catastrophic necroptosis.

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THEORY AND APPLICATION OF MODERN FLEA CONTROL

Michael W. Dryden DVM, MS, PhD, DACVM (parasitology)

College of Veterinary Medicine, Kansas State University, Manhattan KS 66502

Fleas are a common and important external parasite of dogs and cats. The most common flea species infesting dogs and cats in North America and in many areas of the world is *Ctenocephalides felis felis*, the cat flea.[1,2] Cat fleas are voracious blood feeders consuming up to 15 times their body weight in blood daily and female fleas use that blood to produce up to twice their bodyweight in eggs daily.[1-3] So it does not take long before a flea infestation can get completely out of hand. These fleas can cause allergic skin disease (FAD), produce anemia through their blood feeding activities, transmit tapeworms and bacterial pathogens.[1,2] It is therefore important for the health and well-being of our pets that we control these harmful parasites.

It must be understood that it often takes several weeks to eliminate a flea infestation. That is because all flea infestations of dogs and cats originated from a flea-infested environment and it takes time to eradicate the immature stages living in the carpet or outdoors. Once these fleas jump on a dog or cat they will feed, mate and female fleas will begin laying eggs within 24 hours.[1-3] Then within a few days each female flea will be producing 40 – 50 eggs per day, with hundreds and potentially thousands of eggs being deposited into the home or yard.[3] The in-home and potentially outdoor premises rapidly becomes infested with egg, larvae, pupae and emerging adult fleas, often referred to as the flea biomass.

Historically veterinarians and pet owner treated the premises directly through the application of insecticides and insect growth regulators into the carpet and yard.[4] This was done in an attempt to kill emerging fleas and prevent development of eggs & larvae. Premises treatments were considered necessary to break the flea lifecycle. The primary reason that premises treatments were necessary was that prior to the mid-1990s topical products (dips, sprays, collars, powders etc..) had no substantial duration of residual activity.[5] Premises treatments were difficult to conduct, time consuming, expensive, environmentally unfriendly and often ineffective.

However, with modern topical and systemic residual flea products, control of infestations in the premises is now achieved by preventing flea reproduction.[6,7] Reproduction is halted either through the use of highly effective residual adulticides that kill most newly acquired fleas before they begin reproduction (killing fleas within 24 hours after jumping on treated pet) or through the use of insect growth regulators (or insecticides) with ovicidal activity to kill any eggs that might be produced.[6,7] Simply if you cannot reproduce as a species you go extinct in the local premises.

When focusing on residual adulticides it is the residual speed of kill of a product that is of utmost importance.[8] Residual speed of kill relates to how rapidly a product kills newly acquired fleas at some time point (days or weeks) after administration. If a product can kill newly acquired fleas fast enough it can prevent flea reproduction and markedly reduce the amount of salivary proteins injected by the fleas, thus minimizing or eliminate FAD symptoms.[5,8]

Although modern residual flea adulticides provide prolonged adulticidal activity, it has been determined that efficacy and speed of kill of most products will decrease over time following administration.[5,9-12] As the residual efficacy decreases and fleas are not killed within 24 hours, female fleas can live long enough to produce eggs.5,9,10 Therefore, it is important to use a product that is effective enough to suppress reproduction between scheduled reapplications. Residual efficacy can also be affected by under-dosing by clients, bathing or swimming that can reduce insecticide levels of topical formulations, poor G.I. absorption with oral products and natural variability in susceptibility or outright resistance.[13]

Proper administration of effective residual flea products to all dogs and cats means no more fleas reproducing and no more eggs dropping into the environment. Therefore, within 2 to 7 days, eggs that were previously deposited have developed into larvae, within 1 to 2 weeks the larvae have now developed into pupae, and 1 to 4 weeks later those pupae are now adult fleas. As these fleas emerge and jump on treated pets they are hopefully being killed by the flea product. Therefore within 3 to 8 weeks or occasionally as long as 3 months, all the adult fleas and immature life stage biomass should be gone.[6,7]

How bad a flea infestation becomes and how rapidly a flea infestation is eliminated is not only affected by the product used, but also by environmental conditions. Relative humidity in the microenvironment is primary determining factor in flea populations. This is because flea larvae are weak link in the life-cycle chain and are very susceptible to heat and desiccation.[1,2] In addition, the rate of flea development and therefore how rapidly the biomass is exhausted can be very temperature dependent.[1,2] If a flea infestation continues beyond the expected 3 to 8 weeks (or longer), a commonly encountered problem is an untreated flea host in the home or maybe the product itself is not stopping reproduction. An interesting assessment of product performance entails the evaluation of gender structure of newly emerged fleas in these homes.[14] While most insect species exhibit protrandry (males tend to emerge before females), *C. felis* belong to a much smaller group that exhibits protogny (females tend to develop before males).[15] The first fleas to emerge from a cohort of eggs are females, followed by both males and females and then lastly almost exclusively males. It has been demonstrated that if flea reproduction is inhibited by insecticidal treatments administered to a pet, then a gender shift in premises flea population takes place overtime from a female dominated population towards a more male dominated population.[14] In a recent field investigation, 60% of the unfed fleas collected in premises light traps on day 0 were female, whereas by 28-30 days following treatment with oral afoxolaner, 78% of the unfed fleas collected in the traps were male. This was a clear and rapid gender shift, indicative of cessation of flea reproduction.

Another issue that must be expected in a small number of homes is that the flea infestation may get worse before it gets better.[7] These cases are referred to as "Red-line homes". By definition a red-line home is a house were premises trap flea counts increase > 20% over day 0 trap counts within 1 to 4 weeks post-treatment.[14] These surges in emerging fleas occurs because of a large preexisting biomass in the indoor premises. Such surges in emerging fleas and resulting increase in flea numbers on household pets can give the perception of product failure. Often extensive and frequent mechanical intervention (vacuuming, washing pet bedding and area and throw rugs, etc..) and even application of insecticides into the premises may be necessary in these cases.

Fluralaner, afoxolaner and sarolaner are recently introduced oral flea and tick adulticides in the isoxazoline class of drugs. These drugs work as GABA-Chloride antagonists causing over excitation of the insect and arachnid nervous system and rapid ectoparasite death.[16-18] These compounds have demonstrated rapid and persistent efficacy against fleas and multiple species of ticks].

Following the administration of a fluralaner chew, efficacy has been maintained against fleas in both field and laboratory studies for 12 weeks against fleas. A single dose of a fluralaner chew killed newly acquired female fleas rapidly enough that no eggs were laid after repeated infestations for 120 days.[19] In field studies evaluating dogs not managed with associated medications, afoxolaner, fluralaner and sarolaner not only managed flea infestations, but also managed clinical signs associated with FAD and pruritus.[20,21]

Following administration of fluralaner or afoxolaner, flea populations on pets were reduced by 99.0% and 99.3%, respectively within 7 days.[20] Flea populations on the fluralaner treated dogs were 0 (100% efficacy) on days 54-60 and 82-86 after the administration of a single dose on day 0. Administration of 3 monthly doses of afoxolaner reduced flea populations by 100% on days 82-86. Flea numbers in indoor-premises were markedly reduced in both treatment groups by days 82-86, with 100% and 98.9% reductions in flea trap counts in the fluralaner and afoxolaner treatment groups, respectively. Marked improvement was observed in FAD lesion scoring, Atopic Dermatitis lesions scoring (CADESI-4) and pruritus scores with both formulations.

Spinosad first became available as an oral treatment for the control of flea infestations on dogs in late 2007. A multi-clinic, investigator-blinded study was undertaken in client-owned dogs to investigate and compare the flea control provided by 3 consecutive monthly treatments of oral spinosad (SPN) or fipronil/(s)—methoprene topical (FSM) spot-on.[22] The first household dog meeting enrollment criteria and with at least 10 fleas (whole-body flea count) served as the index dog in a household against which primary objectives were set. Allocation was based on pruritus scores at the enrollment visit and on single or multiple pet household. Index pets were randomized to treatment with either SPN or FSM, dispensed on day 0 for at-home administration by owners. All other household dogs and cats, maximum 4 pets per household, were dispensed the same treatment as the index dog (spinetoram was dispensed for cats in SPN households). Subsequent treatments were dispensed when index dogs were returned for whole-body flea counts and pruritus-scoring at visits on days 30 and 60, with final assessments on day 90 (± 5 days on each occasion). Primary

endpoints were the number of flea-free index dogs in each group one month after the final treatment, the reduction in owner-reported pruritus, and the reduction from baseline in mean flea counts. One hundred twenty-eight index dogs were enrolled (65 in the SPN arm; 63 in the FSM arm) at 10 clinics in Florida (6), North Carolina (2), Louisiana (1), and Texas (1). On day 0, geometric mean flea counts were 57.7 (range: 10-1,469) and 44.8 (10-717) for the SPN and FSM groups, respectively. On Day 90, 55 of 58 (95%) and 21 of 55 (38%) index dogs completing the study were flea-free in SPN and FSM groups, respectively; mean SPN pruritus scores declined to 0.92 (6.67 on day 0), and to 3.83 (6.33 on day 0) for FSM; geometric mean flea counts (% control) were 0.08 (99.9%) and 5.19 (88.4%), for SPN and FSM groups, respectively.

Flea allergy dermatitis or flea bite hypersensitivity is the most common dermatologic disease of domestic dogs. Cats are also afflicted with FAD, which is one of the major causes of feline miliary dermatitis. Historically, it has been said that one flea is all that is necessary to maintain theclinical signs of FAD and therefore total flea eradication is necessary. Newer adulticides such as fipronil, imidacloprid, metaflumizone,nitenpyram, selamectin, and spinosad have had a positive clinical effect on dogs and cats with FAD. However, data on flea biology and the effect ofthese products on flea feeding bring into question theonce perceived dogma of the 'one flea bite'.[5] Adult catfleas begin feeding almost immediately once they find a host, with many fleas feeding within minutes. In onestudy, 25–60% of fleas were blood fed within 5 min andin another study the volume of blood consumed by fleas was quantifiable within 5 min Feeding is so rapid that partially digested blood can be defaecated in as little as2–6 min after fleas acquire a host. After rapid transitthrough the flea, the excreted blood dries within minutes into reddish black faecal pellets or long tubular coils (fleadirt). While initiation of feeding is rapid, daily blood consumptionis voracious. Female cat fleas can consume upto ten times their body weight in blood the very first day. They are on the host and peak consumption occurs withina few days at 15 times their body weight (13.6 lL) daily.With such rapid and voracious blood feeding, is it reasonableto assume that residual insecticides can truly preventflea biting and feeding?

A study was conducted at Kansas State University, Manhattan KS, USA to evaluate the residual activity of fipronil and imidacloprid on egg production and blood feeding. There were two objectives to these studies 1) to evaluate if these compounds will kill newly acquired fleas prior to them feeding and 2) to determine how long these compounds will prevent viable egg production after application.In the first experiment six cats were treated with either a fipronil spray (0.29% w/w) formulation, an imidacloprid spot-on (9.1% w/w) formulation at labeled rates or were left as untreated controls. Surprisingly when 100 *Ctenocephalides felis* were placed on cats 6 days after treatment with imidacloprid or fipronil, the percent of fleas that fed and consumed blood was 89 and 92%, respectively.[5] While the adulticidal efficacy of the products was 100%, neither product killed fleas before the vast majority could bite, feed and consume at least some quantity of blood.

In another study conducted in Europe it was determined that the topical application of imidacloprid or fipronil to cats did not prevent fleas from biting and feeding. Unconfined fleas placed on cats treated with imidacloprid and fipronil had reductions in the percent of fleas blood feeding of 49.6 and 39.5%, respectively, on day seven; while reductions in percent of fleas feeding on day 28 was 0 and 3.4%, respectively. While topical applications of dichlorvos/fenitrothion or permethrin did reduce the percent of fleas feeding by greater than 80%, these compounds also did not completely prevent flea bites or feeding. The data on percent of fleas feeding on imidacloprid and fipronil treated cats in the European study differ from the data in the Kansas State University Study. This likely occurred due to the known reduced susceptibility of the KSU flea strain to imidacloprid and fipronil.

Another study conducted at KSU using dogs evaluated the ability of a 65% permethrin spot-on, a 13.8% fenthion spot-on and an 8% Chlorpyriphos collar to reduce blood feeding by fleas.[5] At two weeks post-treatment evaluation of the blood fed status of fleas revealed that an average of 66.7% of fleas from permethrin treated dogs had fed. Fleas from chlorpyrifos collared dogs and fenthion treated dogs averaged 53.0 and 37% blood fed status, respectively. In this study the percent of fleas feeding on organophosphate and pyrethroid treated dogs was considerably higher than in the study conducted in Europe. It was later determined that the flea strain used in the KSU study was tolerant/resistant to certain organophosphates and pyrethroids.

Additional research has now been conducted to quantify the amount of blood consumed by fleas on insecticide treated cats.[23] In this study fleas were confined for 24 hours in confinement feeding chambers attached to treated

cats once a week for four 4 weeks. Confinement feeding chambers were used so that fleas and their feces could be collected for quantification and analysis. Cats were treated on day 0 with fipronil, imidacloprid, selamectin at label rates or were left untreated. In addition, another group of cats was administered nitenpyram one hour prior to each weekly infestations. After each 24 hour infestation fleas and feces were removed, microcell removed and the quantity of blood consumed and excreted was determined spectrophotometrically using the Drabkin's Reagent Method.Fleas placed on imidacloprid and fipronil-treated cats seven days post-treatment had reductions in blood consumption of 90.78 and 69.77%, respectively. Whereas, at 14 days post-treatment fleas on fipronil-treated cats had no statistically significant reduction in blood consumption as compared to fleas on untreated controls while fleas on imidacloprid-treated cats consumed 55.73% less blood as fleas on controls. Then by three weeks post-treatment fleas on imidacloprid-treated cats had no statistically significant reduction in blood consumption as compared to fleas on untreated controls. Of particular interest was that fleas placed on cats treated orally with nitenpyram never consumed more than 1.63% (98.37% reduction) as much blood as fleas placed on control cats. Topically applied, but transdermally absorbed selamectin also had a pronounced effect upon blood consumption of fleas. Even on day 28 post-treatment there was an 88.9% reduction in blood consumption as compared to fleas on untreated controls.

As stated previously compounds such as fipronil, imidacloprid, metaflumizone, and selamectin and spinosad have had a major impact on reducing the occurrence of FAD. However, the data from the qualitative and quantitative studies demonstrates that these compounds do not stop flea bites nor completely stop flea feeding. Therefore, it appears their role in managing FAD is likely related to a decrease in prolonged flea feeding and thereby the amount of salivary protein delivered to the pet and in the long term reducing flea numbers. It is this author's opinion that FAD is related to the degree of hypersensitivity of an individual animal, the numbers of fleas feeding and amount of antigen injected. This certainly brings into question the old dogma of a single flea bite eliciting an FAD reaction, at least in the majority of clinically afflicted animals. If a single flea bite was responsible, it appears no flea product would provide much relief, at least not until the flea population was eradicated. Also of importance to note is that regardless rather as to whether an insecticide works topically or systemically may be irrelevant in the management of fleas or FAD, since in the one study the systemically active compounds had a pronounced effect on blood feeding.[5]

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Introduction to Hyperbaric Oxygen Therapy in Veterinary Dermatology

Angus JC

Animal Dermatology Clinic
Pasadena CA 91001

INTRODUCTION

Hyperbaric Oxygen Therapy (HBOT) is not a new therapy, but only relatively recently been used specifically for veterinary cases.[1] The basic concept of HBOT is to achieve saturation of compromised tissue in near 100% oxygen under pressure. At these concentrations and pressure, oxygen rapidly exceeds the saturation point of hemoglobin and begins to diffuse directly in plasma as well as absorption across skin directly into tissues. HBOT impacts cellular function, cytokine production, stem cell mobilization, neutrophil phagocytosis, and other systems that impact recovery from injury, infection, and illness.[2]

Medicare endorsed indications for HBOT with the strongest evidence for efficacy in human medicine include: complicated diabetic wound, necrotizing fasciitis, skin graft, osteomyelitis, radiation necrosis, actinomycosis, crush injury, carbon monoxide poisoning, cyanide poisoning, gas embolism, gas gangrene, and decompression illness. Other disease states HBOT has been applied include: traumatic brain injury (TBI), concussion, stroke, dementia, cerebral palsy, autism, sudden blindness, sudden deafness, sports injury, postsurgical recovery, and snake bite … to name a few.In veterinary dermatology the principle focus is on wound-healing and infection.

WOUND HEALING

Nine of the 14 Undersea and Hyperbaric Medical Society (UHMS) accepted indications for HBOT are applications for wounds. Oxygen is an essential co-factor for normal wound healing, involved in almost every aspect from fibroblast activity, collagen synthesis and formation, macrophage function, bacterial phagocytosis, angiogenesis and epithelial cell division.Partial pressure of oxygen >40mmHg is required for wound healing (normoxic); between 31-40mmHg is mild hypoxia and anything less than 30mmHg is considered moderate to severehypoxia and can be associated with significantly delayed healing.[3] The partial pressure of oxygen at the site of wounds is determined by the oxygen concentration in room air, respiration, cardiac output, oxygen carrying capacity of hemoglobin and finally tissue perfusion. Since oxygen diffusion is determined by gradients from high to low, at each step between room air and the wound, the partial pressure of oxygen is reduced, including the step between normal tissue and the wound itself. In healthy adult humans, skin wounds generally have pO2 levels 20mmHg lower than comparable control tissue control tissue, which can range from 40-70mmHg, depending on location on the body and normal vascular perfusion. Common conditions that further compromise oxygen levels at wound sites include respiratory/cardiac function, anemia, occlusion of the wound from room air, and poor perfusion due to compression, edema, skin over bone, thrombosis, or other vascular compromise. Even under normal conditions breathing room air at sea level, the partial pressure of oxygen in a wound can be as low as 20-40mmHg. In other words, perfectly healthy animals may heal fine at normoxic conditions, but any compromise at any point or any stage of healing can result in poor, delayed, or total failure. The pO2 can increase to 100-300 mmHg by simply increasing inhaled oxygen or flowing 100% oxygenover tissues; however, in hyperbaric chamber pO2 levels can exceed 350-500 mmHg at the wound site. Even in the case of complete vascular obstruction, HBOT will diffuse oxygen into tissues around the occluded vessel completely independent of red blood cells and blood flow. After exiting the chamber, partial pressure of oxygen at wound site remains above 40mmHg for up to 3 hours after returning to normal room air. However, return to room air and breaks between sessions is important, as prolonged hyperoxic states can inhibit angiogenesis and delay healing.

HBOT not only overcomes anoxia and hypoxia to restore typical healing but has been shownto accelerate wound healingabove that achieve in normoxic states.[4] One factor is that optimal fibroblast proliferation is achieved at 80mmHg, twice what is typical under normal conditions.5Other mechanisms include modification of growth factors, cytokine effects, and nitric oxide production. The impact can be measured in clinical cases. A human study of wound

closure in diabetic foot ulcersevaluated the impact of oxygen in different treatment groups. The study compared daily treatments with 100% topical oxygen flowing over the wounds at normal atmospheric pressure vsdaily HBOT sessions for similar lengths of time. The 100% oxygen flow group healed at a rate of 0.004 cm3/day vs 0.064 cm3/day for the HBOT group.The HBOT treated wounds healed at a rate 15 times higher than the oxygen treated group.[6] Additionally, HBOT has been demonstrated to have an impact on adult stem cells, doubling circulating stem cells after treatments. HBOT mobilizes stem/progenitor cells by stimulating NO synthesis,7Specifically vasculogenic stem cells are mobilized from bone marrow of diabetic patients, recruited and recruited to skin wounds.[8]

INFECTION

HBOT is also useful adjunctive therapy in the management of deep infection by Staphylococcus, atypical bacteria, and fungal organisms. This is achieved primarily through increased neutrophil efficiency at phagocytosis and oxidative killing of bacteria and other organisms. Neutrophil phagocytosis of Staphylococcus is compromised when the partial pressure of Oxygen drops below 30-35 mmHg.[9] Remember that normoxic wounds are often in the range of 20-40mmHg, so any wound is more susceptible to infection than normal tissue, hypoxic wound more so.

In addition to optimized neutrophil action HBOT has direct bacteriostatic and bactericidal effects against gram positive, gram negative aerobic and anaerobic organisms. Clostriadial toxin production is reduced and inactivated. Perhaps most interestinglyHBOT has been shown to have synergism with oxygen dependent antibiotics, including potentiated sulfonamides, cephalosporins, fluoroquinolones, and aminoglycosides. Recently HBOT has been investigated for treatment of biofilm forming pneumonia in cystic fibrosis patients. In vitro evidence shows increased uptake of ciprofloxacin in oxygen-depleted Pseudomonas aeruginosa biofilms.[10]

COMPLICATIONS, SIDE EFFECTS, AND CONTRAINDICATIONS

Mostpatients exhibit normal relaxedbehavior typical of a dog or cat in a quiet kennel. Confinement anxiety is a rare event but can occur. Patients with separation anxiety or that exhibit excessive fear, anxiety or stresswhen left alone a kennel may require some intervention or may not be good candidates for HBOT. Light administration of antianxiety medications such as trazadone, Alpha-2-agonists, or benzodiazepams are permissible. **Opiods are contraindicated in HBOT patients.**

Complications can occur with HBOT in dogs and cats. Barotrauma of middle ear, sinus, dental, or intestinal "squeezes" or "blocks" are typically the result of failure to equalize pressure during descent or ascent. Monitoring patient behavior and modifying the pressure and time is the best method to avoid these complications. Potential risk factors include airway obstruction, brachycephalic breeds, history of trauma, or history of blockages. More severe barotrauma to gas filled spaces would include pulmonary barotrauma due to overexpansion of alveoli during too rapid ascent; signs could include, subcutaneousemphysema, mediastinal emphysema, or in most severe event a spontaneous pneumothorax

Oxygen Toxicity occurs when there is an imbalance between production of reactive oxygen radicals and the bodies ability to compensate. Extreme activation of sympathetic nervous system or high levels of sympathomeimetics (stress, adrenaline), high fever, hyperthermia, or hyperthyroidism have been associated with increased risk for oxygen toxicity. Seizures associated with Oxygen Toxicity are reported in humans and animals. Seizures can occur acutely but are often preceded by facial muscle twitching and agitation. Although most common at very high pressures (3ATM) used to treat gas gangrene and necrotizing fasciitis, seizures may occur at normal treatment pressures for wound healing and other indications (1.5 – 2.0ATM). Seizures are reversible with return to normal oxygen and pressure. Other signs of oxygen toxicity observed in humans (airway toxicity, tracheal/bronchial irritation, myopia) are not observed frequently in animals except in longer term administration situations.

Absolute contraindications for HBOT in animals include, untreated pneumothorax and specific anticancer therapy (Doxorubicin, bleomycin, cis-platin) which can potentiate cardiac and pulmonary toxicity of the drugs. Some relative contraindications reported for animals are congestive heart failure, arterial hypertension, severe airway disease, epilepsy, high fever, recent thoracic surgery, optic neuritis, obstructive sinus or tympanic bulla disease. Patients should not receive opiod narcotics during HBOT sessions due to potential to suppress normal respiration; consult expert regarding withdrawal times.

Table 1: Indications for Hyperbaric Oxygen Therapy in Dogs/Cats

Dermatologic	Non-Dermatologic
• Infected Wounds • Non-healing Wounds • Pressure sores / Decubital Ulcers • Thermal/Caustic Injury • Vasculitis • Ischemic Dermatopathy • Deep tissue infections / Cellulitis • Mycobacterial infections • Infected Acral Lick Granuloma • Severe Pododermatitis • Post-radiation therapy (oncology)	• Osteo-arthritis, Orthopedic sx / recovery • Post-surgical edema, bruising, pain • Neurology - Intervertebral Disc / Ischemic • Osteomyelitis • Diskospondylitis • Severe dental disease / stomatitis • Saddle Thrombi / FCE/ Ischemic events • Snake envenomation • Gastric ulcer, ileus, pancreatitis, peritonitis • Acute Deafness / Ototoxic injury

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THE PHYSICS AND MYTHS OF SURGICAL LASERS

Peter Vitruk, PhD, MInstP, CPhys, DABLS

Aesculight LLC (dba LightScalpel LLC), 11818 N Creek Pky N , Bothell, WA, 98011, USA
American Laser Study Club, 20029 99TH Ct NE, Bothell, WA, 98011, USA

Photo-Thermal Laser-Tissue Interaction. The key to the success of soft tissue lasers is their ability to cut and coagulate the soft tissue at the same time, which makes many soft tissue procedures much simpler and far more enjoyable for practitioners[1-11]. Figure 1 presents the known optical absorption coefficient spectra of the soft tissue's four main chromophores[3-6] – water, hemoglobin (Hb) and oxyhemoglobin (HbO_2), which are needed to understand the photo-thermal ablation (or photovaporolysis[3,4]) and photo-thermal coagulation (or photopyrolysis[3,4]) efficiencies for the soft tissue lasers7 on the market today: *Near-IR diodes at 808 - 1,064 nm; Mid-IR Erbium lasers at 2,780 nm and 2,940 nm; and IR CO_2 laser at 9,300 nm and 10,600 nm.*

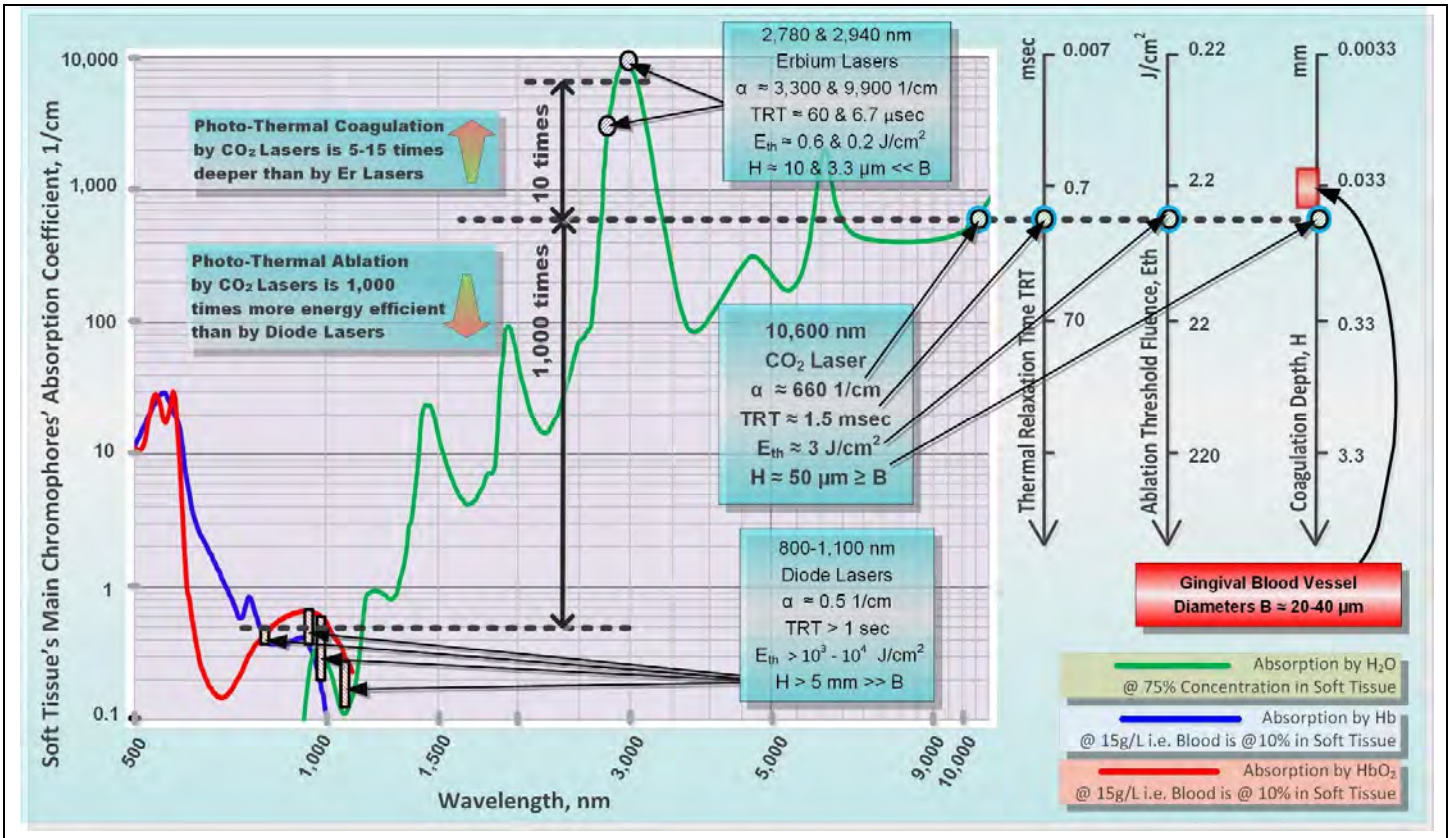

Figure 1. Spectra of Absorption Coefficient, 1/cm, at histologically relevant concentrations of water, hemoglobin (Hb), oxyhemoglobin (HbO_2) in sub-epithelial and sub-epidermal soft tissue, and: Thermal Relaxation Time, ***TRT***, msec; short pulse Ablation Threshold Fluence, E_{th} , J/cm^2 ; and short pulse Photo-Thermal Coagulation Depth, ***H***, mm. ***B*** is gingival blood vessel diameter.

Both Erbium laser (approx. 3,000 nm) and CO2 laser (approx. 10,000 nm) wavelengths are highly efficiently absorbed by the soft tissue and are efficient at cutting and ablating the soft tissue purely radiantly (non-contact). At the same time, diode lasers (approx. 1,000nm) are highly inefficiently absorbed by the soft tissue.

During photo-thermal laser-tissue interaction, the laser beam energy is absorbed (by tissue's main chromophores

- absorption centers) and heats the tissue inside the irradiated volume, which can result in tissue ablation and coagulation. Incident laser beam intensity is exponentially attenuated inside the tissue: $I = I_0 Exp [-x/A]$, where $1/A$ is absorption coefficient (or attenuation coefficient if light scattering is taken into account) from Figure 1.

Thermal Relaxation Time. Soft tissue ablation and coagulation efficiencies are influenced not only by absorption/attenuation spectra, but also by laser pulse duration and tissue' thermal conductivity. The rate of how fast the irradiated tissue diffuses the heat away is defined through the thermal diffusion time, or Thermal Relaxation Time, as $TRT = A^2/K$,[5,6] where A is optical absorption (or Near-IR attenuation) depth discussed above. The physics behind thermal diffusivity process is similar to diffusion and Brownian motion first described by Einstein. Coefficient K is tissue's thermal diffusivity; $K = \lambda /(\varrho\ C) \approx 0.155\ (+/-0.007)\ mm^2/sec$ (derived from heat conductivity $\lambda \approx 6.2\text{-}6.8\ mW/cm\ ^oC$; specific heat capacity $C \approx 4.2\ J/g\ ^oC$, and density $\varrho\ \approx 1\ g/cm3$ for liquid water for temperatures in $37\text{-}100^oC$ range5).

The most efficient heating of the irradiated tissue takes place when laser pulse energy is high and its duration is much shorter than ***TRT***. The most efficient cooling of the tissue adjacent to the ablated zone takes place if time duration between laser pulses is much greater than ***TRT***. Short laser pulse allows for the most efficient ablation of the irradiated tissue with minimum coagulation and hemostasis underneath the ablated tissue.

The least efficient heating of the irradiated tissue takes place when laser pulse energy is low and its duration is much longer than ***TRT***. The least efficient cooling of the tissue adjacent to the ablated zone takes place if time duration between laser pulses is much shorter than ***TRT***. For instance, long pulse and continuous wave (CW) CO_2 lasers are less efficient cutters but provide for greater depth of coagulation for excising/incising in highly vascular and inflamed tissues like hemangioma.

Photo-Thermal Ablation/Cutting Efficiency. Just because water is the most prevalent and the most concentrated soft-tissue' chromophore, the most efficient soft tissue photo-thermal ablation (or photovaporolysis[3,4]) is a process of vaporization of intra- and extra-cellular water.[3-6] For a fixed laser beam diameter (or spot size), the volume of the tissue exposed to the laser beam is proportional to the optical absorption (or Near-IR attenuation[5]) depth. The shorter the absorption (or attenuation) depth – the less energy is required to ablate the tissue. The longer the optical penetration depth - the greater the volume of irradiated tissue and, therefore, more energy is required to ablate the tissue within the irradiated volume of tissue.

The minimum energy density requirement to vaporize the irradiated soft tissue can be calculated from the spatial distribution of laser light intensity inside the irradiated tissue for different wavelengths that are relevant to practical soft tissue l Near-IR Diode, Mid-IR Erbium and IR CO_2 lasers, for the steady-state conditions[6] that are the most suited for high efficiency photo-thermal ablation (pulse duration $\leq TRT$) with minimum collateral damage to the surrounding tissue (pulse repetition rate $<< 1 / TRT$).

The Near-IR wavelengths 800-1,100 nm are characterized by 100s-1,000s times greater photo-thermal ablation threshold energy densities than Mid-IR and IR wavelengths because of weak Near-IR absorption by the soft tissue's chromophores, which results in multi-millimeter depth of laser energy penetration into the soft tissue. Such multi-millimeter ambiguity in tissue removal spatial accuracy at Near-IR wavelengths (often cited3-6 as "poor scalpels" and as "not conducive to precise ablation") increases the collateral damage risk of overheating if photo-thermal ablation is attempted. Such risk is referred to in8 as "vital structures … may be heavily damaged before tissue ablation at the surface initiated".

Unlike Near-IR wavelengths, the Mid-IR wavelengths (Erbium lasers) and IR wavelengths (CO_2 lasers) exhibit much shorter absorption depths, which makes Mid-IR and IR lasers far more spatially precise and safer in soft-tissue ablative applications. The ablation threshold energy density[5,6] E_{TH} for 75% water-rich soft tissue is 3 J/cm^2 at 10,600 nm CO_2 laser wavelength.

Depth of Laser Vaporization/Ablation/Incision. For a laser scalpel, e.g., CO_2 or Erbium lasers, the power density of the focused laser beam is equivalent to the mechanical pressure that is applied to a cold steel blade. Greater laser fluence (i.e., power density times the duration it applied to the target) results in greater depth and rate of soft tissue removal. For short pulse steady state ablation conditions ($xa << A$),[2,5,6] the ablation depth is: $A\ (E - E_{th}) / E_{th}$, where A is

the absorption depth and E^{th} is the ablation threshold, and ***E*** is the fluence delivered to the tissue. For repetitive pulses that are scanned across the soft tissue, the depth of incision is proportional to laser average power, and is inversely proportional to focal spot diameter and the surgeon's hand speed.

Efficiency and Depth of Laser Coagulation / Hemostasis. Coagulation occurs as a denaturation of soft tissue proteins that takes place in the 60-100°C temperature range[3-5] leading to a significant reduction in bleeding (and oozing of lymphatic liquids) on the margins of ablated tissue. Photo-thermal coagulation is also accompanied by hemostasis due to shrinkage of the walls of blood and lymphatic vessels due to collagen shrinkage at increased temperatures. Since blood is contained within and transported through the blood vessels, the diameter of blood vessels ***B*** (20-40 μm[5]) is a highly important spatial parameter in considering the efficiency of photo-thermal coagulation.

During soft tissue laser vaporization with pulses that are comparable to or shorter that Thermal Relaxation Time ***TRT***, the coagulation depth ***H*** is proportional to the absorption depth of light in the soft tissue.[5]

For ***H<<B*** (see Erbium laser wavelengths), optical absorption and coagulation depths are significantly smaller than blood vessel diameters; coagulation takes place on relatively small spatial scale and cannot prevent bleeding from the blood vessels severed during tissue ablation.

For ***H>>B*** (diode laser wavelengths), optical absorption (Near-IR attenuation) and coagulation depths are significantly greater than blood vessel diameters; coagulation takes place over extended volumes - far away from ablation site where no coagulation is required.

For ***H ≥ B*** (CO_2 laser wavelengths), sub 100-μm[5,7] coagulation depths extend just deep enough into a severed blood vessel to stop the bleeding; the coagulation is more efficient then for diode (***H>>B***) and Erbium (***H<<B***) laser wavelengths.

Near-IR Diode Laser Soft Tissue Ablation and Coagulation. Near-IR diode laser light circa 1,000 nm is not used to optically ablate the soft tissue; instead, the diode laser optical energy is used to heat up the charred distal end of the fiber glass tip to 500-900^{O}C,[9] which then heats up the soft tissue through heat conduction from hot glass tip: soft tissue is burned off on contact with the hot charred glass tip, while the margins of the burn are coagulated. Unlike non-contact surgical lasers (such as CO_2 or Erbium), the soft tissue ablative diodes are contact thermal non-laser wavelength-independent devices.[9]

Summary. *A combination of the CO^2 laser wavelength, and SuperPulse settings, and tightly focused laser beam* allows for:
- a char-free and bloodless surgery (i.e., approximately 1,000 times more photo-thermal cutting efficiency than diodes, and for approximately 10 times more photo-thermal coagulating efficiency than erbium lasers;

- *Sub-100 μm coagulation/hemostasis depth, which closely matches the blood capillary diameters.*[5] It allows, unlike with erbium lasers, for an instant hemostasis during high speed ablation / cutting. It affords the clinician with the improved visibility of the surgical field and therefore allows for more precise and accurate tissue removal;[7]

- *Highly controllable speed and depth of incision* with dynamic range from micrometers to millimeters. The depth is proportional to laser power and inversely proportional to laser beam diameter and handspeed; [1,2,11]

- *Minimal post-operative pain, discomfort, swelling and edema, significantly reduced post-surgery production of myofibroblasts, diminished wound contraction and scarring*. [1,2,11] As observed in our surgeries, healing with the CO_2 laser is markedly different from the other surgical modalities, it is uncomplicated and predictable.

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COMPOUNDED VETERINARY MEDICATIONS: CONTROVERSIES AND GUIDELINES

Papich, M.G.

College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

INTRODUCTION

Compounding for veterinary patients has received a lot of attention in the past few years. Because of problems with compounded preparations for human patients, there have been calls for more regulations on compounding. The recent problems that resulted from some human compounded formulations, including 74 human deaths, were highly publicized. Subsequently, the U.S. Congress held hearings and proceeded with legislation to help prevent problems in the future. This act, named the "The Drug Quality and Security Act" was signed into law on November 27, 2013. It was intended to ensure better quality of compounded products. Unfortunately – or fortunately, depending on one's perspective – regulation of veterinary compounding was not included in the federal legislation that resulted from this action. The new federal legislation is somewhat limited in its scope and is intended to primarily improve quality of sterile compounded products.Some critics of the legislation suggest that it comes up short of ensuring safety and quality of compounded products. According to one perspective of the act,[1] "Traditional compounders can now operate without fear of federal enforcement". Because of concerns with veterinary compounding – particularly compounding from bulk chemical substances – the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) has attempted to provide guidance on compounding for veterinary use, but at this time.

CURRENT STATUS OF VETERINARY COMPOUNDING

Until recently, veterinary drug compounding was covered in Food and Drug Administration (FDA) Guidance issued in 2003. This guidance, called "Compounding of Drugs for Use in Animals, or Guidance 608.400" [2] addressed the areas in which the FDA would use regulatory discretion to enforce the Federal Food, Drug, and Cosmetic Act. However, many parts of the guidance, particularly those involving the use of bulk chemicals (bulk powder) were not actively enforced. This guidance was withdrawn, and the agency attempted to replace it with a new "Guidance for Industry" (GFI #230). However, because of additional concerns, and comments received by the FDA, the GFI was withdrawn in the fall of 2017. Until a replacement is issued there are no FDA policies for compounded medications for veterinary use.

The basis for addressing this issue is primarily to address compounding from bulk chemicals. The FDA recognizes that there are limited circumstances when an animal drug compounded from bulk drug substances may be necessary. The agency intends (in the future) list specific conditions under which the agency generally would not take regulatory action against state-licensed pharmacies or veterinarians when drugs are compounded for animals from bulk drug substances. According the FDA, "there are circumstances where there is no approved drug that can be used or modified through compounding to treat a particular animal with a particular condition. In those limited situations, an animal drug compounded from bulk drug substances may be an appropriate treatment option". It is anticipated that a new guidance will limit compounding for animals to FDA-approved products, or bulk chemicals when no other reasonable option exists, but the exact language that will appear in the guidance is not yet known.

Compounding, defined by the United States Pharmacopeia (USP) as "the preparation, mixing, and assembling, packaging, and labeling of a drug or device in accordance with a licensed practitioner's [the veterinarian] prescription of medication or under an initiative based on the practitioner/patient/pharmacist/compounder relationship in the course of professional practice."[3] Drug compounding has always been a part of veterinary medicine. Historically, veterinarians have been known for preparing concoctions, mixtures, and remedies for their patients because there were few approved formulations on the market for animals. Today, there are more available drugs for animals, and a better understanding of the risks of drug instability and incompatible mixtures. Questions concerning this widespread practice have been raised, particularly with respect to the drug's stability, purity, and potency when the original dose form is altered, or when compounding is performed from the bulk drugs. "Bulk chemicals" or "Bulk drugs" are defined as active ingredients used in the manufacture of finished dosage forms of the drug.[3, 4] Bulk drug

substances are also referred to as active pharmaceutical ingredients (APIs). The bulk drug substances are sometimes used because it does not contain excipients, which are the inactive ingredients added as a preservatives, stabilizers, buffers, or to enhance solubility.

Compounding is performed for the purpose of ease of administration or because the original dosage form is unsuitable for the purpose intended. It is common for veterinary dermatologists to rely on compounded medications for topical treatments, as in the treatment of otitis externa, where specialized mixtures may be needed. Compounding does not include the preparation of a drug by reconstitution or mixing that is according the manufacturer's instructions on an approved human or veterinary drug product. (For example, preparing a vial for injection.)

Veterinary medicine has been debating the practice and regulation of compounding for over 20 years. In 1993, a symposium on Compounding in Veterinary Medicine was held by the American Academy of Veterinary Pharmacology and Therapeutics (AAVPT)[5]. This symposium had representatives from AVMA, FDA/CVM, pharmacology and pharmacy groups, and USP. The symposium heard various views from practitioners, pharmacologists, regulatory officials, pharmacists, and lawyers. This symposium issued a Task Force Report that summarized the presentations and resulted in the Compliance Policy Guide published in 1996. The proceedings from this symposium are very informative and contained 115 pages of presentations, which cannot be adequately summarized here. The FDA followed several years later with arevised Compliance Policy Guide (CPG) of 2003 for compounding drugs that was cited above.

The FDA recognizes the importance of compounding in veterinary practice, but also must ensure that compounded drugs do not cause harm to the treated animals, produce ineffective potency, or residues in food animals. FDA regulations permit the compounding of formulations from approved animal or human drugs under the current federal code: 21 CFR 530.13.[3] The FDA is concerned that allowance of some compounding on a patient-by-patient basis has gone over the limits intended in the original federal regulation and has been expanded to result in large-scale production and labeling of some products that may constitute manufacturing and distributing of new animal drugs.

THE NEED FOR COMPOUNDED DRUGS IN VETERINARY MEDICINE

The palatability, ease of administration, and dispensing factors are among the considerations when formulating drugs for animals. Drugs intended specifically for animals are designed with great care. Pastes and dosage syringes are available for some drugs used in horses. Flavored tablets are used commonly in dogs for tablets that are given by pet owners. Transdermal medications are available for dogs and cats to avoid the necessity of frequent administration to a pet that may be difficult to medicate. Sometimes compounding is a necessity. Despite the advances in new drugs available for animals, many needs are still not met. Therefore, various drugs are use in an animal species not listed on the approved label, or are human drugs administered to animals. Some drugs require compounding simply because no approved form of the drug exists in the U.S. Drugs that are often compounded for veterinary medicine because approved forms are not available include: potassium bromide, metronidazole benzoate suspension, methimazole transdermal, diethylstilbestrol, cisapride, various antidotes, and other products that have either been discontinued in human medicine, or because of shortages in availability.

CONCERNS FROM COMPOUNDED VETERINARY FORMULATIONS

Many compounded formulations that are prepared by reputable pharmacies that result in high quality medications with assurances of potency, stability, and beyond-use-dates (BUD). However, there are also concerns. Beyond-use-dates are provided in the USP general chapter on compounding (USP <795>).[4] The BUD for aqueous (water-containing) oral formulations stored at controlled cold temperatures is 14 days to maintain the potency within 90-110% of the nominal formulation strength (+/- 10% is the USP standard for strength of formulations). Compounding pharmacies sometimes go beyond this date without studies to support their claim. There is evidence from published articles that some drugs are not suitable for compounding because they result in rapid inactivation, loss of potency, or lack of systemic absorption when administered to an animal. We have evidence that administration of antibiotics from some compounded formulations are sub-therapeutic and can increase the risk of bacterial drug resistance, which is a risk both to the pet and animal owner.In some cases the compounded products may actually contain levels well above the labeled amount which presents a risk of toxicity for the pet, as well as the animal owner handling the medication.

Examples of Potential Problems

There are several relevant published examples in which drug stability and efficacy has been compromised through compounding.[3] When protective coatings are disrupted, and the vehicles altered, the stability of the product may be compromised. When the formulation is prepared from the bulk API without excipients or preservatives added to maintain stability, strength, or to maintain a desired pH, the quality and strength of the drug may be compromised. In some instances, only a slight alteration of pH can affect the drug. According to the USP-NF, "improper pH ranks with exposure to elevated temperature as a factor most likely to cause a clinically significant loss of drug. A drug solution or suspension may be stable for days, weeks, or even years in its original formulation, but when mixed with another liquid that changes the pH, it degrades in minutes or days. It is possible that a pH change of only one unit could decrease drug stability by a factor of ten or greater." Addition of a water-based solution to a product to make a liquid solution or suspension can hydrolyze some drugs (beta-lactams, esters). Some drugs undergo epimerization (steric rearrangement) when exposed to a pH range higher than what is optimum for the drug (for example this occurs with tetracycline at a pH higher than 3). Other drugs are oxidized, catalyzed by high pH, which renders the drug inactive. Drugs most likely to be subject to oxidation are those with hydroxyl group bonded to an aromatic ring structure. Oxidation may occur from exposure to light and oxygen during reformulation and mixing. Oxidation is catalyzed by high pH and usually leads to drug inactivation.

For example, when omeprazole was compounded for oral use in horses, it was not as effective for treating gastric ulcers as the commercial formulation registered for horses (*Gastrogard*). Omeprazole is known for its instability unless administered in the original formulation intended for horses or people. Itraconazole is notorious for its instability and variable oral absorption. When the brand name itraconazole (Sporanox) and generic itraconazole were compared in research dogs, they produced similar plasma concentrations. But by comparison, the plasma concentrations from a compounded product were negligible[6]. Plasma concentrations from compounded itraconazole administered to cats was barely detectable compared to concentrations produced from the brand name capsule or solution[7]. When doxycycline hyclate was examined in a compounded aqueous formulation made from crushed tablets, strength of the preparation depleted drastically after 7 days[8]. Other compounded formulations known to produce formulations of poor strength and quality are pergolide, trilostane, pimobendan, clenbuterol, boldenone, amikacin, minocycline, and ketoprofen.

On the other hand, some compounded formulations made from extemporaneous preparations have been shown to retain their strength for storage times of at least 28 days and longer. These include enrofloxacin, carprofen, meloxicam, potassium bromide, sodium bromide, and metronidazole benzoate. Enrofloxacin prepared in an oral flavored suspension for use in exotic animals [9]. The oral suspension retained potency within the USP acceptance criteria for 56 days. Despite the problems cited above for compounded omeprazole, if one starts with the FDA-approved equine formulation of GastroGard and compounds it with corn oil to a concentration of 10 or 40 mg/mL (appropriate for cat and dogs) it retained the original strength for 6 months (author's observation; not published). Antibiotics reconstituted in a vial and stored in individual aliquots in plastic tuberculin syringes in the freezer (eg, ceftazidime, meropenem) were stable for 25 days (author's study; publication in press). Ceftazidime stored in this manner in the refrigerator retained potency for 5 days; meropenem retained potency for 48 hours.

Veterinarians and pharmacists are obligated to be cognizant of the potential for interactions and interferences with stability. Oxidation is often visible through a color change (color change to pink or amber for example). Loss of solubility may be observed through precipitation. Some drugs are prone to hydrolysis from moisture. A rule-of-thumb for veterinarians is that if a drug is packaged in blister packs or moisture proof barrier, it is probably subject to loss of stability and potency if mixed with aqueous vehicles. If compounded formulations of solid dose forms show cracking or "caking", or swelling, the formulation has probably accumulated moisture and may have lost potency. Another rule-of-thumb is that if the original packaging of a drug is in a light-protected or amber container it is probably prone to inactivation by light. Vitamins, cardiovascular drugs, tetracyclines, and phenothiazines are labile to oxidation from light during compounding. Also, as a general rule, if an antibiotic is available in a powder that must be reconstituted in a vial or oral dispensing bottle prior to administration, it should not be mixed with other drugs.

Many drugs intended for one species (or humans) are frequently compounded for another veterinary species. In these

instances, it is not only the compounding practice that may affect drug absorption, but also the species differences. Although one assumes that absorption may be similar, differences can exist that may result in poor efficacy. Grass & Sinko concluded that there is no apparent relationship when comparing bioavailability of orally-administered drugs, between humans, dogs, primates, and rodents[10]. Therefore, for drugs administered orally, it is very difficult to broadly extrapolate from studies performed in people to veterinary species. Specific studies are usually needed, unless it is known that the drug is highly stable, soluble, and well absorbed under a variety of conditions.

The Problem with Compounded Transdermal Gels

Several published studies, or studies presented at conferences in abstracts have demonstrated poor or unreliable systemic absorption from transdermal gels (summarized in [3]). To the author's knowledge, only a few drugs were shown to produce therapeutic effects when compounded into a transdermal gel and applied to the ear of cats: methimazole for feline hyperthyroidism, mirtazapine for appetite stimulation in cats, and amlodipine for feline hypertension. The skin is an efficient barrier and it is very difficult to facilitate the absorption of most drugs through the skin. Drugs have been combined with penetration enhancers to facilitate transdermal absorption. One popular example of a penetration enhancer, is pleuronic lecithin organogel (PLO), which is lecithin (derived from eggs or soybeans) mixed with isopropyl palmitate and a poloxamer (Pluronic). The ingredients in PLO are intended to act as surfactants, emulsifiers, and solubilizing agents. Although the use of PLO is popular among the veterinary compounding pharmacies, there are no successful commercial formulations that have combined PLO with systemic drugs.

The list of drugs that have been shown to be absorbed poorly or highly inconsistently in this form includes: cyclosporine, atenolol, glipizide, dexamethasone, buspirone, amitriptyline, fentanyl, morphine, fluoxetine, diltiazem, ondansetron, theophylline, gabapentin, and enrofloxacin. Despite the lack of assurance of systemic absorption from transdermal gels, some veterinary compounding pharmacies advertise and promote transdermal gels on their web sites. One compounding pharmacy lists over 10 pages of compounded transdermal gels for cats. Development of transdermal technology in animals is very difficult and assurance of stability, potency, and systemic absorption for the vast majority of these products has not been demonstrated. Most compounded transdermals for animals are at best placebos. Because of the lack of effective absorption when these products are applied to the ears of cats, it presents a risk of exposure to the family of these pets, especially children. It is a common habit of cats to rub their ears against their owners and on furniture and bedding. Topical irritation and dermatitis have been observed from some transdermal medications applied to the ear of cats because of direct irritation or because the agent is photoreactive and produces a reaction when the pet is exposed to sunlight.

CURRENT REGULATIONS AND POLICIES

Until the FDA-CVM defines its regulatory policy, there is no current guidance for veterinary pharmacies to follow. However, there are still some restrictions that apply, and each state may have more restrictive requirements than Federal Law. The source of the drug should be a USP or an NF grade substance. Drugs should be compounded from the original formulation, if an approved product exists. Compounding from bulk drugs is acceptable if there is not a proprietary approved formulation available that meets therapeutic needs. If bulk drugs are used because there is no other available form, the pharmacist should use bulk substances registered with FDA, and accompanied by a valid certificate of analysis.

It is the responsibility of the veterinarian and pharmacist to ensure that regulations and guidelines are being followed for confidence in the compounded medication. The USP-NF lists specific standards in the General Chapter on Pharmaceutical Compounding <795> and <797>, (USP-NF, http://www.usp.org).[4] Often overlooked in compounding practices is the guideline to ensure that the compounded formulation is not less than 90.0% and not more than 110% of the theoretically calculated and labeled quantity of active ingredient per unit weight or volume. There are also guidelines available for Quality Assurance in Pharmaceutical Compounding (USP <1163>)[4].

Compounding pharmacies have a responsibility to provide veterinarians and their clients with assurances that their formulations meet compendial standards and are stable and potent for the specified beyond-use-date. Pharmacies can be accredited by the Pharmacy Compounding Accreditation Board (PCAB). This is a voluntary program for improving the quality of compounding operations and preparations. An accredited pharmacy that adheres to USP compounding standards provided in General Chapters<795> and <797>[4] will have significant checks in place to

ensure that compounded products meet a high standard of quality. On the other hand, some pharmacies may not follow these standards and instead, provide veterinarians and their clients with misleading information on the quality of their products. For example, some products may be promoted as meeting "stability standards" for 30, 60, 90, or 180 days after preparation. But, there is a difference between stability testing and potency testing [11].

SUMMARY

Compounding for veterinary patients is in a state of flux until the FDA defines its new policy. It is not known at this time how closely the new Guidance will be enforced. Until now, compounding for veterinary patients has largely unregulated and without federal enforcement. Changes can also be enacted by specific states that could restrict some forms of compounding for animals. In the meantime, veterinarians are encouraged to be skeptical of broad claims of stability, quality, and systemic absorption from compounded products unless the pharmacy can provide some assurances based on studies conducted, or adherence to compendial standards.

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ANALGESIC MEDICATIONS FOR SMALL ANIMALS: WHAT TO USE BEYOND NSAIDS?

Papich, M.G.

College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

INTRODUCTION

There has been a tremendous amount of information published on NSAIDs in the last 10 years. There is far less information on alternatives beyond NSAIDs. There are no other agents approved for chronic treatment of pain and osteoarthritis in dogs. Nothing is approved for chronic treatment in cats. There are a few opioids approved for short-term use but these are impractical, or ineffective for long-term use. Moreover, prescribing opioid medications for chronic treatment is under more scrutiny because of the opioid abuse problem in people. What are the alternatives? (Dietary supplements such as n-3/n-6 fatty acids [omega fatty acids], glucosamine-chondroitin sulfate, green-lipped mussel, and others are used for chronic arthritis, but because of space limitations, they will not be included here. See Vandeweerd, et al,[1] for a more detailed review.)

GRAPIPRANT

Grapiprant (Galliprant®) is a new class called "piprants" that can technically be considered an NSAID (it is "anti-inflammatory" and not a steroid). It is listed here because some veterinarians consider this agent an alternative to NSAIDs. Grapiprant is the first from this group approved for any animal or human. Grapiprant is approved for dogs for daily administration (2 mg/kg oral) to treat osteoarthritis. There is no approval at this time for cats, but new studies are emerging that may help for treatment of cats in the future[2, 3] .

Grapiprant is a non-cyclooxygenase (COX) inhibiting agent. Instead of inhibiting production of prostaglandins, it blocks the receptor. After inflammation is triggered and prostaglandins are synthesized, Prostaglandin E_2 (PGE2) exerts its effects via four receptors (EP_{1-4}). The EP4 receptor is responsible for some of the sensitization of sensory neurons and signs of inflammation. Although other receptors are activated during inflammation, the EP4 receptor has been identified as the primary receptor responsible for mediating pain and inflammation associated with osteoarthritis. In dogs, grapiprant has high binding affinity for the EP4 receptor and binds competitively at a single site[4]. The effective dose in dogs, based on pharmacokinetic-pharmacodynamic (PK-PD) analysis, and extrapolation from laboratory animal models of pain, is 1.7 mg/kg[4], which is almost the same as the FDA-approved dose of 2 mg/kg once daily.

In a pivotal clinical study[5] grapiprant showed effectiveness comparable to traditional cyclo-oxygenase inhibiting drugs. In dogs with osteoarthritis, 48% were classified as treatment successes compared to 31.3% of placebo-treated dogs (131 dogs in each group). Adverse reactions in dogs have been minimal and are similar to those typically encountered for traditional NSAIDs.[5,6] These may include vomiting, diarrhea and decreased appetite. These events have been mild and usually transient. In a safety study with research dogs, they tolerated a dose equivalent to 30 mg/kg of the tablet for 9 months with adverse effects attributed to the gastrointestinal tract as mild and infrequent6.

TRAMADOL

Tramadol (*Ultram*, and generic) is a unique oral analgesic drug. The popularity grew in veterinary medicine when a generic formulation became available at a low price. It was not controlled at that time, which made it easy to prescribe. The status has since been changed to a DEA-Controlled Substance (Schedule IV). The change in controlled status may have altered the prescribing habits of some veterinarians; however, the generic formulation is still inexpensive andwidely available. The most common form is the 50 mg tablets (Ultram, and generic), but is also available as 100-, 200-, and 300- mg extended-release tablets. The exact mode of action for tramadol is still uncertain and there is probably more than one mechanism that contributes to the clinical effects. Tramadol has some mu opioid receptor action, and it inhibits the reuptake of norepinephrine (NE), and serotonin (5 HT). The parent drug has weak binding affinity to the μ-opiate receptor, with an affinity that is 4,000x less than morphine. There is even less affinity for the κ- and δ- opiate receptors. The *O*-desmethyl (+ isomer) metabolite (also known as M1) has μ-opiate receptor

binding affinity that is 400-fold higher than the parent drug, but this is still only about 1/10 that of morphine. In people and laboratory animals, opiate-receptor antagonism with naloxone partially, but not completely, can block analgesic effects of tramadol. Inhibition of the α_2-adrenergic receptor with yohimbine partially blocks analgesic effects, and serotonin type-2 and -3 (5-HT_2, 5-HT_3) receptor blockers also can partially block analgesic effects.Taken together, the effects of tramadol may be explained via inhibition of serotonin reuptake (similar mechanism as fluoxetine and other antidepressant drugs), action on alpha 2 receptors (similar mechanism as dexmedetomi¬dine), and activity for opiate mu recep¬tors (similar mechanism, but less potent than morphine). These effects have beenrecognized in people, and in laboratory rodents, but the mechanisms are far less studied in dogs, cats, and horses. In people, even when there is efficient conversion of tramadol to the active M1 metabolite, the analgesic efficacy is approximately equivalent to codeine. Metanalysis of clinical studies in people with low back pain shows that monotherapy with tramadol is not always effective and some patients may not respond. In people with osteoarthritis the effects are modest with fair evidence of efficacy. The Oxford League Table of Analgesic Efficacy(http://www.bandolier.org.uk/booth/painpag/Acutrev/Analgesics/lftab.html) ranks tramadol near the bottom for analgesic efficacy in humans, compared to oral opioids, NSAIDs, acetaminophen and their combinations.

Pharmacokinetics

The Cytochrome P-450 2D6 enzyme (CYP2D6) in people converts tramadol to the active M1 (O-desmethyl-) metabolite. One of the other metabolites, N,*O*-didesmethyl tramadol (also known as M5) has opiate receptor activity but does not cross the blood brain barrier and lacks central analgesic properties. In all, there are 24 identified tramadol metabolites7. Except for M1, the other metabolites are essentially inactive. In people who are poor metabolizers for converting tramadol to the active M1 metabolite, the analgesic effects are diminished[8-10].

Several pharmacokinetic studies are available for dogs, horses, cats, and some exotic animals. Tramadol is moderately well-absorbed orally in dogs and was well-tolerated. The pharmacokinetic studies in dogs show very high inter-individual (between subject) variation in both the parent drug and metabolite concentrations. Dogs have low CYP2D6 activity; therefore, another anzyme, CYP2D15, is responsible for converting tramadol to the M1 metabolite[11]. However, conversion to M1 in dogs is slow, and tramadol is preferentially converted by other enzymes (CYP-2B11 and-3A12) to the inactive M2 metabolite. Conversion to the inacative metabolite may occur at the site of absorption in the intestine.In addition to being poor metabolizers of tramadol to M1, the metabolite formed is converted to a glucuronic acid conjugate and rapidly excreted[12, 13]. Low M1 concentrations in dogs may limit the analgesic effectiveness. Tapentadol, a drug with a similar mechanism of action as tramadol, does not require metabolism to an active metabolite. When it was compared to tramadol in dogs, there were no significant antinociceptive effects from tramadol, and no detectable concentrations of M1, but significant antinociceptive effects from tapentadol[14].

Overal systemic clearance in dogs is higher than in people, which necessitates a higher dose for dogs to reach similar plasma concentrations of tramadol. The half-life is short in dogs (1-2 hours), which requires frequent dosing. Note that the dose in humans is less than 1 mg/kg, but in dogs, we routinely administer 5 and 10x this dose without producing adverse effects. With repeated administration – such as might be expected for chronic pain – tramadol clearance increases and plasma/serum drug concentration decreases. After 7 consecutive days of oral dosing to dogs, clearance increased by 7x, which decreased the serum tramadol concentration, but concentrations of the inactive metabolite, M2, increased[15]. Likewise, in a clinical study, after 22 days of oral tramadol dosing, the tramadol concentration decreased by an average of 82% compared to measurements on day 816. Metabolite concentrations were not measured.

Is Tramadol Effective?

Safety and efficacy studies have not been sufficient to provide clinical dosing recommendations. Dosages have been based on pharmacokinetic studies and extrapolation. Doses in clinical and experimental animals have ranged from 2-10 mg/kg, every 8-12 hours. Doses of 5-10 mg/kg every 6 to 8 hours orally have been well tolerated in dogs.

Definitive studies are needed with adequate design and controls to determine if tramadol is effective for pain in dogs. Since an assessment of the efficacy in 2013[17], at least 9 additional studies have been published to evaluate efficacy. There are also at least 5 other clinical studies and 2 experimental studies published. A close examination of those studies is needed before making conclusions. Three of the studies used epidural administration. Seven of

the published studies used either SC, IV, or IM injections. There are no approved injectable formulations available in North America. A formulation for injection requires compounding in a sterile formulation, which is unlikely because of the regulatory requirements for sterile injectable-compounded formulations. The studies that examined effectiveness of injectable administration were surgical conditions in which there was no placebo control group and all animals received anesthetic agents prior to, and during surgery, which confounds the assessment of post-operative pain. Some of the studies compared tramadol to low doses of another analgesic (eg, morphine at 0.2 mg/kg, or buprenorphine at 0.02 mg/kg). These low doses may not produce a useful comparison.

For threatment of chronic pain in North America, it is only practical to consider the studies that used oral administration to evaluate efficacy. As mentioned above, the pharmacokinetics and metabolite profile are different between injectable and oral administration because of the metabolism to inactive metabolites that occurs in the intestine and liver because of high first-pass effects.Of the studies that examined efficacy in canine clinical patients using oral tramadol, 3 involved short-term administration associated with orthopedic or enucleation surgery[18-20]. None of the surgical studies included a placebo control group. In one study, oral tramadol was not as effective as firocoxib, and firocoxib-tramadol combination[19]. In another study carprofen was more effective than tramadol[20]. The third study compared oral tramadol to oral hydrocodone-acetaminophen combination[18]. The percentage of dogs with treatment failure in both groups was considered by the authors as unacceptable. There are only 3 studies that have evaluated the effectiveness of oral tramadol in dogs from multiple doses. In a study that examined 4 outcome measures in dogs with osteoarthritis, tramadol (4 mg/kg q8h x 22 days) was compared to other agents, including a placebo[16]. There were treatment effects in all groups, including the placebo treatment group. Owners reported improvement from administration of tramadol, but the kinetic gait analysis did not show improvement. Tramadol treatment group needed more rescue medication than the other two positive treatment groups. In another study, tramadol (2.3-4.8 mg/kg q8h x 14 days) in combination with an NSAID (dipyrone) was compared to other treatments with, and without, another NSAID for cancer pain[21]. There was no placebo group. The tramadol-NSAID combination was more effective than the NSAID-treated group that did not include tramadol. In the most recent, and perhaps best controlled study to date, oral tramadol (5 mg/kg oral, q8h) was compared to carprofen and placebo treatments in 40 dogs with osteoarthritis.[36] Each treatment was administered for 10 days. Carprofen performed better than placebo and tramadol and there was no clinical benefit observed from tramadol in these dogs.

Except for the most recent study,[36] these limited clinical studies in which oral tramadol was administered for multiple doses are inadequate to fully evaluate effectiveness of tramadol in the manner that is used by most veterinarians – long-term treatment for osteoarthristis or other chronic pain. Without a placebo treatment group included in the studies, differences attributed to tramadol treatment are difficult to assess. It was estimated that in studies to evaluate lameness from osteoarthritis in dogs, a placebo effect accounted for 39.7% and 44.8% of the positive response evaluated by pet owners and veterinarians, respectively[22].

There are no clinical safety studies; only observations from the brief clinical experiences cited above. In these reports, adverse effects have been minimal. There are anecdotal accounts of clinical signs that may represent serotonergic effects, but these have not been verified. In toxicocologic studies, dogs have tolerated single doses of 450 mg/kg [15]. At doses up to 40 mg/kg twice-daily for 12 months, the only treatment-related sign was mydriasis and reduced weight gain. At a dose of 25 mg/kg per day, signs attributable to central nervous system toxicity are possible (sporadic seizures)[15].

In cats the clearance and metabolism are much different than in dogs. The efficacy of tramadol as an analgesic in cats maybe consistent with opioid-mediated analgesia, but there have only been limited studies to measure antinociceptive effects in experimental cats. Two of the studies available have shown at least some antinociceptive effects during short-term administration[23, 24], but in another, it was limited[25]. There are no clinical studies to measure the effects from repeated administration.

The clearance of the desmethylmetabolite (M1), which is conjugated to glucuronic acid for elimination in other animals, is much slower in cats and high M1 concentrations have been detected[26]. Because the M1 metabolite is associated with greater opiate-mediated effects than the parent drug, opiate effects have been observed in cats. Dosage recommendations for cats have been in a range of 2-4 mg/kg PO q 12 hours, but a dose of 4 mg/kg orally

produced dysphoria and mydriasis in some experimental cats (authors observations). One study concluded that a dose of 4 mg/kg q6h, oral is needed to produce a consistent maximum antinociceptive effect. Repeated oral dosing may be a challenge in cats because of the unpleasant taste.

What about Tapentadol?

Tapentadol has some similarities to tramadol, but some distinct advantages. Like tramadol, it is a norepinephrine reuptake inhibitor. But in contrast to tramadol is also a direct μ-opiate receptor agonist that does not require metabolism to another metabolite for opiate receptor activity. The opiate receptor affinity is 50x less than morphine, but may produce analgesia through the synergistic effects as a reuptake inhibitor. It is rapidly metabolized in dogs[37], which may limit its oral efficacy. It has produced analgesia in research dogs after IV administration[14], but at this time, clinical studies are not available to assess efficacy from oral administration in dogs.

GABAPENTIN

Gabapentin (*Neurontin*, and generic) is ordinarily used as an anticonvulsant but may have analgesic properties as well. Gabapentin is a structural analogue of gamma aminobutyric acid (GABA). Although it is structurally related to GABA, it does not interfere with sodium-dependent channels or exhibit affinity for other neurotransmitter receptors, such as those affected by benzodiazepines (i.e., glutamate, dopamine, NMDA). It is not controlled by the DEA. Gabapentin inhibits the alpha2-delta subunit of the N-type voltage-dependent calcium channel on neurons. After binding to this subunit, it reduces the calcium influx needed for release of neurotransmitters – specifically excitatory amino acids – from presynaptic neurons. This channel becomes up-regulated when nerves are stimulated, such as in epileptic or neuropathologic conditions. Blocking the channels has little effect on normal neurons, but appears to suppress stimulatedneurons; therefore, gabapentin is associated with few adverse effects in dogs except for sedation.

Gabapentin and a related drug, pregabalin (Lyrica), have been used in humans to treat many pain states, including fibromyalgia, inflammatory pain, diabetic neuropathy, malignant pain, central pain, complex regional pain syndrome, and trigeminal neuralgia. These types of pain may occur in animals, but the existence has not been confirmed.

Gabapentin is available in 100-, 300-, and 400-mg capsules; 100-, 300-, 400-, 600-, and 800-mg scored tablets; and a 50-mg/ml oral solution. The oral solution contains xylitol, which is toxic to dogs if administered at high concentrations. Gabapentin pharmacokinetic have been reported for dogs and cats[17, 27]. It is excreted predominantly by the kidneys and thus has no hepatic interactions. Pregabalin (*Lyrica*) is not as commonly used. It is more expensive and is a controlled drug.

For rescue analgesia (when other drugs have not been effective), gabapentin has been administered in both dogs and cats (and case-reports in horses); however, the dose range is wide. The lower end of the recommended dose is used initially, and the dose may be increased gradually. Sedation is more likely as the dose increases.

Is Gabapentin Effective?

A commentary published in the New England Journal of Medicine in 2017 pointed out that gabapentin was being prescribed to human patients for treatment of pain at increasing rates[28]. The increased use was attributed to finding alternative agents to substitute for oral opioids in people. The only approved use of gabapentin in people is postherpetic neuralgia. Pregabalin is approved for other neuropathic conditions – fibromyalgia, and neuropathic pain associated with diabetes or spinal cord injury. Goodman & Brett[28] point out that gabapentin has not been shown to be effective in people for osteoarthritis or low back pain, despite the frequent prescribing for this use. Pain associated with osteoarthritis, or other musculoskeletal conditions has not been attributed to neuropathic disease; therefore, it is questionalble that these conditions will respond to gabapentin.

Despite the anecdotal references to the use of gabapentin in animals, there are no studies in dogs or cats available to demonstrate convincing efficacy. When administered to dogs for control of pain after intervertebral disc surgery (10 mg/kg every 12 hours) there was no detectable reduction in pain behavior compared to the other drugs[29]. In cats, at doses of 5, 10, or 30 mg/kg, it did not affect thermal threshold anti-nociception in cats, and did not provide thermal antinociception[30]. It did not produce analgesic effects in dogs when administered prior to surgery for limb amputation [31]. There are other isolated anecdotal accounts or case reports on the use of gabapentin for post-

operative pain, but these are uncontrolled and not relevant to the common long-term use in veterinary medicine for chronic pain.

The pharmacokinetic studies show that the half-life in dogs and cats is short (3-4 hours). This indicates that to maintain concentrations believed to be therapeutic in people, doses in dogs should be in the range of 10-20 mg/kg every 8 hours, a dose that is untested in clinical studies. At these doses, adverse effects are possible, and complinance may be a challenge.

Gabapentin is not without adverse effects. Sedation and ataxia are possible, especially as doses are increased. Cognitive dysfunction my occur in people. Human patients taking gabapentin for long periods should have their dose reduced gradually when discontinuing treatment. The reduction should occur over 2 to 3 weeks to prevent seizures (reported in humans) and a rebound pain phenomenon. Whether or not this slow tapering of the dose is needed in dogs and cats is undetermined.

AMANTADINE

Amantadine is an antiviral drug that also has been used to treat some pain syndromes. The proposed mechanism of action for treating pain in animals is via NMDA-receptor antagonism. Oral absorption of amantadine is good, but the precise duration of action and dosing regimens have not been fully investigated in animals. Oral amantadine was combined with meloxicam in a study using dogs with osteoarthritis[32]. At a dose of 3 to 5 mg/kg PO administered once daily with meloxicam, dogs responded better than if meloxicam were given alone. Other doses that have been cited for pain are 2 to 10 mg/kg PO q 8 to 12 hr in dogs and 2 mg/kg orally q 24 hr in cats. Amantadine is available in a 100-mg capsule or in a foul-tasting 10-mg/ml liquid. For rescue analgesia, amantadine is often given with other drugs and may take days to weeks to reach full effect.

ORAL OPIOIDS

Hydrocodone (5 mg) + acetaminophen (500 mg) is combined in a tablet and used for analgesia in people (Vicodin, and generic). Because of the acetaminophen in these products, they should never be administered to cats. In dogs, high acetaminophen exposure may result from the high doses necessary. There is new FDA approved product (*Zohydro E*R) that contains only hydrocodone in an extended-release oral formulation. The use of this product has not been reported in animals. Recently, hydrocodone was rescheduled by the DEA from Schedule III, to Schedule II because of high abuse of this drug in people. This restricts prescribing for animal use (written prescription only), and in some states the duration of use may be limited.

There are no studies that have documented analgesic efficacy from oral hydrocodone in dogs. There is limited evidence that dogs produce the active metabolite from oral dosing (hydromorphone), but it is possible that other metabolites may have activity. In one clinical study, the authors concluded that the number of treatment failures from oral hydrocodone-acetaminophen was unacceptable[18].

Oral morphine is available as syrup, tablets, and prolonged-release oral medication. Despite the advantages of oral morphine formulations, pharmacokinetic studies to demon¬strate effective levels are lacking. Oral administration is associated with high first-pass effects and negligible concentrations of the parent drug[33]. Dogs do not appear to produce the active metabolite (M6G) after oral administration. Therefore, the efficacy of oral morphine in dogs is questionable due to high clearance and poor oral absorption.

CANNABINOIDS

The most popular source of cannabinoids is the cannabis plant (marijuana). There is tremendous interest in the pharmacological use of cannabinoids for treating disorders such as nausea, depression, pain, and seizures. Many states in the U.S. allow medical use of marijuana for these uses. In the states where medical marijuana is allowed, there has been increased administration of marijuana to dogs, cats, and horses. The AVMA has published information to inform veterinarians on the administration of cannabinoids to animals (see: https://www.avma.org/KB/Resources/Reference/Pages/Cannabis-Use-Pets.aspx). Even in states where human medical or recreational cannabis is allowed, it is still illegal for veterinarians to prescribe cannabis or its deravitives to animals.

There is substantial experimental evidence for the effects of cannabis for alleviation of pain caused by osteoarthritis.[38] However, it is undetermined at this time if these experimental results will translate to clinical efficacy in animals.

Clinical studies have not been published on the use in veterinary patients, but clinical studies are underway to investigate the use of cannabinoids for treatment of pain and other conditions in animals. Results are not available at this time.

There are approximately 100 cannabinoids in the cannabis plant, but Δ-9-THC (delta-9-THC) is believed to be the most pharmacologically active. Another cannabinoid is cannabidiol (CBD), which has pharmacological properties, but does not have psychoactive effects compared to the other commercial cannabinoids. There are two important cannabinoid receptors: CB1 and CB2. The receptor CB1 is found in the brain and responsible for the CNS effects, including appetite stimulation, antinausea, and antiemetic properties. The receptor CB2 is found outside the CNS and may have a role in immunity. The effect on nausea and vomiting may occur from binding to the CB1 cannabinoid receptor in the emetic center.

In addition to botanical sources, there are two pharmaceutical formulations of cannabinoids. Synthetic marijuana (THC) is available as a pre¬scription drug (dronabinol) for antiemetic therapy. It is marketed as *Marinol* (and generic) in 2.5, 5, and 10 mg capsules and classified as a Schedule III controlled substance. Nabilone (*Cesamet*) is a synthetic analogue of THC. It is a Schedule II controlled substance available as a 1 mg capsule. Both are approved for treatment of nausea and vomiting in people associated with cancer chemotherapy. Dronabinol is also approved for anorexia associated with weight loss in patients with AIDS. There is a cannabis extract that contains a mixture of THC and CBD called *Nabiximols*. It is not available in the U.S. but is widely available in Europe and Canada as an oral mucosal spray (*Sativex*) for treatment of cancer pain in people. Its use has not been reported for animals. CBD has been marketed on internet sites for pets, but the pharmacokinetics and clinical effects have not been reported.

MONOCLONAL ANTIBODIES

It is obvious that new analgesic medications are needed for dogs and cat, especially for chronic out-patient treatment. Among the new treatments are monoclonal antibodies directed against the nerve growth factor. Nerve growth factor (NGF) is important for nerve growth and differentiation. It can be increased during inflammatory diseases and processes that produce chronic pain.

The anti-NGF monoclonal antibodies suppress the pain signals and these products have undergone initial trials for treatment in dogs and cats. Two such products are ranevetmab and frunevetmab. These products were developed by Nexvet, which has now been acquired by Zoetis.

Ranevetmab is a canine-specific monoclonal antibody for nerve growth factor. Ranevetmab, while not commercially available at this time, is designed as a once monthly subcutaneousinjection for the control of pain associated with osteoarthritis in dogs. Trials so far have been promising without adverse effects[34]. Frunevetmab is a feline-specific monoclonal antibody with a similar indication for cats with chronic pain associated with osteoarthritis[35]. The trials in cats have been promising and showed a 6-week duration analgesic effect from a single injection in cats with pain associated with degenerative joint disease. At this time, neither drug is available for use, but they may be available in the future. The advantage of these agents is a relative lack of adverse effects with a single injection that may last weeks.

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Photos: David Bird, DVM

One and done.

In a U.S. efficacy study, 86% of dogs needed only one injection to resolve their skin infection.[1]

Baseline

8 days post-injection

Recommend CONVENIA® (cefovecin sodium) for first-time resolution of bacterial skin infections.

recommendconvenia.com

Eleven-month-old Labrador Retriever treated only with CONVENIA 8 mg/kg. Case included an initial skin cleansing with a dilute topical antiseptic.

IMPORTANT SAFETY INFORMATION: People with known hypersensitivity to penicillin or cephalosporins should avoid exposure to CONVENIA. Do not use in dogs or cats with a history of allergic reactions to penicillins or cephalosporins. Side effects for both dogs and cats include vomiting, diarrhea, decreased appetite/anorexia and lethargy. See Brief Summary of full Prescribing Information on page XX.

[1]Six R, Cherni J, Chesebrough R, et al. Efficacy and safety of cefovecin in treating bacterial folliculitis, abscesses, or infected wounds in dogs. *J Am Vet Med Assoc.* 2008;233(3):433-439.

zoetis

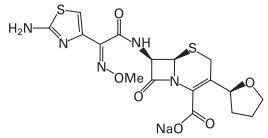
convenia®
(cefovecin sodium)

Antimicrobial for Subcutaneous Injection in Dogs and Cats Only

CAUTION: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

DESCRIPTION: Cefovecin sodium is a semi-synthetic broad-spectrum antibacterial agent from the cephalosporin class of chemotherapeutic agents. Cefovecin is the non-proprietary designation for (6*R*,7*R*)-7-[[(2*Z*)-(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[(2*S*)-tetrahydro-2-furanyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt.

Figure 1: Chemical structure of cefovecin sodium.



Each mL of CONVENIA reconstituted lyophile contains cefovecin sodium equivalent to 80 mg cefovecin, methylparaben 1.8 mg (preservative), propylparaben 0.2 mg (preservative), sodium citrate dihydrate 5.8 mg and citric acid monohydrate 0.1 mg, sodium hydroxide or hydrochloric acid as required to adjust pH.

INDICATIONS:

Dogs
CONVENIA is indicated for the treatment of skin infections (secondary superficial pyoderma, abscesses, and wounds) in dogs caused by susceptible strains of *Staphylococcus intermedius* and *Streptococcus canis* (Group G).

Cats
CONVENIA is indicated for the treatment of skin infections (wounds and abscesses) in cats caused by susceptible strains of *Pasteurella multocida*.

DOSAGE AND ADMINISTRATION:

Dogs
CONVENIA should be administered as a single subcutaneous injection of 3.6 mg/lb (8 mg/kg) body weight. A second subcutaneous injection of 3.6 mg/lb (8 mg/kg) may be administered if response to therapy is not complete. The decision for a second injection for any individual dog should take into consideration such factors as progress toward clinical resolution, the susceptibility of the causative organisms, and the integrity of the dog's host-defense mechanisms. Therapeutic drug concentrations after the first injection are maintained for 7 days for *S. intermedius* infections and for 14 days for *S. canis* (Group G) infections. Maximum treatment should not exceed 2 injections.

Cats
CONVENIA should be administered as a single, one-time subcutaneous injection at a dose of 3.6 mg/lb (8 mg/kg) body weight. After an injection of CONVENIA, therapeutic concentrations are maintained for approximately 7 days for *Pasteurella multocida* infections.

General Dosing Information
A sample of the lesion should be obtained for culture and susceptibility testing prior to beginning antimicrobial therapy. Once results become available, continue with appropriate therapy. If acceptable response to treatment is not observed, or if no improvement is seen within 3 to 4 days, then the diagnosis should be re-evaluated and appropriate alternative therapy considered.

CONVENIA may persist in the body for up to 65 days. The effect of remaining concentrations of cefovecin on any subsequent antimicrobial therapies has not been determined. Fluoroquinolone and aminoglycoside antimicrobials have been reported to be compatible with cephalosporin antimicrobial agents.[1,2,3]

Table 1: Dose Table for CONVENIA at 8 mg/kg Body Weight.

Weight of Animal	Volume of CONVENIA (3.6 mg/lb or 0.045 mL/lb)
5 lb	0.23 mL
10 lb	0.45 mL
15 lb	0.67 mL
20 lb	0.90 mL
40 lb	1.80 mL
80 lb	3.60 mL

PREPARATION OF SOLUTION FOR INJECTION: To deliver the appropriate dose, aseptically reconstitute CONVENIA with 10 mL sterile water for injection. Shake and allow vial to sit until all material is visually dissolved. The resulting solution contains cefovecin sodium equivalent to 80 mg/mL cefovecin. CONVENIA is light sensitive. The vial should be stored in the original carton and refrigerated when not in use. Use the entire contents of the vial within 56 days of reconstitution.

CONTRAINDICATIONS: CONVENIA is contraindicated in dogs and cats with known allergy to cefovecin or to β-lactam (penicillins and cephalosporins) group antimicrobials. Anaphylaxis has been reported with the use of this product in foreign market experience. If an allergic reaction or anaphylaxis occurs, CONVENIA should not be administered again and appropriate therapy should be instituted. Anaphylaxis may require treatment with epinephrine and other emergency measures, including oxygen, intravenous fluids, intravenous antihistamine, corticosteroids, and airway management, as clinically indicated. Adverse reactions may require prolonged treatment due to the prolonged systemic drug clearance (65 days).

WARNINGS: Not for use in humans. Keep this and all drugs out of reach of children. Consult a physician in case of accidental human exposure. For subcutaneous use in dogs and cats only. Antimicrobial drugs, including penicillins and cephalosporins, can cause allergic reactions in sensitized individuals. To minimize the possibility of allergic reactions, those handling such antimicrobials, including cefovecin, are advised to avoid direct contact of the product with the skin and mucous membranes.

PRECAUTIONS: Prescribing antibacterial drugs in the absence of a proven or strongly suspected bacterial infection is unlikely to provide benefit to treated animals and may increase the risk of the development of drug-resistant animal pathogens.

The safe use of CONVENIA in dogs or cats less than 4 months of age (see **Animal Safety**) and in breeding or lactating animals has not been determined. Safety has not been established for IM or IV administration. The long-term effects on injection sites have not been determined. CONVENIA is slowly eliminated from the body, approximately 65 days is needed to eliminate 97% of the administered dose from the body. Animals experiencing an adverse reaction may need to be monitored for this duration.

CONVENIA has been shown in an experimental *in vitro* system to result in an increase in free concentrations of carprofen, furosemide, doxycycline, and ketoconazole. Concurrent use of these or other drugs that have a high degree of protein-binding (e.g. NSAIDs, propofol, cardiac, anticonvulsant, and behavioral medications) may compete with cefovecin-binding and cause adverse reactions.

Positive direct Coombs' test results and false positive reactions for glucose in the urine have been reported during treatment with some cephalosporin antimicrobials. Cephalosporin antimicrobials may also cause falsely elevated urine protein determinations. Some antimicrobials, including cephalosporins, can cause lowered albumin values due to interference with certain testing methods.

Occasionally, cephalosporins and NSAIDs have been associated with myelotoxicity, thereby creating a toxic neutropenia[4]. Other hematological reactions seen with cephalosporins include neutropenia, anemia, hypoprothrombinemia, thrombocytopenia, prolonged prothrombin time (PT) and partial thromboplastin time (PTT), platelet dysfunction and transient increases in serum aminotransferases.

ADVERSE REACTIONS:

Dogs
A total of 320 dogs, ranging in age from 8 weeks to 19 years, were included in a field study safety analysis. Adverse reactions reported in dogs treated with CONVENIA and the active control are summarized in Table 2.

Table 2: Number of Dogs* with Adverse Reactions Reported During the Field Study with CONVENIA.

Adverse Reaction	CONVENIA (n=157)	Active Control (n=163)
Lethargy	2	7
Anorexia/Decreased Appetite	5	8
Vomiting	6	12
Diarrhea	6	7
Blood in Feces	1	2
Dehydration	0	1
Flatulence	1	0
Increased Borborygmi	1	0

*Some dogs may have experienced more than one adverse reaction or more than one occurrence of the same adverse reaction during the study.

Mild to moderate elevations in serum γ-glutamyl transferase or serum alanine aminotransferase were noted post-treatment in several of the CONVENIA-treated dogs. No clinical abnormalities were noted with these findings.

One CONVENIA-treated dog in a separate field study experienced diarrhea post-treatment lasting 4 weeks. The diarrhea resolved.

Cats
A total of 291 cats, ranging in age from 2.4 months (1 cat) to 21 years, were included in the field study safety analysis. Adverse reactions reported in cats treated with CONVENIA and the active control are summarized in Table 3.

Table 3: Number of Cats* with Adverse Reactions Reported During the Field Study with CONVENIA.

Adverse Reaction	CONVENIA (n=147)	Active Control (n=144)
Vomiting	10	14
Diarrhea	7	26
Anorexia/Decreased Appetite	6	6
Lethargy	6	6
Hyper/Acting Strange	1	1
Inappropriate Urination	1	0

*Some cats may have experienced more than one adverse reaction or more than one occurrence of the same adverse reaction during the study.

Four CONVENIA cases had mildly elevated post-study ALT (1 case was elevated pre-study). No clinical abnormalities were noted with these findings.

Twenty-four CONVENIA cases had normal pre-study BUN values and elevated post-study BUN values (37 – 39 mg/dL post-study). There were 6 CONVENIA cases with normal pre- and mildly to moderately elevated post-study creatinine values. Two of these cases also had an elevated post-study BUN. No clinical abnormalities were noted with these findings.

One CONVENIA-treated cat in a separate field study experienced diarrhea post-treatment lasting 42 days. The diarrhea resolved.

FOREIGN MARKET EXPERIENCE: The following adverse events were reported voluntarily during post-approval use of the product in dogs and cats in foreign markets: death, tremors/ataxia, seizures, anaphylaxis, acute pulmonary edema, facial edema, injection site reactions (alopecia, scabs, necrosis, and erythema), hemolytic anemia, salivation, pruritus, lethargy, vomiting, diarrhea, and inappetance.

For a copy of the Material Safety Data Sheet (MSDS) or to report a suspected adverse reaction call Zoetis Inc. at 1-888-963-8471.

CLINICAL PHARMACOLOGY:

Pharmacokinetics
Cefovecin is rapidly and completely absorbed following subcutaneous administration. Non-linear kinetics is exhibited (plasma concentrations do not increase proportionally with dose). Cefovecin does not undergo hepatic metabolism and the majority of a dose is excreted unchanged in the urine. Elimination also occurs from excretion of unchanged drug in the bile. Cefovecin is a highly protein-bound molecule in dog plasma (98.5%) and cat plasma (99.8%) and may compete with other highly protein-bound drugs for plasma protein-binding sites that could result in transient, higher free drug concentrations of either compound. Pharmacokinetic parameters following subcutaneous dosing at 8 mg/kg in the dog and cat are summarized in Table 4.

Table 4: Pharmacokinetic Parameters Reflecting Total Drug Concentrations in Plasma (mean ± standard deviation or range) Following an 8 mg/kg Intravenous or Subcutaneous Dose of Cefovecin in Dogs and Cats.

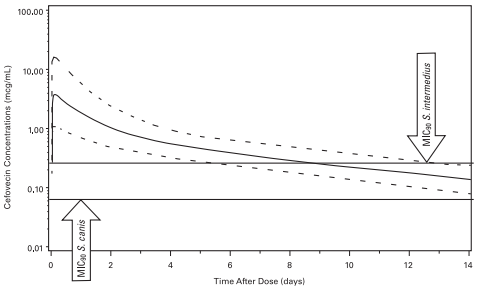
PARAMETER	MEAN ± SD[1] or (Range)	
	Dogs	Cats[p]
Terminal plasma elimination half-life, $T_{1/2}$ (h)*[h]	133 ± 16	166 ± 18
$AUC_{0\text{-}inf}$ (μg·h/mL)*[g]	10400 ± 1900[p]	22700 ± 3450
Time of maximum concentration, T_{max} (h)*[h]	6.2 (0.5-12.0)	2.0 (0.5-6.0)
Maximum concentration, C_{max} (μg/mL)*[a]	121 ± 51	141 ± 12
Vd_{ss} (L/kg)**[g]	0.122 ± 0.011	0.090 ± 0.010
CL_{total} (mL/h/kg)**[g]	0.76 ± 0.13[p]	0.350 ± 0.40

[1] SD = standard deviation
[p] = a phase effect was observed, only data for the first phase are provided (n=6); all other data provided are derived from 12 animals
* = SC
** = IV
[a] = arithmetic mean
[h] = harmonic mean
[g] = geometric mean

Population Pharmacokinetics

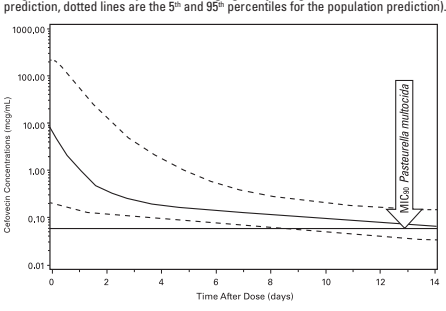
Dogs
Cefovecin plasma concentrations in the dog have been characterized by the use of population pharmacokinetic (PPK) data. Plasma cefovecin concentration data were pooled from 7 laboratory pharmacokinetic studies, each involving young, normal healthy Beagle dogs. The final dataset contained 591 concentration records from 39 dogs. The simulations from the model provide the mean population estimate and the 5th and 95th percentile of the population estimates of total and free cefovecin concentrations over time. Figure 2 shows the predicted free plasma concentrations following administration of 8 mg/kg body weight to dogs. Based upon these predicted concentrations, 95% of the canine population will have active (free) drug concentrations > the MIC_{90} of *S. canis* (0.06 μg/mL) for approximately 14 days and free concentrations > the MIC_{90} for *S. intermedius* (0.25 μg/mL) for approximately 7 days following a single 8 mg/kg subcutaneous injection of cefovecin. (See **MICROBIOLOGY**).

Figure 2: Population Predicted Free Concentration of Cefovecin in Plasma Following a Single Subcutaneous Injection of 8 mg/kg Body Weight in Dogs (solid line is population prediction, dotted lines are the 5th and 95th percentiles for the population prediction).



Cats
Cefovecin plasma concentrations in the cat have been characterized by the use of PPK data. Plasma cefovecin concentration data were pooled from 4 laboratory pharmacokinetic studies. The final dataset contained 338 concentration records from 22 cats. The simulations from the model provide the mean population estimate as well as the 5th and 95th percentile of the population estimates of total and free cefovecin concentrations over time. Figure 3 displays the predicted free plasma concentrations following administration of 8 mg/kg body weight to cats. Based upon these predicted concentrations, 95% of the feline population will have active (free) drug concentrations > the MIC_{90} of *Pasteurella multocida* (0.06 μg/mL) for approximately 7 days when administered a single 8 mg/kg subcutaneous injection of cefovecin. (See **MICROBIOLOGY**).

Figure 3: Population Predicted Free Concentration of Cefovecin in Plasma Following a Single Subcutaneous Injection of 8 mg/kg Body Weight in Cats (solid line is population prediction, dotted lines are the 5th and 95th percentiles for the population prediction).



MICROBIOLOGY: CONVENIA is a cephalosporin antibiotic. Like other β-lactam antimicrobials, CONVENIA exerts its inhibitory effect by interfering with bacterial cell wall synthesis. This interference is primarily due to its covalent binding to the penicillin-binding proteins (PBPs) (ie, transpeptidase and carboxypeptidase), which are essential for synthesis of the bacterial cell wall. For *E. coli*, the *in vitro* activity of CONVENIA is comparable to other cephalosporins, but due to the high-affinity protein-binding, the *in vivo* free concentration of cefovecin does not reach the MIC_{90} for *E. coli* (1.0 μg/mL). CONVENIA is not active against *Pseudomonas* spp. or enterococci.

Dogs
The minimum inhibitory concentration (MIC) values for cefovecin against label-claim pathogens isolated from skin infections in dogs enrolled in a 2001-2003 field effectiveness study are presented in Table 5. All MICs were determined in accordance with the Clinical and Laboratory Standards Institute (CLSI) standards.

Table 5: Activity of CONVENIA against Pathogens Isolated from Dogs Treated with CONVENIA in Field Studies in the US During 2001-2003.

Disease	Pathogen	Microbiological Treatment Outcome	Number of Isolates	Sample Collection (Time Relative to Treatment)	MIC_{50} μg/mL	MIC_{90} μg/mL	MIC Range μg/mL
Skin Infections	*Staphylococcus intermedius*	Success	44	Pre-Treatment	0.12	0.25	≤ 0.06 - 2
		Failure	4	Pre-Treatment			0.12 - 2
	Streptococcus canis (Group G)	Success	16	Pre-Treatment	≤ 0.06	≤ 0.06	≤ 0.06
		Failure	2	Pre-Treatment			≤ 0.06

Cats
The MIC values for cefovecin against *Pasteurella multocida* isolated from skin infections (wounds and abscesses) in cats enrolled in a 2001-2003 field effectiveness study are presented in Table 6. All MICs were determined in accordance with the CLSI standards.

Table 6: Activity of CONVENIA against Pathogens Isolated from Cats Treated with CONVENIA in Field Studies in the US During 2001-2003.

Disease	Pathogen	Microbiological Treatment Outcome	Number of Isolates	Sample Collection (Time Relative to Treatment)	MIC_{50} μg/mL	MIC_{90} μg/mL	MIC Range μg/mL
Skin Infections	*Pasteurella multocida*	Success	57	Pre-Treatment	≤ 0.06	≤ 0.06	≤ 0.06 - 0.12
		Failure	1	Pre-Treatment			≤ 0.06

EFFECTIVENESS:

Dogs
In a double-masked, 1:1 randomized canine field study conducted in the United States, the effectiveness of CONVENIA was compared to a cephalosporin active control. In this study, 320 dogs with superficial secondary pyoderma, abscesses, or infected wounds were treated with either a single injection of CONVENIA (n=157) at 3.6 mg/lb (8 mg/kg) body weight or with an oral active control antibiotic (n=163), administered twice daily for 14 days. In this study, dogs could receive a second course of therapy 14 days after the initial treatment. Of the 320 enrolled dogs, 22 of 157 dogs received 2 treatments of CONVENIA and 35 of 163 dogs received 2 courses of treatment with the active control. In the study, 118 of the 157 enrolled cases were evaluable for effectiveness for CONVENIA, and 117 of the 163 enrolled cases were evaluable for effectiveness of the active control antibiotic. CONVENIA was non-inferior to the active control. Table 7 summarizes the clinical success rates obtained 28 days after the initiation of the final course of therapy.

Table 7: Clinical Success Rates by Treatment Group 28 Days after the Initiation of the Final Course of Therapy.

Type of Infection	Dogs	
	CONVENIA (n=118)	Active Control (n=117)
Skin (secondary superficial pyoderma, abscesses, and infected wounds)	109 (92.4%)	108 (92.3%)

CONVENIA was administered concomitantly with other commonly used veterinary products such as heartworm preventatives, flea control products, sedatives/tranquilizers, anesthetic agents, routine immunizations, antihistamines, thyroid hormone supplementation, and non-steroidal anti-inflammatory drugs during the field study.

Cats
In a double-masked, 1:1 randomized cat field study conducted in the United States, the effectiveness of CONVENIA was compared to an active control. In this study, 291 cats with infected wounds or abscesses were treated with either a single injection of CONVENIA (n=147) at 3.6 mg/lb (8 mg/kg) body weight or with an oral active control antibiotic (n=144), administered once daily for 14 days. CONVENIA was non-inferior to the active control. The clinical success rates were obtained 28 days after the initiation of therapy and are presented in Table 8.

Table 8: Clinical Success Rates by Treatment Group 28 Days after the Initiation of Therapy.

Type of Infection	Cats	
	CONVENIA (n=89)	Active Control (n=88)
Skin (wounds and abscesses)	86 (96.6%)	80 (90.9%)

CONVENIA was used concomitantly with other commonly used veterinary products such as heartworm preventatives, flea control products, sedatives/tranquilizers, anesthetic agents, and vaccines during the field study.

ANIMAL SAFETY:

Dogs
CONVENIA administered to healthy 4-month-old dogs at doses of 12 mg/kg (1.5X), 36 mg/kg (4.5X), and 60 mg/kg (7.5X) every 7 days by dorsoscapular subcutaneous injections was well-tolerated for a total of 5 doses. Vomiting and diarrhea were seen in all treatment groups, with the incidence of vomiting and the incidence and duration of diarrhea increasing in a dose-related manner. Injection site irritation and transient edema occurred with increasing frequency in a dose-related manner and with repeat injections. Two injection site reactions included a seroma over the shoulder and swelling lasting > 30 days. Dogs dosed at 36 mg/kg had a significant (P=0.0088) increase in BUN (all means remained within the normal range) compared to the controls. One dog dosed at 60 mg/kg exhibited a glomerulopathy on histopathology, and 1 dog in this same group had minimal peliosis hepatis.

At an exaggerated dose of 180 mg/kg (22.5X) in dogs, CONVENIA caused some injection site irritation, vocalization, and edema. Edema resolved within 8-24 hours.

Cats
CONVENIA administered to healthy 4-month-old cats at doses of 12 mg/kg (1.5X), 36 mg/kg (4.5X), and 60 mg/kg (7.5X) every 7 days by dorsoscapular subcutaneous injections was well-tolerated for a total of 5 doses. Vomiting and diarrhea were observed in cats, with the incidence of vomiting and the incidence and duration of diarrhea increasing in a dose-related manner. The mean albumin values for all the CONVENIA-treated cats were significantly lower ($P \leq 0.05$) than the control values (all means remained within the normal range) for all time periods. The mean alkaline phosphatase values in the 60 mg/kg group were significantly higher ($P \leq 0.0291$) than the control values for all time periods. Injection-site irritation and transient edema occurred with increasing frequency in a dose-related manner and with repeat injections. One cat in the 12 mg/kg group had a mild renal tubular and interstitial fibrosis, and 1 cat in the 12 mg/kg group had mild glomerulosclerosis on histopathology.

At an exaggerated dose of 180 mg/kg (22.5X), CONVENIA was associated with injection site irritation, vocalization, and edema. Edema resolved within 8-24 hours. On day 10, cats had lower mean white blood cell counts compared to the controls. One cat had a small amount of bilirubinuria on day 10.

STORAGE INFORMATION:
Store the powder and the reconstituted product in the original carton, refrigerated at 2˚ to 8˚ C (36˚ to 46˚ F). **Use the entire contents of the vial within 56 days of reconstitution.** PROTECT FROM LIGHT. After each use it is important to return the unused portion back to the refrigerator in the original carton. As with other cephalosporins, the color of the solution may vary from clear to amber at reconstitution and may darken over time. If stored as recommended, solution color does not adversely affect potency.

HOW SUPPLIED:
CONVENIA is available as a 10 mL multi-use vial containing 800 milligrams of cefovecin as a lyophilized cake.

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January 2013
PAA035845A&P

ABSRACTS SCHEDULE

THURSDAY, MAY 3, 2018

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12:15 PM	**Dr. Andrea Wright:** Preliminary results from a mobile app monitoring canine pruritus and quality of life in dogs prescribed oclacitinib	111
12:30 PM - 2:00 PM	**LUNCH**	
2:00 PM	**WCVD Clinical Consensus Guidelines**	

Scientific Session Presentations

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Suppression of IL-31 receptor alpha isoform transcription in atopic dogs following acute allergen challenge

N. CRAIG*, K. AHRENS* and R. MARSELLA*

**Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Interleukin-31 (IL-31) is accepted as an important cytokine associated with the sensation of pruritus associated with atopic dermatitis in both dogs and humans. IL-31 signals via a receptor incorporating the cytokine-specific subunit IL-31 receptor alpha (IL-31RA). In dogs, four IL-31RA mRNA splice variants encode two protein isoforms: the full length isoform X1 and the truncatedisoform X2 which lacks the N-terminal signal peptide and parts of the cytokine binding domain. The aim of this study was to examine transcription of these two IL-31RA isoforms in the skin of atopic laboratory dogs following acute allergen challenge. Ten atopic laboratory beagles kept in low-allergen conditions were challenged epicutaneously with *Dermatophagoides farinae* to provoke moderate allergic inflammation. Skin biopsy samples were collected prior to challenge and at 3 h, 3 days and 10 days afterwards. Real-time PCR was used to examine IL-31RAisoform transcription. Assay specificity was confirmed by PCR product sequencing and melt curve analysis. Transcription of both IL-31RA isoforms wassignificantly reduced at 3 h and 3 days following allergen challenge ($P < 0.001$), but was not significantly different from baseline on day 10.Transcription of IL-31RA X2 was greater than that of IL-31RA X1 throughout the study ($P<0.005$). In this study transcription of both IL-31RA isoforms wasinversely correlated with clinical signs of allergic inflammation ($P<0.005$). Modulation of IL-31RA expression has also been demonstrated in human atopic keratinocytes. More studies are needed in dogs to understand the role of cytokines on receptor modulation.

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Conflict of Interest: None declared

Transcription of IL-31 in the skin of atopic beagle dogs following exposure to *Dermatophagoides farinae*

N. CRAIG*, K. AHRENS*, R. SANFORD*, R. MARSELLA*

**Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Interleukin-31 (IL-31) is proposed to play a role in atopic pruritus in both dogs and humans. Transcription of IL-31 mRNA has been reported to be higher in the skin of atopic dermatitis (AD) patients than healthy controls, and was increased in a canine model of acute house dust mite-induced AD lesions. We aimed to examine transcription of IL-31 in the skin of atopic dogs following allergen challenge. Ten atopic laboratory beagles were challenged with *Dermatophagoides farinae* allergen to provoke moderate allergic inflammation. Skin biopsy samples were collected prior to challenge and at 3 h, 3 days and 10 days afterwards, and real-time PCR was used to examine IL-31 mRNA transcription. Assay specificity was confirmed by PCR product sequencing and melt curve analysis. We found that levels of IL-31 mRNA in the skin samples were very low or at the limit of detection in many of the samples, particularly at baseline. On day 3, at the peak of the inflammatory response, transcription of IL-31 significantly correlated with clinical scores of dermatitis ($P = 0.033$; $r = 0.6$). In 3/10 dogs there was a marked increase in IL-31 transcription on day 3 compared to baseline. Due to the large variability this change was not statistically significant. Follow-up studies are required to confirm these findings in a larger number of privately owned atopic dogs with moderate to severe dermatitis. It is possible that the low detection at baseline in this colony may be linked to the minimal allergen stimulation at baseline and low staphylococcal colonization.

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Investigation on the effects of oclacitinib and lokivetmab, a caninized anti-IL-31 monoclonal antibody, on IL-31 serum levels in a colony of atopic beagles

R. MARSELLA,* K. AHRENS,* R. SANFORD,* A. TRUJILLO,* M. DORR*

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: IL-31 has attracted attention as mediator of pruritus in canine atopic dermatitis (AD) and is targeted in current therapies. We aimed to investigate: 1) IL-31 serum levels during allergen challenge and oclacitinib (Apoquel®) or lokivetmab (Cytopoint®); 2) correlation between serum IL-31 and pruritus scores in a colony of atopic dogs. Eleven atopic beagles were challenged with allergen (*Dermatophagoides farinae*) twice weekly for 28 days while randomly assigned to receive either oclacitinib (n=4, oral, 0.5 mg/kg twice daily for 2 weeks, then once daily for 2 weeks), or lokivetmab(n=4, subcutaneous, one injection, on day of first challenge, 2mg/kg) or no treatment (n=3). Blood was drawn on days 0, 14 and 28 to measure serum IL-31 using a validated ELISA for canine IL-31. Pruritus was scored weekly (pruritic acts recorded by cameras over a 30-minute period and global score [PVAS]). No significant change was detected overtime for serum IL-31 during the course of either therapy or in controls even if controls developed average 4.5 PVAS with some recordings of 10 PVAS. No correlation was found between pruritus and serum IL-31 on any day. In this pilot study, serum IL-31 does not appear to correlate with disease severity nor predict clinical response as the dogs in our study clinically responded to lokivetmab injection (0 PVAS on week 3 and 4) and to oclacitinib. It is speculated that tissue concentrations may be more relevant in dogs than serum levels. Larger studies are necessary to continue to evaluate these parameters in canine AD.

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Oclacitinib inhibits canine IL-4 and IL-13-activated JAK-STAT pathways in canine DH82 cells

C. JAVENS, S. DUNHAM, and A.J. GONZALES

Global Therapeutics Research, Zoetis Inc., Kalamazoo, MI, USA

Abstract: Cytokines produced from T-helper type II (Th2) lymphocytes are thought to contribute to the clinical signs of canine allergic skin disease (e.g. IL-4, IL-5, IL-13, and IL-31). These cytokines activate Janus kinases (JAK) and signal transducer and activator of transcription (STAT) pathways in cells. Oclacitinib is a selective Janus kinase (JAK)-1 inhibitor that potently inhibits the ability of canine IL-31 to activate STAT3. Previous reports demonstrated that oclacitinib could also inhibit IL-4 and IL-13 function; however human cytokines and cell systems were used in these experiments. The objective of this study was to determine the ability of oclacitinib to inhibit the function of canine IL-4 and IL-13 in the canine DH82 cell line. When the DH82 cells were treated with these cytokines, phosphorylation (activation) of STAT3, 5 and 6 were detected in cells using AlphaScreen technology (PerkinElmer). When DH82 cells were treated with varying concentrations of oclacitinib (0.0001_{μ}M-10_{μ}M), phosphorylation of each STAT was inhibited in a dose-dependent manner. The concentration at which 50% of the phosphosphorlated STAT signal (induced by IL-4 or IL-13) was inhibited (IC50) by oclacitinib varied depending on the STAT protein analyzed. Oclactinib most potently inhibited IL-4 and IL-13-induced STAT3 and STAT5 (IC_{50}'s = 51- 57nM) and was least potent at inhibiting IL-4 and IL-13-induced STAT6 (IC_{50}'s = 680 and 540 nM, respectively). These data demonstrate that oclacitinib can inhibit multiple STAT pathways in cells treated with IL-4 or IL-13, suggesting this activity may contribute to its efficacy in dogs with allergic skin disease.

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Conflict of Interest: All authors are employees of Zoetis Inc.

Evaluation of circulating interleukin-31 levels in cats with a presumptive diagnosis of allergic dermatitis

S. DUNHAM, J. MESSAMORE, L. BESSEY, S. MAHABIR, and A.J. GONZALES

Global Therapeutics Research, Zoetis Inc., Kalamazoo, MI, USA

Abstract: To assess whether interleukin-31 (IL-31) may play a role in feline allergic dermatoses, serum samples from cats with a presumptive diagnosis of allergic dermatitis (n=73) were collected and the circulating IL-31 concentration was compared to age-matched controls (n=17). Collection occurred at 10 independent clinics spread across the United States. Inclusion criteria for a presumptive allergic dermatitis diagnosis included cats with both flea and non-flea hypersensitivity disorders or allergic disorders of unknown origin. Additional inclusion criteria included the cessation of current treatments such as glucocorticoids or ciclosporin for at least 2 weeks prior to sample collection. The mean circulating IL-31 level was 8,798 fg/mL for cats with allergic dermatitis compared to 205 fg/mL in age-matched controls. These data demonstrate increased IL-31 levels in allergic cats and are suggestive that IL-31 plays a role in feline allergic disease; however, a causative role of IL-31 in feline allergic disease remains to be determined. A similar analysis performed in dogs with atopic dermatitis demonstrated that mean circulating levels in atopic dogs was 13,152 fg/mL compared 465 fg/mL in laboratory beagles.

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Panfungal PCR for identification of fungi in skin cytologic preparations

A. N. MYERS*, C. E. OLDER*, J. E. THOMAS†, A. DIESEL†, A. PATTERSON†, and A. RODRIGUES HOFFMANN*

**Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA*
† Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA

Abstract: Cytologic evaluation of skin lesions is a rapid, non-invasive method for diagnosis of fungal skin infections in animals. However, specific identification of the fungus is often difficult to determine cytologically. Panfungal polymerase chain reaction (PCR) followed by sequencing is a diagnostic tool that allows for specific identification of fungi in stained cytologic preparations. Here we present a clinical case where panfungal PCR on a cytology slide was used to identify the causative agent of a fungal skin infection. A 6-year-old, spayed female Boston terrier was presented with a single, crusted papule on the dorsal lumbar region. Skin impression cytology revealed numerous fungal hyphae. Definitive identification of the fungus was not possible cytologically. PCR targeting the internal transcribed spacer 2 region was performed on material scraped off the Diff-Quik-stained cytology slide, and resulting sequences matched *Microsporum gypseum* with 99% identity. This result allowed the clinicians to provide accurate information about this dermatophyte and more appropriate treatment recommendations to the owner. Panfungal PCR was recently performed on cytology slides from two additional cases where organisms were observed by light microscopy. In both cases, a specific etiology was identified: *Aspergillus fumigatus* in a case of canine mycotic rhinitis and *Histoplasma capsulatum* in a donkey liver. When fungal organisms are identified cytologically, panfungal PCR may provide an exact etiology more rapidly than culture and without more invasive diagnostics. Knowledge of the identity of the fungus may guide the choice of antifungal therapy and provide insight into the pathogenesis and epidemiology of the disease.

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Next-generation sequencing improves identification of fungal pathogens from fixed skin biopsies and enables identification of mixed infections

C. MEASON-SMITH*, A. RODRIGUES HOFFMANN*

**Dermatopathology Specialty Service, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA*

Abstract: Molecular methods have proven valuable for identification of fungi observed on histopathology due to subtle nuances in some fungal morphologies and the fastidious nature of fungal cultivation. A recent panfungal PCR assay on formalin-fixed and paraffin-embedded tissues using conventional sequencing was unable to identify 30% of fungi for which PCR was successful. We hypothesized the inability to sequence PCR product was due to heterogeneity of fungal sequences. The objective of this study was to employ panfungal next-generation sequencing (PNGS) on DNA previously extracted from 17 blocks containing skin biopsies for which conventional sequencing was unsuccessful. The 17 blocks contained skin from a variety of animals with various fungal morphologies on histology. PNGS was performed on an Illumina MiSeq instrument using ITS2 primers. Sequences were processed using bioinformatics software, QIIME, and an ITS sequence database. Heterogeneous populations of fungal DNA were identified in all 17 blocks. The histologically suspected fungus was sequenced in 11/17 blocks. Contaminating fungal DNA was found in 17/17 blocks reconfirming the need to interpret sequencing results in the context of histopathology. The two most common contaminants were *Malassezia* spp. and *Neosartorya* spp. PNGS improved the sensitivity of the conventional assay by 64%. This study is the first known example of successfully using PNGS on formalin-fixed tissues to identify mixed populations of fungi. PNGS will aid both veterinary dermatologists and pathologists in identification of emerging fungal pathogens as well as prompting future investigations into the mechanism and implications of mixed fungal infections of the skin.

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Dose-dependent pruritogenic effects of intradermal injections of histamine, compound 48/80 and anti-canine IgE in healthy dogs

T. DENLEY*, A. BLUBAUGH* and F. BANOVIC[v]

**Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, Athens, GA, USA*

Abstract: Scratching behaviors associated with intradermal injection of pruritogens such as histamine and compound 48/80 into the skin of mice and humans is the commonly used model to advance itch research and drug development. However, the predictive validity of this model is poorly documented in dogs. In the present study, we evaluated the dose-dependenteffects of established pruritogenic substances, each with a different mechanism of action, in 10 healthy beagle dogs. All dogs were video-recorded for 30 min after the intradermal injections of goat anti-canine IgE (4 and 25 µg/site), histamine and compound 48/80 (50, 100 and 200 µg/site); two buffered saline injections served as controls. All dogs showed wheal and erythema at the pruritogen injection site; global wheal scores at 30 min post-injection of each substance significantly increased at all concentrations compared to control (one-way ANOVA, $P \leq 0.05$). A blinded evaluation revealed that all pruritogens induced acute pruritic behaviors at the site of injection in 6/10 dogs. There was no injection site pain seen in any dog. Compared to controls, injections of pruritogens did not significantly affect the pruritic seconds or occurrence of pruritic episodes for any of the substances. There was no dose-dependent increase in pruritic behavior observed for any of the concentrations except for compound 48/80 (one-way ANOVA, $P = 0.11$). These preliminary results suggest that intradermal injections of studied pruritogens can induce itch and inflammation in healthy dogs; but inconsistencies occur in the induction of itch with different concentrations of pruritogen within the same dogs.

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Education level of US pet owners and readability of veterinary health information

K.C. TATER*

** Veterinary Information Network, Davis, CA, USA*

Abstract: Health education is important for changing behaviors and increasing adherence. Pet owners frequently consult online sources of veterinary health information, yet there are limited data on its readability and also whether it is consistent with pet owner education levels. To evaluate the education level of the US pet owning population, an analysis was performed on the questionnaire responses of 10,294 individuals in the National Health and Nutrition Examination Survey (NHANES), a nationally representative cross-sectional sampling. A subpopulation of 4,933 adults, representative of a population of 208,525,282, answered NHANES demographic and pet questions. The age adjusted prevalence of high school graduation for adult pet owners was 85.8% ± 1.33, and higher than that for adults without pets (78.5% ± 1.5, P< 0.0001). To evaluate the readability of veterinary health information, Flesch Reading Ease Scores and Flesch-Kincaid Grade Level Scores were calculated for allergy information at three veterinary health websites (28 articles) and compared to that at three human health websites (26 articles). Veterinary websites offered health information that was more difficult to read (P= 0.0052) and written at a higher grade level (P= 0.0047) than that at human health websites. The average veterinary health information readability score was 45.9 ± 8.7 ("difficult to read") and written at an 11th grade level or above (range: 8th grade - college level). Because literacy skills can lag behind education level, further studies are needed to evaluate reading comprehension of health information by veterinary consumers.

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Conflict of Interest: KT is an employee of the Veterinary Information Network, which owns the Veterinary Partner client education site.

Prescription dermatologic topicals used in the US population and their potential toxicity to dogs and cats

K.C. TATER*, S. GWALTNEY-BRANT*, T. WISMER†

** Veterinary Information Network, Davis, CA, USA*
† Animal Poison Control Center, Urbana-Champaign, IL, USA

Abstract: Dermatologic topical prescription medications can be licked off the pet owner and/or absorbed transdermally by the pet. To summarize the variety of dermatologic topical prescriptions potentially used by US pet owners, an analysis was performed on the questionnaire responses of individuals in the National Health and Nutrition Examination Survey (NHANES), a nationally representative cross-sectional sampling. The study sample consisted of 10,170 individuals from NHANES 2011-2014, representative of 311,065,381 US residents. Almost half (48.56 ± 0.78 %) of the US population reported using a prescription medication (range: 1-23 medications per individual). Prescription dermatologic topical medications were used by 1.33 ± 0.21% of the US population. Fifty different drug preparations were identified, with the ten most common containing triamcinolone, clobetasol, hydrocortisone, clotrimazole, lidocaine, prilocaine, clindamycin, estradiol, diclofenac, and betamethasone. The dermatologic topicals were categorized by their risk of toxicity, utilizing information from the published literature and animal poison control reports: low (minimal signs expected beyond gastrointestinal upset with single exposure); medium (some signs possible, generally reversible and not life-threatening); high (potentially irreversible and/or life-threatening signs possible e.g. seizures, organ failure, etc.). With ingestion by dogs or cats, 56% (28/50) had a medium or high risk of toxicity. With dog or cat topical exposure, 30% (15/50) had a medium or high risk of toxicity. Thus, pets in households with individuals on dermatologic prescription topicals may be at risk for toxicosis.

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Preliminary results from a mobile app monitoring canine pruritus and quality of life in dogs prescribed oclacitinib

A. WRIGHT*, C. ALAMAN*, R.HOLLAND*

** Zoetis, 10 Sylvan Way, Parsippany, NJ, USA*

Abstract: Oclacitinib (Apoquel®) is for treatment of allergic pruritus in dogs. A mobile app (PicorCan) activation code was given by veterinarians to 30 dog owners at the time oclacitinib was dispensed. Owners entered contact details and treatment start date and days of therapy (mean =11.8 days, R =2-30). Owners were prompted to choose emoticons (a representation of a facial expression from frowning to smiling on a scale of 1-5) for their dog and themselves, pruritus scores and Canine Dermatitis Quality of Life (QoL) and Treatment Satisfaction Questionnaire (CDQoL-TSQ) measuring QoL for themselves and their dog before and 24 h after treatment completion. Veterinarians accessed a portal (VetSupport+) to monitor the response to oclacitinib. Ninety percent (27/30) of owners started their surveys within 1 day. Twenty percent (6/30) of owners selected the human emoticon for themselves on day 0 increasing to 100% post-treatment, with an average score 4/5. One hundred percent (30/30) of dog owners selected the dog emoticon with an average initial score of 2/5, rising to 4/5 post treatment. The average pruritus score on a scale of 1-10 was 6.73 on day 0 decreasing to 3.13 post-treatment. The dogs' average calculated CDQoL-TSQ score before treatment was 54/100 and improved to 78/100 post treatment. The owners' CDQoL-TSQ score pre-treatment was 40/100 and improved to 54/100 post treatment. Preliminary results show that a mobile application is accepted by owners for monitoring pruritus and response to oclacitinib and has potential to enhance communication between veterinarians and dog owners.

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PEMPHIGOID DISEASES: FROM PATHOPHYSIOLOGY TO NOVEL THERAPEUTIC OPTIONS

Michael Kasperkiewicz, MD

Department of Dermatology, University of Lübeck, Lübeck, Germany

PEMPHIGOID DISEASES

Autoimmune bullous diseases are a heterogeneous group of disorders that can be subdivided according to the level of split formation in the intraepidermal blistering pemphigus diseases and subepidermal blistering pemphigoid diseases.

The pemphigoid group of diseases is characterized by subepidermal blisters due to autoantibody-induced disruption of the components of the dermal–epidermal anchoring complex. Pemphigoid diseases include bullous pemphigoid, mucous membrane pemphigoid, pemphigoid gestationis, linear IgA dermatosis, lichen planus pemphigoides, anti-p200 pemphigoid, and epidermolysis bullosa acquisita. In particular, disorders with anti-BP180 (type XVII collagen) reactivity, including bullous pemphigoid, pemphigoid gestationis, linear IgA dermatosis, lichen planus pemphigoides, and a subgroup of mucous membrane pemphigoid may form a continuous spectrum of subepidermal autoimmune blistering dermatoses. Bullous pemphigoid is not only the most common disorder within the pemphigoid group, but also represents the most frequent autoimmune blistering disease in general. It is a disease of the elderly, with a mean age of onset of 76 years. The incidence of bullous pemphigoid has been estimated between 4.5 and 14 new cases/million/year in central Europe. A higher incidence of 42.8/million/year has been reported in Great Britain based on a data registry established on the general practitioner level. The reported incidence in Germany and Great Britain has more than doubled in the past decade. This development may be due to the increasing age of the general population and/or an increased awareness leading to further diagnostic steps.[1-4]

By the use of different in vitro systems and experimental animal models, the pathogenic relevance of these autoantibodies has been demonstrated. Binding of pemphigoid autoantibodies leads to the separation of the epidermis and dermis by a complex, yet fairly well understood process.[1,2]

Pemphigoid diseases share some clinical characteristics, such as tense blisters and erosions and, by contrast with pemphigus, a negative Nikolsky sign—i.e., friction of nonlesional skin does not lead to intraepidermal disruption and visible erosion. The disorders are, however, heterogeneous with respect to overall clinical presentation, target antigens, and autoantibody isotype. Importantly, prognosis and treatment can vary substantially, so exact diagnosis is necessary. However, the different diseases cannot usually be distinguished on clinical grounds alone, so an assessment of skin-bound or mucous membrane-bound autoantibodies and serum autoantibodies is needed.[1,2,5]

For the correct diagnosis of pemphigoid diseases, the detection of tissue-bound autoantibodies and/or complement C3 by direct immunofluorescence microscopy as well as circulating autoantibodies is pivotal. Although direct immunofluorescence microscopy differentiates pemphigus from pemphigoid disorders, serological analyses are necessary to separate pemphigoid diseases from each other. In fact, while direct immunofluorescence microscopy is still the gold standard for the diagnosis of pemphigoid diseases, in the great majority of patients, diagnosis can be made serologically today.[1,2,5]

Conventional serologic diagnosis of autoimmune bullous diseases is usually performed as multistep procedure and includes an initial screening step by indirect immunofluorescence microscopy, followed by target antigen identification using enzyme-linked immunosorbent assay (ELISA) and/or immunoblotting. By indirect immunofluorescenc microscopy on 1M NaCl-split human skin, anti-laminin 332 mucous membrane pemphigoid, anti-p200 pemphigoid, and and epidermolysis bullosa acquisita can be distinguished from bullous pemphigoid, linear IgA dermatosis, pemphigoid gestationis, and anti-BP180 mucous membrane pemphigoid, but final diagnosis can only be made by more sophisticated methods, i.e. use of cell-derived or recombinant forms of the target antigens. Autoantibodies against the NC16A domain of BP180 (in bullous pemphigoid) are correlated with disease activity. The

corresponding ELISA is, therefore, suitable tests for monitoring disease activity over time and can be a useful aid in setting the optimal dose of the immunosuppressive medication used to treat the disease.[1,2,5]

To facilitate the diagnosis of pemphigoid diseases, new diagnostic tools have been developed in the recent years. A new automated direct immunofluorescence analysis has been shown to improve diagnostic accuracy and to save reagents. In addition, new serological analyses by both a biochip and multivariant profile ELISA have yielded high diagnostic accuracy similar to standard ELISA and are comparatively faster as well as easier to use (reactivity with different antigens at once).[6-8]

In the majority of pemphigoid disorders, disease activity can be sufficiently controlled by corticosteroids in combination with further immunosuppressants/immunomodulants such as dapsone, doxycycline, methotrexate, azathioprine, or mycophenolate mofetil. In some cases, however, treatment is challenging and only in a minority of patients, conventional immunosuppressive therapy induces clinical remission. The monoclonal anti-CD20 antibody rituximab, immunoadsorption, and high-dose intravenous immunoglobulins have been established as newer therapeutic options for treatment-refractory patients.[1,2,5,9]

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AUTOIMMUNE BLISTERING SKIN DISEASES – BASEMENT MEMBRANE AUTOIMMUNITY

Bizikova P

North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences, Raleigh, NC, USA

AUTOIMMUNE SUBEPIDERMAL BLISTERING DISEASES (AISBDs)

Introduction

Spontaneously occurring autoimmune subepidermal blistering diseases (AISBDs) were described in dogs almost 40 years ago.[7, 8] The common pathomechanism shared by these diseases is the autoimmune response against structural proteins of the dermo-epidermal junction. Six diseases have been recognized in dogs based on the clinical phenotype and targeted antigen (Table 1).[4] Some of these entities have been described in other animal species as well (Table 1). In contrast to people, the most common AISBD in dogs is mucous membrane pemphigoid (MMP; 48% of all AISBDs) followed by epidermolysis bullosa acquisita (EBA; 26% of all AISBDs). Bullous pemphigoid (BP), the most common AISBD in people is rarely seen in dogs (10% of all AISBDs).[4]

Table 1: Autoimmune subepidermal blistering skin diseases in dogs

Disease	Percentage of dog with other AISBDs*	Breed Predisposition	Age Predisposition	Characteristic Skin Lesions	Characteristic Lesion Distribution	Major Autoantigen	Minor Autoantigen	Histopathology	Other Species
Mucous Membrane Pemphigoid	48	German shepherd	middle-aged (median: 6 years)	tense vesicles (rare), deep erosions, ulcers, scarring, depigmentation	mucocutaneous junctions, mucosae	collagen XVII	laminin-332, BP230	subepidermal vesiculation without or with minimal inflammation (eutrophilic and/or eosinophilic)	human, cat
Epidermolysis Bullosa Acquisita (EBA)	26	Great dane	young (median: 1.2 years)	erythematous macules and papules; tense vesicles; deep erosions, ulcers	haired skin (footpads sloughing, friction areas) and mucosae / mucocutaneous junctions	collagen VII	nd	microscopic subepidermal vesiculation with variable degree of predominantly neutrophilic inflammation (intermixed eosinophils may be seen)	human
Bullous Pemphigoid	10	nd	middle-aged (median: 5 years)	erythematous macules and papules; tense vesicle; deep erosions, ulcers, crusts	haired skin predominant (concave pinnae, trunk (footpad sloughing not typical)) and mucosae/mucocutaneous junctions	collagen XVII	BP230	microscopic subepidermal vesiculation with variable degree of neutrophilic and/or eosinophilic inflammation	human, cat, horse, pig, (macaque?)
Junctional EBA	6	nd	nd	erythema, vesicles, deep erosions, ulcers and crusts	haired skin (footpads sloughing, friction areas) and mucosae / mucocutaneous junctions (similar to EBA)	laminin-332	nd	microscopic subepidermal vesiculation without inflammation or with variable neutrophilic and/or eosinophilic inflammation	human (different nomenclature)
Mixed AISBD	4	nd	nd	erythema, vesicles, deep erosions, ulcers and crusts	haired skin predominant (concave pinnae, trunk (footpad sloughing not typical)) and mucosae/mucocutaneous junctions (similar to BP)	collagen VII, laminin-332	nd	microscopic subepidermal vesiculation with mixed neutrophilic and eosinophilic inflammation	human (different nomenclature)
Linear IgA Disease (LAD)	3	nd	nd	erythematous papule, erosions, ulcers and crusts	mucosae (oral cavity) and haired skin (ears, nasal planum, extremities)	collagen XVII (secreted)	nd	microscopic subepidermal vesiculation without or with minimal neutrophilic inflammation	human
Bullous Systemic Lupus Erythematosus	1	nd	nd	erythema, vesicles, deep erosions, ulcers and crusts	haired skin, footpads and mucosae/mucocutaneous junctions	collagen VII	nd	microscopic subepidermal vesiculation without inflammation or with variable, predominantly neutrophilic inflammation	human

* Reference [4]; nd - not determined (due to insufficient number of reported cases); major autoantigen - antigen recognized by more than 50% of affected individuals

The mechanism of a blister formation is complex and, depending on the type of disease, the process involves antibodies (IgG and/or IgE and/or IgA), complement and/or various components of the immune system (neutrophils, eosinophils, mast cells, etc.). While the target autoantigens in AISBDs have been identified in dogs and, to some extent, in other species, little is known about the pathomechanism(s) of these diseases in domestic animals (antigen summarized in [4]). Moreover, a commercially available array of antigen-specific immunoserological tests that could assist in distinguishing some of the diseases from each other does not exist in veterinary medicine. Therefore, the diagnosis of a particular AISBD in veterinary dermatology relies on a detailed clinical assessment and histopathology.[4]

Detection of tissue-bound anti-BMZ antibodies or serum anti-BMZ antibodies, a test also not commercially available but fairly simple to perform, is unable to confirm the definitive diagnosis reliably, though it could inform us about the depth of the targeted antigen to some extent. Together with the clinical phenotype and histopathology, this information could further increase our confidence in the diagnosis made based on clinical features and histopathology. However, the diagnosis shall never be made based on a blood test only.[4]

The text below summarizes the most common AISBDs in dogs. A note will be made at the end of each section to indicate other species in which the particular disease was recognized. In the lecture, treatment approach will be discussed for each particular disease.

1. MUCOUS MEMBRANE PEMPHIGOID (MMP)

Epidemiology and signalment

Mucous membrane pemphigoid is the most common AISBD recognized in dogs (48% of all AISBDs).[4, 9] The disease occurs equally between females and males, and German shepherd dog and its crosses appear to be overrepresented (18/58 dogs; 31%).[9, 10] The median age of onset is 6 years with most dogs developing MMP during their mid-adulthood (4-7 years; 50%) or at older age (≥ 8 years; 25%).[9, 10]

Clinical signs

The current knowledge about the clinical aspect of canine MMP is based on 50 cases[9, 10] Canine MMP affects primarily mucosa and mucocutaneous junctions in which the primary, though transient lesion is a vesicle and/or bulla. Rupture of these lesions leads to deep erosions and/or ulcers, found in most dogs (98%), often in a bilaterally symmetric pattern. Scarring is not reported frequently in dogs (16%), possibly due to the frequent oral cavity involvement (62%), which, like in people, is infrequently accompanied by obvious scarring signs.[11] Other frequently affected areas in dogs include nasal planum (34%), ocular/periocular area (20%), genital/perigenital area (16%) and concave ear pinnae (16%). About half of the reported cases exhibited systemic signs, like lethargy, as well as pain with eating, halitosis and hypersalivation. A loss-of-function of affected organs due to chronic scarring, as described in people, was not reported in dogs.[10, 11]

Histopathology

Histopathological features of canine MMP are similar to that reported in people.[11] Subepidermal or submucosal vesicles, if not ruptured, were usually devoid of inflammation.[10] Dermal and submucosal inflammation is variable and mild to moderate amount of both neutrophils (73%) and eosinophils (55%) can be seen below the vesicles or intact epithelium.[10] Rowing of individual neutrophils and/or histiocytes along the basement membrane was less common than that seen in canine epidermolysis bullosa acquisita.[10, 12]

Immunopathology

Most dogs affected with MMP possessed tissue-bound autoantibodies, predominantly IgG (92%), and complement C3 (63%) deposited along the basement membrane zone. Circulating autoantibodies, predominantly IgG and less frequently IgE, could be detected using salt-split canine buccal mucosa tissue in 71% and 32% of dogs, respectively.[9, 10] Like dogs, people affected with MMP, especially those with lesions confined to the oral cavity, do not always possess detectable anti-basement membrane autoantibodies.[11] Similarly to people, canine MMP has been shown to be immunologically heterogeneous with autoantibodies targeting proteins of the basement membrane such as collagen XVII, BP230 or laminin-332.[9] Autoreactivity against other basement membrane proteins such as alpha6/beta4 integrin, laminin-311 or collagen VII, as seen in some affected people, has not been confirmed in dogs yet.

Summary

Canine MMP is a naturally occurring chronic and recurrent AISBD that preferentially affects mucosae and mucocutaneous junctions. Although a fairly rare disease, MMP is the most common AISBD in dogs and it represents a disease homologue to its human counterpart.

Other animal species:

A naturally occurring MMP has been described in two cats (one of the two cats from an older publication on bullous pemphigoid (case #1) fits clinically, histopathologically and immunologically for MMP).[13, 14] Both cats exhibited vesicles and/or erosions and ulcers on mucosae and mucocutaneous junctions (eyelids (1), lips (2), soft palate (1)), and

concave pinnae (2). Histopathology revealed dermo-epidermal separation with none to minimal dermal inflammation composed of dendritic/histiocytic cells and occasional neutrophils and eosinophils.[13, 14] Immunotesting revealed autoantibodies targeting collagen XVII in one and laminin-332 in another cat.[13, 14]

2. EPIDERMOLYSIS BULLOSA ACQUISITA (EBA)

Epidemiology and signalment

Epidermolysis bullosa acquisita is the second most common AISBD in dogs (26% of all AISBDs).4 Most affected dogs were young (median: 1.2 years) males (male:female = 2.3:1) with lesions developing before one year of age in almost half of them (45%).[12] Interestingly, while childhood EBA has been recognized in people, this disease affects mostly people in the fourth to fifth decade of their lives.[15, 16] In the largest case series of canine EBA, great danes were overrepresented (55%).[12]

Clinical signs

The current knowledge about the clinical aspect of canine EBA is based on 20 cases.[12] Like in other AISBDs, characteristic skin lesions seen in dogs with EBA were tense vesicles and bullae (90%) progressing with time into deep erosions and ulcers (100%). Additional lesions included erythematous macules and patches (75%) or papules and wheals (40%). Lesions were usually detected in the oral cavity (95%), concave pinnae (80%) and glabrous skin of groin and axillae (50% and 75%, respectively). In contrast to dogs with MMP and BP, dogs with EBA often exhibited footpad sloughing (70%). Pruritus and pain have been reported in 38% and 85% of affected dogs, respectively, and systemic signs such as fever, lethargy, lymphadenopathy and anorexia were seen in most cases (94%).

Histopathology

Detailed histopathological description can be found for 17 dogs with EBA.[12] Like in other AISBDs, dermo-epidermal separation leading to vesicle formation was a typical finding in EBA. Formed vesicles were devoid of any inflammation (76%) or contained variable numbers of neutrophils (94%). In some dogs, eosinophils were seen intermixed with neutrophils (41%). In early lesions, small subepidermal vacuoles as well as rowing of neutrophils and/or histiocytes were seen along the basement membrane zone. Superficial, dermal perivascular to interstitial inflammation composed of neutrophils (100%) was frequently seen. A low number of eosinophils intermixed among neutrophils was seen in 71% of dogs.

Immunopathology

Most dogs affected with EBA possessed tissue-bound autoantibodies, predominantly IgG (81%), deposited along the basement membrane zone. Circulating autoantibodies, predominantly IgG and less frequently IgE, could be detected using salt-split canine buccal mucosa tissue in 90% and 21% of dogs, respectively.[12] Similarly to people, sera from dogs with EBA contained autoantibodies targeting the NC1 domain of collagen VII.[17]

Summary

Canine EBA is a naturally occurring AISBD with collagen VII autoreactivity affecting mucosae as well as haired skin, especially friction and pressure areas. This disease is a clinical, histopathological and immunological homologue of the human EBA.

Other animal species:

A naturally occurring EBA has not been reported in any other animal species.

3. BULLOUS PEMPHIGOID (BP)

Epidemiology and signalment

In contrast to people, BP is rarely seen in dogs (10% of all AISBDs).4 While older reports of canine BP can be found in the veterinary literature[8, 18-23], many of these cases would be today given a different diagnosis such as MMP, EBA or vesicular cutaneous lupus erythematosus after applying the current clinicopathological criteria for these diseases. The first well-characterized canine BP case dates to mid 1990s.[24] Because of the rarity of this disease and the small number of reported cases, a statement about breed- or sex-predilection cannot be made. The disease usually starts, like many other autoimmune diseases, in the middle adulthood (median age of onset = 5 years).[25]

Clinical signs
The only available case series of seven dogs provided insight about the clinical picture of canine BP.[25] In these dogs, skin lesions consisted of erythematous macules, patches or plaques (4/7; 57%), tense vesicles or bullae (3/7 dogs; 43%) erosions or ulcers (6/7; 86%), as well as crusts (5/7; 71%). Lesions were present on the back (4/7; 57%), pinnae (4/7; 57%), axillae (2/7; 29%), and abdomen (2/7; 29%). Footpads were rarely affected in dogs with BP (1/7; 14%), a contrasting finding to that observed in dogs with EBA. Lesions at mucosal or mucocutaneous junction sites were detected in four dogs (57%) and involved predominantly the oral cavity (3/7; 43%) and lip margins (4/7; 57%).[25]

Histopathology
Like in other AISBDs, dermo-epidermal separation leading to vesicle formation was a typical finding in BP. Formed vesicles contained variable numbers of neutrophils (71%) and eosinophils (67%). Similar inflammatory infiltrate was detected in the upper dermis. In some dog, numerous degranulated mast cells were depicted in the upper dermis.[25]

Immunopathology
Three (75%) and two (50%) of the four tested dogs with BP possessed tissue-bound IgG and IgM autoantibodies, respectively, deposited along the basement membrane zone. Tissue-bound complement (C3) was seen in one of the four tested dogs (25%).25 Circulating autoantibodies, predominantly IgG, could be detected using salt-split canine buccal mucosa tissue in 100% of dogs with BP.[26] Similarly to people, sera from dogs with BP contain autoantibodies targeting the NC16A domain of collagen XVII.[24, 25, 27]

Summary
Canine BP is a naturally occurring AISBD with collagen XVII autoreactivity affecting predominantly haired skin. Mucosal and mucocutaneous junction involvement is seen in about 50% of dogs with BP and footpad sloughing, in contrast to EBA, is only a rare feature. This disease is a clinical, histopathological and immunological homologue of the human BP.

Other animal species:
A naturally occurring BP has been described in cats[13], pigs[28], horses[29] and, possibly, in a rhesus macaque.[30]

In cats, lesions of BP appear to be of minimal severity, with vesiculation and erosions occurring predominantly on the ears, trunk and extremities. Mucosal involvement can be seen, but appears to be mild. Like in people and dogs, the BP affected cat produced IgG against NC16A domain of collagen XVII. [13]

In Yucatan minipigs, clear to hemorrhagic tense vesicles progressing rapidly to erosions and ulcers were seen predominantly on the dorsum. In some pigs, erythema preceded vesicle formation. Mucosal involvement was usually not present. Similarly to other described species, sera from these pigs contained IgG against the NC16A domain of collagen XVII.[28]

In horses with BP, vesicles appeared suddenly and progressed rapidly into erosions and ulcers covered with crusts. The lesions were widespread with especially prominent oral ulceration. Systemic signs such as lethargy and anorexia accompanied the skin lesions. Euthanasia was elected for humane reasons due to the severity of their disease. Sera from horses with BP contained IgG against the NC16A domain of collagen XVII.[29]

In the single case of *macaque rhesus* with BP (an animal undergoing experimental pancreatic transplantation), tense, clear vesicles appeared on the nipples, shoulders and scalp. No mucosal lesions were reported. The dermo-epidermal separation was above the PAS stained lamina densa, and direct immunofluorescence detected anti-BMZ IgG bound to the roof of the blister. The animal showed spontaneous resolution of clinical signs in two weeks.[30]

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PEMPHIGOID DISEASES: FROM PATHOPHYSIOLOGY TO NOVEL THERAPEUTIC OPTIONS

Michael Kasperkiewicz, MD

Department of Dermatology, University of Lübeck, Lübeck, Germany

PEMPHIGOID DISEASES

Autoimmune bullous diseases are a heterogeneous group of disorders that can be subdivided according to the level of split formation in the intraepidermal blistering pemphigus diseases and subepidermal blistering pemphigoid diseases.

In pemphigus diseases, the autoantibodies are directed against desmosomal proteins, and cell contact within the epidermis is lost. Pemphigus can be classified into three major forms: pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic pemphigus. Pemphigus vulgaris and pemphigus foliaceus are the originally characterized, classic forms of pem¬phigus. Pemphigus vulgaris is the most common subtype of pemphigus in Europe, the United States, and Japan. It preferentially affects women, and most of the patients are 50–60 years of age at disease onset. Incidences range from 0.5 cases per million population in Germany to 50 cases per million in Iran. Pemphigus foliaceus is the most common type observed in South America and North Africa owing to the endemic form. Paraneoplastic pemphigus is a rare pemphigus variant distinguished by the presence of a known-associated or occult-associated neoplasm, usually of lymphoid tissue.[1,2]

In pemphigus, IgG autoantibodies are characteristically directed against desmogleins (desmoglein 1 and desmoglein 3), which are part of the cadherin family of cell–cell adhesion molecules that are found in desmosomes, which are the structures primarily responsible for maintaining intercellular adhesion in stratified squamous epithelia, such as the skin and oral mucosa. The sites of blister formation can be physiologically explained by the anti-desmoglein autoantibody profile and tissue-specific expression pattern of desmoglein isoforms. The pathophysiological roles of T cells and B cells have been characterized in mouse models of pemphigus and patients, revealing insights into the mechanisms of autoimmunity.[2,3]

In pemphigus, since cleavage occurs within the epidermis, blisters have a relatively thin roof and are loose and fragile. Thus, skin erosion, rather than blistering, tends to be the predominant finding in pemphigus vulgaris and the nearly exclusive finding in pemphigus foliaceus. The main difference between these two conditions, however, lies in the degree of involvement of the mucous membranes. In pemphigus vulgaris, the mucous membranes are always involved, while the skin may or may not be affected. In pemphigus foliaceus, the mucous membranes always remain normal. Paraneoplastic pemphigus is characterized by the associated neoplasia, marked stomatitis, and polymorphic skin changes: not just blisters and erosions, but also lichen ruber-like plaques and pustules.2,3

The current gold standard of diagnostic testing for pemphigus diseases is direct immunofluorescence microscopy to demonstrate tissue-bound autoantibodies and/or complement C3 in the patient's skin or mucous membranes.[2,3]

Indirect immunofluorescence microscopy of the patient's serum can be used as a screening test for circulating antibodies. Indirect immunofluorescence microscopy on monkey or guinea pig esophagus has become an established mode of testing for serum antibody in pemphigus. For the diagnosis of pemphigus vulgaris and pemphigus foliaceus, sensitive and specific commercial enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies against desmoglein 1 and 3 are available. The great majority of patients with paraneoplastic pemphigus show reactivity to envoplakin and/or periplakin, which can be detected by immunoblotting with extract of cultured human keratinocytes, or else in a recently developed ELISA. Autoantibodies against desmoglein 1 (in pemphigus foliaceus) and desmoglein 3 (in pemphigus vulgaris) are correlated with disease activity. The corresponding ELISAs are, therefore, suitable tests for monitoring disease activity over time and can be a useful aid in setting the optimal dose of the immunosuppressive medication used to treat the disease.[2,3]

To facilitate the diagnosis of pemphigus diseases, new diagnostic tools have been developed in the recent years. A new automated direct immunofluorescence analysis has been shown to improve diagnostic accuracy and to save reagents. In addition, new serological analyses by both a biochip and multivariant profile ELISA have yielded high diagnostic accuracy similar to standard ELISA and are comparatively faster as well as easier to use (reactivity with different antigens at once).[4-6]

Corticosteroids in combination with corticosteroid-sparing immunosuppressive agents remain the first-line therapy for pemphigus. Azathioprine and mycophenolate mofetil are most frequently used as corticosteroid-sparing agents. As our understanding of pemphigus diseases has progressed within recent years, the therapeutic arsenal has been extended by newer drugs and interventions, which have changed the prognosis of these diseases. These include the monoclonal anti-CD20 antibody rituximab, immunoadsorption, and high-dose intravenous immunoglobulins.[2,3,7]

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DESMOSOME AUTOIMMUNITY

Bizikova P

North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences, Raleigh, NC, USA

MECHANISM OF BLISTER FORMATION:

An intact skin is a critically important organ that functions as a first-line defense mechanism against physical and chemical damage. Its integrity is dependent on complex structures maintaining cell-cell and cell-matrix adhesion.[1,2] Several autoimmune skin diseases disrupting this cohesion have been recognized. The mechanism by which this adhesion is disrupted varies depending on the type of disease.

a. *Disruption of basement membrane adhesion* – subepidermal blister formation due to dermo-epidermal separation (bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), epidermolysis bullosa acquisita (EBA), linear IgA disease (LAD), mixed autoimmune subepidermal blistering disease (mixed AISBD))
b. *Disruption of desmosome adhesion* – intraepidermal blister formation due to desmosome dissociation (pemphigus foliaceus (PF), pemphigus vegetans (PVeg), pemphigus vulgaris (PV), paraneoplastic pemphigus (PNP))
c. *Keratinocyte injury and interface dermatitis* – keratinocyte-targeting diseases causing disruption of epidermal cohesion (cutaneous lupus, erythema multiforme complex, etc.)

DISRUPTION OF DESMOSOME ADHESION

PEMPHIGUS DISEASES

Introduction

Veterinarians have been aware of the existence of a naturally occurring pemphigus in domestic animals for decades.[3,4] Canine pemphigus, like in people, encompasses four variants: pemphigus foliaceus (PF), pemphigus vulgaris (PV), pemphigus vegetans (PVeg) and paraneoplastic pemphigus (PNP).[5] Pemphigus erythematosus, a facial-restricted variant of pemphigus foliaceus, is an entity characterized by subcorneal acantholysis accompanied by a lichenoid interface dermatitis.[6] Pemphigus foliaceus is the most common pemphigus variant in dogs, while PV, the most common variant in people, is seen only rarely.[6,7]

Due to the time-constrain, this lecture will provide an update on selected topics on desmosomal autoimmunity in domestic animals with focus on the most common autoimmune skin disease in all domestic species – the PF. We will discuss the pathomechanism of this disease as well as clinically relevant topics such as i) acantholysis in pemphigus and pyoderma, ii)atypical PF or pyoderma?, iii) high-dose pulse therapy in canine and feline PF, and others.

Specific diseases and their main characteristics are summarized in Table 1.

Table 1: Pemphigus diseases in domestic animals and people

	Disease	Affected Species	Over-represented Breeds	Age Predisposition	Characteristic Skin Lesions	Characteristic Lesion Distribution	Target Autoantigen	Histopathology	Human Counterpart (major antigen)
Superficial Pemphigus	Pemphigus Foliaceus (PF)	dog	chow-chow, akita	middle-aged (median: 6 years)	pustules (rare in cats and horses), shallow erosions, thick crusts, scale-crusts (common in horses), alopecia	face (nasal planum, dorsal muzzle, eyelids), concave pinnae, footpads; no mucosal involvement	DSC1 (major), DSG1	subcorneal epidermal or follicular (infundibulum) pustules rich in neutrophils (eosinophils may be present) containing acantholytic keratinocytes	PF (DSG1)
		cat	nd (DSH most common)	middle-aged (median: 6.5 years)		concave pinnae, face (nasal planum, dorsal muzzle, eyelids), limbs; no mucosal involvement	nd		
		horse	nd	median: 7 years		concave pinnae, face, neck, limbs, ventrum (sheath in males); no mucosal involvement			
		small ruminants	nd	young-aged (median: 1.5 years)		face, trunk limbs, perineum and tail (udder and teats in females)			
Deep Pemphigus	Pemphigus Vulgaris (PV)	dog	German shepherd, collie	middle-aged (median: 6 years)	flaccid vesicle, deep erosions and ulcers	mucosae and mucocutaneous junctions (oral cavity frequent) with or without haired skin involvement	DSG3, DSG1 (in muco-cutaneous form)	suprabasal acantholysis and clefting	PV (DSG3, DSG1)
		cat	nd	median: 5 years			nd		
		horse	nd	nd			DSG3, DSG1		
		macaque	nd	nd		haired skin only (friction areas, pressure points)	nd		
	Pemphigus Vegetans	dog	nd	nd	mucosal erosions and hyperplastic/verrucous lesions on haired skin (Hallopeau-type Pveg)	oral cavity, prepuce and anus + haired skin (pinnae, trunk, feet)	DSG1	hyperplastic epidermis with neutrophils and eosinophilic acantholytic pustules at multiple epidermal layers + suprabasal (PV-like) acantholysis	human (DSG3; also DSG1, DSC)
	Paraneoplastic Pemphigus	dog	nd	nd	flaccid vesicles, deep erosions and ulcers	mucosae and mucocutaneous junctions + haired skin	DSG3 + plakins (e.g. evoplakin, periplakin)	suprabasal acantholysis and clefting and single cell apoptosis at multiple layers of the epidermis	human (DSG3, plakins; DSG1, etc.)
		cat	nil	nd		haired skin, concave pinnae	nd		

Abbreviations: nd - not determined (due to insufficient number of reported cases); DSG - desmoglein; DSC - desmocollin; major autoantigen - antigen recognized by more that 50% of affected animals

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CORTICOSTEROIDS

Papich, M.G.

College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

ANTI-INFLAMMATORY MECHANISMS

Glucocorticoids are the most consistently effective drugs available for the treatment of various forms of inflammation in animals. However, their potent anti-inflammatory effects and immunosuppressive actions must be balanced by the multiple side-effects produced by these drugs.

Corticosteroids are bound to corticosteroid-binding globulins in the blood. These protein bound forms circulate in the blood. Only 10% (approximately) is unbound. The free cortisol is the active form When corticosteroids reach the cell, they bind to the glucocorticoid receptor (GR)[1,2] . Glucocorticoids bind with high affinity to the receptor, and the GR is found in almost all tissues of the body. Once in the cell, glucocorticoids bind to receptor sites on responsive genes where they modulate the transcription of glucocorticoid-responsive genes[1-4] .Their action occurs in three ways: (1) The bound receptor moves to the nucleus of the cell where it binds directly to DNA glucocorticoid-responsive elements. This action regulates transcription, with the effects largely dependent on what cell type is affects. (2) The bound glucocorticoid-receptor complex regulates other responsive genese to inhibit transcription factors such as activator protein-1 (AP-1) and nuclear factor kappa-B (NF-κB). These effects may be the most important to suppress inflammatory reactions. (3) The third effect is through non-genomic pathways that regulate membrane-associated and second messenger pathways.

Transactivation vs Transrepression:

The ability of glucocorticoids to suppress inflammatory mechanisms through, for example inhibition of AP-1 and NF-κB, effectively inhibits proinflammatory factors such as cytokines (IL-6, IL-1β, and TNF-α), enzymes (COX-2, iNOS, MMP), and other mediators. This process is referred to as *transrepression* and is generally considered the beneficial property of these agents. On the other hand, the multiple adverse effects and side effects from glucocorticoids arise through the action of the GR to activate genes involved in regulation of sugar, fat, protein, muscle, and bone. This process is referred to as *transactivation*. An ideal anti-inflammatory corticosteroid would be one that can produce the transrepression effects while minimizing transactivation. Work continues to achive this goal [2].

ANTI-INFLAMMATORY EFFECTS

Leukocytes:

Corticosteroids increase the circulating numbers of mature neutrophils. There is a release of cells from the marginal pool of neutrophils and decreased migration and egress into inflammatory tissue. This effect is attributed to decreased expression of adhesion molecules, reduced adherence to the vessel endothelium, and reduced diapedesis from the vessels. Subsequently, there is decreased movement of neutrophils into tissues in response to chemotactic stimuli. Glucocorticoids affect leukocyte traffic more than cellular function. At anti-inflammatory doses, glucocorticoids have little effect on lysosomal stability and phagocytosis.

Eosinophils:

Glucocorticoids inhibit migration of eosinophils into inflamed sites. Glucocorticoids also decrease circulating numbers of eosinophils and basophils.

Macrophages:

Glucocorticoids suppress the function of macrophages. In macrophages, they decrease the inflammatory response, generation of cytokines, and the ability to process antigens. They can produce downregulation of Fc-receptors on macrophages, which inhibits phagocytosis function of macrophages.

Lymphocytes:

Corticosteroids decrease the numbers of lymphocytes in the peripheral circulation – caused by a redistribution of circulating lymphocytes – depress lymphocyte activation, and decrease participa-tion of lymphocytes in inflammation. T-cells are affected more than B-cells. B-cells are generally resistant to the immunosuppressive effects

of glucocorticoids, and there is minimal effect on immunoglobulin synthesis. However, high corticosteroid doses may decrease immunoglobulin levels, probably because of increased catabolism, as well as through the secondary effects to suppress accessory cells (Th-2 cells) and cytokine synthesis. At anti-inflammatory doses, glucocor-ticoids do not decrease an animal's ability to mount a normal immune response (eg, from vaccinations).

Effects on cytokines:
Glucocorticoids inhibit release of inflammatory cytokines from leukocytes (eg, IL-1, TNF-α, prostaglandins), and decrease expression of cytokines from lymphocytes (eg, IL-2).

Effects on vessels:
Corticosteroids improve microvascular integrity, decrease vessel permeability, and improve microvascular circulation. Some of the stabilizing effect on vessels may be attributable to an antagonism of vasoactive substances (eg, histamine, 5-HT), or decreased synthesis of inflammatory mediators (eg, prostaglandins, TNF-α).

Effects on arachidonic acid metabolism:
Corticosteroids can inhibit prostaglandin production by 3 mechanisms. They inhibit the synthesis of the isoform of cyclo-oxygenase (PG synthase-2, also called COX-2) responsible for prostaglandin synthesis during inflammation. Corticosteroids also may produce a dual blockade of the arachidonic acid cascade, through an indirect mechanism inhibiting phospholipase A_2 (PLA_2), resulting in lower concentrations of prostaglandins, prostacyclin, thromboxane, and leukotrienes. Despite this evidence, it is not fully understood if the glucocorticoid effect on arachidonic acid metabolism, prostaglandin synthesis inhibition, or leukotriene metabolism is clinically relevant5.

DIFFERENCES AMONG GLUCOCORTICOIDS
Structure-activity relationships can explain the differences among corticosteroids with respect to mineralocorticoid effects, anti-inflammatory potency, and duration. A double bond between C_1 and C_2 are essential for anti-inflammatory and glucocorticoid activity. Methylation at C_6 decreases the mineralocorticoid effects (methylprednisolone) and a fluorine at C_9 greatly increases the anti-inflammtory potency (dexamethasone). The addition of the acetonide group to triamcinolone greatly increases the anti-inflammatory potency to 5.5 x more than methylprednisolone [6].

The potency and duration of action for exogenous corticosteroids can be compared to cortisol, but assigning cortisol (the natural glucocorticoid) a value of 1, and raning the anti-inflammatory of the others by comparison (**Table 1**). Thus, prednisolone has a potency of approximately 4, and dexamethasone approximately 25-30, compared to cortisol. Generally, doses administered to patients account for this difference in potency.

How were these potencies derived? The original ranking of potencies were derived from 4 rather crude tests performed in rats in the 1960s[7]. These four tests are (1) degree of involution of the thymus gland, (2) measurement of the size of a granuloma from an implanted irritant in a rat, (3) suppression of inflammatory exudate in subcutaneous ouches after injection of an irritant, and (4) measurement of edema in the paw of a rat after injection of an irritant. Other tests can be used to measure the gluconeogenic effects (measurement of glucose and glycogen). The potencies listed in various textbooks (eg, Table 1) reflect the results of these studies. Today more sophisticated methods can be used to measure relative potency of corticosteroids[8]. The lymphocyte proliferation assay, and the whole blood modification of the assay is one of the most widely used. These studies show that the potency of glucocorticoids is directly related to the relative binding affinity (RBA) of the corticosteroid to the cytosolic glucorticoid receptor.

CLINICAL USE
Planning Corticosteroid Therapy
For short-term therapy (less than two weeks), glucocorticoids can be administered daily at anti-inflammatory doses without serious long-term side effects. If long-term therapy is not needed, the medication can be discontinued abruptly with little chance of adverse effects caused by adrenal suppression or abrupt withdrawal. For long-term, chronic therapy, glucocorticoid doses should be titrated to the lowest effective dose and, if possible, administered every other day (EOD).

Choice of a Corticosteroid

Prednisolone, prednisone, or methylprednisolone are the most common choices because they are intermediate-acting steroids and can be used on an every-other-day (EOD) schedule. Initial (induction)

Comparison of Corticosteroid Bases			
Drug	**Duration of Action (hr)[a]**	**Comparative potency[b]**	**Mineralocorticoid effects**
hydrocortisone (cortisol)	8-12	1	2
prednisolone	12-36	4	1
prednisone	12-36	4	1
methylprednisolone	12-36	5	2
triamcinolone	12-36	5	0
flumethasone	32-48	15	0
dexamethasone	32-48	25	0
triamcinolone acetonide	Not reported	25	0
betamethasone	32-48	25	0
deoxycorticosterone (DOCA)	0	0	200
fludrocortisone (Florinef)	Not applicable	10	250

(Table modified from: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11h Ed. McGraw-Hill)
[a]based on duration of HPA-axis suppression.
[b]Potency is listed by arbitrarily assigning cortisol with a potency of 1.0 and as the value increases, so does the potency.
[c]*Note the difference between triamcinolone and triamcinolone acetonide (Vetalog)*

dosages (prednisone or prednisolone) for anti-inflammatory activity are approximately 0.5-1 mg/kg/day for 5 to 10 days, then the dose is gradually lowered (tapered) to a lower dose every-other-day for another 5 to 10 days and eventually to 0.5-0.3 mg/kg, EOD. These are common anti-inflammatory maintenance dosages, although some patients may respond to higher or lower dosages. For example, some animals with inflammatory skin diseases (eg, atopic dermatitis) often respond to doses of 0.25-0.3 mg/kg every other day. If doses of prednisolone in dogs can be maintained at a dose below 0.5 mg/kg per day, this can be tolerated with minimal adverse effects (Lee, et al, 2017). However, there can be wide variation of response among individuals and doses should be titrated to the appropriate response for each patient.

Differences among Species

Cats often require higher dosages than dogs, sometimes twice as much as dogs, possibly owing to differences in receptors[9]. Prednisolone is preferred over prednisone in cats. In cats, some evidence indicates that either oral absorption of prednisone is poor, or once it is absorbed there is a deficiency in the ability to convert to prednisolone[10]. Horses are also deficient in their ability to convert prednisone to prednisolone[11].

Methylprednisolone 1-2 mg/kg per day, tapered to 0.5 mg/kg q48h) and triamcinolone acetonide (0.2 mg/kg per day tapered to 0.1 mg/kg q48h) also has been used in cats and shown to be effective for treatment of allergic pruritus. A common dose of triamcinolone acetonide for cats is 0.5 mg per cat (one tablet) every other day, which is a convenient dose for an average size cat. When prescribing triamcinolone, it is important to use triamcinolone acetonide instead of triamcinolone. The approved veterinary formulation is triamcinolone acetonide which is approximately 6x more potent than triamcinolone.

Cats are also more resis¬tant to the adverse effects than are dogs, but that does not alleviate all concerns about the long-term metabolic effects (increased risk of diabetes, liver changes, catabolic effects, increased risk of heart disease)[12].

Rationale for Every-Other-Day Therapy (EOD):
When an intermediate-acting glucocorticoid is used (duration of action of 12-36 hours), the hypothalmic-pituitary-adrenal (HPA) axis has an opportunity to recover before the next dose. It is important that one chooses a glucocorticoid that does not have a long duration of action for EOD therapy – for example, dexamethasone is not recommended because its duration is at least 36-48 hours. After 1 mg/kg every 48 hours of prednisolone ACTH was suppressed in dogs for 18 to 24 hours, and returned to normal until the next scheduled dose[13]. EOD therapy will minimize, but *will not* prevent adrenal atrophy, and other adverse effects such as those on the immune system, and the effects on metabolism.

Adrenal Recovery Following Glucocorticoid Therapy
After short-term glucocorticoid therapy, HPA axis recovery occurs quickly, and the medication can be discontinued abruptly. After long-term therapy it may take longer for adrenal gland recovery from suppression and a withdrawal syndrome may be observed in animals after glucocorticoids are discontinued. In people, adrenal recovery may take months. In animals, after repeated administration adrenocortical recovery can occur within a few weeks. In healthy dogs, complete recovery of the HPA-axis was evident two weeks after cessation of daily prednisone administration[14]. In another study, recovery occurred one week following discontinuation of every-other-day prednisolone administration[13].

Because recovery of adrenal function after glucocorticosteroid therapy can vary among patients, veterinarians should advise animal owners of the possibility of adrenal suppression following discontinuation of corticosteroid therapy. Patients should be monitored for signs of adrenocortical insufficiency (lethargy and weakness, for example), and supplement animals with physiologic dosages of a short to medium-acting corticosteroid as needed, especially at times of stress[15]. Because the usual physiologic secretion of cortisol is 1 mg/kg/¬day, this translates to a dose for glucocorticoid supplementation of 0.2 to 0.25 mg/kg of prednis¬olone/day.

Formulations
Phosphate and succinate esters: These esters are highly soluble and associated with a rapid onset of action. These esters may be given IV to achieve rapid, high serum concentrations. Examples include prednisolone sodium succinate (Solu-Delta-Cortef), methylprednisolone sodium succinate (Solu-Medrol) and dexamethasone sodium phosphate (Azium-SP, and other brands).

Acetate esters: These esters are poorly soluble and are given IM, SC, or intra-articular for a pro-longed effect. Absorption occurs slowly, in days to weeks. Examples include methylprednisolone acetate (Depo-Medrol).

Aqueous suspensions: These forms are rapidly absorbed after IM or SC injection.

Solutions: These forms are solutions in propylene glycol or alcohol such as dexameth¬asone solution.

SUMMARY OF ADVERSE EFFECTS OF GLUCOCORTICOIDS

System	Effects
Behavioral	Polyphagia, euphoria
Metabolic	Hyperlipidemia, lipolysis, protein catabolism, fatty infiltration of liver, steroid hepatopathy (dogs)
Musculoskeletal	Osteoporosis, myopathy, fibroblast inhibition, decreased intestinal calcium absorption.
Endocrine	Anti-insulin effects, increased glucose, HPA-axis suppression, decreased thyroid synthesis, increased parathyroid hormone synthesis.
Gastrointestinal	Diarrhea, increased risk of ulcers, pancreatitis, colonic perforation.
Host defenses	Decreased bacterial killing, decreased non-specific immunity, increased risk of septicemia, recurrent bacterial cystitis.
Fluid Balance	Sodium and fluid retention, polyuria/polydipsia
Cardiovascular	Fluid overload (water retention), increased risk of cardiovascular disease in cats.

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VIRAL SKIN DISEASES

Wayne Rosenkrantz

Animal Dermatology Clinic, Tustin, California

INTRODUCTION

Viral skin disorders are considered rare in dogs and cats, and many that have been reported are associated with the natural habitat or are a result of the environment where the pet spends time. Cutaneous viral dermatoses are often underdiagnosed in dogs and cats because it is difficult to identify an exact causative agent. However, most of the recognized viral dermatoses have a characteristic clinical appearance unlike that of other common diseases seen in everyday practice. Diagnosis of these disorders is essentially based on clinical features with or without histopathology. Immunohistochemistry and/or polymerase chain reaction (PCR) can be useful for identifying the infection. The current discussion will emphasis the most common dermatoses, particularly papillomavirus-associated dermatoses and feline herpesvirus. In addition, brief discussions on feline calicivirus, FeLV and feline poxvirus will be covered. Additional details can be found in the references.[1,2]

PAPILLOMARIRUS-ASSOCIATED DERMATOTSES

The papillomaviruses (PVs) are grouped with the polyomaviruses to make up the papovaviruses. PVs are small, naked viruses containing double-stranded circular DNA; they lack a lipid envelope, which may account for their relative resistance to physical and chemical destruction. PV infections appear to be limited to the epidermis and epithelium. At least 20 canine and several feline papillomaviruses have been reported.[2,3] PVs are thought to cause oral papillomatosis, cutaneous papillomas and canine viral pigmented plaques, and are rarely associated with the development of oral and cutaneous squamous cell carcinomas in dogs. In cats, PVs are currently thought to cause oral papillomas, feline viral plaques, Bowenoid in situ carcinomas and feline sarcoids. There is increasing evidence that PVs may also be a cause of cutaneous squamous cell carcinomas and basal cell carcinomas in cats.

Canine Oral Papilloma: Canine oral papilloma is a typically a self-limited infectious disease that is normally confined to the mucosal tissue of the oral cavity or lips in young dogs, but it occasionally produces papillomas on the conjunctiva and external nares.The lesions begin as white, flat, smooth, shiny papules and plaques, and progress over 4 to 8 weeks to whitish gray, pedunculated, or cauliflower-like hyperkeratotic masses. Light microscopy reveals papillomatous proliferations of thick squamous epithelium in which some cells are swollen with vesicular cytoplasm. Canine oral PV–induced generalized papillomas may occasionally be the presenting sign in immunosuppressed dogs or those receiving cyclosporine or oclacitinib.[4,5] The lesions regress spontaneously over another 4 to 8 weeks in most cases, although in some cases they may be persistent. Malignant transformation into carcinoma has been seen in rare situations.

Cutaneous Exophytic Papilloma: Cutaneous exophytic papilloma can develop in dogs of any age, but it is more common in younger and elderly dogs. Single or multiple skin lesions may be present, mainly found on the head, eyelids, and feet. They present as white, pink, or pigmented papillated masses that may be sessile or pedunculated. Lesions are typically <1 cm in diameter with a fimbriated surface. Microscopically, cutaneous exophytic papillomas consist of marked epithelial proliferations on numerous thin fibrovascular stalks. These lesions may persist for 6 to 12 months; many, but not all, regress spontaneously over a period of weeks to months. They may occur at a higher incidence in dogs on cyclosporine or oclacitinib.

Cutaneous Inverted Papilloma: Cutaneous inverted papillomas present as a single mass or as multiple small masses. They are unpigmented, raised, firm, and covered by skin with a central pore opening to the surface.[6] The disorder is usually seen in dogs younger than 3 years, although older dogs may also be affected. Masses are most commonly found on the ventral abdomen and groin but may appear on the distal extremities, including the digits and footpads. Lesions are <2 cm and are supported by thin fibrovascular stalks. Light microscopy reveals an inverted flask-like structure below the level of the normal epidermis. Cutaneous inverted papillomas do not usually undergo spontaneous regression, although spontaneously regressed casesmay be seen.

Canine Pigmented Plaques: Canine pigmented plaques are mainly found in pugs and miniature schnauzers during young adulthood, [7,8] but can be seen in other breeds. There may be an inherited autosomal dominant trait, and immunocompromised individuals are also suspected to have an increased incidence.[9] Lesions are multiple, scaly, deeply pigmented macules, plaques, and sometimes papules, commonly located on the ventral neck, ventral trunk, abdomen, and extremities. Histopathologically, they are characterized by demarcated, irregularly psoriasform acanthosis with marked hyperkeratosis and hyperpigmentation. Canine pigmented plaques develop progressively over time and generally do not regress, which is very different from other canine papillomavirus–associated dermatoses.9 The potential for transformation to squamous cell carcinoma (SCC) has also been seen.The presumed familial nature of canine pigmented plaques suggests that they may be equivalent to epidermodysplasia verruciformis (EV) in humans, whichis genetically determined and caused by an unusual susceptibility to EV-specific human PV infections.

Canine Pigmented Papules: Canine pigmented papules are rare and have been reported in a boxer undergoing long-term corticosteroid therapy.[10] The lesions appear as multiple black, rounded papules up to 2 mm in diameter over the ventral skin. A single lesion has been reported at the concave aspect of the pinna of a Rhodesian ridgeback.[11] Histologically, the lesions consisted of well-demarcated, cup-shaped foci of epidermal endophytic hyperplasia with marked parakeratosis and no papillary proliferations. The lesions do not recur following surgical removal and may regress spontaneously after the cessation of corticosteroid therapy.

Feline Viral Plaques and Bowenoid in situ Carcinomas: Traditionally viral plaques and Bowenoid in situ carcinomas (BISCs) have been described as separate diseases. However, since both are predominately caused by *Felis catus* papilloma virus (FcaPV-2) they are likely clinical variations of the same disease. There are also reports of transitional lesions between viral plaques and BISCs.[12] Classically viral plaques (verruca plana, feline cutaneous papilloma) appear as multiple, scaly plaques of variable size that are sometimes hyperpigmented.[13-15] The hyperpigmented lesions more closely resemble Bowenoid lesions and again are likely the same but earlier lesions. They can develop anywhere on the body but are predominantly found on the trunk.Persian cats may be predisposed. Although immunosuppression can predispose an animal to this disorder, feline viral plaques have also been reported in cats without any immunocompromised status.[16] The author has seen one case secondary to cyclosporine therapy that resolved with discontinuation of the drug. The histopathology of plaques shows well-demarcated foci of acanthosis with an undulating configuration and laminated hyperkeratosis. The viral plaques also affect the follicular infundibula, which are plugged with cornified debris. Keratinocytes within the plaques often undergo PV cytopathic changes, such as nuclear shrinkage, koilocytosis, and increased quantities of blue-grey foamy cytoplasm. The histologic appearance of plaques containing prominent PV cytopathic changes is similar to that of viral plaques seen in people with EV.[16] PV antigen was detected in a high proportion of feline viral plaques by immunohistochemical analysis.[12] Although FcaPV-1 has been isolated from feline plaques,[17,18] as mentioned,FcaPV-2 is more constantly present within the plaques and is the likely etiologic agent.[19]

Bowenoid in situ carcinoma(BISC) is clinically typically characterized by heavily scaled, crusted, and pigmented papules and plaques with some erosion.[20] Lesions can present in any location but mostly appear on the face, shoulders, and limbs.[21] They are usually multicentric and discrete, and the affected area may or may not be exposed to the sun. There is also one report of metastatic involvement. [22] The lesions are distinctive, but differentiation from actinic keratosis may be required if they are limited to sun-exposed areas. The lesions are mostly seen in cats older than 10 years, and 22% of reported feline cases were positive for either FIV or FeLV.[21,23,24] The lesions show moderate to severe, irregular, epidermal and follicular hyperplasia with hyperkeratosis. There is full-thickness dysplasia manifested by marked loss of nuclear polarity and disruption of normal epithelial stratification. Groups of cells often have dorsoventrally elongated nuclei that are tilted in one direction. Koilocytosis and clusters of large round keratohyaline granules may also be detected. Viral plaques and BISCs can spontaneously resolve, persist without progressing or slowly increase in size and number. BISCs are pre-neoplastic and should be monitored for progression to a SCC. Bowenoid in situ carcinomas in Devon Rex and Sphinx cats appear to be predisposed to rapid progression and the resultant invasive SCCs also demonstrate high metastatic potential.

Squamous cell carcinomas: Papillomaviruses (FcaPV-2) can be associated with feline cutaneous squamous cell carcinomas. FcaPV-2 has been directly associated with SCCs by the presence of increased p16 and decreased pRb in SCCs that contain FcaPV-2 DNA. There is also evidence that SCCs that contain PV DNA have a different biological behavior to those that do not contain PV DNA. FcaPV-2RNAcanalso have transforming properties and the virus has the potential to influence cell behavior and to contribute to tumor formation.[2] An association with PV infection is observed most frequently in SCCs that develop in areas of the body protected from UV light, such as haired or pigmented skin. However, PV DNA and p16 immunostaining are also detectable in a proportion of SCCs from sun-exposed skin, suggesting that PVs could also act as a co-factor with UV light.[14]

Basal cell carcinomas: An association between cutaneous basal cell carcinomas (BCCs) and PVs has been reported with BISC-like changes in the epidermis overlying some BCCs. [25]

Feline Sarcoid: The term "sarcoid" refers to PV-induced fibroblastic proliferations, which have been well documented in horses. Feline sarcoids, previously called *feline cutaneous fibropapilloma* or *papillomavirus-associated cutaneous fibrosarcoma*, are induced by the feline sarcoid–associated PV (FeSarPV).[26,27] The current thought is felinesarcoidsaredue to across-species infection by bovine papillomavirus (BPV)[27,28] Feline sarcoidsmost commonly develop in free-roaming young cats in rural areas, and half of the reported cases were known to have been exposed to cattle because of the cross-species infection.[29] The lesions are slow-growing, solitary or multiple firm nodules that may measure up to 2 cm in diameter. The exophytic masses may be pedunculated and are often ulcerated. They are most commonly located on the planum, head, neck, tail, and digits. The author has seen one case with not only nasal but oral involvement. Histopathologically, the lesions show a dense proliferation of fibroblastic cells with epidermal hyperplasia in a rete-peg configuration.

Treatment of Papilloma-associated Dermatoses: The search for an effective treatment for PV-associated skin diseases has been frustrated by the nature of PV immunity. Fortunately, routine treatment for many of these lesions is not crucial. Many PV infections regress spontaneously after the development of a cell-mediated immune response because of previous exposure to the virus.[30] The disappearance suggests a cause such as stress, and it should be kept in mind that in some cases glucocorticoids, cyclosporine or oclacitinib can trigger and exaggerate the virus expression. Lack of regression of the papillomas, functional interference, cosmetic concerns, or risk of malignancy may indicate more vigorous therapy. There are many treatment options that exist and some of the more common options include 5% imiquimod, surgery, cryotherapy, autologous or recombinant papillomavirus vaccine and laser therapy. In the past synthetic retinoids have been used with variable success but due to effectiveness of other options, availability, expense and side effects, retinoids have not been used recently. There are reports of azithromycin having some efficacy for oral and cutaneous papillomatosis in dogs and cats at an oral dose of 10 mg/kg once daily for 10 days.[31,32] The exact mechanism of how azithromycin functions as an antiviral agent is not known, but one potential mechanism proposed in a study with human bronchial epithelial cells is that it increases the production of interferon-stimulated genes. The author has seen limited success using this form of therapy. The immune response modifier 5% imiquimod (Aldara, 3M Health Care Limited and Generics) has also been reported to be effective when applied topically three times a week in both dogs and cats.In one study twelve cats with a histologic diagnosis of bowenoid in situ carcinoma were treated with 5% imiquimod cream.[33] Initially, all the cats responded, but most cats (75%) developed new lesions. Five cats (41%) experienced adverse effects including local erythema (25%), increased liver enzyme levels and neutropenia (8%), and partial anorexia and vomiting (8%). The author commonlyutilizes this product as a first-round option for many cutaneous papilloma lesions in both dogs and cats. Interferon (IFN) has also been evaluated. It is produced in the body and exerts a biologic action to protect cells from viral infection. There are three main classes of human IFNs: IFN-α, IFN-β, and IFN-γ. IFN-α elicits broad activities that inhibit virus replication. An intracellular mechanism by which IFN-α-2a inhibits human papillomavirus (HPV)–transformed cell proliferation, and presumably HPV-induced papillomas, operates through the suppression of viral oncoprotein expression and cytostatic arrest of cell cycling. Previous reports utilizing interferon in dogs with papillomas and canine pigmented plaques exist.[30] Depending on the type of IFN used there are many different protocols in the literature. IFN-α-2a (Roferon-A, Hoffman-LaRoche) can be used at 1.5 million units (MU) to 2 MU/m^2subcutaneously three times a week or given orally at 1000 units once daily on a 21-day-on, 7-day-off schedule).IFN-α-2b (Intron A, Schering-Plough) has been administered orally at 30 units/mL with similar frequencies. Recombinant type 1 ω IFN of feline origin (rFeIFN-ω;

Virbagen Omega, Virbac; Intercat, Toray)also has variable dosing and frequency protocols.There is one report of using rFeIFN-ω at 1 MU/kg SQ daily for 5 consecutive days repeated in 14 days with complete remission on a young pug with severe facial and oral papilloma virus.[34] In some instances, combining IFN with other treatments could increase the likelihood of effective treatment response. At the author's practices, the availability and expense of interferon has made this option less commonly used. Cryotherapy is a minimally invasive procedure that uses liquid nitrogen (-195.8°C) to achieve temperatures of at least -50°C to selectively freeze and destroy abnormal tissue. Cryotherapy has also been used with variable success,however in a recent paper it was found to be an excellent option in more refractory cases when used with more than just three freeze thaw cycles. In this paper, utilizing five to six freeze-thaw cycles (15 to 30 seconds) proved to be highly effective in three cases with refractory papillomas.[35] The author has also used autologous vaccines with some success but this requires sampling up to one gram of tissue and sending to outside laboratories for formulation.Virus like particle (VLP) vaccines for COPV (CPV1) and CfPV2 (CPV2) have also been developed.The canine papillomavirus VLP vaccines are purified recombinant canine papillomavirus L1 protein.[36] The VLPs are lacking the viral genes, while autologous vaccine still have viral genes which may cause disease in animals, so the VLPs are safer to use. Lastly CO2 laser therapy has been shown to be of value as a sole or combination therapy in more refractory cases.

FELINE HERPES VIRUS-ASSOCIATED DERMATOSES

Feline herpesvirus-1 (FHV-1; felid herpesvirus 1 [FeHV-1[37]) is a member of the *Varicellovirus* genus of the herpes virus subfamily Alphaherpesvirinae, which is closely related to canine herpesvirus-1. Like other herpesviruses, FeHV-1 contains double-stranded DNA and has a glycoprotein-lipid envelope. It is therefore relatively fragile in the external environment. After airborne infection, the virus multiplies in the upper respiratory mucosa and tonsils and remains latent in the trigeminal ganglion. Viral reactivation may be spontaneous but is most likely after a stress such as moving, entering a multicat household, boarding, pregnancy, surgery, or receiving glucocorticoids.FeHV-1 infection generally causes acute upper respiratory disease (feline rhinotracheitis) as well as conjunctivitis and stomatitis. However, the epidermis and hair follicle epithelium can also be affected. FeHV-1-associated dermatitis (ulcerative facial and nasal dermatitis and stomatitis syndrome, ulcerative and necrotizing facial dermatitis)has been described in cats and the role of FeHV-1 has been demonstrated.[37,3839] Cats of all ages and both sexes can be similarly affected with or without a history of respiratory disease. Typical lesions consist of vesicles, crusts, and ulcers on the nasal planum or haired skin of the face such as the bridge of the nose, perinasal skin, and periocular skin. Skin lesions can also appear anywhere on the body, and ulcerative dermatitis with lesions located on the flanks has also been reported in the absence of facial lesions.Microscopically, the lesions are vesicular, ulcerative, and necrotizing. The epidermis appears hyperplastic with necrotic zones. Mixed inflammation, often with numerous eosinophils, is noted in the dermis. Some adnexa, including sweat glands, are destroyed. Free hair in the dermis is associated with many eosinophils, so the lesions can commonly be misinterpreted as allergic dermatitis or eosinophilic granuloma complex. Intranuclear inclusion bodies are present in the surface and adnexal epithelium in variable numbers and are often close to necrotic tissues. The inclusion bodies are amphophilic or glassy, fill the nucleus, and are associated with marginated chromatin and cytoplasmic swelling. Immunohistochemical analysis or PCR is the most commonly used technique for obtaining evidence of infection.[40] A number of antiviral agents, including famciclovir, ganciclovir, and cidofovir, have shown efficacy against FeHV-1 in vitro.[41] Oral famciclovir (Famvir, Novartis) can be effective at 62.5 to 90 mg/kg once or twice daily or 125 mg/cat three times daily.[42] In vitrostudies have also shown that FeHV-1 is susceptible to feline IFN or recombinant human IFN-α, and doses of IFN-α vary widely from 1 MU/m^2 subcutaneously three times a week to 0.01 to 1 MU/kg once daily for up to 3 weeks.rFeIFN-ω (1.5 MU/kg perilesionally and subcutaneously) is also a reported option, but no controlled trials have been performed.[43] The effect of L-lysine on FeHV-1 replication has been explored both in vitro and in experimental cat studies.[44] L-lysine inhibits virus replication by blocking the availability of the amino acid arginine. Its effectiveness in clinical cases remains highly controversial. Topical imiquimod (2 to 3 times a week, as listed under papilloma virus) has also been proposed, and clinical responses have been seen, but imiquimod can cause irritation especially when applied to open ulcerative lesions.[43,44] Control of secondary infection may be needed in many cases. Topical and oral glucocorticoids and cyclosporineshould be used carefully as they could precipitate clinical lesions.

FELINE CALICIVIRUS-ASSOCIATED DERMATOSES

FCV is a small, unenveloped, single-stranded RNA virus belonging to the family Caliciviridae, which includes important human pathogens such as noroviruses, the most common causes of infectious gastroenteritis in humans. There are many different strains of FCV, which spreads by direct contact with an infected cat that sheds the virus on a regular basis.[45] The most common clinical signs are vesiculoulcerative stomatitis and conjunctivitis, and some strains induce lameness caused by arthropathy.[46] Pustular dermatitis was also reported on the abdomens of two cats after routine ovariectomy.[46] The histopathologic diagnosis was panepidermal pustulosis and necrotizing dermatitis. Positive immunohistochemical staining consistent with the FCV antigen was detected in epithelial cells within the pustular lesions. Highly virulent strains of FCV have emerged and are associated with outbreaks of disease with high mortality and have created a new range of clinical features (FCV-associated virulent systemic disease [VSD]).[47] Cats affected by FCV-associated VSD show subcutaneous edema of the face and limbs and variable levels of ulceration of the skin, particularly on the pinnae, footpads, and nares, along with varying degrees of pyrexia, anorexia, and jaundice. Up to 50% of cats die or are euthanized in extremis. Viral isolation, reverse-transcriptase PCR assays, and fluorescent antibody testing may be used to identify viral antigens in swabs from the oropharynx or conjunctiva, blood samples, skin scrapings, or lung tissue. Positive PCR results should be interpreted with caution as they may be a consequence of low-level shedding by persistently infected carriers. The prognosis is usually good foracute infection if the cat receives general supportive and nursing care and regular cleaning of discharges. Mucolytic drugs or nebulization with saline may offer relief.rFeIFN-ω is registered for control of FCV disease in Japan and Australia. Three injections of rFeIFN-ω (5 MU/kg IV every other day) can reduce the duration and severity of fever and oral ulceration associated with FCV. FCV can persist in the environment for about 1 month and is resistant to many common disinfectants.

FELINE LEUKEMIA VIRUS-ASSOCIATED DERMATOSES

FeLV is an immunosuppressive, oncogenic retrovirus. It can induce skin tumors, such as lymphoma and fibrosarcoma, but most commonly affects the skin by its cytosuppressive action. Clinical signs may include chronic or recurrent infections, including pyoderma, dermatophytosis, demodicosis, and *Malassezia* dermatitis, as well as poor wound healing, seborrhea, and generalized pruritus. In addition, crust, scale, alopecia, erythema and erosionscan be seen, especially on the muzzle, lips, perioral area and head.[48] An affected cat can have lesions on the pinnae that have progressed to nasal and perioral sites as well as to the ventral neck, mammary region, and toes. Histopathologically, irregular acanthosis is accompanied by numerous massive, multinucleated giant cells throughout the epidermis and hair follicles. Single or multicentric cutaneous horn(s) in the centers of the digital, central, or metacarpal/metatarsal pads, and sometimes on the face (e.g., nasal planum and eyelids), as well as severe necrosis caused by vasculitis on the pinnae and tail, are rarely seen.Immunohistochemical staining and PCR may be used to confirm the presence of a gp70-positive FeLV antigen. Over time, affected cats often show signs of internal disease. To date, no treatment has proven effective in eliminating FeLV.

FELINE POXVIRUS-ASSOCIATED DERMATOSIS

Poxvirus infections in cats are most commonly caused by the cowpox virus, a member of the *Orthopoxvirus* genus. These infections have been reported in many animals and are endemic to Europe and Western Asia. Rodents are the natural hosts, so the virus is found in rural hunting cats. It is most common during summer and autumn, coinciding with the most active and breeding seasons of the rodents.The initial skin lesion is a bite wound, commonly on the head, neck, or forelimb. Widespread, randomly distributed, pruritic, erythematous macules, papules, and nodules may develop. Some cases show ulceration in the oral cavity and on the tongue, along with systemic signs such as fever, anorexia, depression, and diarrhea during the viremic period 1 to 3 weeks after infection. The varying clinical features might be related to the genetic diversity of the feline cowpox virus. Histopathology reveals hydropic degeneration of the keratinocytes and the presence of intracytoplasmic eosinophilic inclusion bodies. Virus isolation or PCR analysis are preferred for a conclusive diagnosis. The prognosis is good. Skin lesions heal slowly over 3 to 8 weeks. The feline cowpox can be transmitted to dogs as well as cats and has zoonotic potential.[49] All infected cats should be isolated and handled carefully.

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ECOLOGY AND CONTROL OF TICKS

Michael W. Dryden DVM, MS, PhD, DACVM (parasitology)

College of Veterinary Medicine, Kansas State University, Manhattan KS 66502

While often the same products are used to combat ticks as are used to combat fleas, there are substantial differences between flea and tick control. One of the major differences is in the number of species that confront a dog. While there is one predominant flea species that infests dogs in North America, the Cat flea (*Ctenocephalides felis*), there are at least 10 different tick species that may be encountered. There can be remarkable regional variability in the number and diversity of tick species that infest dogs.[1] While practitioners in Hawaii may only deal with one tick species infesting dogs (Brown Dog tick, *Rhipicephalus sanguineus*), practitioners in New Mexico may encounter three different species, in California six different species and in Kansas up to seven different tick species. This wide diversity in tick species means that ticks occur at different times of the year, are associated with different reservoir hosts and carry and transmit different diseases.

Over the past few decades there has been a change in the distribution and abundance of certain tick species in North America.[1-4] Two of the best documented are *Amblyomma americanum* and *Ixodes scapularis*.[2-4] Since both these ticks are important vectors of human and animal pathogens these changes in distribution and abundance have had a marked effect upon both human and animal health. Various factors have contributed to tick population movement including; changes in agricultural practices, reforestation, wildlife conservation, relocation and restocking, climate fluctuations and decreased environmental pesticide application.

Specific factors that have contributed to the increased range of *A. americanum* include increased habitat via reforestation and its wide host range that includes deer, small mammals, birds and man[3,4]. The White-Tailed Deer is considered a preferred host for *A. americanum*, and all life stages will feed successfully upon White-Tailed Deer. Another species that utilizes similar habitats and is an excellent host for larvae and nymphs is the wild turkey. Areas with high White-Tailed Deer and wild turkey populations can have remarkably large populations of *A. americanum*. Similar to *A. americanum* the distribution of *I. scapularis* is linked to the distribution and abundance of the white-tailed deer.[2]

Ixodes scapularis is widely distributed in the Eastern and Central U.S. in at least 35 states.[5,6] Its distribution is from Florida to Maine, west into far eastern South Dakota, and south through eastern Kansas into central Texas. *Ixodes scapularis* is also located in central and eastern Canada.

Seasonal activity varies by geographic region, but larval activity is generally highest in August and September. Larvae attach to and feed on a wide variety of small mammals, including mice, chipmunks and shrews. Larvae also feed on birds and lizards. The white-footed mouse (*Peromyscus leucopus*) is of particular importance in the tick life cycle and disease transmission, because it serves as a good host for larval *I. scapularis* and it is a major reservoir of *Borrelia burgdorferi*.

Immature ticks typically engorge for 2 to 4 days before dropping off to molt in moist protected areas such as under leaf litter in forested habitats. Larvae over-winter and then molt to nymphs in the spring. Nymphs will feed for 3 – 4 days on a variety of hosts including mice, squirrels, chipmunks, raccoons, opossums, skunks, shrews, cats, birds, and humans. Nymphs occur primarily from May through July in the North. Adults occur most commonly from October through December. Adults that do not find a host will quest again, typically from March to May. Adults feed for 5 – 7 days, primarily on white-tailed deer, but also on bobcats, cattle, coyotes, dogs, foxes, horses, humans, opossums, raccoons and other mammals.

While recent pharmaceutical advances have been made in control of flea reproduction, such advances in the area of tick control are lacking. With the exception of the brown dog tick *Rhipicephalus sanguineus*, our ability to manage tick reproduction is limited, if not almost non-existent. As discussed previously in most flea infestations we have the opportunity to control flea reproduction by either killing fleas before they can reproduce or killing flea eggs. However, it is not just because we have effective residual insecticides, insect growth regulators or insect development inhibitors

that we are successful, it is also due in large part to the fact we can often target the primary reproductive host, the flea infested dog or cat. And interestingly, failures in flea control often occur when flea infested feral pets or flea infested urban wildlife invade the owners' yards.

But when dealing with most 3-host ticks the problem is that the majority of the reproducing ticks are not on the dogs or cats, but on their nature wildlife hosts. Since we are limited in our ability to manage ticks on wildlife, reinfestations are a common occurrence and protracted use of acaracides as preventives is routine in many areas.

Since tick control can be extremely difficult and because they are vectors of a variety of bacterial and protozoal diseases veterinarians should have an understanding of the ecology of the tick(s) encountered in the area in which they practice. Veterinarians need to be educated on the various aspects of tick ecology, disease transmission and control methodologies so that they can then educate their staff and pet owners.

Numerous studies demonstrate the high level of efficacy of the various acaracides, but the residual activity is rarely 100% and the efficacy of products varies between and as well as within species, even in the same laboraotry.[9-16] Evaluations of acaracides under natural or field conditions further illustrates that while efficacy is good it is not 100%.

In a field efficacy trial conducted in Kansas U.S.A, an imidacloprid (8.8% w/w)-permethrin (44.0% w/w) formulation was evaluated on dogs against naturally occurring populations of *Amblyomma americanum*. When dogs were walked in a naturally tick infested environment the 48-hour post-exposure efficacy of imidacloprid-permethrin formulation was 93.5%, 98.9%, 94.6%, 94.1% and 96.6% on days 3, 7, 14, 21 and 28 respectively, post-treatment.[14]

Variation in product efficacy occurs. In two studies conducted at K-State, different results were found when evaluating the efficacy of acaricides against Dermacentor variabilis infestations in dogs from two different regions of the USA.[9,12] In the first study, the efficacy of imidacloprid–permethrin and fipronil–(s)-methoprene formulations were evaluated against a D. variabilis isolate from California. The 48-h post-infestation efficacy on day 30 post-treatment was 92.0% and 83.2%, respectively, for the imidacloprid–permethrin and fipronil–(s)-methoprene formulations. In the second study, the 48-h post-infestation efficacy on day 30 for the imidacloprid–permethrin and fipronil–(s)-methoprene formulations against a *D. variabilis* isolate from Oklahoma was17.5% and 75.7% respectively.

One combination spot-on product that produces more prolonged and pronounced efficacy is fipronil-amitraz. In a study conducted at K-State, the efficacy against Dermacentor variabilis 30 days after treatment was 99.4%.[13]

Recently a new class of insecticide/acaracide has provided the first orally administered approach to tick control. Afoxolaner, fluralaner, lotilanerand sarolaner are members of the isoxazoline class and work by inhibiting insect GABA and Glutamate-gated chloride channels leading to hyper-excitation and death of insects and arachnids. Various studies have demonstrated these compounds remarkable residual speed of tick kill[17-20] and a few have demonstrated their ability to prevent tick transmitted diseases, such as Lyme, Anaplasmosis and Babesiosis.[21-24]

While product efficacy is often excellent in most studies, significant variation in efficacy can occur and 100% control is rarely achieved. Therefore, it can be expected that under natural conditions in areas where dogs are being frequently exposed to ticks, pet owners will see ticks on treated dogs. We might also expect that efficacy in real world situations might be lower due to such factors as bathing and swimming, differences between dog breeds and haircoat types and frequency and correctness of product application.

Since 100% tick kill is rarely achievable, perceived efficacy of acaracides may be directly related to the numbers of ticks to which dogs are exposed. If a dog is treated with one of these highly efficacious acaracides and encounters just a few ticks it is likely all those ticks will be killed. However, if tick exposure is considerably larger, we can expect a few ticks to be observed on these dogs and pet owners may perceive a lack of efficacy. Therefore, in areas where tick populations are increasing the perception may be that the products are not as effective as they once were.

Pet owners often view tick infestations of their pets differently than flea infestations.[12] Whether this is due to concerns about tick transmitted diseases or simply a phobia, the presence of a couple of ticks on the pet often elicits a more pronounced negative reaction than the presence of a couple of fleas. A 95% effective flea product may provide great client satisfaction while a similarly effective tick product may be perceived as a failure. Therefore, it is not uncommon that label recommended application of a product does not appear to control the problem. This may be real or

perceived, based upon pet owner expectations of product performance. Given pet owner concerns, a need to reduce tick borne disease and lack of 100% efficacy; occasionally additional control measures are needed.[12,14] If additional control measures are deemed necessary, pet owners need to be educated as to why additional control measures are necessary and notations made in the pets record before extra label uses are conducted.

One of the most common practical attempted solutions to this problem in dogs is to increase the frequency of application. Here increased residual efficacy is the expected outcome, since you are increasing the residual acaracides levels with the shorter application intervals. Additionally, with many 3-host ticks destruction of tick habitat can reduce exposure pressure. Areas that serve as refuge for ticks and wild mammals such as grass, weeds, and brush piles, between runs and along buildings, can be eliminated or treated with an approved acaricide.

In some situations, especially in tropical and subtropical regions and in climate controlled kennels brown dog ticks may infest buildings with ticks crawling up walls, curtains and throughout the home or kennel.[14-15] In these situations acaracides may need to be sprayed indoors into cracks and crevices, behind and under furniture or cages and along walls and the ceiling. Following application, make sure the acaricide is dry before you allow animals or humans back into the premises to minimize toxicity problems. Finally, restricting pet access from tick-infested environments may be necessary.

It is apparent that the range and local density of certain tick species has increased in many areas. Whatever the factors it must be recognized that tick infestation pressure may be much higher and associated tick transmitted diseases may be more prevalent in some locations today than in the past. The increase in tick populations means that pets are encountering ticks more frequently, are exposed to more ticks per encounter and clients may be seeing more ticks on their pets than in the past. Since tick products do not kill or repel all ticks instantly, clients may get the false impression that the products are not performing as well as in the past. These situations necessitate that veterinarians set client expectations, before clients set their own unrealistic expectations of control.

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IMPORTANT SAFETY INFORMATION: People with known hypersensitivity to penicillin or cephalosporins should avoid exposure to CLAVAMOX. Do not use in animals with a history of allergic reactions to penicillins or cephalosporins. See Brief Summary of full Prescribing Information on page XX.

*Not actual size.
[1]Zoetis Data on File, 2005–2015. 50 million CLAVAMOX Tablets estimated from units sold and dispensed at label dose.

zoetis

CLAVAMOX® CHEWABLE

(amoxicillin and clavulanate potassium tablets)

Chewable Tablets

Antimicrobial For Oral Use In Dogs And Cats

CAUTION: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

DESCRIPTION: CLAVAMOX CHEWABLE Tablet (amoxicillin and clavulanate potassium tablets) is an orally administered formulation comprised of the broad-spectrum antibiotic Amoxi® (amoxicillin trihydrate) and the β-lactamase inhibitor, clavulanate potassium (the potassium salt of clavulanic acid).

Amoxicillin trihydrate is a semisynthetic antibiotic with a broad spectrum of bactericidal activity against many gram-positive and gram-negative, aerobic and anaerobic microorganisms. It does not resist destruction by β-lactamases; therefore, it is not effective against β-lactamase-producing bacteria. Chemically, it is D(-)-α-amino-p-hydroxybenzyl penicillin trihydrate.

Clavulanic acid, an inhibitor of β-lactamase enzymes, is produced by the fermentation of *Streptomyces clavuligerus*. Clavulanic acid by itself has only weak antibacterial activity. Chemically, clavulanate potassium is potassium z-(3R,5R)-2-β-hydroxyethylidene clavam-3-carboxylate.

INDICATIONS: CLAVAMOX CHEWABLE Tablets are indicated in the treatment of:

Dogs: Skin and soft tissue infections such as wounds, abscesses, cellulitis, superficial/juvenile and deep pyoderma due to susceptible strains of the following organisms: β-lactamase-producing *Staphylococcus aureus*, non-β-lactamase-producing *Staphylococcus aureus*, *Staphylococcus* spp., *Streptococcus* spp., and *E. coli*.

Periodontal infections due to susceptible strains of both aerobic and anaerobic bacteria. CLAVAMOX CHEWABLE has been shown to be clinically effective for treating cases of canine periodontal disease.

Cats: Skin and soft tissue infections such as wounds, abscesses, and cellulitis/dermatitis due to susceptible strains of the following organisms: β-lactamase-producing *Staphylococcus aureus*, non-β-lactamase-producing *Staphylococcus aureus*, *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, and *Pasteurella* spp.

Urinary tract infections (cystitis) due to susceptible strains of *E. coli*.

Therapy may be initiated with CLAVAMOX CHEWABLE prior to obtaining results from bacteriological and susceptibility studies. A culture should be obtained prior to treatment to determine susceptibility of the organisms to CLAVAMOX. Following determination of susceptibility results and clinical response to medication, therapy may be reevaluated.

DOSAGE AND ADMINISTRATION:

The dose should be prescribed using a combination of whole tablet strengths (62.5 mg, 125 mg, 250 mg, 375 mg). Do not remove from foil strip until ready to use. Even if the tablet is broken, the entire tablet should be consumed.

Dogs: The recommended dosage of CLAVAMOX CHEWABLE Tablet is 6.25 mg/lb of body weight twice a day.

Skin and soft tissue infections such as abscesses, cellulitis, wounds, superficial/juvenile pyoderma, and periodontal infections should be treated for 5–7 days or for 48 hours after all symptoms have subsided. If no response is seen after 5 days of treatment, therapy should be discontinued and the case reevaluated. Deep pyoderma may require treatment for 21 days; the maximum duration of treatment should not exceed 30 days.

Cats: The recommended dosage of CLAVAMOX CHEWABLE Tablet is 62.5 mg twice a day.

Skin and soft tissue infections such as abscesses and cellulitis/dermatitis should be treated for 5–7 days or for 48 hours after all symptoms have subsided, not to exceed 30 days. If no response is seen after 3 days of treatment, therapy should be discontinued and the case reevaluated.

Urinary tract infections may require treatment for 10–14 days or longer. The maximum duration of treatment should not exceed 30 days.

CONTRAINDICATIONS: The use of this drug is contraindicated in animals with a history of allergic reaction to any of the penicillins or cephalosporins.

WARNINGS: Store CLAVAMOX CHEWABLE out of reach of dogs, cats, and other pets in a secured location in order to prevent accidental ingestion or overdose.

HUMAN WARNINGS: Not for human use. Keep this and all drugs out of reach of children. Antimicrobial drugs, including penicillins and cephalosporins, can cause allergic reactions in sensitized individuals. To minimize the possibility of allergic reactions, those handling such antimicrobials, including amoxicillin and clavulanate potassium, are advised to avoid direct contact of the product with the skin and mucous membranes.

PRECAUTIONS: Prescribing antibacterial drugs in the absence of a proven or strongly suspected bacterial infection is unlikely to provide benefit to treated animals and may increase the risk of the development of drug-resistant animal pathogens. Safety of use in pregnant or breeding animals has not been determined.

ADVERSE REACTIONS: CLAVAMOX CHEWABLE contains a semisynthetic penicillin (amoxicillin) and has the potential for producing allergic reactions. If an allergic reaction occurs, administer epinephrine and/or steroids.

To report suspected adverse events, for technical assistance or to obtain a copy of the SDS, contact Zoetis Inc. at 1-888-963-8471 or www.zoetis.com.

For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or online at http://www.fda.gov/AnimalVeterinary/SafetyHealth.

ACTIONS: The 2 components are rapidly absorbed resulting in amoxicillin and clavulanic acid concentrations in serum, urine, and tissues similar to those produced when each is administered alone.

Amoxicillin and clavulanic acid diffuse readily into most body tissues and fluids with the exception of brain and spinal fluid, which amoxicillin penetrates adequately when meninges are inflamed. Most of the amoxicillin is excreted unchanged in the urine. Clavulanic acid's penetration into spinal fluid is unknown at this time. Approximately 15% of the administered dose of clavulanic acid is excreted in the urine within the first 6 hours.

CLAVAMOX CHEWABLE combines the distinctive properties of a broad-spectrum antibiotic and a β-lactamase inhibitor to effectively extend the antibacterial spectrum of amoxicillin to include β-lactamase-producing as well as non-β-lactamase-producing aerobic and anaerobic organisms.

MICROBIOLOGY: Amoxicillin is bactericidal in action and acts through the inhibition of biosynthesis of cell wall mucopeptide of susceptible organisms. The action of clavulanic acid extends the antimicrobial spectrum of amoxicillin to include organisms resistant to amoxicillin and other β-lactam antibiotics. Amoxicillin/clavulanate has been shown to have a wide range of activity which includes β-lactamase-producing strains of both gram-positive and gram-negative aerobes, facultative anaerobes, and obligate anaerobes. Many strains of the following organisms, including β-lactamase-producing strains, isolated from veterinary sources, were found to be susceptible to amoxicillin/clavulanate *in vitro* but the clinical significance of this activity has not been demonstrated for some of these organisms in animals.

Aerobic bacteria, including *Staphylococcus aureus**, β-lactamase-producing *Staphylococcus aureus** (penicillin resistant), *Staphylococcus* species*, *Staphylococcus epidermidis*, *Staphylococcus intermedius*, *Streptococcus faecalis*, *Streptococcus* species*, *Corynebacterium pyogenes*, *Corynebacterium* species, *Erysipelothrix rhusiopathiae*, *Bordetella bronchiseptica*, *Escherichia coli**, *Proteus mirabilis*, *Proteus* species, *Enterobacter* species, *Klebsiella pneumoniae*, *Salmonella dublin*, *Salmonella typhimurium*, *Pasteurella multocida*, *Pasteurella hemolytica*, *Pasteurella* species*.

* The susceptibility of these organisms has also been demonstrated in *in vivo* studies.

Studies have demonstrated that both aerobic and anaerobic flora are isolated from gingival cultures of dogs with clinical evidence of periodontal disease. Both gram-positive and gram-negative aerobic and anaerobic subgingival isolates indicate sensitivity to amoxicillin/clavulanic acid during antimicrobial susceptibility testing.

SUSCEPTIBILITY TEST: The recommended quantitative disc susceptibility method (FEDERAL REGISTER 37:20527-29; Bauer AW, Kirby WMM, Sherris JC, *et al:* Antibiotic susceptibility testing by standardized single disc method. *Am J Clin Path* 45:493, 1966) utilized 30 mcg Augmentin® (AMC) discs for estimating the susceptibility of bacteria to CLAVAMOX CHEWABLE Tablets.

PALATABILITY: The palatability of CLAVAMOX CHEWABLE Tablets was evaluated in a multi-location field trial. One hundred twelve (112) client-owned dogs were dosed with CLAVAMOX CHEWABLE Tablets at 6.25 mg/lb (12.5 mg/kg) twice daily for 7 days and evaluated for palatability of the product. Dogs freely consumed 83% of their doses within 5 minutes of offering from an empty bowl or owner's hand. Of the 17% of doses unconsumed after 5 minutes, 16% were administered with a treat/food or forced intake and 1% of doses were refused.

STORAGE INFORMATION: Store in a dry, cool place at temperatures not above 25°C (77°F). Do not remove from foil strip until ready to use.

HOW SUPPLIED: CLAVAMOX CHEWABLE Tablets in the following strengths are supplied in strip packs. Each carton holds 10 strips with 10 tablets per strip (100 tablets per carton).

Each 62.5-mg tablet contains amoxicillin trihydrate equivalent to 50 mg of amoxicillin activity and 12.5 mg of clavulanic acid as the potassium salt. For use in dogs and cats.

Each 125-mg tablet contains amoxicillin trihydrate equivalent to 100 mg of amoxicillin activity and 25 mg of clavulanic acid as the potassium salt. For use in dogs only.

Each 250-mg tablet contains amoxicillin trihydrate equivalent to 200 mg of amoxicillin activity and 50 mg of clavulanic acid as the potassium salt. For use in dogs only.

Each 375-mg tablet contains amoxicillin trihydrate equivalent to 300 mg of amoxicillin activity and 75 mg of clavulanic acid as the potassium salt. For use in dogs only.

Dispense according to recommendations outlined in Dosage and Administration section.

NADA #55-099, Approved by FDA

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ABSRACTS SCHEDULE

FRIDAY, MAY 4, 2018

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A description of a local anesthetic technique to block the ear canal in dogs – a cadaveric study

E.A. MARTINEZ*, P.D. WALLACE*, M.L. LEPIZ*

**Department of Veterinary Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA*

Abstract: Otoscopy, bullae flush, and total ear canal ablation are common procedures for dogs with otitis externa. We investigated the anatomy of ear canal innervation in dogs and describe an approach for a local anesthetic technique that can be used to desensitize the ear canal. Eight canine cadavers (two fresh, six thawed), for a total of 16 ears, were used. An anatomical study of the nerves supplying the ear canal was performed in two ears. For the remaining 14 ears (seven left, seven right), the cadavers were placed in lateral recumbency and injections of dye were performed using methylene blue (1:1 ratio sterile saline) using a volume of 0.1 ml/kg (maximum 3 ml) for each injection site. For each ear, two dye injections were performed targeting the auriculotemporal nerve (AT), rostral to the ear canal and caudal to the zygomatic arch, and the greater auricular nerve (GA), ventral to the wing of the atlas and caudal to the tympanic bulla. Bilateral dissection was performed following the injections and the accuracy of dye deposition was determined. Successful nerve staining was defined as nerves stained for a length > 6mm. Occurrence of facial nerve (FN) staining was also recorded. Nerve staining was 93% and 100% successful for AT and GA, respectively. Staining of FN was 71%. Based on our nerve staining criteria, the described approach should provide acceptable sensory blockade of the canine ear canal. Due to the high prevalence of FN staining, eye lubrication may be needed until the effect of blockade has resolved.

Sources of funding: This study was supported by the GINN Fund, Department of Veterinary Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University.

Conflict of Interest: None declared

Clinical and histopathologic features of *Burkholderia cepacia* complex cutaneous infections in immunocompetent and immunosuppressed dogs: five cases

C.L. CAIN*, S.D. COLE†, C.W. BRADLEY†, M.S. CANFIELD‡, E.A. MAULDIN†

**Departments of Clinical Sciences and Advanced Medicine and †Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
‡Animal Dermatology South, New Port Richey, FL, USA

Abstract: The *Burkholderia cepacia* complex (Bcc) is an emerging cause of opportunistic infections. Deep pyoderma associated with Bcc infection has been previously reported in dogs receiving ciclosporin. The objective of this case series was to report the clinical and histopathologic features of five additional Bcc skin infections in dogs, one of which progressed to terminal sepsis. Medical records of five dogs with a skin culture yielding growth of Bcc and skin biopsies for histopathology were reviewed. Four different breeds and one mixed breed dog were represented. Two dogs were receiving ciclosporin and one was receiving oclacitinib; 2/5 dogs had no evidence of immunosuppression. Two dogs were bathed prior to onset of skin lesions. Four dogs presented with dorsally oriented ulcers, crusts, and draining tracts; one dog had post-incisional infection. The main histologic feature from skin biopsies was severe neutrophilic dermatitis with folliculitis and furunculosis. Intracellular gram negative and Warthin-Starry positive rods were present in 3/5 cases. Four dogs were successfully treated with systemic fluoroquinolones or trimethoprim/sulfamethoxazole with or without topical antimicrobials. The Bcc isolate from one dog, which was receiving ciclosporin, was resistant to all tested systemic antimicrobials. This dog developed septicemia and was euthanized; sepsis was confirmed via polymerase chain reaction for *Burkholderia* spp. performed on DNA extracted from skin and liver. This case series illustrates that Bcc skin infections can occur in both immunocompetent and immunocompromised dogs. Bcc isolates may be extensively antimicrobial resistant, presenting a challenge for successful clinical management. Cutaneous infection may progress to life-threatening sepsis.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Localized erythema multiforme associated with a clipper burn in a dog

H.L. THAM*, O. LANZ*, K. LINDER†

**Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA*
†Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Abstract: Erythema multiforme (EM) has been reported in humans, dogs and cats. In humans, a localized form of EM has been associated with lymphatic obstruction, Anthrax vaccination and radiation port site. An 8-year-old female spayed German shepherd mixed breed dog presented with skin lesions that developed 5 days after surgery for a ruptured right cruciate ligament. The lesions were multifocal raised annular and "targetoid", some with a center erosion, others with a center crust, and a few with hair and underlying normal appearing skin. All lesions were localized to the right hip region that was subjected to a clipper burn incurred during a routine aseptic skin preparation that involved clipping with a size 40 blade, followed by alternating scrubs of povidone iodine (7.5% iodine) and isopropyl alcohol (91%) for a total of three times each, then a final iodine solution (10% iodine) was used. The adjacent region that was also clipped and similarly aseptically prepared but without thermal burn appeared clinically normal. Histopathology of the lesions showed lymphohistiocytic interface dermatitis with diffuse, moderate individual cell necrosis at multiple epidermal levels consistent with EM disease complex. The skin lesions resolved spontaneously without treatment. Proposed pathogeneses of localized EM in this dog include direct viral infection of keratinocytes by clipper blade transfer through epidermal injury, leading to cytotoxic lymphocytic reaction or a dysimmune cutaneous reaction due to thermal burn-associated disruption of lymph circulation and peripheral nerve fiber damage (known as immunocompromised cutaneous district in humans).

Sources of funding: Self-funded.

Conflict of Interest: None declared

Bromelain-based enzymatic debridement of deep burns on the paws of a cat

L. WALDMAN

Veterinary Dermatology and Allergy Clinic, Haifa, Israel

Abstract: A bromelain-based proteolytic enzyme preparation (NexoBrid™) is used for the treatment of moderate and deep burns in humans. It removes eschar while preserving healthy dermis, which improves healing and reduces the need for repeated general anesthesia, surgery and autografting. A 13-year-old male, neutered, domestic medium hair cat suffered smoke inhalation, feline lower urinary tract disease and severe burns to all paws in a forest fire. After cleaning the paws, the bromelain-based proteolytic enzyme preparation was applied under occlusive dressing for 4 hours resulting in complete debridement down to healthy dermis. To prevent drying, the wounds were treated, without dressings, with sea buckthorn oil thrice daily for 6 weeks. The day after the bromelain-based proteolytic enzyme preparation treatment, the cat began to walk cautiously to the litter tray. Pseudoeschar formed within 2 days. Granulation tissue appeared after 6 days and clobetasol propionate 0.05% cream (Dermovate®) was then applied thrice daily for 6 weeks to inhibit further granulation tissue formation. Pain was controlled with oral buprenorphine 0.025mg/kg four times daily for the first 2 weeks, then oral meloxicam 0.2mg/kg for 1 day followed by 0.05mg/kg once daily for 6 days. A week after debridement, the cat was alert, walking in the house and eating well, despite signs of painful paws. Five weeks later, adhesions in the left hind paw were surgically released. In conclusion, a bromelain-based proteolytic enzyme preparation developed for the treatment of human burns successfully treated deep paw burns in a cat without the need for intensive wound care.

Sources of funding: NexoBrid™ donated by MediWound Ltd; otherwise self-funded.

Conflict of Interest: None declared

Successful treatment of exfoliative cutaneous lupus erythematosus in a German shorthair pointer with mycophenolate mofetil

A. FERRIGNO* and F. BANOVIC*

**Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA, USA*

Abstract: Immune-modulating drugs show limited therapeutic efficacy in canine exfoliative cutaneous lupus erythematosus (ECLE); over half of ECLE dogs are eventually euthanized for their lack of response to therapy. We report herein a case of generalized ECLE in a dog in which mycophenolate mofetil (MMF) treatment achieved complete remission. A 3-year-old, male castrated German shorthair pointer was presented with a 3 month history of generalized scaling, follicular casts and hypotrichosis affecting the head, trunk, ventrum and medial aspects of all limbs. Severe erythema and well-demarcated annular to polycyclic, hyperpigmented macules and plaques were present on the ventral abdomen, axillae, inguinal area and limbs. The patient exhibited lameness and stiff gait. Histologically, skin biopsy specimens revealed lymphocyte-rich interface dermatitis, infundibular interface mural folliculitis and periglandular lymphocytic infiltrate. Complete blood count, serum chemistry profile, urinalysis, urine protein/creatinine ratio and serum antinuclear antibody test were unremarkable. The absence of systemic signs and unremarkable laboratory tests excluded concurrent systemic lupus erythematosus. Treatment of ECLEwas initiated with oral MMF (22 mg/kg, twice daily); MMF dosage was decreased (10 mg/kg; twice daily) after 7 days when the patient developed diarrhea. Within 3 weeks of starting MMF therapy, a marked improvement in lameness and a moderate decrease in erythema and scaling was observed. After 3 months, erythema, scaling and follicular casts completely resolved and the patient's ECLEremains in complete remission with twice daily MMF. To the authors' knowledge, this is the first reported case of successful treatment of ECLE with mycophenolate mofetil as a single-agent therapy.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Comparison of clinical and epidemiological features of canine atopic dermatitis and atopic-like dermatitis: a retrospective study

L. S. BOTONI*, S.M.F. TORRES†, S.N. KOCH†, M.B. HEINEMANN‡, A.P. COSTA-VAL*

**Department of Veterinary Clinical Sciences and Surgery, Universidade Federal de Minas Gerais, Brazil*
†Department of Veterinary Clinical Sciences, University of Minnesota,USA
‡Department of Preventive Veterinary Medicine and Animal Health, Universidade de São Paulo, Brazil

Abstract: Canine atopic-like dermatitis (ALD) has identical clinical manifestations to atopic dermatitis *sensustricto* (AD); however, allergen-specific IgE cannot be detected in this disease subset. The primary study aim was to compare clinical and epidemiological features of dogs with ALD and AD. Atopic dogs with available intradermal and serum allergy test results were retrospectively selected. Inclusion criteria were met by 253 dogs. Dogs were enrolled in the ALD group if both allergy tests were negative and in the AD group if at least one test was positive. In addition, dogs in the ALD group that were positive on a food elimination trial were excluded from the study. Epidemiological data, disease severity measured by pruritus level, number of body sites affected and maintenance therapy in addition to response to therapy were compared between the groups. Two hundred and sixteen (85.38%) dogs were included in the AD group and 37 (14.62%) in the ALD group. No significant differences were noted between the groups regarding the epidemiological variables evaluated. There were no differences in the mean pruritus scores and number of affected body sites at the first visit (P =0.433 and P =0.474, respectively) or during treatment (P =0.949 and P =0.093, respectively) between groups. Moreover, no differences in the reduction of pruritus (P =0.061) and number of body sites affected (P =0.368) were observed during treatment between groups. In conclusion, no significant differences in the epidemiological data and clinical features evaluated were noted between dogs with ALD and AD.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Association of administration of oclacitinib with improvement of quality of life of acutely pruritic dogs and their owners in 7 days

A. WRIGHT*, S. COOPER, A. PAVLOCK†

**Zoetis, 10 Sylvan Way, Parsippany, NJ 07054 USA*
†AMP Research Solutions, PO Box 90, Parker Ford, PA, USA

Abstract: Oclacitinib (Apoquel®) is prescribed for the treatment of pruritus associated with allergic dermatitis and atopic dermatitis in dogs. This study gathered signalment, diagnosis and Days 0 (pre-) and 7 (post-treatment) pruritus visual analogue scale (VAS) scores from 64 dogs in general practice treated with oclacitinib for acute pruritus. An owner-completed Canine Dermatitis Quality of Life (CDQoL-TSQ) questionnaire (includes separate scales for dog and owner quality of life [QoL]) was completed and scored on day 0 (pre-) and 7 (post-treatment). Associations between dose, study day and other predictors with QoL scores in dogs and owners were tested using generalized linear mixed models and fitted with a beta distribution, logit link and residual pseudo-likelihood in SAS (SAS 9.4, SAS Institute Inc.). Independent variables tested included: dose, study day, pruritus score, dog's age, weight, sex, and diagnosis (categorical; 1 = allergic dermatitis, 2 = acute flare of atopic dermatitis, and 3 = other [flea allergy undergoing flea control trial, food allergy undergoing diet trial, allergy testing, ongoing allergen-specific immunotherapy, and other]). Based on unconditional analysis, only study day and pruritus score were significantly associated ($P < 0.001$) with QoL of dogs. Results from uni-variable models indicate that, as for QoL of dogs, only study day ($P < 0.001$) and pruritus score were significantly associated ($P < 0.001$) with owner QoL. Owner QoL significantly increased from day 0 to day 7; QoLsignificantly decreased as the pruritus score increased. QoL of acutely pruritic dogs and their owners improved in 7 days with oclacitinib.

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THE HAIR FOLLICLE: A FASCINATING MINIORGAN

Welle, MM

Institute of Animal Pathology, Dermfocus, Vetsuisse Faculty, University of Bern, Bern, Switzerland

The base of each hair shaft (HS) resides in a multicellular mini-organ called hair follicle (HF). The HF has a wide range of functions including thermoregulation, physical and immunological protection against external insults, sensory perception, social interactions and camouflage.

Most omnivores and herbivores (horses, cattle, pigs, rats, mice *e.g.*) have simple hair follicles (HF)s, in which primary and secondary HFs are arranged individually and each infundibulum contains one hair shaft (HS). Carnivores (dogs, cats *e.g.*) have compound follicles. In the dog a follicular compound consists of a group of up to 15 HFs. A group is composed of a large central primary hair surrounded by up to five smaller lateral primary hairs. The primary hairs are the guard hairs. Each primary hair is associated with an arrector pili muscle, sebaceous glands and sweat glands. The lateral primary hairs are surrounded by smaller secondary hairs. The secondary hairs have a narrower medulla and a thicker cuticle. Whereas the central primary hairs in some breeds emerge through separate epidermal openings, the lateral primary hairs and the associated secondary hairs share a common infundibulum. While primary hairs are shed all year round, the undercoat is shed seasonally. Hair density varies significantly between dog breeds and ranges from 100-600 follicular compounds/cm2 skin containing 1500-4000 hairs. There are dog breeds that do not have an undercoat e.g. poodles, Yorkshire terriers or whippets, however, as compared to the wolf any dog breed has less secondary HF ranging from 10% to 80%(1).

FOLLICULAR ANATOMY

Extending vertically from the epidermis to the base of the HF, the anagen HF can be divided into three major anatomic regions.

1. The infundibulum which extends from the opening of the HF to the point at which trichilemmal cornification begins (approx. the opening of the sebaceous gland duct)
2. The isthmus which extends from the distal end of the infundibulum to the interface between the completely cornifiedinner root sheath (IRS) and the first non-cornified cell of Huxley`s layer (Adamson's fringe;approx. the insertion of the arrector pili muscle)
3. The inferior portion is composed of a suprabulbar and a bulbar region and extends from the last cell in which trichohyalin granules can be seen in the IRS to the base of the HF. The suprabulbar region is characterized by the non-cornified IRS surrounded by the outer root sheath (ORS). The bulbar region is composed of matrix cells with intermingled melanocytes and the DP.

The infundibulum and the isthmus constitute the permanent portion of the HF, whereas the inferior segment is transitory and undergoes regression during catagen and is absent during telogen.

From the outside to the inside the anagenHF can be conceptualized as eight concentrical layers (2) (Figure 1).

The outermost layer is the ORS. The ORS is contiguous with the basal layer of the interfollicular epidermis. In the infundibulum it undergoes keratinization in the same fashion as in the interfollicular epidermis (infundibular cornification). In the isthmus of a telogen follicle the ORS undergoes trichilemmal cornification (a telogen follicle has no IRS).

Between the ORS and the IRS histologically barely visible cells, called the companion layer are located. The IRS is composed of three concentrical layers. The innermost layer is the cuticle and interlocks with the cells of the hair cuticle. The outer two layers contain trichohyalin granules and produce the IRS keratins. The inner layer of these two layers is called Huxley`s layer. The outer layer is called Henle`s layer. IRS cornification is complete at the interface inferior portion / isthmus. This region is also called Adamson`s fringe. The ORS, the companion layer and the IRS surround the HS. The HS consists of the hair cuticle (forms the hair surface), the cortex (forms the bulk of the hair and is composed of keratinized cells which contain pigment from the melanocytes of the bulb) and the innermost medulla.

Together with the IRS and the companion layer the HS arises from the germinative cells of the bulb known as matrix cells (3). Within a concavity of the matrix cells the DP is located,surrounded by a thin basement membrane. The HF is surrounded by a basement membrane and a connective tissue sheath containing dermal sheath cells. Some of these are capable of regenerating the DP (reviewed in (4)).

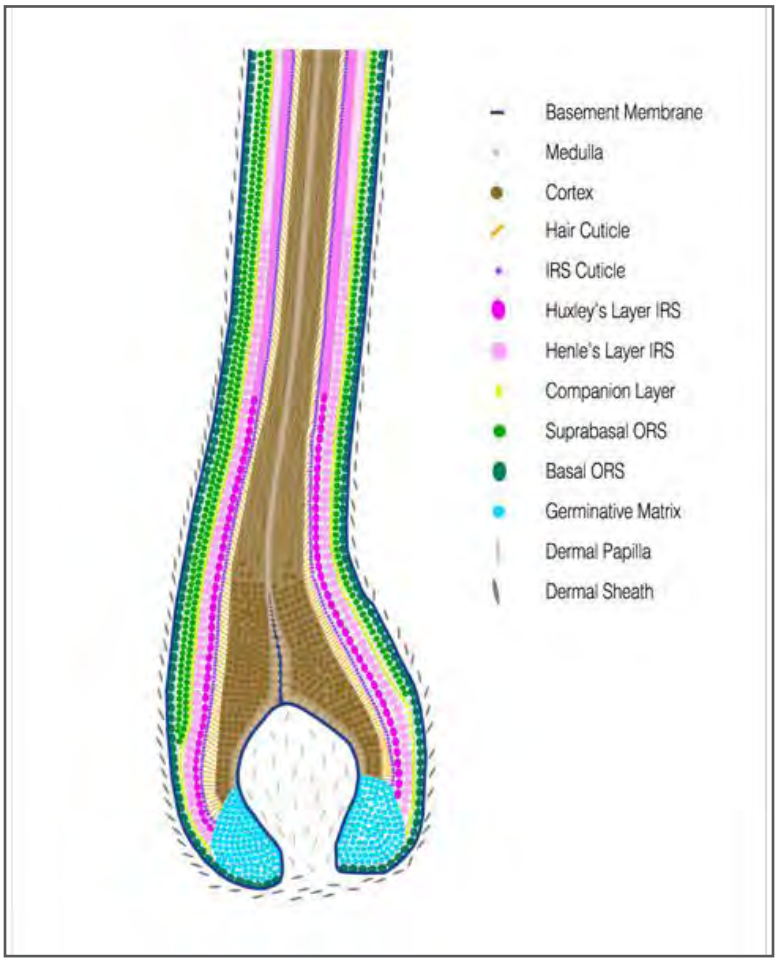


Figure 1:
Concentric layers of the hair follicle

HAIR FOLLICLE MORPHOGENESIS

The mammalian skin and its appendages are derived from ectoderm and mesoderm during embryogenesis. The embryo surface emerges during early embryonic life as a single layer of epithelial cells which gives rise to the epidermis. The dermis is formed from the underlying mesoderm composed of mesenchymal cells. Subsequently, the epidermal-dermal interaction results in HF morphogenesis (5). The stages of HF morphogenesis are broadly classified into: induction, organogenesis and cytodifferentiation(6). Morphologically, HF induction is characterized by a local thickening of the epidermis, known as hair placode. Upon successful initiation of the epithelial placode, organogenesis starts with a condensation of mesenchymal cells underneath the placode, leading to the formation of the hair germ, also named bud. The hair germ proliferates and invaginates into the dermis to form the hair peg and the bulbous peg (5). In cytodifferentiationthe edges of the down-growing follicular epithelium gradually engulf the dermal condensate which from thereon is called the dermal papilla(DP). Consecutive signals from the DP to the adjacent epithelial cells induce their differentiation into the inner root sheath (IRS) in which the future HS will develop. When the IRS is formed, the epithelial cells surrounding the DP (also known as matrix cells) surrounding the DP start to differentiate into distinct lineages to form the different components of the HS that will grow inside the IRS and eventually protrude through the epidermis. HF morphogenesis depends on Wnt, Shh, Notch, BMP and other signaling pathways. The Wnt pathway plays an essential role during hair follicle induction, Shh is involved in morphogenesis and

late stage differentiation, Notch signaling determines stem cell fate while BMP is involved in cellular differentiation. For an accurate HF morphogenesisafinely tuned interplay between signaling molecules and transcription factors which are either expressed or activated in keratinocytes or dermal fibroblasts is mandatory(6).

The coat type in dogs, an essential characteristic of the breed is determined by distinct mutations and their combinations in three genes, namely *RSPO2, FGF5,* and *KRT71* (encoding R-spondin–2, fibroblast growth factor–5, and keratin-71, respectively) (7). In cats coat types, such as hairlessness, curls or long hair are determined by variants of *KRT71, LPAR6, FGF5* genes(8-10).

ALOPECIA ASSOCIATED WITH ABNORMAL DEVELOPMENT OF THE HAIR FOLLICLE OR THE HAIR SHAFT

As in many organs, formation defects can result from failure of induction, morphogenesis or differentiation. **Abnormal development (dysplasia)** of HFs or HSs can develop during organogenesis or during postnatal life when the follicles is already cycling and thus may results in congenital alopecia or alopecia which develops early in life. Dysplasia can be of ectodermal or neuroectodermal origin. During organogenesis ectodermal dysplasia with and without HS formation can be identified. The neuroectodermaldysplasias occurring during organogenesis result in pigmentation disorders and are not associated with alopecia (e.g Albinism, Piebaldism, Waardenburg Syndrome). In postnatal life both ectodermal and neuroectodermaldysplasias occur and both are associated with alopecia.

Follicular dysplasia may result in the absolute absence of HFs (**aplasia**), in the formation of malformed HFs, or in the insufficient formation of HSs. Some forms of dysplasia are wanted by the breeders such as in hairless dog and cat breeds (e.g. Chinese crested dog, Mexican hairless dog, American hairless terrier, Sphinx cat) whereas in the majority of cases aplasia or dysplasia is an unwanted genetic disorder in specific breeds (11).In rodents und humans several genes resulting in follicular dysplasia have been identified(12, 13). In our domestic animals in most cases the definitive cause of these diseases is still unknown. In the following examples of well-known forms of dysplasia in domestic animals are listed.

Ectodermal dysplasia

- ***Aplasia of HFs:*** In these cases early placode formation during organogenesis does not occur. The mechanisms that regulate placode formation in the mammalian ectoderm are extremely complex and insufficiently understood.
  - X-linked HF aplasia and dental dysplasia (anhidrotic ectodermal dysplasia). The X-linked recessive inheritance in dogs has been confirmed by pedigree analysis (14). Mutations in the ectodysplasin 1 (*ED1*) gene are responsible for X-linked ectodermal dysplasia (XLED) in humans (15), cattle 2, (16-22) and mice (23). Mutation in the ectodysplasin (*EDA*) gene on the X chromosome in a colony of German shepherds has been described (24). Furthermore a splice defect in the *EDA* Gene in three mongrel dogs affected with an X-Linked Hypohidrotic Ectodermal Dysplasia has been described (25).
  - Aplasia of HFs without dental dysplasia. This from of aplasia occurs rarely.
- ***Dysplasia of HFs without/reduced hair shaft formation:***
  - Chinese crested dogs
  - Mexican hairless dogs
  - Hairlessness is inherited as a monogenic autosomal semi-dominant trait.
  - Hairless dogs are always heterozygous. Whereas the Mexican hairless is truly hairless some HFs develop in the Chinese crested. Mutation in the *FOXI3* gene are responsible for the hairless phenotype in the Chinese crested dog (26). It has been shown in mice that mutations in *FOXI3* result in the downregulation of several stem cell signature genes(27). This finding may also explain the phenotype in dogs.
  - AkhalTeke horses
  - Naked foal syndrome is inherited as a monogenic autosomal recessive trait and a nonsense variant of the *ST14* gene is responsible for this phenotype (28)
  - Congenital hyptrichosis and short life expectancy in Birmian cats
    This autosomal recessive syndrome is causes by a loss of function allele in the *FOXN1* (forkhead box N1) gene(29).
- ***Dysplasia of HFs with hair shaft formation:***I n the majority of these dysplastic disorders a HF is formed during organogenesis and the clinical picture first becomes evident when the HF starts to cycle (usually within the first

1 to 3 years of life). A genetic basis for these diseases is assumed, but the underlying genetic variant has not yet been identified. In the majority of these cases a HS of insufficient structural quality or an insufficient HC is described histologically. Known diseases associated with follicular dysplasia are:

- Alopecia of the Portuguese water dog (30)
- Alopecia of the Curly coated retriever (31)
- Alopecia of the Chesapeake Bay retriever (32)
- Alopecia in the Irish water spaniel (33)
- Alopecia in the Pont Audemer spaniel (34)

Neuroectodermal dysplasia

In this form of dysplasia the neuroectoderm-derived follicular melanocytes contribute to the alopecia. The definitive cause for these dysplasias is not yet understood.

- Color dilution alopecia: Coat color dilution in dogs is a specific pigmentation phenotype caused by a defective transport of melanosomes leading to large clumps of pigment. It is inherited and a transition in the melanophilin gene (*MLPH*) has been identified as the causative mutation for the dilute phenotype. However, this mutation is not necessarily associated with the development of alopecia (35). It has been suggested, that HSs with large melanosomal aggregates results in fracture of the HSs.
- Black hair follicular dysplasia: It is inherited in an autosomal recessive mode. Scanning electron microscopy has shown that the cuticle is absent over large areas of the hair fiber.

THE HAIR CYCLE

After the initial follicular morphogenesis, the HF is maintained by cycling through periodic stages which include a growth phase (anagen), a regression phase (catagen), and a quiescent phase (telogen).Thereafter a new cycle starts again (36, 37). During this next cycle phase the old club hair is shed in a process called exogen(38). Depending on the hair type and the species, the shedding of the hair fiber can occur at any time of the subsequent HC and thus is independent of the other cycle phases(39, 40). Therelease of the club hair is driven proteases(39, 41,42). Another term which has been introduced in HC terminology is "kenogen": It applies to HFs which have passed the telogen stage, lost their hair fiber (exogen) and remain empty for a certain time before a new anagen phase is initiated(39). Kenogen is known in veterinary literature as hairless telogen. Each of the HC phases has several subphases. The subphases of anagen, catagen and telogen have been described morphologically in the mouse and in the dog (43, 44).

The length of the HC phases determines the length of the HS and its replacement rate during shedding (45). The duration and length of the different cycle phases vary depending on the genetic background, the age, sex, body region, hormonal influence, neurogenic stimulation, composition of the extracellular matrix, nutrition, status of health, numerous environmental factors (day length or photoperiod, grooming, ambient temperature, friction and trauma), intrinsic factors (growth factors and cytokines), and drug therapy.

Hair growth in companion animals is not synchronized. Therefore distinct stages occur simultaneously in different follicles. Nevertheless in canine breeds that need clipping (such as poodles) the anagen stage predominates, whereas the percentage of telogen follicles in most other dog breeds is up to 34% (44, 46).

HAIR FOLLICLE STEM CELL DYNAMICS

To sustain cyclic regeneration, each HF relies on its epithelial SCs. SCs reside in niches that provide spatially distinct microenvironments for their maintenance and function. Consequently HF-SCs are not a single multipotent entity but that numerous SC populations and subpopulations, characterized by different markers exist (2, 4, 47, 48).

The current view is that HF-SCs exist as two functionally distinct pools: the activated hair germ cells with a cell populations which is more prone to proliferation and thus can more quickly respond to the environmental stimuli to engage in a new growth and the quiescent bulge stem cells, which maintain the long term stem cell pool (49, 50). The bulge is located close to the attachment of the arrector pili muscle (51-53). The secondary hair germ is located directly below the bulge in close vicinity to the dermal papilla, which provides key signals for HF development and regeneration.

The different SC populations have differing abilities to contribute to structures including the HF, the interfollicular epidermis, and the sebaceous glands (54). At late telogenLGR5-positive cells of the hair germ get activated and are the first cells that start to proliferate at anagen onset (49, 55). They give rise to transit amplifying cells, which form the germinative layer (matrical cells), that surrounds the DP before they terminally differentiate to form the HS, the IRS and the companion layer (3). It has been shown by clonal analyses, that the cells of the germinative layer are clonally related to each other (56, 57).

Several days after the activation of the hair germ (late telogen / early anagen) the HF-SCs in the mid and upper bulge proliferate briefly to self-renew (4). The HF-SCs of the lower bulge form the upper ORS. A third clonally distinctive cell population is named the lower proximal cup. These cells abut the DP at the proximal end of the HF and surround as a single cells layer the germinative matrix cells as outermost layer of the bulb. They extend distally and give rise to the lower ORS(3, 4). As the cells derived from the hair germ and those derived from the bulge grow downward, the DP is pushed further away from the bulge, thus prompting the HF-SCs to return to quiescence. In contrast the matrix cells still surround the DP and thus maintain contact. This is important because signals from the DP stimulate the matrix cells to continuously proliferate and to finally expand as a column upward, differentiate and generate the concentric layers of the IRS and the HS by expanding (reviewed in (4)). Each cell lineage induces the expression of distinct keratins.

Interspersed with the matrix cells are also melanocytes, which produce melanin granules that are transferred to the matrix cells, thus pigmenting the HS (58). The SCs that support the production of new melanocytes in each HC are neural crest-derived melanocyte SCs. They also reside in the bulge (59) and if they are not maintained graying of hair results (60).

Once a new HS grows out to a certain length, catagen is induced. During catagenmatrix cells and the cells of the lower ORS undergo apoptosis and push the HF into the involution phase of the HC. As regression proceeds, the bulb loses its volume due to the apoptosis and forms a narrow epithelial strand that retracts upward. At its end the DP is trailing. Simultaneously to the apoptosis of the bulb the ORS cells of the upper isthmus repopulate a new SC niche. By the end of catagen the HF is remodeled back into its resting phase (telogen) and the HC has been completed. At this stage, the HS is named club hair which is anchored by trichilemmal keratin, composed of Krt6 which is produced by the inner layer of the ORS.

As mentioned already above HF-SCs are not a single multipotent entity but extensive cellular heterogeneity exists within the mature pilosebaceous unit. The SC associated marker expression has been the topic of a number of excellent recent reviews and differences between human and murine SC markers have been outlined (4, 61, 62) .

HAIRCYCLE CONTROL

The coordination of the HC phases and the SC activity is dependent on complex interactions between signals of the follicular (niche components) and dermal microenvironment (*e.g.* keratinocytes, fibroblasts, adipocytes, immune cells, nerve fibers), macroenvironmental factors (*e.g.* hormones, genetics, age) and of environmental factors (*e.g.* day light, nutrition, circadian rhythm) (63-65). In the mouse the signaling crosstalk between between SCs and their niche has been well characterized by now. The state of the murine HF is mediated by a complex, delicately balanced interplay between transcription factors implicated in, as well as proteins belonging to, several signaling pathways, which constantly compete with each other. The pathways include Hedgehog, Wnt/β-Catenin, transforming growth factor (TGF)-β, Fibroblast growth factor (FGF), bone morphogenic protein (BMP) and Notch signalling(reviewed in (4, 66). However, despite extensive research in the field the complete understanding how the niche elements work together and how they interact with macroenvironmental and environmental factors to initiate and promote a new HC and thus to regenerate a new HF is still not complete. In general it can be noted that BMP signals derived from dermal, adipose and epithelial tissue repress anagen induction while Wnt signals promote SC activation and entry into anagen. In more detail bulge SCs maintain a slow-cycling behaviour due to an overabundance of BMP, TGF-β, FGF18 signallingas well as the inhibition of Wntsignaling. If the balance of inhibiting signals is tipped towards more activating signals new anagen is initiated. Wnt signaling is also important for the proliferation of matrix cells and thus anagen progression. BMP ligands and receptors regulate the terminal differentiation program of the IRS and HS precursors. In addition Notch signaling is critical for differentiation into the mature HF (reviewed in (4)). The induction of catagen

is promoted by molecules such as neurotrophins, FGF5, p75, p53, TGFβ1, Edarsigalling and BMPRIa(Botchkarev et al., 1999; Botchkarev et al., 1998;(67, 68). Gasdermin A3 has been recently shown to be important for the transition from catagen to telogen by balancing the Wntsignalling pathway (69).

ALOPECIA ASSOCIATED WITH AN IMPAIRED HAIR CYCLE

All alopecic disorders of this group are associated with a deregulation of the HC but as said before the pathogenesis in none of these diseases is understood.

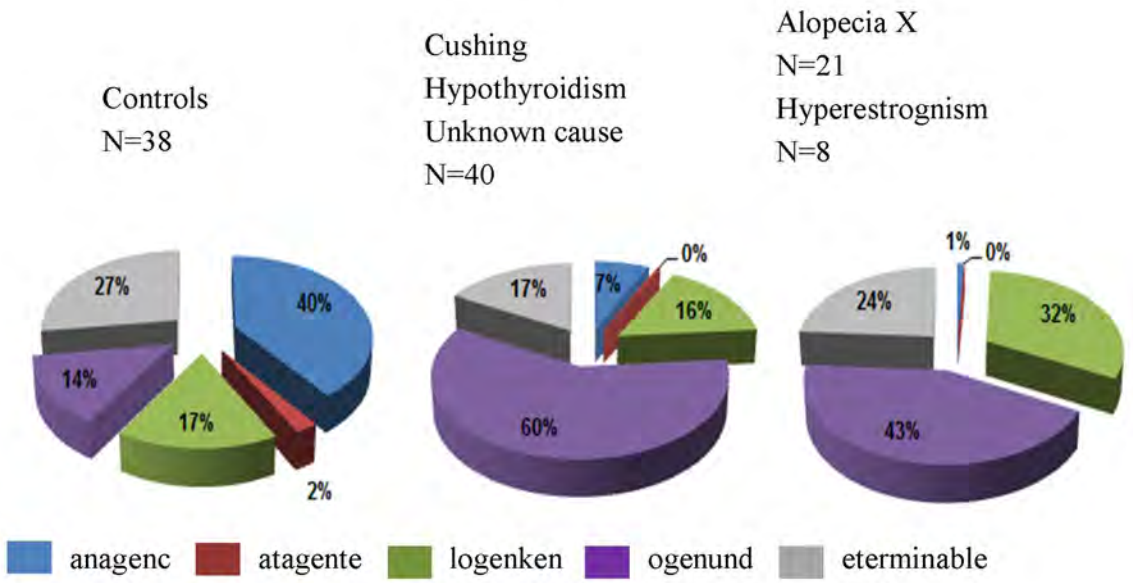
The following diseases in dogs are associated with and impaired HC:

- Endocrinopathies
  - Hyperadrenocorticism
  - Hyperestrogenism
  - Hypothyroidism
- Alopecia X
- Recurrent flank alopecia
- "Post inflammatory alopecia" and HC arrest of unknown cause
- Telogen effluvium
- Post clipping alopecia
- Alopecia associated with miniaturization of the HFs
  - Canine pattern alopecia

In all of these diseases it is most likely that extrinsic or intrinsic factors are missing resulting in a disturbance of the normal HC. In a study investigating some of the HC arrest disorders (endocrinopathies, alopecia X, alopecia of unknown origin) we could show that in all of these diseases a 3-4 fold increase of kenogen follicles is seen histologically whereas the number of anagen follicles decreases dramatically. There is no significant increase in telogen follicles, with the exception of alopecia X and hyperestrogenism(46) (figure 2).

The increased amount of kenogen follicles indicates either that exogen has occurred prematurely and the empty telogen follicle is not yet competent to enter new anagen or that the induction of a new anagen phase does not occur after the telogen phase has been finished and the old club hair has been shed during exogen. A third possible cause may be that signals which are maintaining the anagen phase (anagen promotion) are missing, the HFs enter catagen prematurely and thus enter the subsequent telogen phase earlier. At the same time the initiation of new anagen is not more effective which results in a higher number of telogen follicles which lose the hair shaft and thus stay in kenogen until adequate signals initiate new anagen (e.g. alopecia X).

Figure 2: Percentage distribution of HC stages in dogs with different alopecic disorders compared to control beagles (adapted from (46))



If a HF stays in kenogen for a prolonged time this follicle may also get atrophic.The above-mentioned increased amount of kenogen follicles in alopecia is also observed on the human scalp (70, 71).

No matter which of the above mentioned possibilities (namely premature exogen, lack of anagen promotion or lack of anagen induction) is causative for the different forms of canine alopecia associated with HC arrest they are all the result of a deregulation of the HC. As discussed before the HC is dependent on a fully functional stem cell (SC) compartment and well balanced signaling events that may be influenced by the follicular micro-and macroenvironment which stimulate the activation and differentiation of SC cells and their progeny. Because the activation of the follicular SCs is depending on input from both, activators and inhibitors, the maintenance of the HC is not a "yes or no" decision and cycle after cycle, gradually more and more HFs fail to enter anagen and alopecia becomes more apparent (72). This is one of the reasons why also histologically HC arrest disorders vary largely.

In the following some mechanisms will be outlined which might be associated with the above mentioned HC arrest disorders of dogs based on experimental evidence from mice and humans (summarized in Figure 3). However, the understanding of the detailed pathomechanisms which are causative for the disturbance of the tightly controlled growth phases of the HC resulting in HC arrest disorders are still missing.

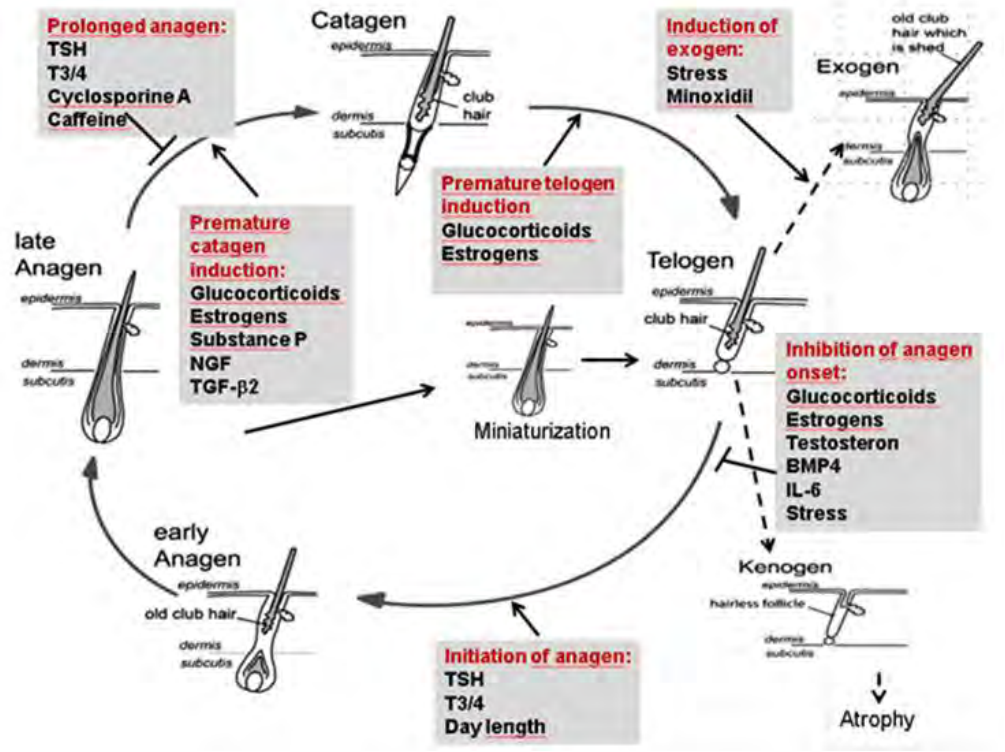


Figure 1: Summary of inhibiting and stimulating factors which might be involved in involved in hair cycle disturbances. Data are derived from mice and human.

In the following some possible pathomechanisms leading to an impaired HC in different disorders are outlined but due to space and time reasons not complete.

Hyeradrenocorticism:

- Impaired anagen onset:
  It has been shown that glucocorticoids delay anagen onset (human), reduce the number of dermal papilla cells (human) and downregulate expression of Ki-67, VEGF and HGF (human) (73).
- Shortenedanagen:
  In mice steroids reduce mitotic activity of matrix cells and stimulate differentiationof matrix cells resulting in premature catagen induction (74).

Hyperestrogenism:

- Inhibition of anagen onset:
  In mice estrogens upregulate BMP4 prevents the transition of HFs from telogen to anagen(75)
- Premature catagen induction:
  Estrogens induce apoptosis by upregulation of HC relevant genes and signaling pathways (MAPK and BMP/TGF) in mice(reviewed in (76)).In humans estrogen inhibited matrix keratinocyte proliferation and enhanced their entry into apoptosis (77)

Hypothyroidism:

- Shortened anagen
  In humans T3/T4 downregulate apoptosis of matrix cells, T4 upregulates proliferation of matrix cells and prolongs duration of anagen(78).
  In mice and rats topically applied thyroid hormone (T3) enhances both skin and hair growth (79).

Alopecia X:

- The strong predisposition of the disease for breeds with a plush undercoat such as Pomeranians and Nordic breeds but also for miniature and toy poodles, pedigree analysis of affected dogs and the onset of the disease at a relatively young age suggests a hereditary background (80-82). However, despite extensive research the pathogenesis is still unknown.
- Mutations in the canine 21-hydrolase gene, the *CTSL2* gene and the *PTCH2* gene have been excluded to be causative for alopecia X (80, 83,84).
- Abnormalities of sex hormones or adrenal steroid intermediates are noted in some affected dogs, others are normal in this aspect (85-87).
- Treatment with melatonin, growth hormone, medroxyprogesterone acetate, trilostane, and an GnRH analogue, respectively was some but not all dogs (86-90)
- In an own study we found a downregulation of key regulator genes of the WNT and SHH pathways, both important HC regulators and a downregulation of some SC markers. Furthermore, genes relevant for GnRH, melatonin and estrogen metabolism were dysregulated in AX, which is in agreement with the suspected but still unproven pathomechanism for this disease (91)

Recurrent flank alopecia:

- The high incidence in some breeds and the familial character in some breeds suggest a genetic predisposition. Studies toprovethisare not available(34).
- Seasonality and response to melatonin suggests an association with the photoperiod. It has been shown that long daylight hours initiate short periods of daily melatonin secretion resulting in summer coat, while short day-length increases melatonin secretion and stimulates a longer, warmer pelage (92, 93).
- Expression of FGF18 in the HF is lower in dogs with RFA. However, since FGF 18 is expressed mostly in the anagen follicle and the number of anagen follicles is reduced in recurrent flank alopecia this result is not unexpected (94).
- Lesional skin grafts of dogs with recurrent flank alopecia on the back of athymic mice resulted in hair regrowth faster than in the donor dogs. This suggests that the pathogenesis of recurrent flank alopecia may be mediated by systemic rather than local factors (95).
- A recent study in mice has shown that the circadian clock influences the mitotic activity of matrix cells. The mitotic activity is higher in the morning than in the evening resulting in faster hair growth in the morning (96). These data may at least partially explain the reduced hair growth in periods of shorter day length.

"Post inflammatory alopecia" and HC arrest of unknown cause:

We receive many skin biopsies in which a non-inflammatory alopecia associated with HC arrest is visible but the underlying cause remains unclear even after a thorough clinical workup. We also see often histological features of a HC disturbance in dogs with a perivascular dermatitis. Examples of possible mechanisms resulting in the above pathology are:

- In mice sonic stress results in the recruitment of substance P and nerve growth factor which results in perifollicular inflammation, HF keratinocyte apoptosis, premature HF regression (catagen induction) and inhibition of HF keratinocyte proliferation (reviewed in (97)).
- In systemic stress conditions plasma glucocorticoids are usually elevated through activation of the hypothalamo-pituitary-adrenal axis (98). The effects of steroids have been discussed earlier.
- Chronic restraint stress increases the number of substance P immunoreactive nerve fibers and activates mast cells (99)
- Chronic restraint stress inhibits hair growth by prolonging the telogen stage and delaying the subsequent anagen and catagen stage (99).
- Mast cell degranulation triggers catagen induction (100).
- Stress is associated with premature catagen development in mice (100).
- Data from mice suggest that IL-6 family members inhibit hair growth in a dose dependent manner. Since increased levels of IL-6 are present in dermal inflammation IL-6 may play a partial role hair growth inhibition seen in inflammatory disease (101).

These data suggest that also in dogs stress or inflammatory mediators released by mast cells or lymphocytes may result in HC arrest disorders.

Telogen effluvium:
Telogen effluvium is the result of an excessive premature shedding of the hair shafts (exogen). Thereafter synchronized initiation of new anagen occurs. Telogen effluvium in humans usually occurs 2 to 4 month after the initial trigger.

In humans telogen effluvium is subdivided into five different types, namely immediate anagen release, delayed anagen release, short anagen syndrome, immediate telogen release, delayed telogen release(34).

In our domestic animals this distinction is not made. The exact underlying causes for the synchronization of the HC are not known. It has been shown however, that in mice psychoemotional stress prematurely terminates the normal duration of the anagen phase in vivo (100).

Post-clipping alopecia:
Post clipping alopecia is characterized by a lack of hair regrowth after clipping. The underlying cause is unclear but since it occurs more often in dogs with a prolonged telogen HC (e.g. Nordic breeds) it may well be that hair regrowth in these dogs takes longer if they are clipped during an early telogen phase just because of the cycle length.Other factors that have been proposed are (34):

- Vascular perfusion changes in response to cutaneous temperature changes after clipping
- Time of the year when hairs are clipped
- Geographic region

Canine pattern alopecia (miniaturization of HFs):
Canine pattern alopeciais histologicallysomewhat similar to human androgenetic alopecia because in both alopecic disorders a miniaturization of HFs is present.It has been suggested that the miniaturization of HF is the result of a shortened anagen phase (102). However, unlike in human pattern baldness, an androgen receptor dysfunction has not been proven in dogs and therefore the pathogenesis is still unknown. There is a strong breed predisposition suggesting a genetic background like in humans. Some of the findings in humans with androgenetic alopecia are:

- Like in dogs a genetic predisposition has a key role in the development of androgenetic alopecia and mutations in four genetic risk loci have been identified in man (103).
- The CD200-rich and CD34- positive HF progenitor cells in the bald scalp are reduced suggesting a defective formation of the hair germ (104).
- Extrafollicular factors must be involved in adrogenetic alopecia as it is age and sex dependent (64, 105)
- A correlation between hair size and the number of DP cells has been noted in human HFs. (106).

- Men with androgenetic alopecia typically have higher 5-alpha-reductase, lower total testosterone, higher unbound/free testosterone and higher free androgens, including Dihydrotesterone

In summary despite substantial progress in understanding the HF physiology and pathology there is still substantial lack in the knowledge about human HFbiology and only few studies are available for domestic animals. Partially this is due to the fact that no in vivo studies can be performed. However, new genetic and in vitro research tools are getting more available whick will allow to perform functional studies.

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Lymphocytic isthmic mural folliculitis with hair regrowth in a dog

J. M. NOVAK*, V. K. AFFOLTER+, B. G. MCMAHILL*

**IDEXX Laboratories, Westbrook, ME, USA*
+Department of Pathology Microbiology and Immunology, School Veterinary Medicine, University of California Davis, CA, USA

Abstract: An eight-year-old male neutered white Havanese, presented with chronic unresolved skin problems,which included marked to severe pruritus, alopecia, and ulcerations.Physical exam revealed generalized patches of erythema, scaling and alopecia on head, neck, back, and forelimbs. There was yellow scaling and crusts on the tips of both pinnae. Mild erythema was present around both eyes. Clinical differential diagnoses included: chronic allergic dermatitis/atopic dermatitis, dermatophytosis and scabies. Skin scrape was negative for mites. Dermatophyte DTM culture negative. HESKA allergen panel revealed various environmental sensitivities. Patient was seen multiple times over the course of the following 3 months and treated with a combination of antibiotics (cefpodoxime, cefovecin, cephalexin), antifungal (ketoconazole), shampoo bath (VetraSeb+PS), ivermectin (injection and oral), prednisone and dexamethasone(oral and injection), Apoquel, Temaril-P, and omega-3fatty acids.Due to poor response to treatment, histopathology was elected. Five punch biopsy specimens were taken from neck, hind legs, and abdomen. Histologic findings included lymphocytic mural and perifollicular inflammation at the level of the isthmus and infundibulum. Fewintraepidermallymphocytic infiltrates were also present. Sebaceous glands were diffusely absent, with only raresmall lobules remaining. There was mild to moderate follicular infundibular hyperkeratosis, acanthosis and epidermal lamellar hyperkeratosis and parakeratosis. Special stains did not reveal presence of dermatophytes and there was no evidence of intrafollicular Demodex mites. Histopathologic differential diagnosis included: allergic reaction, pseudopelade, and possible drug reaction. The patient was treated with cyclosporine, omega-3 fatty acids, and shampoo baths (Ketohex). Over a period of 3 months, complete resolution of the cutaneous problems was achieved with hair growing back in a different color.

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Non-infectious mural folliculitis in a dog

T. OSUMI*, Y. KAWAMURA†, I. MITSUI†, K. NISHIFUJI*

**Animal Medical Center, Tokyo University of Agriculture and Technology, Tokyo, Japan.*
†No Boundaries Animal Pathology, Tokyo, Japan.

Signalment: Estimated 10-year-old (owned from dog shelter), castrated-male Golden retriever.

History and signs: The dog presented with a 9-month history of non-pruritic, well-demarcated macular alopecia initially recognized on the left cubital flexor area that spread to the neck and periauricular areas. The dog also had a gastrointestinal stromal tumor that was diagnosed 2-month before and surgically excised. Trichogram, skin scraping and cytology revealed no microorganisms or parasites on the skin lesions.

Our histopathological differential diagnoses:

- Eosinophilic mucinotic mural folliculitis
- Isthmus mural folliculitis (pseudopelade)
- A disease resembling multifocal, spontaneous noninfectious alopecia in Norwegian puffin dogs (Bergvall KE et al, Vet Dermatol 25:112-119, 2014)

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Extensive reticular atrophic alopecic lesion in a dog due to dermatophytosis.

J. WERNER* and S. R. T. DALTRO.**

**Laboratorio de Patologia Veterinária Werner&Werner, Curitiba, Paraná, BRAZIL.*
***Clínica Veterinária Doutor Derme – Medicina Diagnóstica, Salvador, Bahia, BRAZIL.*

Abstract: The purpose of this report is to describe a dermatology case with unusual clinical and histopathologic pattern and interesting resolution. A one-year-old female Shih Tzu dog was presented for evaluation of an alopecic lesion in the ventral region of the neck, which had an extensive area of multifocal lightly erythematous atrophic alopecia interspersed with normal skin, which caused a reticular pattern to the lesion. Irregular thickenings were palpable. No other cutaneous lesions were present elsewhere in the body. The patient was treated for the same lesion before with oral corticosteroids for two weeks and antifungal medication in spray for 20 days, but the lesion did not improve. There was no history of prior cutaneous traumaor use of harness or collar. Clinical differential diagnoses were dermatophytosis, atrophodermia, and ischemic scarring. Histopathologically, the epidermis was normal. There were multifocal areas of mid and deep dermal fibrosis that matched the clinical atrophic alopecic lesions. Between the areas of fibrosis, besides normal pilosebaceous units, there were granulomas at follicular and perifollicular regions. Hair shaft fragments were observed in the center of some granulomas and one of such fragment was infiltrated by tubular structures compatible with fungal hyphae, which stained positively with periodic acid-Schiff. No spores were noted. The follicles and sebaceous glands, when not obliterated by the inflammatory reaction, were normal. A histopathological diagnosis of dermatophytosis was made and the dog was treated with topic terbinafine 1% twice a day until the last contact with the veterinarian. The lesions regressed considerably over the course of 21 days. Hair regrowth started after two weeks of treatment and, surprisingly, the atrophic scars regressed with resolution of the reticular pattern.

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CUTANEOUS AMYLOIDOSIS - SEARCHING FOR AN UNDERLYING CAUSE

Affolter VK*, Woods L†, Harrison L§

** Department of Pathology, Microbiology, Immunology, School of Veterinary Medicine, UC Davis, Davis CA, USA*
† California Animal Health and Food Safety Laboratory, Davis, CA USA
§ Willow Oak Equine, Woodland CA, USA

Abstract: A 6 year-old Quarter horse mare was presented for development of multiple skin nodules that had been present for 6 months and were originally diagnosed as urticaria. There was no response to treatment with antihistamines. At the original initial visit, the mare also exhibited upper respiratory signs. These were thought to have an infectious etiology as the mare had recently been participating at a competition. The respiratory signs resolved, however over the next 5 months the number as well as the size of the skin nodules increased. Waxing and waning of the lesions was not observed and the mare did not show signs of pruritus. The mare also appeared increasingly less active and had decreased appetite. On presentation at the time of skin biopsy, numerous skin nodules were noted all over the body, except for distal extremities, ranging in size from 1-3 cm in diameter. The overlying skin was intact and the nodules were not painful. Some nodules appeared to affect deeper tissues. Enlarged inguinal lymph nodes were palpated. Several nodules were biopsied and submitted for histology.

All samples revealed prominent deposition of a pale glassy eosinophilic material suggestive of amyloid with associated lymphoid, plasmacellular and histiocytic infiltrates. Histologic features as well as results from ancillary testing and clinical follow-up will be presented.

EQUINE DERMATOLOGY - CORRELATION OF CLINICAL PRESENTATION WITH HISTOPATHOLOGY

Affolter VK*

** Department of Pathology, Microbiology, Immunology, School of Veterinary Medicine, UC Davis, CA, USA*

I. NON-INFLAMMATORY ALOPECIA WITH/WITHOUT SCALING

Infectious folliculitis causes alopecia, but it would be rare to not have other lesions such as papules, nodules and crusts accompany bacterial folliculitis, dermatophytosis, or dermatophilosis. The diseases in this first group can cause skin lesions that clinically lack any visibleevidence of inflammation or have very mild clinical signs of inflammation, at least early in the disease process. As some diseases can be associated with pruritus or may develop secondary pyoderma, signs of obvious inflammation may develop secondarily.

Alopecia areata (AA): [1-4] well-circumscribed areas of alopecia not associated with scaring and or obvious features of inflammation (erythema or crusting). Possible predilection for Appaloosas and Palominos. Clinical presentation: Face, neck and trunk are predilection sites. Lesions are non-pruritic. Mane and tail can be affected ("mane and tail dysplasia/ dystrophy of Appaloosas" is likely a form of AA). Alopecia universalis is a more widespread form affecting the entire body. Occasionally,hoof quality can be affected by the process. Pathogenesis: Immunologic attack on anagen hair follicles characterized by antibodies against trichohyalin, inner and outer root sheath as well as CD4+and CD8+ T cells and CD1+ dendritic. Diagnosis: Requires biopsy. Histopathology: Step sections are often required to find affected follicles with peribulbar lymphocytic dermatitis, bulbitis (CD4+ and CD8+ T cells) and peribulbar fibrosis. Hair follicles may appear dysplastic. Clinical follow up: Lesions may regress, and then reoccur in a waxing and waning pattern or they may persist. Corticosteroids, Minoxidil and cyclosporin have been used for management of AA.

Linear alopecia:5 Quarter horses are predisposed. Clinical presentation: linear, vertically arranged alopecia most commonly occuron the neck, shoulder and thorax. The skin may be hyperpigmented and scaly, no pruritus or pain is noted. Occasionally,lesions are covered by a thick layer of keratin (linear keratosis). Pathogenesis: The etiology of this condition is not known. Early stages maybe characterized by a granulomatous mural folliculitis, a process that has been associated with drug reaction in other species. Diagnosis: Clinical presentation is usually fairly diagnostic. Histopathology: Early stages reveal lymphocytic infiltrative mural folliculitis that may be accompanied by keratinocyte apoptosis. This tends to transition into a granulomatous mural folliculitis with destruction of hair follicles. Special stains and cultures are consistently negative. The chronic stage is characterized by loss of hair follicles. Marked hyperkeratosis can be observed. Clinical follow up: Hair regrowth is usually not observed. Differential diagnosis: Linear epidermal nevus.

Telogen effluvium:[6] Clinical presentation: Sudden hair loss, often generalized. Pathogenesis: Various underlying causes (fever, pregnancy, shock, severe illness etc.) result in sudden cessation of anagen hair follicles with simultaneous transition into catagen and telogen. A few weeks after the incidence there is sudden hairloss with a new anagen phase starting. Diagnosis/histopathology: Careful evaluation of the history may elucidate presence of telogen effluvium. Evaluation of hair shafts reveals presence of club hairs. Biopsies show presence of numerous anagen hair follicles and absence of inflammation. Clinical follow up: Normal hair coat will develop as germinal cells are not affected.

Selenosis:[7] Clinical presentation: There is progressive hair breakage and loss of mane and tail hair. Alopecia can become generalized. Additional lesions include hoof deformities. Pathogenesis: Soil rich in selenium results in accumulation of selenium in certain plants that are ingested. Selenium may interfere with oxydative enzyme system (affecting sulfur-containing amino acids in the keratins) Diagnosis: Measurement of selenium levels in blood (2ppm) and hair shafts (10ppm m). Histopathology: High levels of selenium can also induce transition into catagen and telogen. Hair follicles become atrophic and devoid of hairshafts. There may bedyskeratosis and mild superficial follicular keratosis. Clinical follow up: effect is reversible but it is a long recovery.

Anagen defluxion:[6] Clinical presentation: Sudden hair loss, often generalized. Pathogenesis: Underlying causes (toxic, fever, metabolic) interfere with anagen stage. The hair follicles shutdown. Hair shaft abnormalities can be observed.

Diagnosis: History and biopsy. Histopathology: There is matrical cell apoptosis. Clinical follow up: Normal hair coat will develop as germinal cells are not affected.

Sarcoidosis:[8] Clinical presentation: There is marked alopecia and exfoliation often starting on the face, neck progressing to trunk. Pruritus and pain may be present. Over time the lesions may becomeovertly crusting. There is no breed or age predilection, geldings may be predisposed. Affected horses often exhibit weight loss and internal lesions may develop (granulomatous inflammation in lungs and liver, osteopenia). Pathogenesis: No infectious etiologies have been identified despite studies where tissue culture,special stains, immunohistologyand PCR have been performed on lesional skin. Diagnosis: Biopsy is required. Histopathology: There is a nodular to coalescing, randomly arranged dermal infiltrates predominated by histiocytes and admixed multinucleated giant cells. Lymphocytes and other inflammatory cells may be present, but are not a prominent feature. Lesions are often referred to as a "naked granulomas". Clinical follow up: Prognosis for horses with internal lesions is poor. Horses with lesions limited to the skin may wax and wane with some lesions resolving but lesions may recur.

Epitheliotropic T cell lymphoma (ETCL), pagetoid form: [9,10] Very rare in horses. Clinical presentation: The alopecia with variable amount of scaling tends to be most prominent when neoplastic cells are infiltrating hair follicle epithelia. With epidermis involved, the skin tends to be erythematous and can become erosive and ulcerative. Documented cases of ETCL in the horse have lesions involving the face, muco-cutaneous junctions and; in one horse the lesions were generalized. Pathogenesis: Neoplastic T lymphocytes are homing and invading epidermis as well as hair follicles and sweat glands. The T cells form intraepithelial aggregates. Diagnosis: biopsy: In many cases immunohistochemistry and sometimes clonality are required to confirm the diagnosis. Histopathology: Biopsy is required. CD3+, CD8+ T lymphocytes (personal observation) are dispersed within the epithelia and form intraepithelial aggregates. Confirmation is done by immunohistochemistry and clonality testing for TCR arrangement. This is particularly important in very early stages, which can mimic inflammation. Clinical follow up: Not many cases have been documented, but lesions are progressive.

Onchocerciasis:[11,12] Adult filarial Onchocercacervicalis live in the nuchal ligament and can initiate a granulomatous response. The microfilaria initiate the cutaneous lesions. With routine prophylactic use ofavermectins cases of cutaneous onchocerciasis have not been observed in most countries. However, as occurrence of cutaneous habronemiasisis clinically increasing in some areas, perhaps onchocerciasis has the potential to do the same. Clinical presentation: Typically irregular, often circular areas of alopecia are associated with some degree of scaling as well as leukoderma. Over time there is marked lichenification, and erosions,and ulcerations may develop. Lesions may be limited to head and neck; another common location is the ventral abdomen. Some horses present with severe ocular lesions. Pathogenesis: Adult parasite is located in the nuchal ligament from which the microfilara (2000-240um) migrate to the skin, typically to the umbilical areas as well as head and neck. Larvae are then picked up by Culicoides spp., gnats and potentially mosquitos, which all can act as intermediate hosts. Diagnosis: the microfilara can be identified by mincing a skin biopsy an immersing it in physiological saline at room temperature. The microfilara tend to migrate out of the tissue into the fluid and within 30 minutes of incubation can often be observed under the microscope. Histopathology: Variable perivascular to interstitial infiltrates which is predominated by eosinophils, but may be accompanied by lymphocytes and plasma cells. The microfilara are seen amidst the inflammatory cells. Clinical follow up: Leukoderma tends to be irreversible as well as alopecia due to scaring.

Equine sarcoid, flat/occult form:[13-15] Clinical presentation: Predilection sites are perioral, periorbital, area and neck. Most commonly clinical presentation is circular areas of alopecia, which may be associated with scaling. On palpation the skin is slightly thickened. Occasionally small papules develop. Pathogenesis: Previous trauma to the area has always been discussed as an underlying factor laying the ground for infection with bovine Papilloma virus 1 and 2, which both have been associated with equine sarcoid. Early genes E5, E6, E7 are associated with the malignant transformation of fibroblasts by down-regulating mammalian suppressor genes. Diagnosis: Biopsy is required and in questionable cases in situ hybridization for Papilloma virus. Careful: positive PCR for Papilloma virus can be found with other lesions, as Papilloma virus is ubiquitous. Histopathology: A band of proliferative spindle and stellate cells abut the variably hyperplastic epidermis. Although not invading follicular epithelia, the follicles tend to be atrophic in that area. In situ hybridization visualizes presence of Papilloma virus. Clinical follow up: If not traumatized, these sarcoids

tend to either stay static or grow slowly. If traumatized they tend to transition into a verrucous or fibroblastic sarcoid.

II. THE PRIMARY LESION IS DEPIGMENTATION

Acquired loss of pigment can occur in association with inflammatory processes. Leukoderma and leukotrichia is a consistent feature of interface dermatitis, a process characterized with cytotoxicity of keratinocytes and resultant pigmentary incontinence. Some examples include cutaneous lupus erythematosus and interface drug reactions. Primary depigmentation with a clinical lack of evidence of inflammation is rare.

Vitiligo:[16] It can occur at any age, but most commonly in young horses. Arabians are predisposed. Grey horses are more commonly affected. Clinical presentation: Annular leukoderma (depigmented maculae and patches) most commonly occurring on the lips, muzzle, around the eyes, anus, vulva or prepuce. Other body areas and hooves can be affected as well. Pathogenesis: Several mechanisms have been proposed. Anti-melanocyte antibodies have been identified in Arabians. Alternatively, autotoxicityhas been proposed by melanocytes being susceptible to either melanin precursors (dopachrome) or inhibition of free radical scanvenger (thioredoxinreductase). Diagnosis: History and biopsy. Histopathology: At an early stage there is mild lymphocytic infiltration with mild multifocal lymphocytic exocytosis. At a later stage there is complete absence of melanocytes. Clinical follow up: Loss of melanocytes is permanent.

Spotted leukotrichia:[16] More commonly seen in Arabians, Thoroughbreds and Shires. Clinical presentation: 1-3cm in diameter areas of leukotrichiaare typically present. Associated leukoderma is unusual. Pathogenesis: Not known. Diagnosis: Clinical presentation and biopsy. Histopathology: Absence of melanocytes and pigment in hair bulbs and hair shafts. Clinical follow up: Both spontaneous remission as well as permanent lesion are seen.

Hyperestheticleukotrichia:[17] There may be a predilection for Arabian and paint horses. Most cases are seen during spring and summer. Clinical presentation: Typically a reticulated pattern of leukotrichiaover the back is present. The lesionsare extremely painful and may be associated withcrusts as well. Pathogenesis: Etiology and pathomechanism are unknown. Herpes virus has been suggested as a possible triggering factor. Diagnosis: Clinical presentation of depigmentation associated with pain. Histology can support the diagnosis. Histopathology: Typically there is dermal edema and presence of large stellate cells, which may contain hemosiderin. Spongiosis may lead to spongiotic vesicles. There may be features similar to erythema multiforme with keratinocyte apoptosis. However, lymphocyte satellitosis is not a prominent feature. Clinical follow up: There is spontaneous regression, however, lesions may reoccur.

III. LESIONS PRIMARILY AFFECTING LEGS

Scratches / pastern dermatitis / mud fever/ greasy heel:[18] These terms are used interchangeably to describe a variety of diseases affecting the pastern areas, mostly occurring in adult horses; there is no breed or sex predilection except for draft horses (please refer to chronic progressive lymphedema below). Photoaggravatedvasculitis is discussed separately. Clinical presentation: Erythema, exudation, crusting with is predominantly seen in palmar and plantar areas of the pastern region, but may extend cranially as well as further proximal to fetlock. There may be swelling of the lower extremities (referred to as "stocking up"). The lesions can be painful as well as pruritic. Pathogenesis: Predisposing factors are mud, moisture, contact hypersensitivity, insect bites and potentially minor small traumas. The skin gets infected with either bacteria (Staphylococci, Dermatophilus) or dermatophytes (Trichophyton). Chorioptes infestation can be a primary cause for pastern dermatitis. Diagnosis: Skin scrapings, cytology as well as bacterial and fungal cultures to identify infectious etiologies. Biopsy can assist in recognizing other etiologies for skin disease in this location. Histopathology: Depending on the initiating factors there is severe superficial pleocellular dermatitis with crusting and luminal folliculitis. Clinical follow up: Lesions usually respond to appropriate therapies, except for draft horses where an underlying lymphedema persists (see below).

Chronic progressive lymphedema:[19] This is a disease affecting many draft horse breeds, including Shires, Clydesdales, Belgians, some German draft horses, Percherons, Friesian, and Gypsy Vanners. Clinical presentation: Early on(lesions may develop as early as 2 years of age) there is slight edema of the lower legs, which often is not detected due to the feathering in these breeds. Tendons and canon bone are no longer distinct and the lower legs have a more cone-like shape. The heavy feathers together with the prominent keratin layer and large numbers of sebaceous glands predispose for bacterial infections and mite infestation (Chorioptes). With each inflammatory event the lymph edema

is increasing and with chronicity there is induration of the edema, formation of folds as well as proliferative nodules. Decreased perfusion and persistent stagnation of lymphatic drainageincrease the risk for development of repetitive infections. The lesions may progress up to involve the hock and/or carpi. Pathogenesis: Given the extremely high incidence within these breeds a genetic predilection has to be considered. Currently, the exact genetic underlying factors have not been identified. Diagnosis: The clinical presentation tends to be very suggestive for the diagnosis. Routine skin punch biopsies are often not diagnostic (characteristic lymphatic lesions tend to be in the deeper tissues). Lymphangiography demonstrates the changes of the lymphatics. Histopathology: A helpful indication is the disarray of the dermal elastin fiber network. There is dermal fibrosis and various signs of secondary infections may be seen histologically. Clinical follow up: It is a progressive disease. Withelaborate management the horses can be kept comfortable, but there is no successful permanent treatment for the lymphedema.

Phototaggravatedvasculitis:[20] Clinical and histologic lesions cannot be distinguished from a true photosensitization and clinical work up (other depigmented areas on the body affected, history of certain drug or plant exposure, evidence of liver diseases). Clinical presentation: Typically affects non-pigmented areas of the distal legs. It can be limited to one leg despite having multiple non-pigmented legs. Rarely, pigmented legs may also be affected. Erythema, oozing or exudative erosions and ulcerations are observed on affected legs, the affected legs may also be edematous. Lesions tend to be painful rather than pruritic. Pathogenesis: Likely an immune-complex associated process, the trigger of which is still unknown. Percutaneous absorption of antigens as well as drugs have been discussed as possible triggers. Diagnosis: Biopsy. Histopathology: Typically there is a leukocytoclasticvasculitis within the superficial dermis with micro-hemorrhage, serum exudation. There is often fibrinoid necrosis of vascular walls. As lesions progress there will be fibrosis and chronic change of vascular walls, characterized by hyalinizationof vascular walls and loss of endothelial cells. Clinical follow up: Exposure to sunlight should be avoided.

Cannon keratosis ("stud crud"):[21] Cranial aspect of cannon bone area is affected, more often affecting hind legs thanfront legs. Predominant clinical lesion is hyperkeratosis associated with alopecia. Pathogenesis: The etiology of this condition is not known. Alopecia develops as hyperkeratosis extends into the hair follicles. Diagnosis: Clinical presentation is usually diagnostic. Lesions can become secondarily infected if fissures develop. Histopathology: Marked distention of infundibula with follicular and superficial compact hyperkeratosis, parakeratotic caps and a mild perivascular dermatitis. Clinical follow up: KY jelly, anti-seborrheic shampoos, tacrolimus, propylene glycol are used for management.

Cutaneous habronemiasis:[22] Despite good response to avermectins, it appears that cutaneous habronemiasis is seen with increasing frequency. Clinical presentation: Typically, when lesions develop on the legs,they are localized to the distal extremities. There is marked ulceration with thickening of the underlying dermis and subcutis. Within the granulation tissue, firm granular material ("sulfur granules") can often be expressed. Pathogenesis: H. muscae, H. majus, Draschiamegastoma. Larvae are deposited on the skin by house flies (Muscadomestica). With minor trauma present, developing larvae may enter the dermis and a marked hypersensitivity reaction to the larvae leads to severe inflammation. Diagnosis: Impression of eroded lesions reveals numerous eosinophils and possible small sulfur granules with entrapped larvae. Histopathology: A nodular to diffuse predominantly eosinophilic dermatitis has entrapped foci of eosinophilic material centered around Habonema larvae. Typically the overlying epidermis is ulcerated. Clinical follow up: As it is a hypersensitivity reaction lesion,it may reoccur with new seasonal infections.

Coronary band dysplasia/dystrophy:[23] This entity is seen in adult horses. There may be a predilection for draft horses; however; this maybe because of an association with CPL. Clinical presentation: All four legs are affected with marked thickening, scaling, can be accompanied by erythema, crusting, fissuring and cracks. Secondary infections may occur. The quality of the hoof wall may change as a result. Chestnuts may show similar changes. Pathogenesis: Etiology and pathomechanisms are not known. Potential genetic factors have to be considered. Diagnosis: Biopsy is requested for diagnosis and differential diagnosis to pemphigus foliaceus, contact dermatitis, selenium toxicity, eosinophilicexfoliative dermatitis.Histopathology: Marked epidermal hyperplasia is associated with papillomatosis, compact hyperkeratosis and focal parakeratotic hyperkeratosis over dermal papillae ("papillary squirting"). Inflammation is observed particularly when there are secondary infections. Clinical follow up: This is a chronic condition requiring palliative management.

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UPDATE ON EQUINE DERMATOLOGY

Wayne Rosenkrantz

Animal Dermatology Clinic, Tustin, California

INTRODUCTION

The knowledge and experience in dealing with equine skin disease has expanded in the last decade and has become a very important subspecialty in veterinary dermatology. Updates on allergic hypersensitivities (insect, adverse food reactions and atopic dermatitis) and common equine infectious diseases (pyoderma, dermatophyte and *Malassezia*) will be covered. A case orientated approach will be given with emphasis on clinical, diagnosticand therapeutic options.

ALLERGIC HYPERSENSITIVITIES

The horse suffers from a variety of allergic hypersensitivity conditions. Insect hypersensitivity is the best defined and understood, particularly *Culicoides* hypersensitivity. Atopic dermatitis is also becoming a more commonly recognized entity in the horse. Food allergies are occasionally seen but are difficult to identify with certainty. Some conditions can present with either pruritus and/or hives and may create other generalized skin eruptions such as papules, scales and crusting.

Horses can mount an immediate hypersensitivity response and equine immunoglobulin IgE has been identified and characterized.[1-5] In horses, a single gene encoding the IgE heavy chain constant region (IGHE gene) exists per haploid genome and several allelic variants of the equine IGHE gene were found. IgE occurs in its soluble form in equine serum and physiological concentrations of total IgE are around 1000-fold higher in normal horse than in normal human serum. Maternal IgE is enriched in the colostrum and transferred to the neonatal foal after birth. Foals do not produce detectable concentrations of endogenous IgE for several months after birth. IgE-mediated mechanisms have been implicated in the pathogenesis of several allergic diseases in horses. The findings are mainly based on the induction of immediate skin reactions after intradermal testing with allergen extracts.[4]

Insect hypersensitivity: Insect hypersensitivity is the most common cause of equine pruritus. It is generally a seasonal highly pruritic dermatitis that can also involve urticaria. It is usually due to a hypersensitivity to salivary antigens administered by the biting insect. The most common insects involved include *Culicoides*, black flies, horn flies and stable flies. Occasionally we can also see reactions from mosquitoes, deer flies and horse flies. Like canine allergic dermatitis, both inhalation and percutaneous absorption of insect allergens most likely exist. Black ants, housefly, caddisfly and mayfly, dust and storage mites are non-biting insects that may create this type of hypersensitivity. Clinical evidence strongly suggests that this disorder has a familial and therefore genetic tendency. Insect hypersensitivity has been shown to have IgE-induced immediate and late-phase reaction as well as cell-mediated delayed reactions. A complex interaction of inflammatory cells and their mediators are involved. Eosinophils and lymphocytes constitute the major inflammatory cells with increased numbers of CD5+ and CD4+ T lymphocytesandLangerhans cells present in affected lesions. The presence of LTB4 and LTD4 have also been documented. IgE, IgE mRNA positive cells and tryptase positive mast cells are also present in lesional skin biopsies. One study found that horses affected by insect bite hypersensitivity had significantly more IgE-bearing cells in skin biopsies than healthy horses. [6]

Culicoides hypersensitivity has been studied the most extensively and is considered a type I and IV hypersensitivity from the bites of *Culicoides* spp. A Th2 polarized response has been detected in horses that develop this type of hypersensitivity and appears to be linked to a decrease in IL-10 production compared to non-allergic horses.[7] More than 1000 species of *Culicoides* exist and depending on the geographic area, 30 -40 species of these can be active and feeding. Many different species may contribute to the clinical lesions of the disease with specific species having distinct feeding patterns on the body, feeding dorsally on the mane, tail, while others feed ventrally.[8] The main allergens are proteins found in the saliva. More than 10 potential allergens from *Culicoides nubeculosus* salivary extracts showed a variety of IgE binding patterns in immunoblots. In addition, IgGa and IgGT but not IgGb werefound. Both IgE and IgG were found in much higher levels in insect hypersensitive horses than healthy horses.[9] It has also

been reported that healthy horses attract more biting midges than horses with insect hypersensitivity.[10] Cross reactivity of *Culicoides* allergens in horses with hypersensitivity is controversial.There are many reports from all over the world supporting that species that are not native to a specific area can create IDT reactivity, whereas other studies show hypersensitivity requires a specific local indigenous species.[8,12-22] There are also studies that show thatdifferent species of insects can exhibit cross reactivity with *Culicoides*. For example, IgE cross-reactivity and co-sensitization against flies of genera *Culicoides* and *Simulium* has been documented.[23] In addition to *Culicoides* spp. having specific feeding patterns, other insect hypersensitivity lesions also tend to reflect the insect feeding patterns. *Culicoides* spp. have three main feeding patterns: dorsal, ventral and a combination of these. The dorsal pattern usually creates lesions over the face, pinnae, head, mane, withers and tail head. The ventral distribution can affect the intermandibular areas, thorax, abdomen, axillae, ventral midline and groin. *Haematobia irritans*, *Simulium* and some *Culicoides* spp. prefer to feed in these locations. *Haematobia irritans* (Horn flies) tends to favor a focal ventral umbilicus location. The preferred feeding sites of *Stomoxys calcitrans* (Stable flies) and *Aedes* (Mosquitoes) are the caudal lateral aspects of both the front and hind limbs. The affected sites are characterized by intense self-trauma, crusting, and alopecia with more chronic lesions also exhibiting lichenification and scarring.

Atopic dermatitis: Atopic dermatitisis an inherited predisposition to form sensitizing antibodies to environmental allergens such as the pollens of grasses, weeds, trees, molds and dust. Sensitizing antibodies (IgE) will bind to mast cells in the skin or respiratory tracts and ultimately end up creating a mast cell release of inflammatory mediators. To call the disease atopic dermatitis we must verify that horses make IgE in response to environmental allergens, that they have an imbalance between Th2 and Th1 cells, that they absorb allergens through the skin and that they have an impaired skin barrier. It is clear that horses make IgE and that allergen-specific IgE can be detected using intradermal and/or serum testing. Based on what we know about mammalian IgE, we can assume that horses, like other allergic mammals, use the same immunologic mechanisms. While we lack evidence about pollens, molds, dusts or danders, there is good evidence that a Th2/Th1 imbalance is involved in horses with *Culicoides* hypersensitivity as described above and that this insect bite hypersensitivity shares many features with atopic dermatitis. As seen in other species, it is likely that horses have a familial predisposition to atopic dermatitis and thatmany polymorphic genes are involved that influence the function of the innate and acquired immune responses as well as the structure and function of the skin barrier. Similar immunology also likely occurs in horses with an immune response that is skewed toward a T helper 2 response, increased IgE production and involvement of a variety of cytokines, including IL-4, IL-5, IL-6, IL-13, and IL-31. An eosinophilic inflammatory infiltrate and pruritus are significant features of the atopic response in the skin of horses. There is a complex interplay between the immune system and the nervous system which promotes the sensation of itch. Th2 cytokines, particularly IL-31, directly stimulate itch by binding to their receptors on nerve fibers which is thought to be important in equine atopic dermatitis. Other pruritogenic mediators likely play a part as well (histamine, proteases, substance P, opioids, neurotrophins and other neuroactive peptides). Secondary infections with *Staphylococci* and *Malassezia* yeast can alsofurther aggravate the level of pruritus. Specific studies in the horse regarding Th-2 cells and allergies have been seen with COPD horses where increased numbers of T helper cells were found in bronchoalveolar lavage fluids, including increased numbers of lymphocytes expressing mRNA for IL-4 and IL-5 and reduced numbers of cells positive for IFN-gamma mRNA.24Heimann and colleagues used immunohistochemical staining to compare the distribution of CD4+, CD8+, and FoxP3+ T regulatory cells between normal horses and those with insect hypersensitivity. There were increased numbers of T cells in the affected horses, but ratios of FoxP3+ T cells/CD4+ were significantly lower in affected horses compared to normal horses.[25] Affected horses showed elevated mRNA levels for IL-13 in lesional and nonlesional skin and lower mRNA levels for IL-10 in lesional skin. These data could support the hypothesis that insect hypersensitivity in horses is associated with imbalances in the ratio of T helper 2 cytokines and those produced by regulatory T cells. No studies have been evaluated to determine the effects of IL-31 in the horse but it is certainly speculated to be an important cytokine in equine atopic dermatitis and insect hypersensitivity.

Barrier dysfunction is considered an integral part of the pathogenesis of atopic dermatitis. In fact, the skin barrier and the immune response are believed to be tightly linked. We know very little about the skin barrier of horses; one study established that some of the ultrastructural changes associated with barrier defects in humans and dogs were seen in the skin of one atopic horse.[26] This finding supports continued study into the barrier function of horses and whether

barrier repair will become part of a multimodal approach to the management of atopic dermatitis in horses.

Many of the affected horses with atopic disease will present with similar findings as those seen with insect hypersensitivities. It is the authors' experience that it is extremely common to have both insect and atopic disease in the same horse. Pruritus with secondary lesions of alopecia, excoriations, lichenification and hyperpigmentation may be present on the ears, face, ventrum and legs. In some of these horses, the pruritus may be accentuated by insect hypersensitivity. Urticarial lesions are also common in horses with atopic disease and in some cases pruritus and urticarial lesions can be seen in the same individual. Laminitis and head tossing are ill-defined clinical signs that have been anecdotally reported with atopic dermatitis.

Head tossing: Head tossing is an interesting and often frustrating disorder. Allergies are but one of the proposed causes of this syndrome. Other reported causes include middle ear disorders, ear mites, rider ineptitude, auditory tube diverticulum (guttural pouch) mycosis, periapical dental osteitis, equine protozoal myeloencephalitis (EPM) and vasomotor rhinitis. Many cases exhibit seasonality, whichcould suggest that photoperiod and associated neurohumoral changes, ambient temperature and humidity could play a role, as well as exposure to allergens or other environmental triggers.The author has only seen a limited number of these cases but has seen ASIT be effective in one case. There are other reports supporting allergy testing and allergen specific immunotherapy as well as many other treatments including antimicrobials, glucocorticoids (systemic and inhaled), antihistamines, gabapentin, alpha-2-agonists, fluphenazine, phenytoin and phenobarbitol, melatonin, sodium cromoglycate eye drops,fly control, acupuncture and cyproheptadine (0.3mg/kg BID).

Food intolerances or adverse food reactions: In general, food intolerances and adverse food reactions in the horse are difficult to define and their true incidence is not known. Food intolerance or adverse food reactions in the horse is an allergic or idiosyncratic reaction to dietary grains, grasses, food additives or dietary supplements. Specific food groups that induce pruritus in the horse have been associated with wheat, oats, concentrates, barley, bran, alfalfa, and feed supplements. Allergic reactions to foods are thought to be caused by multiple immunologic mechanisms including type I, II, III and IV reactions. Other non-immunologic reactions can trigger mast cell release from mechanisms that are unknown. Food allergies can trigger urticarial symptoms and can present as non-seasonal pruritus. Pruritus and urticaria may be seen on parts of the body less likely to be affected by an insect hypersensitivity such as the lateral caudal thorax and flanks. Pruritus limited to the base of the tail would also make one concerned with food hypersensitivity. The author has seen one case with concurrent gastrointestinal symptoms in the form of diarrhea or soft stools.

Diagnosis: The diagnosis of an allergic skin disorder relies extensively on history and physical findings. Seasonal history is often seen initially, particularly with atopic disease and insect hypersensitivities. Food allergy typically has more of a year-round history. Physical findings are listed as above. Other tools that can aid in the diagnosis and management of allergic skin disorders include dermatohistopathology and intradermal allergy testing.

Dermatopathology; Dermatopathology may not be particularly rewarding in allergic horses as it is relatively nonspecific, but it can rule out other infectious, neoplastic and autoimmune disorders. Many allergic horses will exhibit mixed eosinophilic perivascular infiltrates with variable degrees of surface crusting, erosions and ulcerations. Other features could include spongiosis, exocytosis and patchy areas of hyper- and parakeratosis. Focal areas of eosinophilic folliculitis and eosinophilic granulomas can be seen, more often in insect hypersensitivity.

Allergy Testing: Allergy testing in the form of intradermal testing (IDT) has been studied extensively in the last decade. It has been primarily used for selection of allergens for ASIT in humans, dogs, cats, horses and other mammalian species, but some clinicians feel it may aid in diagnosis. IDT is an in vivo test that requires intradermal injection of allergies that in theory bind and bridge reaginic IgE antibodies on the surface of mast cells and result in mast cell degranulation resulting in wheal and flare reactions. An alternative to IDT is serum invitro allergy testing (SIAT). Several laboratories offer equine SIVT in the United States including ALK-ACTT (Port Washington, NY), Heska Corporation (Fort Collins, CO), Greer, Idexx Laboratories (Westbrook, Maine),Biomedical Laboratory (Austin, TX), and Spectrum Laboratories (Phoenix, AZ). To date, the value of these tests and ASIT based on these tests has been controversial in the horse. Problems related to technique, non-specific binding, lack of standardization between labs, allergen preparation, and sample handlings are concerns. Most are using a polyclonal anti-IgE reagent; the specificity

and the affinity of the reagents vary between labs. The in vitro tests are also expensive, often costing more than comparative canine assays. Lorch, et al found a sensitivity of 47.3% and a specificity of 81.7% with a positive predictive value of 68.7% and a negative predictive value of 64.7% in horses with atopic disease and horses without atopic disease using IDT as a criterion standard.[27] This study used three different allergen-specific assays and found that none produced the results similar to those obtained by IDT. Poor correlations were shown between IDT and an ELISA using a monoclonal antibody specific for horse IgE, with only 2/61 allergens (Timothy and Quack) havingsubstantial agreement between IDT and IgE ELISA.[28] A recurrent airway obstructive disease (RAO) study compared aserological IgE ELISA test (Allercept), an in vitro sulfidoleukotriene (sLT) release assay (CAST) and intradermal testing (IDT). In all three tests, the majority of the positive reactions were observed with the mite extracts (64%, 74% and 88% of all positive reactions, respectively) but none of the tests showed a significant difference between RAO-affected and control animals.[29] Another study evaluated and compared levels of allergen-specific IgE using an ELISA method in Icelandic horses, with and without insect bite hypersensitivity (IBH). The investigators also looked at patterns of allergen-specific IgE to insects, pollens, molds and mites in those groups of horses and examined the clinical significance of employing two different cut-off levels for the ELISA. The use of two cut-off levels, 150 EA and 300 EA, did not eliminate the false positives. Horses with IBH had a higher number of positive reactions than healthy controls and this was borderline significant (P=0.053). This study showed that serological testing with a high-affinity IgE receptor (FcepsilonR1alpha) is presently not suitable as a tool for establishing a diagnosis of IBH or equine atopy.[30] One study supported the value for SIVT where 27 horses that were reported to benefit from ASIT, 13 had their ASIT formulated based on the results of IDT, nine had their ASIT based on a serum test and five had both an IDT and a serum test. The success proportions of ASIT between skin tests, serum tests and both showed no statistical difference between the three groups. [31]These results likely reflect the impact of how well the clinician correlates positive reactions with the history of exposure to positive allergens and clinical symptoms. The author has also seen more positive ASIT outcomes within vitro tests in the last few years and, as in the interpretation of canine SIVT,results should be interpreted in light ofhistory and physical exam for selection of allergens for immunotherapy.

Of course, IDT is not without its share of problems but it has been shown that allergic horses react more frequently to IDT then healthy horses.[32-34] IDT is not readily available for all practitioners and it is often not financially practical to maintain the extracts and perform testing themselves. Specialists are not always available to do testing. Even when available there can be problems with false positive and false negative reactions. These can be minimized by the expertise of the allergist. The allergens to be utilized depend upon the geographical region, although many allergens are found worldwide. Most specialists utilize similar allergens to what is used in small animals with the addition of more insects and molds. Table I includes the test and allergen concentrations that the author utilizes at the Animal Dermatology Clinics of Southern California. There are concerns about testing concentrations for many allergens, particularly insect allergens. Work has been performed looking at the irritant threshold concentrations for many of the commonly used insect allergens.[35,36] Based on these studies the author has modified some of the insect testing concentrations, and these are reflected in Table I. Since molds are so ubiquitous and do not vary significantly between geographic locations, it can be difficult to choose which are important to include for IDT. However, moist and humid climates will have higher mold counts. Molds may also be more important in cases with airway disease. Once antigen selection has been made, they need to be obtained and prepared for testing. Allergenic extracts should be from a reputable supply company. The author uses aqueous allergens from Greer Laboratories, Lenoir NC 28645 or ALK, Port Washington, NY 11050. Standard concentration of most pollen and mold allergens for testing is 1,000 PNU/ml (protein nitrogen units/ml) with insects having more variable testing concentrations (see above or Table I). Some allergens are also supplied in a weight to volume (W/V) and require alternative dilutions. Dilution schedules can be obtained from your allergen supply company. Solutions for skin testing should be made up fresh every 4 weeks to maintain appropriate potency.

To obtain optimal results with IDT, the horse should be withdrawn from antihistamines for several days (5 -10) and oral glucocorticoids for 10-14 days prior to testing. Longer withdrawal periods may be needed if oral glucocorticoids have been used for extended periods of time or if long-acting injectable glucocorticoids have been used. A study evaluated intradermal testing and withdrawal times from hydroxyzine (after 500mg BID for 7 days) and dexamethasone (after 20mg/d for 7 days) in five horses without allergic symptoms before and after treatment with these drugs. Testing

was repeated in 3 -4h, 7 days and 14 days after drug withdrawal. This study concluded that treatment of horses with dexamethasone or hydroxyzine for7 days had no effect on testing results but did decrease IDT wheal diameters. Based on findings of this study, withdrawal times of 14 and 7 days for dexamethasone and hydroxyzine, respectively, prior to IDT can be recommended.[37] Skin testing usually requires sedation and shaving. The author has had good success utilizing xylazine hydrochloride intravenously. Phenothiazine tranquilizers should be avoided as they may inhibit IDT reactions. The best site for testing is the lateral cervical region above the jugular furrow, between the jaw and the shoulder. Stay below the mane as the skin is thicker and more difficult to inject in this location. The site should be clipped with a number 40 blade and sites ink marked for reference of antigen identification. Approximately .05 to .1cc of the antigen is injected intradermally. Injections should be made 2 cm apart to avoid overlapping of reactions and misinterpretation of results. Reactions should be evaluated at 15-30 minutes and if possible at 45 minutes, 4-6 hours and at 24 to 48 hours. It may be impractical to do 24 - 48 hr reactions in many clinical situations. Owners can be advised to observe for late onset or delayed reactions (swellings) and can measure and report these via the phone. Reactions are subjectively interpreted as with small animals, scoring reactions 0, 1, 2 3, and 4. Grading is based upon size, demarcations, depth and turgor of the wheals compared to a positive control (histamine 1:100,000 dilution) and a negative control (saline). Reactions greater then 2/4 are considered positive.

There is no accurate in-vitro or in-vivo test for food allergies. The only accurate way to diagnose an adverse food reaction or intolerance is food avoidance. In small animals, we now know that it takes between 6-8 weeks or longer to document this, however the time limits have not been confirmed in the horse. The four weeks that is currently recommended may be too short and the author is currently recommending 6 weeks in the horse. It can be difficult to convince an owner to do an elimination diet in a horse. Selection of a protein source that is foreign or not commonly fed is recommended. The author has had success with timothy or barley if not routinely fed. In addition, elimination of unnecessary supplements, vitamins and other drugs should be discontinued for this time. At the end of the dietary trial the horse should be re-challenged with the previous diet and/or supplements. Generally adding back one item every 5-7 days is recommended to determine which food group or protein is responsible.

Other treatment options: Treatment for allergic skin disorders is often best determined through appropriate rule outs and diagnostics. Avoidance or reduced allergen exposure is often the best method of management, however, many times it is impractical. Avoidance is also attempted when we suspect or can diagnose that we are dealing with an insect hypersensitivity. By knowing the specific type of insects involved, attention can be focused on feeding sites on the horse as well as locationin the environment for therapeutic disruption of the insect's life cycle. For example, if *Tabanus, Chrysops, Haematobia irritans* and /or *Stomoxys calcitrans* is identified, the horse should be stabled during the day as these flies are all daytime feeders. Moving affected horses away from stagnant water can also be helpful. *Simulium* or black flies tend to favor moving water, such as nearby steams or washes. Using a fan in a box stall and using 32 fine meshing netting or screening can help protect against *Culicoides* spp. Reducing exposure is critical and may not necessarily be complete for control, but may aid along with other treatments. The author favors fly repellents with permethrin (3-7%) as the primary insecticide and repellent. N,N-diethyl-m-toluamide (DEET) is also a good repellent but has no insecticidaleffects. However,one study did not show statistically significant reduction of *Culicoides* 24h post treatment with a topical insecticide containing permethrin (3.6%).20Fipronil, a commonly used flea control product (Frontline, Merial,) has also been used for insect control in hypersensitive horses. It is applied in the spray formulation at common fly feeding sites i.e., mane, tail head, legs and ventrum on a 2 – 3x a week basis. A useful insect eliminating device is the Mosquito Magnet ®. This kills mosquitoes, black flies, *Culicoides,* sand flies and other biting insects. It utilizes platinum beads to convert propane into carbon dioxide with a counter-flow technology that emits a plume of carbon dioxide, heat and octenol attractant and moisture from the inner attractant tube. The insects are attracted butdo not fly across the plume and so are caught in a vacuum and dehydrate and die. Fly baits can also be helpful in reducing fly numbers. Older products utilized organophosphates and more recently imidacloprid. A recent product that utilizes a new class of insecticide, spinosyn (Elector Bait®, Elanco), has a delayed mode of action and flies die away from the bait and is extremely safe. Protective blanketing and fly shields can also be used successfully to protect against insect bites. Since dust mites can be recovered from horse blankets, washing blankets in hot water can also be of value in cases with dust mite allergies. Dust mite and storage mite numbers can also be reduced in the stalls by using a borate based product (DUSTMITE, Ecology Works) that can keep mite levels suppressed for 3-4 months.

For mold allergies, environmental mold control can be of some value and changing bedding types in stalls may be beneficial.

Besides avoidance, other treatment options include topical (emollient, moisturizing and anti-pruritic) and systemic therapy (antihistamines, phosphodiesterase inhibitors, fatty acids, ASIT and glucocorticoids). Many of the small animal products can be used that provide emollient, moisturizing and anti-pruritic effects. The author prefers products that contain colloidal oatmeal, essential fatty acids, pramoxine and hydrocortisone. Additional antiseborrheic and antimicrobial agents can be used if secondary scaling, flaking and infections are present. There are several small animal products available through Bayer, Virbac, Vetoquinol, Ceva, Dechra and Vet Biotek.

Antihistamines have classically been defined as chemicals that block the action of histamines at receptor sites. However, they may also have antipruritic effects and reduce urticarial reactions by stabilizing mast cells and having anti-serotonin properties. Although exact dosing and pharmacokinetics are lacking in the horse, many practitioners use these drugs. They typically have fewer side effects than glucocorticoids although they are not nearly as effective. One antihistamine used by the author and others is pyrilamine maleate. It is given parenterally at a dose of 1 mg/kg. However, one study showed pyrilamine is poorly bioavailable orally (18%), and can be detected by sensitive enzyme-linked immunosorbent assay tests in urine for up to 1 week after a single administration. Despite this, data suggests that the withdrawal time prior to performing IDT for pyrilamine after repeated oral administrations is likely to be at least 1 week or longer.*38* The author's favorite antihistamine is hydroxyzine pamoate at a dose of 1-1.5 mg/kg every 8 hours. This has been shown to be more effective for urticaria than pruritus. Other antihistamines used with limited success include diphenhydramine 0.75-1 mg/kg BID, doxepin hydrochloride 0 .5 -0.75 mg/kg BID and chlorpheniramine 0.25 mg/kg BID. Side effects are minimal and include light sedation, although occasional personality changes may be seen that may require reduction of dosages or discontinuation of the drug. The American Quarter Horse Association recommends a 10-day withdrawal prior to any shows or competition.

The author has used pentoxifylline (PTX), a methylxanthine derivative that is a potent inhibitor of phosphodiesterasewith strong anti-inflammatory properties, for control of equine atopic dermatitis.It has been used in the equine for vascular diseases, laminitis and for treatment of airway obstruction. The current dosing is ~15 mg/kg BID. Controversy exists on the pharmacokinetics of the drug in the horse and exact dosing is not known. Results indicate PTX is rapidly absorbed and metabolized. Higher serum PTX concentrations, area under the curve, and bioavailability were observed after the first oral dose, compared with the last dose. Serum concentrations of both PTX and the major metabolite (M1) reach serum concentrations considered to be therapeutic in humans and horses with endotoxemia. Some studies suggest increasing the dose rate to 30 mg/kg/day by either increasing the dosage with twice daily administration or by increasing the dosing frequency to three times daily.[39] It can be tried in atopic dermatitis and urticaria.

Essential fatty acid (EFA) supplementation has had increased use in the horse. It is aimed at modifying the arachidonic acid cascade and thereby reducing pruritus and urticaria associated with inflammatory mediators resulting from this cascade. In one study, thecirculating fatty acid profiles and the acquisition and washout of fatty acids in response to n-3 supplementation were determined for horses fed a supplement high in eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid. The results of this study showed that the circulating fatty acids in horses can be influenced through targeted supplementation.[40] In another study, 14 horses with seasonal *Culicoides* hypersensitivity were given 20 grams daily of evening primrose oil and cold-water marine fish oil in an 80 to 20 ratio. The results were that 4 horses were no better, 5 horses were better and 5 horses were much improved. In yet another study, horses were fed 200 ml of linseed oil per day for a 6-week period, and showed no significant change in pruritus or lesional surface areas. However, this time frame may have been too short to completely evaluate the potential benefits of n-3 fatty acids.*41* The author has seen limited success using similar fatty acid combinations but, when used, recommends a balanced high concentration EFA supplement found in Platinum Performance Equine products (Platinum Performance, Inc.). EFA can be part of adjunctive therapy with other forms of therapy utilizing a multimodal approach to manage equine hypersensitivities.

Allergen Specific Immunotherapy (ASIT): There are now numerous studies demonstrating value for immunotherapy in both insect and environmental allergens. However, most studies have not been controlled and included only

small numbers of horses. One study on *Culicoides* hypersensitivity evaluated ASIT in adouble-blinded control fashion with poor results. [42] However, in another trial, all 10 horses with Culicoides hypersensitivity improved during immunotherapy, and seven of these horses deteriorated again after cessation of therapy.[14] Most authors reported a 60% to 71% good to excellent response to ASIT based on the results of intradermal testing.[15,32, 43-47] Reports evaluating the influence of multiple concurrent positive reactions to insects on the outcome of ASIT show conflicting results.[47-48] It may require a longer period of treatment to see positive results. In a placebo controlled study by the author, 64% of the horses treated with antigens showed a 50% or greater improvement compared to only 23% with placebo. These cases included both insect and pollen reactive horses.[47] In a large retrospective study, 41 horses seen over a 17-year period were treated with ASIT and according to the owners surveyed, the overall response rate to ASIT was 84%.[31] This percentage of success, as well as one other report of 92% success using ASIT in the management of equine urticaria,[48] appear higher compared to what is reported in other studies. It is likely that owner assessment was skewed by placebo effect or because of concurrent medications. In the UCD study where the success of ASIT was evaluated as a sole therapy, 59% of the cases were well controlled with a further small percentage (9%) of horses being considered partial responders (i.e. concurrent medications continued to be administered with ASIT, but glucocorticoids could be discontinued). This would give a total response rate of 69% (22 of 32 horses) putting the success rate closer to previous reports. Also of interest was that of the 30 owners who reported using antipruritic medications prior to beginning ASIT, 57% (17 of 30) reported being able to discontinue those medications with the addition of ASIT.[31] Another study looked at the benefits of ASIT over an extended period of time in a prospective clinical and immunological study. Nineteen horses received ASIT for up to 24 months. Horses were randomized to one of three treatment groups: ASIT based upon intradermal test (IDT) results (n = 7); allergen-specific IgE results by ELISA (n = 6); or a combination based on IDT and ELISA results (n = 6). There was excellent agreement between allergen-specific IgE concentrations (time 0) and both immediate and delayed IDT results, and between immediate IDT and IgG results. Specific concentration of serum IgE and IgG decreased significantly for 13% and 38% of allergens, respectively, that were included in ASIT.[49] These results suggest that ASIT provides significant clinical benefit and supports roles for both allergen-specific IgE and IgG in the pathogenesis of equine AD. This data also suggests that the clinical benefits from ASIT may result from reduction of allergen-specific IgE and IgG concentrations in serum. The most recent report on ASIT was based on a retrospective phone survey in 34 cases with urticaria and pruritus (n=11), non-pruritic urticaria (n=7), pruritus (n=6), RAO (n=9) and RAO with urticaria (n=1). In 33/34 on ASIT, the number of ASIT refills ranged from 0 to 11 (mean of 3.7 and median of 3) with intervals between refills ranging from 3 to 12 months. Eighteen cases resulted in complete phone interview follow up. In 6/18 cases 50–100% horses improved andremained on ASIT, 4/18 improved 75% or more andowner stopped ASIT with minimal return of clinicalsigns, 5/18 reported no improvement and discontinued ASIT. Three horses with RAO (one with concurrent urticaria) had improved.This study suggestedbenefits of ASIT and that a small number of affectedhorses may eventually be able to be weaned off ASIT without recurrence of clinical signs.[50]

The ASIT technique is similar to what is used in small animals (see attached Equine ASIT schedule). Most horses required antigen booster injections at 7 to 14 day intervals, with volumes ranging from .5 to 1 cc. Injectionsare given subcutaneously over the lateral cervical area. Antigen reactions are uncommon, with swelling at injection sites being the most common, which generally resolves within 1-2 days. Angioedema and anaphylaxisare extremely rare in the author's experience. Oral ASIT can also be used in the horse. The author has only utilized this in a limited number of cases (4 to date) but is aware of 2 horses currently doing well on this. There one report by Scholz, et al, 2016 of severe angioedema in a horse on oral ASIT, that when switched to injectable ASIT had no further reactions and was successfully managed.

Glucocorticoids: Systemic glucocorticoids are often required for short term relief and in some cases for longer term control. They are very frequently prescribed and certainly need to be used judiciously and in appropriate dosing and intervals. It is essential to make an accurate diagnosis before using glucocorticoid therapy to decide on the type, duration and the dose of therapy required. Therapeutic dosages are not determined for any glucocorticoid in any equine dermatoses and each case needs to be treated individually. Recommended dosages are merely guidelines. The author relies primarily on prednisolone and dexamethasone in practice. Prednisone and prednisolone do not appear to be equal in the horse. Possible reasons why horses do not respond as well to oral prednisone are poor absorption, rapid excretion, failure of hepatic conversion to prednisolone or combination of all of these.[51] Depending

on the severity of the case, dosages may need to be at the high or low end of anti-inflammatory levels to control most allergic hypersensitivity conditions. Most induction periods range from 7-14 days followed by a tapering period of 2-5 weeks and then a maintenance period that may be used for as short a time as a few months or indefinitely, depending on the severity of the case and the seasonality. Induction dosages for prednisolone are 0.5-1.5 mg/kg per day with maintenance dosages at 0.2-0.5 mg/kg every 48 hours. Some cases will be resistant to prednisolone and may respond to either injectable or oral dexamethasone. Often an initial loading dose of dexamethasone is needed at .02-0.1 mg/kg, which may be followed by an oral maintenance dosage of .01 -. 02 mg/kg every 48 to 72 hours. This regime is particularly helpful in more refractory cases. When using oral glucocorticoids, writing out the induction, tapering and maintenance dosages on a day-to-day basis is extremely helpful (see attached client handout schedule). Such a schedule allows safer administration at a "threshold dose" so that the case remains disease free.

TABLE I - EQUINE SKIN TEST

Animal Dermatology Clinic

SOUTHERN CALIFORNIA EQUINE
INTRADERMAL ALLERGY TEST

Date: Dr.: Patient: Client:

CONTROLS			**GRASSES**
1. Saline			32. Bermuda *Cynodon dactylon*
2. Histamine			33. Seven Grass Mix +
INSECTS			34. Brome *Bromus inermis* +
3. Mosquito *Culicidae*	1,000		35. P. Rye *Lolium perenne* +
4. Mosquito *Culicidae*	250		36. Orchard *Dactylis Glomerata* +
5. Deer Fly *Chrysops sp.*	1,000		37. Timothy *Phleum pratense* +
6. Deer Fly *Chrysops sp.*	250		38. Alfalfa *Medicago sativa*
7. Black Ant *Camponotus pennsylvanicus*	125		**TREES**
8. Flea *Ctenocephalides canis/felis*	1,000w/v		39. Box Elder *Acer negundo*
9. Flea *Ctenocephalides canis/felis*	400w/v		40. Palm *Arecastrum romanzoffianum*
10. Storage Mite *Acarus Siro*			41. West. Juniper *Juniperus occidentalis*
11. Storage Mite *Tyrophagus Putrescentiae*			42. Acacia *Acacia spp.*
12. Culicoides *Variipennis*	1:1,000		43. Western Oak Mix *Quercus spp.*
13. Culicoides *Variipennis*	1:10,000		44. Wester Walnut Mix *Juglans spp*
14. Horse Fly *Tabanus ssp.*	1,000		45. Olive *Olea europaea* +
15. Horse Fly *Tabanus ssp.*	250		46. Melaleuca *Melaleuca quinquenervia*
16. House Fly *Musca domestica*	1,000		47. Eucalyptus *Eucalyptus globulus*
17. House Fly *Musca domestica*	250		48. Orange *Citrus sinensis*
18. Moth *Lepidoptera*			49. CA Cottonwood *Populus fremontii* ++
19. Dust Mite *D. Farinae*	250		50. Arroyo Willow *Salix lasiolepis* ++
20. Dust Mite *D. Farinae*	62.5		51. White Mulberry *Morus alba*
21. Caddisfly *Trichoptera*			52. Pepper Tree *Schinus spp.*
22. Mayfly *Ephemeroptera*			53. Salt Cedar *Tamarix gallica*
EPITHELIA			**WEEDS**
23. Cat Dander *Felis catus*			54. Pigweed Mix *Amaranthus spp.*
24. Feather *Chicken, Duck, Goose*			55. Lambs Quarter *Chenopodium album*
25. Mouse *Mus musculus*			56. Russian Thistle *Salsola kali*
26. Rat *Rattus norvegicus*			57. Firebrush *Kochia scoparia*
27. Pyrethrum *Chrysanthemum cinerariifolium*			58. Western Ragweed *Ambrosia spp.*
MOLDS			59. Sage Mix *Artemisia spp.*
28. Curvularia *spicifera*			60. Dandelion *Taraxacum officinale*
29. Fusarium Mix			61. Baccharis *Baccharis spp.*
30. Mucor Mix			62. Mustard *Brassica spp.*
31. Penicillium Mix			63. Dock/Sorrel Mix *Rumex spp.*
Scoring Legend			64. English Plantain *Plantago lanceolata*
0 or left blank = no reaction			65. Nettle *Urtica dioica*
1 = low reaction			
2 = moderate reaction			If a "+" is beside a score = slightly larger than the score/number.
3 = strong reaction			
4 = very strong reaction			

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ALLERGEN SPECIFIC IMMUNOTHERAPY (ASIT) Table 2

ASITis one type of treatment for allergies in horses. The major benefit is its relative lack of side effects. Theoretically, it helps by creating tolerance, which allows your horse to be exposed to higher levels of allergens without developing symptoms such as hives, itching, rubbing, chewing, etc. ASIT is not always effective. Approximately 60% of the horses will be controlled. Of this 60% approximately 50% will be controlled without the use of other drugs. The additional 10% are helped, though they are not totally controlled and may require the use of other medications.The response to ASIT can be slow and gradual. Most horses do not respond until they have been on the injections for 3-6 months. Some may take as long as 9 months. Once they have responded, treatment will usually be needed for life. Your horse must be re-evaluated after 4-5 months and some adjustments may need to be made in his/her treatment.Side effects are rare. If swelling, itchiness, or hives appear within an hour of giving an injection, call the clinic. More serious side effects are very rare, but would include colic, diarrhea, respiratory difficulties, angioedema or collapse associated with the injection. Call our clinic or an emergency clinic at once if this occurs. More serious side effects generally occur within the first few months of therapy; therefore, you should give these injections when you will be with your horse for a minimum of 1-2 hours.

IMPORTANT

- Antigens must be refrigerated
- Your horse must be re-evaluated during the 10-day injection intervals after 4- 6 weeks and again in 6 months
- It may take 6-9 months to show a response to antigens
- In most cases, antigen injections will be life-long

EQUINE ASIT SCHEDULE

DAY	DATE/SYMPTOMS†	AMOUNT	DAY	DATE/SYMPTOMS†	AMOUNT
VIAL #1			25		1.0cc
1		0.1cc	10 DAY INTERVALS (Recheck)‡		
3		0.2cc	35		1.0cc
5		0.3cc	45		1.0cc
7		0.4cc	55		1.0cc
9		0.6cc	14 DAY INTERVALS		
11		0.8cc	69		1.0cc
13		1.0cc	83		1.0cc
VIAL #2			97		1.0cc
15		0.2cc	111		1.0cc
17		0.3cc	20 DAY INTERVALS		
19		0.4cc	131		1.0cc
21		0.6cc	151		1.0cc
23		0.8cc	171	Recheck‡	1.0cc

†Record date and any increase or reduction in clinical signs

‡Call for a recheck appointment prior during 10-day cycle, some horses require volume and interval adjustments. A 6 month recheck should also be scheduled

DO NOT STOP INJECTIONS WITHOUT NOTIFYING YOUR Veterinarian

INFECTIOUS DISEASES

Dermatophytosis and *Malassezia* Dermatitis: Dermatophytosis is a common, contagious superficial fungal infection of keratinized tissues. This usually includes the epidermis and hair and rarely the hooves. It is more common in younger horses or in debilitated or immunosuppressed horses. The incidence also increases in hot, humid climates or in horses kept in close contact with dark moist environments. More cases are seen in the fall and winter months. The most common species identified in the horse include *Trichophyton* spp. or *Microsporum* spp. *T. equinum var. equinum* and *var. autotrophicum* and *T. mentagrophytes*. Most of these are zoophilic dermatophytes and transmission requires direct contact with infected animals or contact with infected hair or crusts in the environment. Hair loss with associated scale and crusting is the most common clinical sign. The alopecia is a result of hair shafts that are weakened by the fungus and then break or as a result ofdeeper folliculitis. Lesions are usually multiple and vary in size and distribution. Typical lesions are 2-4mm in size and occur over the truncal, facial, head, axilla and chest locations. These sites are often where tack, blankets or saddles sit allowing the infection to more readily occur. Pruritus is not typically seen but can be present in some cases. Good review articles on equine dermatophytosis have been published.[52,53]

Malassezia dermatitis in the horse most commonly causes pruritus in the caudal intermammary area and tail head in mares but can create symptoms in male horses often over the prepucial area.[54] The exact species of *Malassezia* growing on horses needs further investigation. In one study, the *Malassezia* spp isolated were identified as *M. pachydermatis, M. furfur, M. slooffiae, M. obtusa, M. globosa,* and *M restricta*.[55] Often there is a dry, greasy exudate in the intermammary folds. This often elicits a pruritic response. Treatment with 2%miconazole-chlorhexidine shampoos are usually effective.

Diagnosis of dermatophyte is made by direct hair exams, cytology, fungal culture or biopsies. Optimally, microscopic examination of hyphae and macroconidia is needed for complete identification. For *Malassezia*, cytology is adequate.

Treatment is not always necessary and many cases are self-limiting, often disappearing within 1-3 months. Infected horses should be isolated and all tack and grooming items should not be shared. Shaving the affected sites is of controversial value and may speed the healing process and reduce environmental contamination. Care should be taken when handling suspect or confirmed cases as this is a zoonotic disease and can create lesions in humans. Topical therapy in the form of shampoos and spray on rinses can be beneficial. The author prefers a poultry premise spray containing13.8% enilconizole (Clinafarm EC, Schering Plough and Imaverol, Jannsen). This can be used as a 2% spray on 2-3 times a week to the affected sites. Other topical therapies include 4 % lime sulfur, 2% chlorhexidine, 0.5% povidone-iodine, 0.5% sodium hypochlorite. Systemic therapy with griseofulvin has been used with a wide range of dosing 10-100mg/kg q 24h for 14-21 days. It can be tried in more resistant cases. It has limited availability and is a teratogenic drug soshould be avoided in pregnant horses. Itraconazole and fluconazole have been used to treat other mycotic infections in the horse such as coccidioidomycosis and aspergillosis at 2-5mg/kg q 12h and can also be tried but cost can be prohibitive.[56] 20% NaI may be given IV (250mg/500kg every 7 days for 1 -2 treatment courses. This also is contraindicated in pregnant mares as it may cause abortion. Other possible options include using ethylenediamine dihydroiodide (EDDI) at a dose of 1 gram/day as used in cattle. Others have used EDDI as a feed additive formulation (Neogen Corporation, Lexington Kentucky) and dosed at 1 -2 mg/kg once to twice daily for first week then reduced to .5 to 1mg/d daily for next 2 – 3 weeks. Environmental treatment and disinfecting the tack, blankets and grooming equipment with one of the above mentioned topicals should also be performed.

Staphylococcal Folliculitis: Although many bacterial skin infections in the horse appear as nodular or papular eruptions there are times when lesions can present in a more scaling and crusting pattern. Folliculitis due to *Staphylococcus* species is common in the horse and appears more frequently during the warmer summer months and in sites where tack, blankets or saddles rub or irritate the skin surfaces. The higher incidence in these locations and during the summer months has given such synonyms as summer rash or scabs, saddle sores or scabs or sweating eczema to describe this syndrome. Horses that are not properly rinsed and bathed after being worked may be predisposed. The author also believes that insect bites either aggravate or possiblycouldbe a source of vector inoculation of *Staphylococcus* in some cases. The distal limbs can also be affected. The lower limbs and the pastern area can be significantly affected and can be a major differential when evaluating pastern dermatitis.The most common isolates are *Staphylococcus pseudintermedius* and less commonly *Staphylococcus hyicus*. In one study,128

strains of *Staphylococcus* from lesions, mostly from the skin, were identified and compared with 29 strains isolated from the healthy skin. The pathogenic species *Staphylococcus aureus*, *S. intermedius* and *S. hyicus* were found almost exclusively in lesions. Methicillin-resistant coagulase negative *Staphylococci* (MR-CoNS) species such as *S. xylosus, S. lentus, S. epidermidiis, S. hemolyticus, S. capitis* and *S. sciuri* can cause disease but are more frequently found on healthy than lesional skin.[57-60]

There are increasing worldwidereports of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and colonization in horses and evidence that MRSA can be transmitted between horses and humans.The majority of nosocomial infections in horses is associated with particular MRSA clonal lineages. Clonal lineages belonging to clonal complex (CC) 8 appear to be diminishing whereas MRSA attributed to CC398 is becoming increasingly more prevalent. Mostof the CC398 isolates belong to a subpopulation which is particularly associated with equine hospitals as indicated by molecular typing. When emerging in equine clinics, MRSA from horses were also found as nasal colonizers in veterinary personnel. MRSA exhibiting the typing characteristics of MRSA from equine clinics are rare among MRSA from infections in humans. Although rare, so far epidemic MRSA from human hospitals (HA-MRSA, e.g., ST22, ST225) have been isolated from nosocomial infections in horses and need attention in further surveillance. [61] More details are available in the attached references.[62-73]

Risk factors for MRSA infections havealso been investigated in horses. One study looked at the evolution of antibiotic resistance patterns before and after preventative pre- and postoperative penicillin treatment. In this study staphylococci were isolated from skin and wound samples at different times during hospitalization. Hospitalization and preventive penicillin use were shown to act as selection agents for multi-drug-resistant commensal staphylococcal flora.[71] In another report, risk factors for MRSA colonization and infection showed that administration of ceftiofur or aminoglycosides was associated with the acquisition of MRSA during hospitalization. Other risk factorsfor community-associated colonization include previous identification of colonized horses on the farm, antimicrobial administration within 30 days, admission to the neonatal intensive care unit, and admission to a service other than the surgical service.[58]

Diagnosis is made on history and physical examination, cytology, culture and sensitivities and biopsies. Routine Diff Quik staining from intact papules or impression smears from crusted material is quite valuable. Skin biopsies often reveal bacteria in the surface crusts with occasional folliculitis identified.

Treatments with topical chlorhexidine shampoo is the author's favorite product. Systemic antibiotics based on cytology or culture and sensitivity are also indicated in more severe cases. Trimethoprim sulfa is the main antibiotic used at 25mg/kg q 12h for 14 days but resistance is occasionally documented. Doxycycline can also be used at a dose of 10mg/kg q24hr. Many non-methicillin resistant cases will respond to procaine penicillin 22,000 – 44,000 IU q 12h for 14 days. Prevention is critical and in many cases proper husbandry is needed to keep tack and blankets clean and horses should be rinsed or bathed regularly after working.

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ABSRACTS SCHEDULE

SATURDAY, MAY 5, 2018

Dermatopathology Session (ISVD) Presentations

9:00 AM - 11:00 AM	**ISVD Clinicopathological Correlations**	
	Dr. Erica Noland / Dr. Annette Peterson	190
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Concurrent Session Presentations

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11:30 AM - 12:30 PM	**Amelia White, DVM, MS:** What's your diagnosis? A case-based approach to fungal dermatoses	

Presumed erythema multiforme and subsequent severe widespread cutaneous and systemic dystrophic mineralization after treatment with prednisone

E.L. Noland*, D.G. Sledge*, and A. Petersen†

**Veterinary Diagnostic Laboratory and †Department of Small Animal Clinical Sciences, Michigan State University, East Lansing, United States*

A six-year-old male Labrador retriever dog presented with a two-week history of crusty lesions on the muzzle, abdomen, and dorsal neck; hyperkeratosis of the paw pads; and lethargy and weight loss. The dog also had a one-year history of elevated ALT (450 U/L). Skin lesions progressed despite cefpodoxime and low-dose prednisonetreatment. Skin cytology revealed neutrophils and macrophages, macerated tissue culture yielded few methicillin resistant S. pseudintermediuscolonies, IFA titer for R. rickettsia was 5120, and liver ultrasound was normal. In biopsies, there was full thickness dyskeratosis of the epidermis and follicular epithelium with frequent lymphocytic to lymphohistiocyticsatellitosis and regionally extensive replacement of the epithelium by histiocytes and fewer multinucleate cells with occasional perimembranous E-cadherin immunoreactivity. Erythema multiforme was suspected. Lesions improved with prednisone and doxycycline, but because of PU/PD, cyclosporine modified was started and the prednisone dose (0.6 mg/kg q12h PO) was lowered by 25%. The owner stopped the prednisone and lesions worsened, so the prior dose of prednisone was restarted. Lesions consistent with calcinosis cutis appeared within 8 weeks. Prednisone was reduced by 75% then stopped, but skin lesions progressed and euthanasia was elected. On necropsy, there were generalized regions of calcinosis cutis, epidermal ulceration with crusts containing bacteria, and follicular atrophy and follicular keratosis, which were likely a consequence of prednisone therapy. Interestingly, there was mineralization throughout multiple tissues without evidence of parathyroid or kidney disease; as such, systemic mineralization was considered most likely dystrophic, and possibly a consequence of prednisone therapy or prior systemic insult.

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Conflict of Interest: None declared

EXFOLIATIVE DERMATITIS WITH LYMPHOCYTIC MURAL FOLLICULITIS IN A CAT

Welle MM*,Song MD†

** Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Basel Area, Switzerland*
† Department of Dermatology, VetMed, Phoenix, Arizona, USA

Abstract: A six-year-old, female spayed, cat had been experiencing skin problems for approximately 4 weeks.

The cat was examined and found to have partial alopecia on the head, face, neck and pinna. In addition, there was some crust and scale with a few excoriations dorsal to the right eye and on the nasal planum. On the face and the neck there was some thickening with hyperpigmentation and alopecia. The partial alopecia extended to the front limbs but spared the dorsal aspect of the paws.In addition to the skin lesions, there was general lymphadenopathy. The body temperature, abdominal palpation and thoracic auscultation were all within normal limits. However, there was a possible ocular ulcer associated with feline herpes virus.

Skin scrapings and Wood's lamp examination failed to reveal anything significant. The trichograms were negative for any mites or hyphae and all the hair were in telogen stage of development. Skin cytology revealed some neutrophils, +3 Malassezia sp. and +1 coccoid bacteria. Radiographic examination of the chest was unremarkable.The blood profile displayed leukocytosis, lymphopenia, hypergammaglobulinemia, elevations of the AST, and GLDH. The FeLV and FIV tests were negative. The abdominal ultrasound showed enlargement of all lymph nodes. A fine needle aspirate of the lymph nodes revealed lymphoblasts of greater than 50%, small lymphocytes and plasma cells.

Biopsies of the skin will be discussed.

Unusual patchwork alopecia and pruritus in a Labrador retriever dog

C.L. Cain* and C.W. Bradley†

**Department of Clinical Sciences and Advanced Medicine and †Department of Pathobiology, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, USA*

Abstract: A 7 year-old spayed female Labrador retriever was presented to the dermatology service for evaluation of generalized pruritus and progressive alopecia. The patient had been previously diagnosed with superficial pyoderma and had received multiple systemic antimicrobials. Initial examination findings included multifocal alopecia over the body surface in a "patchwork" pattern with hyperpigmentation, as well as epidermal collarettes and crusts with underlying erosions and ulcers. The patient had been diagnosed with hypothyroidism one year prior to presentation and was receiving supplementation with levothyroxine. A complete blood count, biochemical profile, and urinalysis were performed two months prior to presentation and were unremarkable apart from mild proteinuria. This case necessitated clinical and histopathologic correlation and communication. Further work-up resulted in a confirmed diagnosis and clinical management plan for this patient.

CHRONIC SKIN LESIONS IN A DOG WITH MULTICENTRIC B CELL LYMPHOMA

Affolter VK*,Outerbridge CA†,Nagle T ‡, O'Brien D§

** Department of Pathology, Microbiology, Immunology, School of Veterinary Medicine, UC Davis, CA, USA*
† Department of Medicine & Epidemiology, School of Veterinary Medicine, UC Davis , USA
‡Sacdermvet at Vista Veterinary Specialists, Sacramento, CA, USA
§VCA Sacramento Veterinary Referral Center, Sacramento, CA, USA

Abstract: An approximately 12 year-old, male-neutered Chihuahua mix with severe generalized skin lesions was surrendered to a shelter. Duration of the skin lesions was unknown.

Clinical evaluation of the dog revealed severe, generalized alopecia with markedly thickened skin. The skin thickening was dramatic and severe, causing large, indurated skin folds, most prominently on all for legs. There was marked lichenification with scaling and focal small erosions. Both corneas appeared cloudy. The peripheral lymph nodes were moderately to markedly enlarged.

Deep skin scrapings were negative and cytology from multiple sites demonstrated yeast organisms, neutrophils and small numbers of cocci. Fine needle aspirates of lymph nodes were predominated by large numbers of large lymphocytes, suggestive of lymphoma. The diagnosis of high-grade lymphoma with potential cutaneous involvement was made.Immunocytochemistry on lymph node aspirates revealed a predominance of large CD79a+ B cells. Clonality testing identified a clonal rearrangement of IgH and KDE, supporting the diagnosis of a large B cell lymphoma affecting peripheral lymph nodes.

Subsequent skin biopsies were taken to evaluate for potential lymphoma in the skin versus an unknown unrelated skin disease or a possible paraneoplastic process. The skin biopsies will be discussed.

VIDEO OTOSCOPY AND CT IN THE DIAGNOSIS AND MANAGEMENT OF CANINE AND FELINE OTIC DISEASE: PART 1

Mike Canfield DVM, DACVD[1], Rod Rosychuk DVM, DACVIM[2]

[1]Animal Dermatology South, New Port Richey, Florida
[2]Dept. of Clinical Sciences, Colorado State University, Ft. Collins, Colorado

ANATOMY OF THE CANINE AND FELINE EAR – "STRIPPED DOWN"

Pinna and External Ear Canals

The external ear canal is divided into two sections, the vertical and the horizontal canal. The vertical canal and the first part of the horizontal canal is formed by the auricular cartilage as it "rolls" up to become tubular in shape. On the caudo-lateral entrance to the vertical canal, the inter-tragic incisure is a very helpful "groove" in which to put the tip of a video otoscope cone to facilitate the initiation of an otoscopic examination. The annular cartilage is the relatively short, rolled, tube-like cartilage that extends from the auricular cartilage to the temporal bone. It forms the more proximal part of the horizontal canal. The luminal fold extends down from the dorsal aspect of the canal at the junction of the vertical and the horizontal canal in the dog. The prominence of this fold varies between breeds and between individuals within breeds. With the pinna in its normal position, the "fold" may extend down to significantly compromise visualization/ otoscope advancement in to the horizontal canal. The luminal fold can be minimized by pulling the pinna up and out from the base of the skull. The luminal fold is less prominent in the cat, compared to the dog. Adjacent to the tympanum, the horizontal canal is supported by bone. In this relatively short area, the horizontal canal narrows slightly. In brachycephalic and some other breeds (e.g. St. Bernard, Newfoundland), the horizontal canal may narrow significantly just in front of the tympanic membrane (normal stenosis). In dogs, the length of hairs growing within the vertical and horizontal ear canals varies with the breed (e.g. long, dense hair growth noted in such breeds as the poodle, Giant Schnauzer etc.). Even when significant hair growth is not noted in the horizontal canal, it is common to see a small, more focal area of longer hair growth on the floor of the horizontal canal, just in front of the tympanum. Because these hairs are always found on the floor of the canal, they do serve as a useful landmark for orientation when performing manipulative work in the ear. The volume of the external ear canal correlates with body weight. A 5.4 kg dog has an external ear canal volume of 4.5 cubic cm; a 20 kg dog the volume is 9.6 cubic cm. In the cat, hair follicles are sparse or absent along the canals. It usually takes a video otoscope to see these very fine hairs. Cerumen covers the surface of the lining of the ear canal. Significant wax accumulation should not be noted on the walls of the canals in the normal dog and cat ear. An exception to this rule occurs in the dog. In the normal dog ear, it is quite common to see a small amount of wax on the floor of the horizontal canal, just adjacent to the tympanum. This accumulation is not seen in the normal cat. Anything that causes irritation or inflammation within the ears will result in increased wax production. The degree of hydration of the ear wax will often contribute significantly to its color (e.g. drier wax tends to be darker).

Tympanic Membrane

The tympanic membrane of the dog is made up of the pars flaccida and pars tensa. The pars flaccida is a small area of the dorsol to antero-dorsal region of the tympanum which is relatively flaccid and vascular. This structure is noted to dilate or "bulge", in association with increases in air pressure within the middle ear. The pars flaccida can also dilate and fill with fluid / debris in association with otitis media. Cats do have a pars flaccida, but it is not readily visible and usually does not dilate with pressure changes in the middle ear as seen in the dog. It may dilate when filled with the exudate associated with otitis media. The horizontal junction between the pars flaccida and the pars tensa is marked by a ligamentous structure called the malleolar fold. The majority of what is seen of the tympanum is the large pars tensa. A normal pars tensa is translucent, with striations seen extending from the manubrium of the malleus outward to the periphery. Some of this translucency is lost as the individual ages. The manubrium (handle) of the malleus (striamallearis) is situated within the fibrous layer of the tympanum. The manubrium is significantly curved, with the open end of the curve pointing toward the nose. It is located over the antero – mid portion of the tympanum. It is not readily visible on all otoscopic examinations. In the cat, the manubrium of the malleus is only slightly curved (as

compared to the dog). Tension on the manubrium gives the tympanum a mildly concave outer contour. The area of the tympanum around the manubrium (handle) of the malleus (see below) appears to be very important with respect to regrowth of the tympanum following tympanic membrane perforation. Severe or irreversible damage to this area appears to be associated with a poor prognosis for tympanum regrowth. In the dog, a whitish appearing discoloration can sometimes be seen through the mid to mid dorsal aspect of the tympanum. This is the bony ridge (bulla septum) that partially separates the tympanic cavity from the tympanic bulla. The "dark" area often seen caudo-ventrally and sometimes caudally to this is the opening to the tympanic bulla. The periphery of the tympanum attaches to the *annulus fibrocartilagenous* which is a fibrocartilagenous ring that attaches to the surrounding bone. The tympanum is oriented at about a 30 - 45 degree angle from perpendicular (dorsal to ventral). This means that the dorsal aspect of the tympanum is closer to the otoscopist than the ventral aspect.

Canine Middle Ear

The middle ear, starting from dorsal to ventral is first made up of the epitympanic recess which houses the three small middle ear bones: the malleus which is attached to the tympanic membrane, incus and stapes which is attached to the oval (vestibular) window leading to the inner ear. The oval window is dorsal and fairly central in the epitympanic recess. The tympanic cavity is that area of the middle ear just inside the tympanum (aka mesotympanic cavity). The medial surface of the tympanic cavity is made up of the petrous portion of the temporal bone which includes the barrel shaped cochlear promontory. The cochlear promontory is located opposite to the mid-dorsal aspect of the tympanic membrane. The cochlea sits within this structure. At the caudal end of the promontory is the cochlear (round) window which communicates with the bony labyrinth of the cochlea. This structure is caudo-dorsal in the tympanic cavity. The round window is only covered by a thin epithelial lining. The opening of the auditory tube (Eustachian tube) lies in a rostro-medial location in the tympanic cavity. Beneath the tympanic cavity is the large tympanic bulla. The tympanic cavity and the bulla are separated by a partial bony ridge – the bulla septum. This bony ridge does vary significantly with respect to width from dog to dog. The leading edge of this ridge is often noted to have variably sized, sometimes globular bony projections (spicules). Sympathetic nerves run across the more dorsal aspect of the medial wall of the middle ear, over the upper part of the cochlear prominence. The facial and parasympathetic nerves run over the most dorsal aspect of the medial wall of the middle ear. While they travel within a bony channel through much of the middle ear, but for a short distance, they are covered by only respiratory epithelium. It is this exposure that makes them prone to damage with middle ear disease. The mean middle ear volume of mesaticephalic dogs (intermediate length and width skull) as measured by CT is 1.5 ml. The middle ear cavity volume increases in a nonlinear fashion by body weight.

Feline Middle Ear

The middle ear is divided into two chambers by an almost complete, thin, bony plate. The smaller dorso-lateral chamber consists of the epitympanic recess and tympanic cavity. The ventro-medial chamber is larger and analogous the tympanic bulla of the dog. The communication between the dorso-lateral and ventro-medial chambers is limited to a long, thin "slit" adjacent to the medial wall of the middle ear which becomes a small "hole" at the caudal most aspect of the septum. The round window (cochlear window) is just adjacent to this opening. The sympathetic nerves form a plexus that course over the medial wall / cochlear promontory of the middle ear. These nerves extend more ventrally than in the dog (just above the "half way" line bisecting the medial bony wall of the middle ear). The facial and parasympathetic nerves run dorsally in the epitympanic recess, through a bony channel and is therefore relatively resistant to damage in association with otitis media (unless the process causes bony lysis). The middle ear is covered by a respiratory epithelium that is cililated and contains goblet cells.

The Internal (Inner) Ear

The medial wall of the middle ear is made up of the petrous portion of the temporal bone. The cochlear promontory is part of this bone. The spaces within the petrous temporal bone are called the osseous labyrinth. Suspended within this osseous labyrinth and surrounded by perilymph is the membranous labyrinth: cochlea, saccule, utricle and semicircular canals. The osseous labyrinth is continuous with the subarachnoid space via the cochlear aqueduct. Through this duct, perilymph is continuous with the cerebrospinal fluid in the subarachnoid space around the brain. Inner ear disease can spread via this structure to the meninges. The membranous labyrinth is an interconnecting

system of epithelium lined tubules and spaces filled with a fluid (endolymph). The auditory (hearing) system of the inner ear consists of the cochlea and the associated cochlear branch of the vestibulocochlear nerve which is in turn connected to the central nervous system. The caudal half of the petrous temporal bone contains the vestibular system consisting of the saccule, utricle and semicircular canals. These are fluid-filled, epithelial lined compartments.

COMPUTED TOMOGRAPHY (CT) OF THE NORMAL CANINE AND FELINE EAR

In the normal canine external ear, CT allows for the visualization of the auricular and annular cartilages and the epidermis/dermis/subcutis of the lining of the ear canal; in the middle ear – the tympanic membrane (TM), the auricular ossicles, the epitympanic recess, tympanic cavity and tympanic bulla, the bulla septum (ridge of bone at the junction of the tympanic cavity and tympanic bulla) and variably sized bony spicules that emanate from the bulla septum; the inner ear is enclosed within the petrous temporal bone dorsomedially to the middle ear and due to their small size, there is limited visibility of the structures of the inner ear. The cochlea and vestibular window are usually seen. In most regards, the visible anatomy of the feline ear is similar to the dog, although, in the cat there is an almost complete bony plate that separates the middle ear in to a dorsolateral and larger ventromedial chamber.

DIAGNOSIS AND MANAGEMENT OF EAR CANAL MASSES IN THE DOG AND CAT

Canine:

Neoplasia: It appears that it is more common to see benign tumors in the canine ear canal, but this observation is controversial. Benign tumors, noted in decreasing order of occurrence included sebaceous gland adenomas, ceruminous gland adenomas, papillomas, basal cell tumors, ceruminous gland adenomas, histiocytomas, plasmacytomas, melanomas and fibromas. Malignant tumors, in decreasing order of incidence include ceruminous gland adenocarcinomas, undifferentiated carcinomas, squamous cell carcinomas, sarcomas, malignant melanomas and hemangiosarcoma. Ceruminous adenomas are typically exophytic and pedunculated. The surface of the masses is usually irregular; occasionally eroded or ulcerated. Parts of the mass may be discolored a "bluish" color which is related to the accumulation of ceruminous gland secretions within the mass. In that these masses often do have a narrower base; it is possible to excise the site of origin of the tumor, resulting in lack of re-growth. Depending on the site of origin within the canal, it is also possible to laser the base of the base of the mass through a video otoscope. Ceruminous gland adenocarcinomas are usually broad based, more irregular in contour and more likely to be eroded or ulcerated and more likely to have parts of the mass be a "bluish" color, due to the accumulation of ceruminous secretions within the mass. These tumors are aggressively locally invasive, potentially even extending through the cartilage of the ear canal.

Dilated pars flaccida: In the presence of otitis media, the pars flaccida may fill with inflammatory secretions / debris and dilate to various degrees. It is not uncommon to have it entirely occlude the horizontal canal, appearing as a smooth, often discolored sometimes neovascularized mass that is attached at its dorsal margin, but not ventrally and laterally. It appears very "cyst" like. Its contents may be fluid like or very dense (cholesterol granuloma). Cholesterol granuloma material has a very characteristic yellow-gold color.

Cysts: Epidermal inclusion cysts and ceruminous cysts are only rarely seen in the ear canal of the dog. In the authors' experience, inclusion cysts have been most commonly seen to arise from the floor of the horizontal canal, just in front of the tympanic membrane. They are smooth, rounded, relatively broad based structures that are whitish in color and fluid filled. They are often quite firm. Ceruminous cysts are usually solitary, relatively broad based and may occur at any place within the canal. Ceruminous cysts often take on a slightly bluish color because of the accumulation of ceruminous gland secretions.

Inflammatory Polyps: Aural Inflammatory Polyps in the dog tend to most commonly arise from the walls of the canals (unlike the cat, where polyps primarily arise from the middle ear). They are usually pedunculated, smooth, whitish in color, and firm. They are not fluid filled. Polyps can usually be removed by traction and avulsion. While some polyps have a narrow base and can be completely removed with traction, those with a broader base may have to be removed in pieces. Emphasis is again placed on removing as much of the base of the mass as possible. Following removal, a topical steroid is used in the ear for 4-6 weeks. With complete resection, regrowth is usually not encountered.

Proliferative Lesions: Chronic otitis externa may result in the development of fibroproliferative nodules within the canals. Proliferative nodules are usually multiple, variably sized, with relatively smooth surfaces. They are often

broad based. Ceruminous glands may dilate and fill with ceruminous secretions within these structures to impart a "bluish" discoloration to parts of the nodules. There is usually more diffuse thickening of the walls of the canals that will contribute stenosis of the canals. The ultimate goal for these proliferative ears is to shrink or remove the nodules and "open up" the canals throughout their length (to the tympanic membrane). Medical management directed at shrinking masses typically involves the use of both an oral and potent topical steroid. A more rapid reduction in the size of the masses may be achieved by using intralesional glucocorticoids (see below). Proliferative nodules may also be removed utilizing biopsy forceps or with laser. This is followed up with aggressive oral and topical steroid therapy.

Feline:

Neoplasia: Tumors of the ear canal of cats are more commonly malignant. The most common of the malignant tumors are ceruminous gland adenocarcinomas, followed by squamous cell carcinomas and then carcinomas of undetermined origin. Ceruminous gland adenocarcinomas are generally very broad based, with smooth to irregular surfaces. There is often a bluish discoloration to some of the proliferative tissue, due to the accumulation of ceruminous secretions. It is usually not possible to completely remove these masses from the ear, either with biopsy forceps or laser. Biopsies are generally performed to confirm a diagnosis. Ceruminous gland adenomas are occasionally seen in cats as narrower based proliferative lesions. Excisional biopsies with special emphasis on trying to remove as much of the base of the lesion as possible can be curative.

Feline Ceruminous Cysts/ Ceruminous Cystomatosis

Ceruminous cystomatosis is a non neoplastic disorder wherein ceruminous glands become cystic. These focal, smooth surfaced, blue colored lesions may be solitary or grouped and may originate anywhere from the tympanum, throughout the canals. The concurrent presence of otitis externa does tend to worsen the clinical manifestations of this syndrome (more lesions, larger lesions). Some severe cases (usually multiple, variably sized lesions) may proceed to the development of ceruminous gland adenocarcinoma. Excision / laser ablation for a singular or a few cysts can be curative.

Aural Polyps: Most polyps appear to grow from the epithelial lining of the epitympanic cavity/tympanic cavity, then extend either through the tympanum in to the horizontal canal or down through the auditory canal in to the posterior pharynx. Polyps grow in both directions in about 10% of cases.In the cat, polyps only rarely originate from the wall of the horizontal canal.Clinically they are usually seen as a smooth to rough surfaced, pink colored mass filling the horizontal canal. Diagnosis is by excisional biopsy.

The current therapy of choice for the management of aural polyps include removal by traction/avulsion followed by several weeks of an oral steroid which is expected to have a 95%+ cure rate. Procedure: under anesthesia, an open ended tomcat catheter is used to sample from deep within the horizontal canal (around the protruding mass) for cytology and culture and sensitivity testing. The ear is then thoroughly cleaned. The capsule of the polyp is firmly grasped with biopsy forceps (Storz, cat. number 69133) either blindly or through a larger otoscope cone / surgical otoscope and pulled out. If the polyp has a narrow stalk, the majority of the mass will often come out. If it is "broad based', it may have to be removed in pieces. Residual polyp material (if readily visible) is removed down to the level of the tympanum, just within the entrance to the middle ear (you will be in the middle ear, but not deeply in to the middle ear). Removal of residual polyp tissue from deep within the horizontal canal and to just within the entrance to the middle ear is facilitated by utilizing biopsy forceps directed through a video otoscope. Samples are again taken from the middle ear for cytology and culture and sensitivity testing. This and the sample from the horizontal canal are combined for purposes of culture. If there is ready access to the middle ear, it can be gently lavaged with large volumes of saline. Post avulsion topical therapy is dictated by cytology findings; the product should contain a steroid and be "safer" with respect to potential for ototoxicity. We tend to use "mixes" : for bacteria – enrofloxacin (22.7 mg/ml) : dexamethasone sodium phosphate (4 mg/ml), 1:2; for Malassezia – 1% miconazole : dex. sodium phosphate 1:1 or 1:2; for both bacteria and Malassezia (1% miconazole, enrofloxacin and dex. spp, 2:1:1). 0.3 – 0.4 ml of the mix BID for 1-2 weeks, then once daily for two weeks, then once every other day for two weeks. Post avulsion, the ear is flushed twice weekly. Post avulsion, the cat is treated with oral prednisolone, beginning at 2-3 mg/kg/day for 2 weeks, then 1 – 1.5 mg/kg/day for 2 weeks, then 0.5 – 0.75mg/kg/day for 2 weeks, then 0.5-0.75 mg/kg once every other day for 2 weeks (6-8 weeks of therapy). Oral glucocorticoid therapy is considered mandatory and the likely reason for the high

cure rate associated with this approach. Traction/avulsion may produce a Horner's syndrome (usually resolves within 2 – 3 weeks). The patient is rechecked every 2 weeks post avulsion to assess for resolution of secondary infections and healing of the tympanum. Healing potential for the tympanum tends to vary. A persistent hole in the tympanum is, however, usually well tolerated. Refractory cases are managed by ventral bulla osteotomy.

CT IN THE DIAGNOSIS AND MANAGEMENT OF MASSES WITHIN THE EAR CANALS

Neoplasms / cysts / polyps appear as soft tissue masses that occlude the canal to various degrees. Of particular importance is the ability of CT to evaluate for the potential extension of malignant tumors from the horizontal canal in to surrounding tissues (including through the cartilage of the ear) and in to the middle ear (soft tissue density). These structures will enhance with contrast. Enhancement helps to delineate the margins of the tumor. CT also allows for the better evaluation of complications related to the "mass effect" of having the horizontal canal completely obstructed by a mass. Wax/inflammatory/epithelial debris may accumulate behind the mass to eventually perforate the tympanic membrane and accumulate within the middle ear, producing an otitis media and the CT changes associated with this. Knowledge of the integrity of the tympanic membrane, as obtained on CT examination, may temper the aggressiveness with which a deep ear is performed to remove this debris. In tumors affecting the middle or inner ear, the predominant CT findings are lysis of the contour of the bulla or bulla septum (in the cat) or the petrosal part of the temporal bone, soft tissue swelling around the middle ear and distinct contrast enhancement. Squamous cell carcinomas in cats commonly cause lysis.

CT will show proliferative changes and they will be contrast enhanced.

Proliferative changes narrow and may completely occlude the external ear canal. These changes will enhance with contrast. Proliferative changes may also produce a mass effect re: debris accumulation and eventual perforation of the TM and the development of an otitis media.

A dilated pars flaccida in the dog will appear as a smooth, rounded soft tissue mass filling the canal to various degrees, just in front of where the TM would be. This does not enhance. There are variable concurrent signs of otitis media.

VIDEO OTOSCOPE PROCEDURES IN THE MANAGEMENT OF EAR CANAL DISEASE

Biopsies; Neoplasia, Polyp and Cyst Removal

- Biopsies utilizing the biopsy forceps are best facilitated by grasping tissue and both pulling and turning the biopsy forceps at the same time. Because biopsy samples tend to be small, consideration should be given to placing them in biopsy cassettes for purposes of submission to your pathologist.
- Consider using of a larger pair of biopsy forceps (Storz; Biopsy and grasping forceps catalogue number 69133) through a conventional hand held operating otoscope to remove larger amounts of tissue, then utilizes the smaller biopsy forceps through the video otoscope to remove remaining tissue.
- A 2 cannel adapter can be attached to the video otoscope working channel. The use of the simultaneous passage and use of biopsy or grasping forceps. Gravity flow is usually sufficient, but flow can be enhanced by using a pressure infusion bag around the IV bag. The continuous fluid flow will prevent fogging and debris accumulation on the scope lens, keeping the field of vision clear. It may facilitate flushing away hemorrhage so that you can observe the biopsy site more clearly.
- Note: if bleeding is encountered, suction fluid from the ear; remove scope; put pressure on ear or insert a cotton tipped swab in the ear and wait. It will usually stop within 1-2 minutes. A residual blood clot often has to be removed (can grab through video otoscope) prior to proceeding.

Deep Ear Cleaning:

Flushing, suctioning, "grasping" forceps and ear curettes:
Flushing and suctioning can be done through the working channel of the video otoscope utilizing a 5 F polypropylene urethral catheter (Argyle Suction catheter; Covidien), cut down to 6.5 or 7 inches or a 16 gage, 5.5 inch Teflon catheter (Milacath No. 1611; Mila International Inc.) or a 5 ½ inch, open ended tomcat catheter attached to a 12 cc syringe. Flush in to the ear, breaking up debris, then suction this out and discard. Repeat the process as necessary. Grabbing forceps can be used to grasp larger particulate material as necessary. An ear curette can also be used to break up material to facilitate flushing it out.

The Vetpump II (Storz) – offers the option of flushing and suctioning through the same catheter, utilizing a "one handed" button system (video otoscope held in one hand; flushing/suctioning unit held in the other). A 5 F urethral catheter is attached to the Vetpump and passed through the operating port of the video otoscope. The greatest cleaning action is simply achieved in the "flush" mode… running large amounts of fluid through the ear. Flushing pressures used are generally dictated by where in the ear the flushing is taking place. A large amount of pressure (setting at ¾ to full pressure) is used when working well away from the ear drum area. The pressure is reduced to ¼ to ½ when working close to the eardrum. The magnitude of the suctioning should be closely monitored. Excessive suction will result in removal of too much saline and collapsing of the canals. It is possible to damage the eardrum with excessive suction. The author prefers to use short intervals of suction, followed by re-expansion of the canal with flushing to facilitate cleaning. The optimal benefits of the Vetpump are usually achieved with one individual "running" the flush/suction catheter and another directing the video otoscope. The director of the video otoscope can help move the tip of the catheter around in the ear to facilitate access to various areas, while the individual working the catheter can move it to various depths within the ear. If the tympanum is perforated, the catheter can be passed in to the middle ear, to facilitate cleaning this structure. Flushing pressures are usually reduced (1/4 to ½ pressures). If in the middle ear, it is important that the hole in the tympanum is large enough to facilitate saline coming out of the middle ear, around the catheter. This minimizes the potential to further traumatize the tympanum.

The Auri-Kleen offers similar benefits to the Vetpump II but it eliminates the flushing and suctioning head by incorporating a foot pedal to control suction and irrigation. The majority of operations can be performed by a single clinician as the scope is held in one hand and the suction and irrigation catheter is easily manipulated with the other hand.

Intralesional Steroid Injections: This is often done in conjunction with anesthesia to perform a deep ear cleaning and biopsies of the lesions (to make sure they are not neoplastic). This therapy may lessen the amount of systemic glucocorticoid necessary to reduce proliferative changes. We utilize triamcinolone acetonide (2 mg/ml); spinal needle (3.5 or 6 inch, 22 gauge); 0.1 ml injections into proliferative lesions and/or if 360 degree proliferation, administer in a "ring" of 3 points around wall, with each "ring" 1-2 cm apart. The maximum triamcinolone dosage this author usually uses in a 30 – 40 pound dog is 6 mg. Repeat administration may be considered in 3-4 weeks.

VIDEO OTOSCOPY AND CT IN THE DIAGNOSIS AND MANAGEMENT OF CANINE AND FELINE OTIC DISEASE: PART 2

Mike Canfield DVM, DACVD[1], Rod Rosychuk DVM, DACVIM[2]

[1]Animal Dermatology South, New Port Richey, Florida
[2]Dept. of Clinical Sciences, Colorado State University, Ft. Collins, Colorado

Canine Otitis Media (OM): Pathogenesis

Inflammation within the middle ear may be infectious (bacterial, fungal) or noninfectious (foreign body, ceruminous debris, neoplasia, polyps, trauma, cholesteatoma).

A. Otitis media is most commonly an extension of an otitis externa, through a perforated tympanic membrane (TM).
   - In the dog, severe stenosis of the horizontal ear canal (chronic proliferative disease) has been associated with a concurrent otitis media in 50 – 80% of cases. Debris is unable to exit the ear and instead places pressure on and finally perforates the tympanum. A similar pathogenesis is noted with masses (cysts, polyps or neoplasia) when they totally occlude the vertical or horizontal canal or complete atresia of the canal (congenital or post traumatic / post inflammatory) - i.e. debris builds up behind the mass or area of atresia to eventually perforate the TM.
   - Severe bacterial infection ("rods seen cytologically; usually Pseudomonas spp) has been associated with a higher incidence of perforation.
   - Chronic debris accumulation (ceruminoliths; +/- secondary infection) may put enough pressure on the TM to eventually result in perforation and extension to cause an otitis media.
   - Foreign bodies (specifically grass awns) have been associated with TM perforation and OM.
   - TM perforation associated with deep ear cleaning and the subsequent injection of bacteria in to the middle ear may produce signs of OM, often within a couple of weeks of the "cleaning".
   - It is possible to have a perforated TM "heal" over an active otitis media, although, in our experience, this appears to be uncommon.

B. Extension of infection up the auditory tube does occur, but is uncommon in the dog. It is usually related to pharyngeal disease (inflammation; infection; neoplasia). The tympanum is usually intact in these scenarios, but abnormal (more opaque, thickened, +/- discolored, +/- neovascularized; pars flaccida may be dilated and exudate/fluid/debris filled).

C. Recalling that small amounts of fluid always drain from the middle ear through the auditory tube (natural flushing mechanism of the middle ear), anything that causes auditory tube dysfunction can result in fluid accumulation. Obstruction or dysfunction of the auditory tube due to posterior pharyngeal inflammatory disease or neoplasia may result in the accumulation of secretions within the middle ear. Auditory tube dysfunction may also result in fluid accumulation within the middle ear. This is likely the pathogenesis of mucous/fluid accumulation in Cavalier King Charles Spaniels[1] and other breeds such as the Boxer[2], noted to develop Otitis Media with Effusion (aka Primary Secretory Otitis media). Auditory tube dysfunction as the likely cause for middle ear fluid accumulation has also been noted in dogs with soft palate hypoplasia and trigeminal nerve lesions. Although the fluid accumulations with auditory tube dysfunction are often non inflammatory and sterile, otherwise normal microflora of the middle ear may produce infection.

D. Cholesteatoma is thought to form with a piece or pieces of the TM are displaced into the middle ear and attach to the wall of the middle ear. The TM tissue essentially becomes an epidermoid cyst made up of a multi-layered squamous epithelium producing large amounts of keratin. This keratin forms the matrix of the cholesteatoma. The matrix rests on a perimatrix, which is a stroma of varying thickness that is then attached to the bone of the middle ear. Hyperkeratosis and shedding of keratin debris results in the gradual expansion of the cyst and expansion

of the bulla wall. This may result in bulla wall thinning and eventual lysis. Osteoproliferation may also be seen[3]. Concurrent infection is not uncommon and potentially contributes to lytic and proliferative bulla wall changes. Cholesteatoma is most commonly associated with chronic, proliferative otitis externa wherein severe stenosis may be a significant contributor to debris driving parts of the TM in to the middle ear.

E. Polyps and neoplasia originating from structures within the middle ear are rare. It is more common to see middle ear neoplasia as an extension of neoplasms originating from within the canal and extending in to the middle ear (e.g. ceruminous and sebaceous gland adenocarcinomas).

Canine Otitis Interna (OI): Pathogenesis: otitis interna is most commonly an extension from middle ear disease (bacteria or bacterial toxins; pro-inflammatory cytokines; neoplasia).

Feline Otitis Media: Pathogenesis

As for the dog, inflammation within the middle ear may be infectious (bacterial, fungal, viral?) or noninfectious (ceruminous debris; polyps, foreign body, neoplasia, trauma). Inflammation within the inner ear (otitis interna, OI) is usually an extension from an otitis media. In the cat, it is relatively common to see evidence of otitis interna along with otitis media.

A. Otitis media may be seen an extension from an otitis externa, through a perforated tympanum. This pathogenesis usually involves the accumulation of ceruminous debris (e.g. ceruminolith) that results in pars tensa tears. Occasionally this scenario may be seen in ears that are stenotic due to inflammation (mass effect; see previous). A similar pathogenesis may be seen with any mass that completely occludes the canals (neoplasia, ceruminous cyst).

B. Extension of infection up a likely dysfunctional auditory tube appears to be a relatively common pathogenesis in the cat, compared to the dog. This is likely because cats have a relatively higher incidence of posterior pharyngeal inflammatory disease due to viral infections. With ascending infections, the pars tensa is usually abnormal (thicker, opaque, +/- discolored, +/- inflamed), but intact. Dilatation of the pars flaccida is occasionally seen with more severe accumulations of exudate within the middle ear in the cat. In such cases the dilatation is also due to thickening of the tympanic membrane (myringitis).

C. Serous to slightly mucoid secretions are normally produced in the middle ear and drain through the auditory tube as part of the normal flushing mechanism of the middle ear. Obstruction or dysfunction (i.e. collapse) of the auditory tube will result in the accumulation of secretions within the middle ear. Some insight as to what changes might be expected to occur in the middle ear of an otherwise normal cat with an obstructed auditory tube has been recently explored in an experimental setting. Within a week of obstruction, moderate hyperplasia and a neutrophil rich mild inflammatory cell infiltrate was noted in the epithelial layer lining the middle ear. The middle ear was filled with a serous effusion. At 2 weeks post obstruction, hyperplasia and edema of the epithelium was severe and there were more inflammatory cells within the serous effusion. After 4 weeks of obstruction, the serous effusion became more mucoid, with an increased number of inflammatory cells (plasma and other mononuclear cells)[4].

   In one study, the prevalence of bulla effusion in cats with sinonasal disease (inflammatory or neoplastic) was 28% (13/46 cats)[5]. Bulla effusions are relatively commonly seen as incidental findings on CT/MRI examinations of cats[6]. Most of these cats have no clinical signs of OM. It is assumed the effusions are related to obstruction. In our experience, effusions in such cases are usually sterile and may be non inflammatory or inflammatory. The incidence of bacterial infections in these scenarios (effusion as an incidental finding) is low. In that there may be small numbers of bacteria within the middle ear of some normal cats (10% being culture positive at any given time), it is suspected that this may be the source of the infection in some of these ears. When fluid is only serous to lightly mucoid and not significantly inflammatory, the tympanum is more opaque than normal, but otherwise normal. Affected patients are otherwise asymptomatic. When the middle ear effusions are inflammatory (with or without infection), the TM is very opaque and may be thickened, discolored or inflamed. The pars flaccida may dilate due to thickening (myringitis) and the accumulation of inflammatory debris associated with the otitis media. Discoloration/inflammation/thickening of the tympanum tends to be more common with active secondary infection.

D. An acute otitis media / otitis interna recognized in the cat has been termed "Primary" otitis media because a cause for the otitis has not been established. To date, cultures from the middle ear have been negative, as have efforts to find viruses (e.g. herpesvirus, calicivirus). Affected cats usually have not had histories of sinus or pharyngeal disease (to suggest auditory tube obstruction). They are usually presented with acute signs of inner ear disease (head tilt, ataxia, nystagmus). Inflammation within the middle ear is often neutrophilic to pyogranulomatous. The TM is intact, but abnormal (more opaque; occasionally inflamed). The authors suspect that at least some of these cats do have auditory tube dysfunction (with a pathogenesis of signs as outlined above).

E. Aural polyps: see Part 1 of these proceedings.

VIDEO OTOSCOPIC CHANGES ASSOCIATED WITH OTITIS MEDIA

Canine otitis Media:

1. When the tympanic membrane is intact, the presence of a concurrent otitis media related to the accumulation of inflammatory /ceruminous /epithelial debris is often associated with a TM that is opaque and potentially discolored (darker; brownish) or neovascularized. The pars flaccida may be dilated, more opaque to discolored, neovascularized. Most "bulging" tympana seen in the dog are related to dilated pars flaccidas. With Otitis Media with effusion (aka Primary Secretory otitis Media) in the Cavalier King Charles Spaniel and some other breeds, the pars flaccida may be dilated and more opaque than normal; pars tensa also more opaque1. In Boxers with this syndrome, bulging of the pars tensa (not flaccida) has been noted2.
2. When the tympanum is not intact and the OM is related to inflammation/infection, debris within the middle ears can appear highly variable: purulent; mucopurulent; mucoid; usually darker waxy debris, often admixed with lighter colored epithelial debris; whitish material that is often predominantly epithelial debris.
3. With chronic otitis media, after debris has been removed from the tympanic cavity, it is often possible to visualize the bone making up the medial wall of the middle ear. With chronic otitis media, the lining of the middle ear may be covered with granulation tissue. Granulation tissue may totally obliterate the communication between the tympanic cavity and tympanic bulla. With chronicity in chronic proliferative ears, the lining of the horizontal canal may actually extend in to the middle ear (as evidenced by finding hairs that normally grow from the floor of the horizontal canal, growing within the ventral aspect of the tympanic cavity.
4. Visualization of the middle ear changes associated with a cholesteatoma is often hindered by severe stenosis of the horizontal canal. If it is possible to see in to the middle ear, visualized keratogenous debris is often non specific when compared to otitis media. This debris is often whitish to tan in color. On a rare occasion, a cholesteatoma will appear as a smooth mass within the middle ear.

Visualization of the tympanic bulla: through the regular video otoscope, it is possible to visualize into the tympanic cavity, but not into the bulla. In larger breed dogs (>20 kg), an arthroscope (2.7 mm diameter; with fluid infusion sleeve only) can be used to visualize in to the middle ear because of the angle of the tip (30 degrees) allows for visualization "around the corner" in to the middle ear. Visualization is enhanced by continuous fluid infusion through the arthroscope sleeve. It is not possible to atraumatically pass this in to the middle ear of any smaller individual. Great care must be taken in making sure that you do not "force' the scope into the middle ear – significant trauma may be induced Manipulative procedures (grasping forceps; flushing) cannot be performed through this sleeve.

CT CHANGES ASSOCIATED WITH OTITIS MEDIA/INTERNA

In both the dog and the cat, OM associated with debris accumulation within the middle ear (fluid/exudate/wax/epithelial debris) +/- active bacterial and/or fungal (Malassezia) infection is visualized as a soft tissue density. With chronic otitis media, thickening of the epithelial lining of the middle ear will also contribute to the soft tissue density seen within the middle ear. It is usually difficult to differentiate between fluid/ thick inflammatory debris and tissue proliferation (e.g. fibroplasia) within the middle ear. With chronicity, there may be sclerosis and proliferation and potentially lysis of the bulla wall. There may be contrast enhancement of the lining of the tympanic bulla (thickening of the normal epithelial lining of the middle ear) or contrast enhancement of the contents of the tympanic cavity in the presence of marked fibrous proliferation. Accumulated epithelial debris (keratin) and exudates will not contrast enhance. In dogs, bony spicules that normally originate from the bulla septum may break off and become embedded

within this debris. Especially with infection, there may be lysis of the petrous temporal bone in the region of the inner ear. The development of an otitis interna might be seen as an obliteration of the fluid-filled spaces of the inner ear. Brainstem involvement may be seen on post-contrast images.

In the dog, cholesteatomas are associated with expansion of the tympanic bulla, osteoproliferation, osteolysis and/or osteosclerosis (in decreasing order of incidence) 6. There is soft tissue density within the middle ear that is not contrast enhancing (because a cholesteatoma is composed of keratin debris which is avascular). However, a heterogenous contrast enhancement may be seen which is likely due to increased vascularity within the perimatrix3.

Feline aural polyps are seen as a soft tissue density within the proximal horizontal canal (adjacent to the TM). The middle ear is usually filled with a soft tissue density. There is variable thickening of the bulla wall. There may be lysis of the bulla septum. This appears to be most commonly associated with concurrent bacterial infection of the middle ear, although a component of pressure necrosis may also play a role. There is usually strong homogenous contrast enhancement or poor central enhancement with strong rim enhancement.

In both the dog and cat, tumors affecting the middle or inner ear will produce soft tissue densities. It is more common to see lysis of the contour of the bulla or bulla septum (in the cat) or the petrosal part of the temporal bone. Slower growing, less aggressive tumors may cause bulla expansion. There may be soft tissue swelling around the middle ear. With neoplasia, there is distinct contrast enhancement. Squamous cell carcinomas in cats commonly cause lysis.

With "Primary Secretory Otitis Media" in the Cavalier King Charles Spaniel and rarely in other breeds, the middle ear is filled with a soft tissue density which is actually fluid (thick mucous). A dilated, fluid filled pars flaccida may be seen. There are no bony changes.

DIAGNOSTIC AND THERAPEUTIC TECHNIQUES IN THE MANAGEMENT OF OTITIS MEDIA

MYRINGOTOMY: This procedure is generally performed when there is suspicion of otitis media, but the tympanum is intact. In some of these cases, the otitis media is actually an extension from an otitis externa wherein the otitis media persists after the hole in the tympanum "heals" over. Alternatively, infection may develop within the middle ear due to an ascending infection from the pharyngeal region through the auditory canal. Effusions may also be seen with obstruction of the auditory tube (effusions may be sterile or not).

Prior to performing any myringotomy, the horizontal canal should be thoroughly cleaned and dried. The site for performing the myringotomy is caudo ventral in the pars tensa, over the ventral most part of the tympanum (just above the wall of the horizontal canal). Several equipment options exist for performing the myringotomy: needles/ catheters/culture swabs:

1. 6 ", 22 gage spinal needles are available that are long enough to be passed through the operating port of the video otoscope. Storz makes a long needle that can be used for this purpose. The needles allow for the creation of a very small hole that will heal very rapidly.
2. An open ended tomcat catheter (tip may be cut to an angle to allow for better perforation of the tympanum), or "cut down" 5 F polypropylene urethral catheter (tip can be cut to an angle) or 16 gage Teflon catheter (tip can be cut to an angle) , attached to a 12 cc syringe can be used through the video otoscope. Video otoscopy allows for wonderful visualization and proper placement of a myringotomy needle/catheter.
3. Whichever method is used, once the needle/catheter is through the tympanum, it is advanced until it hits bone and then aspirated. If there is strong suggestion of debris within the middle ear (i.e. based on radiographs/ CT or MRI) but nothing is retrieved on aspiration, then a small amount (1-2 ml) of sterile saline can be infused in to the bulla and then re-aspirated.
4. If an otitis media is encountered, a larger hole should be created to facilitate more thorough flushing (i.e. passing catheter in to the middle ear) and to prevent barotrauma from the increased water pressure within the middle ear. The key to medically managing otitis media is flushing large amounts of fluid through the middle ear.

MIDDLE EAR CLEANING: Prior to cleaning, emphasis is placed on using a catheter to sample from the middle ear (for both cytology and bacterial culture and sensitivity testing). If the tympanum is perforated or is not present, the bulla can be "flushed" and "suctioned" as outlined in "Part 1" of these proceedings. Emphasis is placed on working in

the bottom half of the middle ear (to prevent trauma to the sensitive structures that are in the more dorsal and caudo dorsal aspects of the middle ear). If one is working through a hole in the tympanum, it is imperative that the hole be large enough to facilitate fluid coming back out of the middle ear once it is flushed in. Holes for this purpose should be about 1/3 to ½ the surface area of the pars tensa. If the hole requires enlarging, this can be done with a catheter. For flushing purposes, to facilitate better catheter entry into the tympanic bulla, a slight curve can be given to a 16 gage, teflon catheter by placing a circlage wire through the catheter and bending the tip, then heating the catheter in boiling water. Once it has cooled, the catheter will retain its "curve". The catheter will straighten as it goes through the operating port, but will curve again as it exits the tip of the otoscope. The curved end should be directed down into the bulla. Cardiovascular catheters with curved tips are available that can also provide this better access to the bulla. The prime goal with respect to cleaning the middle ear would be to make sure large volumes of saline have been flushed through the middle ear (e.g. in a black Labrador retriever, we would flush at least 200 – 300 ml through the middle ear).

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Selected Feline Dermatoses: Dermatoses Affecting Digits

Bizikova P

North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences, Raleigh, NC, USA

The lecture will review several case-scenarios in which cats with, at first glance, similar skin lesions end up with different diagnoses. The lecture will also discuss some of the main differential diagnoses for each case scenario and a diagnostic approach to distinguish them apart.

The text below contains basic information about some of the diseases discussed in the lecture.

1. CATS WITH THICKENED ABDOMEN AND EXUDATION

MYCOBACTERIAL PANNICULITIS[1,2] (due to non-tuberculous, rapidly-growing mycobacteria) *Uncommon disease*

Mechanism of lesion formation: wound contamination by mycobacteria present in the environment (soil, water, decaying vegetation); mycobacteria identified: M. fortuitum, M. chelonae-abscesus, M. smegmatis, M. flavescens, M. avium, etc.

Source of infection: environment (soil, water, decaying plant material); infection is usually the result of a traumatic inoculation (outdoor living cats are in higher risk)

Potential zoonotic risk with some species (wound inoculation; especially in immunocompromised people)

Signalment:

- no breed, sex or age predilection
- outdoor cats in higher risk

Main characteristics:

- deep dermal and subcutaneous papules, nodules and/or plaques with or without draining tracts (exudate may appear oily)
- they are commonly localized on the caudal abdomen, but face, extremities, tail base and perineum (areas easy to injure) are also reported

Diagnosis (skin):

- cytology from the deep dermal/subcutaneous masses (FNA) and draining tracts (impression smear): pyogranulomatous inflammation with a chance to find intracellular, negatively stained rods if Dif Quick or Giemsa stain is used (special stain is required to visualize them (Ziehl-Neelsen); cytology has low sensitivity
- histopathology: excision biopsy is recommended due to the depth of the lesion; special stains need to be performed to demonstrate the pathogen (consider Fite-Faraco staining as some nontuberculous mycobacteria do not stain well with classic Ziehl-Neelsen staining)
- culture is challenging, but attempt to culture should be made to determine the sensitivity profile (tissue culture is preferred)
- PCR

Treatment:

- surgery is recommended if local infection can be removed; larger areas can be treated first to reduce the size and then surgically debrided or vice versa (surgically debrided and treatment of the residual lesions); multiple surgeries may be considered
- medical management includes combination of antibiotics (often 3 ATB during the first few months, then reduced to two) such as quinolones (pradofloxacin, moxifloxacin, marbofloxacin), azithromycin, rifampin for 6-9 months (if culture is available, antibiotics should be selected based on the sensitivity profile); topical therapy with clofazimine in petroleum jelly or in silver sulfadiazine with or without DMSO have been used together with oral ATB
- prognosis should be guarded (depends on the type of mycobacterium species, extent and severity of the disease, resistance to ATB)

LYMPHANGIOSARCOMA[3]
Rare disease

Mechanism of lesion formation: malignant neoplasia of lymphatic vessels (predisposing factor has not been identified in cats)

Signalment:
- no breed, sex predilection; usually older cats, but the age of onset range is wide (2 to 15 years)

Main characteristics:
- patchy to diffuse erythema or purpura with poorly defined skin thickening (plaque/mass) usually localized on the caudal abdomen, but may be observed on the ventral thorax or neck
- oozing of serous to serosanguineous exudate (lymphorrhea) is characteristic

Diagnosis:
- histopathology (a deep wedge biopsy or double punch biopsy is recommended due to the depth of the neoplastic process)

Treatment:
- radical surgical excision (usually not possible due to the infiltrative growth)
- chemotherapy (only one case reported): metronomic therapy with toceranib, meloxicam and Chlorambucil (partial remission in 18 days and stable disease was maintained for 36 days)[4]
- prognosis is poor (most cats are euthanized within few weeks to 10 months)

2. CATS WITH EROSIONS/ULCERATIONS AND CRUSTING ON THE MUZZLE

PEMPHIGUS FOLIACEUS[5, 6]
Rare disease (but the most common autoimmune skin disease in cats)

Mechanism of lesion formation: it is believed to be similar to the other species; a disruption of keratinocyte adhesion in the superficial levels of the epidermis leading to an acantholysis and subcorneal pustule formation followed by shallow erosions and crusts

Signalment:
- not confirmed breed or sex predilection; middle-aged cats usually affected; but the range is broad: less than 1 year to 17 years of age)

Main characteristics:
- pustules (rarely seen due to their fragility), erosions and crusts affecting predominantly the ear pinnae, periocular, muzzle regions of the face
- crusting and purulent exudation around nail beds of most digits is highly suggestive of pemphigus foliaceus (may be the only clinical sign in about 11% of cats6
- pruritus is variable
- systemic signs are fairly common (half of the cats) and include lethargy, anorexia and/or fever

Diagnosis:
- combination of clinical and microscopic assessment
  - symmetric, erosive to crusty dermatitis in predisposed areas (crusts on pinnae muzzle and purulent exudation around most nail beds and/or nipples are highly suggestive)
  - cytology (impression smear) from intact pustule or from underneath of a crust-covered erosion showing acantholysis and lack of bacteria
  - histopathology of a pustule or a crust-covered eroded skin (do not scrub the crusts off as they may contain the only evidence of the disease)
- exclusion of other acantholytic diseases; although superficial pyoderma, and particularly impetigo that is known to contain some acantholytic cells, is not a common skin disease in cats

Treatment:

- prednisolone (2-4 mg/kg/day) or triamcinolone (0.4-1 mg/kg/day) often provide a rapid remission (median: 3 weeks) 6, but the disease has tendency to relapse with reduced dosage or discontinuation of the medication. To avoid side effects associated with a long-term use of glucocorticoids, cats requiring long-term treatment are often switched to other drugs such as:
  - cyclosporine (7-10 mg/kg/day) - has been used with success to provide a long-term control in relapsing cases
  - chlorambucil (0.1-0.2 mg/kg daily to every other day) - has been used to achieve remission as well as to provide long-term control

FELINE HERPESVIRUS DERMATITIS
Uncommon disease (while herpesvirus infection is a common disease, the skin involvement is seen infrequently)

Mechanism of lesion formation: cytopathic effect of feline Herpesvirus-1 and immune response of the cat to the infection

Signalment:

- no breed, sex or age predilection
- several stress factors (multi-cat households and/or the use of glucocorticoids) have been suggested to cause a reactivation of a latent herpesvirus infection (respiratory, ocular and/or cutaneous); FIV and FeLV have not been shown to be predisposing factors

Main characteristics:

- vesicular, ulcerative and crusting dermatitis (non-healing) commonly affecting the nasal planum, nose bridge, periocular region (connection with a mucosa is usually noted)
- lesions may or may not be symmetric
- other areas of the skin may be affected rarely (e.g. forelimbs (possibly transmitted during grooming))
- pruritus is variable
- upper respiratory tract infection and/or conjunctivitis may be seen concurrently or may be reported historically (even several months ago)
- ulcerations in the oral cavity may be also present (herpesvirus stomatitis)

Diagnosis:

- characteristic skin lesions and concurrent (if present) upper respiratory tract infection mat be clinically helpful
- cytology (impression smear) from the ulcer may be deceiving (eosinophilic nature may mislead clinician towards allergies; neutrophilic inflammation may be secondary due to secondary bacterial contamination of the ulcer or in cats treated with glucocorticoids)
- sample for histopathology should include area of the ulcer margin (75% ulcer, 25% intact margin) as well as the ulcerated center (immunohistochemistry can be used to visualize the virus better)
- PCR for feline Herpesvirus-1

Treatment[7]:

- stress reduction and discontinuation of any immunosuppressive drugs if given previously
- famciclovir: 40 (preferably 90) mg/kg 3x daily orally) for ~ 4 weeks (but maybe longer)
- recombinant feline IFN omega - perilesional or subcutaneous
- L-lysine: controversial efficacy; lysine, when administered as bolus, reduced viral shedding in latently infected cats and clinical signs in cats undergoing primary exposure to the virus; however, the efficacy of lysine as a dietary supplement in client-owned cats has not been investigated

MOSQUITO-BITE HYPERSENSITIVITY[8, 9]
Uncommon to rare disease

Mechanism of lesion formation: an allergic reaction to mosquito bites with a very distinct clinical presentation (it is not known if other biting insect could cause similar reaction)

Signalment:
- no breed, sex or age predilection
- outdoor life-style
- possibly color preference (darker color)[9]

Main characteristics:
- military dermatitis progressing to more prominent papules and crusting or, in severe cases, to swelling, alopecia, erosions/ulcers and exudation
- lesions are typically present on the muzzle (nasal planum may be involved), pinnae and/or periorbital region
- pruritic
- seasonal pattern (summer) with lesion resolution in the Fall or with an indoor confinement

Diagnosis:
- cytology from the papules/erosions/ulcers: confirms eosinophilic dermatitis (rule out other eosinophilic reactions in this area (e.g. herpesvirus dermatitis in case of nasal planum/muzzle lesions))
- histopathology

Treatment: avoidance of mosquito biting and glucocorticoids (anti-inflammatory dosage)

SQUAMOUS CELL CARCINOMA[10]
Common disease

Mechanism of lesion formation: locally invasive skin neoplasia of keratinocytes

Etiology/Distribution: UV-light exposure (nose, ears, eyelids of white cats) or papilloma virus-associated (anywhere; starting as pigmented hyperkeratotic viral plaques and progressing into Bowenoid in situ carcinoma and SCC)

Signalment:
- no breed, sex or age predilection
- middle-aged to older white cats with access to outdoors or tendency to sunbathe (solar-induced)
- feline viral plaques (younger cats), Bowenoid in situ carcinoma/SCC (older or immunosuppressed cats)

Main characteristics:
- non-healing erosions, ulcers and crusts with some peripheral proliferation at the edge of the lesion; mass formation may become clearer with time
- more or less pigmented, hyperkeratotic papules/plaques with or without ulceration and crusting

Diagnosis:
- cytology from the papule/plaque: fine needle aspiration (in case of a clearly proliferative lesion); impression smear after gently scraping off the crust and exudate from the surface of the ulcerated lesion

*** Cytology may not be diagnostic in all cases, especially if prominent inflammatory response is present***
- histopathology

Treatment:
- complete surgical excision (5mm margins) - invasive SCC (disfiguration possible in case of nasal planum/ear/eyelid lesions)
- radiotherapy – invasive SCC (repeated anesthesia and higher recurrence rate than surgery)
- cryosurgery - superficial SCC
- photodynamic therapy – superficial SCC (photosensitizing agents: 5-aminolaevulinic acid (5-ALA), hematoporhyrin derivate, etc.)
- CO2 laser
- cytotoxic drugs (carboplatin intralesionally)
- imiquimod (early lesions of viral plaque/Bowenoid in situ carcinoma type lesions (not solar-induced SCC); expect large degree of local inflammation with this treatment)

PEMPHIGUS VULGARIS[11]
Very rare disease with less than a handful of cases described in the literature

Mechanism of lesion formation: autoimmune blistering skin disease characterized by a suprabasal acantholysis

Signalment:
- unknown breed or sex predilection
- median age: 5 years (range: 1-14 years)

Main characteristics:
- deep erosions and ulcers in the oral cavity, lips and nasal planum
- halitosis

Diagnosis:
- histopathology (sample from the margin of the ulcer)

Treatment:
- glucocorticoids (immunosuppressive dosage; 2/4 cats showed rapid complete resolution and 1/4 cats showed partial resolution of clinical signs)
- glucocorticoids and gold salts (1/4 cats responded to this combination)

AUTOIMMUNE SUBEPIDERMAL BLISTERING SKIN DISEASES
Very rare diseases with less than handful of cases described in the literature

A naturally occurring mucous membrane pemphigoid (MMP) has been described in two cats (one of the two cats from an older publication on bullous pemphigoid (case #1) fits clinically, histopathologically and immunologically for MMP).[12, 13] Both cats exhibited vesicles and/or erosions and ulcers on mucosae and mucocutaneous junctions (eyelids (1), lips (2), soft palate (1)), and concave pinnae (2). Immunotesting revealed autoantibodies targeting collagen XVII in one and laminin-332 in another cat.[12, 13]

A naturally occurring bullous pemphigoid (BP) has been described in a cat[12] in which lesions appeared to be of minimal severity, with vesiculation and erosions occurring predominantly on the ears, trunk and extremities. Mucosal involvement was mild. Like in people and dogs, the BP affected cat produced IgG against NC16A domain of collagen XVII.[12]

3. CATS WITH DIGITAL SWELLING, EXUDATION AND CRUSTING
PEMPHIGUS FOLIACEUS (see section above)

METASTATIC PULMONARY ADENOCARCINOMA[14]
Rare disease

Mechanism of lesion formation: distant metastases from a pulmonary adenocarcinoma into feline digits is well recognized and highly clinically characteristic entity

Signalment:
- no breed, sex predilection; older cats (average age: 12 years)

Main characteristics:
- single, but usually multiple swollen, erythematous and ulcerated digits with or without nail loss
- osteomalacia (X-ray) and lameness
- respiratory signs are usually not present clinically

Diagnosis:
- cytology from the digit masses (not rewarding usually due to concurrent inflammation and secondary infection)
- histopathology
- chest X ray

Treatment:
- none
- prognosis poor (most cats euthanized shortly after diagnosis is made)

OTHER DISEASES CAUSING DIGITAL SWELLING OR CLEAR MASS FORMATION IN CATS:
most of them are rare

Other diseases can lead to digital swelling in cats. Inflammatory and infectious causes include eosinophilic granulomas, fungal or bacterial infection. Digital neoplasias are rare with exception of SCC. Other, less common neoplasias include fibrosarcoma, adenocarcinoma, osteosarcoma and other.1[5]

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Fungal Dermatoses in Veterinary Dermatology

Amelia White, DVM, MS, DACVD

Assistant Clinical Professor of Dermatology
Auburn University College of Veterinary Medicine

What are fungi?[1]
Fungi are eukaryotic organisms that are ubiquitous in the environment with over 100,000 species known (yikes, how many more are there that we don't know about?). Most fungi live in the soil, but they can also be pathogens of plants and animals. What makes a fungus different than plants and bacteria? Fungi have *chitin* in their cell walls, a long-chain glucose-derived polymer. Chitin is also found in the exoskeletons of some arthropods (shrimp, crabs, insects) and in the scales of fish. Functionally, it is very similar to the protein keratin, and provides structure and support to the cell wall. It can also play a role in phenotypic expression, and thus have a role in signaling and communication between organisms. Fungi have other components of the cell wall including mannoproteins and beta-glucans among other things. The cell membrane contains *ergosterol*.[2] Fungi are different than plants because they cannot synthesize food from light. Instead, they are heterotrophs and must ingest or absorb nutrients for energy (animals are also heterotrophs). They do this by secreting enzymes to digest food externally and then absorbing it through their cell walls.

What do fungi do?[1]
If you hear the word fungus, what immediately comes to mind? I think of a mushroom popping up overnight after a heavy rain. Fungi are thought of as the primary decomposers in ecological systems and important for recycling nutrients back to the environment. Some fungi are edible (truffles…yummy), others are stinky (mould on the food in the back of the fridge), some have medicinal purposes (antibiotics), others are used recreationally (psychometric agents) and others are toxic. No matter the effects fungi may have, they really are just trying to decompose things. They generally are classified based on their normal habitat: geophilic (soil), zoophilic (animals), and anthropophilic (humans).

What do they look like?[1]
Fungi can grow in several different forms: 1) *yeast* (unicellular), 2) *mould* (multicellular/filamentous), or 3) both yeast and mould. Typically, fungi grow as *hyphae* which may branch to form interconnected hyphal networks called *mycelia*. These may be large enough to see with the naked eye, like what we see as fluffy white "mould" on week-old bread. Mycelia also are the colonies we see when we grow a fungal organism in an agar dish (culture). Hyphae can be either *septate* (divided into multiple compartments) or *coenocytic* (noncompartmentalized). The presence of pigment in the cell wall can cause pigmentation of the hyphal structures and mycelia.

How do they reproduce?[1]
Fungi have complex reproduction…but we all know that sex is pretty complicated. So let's break it down. In general, there are two forms of reproduction: 1) asexual and 2) sexual. Asexual reproduction occurs when offspring arise from ONE parent (so there's no fusion of gametes and generally no chromosomes involved). Sexual reproduction occurs when offspring arise from TWO parents (so there IS fusion of genetic info and sharing of chromosomes). Now this is what some people refer to as "bowchickawowow!" for those of us with adolescent mindsets. ☺

A lot of fungi can do both! This is probably why fungi have been around forever, and will continue to be around forever. The sexual state is called the *anamorph* and the asexual state is called the *teleomorph*. Asexual reproduction can occur in a lot of different ways, but fungi usually do so through production of vegetative spores called *conidia* or through fragmentation of the mycelia. Sexual reproduction occurs most commonly via *meisosis* (chromosomal division resulting in four daughter cells with half the number of chromosomes as the parent cell). This form of sexual reproduction is unique and different from the typical "mating" form of sexual reproduction. "Mating" form of sexual reproduction is limited to the fungal taxonomic groups, Ascomycota and Basidiomycota.

Normal Fungal Flora

Dogs and cats are furry creatures, and environmental moulds and pollens along with dirt and anything else "the cat brought in" can cling to the haircoat. This means that many different saprophytic moulds and yeast can be found in their haircoats (an extensive list can be found on pg. 224 of *Muller and Kirk's Small Animal Dermatology*, 7th Ed.).[3] Fungi isolated in dogs may include *Alternaria, Aspergillus, Aureobasidium, Chrysosporium, Cladosporium, Mucor, Penicillium*, and *Rhizopus*. For cats fungi include many of the same genus with the addition of *Rhodotorula* and *Scopulariopsis*.Additionally, dermatophytes (e.g. *Microsporum* sp. and *Trichophyton* sp.) can be harbored in the haircoat of dogs and cats.[3]

Seeking Opportunity

So how does an infection happen? Most fungi are opportunistic pathogens meaning that they only invade the body if the natural defense system of the host permits them to do so. The most important defense system against fungi opportunists is the *innate immune system*. This is the sector of the immune system that is naturally present, nonspecific, and not due to prior sensitization.[4] Examples of innate immunity include physical barriers like intact skin and hair, cutaneous lipid bilayer, moisture, temperature, pH, antimicrobial peptides, and other enzymes.4 The innate immune system also includes leukocytes (white blood cells) such as Natural Killer (NK) cells, mast cells, eosinophils, basophils, macrophages, neutrophils and dendritic cells. These cells recognize conserved regions on pathogens called pathogen-associated molecular patterns (PAMPs) and mount an immune attack.[4] This means that even if the immune system has never been exposed to a particular pathogen previously, it can still mount an attack against it. Invasion and infection with a fungal organism occurs when natural barriers and/or immunity are compromised. The organism invades the tissue and is able to resist the host's immune system. This could happen for numerous reasons such as physical trauma to the skin barrier, surgery, poor husbandry, underlying diseases, immune-compromising medications, and changes in normal microbiota.[2,4] Fungi invade via different mechanisms and then may remain at the site of infection or disseminate (spread) throughout the body. Once inside of the body, they can create different types of reaction patterns including: 1) acute suppurative inflammation and microabscess formation, 2) chronic granulomatous to pyogranulomatous inflammation, and 3) necrosis caused by invasion of blood vessel and subsequent tissue infarction.[3] Once they disseminate, the prognosis for survival of the animal is poor.

Fungal Imposters[3,5-15]

What about the organisms that have similar characteristics to fungi, but are not truly fungi? The most common organisms that can wreak havoc in the skin are in the class Oomycetes which are aquatic organisms. These differ from true fungi because they produce motile, flagellate *zoospores* (infective stage) and lack chitin in the cell wall. Additionally, ergosterol is not a major component of the cell membrane. This comes into play when discussing their lack of susceptibility to azole antifungals which target ergosterol formation. These organisms typically are found in temperate to tropical regions that have high humidity. The biflagellate aquatic zoospores are released into warm, slow-moving or stagnant water. They are attracted to areas of high CO2 which is emitted from damaged skin and gastrointestinal mucosa. Once they penetrate non-intact tissue, they are either recognized by the immune system and eliminated, or they bypass the normal immune response and create infection. Even though they are not true fungi, they will grow on fungal tissue culture given the proper tissue handling and growth requirements, so be sure to notify the laboratory of your suspicion. Additionally, advanced diagnostic techniques such as serology, immunohistochemistry, and PCR are available.

Zoonotic Potential

There is azoonotic (spread of disease from animal to human) potential for every cutaneous fungal disease; however, the risk is higher for some fungi over others. We have all heard that dermatophyte has a high zoonotic potential, but what about the weird opportunistic fungi? One of the biggest zoonotic concerns is for *Sporothrixsp*. This is largely because cats with sporotrichosis shed large numbers of infectious organisms from their draining tracts.[7] A minor abrasion, even microscopic, on your finger can lead to a terrible infectious nodule! Other fungi are much less likely to be zoonotic; however, if abrasions on the skin are present, and the person has an altered immune response, then the risk is greater. A good rule-of-thumb is, **"Treat everything like it's zoonotic!"** Be smart. Put on gloves. Wash your hands well. And don't touch it if it looks bad and you know your immune system is compromised.

Cutaneous Fungal Dermatoses

The ins and outs of how each fungal organism creates infection and is subsequently treated is unique to the organism. For this reason, I have included a table of some of the most common fungal dermatoses we see in dogs and cats (see **Table 1**). Diagnosing and treating each disease is very similar. We will focus on this next, and have case discussions in lecture to review each type of fungal dermatosis.

Diagnosis

Because we know that many fungi reside normally on the skin andhaircoat of dogs and cats, just growing a fungus in culture does not prove the diagnosis of fungal dermatitis. *Muller and Kirk's Small Animal Dermatology* provides helpful criteria for differentiating pathogenic from contaminant fungi[3]

These include:

1. Source of sample collected
2. Number of colonies isolated in culture
3. Species isolated in culture
4. Whether the fungus can be repeatedly isolated from the animal
5. The presence of fungal elements in the tissues (usually assessed via histopathology)

I might also add to this list, 6) sampling technique. Proper specimen collection (site and technique) is important to ensure the diagnosis is correct. A specific diagnosis is generally achieved by a combination of history, dermatological and physical exam findings, cytological findings, tissue culture, histopathology, serological analysis, and PCR or other immunodiagnostic techniques.

A. History
When obtaining a history, important information includes signalment, age of onset, environmental exposure, swimming/water exposure, onset and distribution of clinical lesions, pruritus score, response to previous therapies, presence of respiratory of gastrointestinal abnormalities, and lesions present on other pets/humans in the household.

B. Physical examination**WEAR GLOVES**
The value in a complete physical exam cannot be understated here. Fungal infections spread via lymphatics, so lymph nodes may be palpably firm or enlarged. Some fungal infections invade the eye, and a thorough ocular exam is important. The oral cavity should be evaluated for evidence of masses or other lesions, as well as hydration and perfusion status. The skin, ears and extremities should be thoroughly evaluated for any lesions (see next section). Thoracic auscultation is performed to assess for arrhythmias, murmurs or changes in lung sounds (crackles, wheezes, harsh sounds, or decrease lung sounds), and changes in air passage through the upper airway include nares, nasal cavity, nasopharynx, larynx and trachea. Thorough abdominal palpation may reveal mass-like structures suggestive of gastrointestinal spread (or origin) of disease. Rectal examination is valuable to assess dorsal lumbar lymph nodes, swelling or pain of the prostate in male dogs, and for any changes in rectal mucosa.

C. Dermatological examination
You guys are experts here already! Fungal dermatoses can present with a range of cutaneous lesions depending on the fungal offender including more superficial lesions like alopecia, erythema, papules, pustules, epidermal collarettes, and crusts. However, we also classically think about fungal infections with forms of nodular dermatitis which present as furuncles, nodules, masses, ulcers and fistula. These indicate a deeper form of cutaneous infection and inflammation. Distribution of lesions usually is asymmetrical in nature when dealing with infectious causes of dermatitis; however, lesions can be widespread. Based on the type of fungus, certain areas may be more prone to developing lesions. Examples of this include face and distal extremities for dermatophytosis; lip folds, ventrum, flexure surfaces and intertriginous regions for *Malassezia* dermatitis; head, extremities, and tail base for sporotrichosis in cats; solitary lesions on face, legs, trunk or tailhead for pythiosis in dogs. Refer to **Table 1** for information on each fungal dermatosis.

D. Cytological evaluation
Samples can be collected via different means depending on the nature of the lesion. For exudative lesions, direct

impression smear of the exudate is generally recommended. Direct impression smears or tape cytology from less exudate lesions is appropriate. For nodular lesions, especially non-draining nodules, fine needle aspirate of the lesion using a 22-25 gauge needle and 5 ml syringe to express contents onto a glass slide is generally more rewarding the surface cytology alone. Diff-Quik® is used to stain slides for evaluation at 400-1000 times power.

Fine needle aspirate of regional lymph nodes can also assist in making a diagnosis.

Microscopic evaluation of hairs (trichography) can be helpful to look for invasion of fungal hyphae or spores into and around the hair shaft. Potassium hydroxide (KOH) 10-20% with heat can be used to dissolve keratinous buildup around hair shafts to improve visibility for trichography.[3,7]

E. Wood's Lamp

Screening test for dermatophytosis. Some species of dermatophyte fluoresce an apple-green/yellow-green color including *Microsporumcanis* (50-80% of isolates), *M. distorum, M. audouinii*, and *Trichophyton schoenleinii*. The fluorescence is caused by the fungal metabolite tryptophan.[3,7] Keep in mind that other things can fluoresce to create a false positive result including medications, bacteria, scale/crust, soaps, petroleum, and fabric fibers.[3] Wood's lamp can be very helpful in several aspects from making a diagnosis of dermatophytosis to selecting the best hairs to sample for culture. But remember, just because it does NOT fluorescence does NOT mean it's NOT dermatophyte. I know…that's a lot of NOT's. ☺

F. Dermatophyte Test Medium (DTM)

Hair, claw, or dry skin scrape samples are added to this special agar for growing dermatophyte that contains Sabouraud dextrose agar with added cycloheximide, gentamicin, and chlortetracycline to inhibit secondary growth of other fungi and bacteria. *Phenol red* is added as a pH indicator. When dermatophytes consume protein in the medium and release alkaline metabolites, the pH increases and causes a color change from yellow to red. After dermatophytes have consumed all of the protein, they will use the remaining carbohydrates producing acid metabolites that cause the medium to change color from red back to yellow. The majority of other fungi (non-dermatophytic) consume carbohydrate metabolites first so that no red color change occurs initially if they are the only fungal organisms present; however, after about 10-14 days red color change occurs as they begin to utilize the remaining proteins. Ideally, DTMs should be evaluated daily for concurrent colony growth and color change. Any colonies that grow should be examined cytologically to confirm the most likely organism(s) present by evaluating micorscopic morphology of fungal reproductive structures.[3,7] Macroscopic features of the colony can aid in fungal identification as well.7 Because the cycloheximide (antifungal) in DTM plates can inhibit growth of sensitive fungi such as *Candida* sp., *Aspergillus* sp., *Zygomycota*, and others, there are some cycloheximide-free plate options for in-house dermatophyte testing.

Additional in-house dermatophyte culture plate options include: 1) Sab-Duets - double plate with one side DTM and one side Sabouraud dextrose agar only); 2) enhanced sporulation agar (ESA)/rapid sporulating media (RSM) – contains products to inhibit growth of contaminants and uses bromothymol as pH indicator turning medium a blue-green color at an alkaline pH; 3) Derm-Duet II and DermatoPlate-Duo – double plates of DTM and ESA; 4) DTM bottles – difficult to fit toothbrushes in containers and remember not to screw lid tightly (dermatophytes need O2). When growing the in-house DTMs, keep in mind that dermatophytes prefer to grow in the dark at 30°C with 30% humidity.[3,7] False negative growth may occur if culture environments are suboptimal.

G. Macerated Tissue Culture

This diagnostic is used primarily for subcutaneous mycoses that have invaded beyond the skin and hair into the dermis and subcutaneous tissues such as *Blastomyces* sp., *Cryptococcus* sp., *Sporothrix* sp., *Aspergillus* sp., and *Pythium* sp. Rarely, dermatophyte can invade deeply to create kerions or pseudomycetomas. However, keep in mind that dimorphic fungi like *Blastomyces* sp., *Cryptococcus* sp., *Histoplasma* sp., *Sporothrix* sp., and *Coccidioides* sp. convert into their infectious stage (mycelia) in culture and pose a zoonotic risk to laboratory personnel. Be sure to notify the laboratory personnel of your differential list so that they may take the appropriate precautions when handling the samples.[7]

Prior to performing the biopsy, the best lesion should be selected. It is best to choose a primary lesion (nodule) if

one exists. Avoid performing a biopsy through a draining tract if possible. The site should be surgically prepared to remove any surface contaminants. The area can be numbed with lidocaine. Additionally, sedation may be required if the patient is fractious, overly stressed, or painful. Next, a punch biopsy tool or scalpel blade is used to remove a tissue sample. When sampling for tissue fungal culture, usually samples are also submitted for bacterial cultures to rule out the other differential diagnoses. Keep this in mind so that an adequate quantity of sample is submitted. You may need to take up to 4-5 punch biopsy samples if many difficult cultures are being submitted (along with histopathology). Samples are placed in culture transport medium and submitted to the laboratory as soon as possible. Refrigeration can be used to preserve most fungi in transit; however, some fungal and fungal-like organisms (e.g. *Pythium* sp., *Lagenidium* sp., *Paralagenidium* sp., *Aspergillus* sp., and *Zygomycetes*.[7] If you are not familiar with the handling of the specimen, it is always recommend to consult the laboratory submission guidelines or speak with a laboratory personnel for further guidance. And it is a good rule-of-thumb to get it to the lab ASAP to avoid death of organisms.

In additional to tissue culture, fungal cultures may be obtained from fluid from nodules, blood, or other bodily fluids depending on the fungal organism present and its location in the body. Each sample site will vary in sensitivity. Skin swabs for fungal culture are not recommended.[7]

H. Histopathology

At the time tissue is collected for fungal culture, additional pieces can be collected for histopathological evaluation. This is especially important because documentation of fungal invasion into tissue is needed to ensure that the organism(s) cultured or identified via other diagnostic methods are pathogenic for the patient (and not just a contaminant). If there is NO invasion of fungal structures into a diseased tissue sampled, then you would be more suspicious that the fungal organism identified is NOT the culprit of the dermatitis. Again, collection of primary lesions is most ideal. The surface of skin collected for histopathology usually is not surgically prepared to avoid removing essential components necessary for making the diagnosis.

Special stains are usually ordered to better visualize fungal elements in the tissue. These include Gomori-methenamine silver (GMS) and Periodic acid-Schiff (PAS) stains. For some types of fungal dermatitis, the fungal yeast present in the tissue are unique and diagnostic alone (e.g. blastomycosis, cryptomycosis, sporotrichosis, histoplasmosis, and coccidioidomycosis).[3,7] Other types of fungal hyphal or yeast structures are not unique enough to distinguish on histopathology alone (e.g. hyalohyphomycoses, zygomycoses, pythiosis, lagenidiosis, chromomycoses, etc.).[3,7,9,10]

I. Serological Testing[3,7]

Commercially available tests that can detect antigens and/or antibodies in body fluids and tissue have increased the ease and speed of diagnosing some fungal infections. Below is a list of some of the serological tests available. These are typically available at many university and commercial laboratories.

1. Agar gel immunodiffusion (AGID) test (measures antibody present)
2. Enzyme-linked immunosorbent assay (ELISA) tests(measures antigen present)
3. Antigen detection tests

J. Immunodiagnostic and Other Techniques[3,5-7,16]

These are typically available at many university and commercial laboratories.

1. Panfungal PCR – performed on formalin fixed paraffin-embedded tissue. Screens for many different fungal organisms (Texas A&M)[16]
2. E-testing for susceptibility to antifungal therapies (Auburn University)
3. Immunohistochemistry
4. PCR for individual fungi
5. Immunofluorescence (direct and indirect methods)

K. Other

Once the diagnosis of opportunistic fungal dermatitis is made, the next step is to determine the extent of disease. What other body systems other than the skin are affected? Typically, I tell my clients that these types

of infections are very abnormal (aside from dermatophytosis and *Malassezia* dermatitis). We need to determine why their pet got the infection in the first place. Can we identify a reason why it might be immune-suppressed and reverse this? If the infection has already spread to other body systems then the prognosis is significantly worse, especially considering these infections do not respond very well to traditional antifungal therapies. For this reason, I explain to clients that we treat this much like cancer. It is important to be sure it has not spread elsewhere prior to initiating an aggressive treatment plan. Additional diagnostics might include: FNA of lymph nodes, thoracic radiographs, abdominal ultrasound, FNA of liver and spleen if indicated, and complete bloodwork to assess systemic health.

Treatment

Topical Antifungal Therapy[3,7]

This is helpful when treating superficial mycoses (e.g. dermatophytosis, *Malassezia* dermatitis, or *Candida* sp.) and includes the use of products such as lime sulfur, iodide solution, azoles, terbinafine, chlorhexidine (>2%), acetic acid, naltifine, amphotericin B, and nystatin. These are available in different formulations ranging from concentrated solutions, shampoos, sprays, lotions, mousses, or creams/ointments. Many factors will determine if topical therapy is a best route of treatment and which formulation to use including patient tolerance, haircoat, owner compliance, cutaneous lesion characteristics, product characteristics, and fungal organism present. Topical antifungal therapy can be used as an adjunctive therapy for subcutaneous mycoses, but are not appropriate as sole therapy.

Systemic Antifungal Therapy[3,7-9,17,18]

This therapy is required when subcutaneous/deep mycoses are present. When selecting the most appropriate medication, considerations include fungal organism present, body site affected, species of patient, age of patient, underlying diseases (e.g. renal failure), safety considerations (pet and people), and the cost of treatment.

A. Azoles
   Mechanism of action (MOA): inhibit ergosterol formation in the cell membrane
   Spectrum of activity (SOA): broad spectrum; itraconazole sometimes used in combination with terbinafine for oomycotic infections.
   Side effects: gastrointestinal disturbance, hepatotoxicity, teratogenic, bone marrow suppression, altered drug metabolism, haircoat color changes

   Examples: ketoconazole, itraconazole, fluconazole, voriconazole, posaconazole, enilconazole.

B. Griseofulvin
   MOA: inhibits DNA synthesis and cell wall formation
   SOA: dermatophyte only
   Side effects: gastrointestinal disturbance, bone marrow suppression, teratogenic, hepatotoxicity

C. Terbinafine
   MOA: inhibits ergosterol formation in the cell membrane
   SOA: dermatophyte, *Aspergillus*, and *Sporothrix*; less effective for *Malassezia, Candida*, and dimorphic fungi; sometimes used in combination with itraconazole for oomycotic infections.
   Side effects: gastrointestinal disturbance, bone marrow suppression, teratogenic, hepatotoxicity

D. Amphotericin B and other Polyenes
   MOA: irreversibly binds ergosterol causing altered cell permeability (leakage)
   SOA: broad spectrum; sometimes used in combination with azoles or terbinafine for oomycotic infections
   Side effects: gastrointestinal disturbance, nephrotoxicity, fever, muscle tremors, phlebitis

E. Flucytosine
   MOA: inhibits DNA synthesis of cell wall
   SOA: used in combination with amphotericin B for *Candida, Cryptococcus, Aspergillus* and some phaeohyphomycoses. Sometimes used in combination with azoles and amphotericin B for oomycotic infections.
   Side effects: bone marrow suppression, gastrointestinal disturbance, nephrotoxicity, teratogenic, hepatotoxicity, neurotoxicity

F. Chitin synthesis inhibitors
MOA: inhibits chitin and cell wall synthesis
SOA: adjunctive therapy (lufenuron) or used when other therapies fail (caspofungin for Aspergillus in people)
Side effects: none for lufenuron; infusion site reaction, vomiting and hepatotoxicity for caspofungin
Examples: lufenuron, caspofungin

G. Iodides
MOA: unknown, may work by enhancing phagocytic activity of leukocytes.
SOA: *Sporothrix* most commonly; also *Basidiobolus*, phycomycosis, and rhinosporidiosis.
Side effects: gastrointestinal disturbance, hypersalivation, muscle fasciculations, cardiomyopathy, hypothermia, depression, scaling, dry haircoat, and hypothyroidism.

H. Immunotherapy
Many forms of immunotherapy exists. Vaccines containing whole cells, concentrated soluble antigens, autoantigen, or combinations of exoantigens and cytoplasmic antigens for treatment of *Pythium* sp. infections for use in horses have proven more successful than for use in dogs.In addition, it is proposed that this therapy may work synergistically with systemic antifungal therapy.These vaccines are thought to work by reprogramming the Th1/Th2 cellular balance towards a more productive killing environment. Vaccines for treatment and prevention of dermatophytosis in dogs and cats have minimal efficacy to date. Immune-stimulating therapy for immune-compromised individuals undergoing chemotherapy (for cancer) also includes use of recombinant hemopoietic cytokines such as G-CSF, M-CSF, and GM-CSF to boost neutrophil counts and help prevent opportunistic infections. Human intravenous immunoglobulin (IVIG) therapy may also help to reduce the risk of opportunistic infections in immunocompromised animals.

I. Antifibrotics
Fibrosing (scarring) inflammation may occur as a sequela to fungal infection, and may significantly impact quality of life. For example, pulmonary fibrosis is a known sequel to treatment of systemic fungal disease in people and animals. Use of antifibrotic agents, like pentoxifylline, with antifungal therapy significantly reduces fibrosis in people and mice. Further studies are warranted in dogs and cats.

J. Antibiotics[19-21]
Recent in vitro testing of antibiotics that are protein synthesis inhibitors suggests that some antibiotics may have antimycotic effects. Of particular interest is the effect of these antibiotics, especially synergistically with other antifungals, on the growth of Pythium.

K. Environmental Fungicides
As mentioned early, many of these opportunistic fungal organisms fail to respond to traditional antifungal therapy. This is especially true for oomycotic infections (e.g. pythiosis and lagenidiosis). For this reason, some fungicides used to kill oomycetes pathogenic to plants have been used topically and orally in dogs. Topical use of metalaxyl in some dogs was unsuccessful and caused nausea, vomiting and anorexia.22 Another group found the oral use of mefenoxam in combination with other antifungals successful in a dog with gastrointestinal form of pythiosiswith no side effects.[17]

L. Anti-inflammatory agents
Glucocorticoids have many desirable and undesirable effects. We use them often in dermatology to reduce itchiness and inflammation of the skin with great success. However, it has negative effects on phagocytic cells and inhibits their function. This is one of the reasons glucocorticoids are contraindicated when infections are present. Interestingly, some dermatologists are using low, anti-inflammatory doses (0.5 mg/kg) on tapering schedules at the beginning of therapy for cutaneous pythiosis and lagenidiosis. The thought is that if the inflammation can be reduced, then the antifungal agents might reach higher concentrations at the site of infection and be more effective at treating the oomycotic infection. Additionally, oomycotic infections typically insight an altered TH2/TH2 cellular balance towards a TH2 response. This means that the cells responsible for killing the organisms (e.g. macrophages and neutrophils) are replaced by pro-inflammatory cells (e.g. eosinophils). If the scale can be tipped back towards normal, then the body has a better chance to fight off the

infection. However, glucocorticoids should be used with extreme caution in these patients to avoid worsening the condition and compromising the patient.

M. Hyperbaric Oxygen Therapy (HBOT)
HBOT is a newer therapy in veterinary medicine. It has been used in human medicine for numerous conditions ranging from stroke to head trauma to nonhealing wounds. We do not really understand a lot behind this therapy yet, but groups are starting to study this therapy for use in veterinary medicine. Anecdotally, it has shown to accelerate wound healing and reduce inflammation of tissue in veterinary medicine. HBOT therapy is almost always used in addition to more traditional therapies, so additional studies are needed to delineate the true efficacy of this as an adjunctive therapy for chronic fungal infections among other conditions.

Surgery

Subcutaneous mycoses are more difficult to treat with systemic medications due to their presence within granulomas or abscesses resulting in poor drug penetration at the site. Additionally, when the infection is caused by an environmental commensal that should not normally cause infection, these organisms sometimes do not have high susceptibility to antifungal agents. Prognosis for recovery is guarded to poor. Surgical resection of the diseased tissue followed by aggressive systemic therapy provides the best prognosis for patients as long as the disease has not spread to other body sites. Surgeons will treat subcutaneous mycoses similar to neoplasia and resect wide and deep in order to ensure complete removal of the diseased tissue. In areas not amenable to surgery (e.g. extremities, tail) the best course of action may be amputation. However, the possibility of infection relapse is possible, so close follow-up with the patient and owner is necessary to monitor for recurrence.

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Superficial Mycoses										
Disease	Causative Agent	Organism Characteristics	Habitat	Species Affected	Transmission	Cutaneous lesions	Cutaneous Lesion Distribution	Other Clinical Signs	Treatment	Prognosis
Dermatophytosis	*Microsporum canis*	Hyphae and arthroconidia within and on hairshafts, respectively; macroconidia - canoe-shaped, ≥6 cells, thick walls, terminal knob	Cat	Cat, dog, human most commonly. Many species	Direct and indirect contact	Papules, pustules, crusts, epidermal collarettes, scale, alopecia and erythema. +/- pruritus. +/- claw abnormalities. Kerion (rare)	Face, extremities, trunk, generalized	Pseudomycetoma (rare)	Topical therapy. Systemic therapy - azoles, terbinafine, griseofulvin	Good
	M. gypseum	Hyphae and arthroconidia within and on hairshafts, respectively; macroconidia - rowboat-shaped, <6 cells, thin walls	Soil	See above.	See above.	See above.	See above.	See above.	See above.	Good
	Trichophyton mentagrophytes	Hyphae and arthroconidia within and on hairshafts, respectively; macroconidia - cigar-shaped; spiral hyphae	Rodents	See above.	See above.	See above.	See above, and nasal planum.	See above.	See above.	Good
Malassezia dermatitis	*Malassezia pachydermatis (most commonly)*	3-8 µm yeast, monopolar budding (peanut-shaped, round to oval)	Commensal	Cat, dog	Unlikely	Erythema, lichenification, hyperpigmentation, seborrhea oleosa and sicca, alopecia, pruritus, paronychia	Ears, mucocutaneous junctions, paws, axilla, groin, clawbed, ventral neck, medial thighs, interdigital regions; generalized	N/A	Topical therapy. Systemic therapy if widespread, immunocompromised, or hypersensitive	Good
Candidiasis	*Candida spp.*	2-6 µm yeast +/- budding, pseudohyphae/hyphae	Commensal	Many	Unlikely	Pruritus, ulcer, white plaques, erythema, papules, pustules, ulcers	Mucocutaneous junctions, intertriginous areas, ear, pinna, clawbed, paws	Nodules (rare)	Topical therapy. Systmic therapy is widespread, immunocompromise is severe.	Fair

Subcutaneous and Deep Mycoses

Disease	Causative Agent	Organism Characteristics	Habitat	Species Affected	Transmission	Cutaneous lesions	Cutaneous Lesion Distribution	Other Clinical Signs	Treatment	Prognosis
Sporotrichosis	*Sporothrix schenckii*	2-10 µm yeast	Soil, organic debris	Many: dogs, cats, humans, equine most commonly	Direct implantation - thorns, bite wounds, insect bites, puncture wounds	Nodules, ulcers, draining tracts, plaques, alopecia, crusts, necrosis	Head, pinna, trunk (dog); head, distal extremities, tail base (cat); corded lymphatics, ventrum, distal extremities (horse)	Enlarged lymph nodes, corded lymphatics, respiratory signs	Systemic therapy. Euthanasia? ***(cats are a high zoonotic risk!)***	Guarded to poor
Eumycotic mycetoma	Black grain mycetoma (pigmented fungi): *Cladophialophora, Curvularia, Exophiala, Leptosphaeria, Madurella, Pyrenochaeta, Staphylotrichum, Torula*	Broad (2-6µm), septate, branching pigmented hyphae	Soil, organic debris	Many: dog, cat, horse, human, etc.	Direct implantation - splinter, trauma	Large nodules/masses, tissue grains, draining tracts	Usually solitary: distal extremities, face	Extend to muscle, joint, bone; sinusitis; keratitis; lymphadenitis; pneumonia; dissemination	Surgery + systemic therapy for 2-3 months. Medical therapy alone unsuccessful.	Guarded
	White-grain mycetoma (unpigmented fungi): *Acremonium, Pseudallescheria*	Broad (2-6µm), septate, infrequently branching non-pigmented hyphae	Soil, organic debris, water	Many: dog, cat, horse, human, etc.	Direct implantation - splinter, trauma	Large nodules/masses, tissue grains, draining tracts	Usually solitary: distal extremities, face	Extend to muscle, joint, bone; sinusitis; keratitis; lymphadenitis; pneumonia; dissemination	Surgery + systemic therapy for 2-3 months. Medical therapy alone unsuccessful.	Guarded
Chromomycosis (phaeohyphomycosis & chromoblastomycosis)	*Alternaria, Bipolaris spiciferum, Cladophialophora bantiana, Curvularia, Exophiala, Phialemonium obovatum, Phialophora, Pseudomicrodochium suttonii, Scedosporium, Wangiella, and many others*	Broad (2-6µm), septate, irregular branching and unbranching pigmented hyphae	Soil, organic debris	Many: dog, cat, horse, ruminants, human, etc.	Direct implantation - splinter, trauma, bite, wound contamination	Firm to fluctuant solitary nodules, papules, ulcers, draining tracts, NO TISSUE GRAINS	Usually solitary but can be multifocal: face, lateral trunk, dorsum, extremities	Upper respiratory signs; extend to muscle and bone; dissemination possible	Surgery + systemic therapy for 2-3 months.	Guarded
Hyalohyphomycosis	*Acremonium, Fusarium, Geotrichum, Paecilomyces, Penicillium, Pseudallescheria*	Broad (2-6µm), septate, infrequently branching non-pigmented hyphae; +/- spherical yeast-like cells (2-15µm)	Soil, organic debris, water	Many: dog, cat, horse, ruminant, human, etc.	Direct implantation - splinter, trauma, invasion of mucosa, dissemination from other sites	Solitary to multifocal firm nodules, papules, ulcers, draining tracts	Clawbed, head, rump, ear canal, mammary tissue, paw, lip/mouth, distal extremities	Eyes, joints, lungs, kidneys, liver	Surgery + systemic therapy for 2-3 months. Medical therapy alone unsuccessful.	Grave
Zygomycosis	Mucorales: *Rhizopus, Mucor, Saksenaea, Absidia.* Mortierellales: *Mortierella* Entomophthorales: *Conidiobolus, Basidiobolus*	Broad (6-25µm), thin-walled, occasionally septate, irregularly branching, nonparallel walls	Soil, organic debris, haircoat and skin flora, normal flora of insects, GI flora in amphibians/reptiles	Many: dog, cat, horse, human, pig, etc.	Wound contamination, insect bites, gastrointestinal or respiratory translocation	Solitary to multifocal nodules, ulcers	Extremities, mucocutaneous areas, trunk	Upper respiratory signs, mouth, rarely disseminates	Surgery + systemic therapy for 2-3 months.	Poor to Grave
Oomycosis	*Pythium sp.*	Broad (4-6µm), septate, irregularly branching, nonparallel walls.	Aquatic (fresh, stagnant or slow moving water), organic debris	Many: dog, cat, human, horse, sheep	Consumption water, standing in water (non-intact skin or mucosa)	Solitary to multifocal nodules, masses, ulcers, draining tracts, kunkers	Body, face, extremities, tailhead	Gastrointestinal system, lymph nodes	Surgery + systemic therapy for 2-3 months; immunotherapy.	Guarded
	Lagenidium sp.	Broad (7-25µm), poorly-septate, thick-walled, nonparallel walls.	Aquatic (fresh, stagnant or slow moving water), organic debris	Many: dog, human,	Consumption water, standing in water (non-intact skin or mucosa)	Multifocal firm nodules, ulcers, draining tracts	Body, face, extremities, tailhead	Vasculature, lymph nodes, lungs, cranial mediastinum, usually disseminates	Surgery + systemic therapy for 2-3 months.	Poor to Grave
	Paralagenidium sp.	Broad (6-8µm), septate, irregularly branching, nonparallel walls.	Aquatic (fresh, stagnant or slow moving water), organic debris	Many: dog, human	Consumption water, standing in water (non-intact skin or mucosa)	Solitary to multifocal nodules, plaques, +/- alopecia, +/- ulcers,	Body, face, extremities, tailhead, perineal	Usually localized to skin/SQ (chronic, slowly-progressive)	Surgery + systemic therapy for 2-3 months.	Good to Guarded
Blastomycosis	*Blastomyces dermatitidis*	Round to oval yeast 5-35 µm: broad-based budding, thick refractile double-contoured cell wall	Sandy, acidic soil, organic debris	Many: dog, cat, human, horse	Inhalation of spores most commonly; inoculation into skin	Multiple firm papules, nodules and plaques, ulcers, draining tracts, abscesses	Nasal planum, clawbeds, anywhere	Usually disseminated: lymph nodes, lungs, skin, eyes, bones, SQ tissues, nares, barin, testes	Systemic therapy	Poor to Grave
Coccidiomycosis	*Coccidioides immitis*	Large, round, thick-walled spherules (20-200 µm) filled with endospores (2-5 µm)	Sandy, alkaline soil, organic debris	Many: dog, cat, horse, human	Inhalation of spores	Multiple papules, nodules, abscesses, draining tracts, ulcers over boney areas	Over boney areas	Disseminates to bones, skin, eyes, heart, pericardium, testicles, brain, spinal cord, spleen, liver and kidneys	Systemic therapy	Good to Guarded
Cryptococcosis	*Cryptococcus neoformans*	Pleomorphic, round to elliptical (2-20µm) yeasts, narrow-based budding, clear and refractile thick mucinous capsule (soap-bubble appearance)	Nitrogen-rich, alkaline soil (bird feces)	Many: dog, cat, human, horse, ruminants, dolphins, ferrets, birds, koalas, marsupials	Inhalation of spores most commonly; inoculation into skin	Papules, nodules, ulcers, abscesses, draining tracts	Nose, lips, clawbed - dog. Face, pinna, paws - cat.	Disseminates to lymph nodes, skin, bones, CNS, lungs, eyes	Systemic therapy	Poor to Grave
Histoplasmosis	*Histoplasma capsulatum*	Small (2-4 µm) round yeast with basophilic center and lighter halo (usually found inside phagocytes)	Nitrogen-rich, alkaline soil (bird feces)	Many: dog, cat, humans	Inhalation of spores most commonly; inoculation into skin	Multiple papules, nodules, ulcers, draining tracts	Anywhere	Disseminates to lymph nodes, skin, gastrointestinal tract, eyes, spleen	Systemic therapy	Fair to Good

POSTERS

Residual *in vitro* activity of canine hair against *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* following a single antimicrobial bath

M.R. HOGG*, D.J. BERGER†, J. MOCZARNIK†, A. VIALL‡, A.C. KRULL§, J.O. NOXON†,

**College of Veterinary Medicine, Iowa State University, Ames, IA, USA*
†Department of Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA
‡Department of Veterinary Pathology, Iowa State University, Ames, IA, USA
§Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA

Abstract: Limited information exists comparing antimicrobial activity of shampoos. The study objective was to determine the residual antimicrobial activity on canine hairs treated with a single application of shampoo. Forty-eight privately owned dogs were bathed with a control shampoo 3 days prior to group assignment. Dogs were then randomly assigned to one of four treatment groups: 2% chlorhexidine gluconate/2% miconazole nitrate (Malaseb®); 3% chlorhexidine gluconate/0.5% climbazole (DOUXO® Chlorhexidine); 2% chlorhexidine gluconate/2% miconazole nitrate/tromethamine USP disodium EDTA (MICONAHEX+TRIZ®); 2% chlorhexidine gluconate/2% miconazole nitrate/microsilver (BioHex™) and bathed by a blinded investigator using 10 mLs of shampoo per 10 kgs of body weight. Hair was collected prior to medicated bathing, then 1 h, 2, 4, and 7 days post-bathing, weighed and placed onto Mueller-Hinton with 2% NaCl agar streaked with *Staphylococcus pseudintermedius* or Sabouraud's dextrose agar streaked with *Malassezia pachydermatis*. Duplicate plates were incubated for 24 and 72 h, respectively, and the zone of inhibition measured. Hair collected prior to bathing did not demonstrate microbial inhibition, while after treatment samples exhibited residual antifungal and antibacterial activity throughout the study for all groups. No statistical differences were observed in zones of inhibition between shampoos against *M. pachydermatis*. There were significant treatment differences for *S. pseudintermedius*. The largest zones of inhibition were from hairs bathed with Malaseb® and BioHex™. These results indicate formulation differences appear to affect the residual antibacterial but not the antifungal efficacy of these four shampoos. Further studies are needed to determine if these *in vitro* differences correlate to differences in clinical efficacy.

Sources of funding: Self-funded.

Conflict of Interest: J.O. Noxon has lectured and consulted for Bayer Animal Health.

Zinc-responsive dermatosis in a French bulldog

P. BLOOM

Allergy, Skin and Ear Clinic for Pets, Livonia, MI, USA

Abstract: Zinc responsive dermatosis (ZRD) has been associated with two different etiologies. Type I ZRD is seen almost exclusively in Nordic breeds and thought to occur due to a decrease in zinc absorption. Type II ZRD is seen in rapidly growing large-breed puppies or young adults and is due to inadequate zinc in the diet or due to dietary components inhibiting or reducing zinc absorption. Clinically ZRD is characterized by erythema, thick adherent scaling, crusting, alopecia, and lichenification. Distribution of the lesions most commonly involves the pinnae, periorbital, areas, mucocutaneous junctions and pressure points. Recently ZRD has been reported in Boston terrier dogs. This is a case report of ZRD in a French bulldog (FBD). A 5-month-old intact male FBD presented with a 2.5-month history of a non-pruritic crusting pinnal dermatitis. The puppy was eating a national brand puppy food. On examination there was severe, thick adherent scale/crust along the margins of both pinnae creating a scalloped appearance. Histopathology of the left pinna revealed marked laminar orthokeratotic toparakeratotic hyperkeratosis that distended follicular ostia with large accumulations of serum. There was mild perivascular lymphoplasmacytic dermatitis. Treatment with 2 mg/kg zinc methionine (NutriVed Chewable Zinpro®) once daily per os was begun. There was dramatic improvement by day 60 post treatment and complete resolution of the lesions by day 90. To the author's knowledge this is the first report of Type I ZRD in a FBD.

Sources of funding: Self-funded.

Conflict of Interest: None declared.

Combination therapy of azathioprine, ciclosporine-modified and ketoconazole in a dog with concurrent pemphigus foliaceus and hyperadrenocorticism

T. S. YUN, J. H. KANG, M. P. YANG and B. T. KANG

Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Korea

Abstract: A 10-year-old, spayed female shih-tzu presented with an 85-day history of progressive erythema and multiple crusts.The dog had signs of hyperadrenocortism which was diagnosed 55 days prior to presentation. She then received 55 days of trilostane (Vetoryl®; 10 mg/body, once daily, orally) however this was discontinued due to poor response.In addition to generalized alopecia from hyperadrenocorticism, erythematous plaques and crusts were noted on the trunk, head, and food pads. Lesional impression smears revealed numerous acantholytic cells and non-degenerated neutrophils. Laboratory examination showed neutrophilic leukocytosis and increased alkaline phosphatase activity. Histopathologic findings demonstrated subcorneal pustules with acantholytic cells, as well as epidermal hyperkeratosis and hair follicles containing keratin and cellular debris. Based on these findings, this dog with hyperadrenocorticism was diagnosed with concurrent pemphigus foliaceus (PF). Because of the desire to avoid corticosteroids, the dog was treated with azathioprine (2 mg/kg, once daily, orally), ciclosporine-modified (7 mg/kg, once daily, orally) and ketoconazole (5 mg/kg, once daily, orally). The ketoconazole was chosen to help manage the hyperadrenocoticism and elevate ciclosporine levels. By day 80 post post-treatment, more than 70% of the dermatological signs were improved without adverse events. Although this partial response had been maintained for additional 35 days, the patient was then lost to follow-up. To our best knowledge, this is the first case that describes a dog with concurrent PF and hyperadrenocorticism that partially responded to combination therapy with azathioprine, ciclosporine-modified and ketoconazole. These medications may be considered in other dogs diagnosed with concurrent PF and hyperadrenocorticism.

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Conflict of Interest: None declared.

Complementary effects of white rose petal extracts in the treatment of canine atopic dermatitis

T. YUN, S. J. PARK, Y. JEONG, Y. B. KIM, J. H. KANG, M. P. YANG and B. T. KANG

Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Korea

Abstract: The study purpose was to investigate the therapeutic efficacy of white rose petal extracts (WRPE) in canine atopic dermatitis (CAD). Seventeen atopic dogs and four healthy beagles were enrolled. Experimental dermatitis was induced three times (days 1, 4 and 7) in the beagles by intradermal injections of histamine dihydrochloride, compound 48/80 and substance P acetate hydrate. The atopic dogs had WRPE cream (1%, n=9) or solution (0.5%, n=4 or 1%, n=4), applied once daily; all the beagles had WRPE solution (1%) or WRPE cream (1%) or control (0% WRPE) applied thrice daily. In atopic dogs, clinical assessments were performed by measuring transepidermal water loss (TEWL) and using modified Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4 and pruritus visual analogue scale (PVAS) on days 0, 14 and 28. In the beagles, TEWL and wheal diameter were measured on days 1, 4 and 7. Histopathology was performed before (day 0) and after topical treatments (days 2 and 8). In atopic dogs, 1% solution decreased TEWL on day 28 compared to day 0 ($P<0.05$), whereas PVAS and modified CADESI-4 scores were not changed. In the beagles, TEWL significantly increased with compound 48/80-induced dermatitis ($P<0.001$), but the increases were significantly less in the 1% cream-treated lesions compared to control-treated lesions ($P=0.04$). Wheal diameter of histamine-induced lesions significantly decreased following application of 1% cream ($P<0.001$). Histopathologically, the 1% cream decreased edema and inflammation in histamine-induced lesions. Therefore WRPE may be effective in restoring skin barrier function in CAD and reducing histamine-induced inflammation.

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Conflict of Interest: None declared.

Management of epidermolysis bullosa acquisita (EBA) and meticillin-resistant *Staphylococcus pseudintermedius* in a young great dane

K. E. LOFT* and A. KOID*

**Angell Animal Medical Center, Boston, MA, USA*

Abstract: Canine epidermolysis bullosa acquisita (EBA) is a chronic sporadic autoimmune bullous disease with unknown etiology and no known sex or geographic predisposition. EBA does not have a Mendelian pattern of inheritance, but a genetic component is likely as great danes appear to be over-represented. EBA patients present with acute onset of sub-epidermal blistering associated with autoimmune reactions to collagen (type VII) within anchoring fibrils of the dermal-epidermal junction (DEJ). A 7-month-old 43 kg, castrated blue male great dane presented with a 5 day history of rapid onset of dermatologic signs with mildly pruritic papules progressing to diffuse generalized erosions and ulcerations of the axillary, inguinal, mucocutaneous skin, paw pads, concave pinnae and oral mucosa with lethargy and pyrexia (39.9 C). At presentation, the dog was receiving oral amoxicillin/clavulanic acid (Clavamox®) 375mg twice daily and oral prednisone 1 mg/kg once daily. Histopathology revealed severe multifocal neutrophilic dermatitis with subepidermalclefting, bullae, edema, and ulceration consistent with inflammatory EBA. Aerobic cultures identified meticillin-resistant *Staphylococcus pseudintermedius* susceptible to chloramphenicol. The dog responded completely to chloramphenicol 41mg/kg thrice daily for 21 days, prednisone 2mg/kg/day twice daily, azathioprine 2mg/kg once daily initially, reduced to every 48 h and colchicine 0.3 mg once daily, all administered orally as well as topical chloramphenicol. The prednisone was tapered to 15 mg every 48 h and colchicine was discontinued. The EBA remains in remission for over 60 days at time of writing. This case adds to a limited pool of clinical case material of successful treatment of EBA.

Sources of funding: Self-funded.

Conflict of Interest: None declared.

Serum LPS-binding protein in healthy and atopic golden retrievers and Australian shepherds

N. CRAIG*, K. AHRENS* and R. MARSELLA*

**Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, United States*

Abstract: Atopic dermatitis (AD) is an important chronic allergic inflammatory disease involving alterations in skin barrier function and dysregulation of theimmune system. In humans, AD is associated with increased serum levels of lipopolysaccharide (LPS) and LPS-binding protein (LBP), which is interpreted as an indication of increased translocation of bacterial products from the gut and therefore increased gut permeability. We aimed to examine serum levels of LBP in healthy and atopic dogs. To minimize breed variation and to focus on the effect of atopic trait, we selected golden retrievers (GR) as representatives of a predisposed atopic breed and Australian shepherd (AS) dogs as a non-predisposed breed. We standardized the diet for 10 days prior to sample collection. Recent history of gastrointestinal disease or antibiotic use was an exclusion criteria. We collected serum samples from five healthy GR, eight healthy AS dogs, six atopic GR and four atopic AS dogs. Serum LBP was measured using a canine LBP ELISA kit. Serum LBP varied widely (0.34-8.41 ng/ml), and there was no difference according to group, breed or health status. Due to the large variability and the small sample size, no conclusions can be made. Larger studies are needed to further investigate the relationship between gut permeability and AD in dogs including also other methods of measuring gut permeability.

Sources of funding: Self-funded.

Conflict of Interest: None declared.

Efficacy of oral afoxolaner for the treatment of canine generalized demodicosis in Japan

N. MURAYAMA*, Y. OSHIMA*

**Dermatology Services for Dogs and Cats, Koto-ku, Tokyo, Japan*

Abstract: Canine demodicosis presents with various skin lesions and severe cases can be difficult to control. This study assessed the efficacy of afoxolaner (Nexgard Spectra®) at the dosage of 2.5–6.3 mg/kg given orally every 4 weeks in dogs with juvenile-onset (n=4) and adult-onset (n=11) generalized demodicosis. Fifteen client-owned dogs diagnosed with generalized demodicosis were included in this study. Deep skin scrapings at five affected areas and clinical evaluations including alopecia, erythema, papules, pustules, and crusts/scales were performed every 4 weeks for 3 months. Skin biopsies were performed from representative skin lesions on day 0 in all dogs. Histopathological findings showed lymphocytic mural folliculitis (13/15), follicular hyperkeratosis (9/15), pyogranulomatous dermatitis (5/15), and subcorneal pustules (2/15). The rate of decrease in mite counts was 91.2%, 99.8% and 99.9% on days 28, 56 and 84, respectively. In five dogs with pyogranulomatous dermatitis, the rate of decrease was 76.4%, 99.58% and 100% on days 28, 56 and 84, respectively, while the rate of decrease was 98.7%, 99.9% and 99.9% on days 28, 56 and 84, respectively, in the other 10 dogs. Skin lesionsshowed significant improvement on days 56 and 84. No statistically significant difference was noted in the reduction of mite counts and improvement of skin lesions except papules on day 56 (P =0.008) between dogs with pyogranulomatous dermatitis and those without pyogranulomatous dermatitis. No adverse effects were observed in any dogs. In conclusion, afoxolaner administered every 4 weeks could be a treatment for dogs with generalized demodicosis presenting with various skin lesions.

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Conflict of Interest: None declared.

Comparing the cost of management of canine atopic dermatitis: Canine Atopic Dermatitis Immunotherapeutic versus other therapies

K.K. MWACALIMBA* A. HILLIER* and D. AMODIE*

**Zoetis LLC, 10 Sylvan Way, Parsippany, NJ, USA*

Abstract: ACanine atopic dermatitis (AD) is a commonly diagnosed condition in veterinary dermatology, requiring comprehensive management and frequent rechecks. Furthermore, the cost of medical management may be a concern for both pet owners and veterinarians. An observational retrospective pharmacoeconomics study was conducted to compare the cost of AD management in dogs for the 12 months prior to canine atopic dermatitis immunotherapeutic (CADI) (Cytopoint®) (Period A [PA]), and 12 months while receiving CADI (Period B [PB]). Therapy during both periods was unrestricted and included (amongst others) corticosteroids, antibiotics, allergen-specific immunotherapy, medicated shampoos, prescription diets, and topical therapy. Data were obtained from financial transaction records drawn from patients attending dermatology specialty practices. Descriptive summary statistics were used to characterize the cost of care for all AD management treatments offered over the two treatment periods (PA and PB). The records of 174 dogs (average weight 15.43kg) from 10 dermatology practices were included. The results showed that dogs were presented 39% more often during PB than PA (1756 [PB] versus 1265 visits [PA]); the mean number of visits per dog during PA was 7.3 compared to 10.1 visits during PB. The annual cost for therapy during PA was $1,274, compared to $1,088 during PB, a 15% decrease in annual cost of AD management. Cost savings were in part attributable to decreased visits where corticosteroids were dispensed (59% decrease), blood chemistry profiles were performed (22% decrease), and/or antibiotic were dispensed (9% decrease). Increased visits for CADI treatment offered increased opportunities for patient evaluation without additional cost.

Sources of funding: Zoetis, Inc.

Conflict of Interest: Kennedy Mwacalimba, Andrew Hillier and Deborah Amodie are employees of Zoetis, Inc.

Ex-vivo boosted immune cell therapy for canine chronic ulcerative dermatitis in a dog

S.G. BAE*, J.T. KIM*, T.H. OH*

**Laboratory of Veterinary Dermatology, Kyungpook National University, Daegu, Korea*

Abstract: A 13-year-old, neutered male shih tzu presented after 9weeks of ineffective treatment with topical and systemic antibiotics and anti-inflammatory and immunosuppressive doses of prednisolone for an exudative, ulcerative dermatitis. Physical examination revealed a pruritic, bilaterally symmetrical severely erythematous, exudative and ulcerative dermatitis with superficial crusts affecting the periocular, pinnal, axillary, inguinal, hind limb, and perianal regions. Complete blood count revealed leukopenia. Skin cytology revealed numerous neutrophils and intracellular and extracellular cocci. A diagnosis of canine chronic ulcerative dermatitis with a bacteria dermatitis was made histopathologically. Based on the antibiotic susceptibility results, the dog was treated with oral chloramphenicol (40 mg/kg) and amoxicillin/clavulanate (20 mg/kg) both twice daily for 2 weeks. However, the skin lesions progressed, most notably with worsening of the exudate and pruritus, despite antibiotic therapy. Following consultation with the dog's owner, the decision was made to treat with ex-vivo boosted immune cell(EBIC) therapy and the same previousantibiotics. For EBIC therapy, the dog's peripheral blood mononuclear cells were isolated, cultured, and expanded for 2 weeks. The expanded immune cells were infused intravenously every 2 weeks, for a total of six infusions. No adverse events were observed during therapy. After six EBIC therapy infusions, all skin lesions had resolved completely and no recurrence was noted for 2 years. This is the first report to describe a case of canine chronic ulcerative dermatitis with bacterial dermatitis refractory to several topical and systemic antibiotics and prednisolone that responded to boosted immune cell therapy combined with systemic antibiotics.

Sources of funding: Self-funded.

Conflict of Interest: None declared.

Polyhydroxy acid-based topical treatment for ichthyosis in the golden retriever: histopathological findings

A. PUIGDEMONT*, N. FURIANI†, D. FONDEVILA ‡, L. RAMIÓ-LLUCH §, and P. BRAZÍS§

**Department of Pharmacology, Therapeutics and Toxicology, Facultat de Veterinària, Edifici V, UniversitatAutònoma de Barcelona, 08913 Bellaterra, Barcelona, Spain.*
†Studio di Dermatologia Veterinaria, via Morandi 7/C, 06083, Ospedalicchio di Bastia Umbra, Perugia, Italy.
‡Department of Animal Medicine and Surgery, Facultat de Veterinària, Edifici V, Universitat Autònoma de Barcelona, 08913 Bellaterra, Barcelona, Spain.
§Laboratorios LETI, Edifici Astrolabio, Avinguda Cerdanyola 92, 08172 Sant Cugat del Vallès, Barcelona, Spain.

Abstract: Autosomal recessive congenital ichthyosis (ARCI) in golden retrievers has been associated with a mutated PNPLA1 gene. The polyhydroxy acid gluconolactone promotes stratum corneum acidification improving the epidermal barrier through epidermal lipid maturation and keratinocyte cohesion. This study examined the efficacy of a gluconolactoneand other α and ß hydroxy acids topical treatment (Kerato, Laboratorios LETI, Spain) for the management of ichthyosis in golden retrievers. Two golden retrievers with clinical signs of ARCI and a PCR-confirmed mutated PNPLA1 gene received a shampoo and a lotion containing gluconolactone and other α and ß hydroxy acids twice a week for 14 days and then weekly for 14 days. Biopsy specimens were obtained from the ventrolateral aspect of the thorax before and 30 days after starting treatment. Reduction in the extent and size of the scale on day 14, day 30 and day 90 compared to day 0 was subjectively estimated and expressed as percentage improvement by a veterinary dermatologist.Before treatment, histological findings revealed a compact orthokeratotic hyperkeratosisand multifocal pigmentation. On day 30, orthokeratotic hyperkeratosis was reduced, keratin was re-organized as a laminated woven mesh and reduction in pigmentation was observed. At days 14 and 30, the topical treatment had significantly reduced the presence of scaleby 60% and 90%, respectively. In conclusion, a topical treatment with gluconolactone and α and ß hydroxy acids improved the condition of the stratum corneum and reduced the number of scales in golden retrievers with ARCI after 30 days of treatment.

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Conflict of Interest: This study was self-funded.

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Simparica acts fast—it starts killing fleas within 3 hours and ticks within 8 hours*—and keeps going strong for 35 days* **without losing effectiveness at the end of the month.**

Premium protection without the premium price—with our rebate offers and affordable price, you can compete against OTC brands and bring flea and tick protection back into your practice.

IMPORTANT SAFETY INFORMATION: Simparica is for use only in dogs, 6 months of age and older. Simparica may cause abnormal neurologic signs such as tremors, decreased conscious proprioception, ataxia, decreased or absent menace, and/or seizures. Simparica has not been evaluated in dogs that are pregnant, breeding or lactating. Simparica has been safely used in dogs treated with commonly prescribed vaccines, parasiticides and other medications. The most frequently reported adverse reactions were vomiting and diarrhea. See full Prescribing Information on the back of this page and at **www.zoetisUS.com/SimparicaPI**.

*Studies show Simparica starts killing ticks in 8 hours and is ≥96.9% effective for 35 days against weekly reinfestations of *Ixodes scapularis*, *Amblyomma americanum*, *Amblyomma maculatum*, *Dermacentor variabilis*, and *Rhipicephalus sanguineus*.[1,2]

Learn more about Simparica.
Contact Zoetis Customer Service at
1-888-ZOETIS-1 or **1-888-963-8471**.

References: 1. Six RH, Geurden T, Carter L, et al. Evaluation of the speed of kill of sarolaner (Simparica™) against induced infestations of three species of ticks (*Amblyomma maculatum*, *Ixodes scapularis*, *Ixodes ricinus*) on dogs. *Vet Parasitol.* 2016;222:37-42. **2.** Six RH, Everett WR, Young DR, et al. Efficacy of a novel oral formulation of sarolaner (Simparica™) against five common tick species infesting dogs in the United States. *Vet Parasitol.* 2016;222:28-32.

zoetis

Simparica™
(sarolaner) Chewables

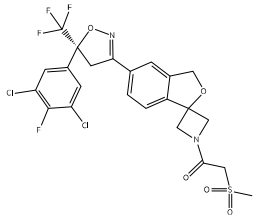
FOR ORAL USE IN DOGS ONLY

CAUTION: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

Description:
SIMPARICA is a flavored, chewable tablet for administration to dogs over 6 months of age according to their weight. Each tablet is formulated to provide a minimum sarolaner dosage of 0.91 mg/lb (2 mg/kg) body weight.

Sarolaner is a member of the isoxazoline class of parasiticides and the chemical name is 1-(5'-((5S)-5-(3,5-Dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydroisoxazol-3-yl)-3'-H-spiro(azetidine-3,1'-(2)benzofuran)-1-yl)-2-(methylsulfonyl)ethanone. SIMPARICA contains the S-enantiomer of sarolaner.

The chemical structure of the S-enantiomer of sarolaner is:



Indications:
SIMPARICA kills adult fleas, and is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis*), and the treatment and control of tick infestations [*Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick)] for one month in dogs 6 months of age or older and weighing 2.8 pounds or greater.

Dosage and Administration:
SIMPARICA is given orally once a month at the recommended minimum dosage of 0.91 mg/lb (2 mg/kg).

Dosage Schedule:

Body Weight	SAROLANER per Tablet (mg)	Number of Tablets Administered
2.8 to 5.5 lbs	5	One
5.6 to 11.0 lbs	10	One
11.1 to 22.0 lbs	20	One
22.1 to 44.0 lbs	40	One
44.1 to 88.0 lbs	80	One
88.1 to 132.0 lbs	120	One
>132.1 lbs	Administer the appropriate combination of tablets	

SIMPARICA can be offered by hand, in the food, or administered like other tablet medications.

Care should be taken that the dog consumes the complete dose, and treated animals should be observed for a few minutes to ensure that part of the dose is not lost or refused. If a dose is missed, administer SIMPARICA and resume a monthly dosing schedule.

SIMPARICA should be administered at monthly intervals.

Flea Treatment and Prevention:
Treatment with SIMPARICA may begin at any time of the year. In areas where fleas are common year-round, monthly treatment with SIMPARICA can continue the entire year without interruption.

To minimize the likelihood of flea re-infestation, it is important to treat all dogs and cats within a household with an approved flea control product.

Tick Treatment and Control:
Treatment with SIMPARICA can begin at any time of the year (see **Effectiveness**).

Contraindications:
There are no known contraindications for the use of SIMPARICA.

Warnings:
Not for use in humans. Keep this and all drugs out of reach of children and pets. For use in dogs only. Do not use SIMPARICA in cats.

SIMPARICA should not be used in dogs less than 6 months of age (see **Animal Safety**).

Precautions:
SIMPARICA may cause abnormal neurologic signs such as tremors, decreased conscious proprioception, ataxia, decreased or absent menace, and/or seizures (see **Animal Safety**).

The safe use of SIMPARICA has not been evaluated in breeding, pregnant, or lactating dogs.

Adverse Reactions:
SIMPARICA was administered in a well-controlled US field study, which included a total of 479 dogs (315 dogs treated with SIMPARICA and 164 dogs treated with active control once monthly for three treatments).

Over the 90-day study period, all observations of potential adverse reactions were recorded.

Table 1. Dogs with adverse reactions

Adverse reaction	sarolaner	sarolaner	active control	active control
	N	% (n = 315)	N	% (n =164)
Vomiting	3	0.95%	9	5.50%
Diarrhea	2	0.63%	2	1.20%
Lethargy	1	0.32%	2	1.20%
Inappetence	0	0%	3	1.80%

Additionally, one female dog aged 8.6 years exhibited lethargy, ataxia while posturing to eliminate, elevated third eyelids, and inappetence one day after receiving SIMPARICA concurrently with a heartworm preventative (ivermectin/pyrantel pamoate). The signs resolved one day later. After the day 14 visit, the owner elected to withdraw the dog from the study.

For a copy of the Safety Data Sheet (SDS) or to report adverse reactions call Zoetis Inc. at 1-888-963-8471. Additional information can be found at www.SIMPARICA.com. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or http://www.fda.gov/AnimalVeterinary/SafetyHealth.

Clinical Pharmacology:
Sarolaner is rapidly and well absorbed following oral administration of SIMPARICA. In a study of 12 Beagle dogs the mean maximum plasma concentration (C_{max}) was 1100 ng/mL and the mean time to maximum concentration (T_{max}) occurred at 3 hours following a single oral dose of 2 mg/kg to fasted animals. The mean oral bioavailability was 86% and 107% in fasted and fed dogs, respectively. The mean oral $T_{1/2}$ values for fasted and fed animals was 10 and 12 days respectively.

Sarolaner is distributed widely; the mean volume of distribution (Vdss) was 2.81 L/kg bodyweight following a 2 mg/kg intravenous dose of sarolaner. Sarolaner is highly bound (≥99.9%) to plasma proteins. The metabolism of sarolaner appears to be minimal in the dog. The primary route of sarolaner elimination from dogs is biliary excretion with elimination via the feces.

Following repeat administration of SIMPARICA once every 28 days for 10 doses to Beagle dogs at 1X, 3X, and 5X the maximum intended clinical dose of 4 mg/kg, steady-state plasma concentrations were reached after the 6th dose. Following treatment at 1X, 3X, and 5X the maximum intended clinical dose of 4 mg/kg, sarolaner systemic exposure was dose proportional over the range 1X to 5X.

Mode of Action:
The active substance of SIMPARICA, sarolaner, is an acaricide and insecticide belonging to the isoxazoline group. Sarolaner inhibits the function of the neurotransmitter gamma aminobutyric acid (GABA) receptor and glutamate receptor, and works at the neuromuscular junction in insects. This results in uncontrolled neuromuscular activity leading to death in insects or acarines.

Effectiveness:
In a well-controlled laboratory study, SIMPARICA began to kill fleas 3 hours after initial administration and reduced the number of live fleas by ≥96.2% within 8 hours after flea infestation through Day 35.

In a separate well-controlled laboratory study, SIMPARICA demonstrated 100% effectiveness against adult fleas within 24 hours following treatment and maintained 100% effectiveness against weekly re-infestations for 35 days.

In a study to explore flea egg production and viability, SIMPARICA killed fleas before they could lay eggs for 35 days. In a study to simulate a flea-infested home environment, with flea infestations established prior to the start of treatment and re-infestations on Days 7, 37 and 67, SIMPARICA administered monthly for three months demonstrated >95.6% reduction in adult fleas within 14 days after treatment and reached 100% on Day 60.

In well-controlled laboratory studies, SIMPARICA demonstrated ≥99% effectiveness against an initial infestation of *Amblyomma americanum, Amblyomma maculatum, Dermacentor variabilis, Ixodes scapularis,* and *Rhipicephalus sanguineus* 48 hours post-administration and maintained >96% effectiveness 48 hours post re-infestation for 30 days.

In a well-controlled 90-day US field study conducted in households with existing flea infestations of varying severity, the effectiveness of SIMPARICA against fleas on Day 30, 60 and 90 visits compared to baseline was 99.4%, 99.8%, and 100%, respectively. Dogs with signs of flea allergy dermatitis showed improvement in erythema, papules, scaling, alopecia, dermatitis/pyodermatitis and pruritus as a direct result of eliminating fleas.

Animal Safety:
In a margin of safety study, SIMPARICA was administered orally to 8-week-old Beagle puppies at doses of 0, 1X, 3X, and 5X the maximum recommended dose (4 mg/kg) at 28-day intervals for 10 doses (8 dogs per group). The control group received placebo tablets. No neurologic signs were observed in the 1X group. In the 3X group, one male dog exhibited tremors and ataxia post-dose on Day 0; one female dog exhibited tremors on Days 1, 2, 3, and 5; and one female dog exhibited tremors on Day 1. In the 5X group, one female dog had a seizure on Day 61 (5 days after third dose); one female dog had tremors post-dose on Day 0 and abnormal head coordination after dosing on Day 140; and one female dog exhibited seizures associated with the second and fourth doses and tremors associated with the second and third doses. All dogs recovered without treatment. Except for the observation of abnormal head coordination in one dog in the 5X group two hours after dosing on Day 140 (dose 6). There were no treatment-related neurological signs observed once the dogs reached the age of 6 months.

In a separate exploratory pharmacokinetic study, one female dog dosed at 12 mg/kg (3X the maximum recommended dose) exhibited lethargy, anorexia, and multiple neurological signs including ataxia, tremors, disorientation, hypersalivation, diminished proprioception, and absent menace, approximately 2 days after a third monthly dose. The dog was not treated, and was ultimately euthanized. The first two doses resulted in plasma concentrations that were consistent with those of the other dogs in the treatment group. Starting at 7 hours after the third dose, there was a rapid 2.5 fold increase in plasma concentrations within 41 hours, resulting in a C_{max} more than 7-fold higher than the mean C_{max} at the maximum recommended use dose. No cause for the sudden increase in sarolaner plasma concentrations was identified.

Storage Information:
Store at or below 30°C (86°F) with excursions permitted up to 40°C (104°F).

How Supplied:
SIMPARICA (sarolaner) Chewables are available in six flavored tablet sizes: 5, 10, 20, 40, 80, and 120 mg. Each tablet size is available in color-coded packages of one, three, or six tablets.

NADA #141-452, Approved by FDA

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Revised: July 2016

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