LOVE at one dose

Try Claro®. The one and only FDA-approved canine otitis externa treatment featuring:

- Single-dose treatment
- Vet administered to ensure compliance
- No work for your clients

BayerDVM.com/Claro

Claro® Otic Solution is approved for the treatment of ear infections in dogs caused by susceptible strains of yeast (Malassezia pachydermatis) and bacteria (Staphylococcus pseudintermedius). CAUTION: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian. CONTRAINDICATIONS: Claro® should not be used in dogs known or suspected to be allergic to Claro® or any of its ingredients.
**CLARO®**

**(florfenicol, terbinafine, mometasone furoate)**  
Otic Solution  

Antibacterial, antifungal, and anti-inflammatory  

For Otic Use in Dogs Only

The following information is a summary of the complete product information and is not comprehensive. Please refer to the approved product label for complete product information prior to use.

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

**PRODUCT DESCRIPTION:** CLARO® contains 16.6 mg/mL florfenicol, 14.8 mg/mL terbinafine (equivalent to 16.6 mg/mL terbinafine hydrochloride) and 2.2 mg/mL mometasone furoate. Inactive ingredients include purified water, propylene carbonate, propylene glycol, ethyl alcohol, and polyethylene glycol.

**INDICATIONS:** CLARO® is indicated for the treatment of otitis externa in dogs associated with susceptible strains of yeast (Malassezia pachydermatis) and bacteria (Staphylococcus pseudintermedius).

**DOSAGE AND ADMINISTRATION:** CLARO® should be administered by veterinary personnel. Administration is one dose (1 dropperette) per affected ear. The duration of effect should last 30 days. Clean and dry the external ear canal before administering the product. Verify the tympanic membrane is intact prior to administration. Cleaning the ear after dosing may affect product effectiveness. Refer to product label for complete directions for use.

**CONTRAINDICATIONS:** Do not use in dogs with known tympanic membrane perforation (see PRECAUTIONS). CLARO® is contraindicated in dogs with known or suspected hypersensitivity to florfenicol, terbinafine hydrochloride, or mometasone furoate, the inactive ingredients listed above, or similar drugs, or any ingredient in these medicines.

**WARNINGS:** Human Warnings: Not for use in humans. Keep this and all drugs out of reach of children. In case of accidental ingestion by humans, contact a physician immediately. In case of accidental skin contact, wash area thoroughly with water. Avoid contact with eyes. Humans with known hypersensitivity to florfenicol, terbinafine hydrochloride, or mometasone furoate should not handle this product.

**PRECAUTIONS:** Do not administer orally. The use of CLARO® in dogs with perforated tympanic membranes has not been evaluated. The integrity of the tympanic membrane should be confirmed before administering the product. Reevaluate the dog if hearing loss or signs of vestibular dysfunction are observed during treatment. Use of topical otic corticosteroids has been associated with adrenocortical suppression and iatrogenic hyperadrenocorticism in dogs. Use with caution in dogs with impaired hepatic function. The safe use of CLARO® in dogs used for breeding purposes, during pregnancy, or in lactating bitches has not been evaluated.

**ADVERSE REACTIONS:** In a field study conducted in the United States, there were no directly attributable adverse reactions in 146 dogs administered CLARO®. To report suspected adverse drug events and/or obtain a copy of the Safety Data Sheet (SDS) or for technical assistance, contact Bayer HealthCare at 1-800-422-9874. 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.

NADA 141-440, Approved by FDA. Bayer, the Bayer Cross and CLARO are registered trademarks of Bayer. ©2016 Bayer HealthCare, LLC. Distributed by Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, Kansas 66201.
• World-renowned Speakers
• Exceptional Round Table Topics and Discussions
• Informative Poster Displays
• Industry Exhibitors
• Lively Receptions
• Two half days to allow for free time
• And much, much, more in store

For more information about North American Veterinary Dermatology Forum 2018, consult our website at navdf.org or call 1-866-854-5525

#NAVDF2018
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LOST DOG

Dex disappeared from his dog house!

Please keep an eye out for him around 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 NAVDF in App Store
2. Click "GET" to download

**Android**
1. Search NAVDF in Google Play
2. Click “Install” to download

LAPTOP OR OTHER DEVICES
Use https://crowd.cc/2017navdf for online version of the app
SHUTTLE SCHEDULE

Shuttle from Animal Kingdom Lodge to Contemporary:
Wednesday: 3:30 pm – 8:30 pm
Thursday: 6:30 am – 6:30 pm
Friday: 6:30 am – 4:30 pm
Saturday: 6:30 am – 6:30 pm

The shuttle will run bottom of each hour (:30) pick up/ drop off at Animal Kingdom Lodge and top of each hour (:00) pick up/ drop off at the Contemporary.

Local Taxi Companies:
Diamond Cab Company: 407-523-3333
Taxi Orlando Cab Service: 321-732-8266
Yellow Cab Orlando: 407-900-5207

App Cars:
Uber
Lyft

Visit Disney's Magical Express for complimentary transportation to and from the airport.

A LA CARTE LUNCH

A la Carte Lunch
12:30 – 2:30 pm
Visit Disney Dining for all the options on Disney Properties:
https://disneyworld.disney.go.com/dining/

Ala Carte Lunches will be available at the Contemporary in the Convention Porte Cochere area during the lunch break time period. All seating is outside under the Porte Cochere.
REGISTRATION & EXHIBIT HALL HOURS

REGISTRATION HOURS

<table>
<thead>
<tr>
<th>Date</th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wednesday, April 26</td>
<td>5:00pm - 7:00pm</td>
</tr>
<tr>
<td>Thursday, April 27</td>
<td>7:00am - 5:30pm</td>
</tr>
<tr>
<td>Friday, April 28</td>
<td>7:30am - 5:30pm</td>
</tr>
<tr>
<td>Saturday, April 29</td>
<td>7:30am - 5:30pm</td>
</tr>
</tbody>
</table>

EXHIBIT HALL & POSTER HOURS

<table>
<thead>
<tr>
<th>Date</th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thursday, April 27</td>
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</tr>
<tr>
<td>Friday, April 28</td>
<td>8:30am - 3:30pm</td>
</tr>
<tr>
<td>Saturday, April 29</td>
<td>8:30am - 11:30am</td>
</tr>
</tbody>
</table>
BLOOD, SWEAT AND

EARS

*BAYTRIL* OTIC

FEATURING ENROFLOXACIN

WITH SPECIAL GUEST

SILVER SULFADIAZINE

VS

THE PSEUDOMONAS OTTITIS OF CHARLIE "JAWS" MALONE

BAYTRIL* OTIC (ENROFLOXACIN/SILVER SULFADIAZINE) ANTIBACTERIAL-ANTIMYCOtic EMULSION

FIGHT NASTY

CAUTION: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian. Federal law prohibits the extra label use of this drug in food-producing animals. CONTRAINDICATIONS: Baytril* Otic is contraindicated in dogs with suspected or known hypersensitivity to quinolones and/or sulfonamides.

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FIGHTNASTY.COM
Baytril® Otic
(enrofloxacin/silver sulfadiazine)
Antibacterial-Antimycotic Emulsion
For Ototopical Use In Dogs

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

Federal law prohibits the extralabel use of this drug in food-producing animals.

PRODUCT DESCRIPTION:
Each milliliter of Baytril® Otic contains: enrofloxacin 5 mg (0.5% w/v), silver sulfadiazine (SSD) 10 mg (1.0% w/v), benzyl alcohol (as a preservative) and cetylstearyl alcohol (as a stabilizer) in a neutral oil and purified water emulsion. The active ingredients are delivered via a physiological carrier (a nonirritating emulsion).

MICROBIOLOGY:
In clinical field trials, Baytril® Otic demonstrated elimination or reduction of clinical signs associated with otitis externa and in vitro activity against cultured organisms. Baytril® Otic is effective when used as a treatment for canine otitis externa associated with one or more of the following organisms: Malassezia pachydermatis, cutisrose-positive Staphylococcus spp., Pseudomonas aeruginosa, Enterobacter spp., Proteus mirabilis, Streptococci spp., Aeromonas hydrophila, Aspergillus spp., Klebsiella pneumoniae, and Candida albicans.

INDICATIONS:
Baytril® Otic is indicated as a treatment for canine otitis externa complicated by bacterial and fungal organisms susceptible to enrofloxacin and/or silver sulfadiazine (see Microbiology section).

EFFECTIVENESS:
Due to its combination of active ingredients, Baytril® Otic provides antimicrobial therapy against bacteria and fungi (which includes yeast) commonly encountered in cases of canine otitis externa.

CONTRAINDICATIONS:
Baytril® Otic is contraindicated in dogs with suspected or known hypersensitivity to quinolones and/or sulfonamides.

HUMAN WARNINGS:
Not for human use. Keep out of the reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation develops or persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolone compounds or antibacterials should avoid handling this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight.

PRECAUTIONS:
The use of Baytril® Otic in dogs with perforated tympanic membranes has not been evaluated. Therefore, the integrity of the tympanic membrane should be evaluated before administering this product. If hearing or vestibular dysfunction is noted during the course of treatment, discontinue use of Baytril® Otic.

Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures. Quinolone-class drugs have been associated with cartilage erosions in weightbearing joints and other forms of arthropathy in immature animals of various species. The safe use of Baytril® Otic in dogs used for breeding purposes, during pregnancy, or in lactating bitches, has not been evaluated.

ADVERSE REACTIONS:
During clinical trials, 2 of 113 (1.7%) dogs exhibited reactions that may have resulted from treatment with Baytril® Otic. Both cases displayed local hypersensitivity responses of the aural epithelium to some component within the Baytril® Otic formulation. The reactions were characterized by acute inflammation of the ear canal and pinna.

For medical emergencies or to report adverse reactions, call 1-800-422-9874. For customer service or to obtain product information, including Material Safety Data Sheet, call 1-800-633-3796.

SAFETY:
General Safety Study:
In a target animal safety study, Baytril® Otic was administered in both ears of 24 clinically normal beagle dogs at either recommended or exaggerated dosages: 10, 30 or 50 drops applied twice daily for 42 consecutive days. A control group of 8 beagle dogs was treated by administering 50 drops of vehicle in one ear twice daily for 42 consecutive days, with the contralateral ear untreated. Erythema was noted in all groups, including both treated and untreated ears in the controls, which resolved following termination of treatment.

Oral Safety Study:
In order to test safety in case of ingestion, Baytril® Otic was administered, twice daily for 14 consecutive days, to the dorsum of the tongue and to the left buccal mucosa of 6 clinically normal dogs. No adverse local or systemic reactions were reported.

DOSAGE AND ADMINISTRATION:
Shake well before each use. Tilt head so that the affected ear is presented in an upward orientation. Administer a sufficient quantity of Baytril® Otic to coat the aural lesions and the external auditory canal. As a general guide, administer 5-10 drops per treatment in dogs weighing 35 lbs. or less and 10-15 drops per treatment in dogs weighing more than 35 lbs. Following treatment, gently massage the ear so as to ensure complete and uniform distribution of the medication throughout the external ear canal. Apply twice daily for a duration of up to 14 days.
# MEETING SCHEDULE

**WEDNESDAY, APRIL 26, 2017**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 am - 4:30 pm</td>
<td><strong>ACVD Residents’ Education Forum</strong> <em>Sponsored by Zoetis</em></td>
<td>Sorcerer’s Apprentice Ballroom 3</td>
</tr>
<tr>
<td>8:00 am - 8:30 am</td>
<td>Breakfast</td>
<td></td>
</tr>
<tr>
<td>8:30 am - 10:00 am</td>
<td>Melanogenesis - Andrew Rosenberg, DVM, DACVD</td>
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<tr>
<td>10:00 am - 10:30 am</td>
<td>Break</td>
<td></td>
</tr>
<tr>
<td>10:30 am - 12:00 pm</td>
<td>Cytokines - Valerie Fadok, DVM, DACVD</td>
<td></td>
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<tr>
<td>12:00 pm - 1:00 pm</td>
<td>Lunch</td>
<td></td>
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<tr>
<td>1:00 pm - 2:30 pm</td>
<td>Fungal Disease - Karen Campbell, DVM, MS, DACVIM, DACVD</td>
<td></td>
</tr>
<tr>
<td>2:30 pm - 3:00 pm</td>
<td>Break</td>
<td></td>
</tr>
<tr>
<td>3:00 pm - 4:30 pm</td>
<td>Equine Dermatology - Wayne Rosenkrantz, DVM, DACVD</td>
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<tr>
<td>8:00 am - 8:00 pm</td>
<td><strong>Cyber Café</strong> <em>Sponsored by Veterinary Information Network</em></td>
<td>Olympus Ballroom</td>
</tr>
<tr>
<td>8:00 am - 5:00 pm</td>
<td><strong>ACVD Exam Committee Meeting</strong></td>
<td>Pastoral Room 1</td>
</tr>
<tr>
<td>9:00 am - 12:00 pm</td>
<td><strong>NAVDF Organizing Committee Meeting</strong></td>
<td>Pastoral Room 2</td>
</tr>
<tr>
<td>9:00 am - 11:00 am</td>
<td><strong>WCUD9 EOC Meeting</strong></td>
<td>Fantasia Ballroom P</td>
</tr>
<tr>
<td>10:15 am - 10:45 am</td>
<td>Break - For Board Meetings</td>
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<tr>
<td>11:45 am - 12:45 pm</td>
<td>Lunch - For Board Meetings</td>
<td></td>
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<tr>
<td>12:00 pm - 12:45 pm</td>
<td><strong>AAVD Executive Board Meeting</strong></td>
<td>Pastoral Room 2</td>
</tr>
<tr>
<td>12:00 pm - 5:00 pm</td>
<td><strong>ACVD Executive Board Meeting</strong></td>
<td>Pastoral Room 3</td>
</tr>
<tr>
<td>1:00 pm - 4:30 pm</td>
<td><strong>Exhibitor &amp; Poster Setup</strong></td>
<td>Fantasia Ballroom H &amp; Fantasia Lobby</td>
</tr>
<tr>
<td>1:00 pm - 5:30 pm</td>
<td><strong>ADVT VTS - Board Examination</strong></td>
<td>Fantasia Ballroom N</td>
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<tr>
<td>1:00 pm - 5:00 pm</td>
<td><strong>ISVD Board Meeting</strong></td>
<td>Fantasia Ballroom Q</td>
</tr>
<tr>
<td>2:00 pm - 5:00 pm</td>
<td><strong>ACVD AOK Committee Meeting</strong></td>
<td>Fantasia Ballroom M</td>
</tr>
<tr>
<td>3:00 pm - 3:30 pm</td>
<td>Break - For Board Meetings</td>
<td></td>
</tr>
<tr>
<td>5:00 pm - 7:00 pm</td>
<td><strong>Registration</strong></td>
<td>Fantasia Lobby – East Registration Desk</td>
</tr>
<tr>
<td>5:00 pm - 7:00 pm</td>
<td><strong>Welcome Reception</strong> <em>Sponsored by Hill's Pet Nutrition</em></td>
<td>Fantasia Ballroom G</td>
</tr>
<tr>
<td>6:00 pm - 8:00 pm</td>
<td><strong>NAVDF Program Committee Meeting</strong></td>
<td>Pastoral Room 2</td>
</tr>
<tr>
<td>7:00 pm - 9:00 pm</td>
<td><strong>Resident Presentation Run-throughs</strong></td>
<td>Sorcerer’s Apprentice Ballroom 3</td>
</tr>
</tbody>
</table>
### MEETING SCHEDULE

#### THURSDAY, APRIL 27, 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:00 am - 8:00 pm</td>
<td><strong>Cyber Café</strong>&lt;br&gt;Sponsored by Veterinary Information Network</td>
<td>Olympus Ballroom</td>
</tr>
<tr>
<td>7:00 am - 5:30 pm</td>
<td><strong>Registration</strong></td>
<td>Fantasia Lobby – East Registration Desk</td>
</tr>
<tr>
<td>7:00 am - 8:30 am</td>
<td>Roundtable Breakfast Buffet (coupon required)</td>
<td>Pastoral Lobby</td>
</tr>
<tr>
<td>7:30 am - 8:45 am</td>
<td><strong>ADVT Membership Meeting</strong></td>
<td>Pastoral Room 3</td>
</tr>
<tr>
<td>7:30 am - 8:45 am</td>
<td><strong>Roundtables</strong></td>
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<tr>
<td></td>
<td>#1 Cytopoint: Experiences and Questions&lt;br&gt;K. Coyner</td>
<td>Fantasia Ballroom K</td>
</tr>
<tr>
<td></td>
<td>#2 Apoquel&lt;br&gt;W. Rosenkrantz</td>
<td>Fantasia Ballroom L</td>
</tr>
<tr>
<td></td>
<td>#3 Flea and Tick Control&lt;br&gt;F. Banovic</td>
<td>Fantasia Ballroom M</td>
</tr>
<tr>
<td></td>
<td>#4 Microbiomes&lt;br&gt;A. Diesel</td>
<td>Fantasia Ballroom N</td>
</tr>
<tr>
<td></td>
<td>#5 Use of Technicians in a Dermatology Practice&lt;br&gt;J. Plant</td>
<td>Fantasia Ballroom P</td>
</tr>
<tr>
<td></td>
<td>#6 Journal Club&lt;br&gt;L. Ferrer</td>
<td>Fantasia Ballroom Q</td>
</tr>
<tr>
<td></td>
<td>#7 Controversies in Immunotherapy&lt;br&gt;D. DeBoer</td>
<td>Pastoral Room 1</td>
</tr>
<tr>
<td></td>
<td>Disney Animal Kingdom – Hospital Tour&lt;br&gt;(7:15 am – 8:45 am)</td>
<td>Disney’s Animal Kingdom</td>
</tr>
<tr>
<td>8:30 am - 4:30 pm</td>
<td><strong>Exhibits &amp; Posters</strong></td>
<td>Fantasia Ballroom H &amp; Fantasia Lobby</td>
</tr>
<tr>
<td>9:00 am - 11:00 am</td>
<td><strong>Scientific Session</strong>: Skin Microbiome – The Little We Know About This Fascinating Microscope World&lt;br&gt;Sheila Torres, DVM, PhD, DACVD</td>
<td>Fantasia Ballroom G</td>
</tr>
<tr>
<td>9:00 am - 10:00 am</td>
<td><strong>Concurrent Session</strong>: Updates On Allergen-Specific Immunotherapy&lt;br&gt;Douglas DeBoer, DVM, DACVD</td>
<td>Sorcerer’s Apprentice 1 &amp; 2</td>
</tr>
<tr>
<td>10:00 am - 11:00 am</td>
<td><strong>Concurrent Session</strong>: Delusional Parasitosis In Veterinary Practice: How To Recognize And What To Do?&lt;br&gt;Katharine Nelson, MD &amp; Sandra Koch, DVM, DACVD</td>
<td>Sorcerer’s Apprentice 1 &amp; 2</td>
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<tr>
<td>Time</td>
<td>Session Description</td>
<td>Location</td>
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<tr>
<td>9:00 am - 10:30 am</td>
<td><strong>Abstract Session</strong>: ACVD Residents’ Short Communications</td>
<td>Fantasia Ballroom J</td>
</tr>
<tr>
<td>11:00 am - 11:30 am</td>
<td>BREAK/ VISIT EXHIBITS &amp; POSTERS</td>
<td></td>
</tr>
</tbody>
</table>
| 11:30 am - 12:30 pm | **Scientific Session**: Pruritus vs. Pain  
Richard Lecouteur, DVM, PhD, DACVIM, DECVN | Fantasia Ballroom G |
| 11:30 am - 12:00 pm | **Concurrent Session**: Understanding The Basics Of Stem Cell Therapy In Companion Animal Dermatology  
Andrea Lam, DVM, DACVD | Sorcerer’s Apprentice 1 & 2 |
| 11:30 am - 12:30 pm | **Concurrent Session**: Taking Biopsies - a Pathologist’s Perspective  
Keith Linder, DVM, PhD, DACVP | Sorcerer’s Apprentice 1 & 2 |
| 11:30 am - 12:30 pm | **Abstract Session**: ACVD Residents’ Short Communications | Fantasia Ballroom J |
| 12:30 pm - 2:00 pm | LUNCH On Your Own                                                                   |                   |
| 12:30 pm - 2:00 pm | **ACVD Residency Mentors Meeting**  
Sponsored by Stallergenes Greer | Sorcerer’s Apprentice 3 |
| 2:00 pm - 3:00 pm | **Scientific Session**: Feline Rush Immunotherapy  
Mandy Burrows, BSc, BVMS, MANZCVS, FANZCVS  
Sponsored by ALK | Fantasia Ballroom G |
| 3:00 pm - 4:00 pm | **Scientific Session**: Feline Pemphigus Foliaceus  
Mandy Burrows, BSc, BVMS, MANZCVS, FANZCVS  
Sponsored by ALK | Fantasia Ballroom G |
| 2:00 pm - 3:00 pm | **Concurrent Session**: Primary Secretory Otitis Media  
Lynette Cole, DVM, MS, DACVD | Sorcerer’s Apprentice 1 & 2 |
| 3:00 pm - 4:00 pm | **Concurrent Session**: Otitis Management  
Lynette Cole, DVM, MS, DACVD | Sorcerer’s Apprentice 1 & 2 |
| 2:00 pm - 3:00 pm | **Abstract Session**: ACVD Residents’ Short Communications | Fantasia Ballroom J |
| 3:00 pm - 4:00 pm | **Abstract Session**: Clinical Short Communications | Fantasia Ballroom J |
| 4:00 pm - 4:30 pm | BREAK/ VISIT EXHIBITS & POSTERS                                                      |                   |
| 4:30 pm - 5:30 pm | **Scientific Session**: Setting Antimicrobial Breakpoints  
Mark Papich, DVM, MS, DACVP | Fantasia Ballroom G |
| 4:30 pm - 5:30 pm | **Concurrent Session**: Veterinary Medicine at Disney  
Geoff Pye, BVSc, MSc, DACZM | Sorcerer’s Apprentice 1 & 2 |
| 4:30 pm - 5:30 pm | **Abstract Session**: Original Short Communications | Fantasia Ballroom J |
| 5:45 pm - 7:15 pm | **ACVD Diplomates’ Business Meeting**  
Sponsored by Dechra Veterinary Products | Fantasia Ballroom G |
| 5:45 pm | **ACVD Residents’ Dinner**  
Sponsored by Dechra Veterinary Products | Odyssey Pavilion followed by Disney fireworks at Mexico Vista at Epcot |
| 7:15 pm | **ACVD Diplomates’ Dinner**  
Sponsored by Bayer HealthCare LLC Animal Health | Four Seasons Resort Orlando at Walt Disney World® Resort 10100 Dream Tree Blvd, Lake Buena Vista, FL |
### MEETING SCHEDULE

#### FRIDAY, APRIL 28, 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:00 am - 8:00 pm</td>
<td><strong>Cyber Café</strong>&lt;br&gt;Sponsored by Veterinary Information Network</td>
<td>Olympus Ballroom</td>
</tr>
<tr>
<td>7:00 am - 8:30 am</td>
<td><strong>Roundtable Breakfast Buffet</strong> (coupon required)</td>
<td>Pastoral Lobby</td>
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<td><strong>Registration</strong></td>
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<tr>
<td>7:30 am - 8:45 am</td>
<td><strong>Roundtables</strong>&lt;br&gt;&lt;br&gt;#8 Perianal and Perineal Dermatitis&lt;br&gt;K. Doerr&lt;br&gt;&lt;br&gt;#9 Behavioral Dermatoses&lt;br&gt;S. Borns-Weil&lt;br&gt;&lt;br&gt;#10 MRS Infection Control&lt;br&gt;S. Weese&lt;br&gt;&lt;br&gt;#11 Otitis&lt;br&gt;A. Rosenberg&lt;br&gt;Sponsored by Royal Canin&lt;br&gt;&lt;br&gt;#12 Probiotics&lt;br&gt;R. Mount&lt;br&gt;&lt;br&gt;#13 Skin Barrier&lt;br&gt;J. Pieper&lt;br&gt;&lt;br&gt;#14 Equine Dermatology (ie. Sarcoids)&lt;br&gt;T. Prange&lt;br&gt;&lt;br&gt;#15 Tech Roundtable - Therapeutic Product Assessment and Comparison for the Treatment of Otitis Externa&lt;br&gt;C. George</td>
<td>Fantasia Ballroom K, Fantasia Ballroom L, Fantasia Ballroom M, Fantasia Ballroom N, Fantasia Ballroom P, Fantasia Ballroom Q, Pastoral Room 1, Pastoral Room 2</td>
</tr>
<tr>
<td>8:30 am - 4:30 pm</td>
<td><strong>Exhibits &amp; Posters</strong></td>
<td>Fantasia Ballroom H &amp; Fantasia Lobby</td>
</tr>
<tr>
<td>8:30 am - 4:30 pm</td>
<td><strong>Disney Animal Kingdom – Hospital Tour</strong>&lt;br&gt;(7:15 am – 8:45 am)</td>
<td>Disney’s Animal Kingdom</td>
</tr>
<tr>
<td>9:00 am - 10:00 am</td>
<td><strong>Scientific Session:</strong> Clinicopathology Correlations provided by ISVD - Inflammatory&lt;br&gt;Derick Whitley and Christina Gentry, Jennifer Ward and Tiffany Tapp</td>
<td>Fantasia Ballroom G</td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td>Location</td>
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<td>--------------</td>
<td>------------------------------------------------------------------------</td>
<td>------------------------</td>
</tr>
</tbody>
</table>
| 9:00 am - 10:00 am | **Scientific Session:** Clinicopathology Correlations provided by ISVD - Neoplastic  
Dunbar Gram, DVM, DACVD & Pamela Ginn, DVM, DACVP, Michael Canfield, DVM, Dipl, ACVD & Jeanine Peters-Kennedy, DVM, Dipl, ACVD, ACVP | Fantasia Ballroom G    |
| 9:00 am - 10:00 am | **Concurrent Session:** Resistant Infections  
Scott Weese, DVM, DVSc, DACVIM | Sorcerer’s Apprentice 1 & 2 |
| 10:00 am - 11:00 am | **Concurrent Session:** Antimicrobial Guidelines  
Mark Papich, DVM, MS, DACVCP | Sorcerer’s Apprentice 1 & 2 |
| 9:00 am - 11:00 am | **Abstract Session:** Original Short Communications | Fantasia Ballroom J    |
| 11:00 am - 11:30 am | **BREAK/ VISIT EXHIBITS & POSTERS** |                       |
| 11:30 am - 12:30 pm | **Scientific Session:** Erythema Multiforme, Stevens-Johnson Syndrome And Toxic Epidermal Necrolysis: Comparative Aspect In Humans And Dogs.  
Frane Banovic, DVM, PhD, DECVD | Fantasia Ballroom G    |
| 11:30 am - 12:30 pm | **Concurrent Session:** Antimicrobial Stewardship / Clinical Perspective Panel  
Sheila Torres, DVM, PhD, DACV  
Scott Weese, DVM, DVSc, DACVIM  
Mark Papich, DVM, MS, DACVCP | Sorcerer’s Apprentice 1 & 2 |
| 11:00 am - 12:30 pm | **Abstract Session:** Original Short Communications | Fantasia Ballroom J    |
| 12:00 pm - 2:00 pm | **ACVD Ethics Committee Meeting** | Fantasia Ballroom L    |
| 12:00 pm - 2:00 pm | **LUNCH On Your Own** |                       |
| 12:30 pm - 2:00 pm | **ACVD Residents Lunch Meeting**  
*Sponsored by Stallergenes Greer* | Sorcerer’s Apprentice 3  |
| 12:30 pm - 2:00 pm | **GVDEG Meeting** | Pastoral Room 1        |
| 12:30 pm - 2:00 pm | **CAVD Executive Committee Meeting** | Fantasia Ballroom Q    |
| 12:30 pm - 2:00 pm | **ICADA Meeting** | Fantasia Ballroom K    |
| 12:45 pm - 1:45 pm | **ACVD Website Committee** | Fantasia Ballroom M    |
| 2:00 pm - 3:00 pm | **Scientific Session:** Infusion Medicine In Dermatology  
Marie Holowaychuk, DVM, DACVECC | Fantasia Ballroom G    |
| 3:00 pm - 3:15 pm | **ACVD Resident Research Awards**  
*Sponsored by Bayer Healthcare LLC Animal Health*  
**ACVD Externship Grants**  
*Sponsored by Hill's Pet Nutrition* | Fantasia Ballroom G    |
| 2:00 pm - 3:00 pm | **Concurrent Session:** Advanced Imaging And Video Otoscopy  
Mike Canfield, DVM, DACVD  
Rod Rosychuk, DVM, DACVIM | Sorcerer’s Apprentice 1 & 2 |
| 2:00 pm - 4:00 pm | **Abstract Session:** Original And Clinical Short Communications | Fantasia Ballroom J    |
| 4:00 pm - 4:30 pm | **BREAK/ VISIT EXHIBITS & POSTERS** |                       |
| 4:30 pm - 5:30 pm | **Abstract Session:** Clinical Short Communications | Fantasia Ballroom J    |
| 6:00 pm | **Reception**  
*Sponsored by Royal Canin Veterinary Diet* | House of Blues  
Featured Entertainment: Yeasty Boys  
Bus transportation provided |
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>6:00 am - 4:00 pm</td>
<td>Cyber Café&lt;br&gt;&lt;i&gt;Sponsored by Veterinary Information Network&lt;/i&gt;</td>
<td>Olympus Ballroom</td>
</tr>
<tr>
<td>7:00 am - 8:30 am</td>
<td>Roundtable Breakfast Buffet (coupon required)</td>
<td>Pastoral Lobby</td>
</tr>
<tr>
<td>7:30 am - 5:30 pm</td>
<td>Registration</td>
<td>Fantasia Lobby – East Registration Desk</td>
</tr>
<tr>
<td>7:15 am - 8:45 am</td>
<td>Disney Animal Kingdom – Hospital Tour</td>
<td>Disney’s Animal Kingdom</td>
</tr>
<tr>
<td>7:30 am - 8:45 am</td>
<td>Tech Roundtable - Demodex&lt;br&gt; M. Streicher</td>
<td>Pastoral Room 1</td>
</tr>
<tr>
<td>7:30 am - 8:45 am</td>
<td>ACVD Residents’ Roundtable&lt;br&gt;&lt;i&gt;Sponsored by Royal Canin&lt;/i&gt;</td>
<td>Sorcerer’s Apprentice 3 Ballroom</td>
</tr>
<tr>
<td>8:30 am - 11:30 am</td>
<td>Exhibits/ Posters</td>
<td>Fantasia Ballroom H &amp; Fantasia Lobby</td>
</tr>
<tr>
<td>9:00 am - 10:00 am</td>
<td>Scientific Session: Demodex&lt;br&gt; Wayne Rosenkrantz, DVM, DACVD</td>
<td>Fantasia Ballroom G</td>
</tr>
<tr>
<td>10:00 am - 11:00 am</td>
<td>Scientific Session: Behavior&lt;br&gt; Stephanie Borns-Weil, DVM</td>
<td>Fantasia Ballroom G</td>
</tr>
<tr>
<td>9:00 am - 11:00 am</td>
<td>Concurrent Session: Dermatologic Markers Of Internal Disease&lt;br&gt; Catherine Outerbridge, DVM, MVSc, DACVIM, DACVD</td>
<td>Sorcerer’s Apprentice 1 &amp; 2</td>
</tr>
<tr>
<td>9:00 am - 11:00 am</td>
<td>ADVT - Educational Session: Structure and function for the veterinary dermatology technician and alopecic diseases.&lt;br&gt; Jason Pieper, DVM, MS, DACVD</td>
<td>Fantasia Ballroom K&amp;L</td>
</tr>
<tr>
<td>9:00 am - 10:00 am</td>
<td>Dermatopathology Session (ISVD): Plenary Part I&lt;br&gt; Melanocyte Biology And Pathology In Animals.&lt;br&gt; Keith Linder, DVM, PhD, DACVP</td>
<td>Fantasia Ballroom J</td>
</tr>
<tr>
<td>10:00 am - 11:00 am</td>
<td>Dermatopathology Session (ISVD): Plenary Lecture Part 2 - Melanocytic Tumor Diagnosis in Humans&lt;br&gt; Michael Tetzlaff, MD, PhD</td>
<td>Fantasia Ballroom J</td>
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<tr>
<td>11:00 am - 11:30 am</td>
<td>BREAK/ VISIT EXHIBITS &amp; POSTERS</td>
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<tr>
<td>11:30 am - 12:00 pm</td>
<td>Scientific Session: Equine Papillomaviruses&lt;br&gt; Keith Linder, DVM, PhD, DACVP</td>
<td>Fantasia Ballroom G</td>
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<tr>
<td>12:00 pm - 12:30 pm</td>
<td>Scientific Session: Diagnosis and Treatment of Equine Sarcoïds&lt;br&gt; Timo Prange, Dr. med. vet. MS, DACVS</td>
<td>Fantasia Ballroom G</td>
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<td>Time</td>
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<tr>
<td>11:30 am - 12:30 pm</td>
<td>Concurrent Session: Marine Mammal Dermatology</td>
<td>Sorcerer’s Apprentice 1 &amp; 2</td>
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<tr>
<td></td>
<td>David Rotstein, DVM, DACVP</td>
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<tr>
<td>11:30 am - 12:30 pm</td>
<td>ADVT - Educational Session: Structure and function for the veterinary</td>
<td>Fantasia Ballroom K &amp; L</td>
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<tr>
<td></td>
<td>dermatology technician and alopecic diseases.</td>
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<td></td>
<td>Jason Pieper, DVM, MS, DACVD</td>
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<tr>
<td>11:30 am - 12:00 pm</td>
<td>Dermatopathology Session (ISVD): Plenary Lecture Part 2 - Melanocytic</td>
<td>Fantasia Ballroom J</td>
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<tr>
<td></td>
<td>Tumor Diagnosis in Humans</td>
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<td></td>
<td>Michael Tetzlaff, MD, PhD</td>
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<tr>
<td>12:00 pm - 12:30 pm</td>
<td>Dermatopathology Session (ISVD): Discussion from Listserv</td>
<td>Fantasia Ballroom J</td>
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<tr>
<td></td>
<td>David Shearer, BVetMed Cert SAD, PhD, CBiol, MSB, MRCVS</td>
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<tr>
<td>12:30 pm - 2:00 pm</td>
<td>LUNCH On Your Own</td>
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<tr>
<td>12:30 pm - 2:00 pm</td>
<td>AAVD Business Meeting – Lunch</td>
<td>Fantasia Ballroom G</td>
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<tr>
<td>2:00 pm - 2:45 pm</td>
<td>ADVT – VTS Informational Meeting</td>
<td>Fantasia Ballroom K &amp; L</td>
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<tr>
<td>2:00 pm - 3:00 pm</td>
<td>Scientific Session: Janus Kinase (JAKs) inhibitors: Theory, Practice</td>
<td>Fantasia Ballroom G</td>
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<tr>
<td></td>
<td>and Prospects… in humans.</td>
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</tr>
<tr>
<td></td>
<td>Massimo Gadina, PhD</td>
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<tr>
<td>3:00 pm - 4:00 pm</td>
<td>Scientific Session: Use Of JAK Inhibitors In Vet Med</td>
<td>Fantasia Ballroom G</td>
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<tr>
<td></td>
<td>Valerie Fadok, DVM, PhD, DACVD</td>
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<tr>
<td>2:00 pm - 3:00 pm</td>
<td>Concurrent Session: Radiation Therapy In Veterinary Dermatology</td>
<td>Sorcerer’s Apprentice 1 &amp; 2</td>
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<tr>
<td></td>
<td>Alison Diesel, DVM, DACVD</td>
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<tr>
<td>3:00 pm - 4:00 pm</td>
<td>Concurrent Session: Achieving Work-Life Balance</td>
<td>Sorcerer’s Apprentice 1 &amp; 2</td>
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<td></td>
<td>Marie Holowaychuk, DVM, DACVECC</td>
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<tr>
<td>2:00 pm - 4:00 pm</td>
<td>Dermatopathology Session (ISVD): What’s your diagnosis - Unknown or</td>
<td>Fantasia Ballroom J</td>
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<tr>
<td></td>
<td>poorly characterized interactive case discussion – Panel Chair:</td>
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<tr>
<td></td>
<td>David Shearer, BVetMed Cert SAD, PhD, CBiol, MSB, MRCVS</td>
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<tr>
<td>4:00 pm - 4:30 pm</td>
<td>BREAK/ VISIT POSTERS</td>
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<tr>
<td>4:30 pm - 5:30 pm</td>
<td>Scientific Session: Monoclonal Antibodies</td>
<td>Fantasia Ballroom G</td>
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<tr>
<td></td>
<td>Valerie Fadok, DVM, PhD, DACVD</td>
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<tr>
<td>4:30 pm - 5:30 pm</td>
<td>Concurrent Session: What if Disney Ran Your Veterinary Practice?</td>
<td>Sorcerer’s Apprentice 1 &amp; 2</td>
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<tr>
<td></td>
<td>Scott Terrell, DVM</td>
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<tr>
<td>4:30 pm - 5:00 pm</td>
<td>Dermatopathology Session (ISVD): Discriminatory Features Of</td>
<td>Fantasia Ballroom J</td>
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<tr>
<td></td>
<td>Osinophilic Dermatitis (Wells-Like Syndrome) And Sterile Neutrophilic</td>
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<tr>
<td></td>
<td>Dermatosis (Sweet’s-Like Syndrome) In Dogs:</td>
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<td></td>
<td>A clinicopathological and immunohistochemical study</td>
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<tr>
<td></td>
<td>Charles Bradley, VMD, DACVP</td>
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<tr>
<td>5:00 pm - 5:30 pm</td>
<td>ISVD Annual General Meeting (AGM)</td>
<td>Fantasia Ballroom J</td>
</tr>
<tr>
<td></td>
<td>Chair: David Shearer</td>
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</tr>
</tbody>
</table>
# ROUNDTABLE SESSIONS

## THURSDAY, APRIL 27, 2017

<table>
<thead>
<tr>
<th>#</th>
<th>Title</th>
<th>Presenter</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Cytopoint: Experiences and Questions</td>
<td>K. Coyner</td>
<td>Fantasia Ballroom K</td>
</tr>
<tr>
<td>#2</td>
<td>Apoquel</td>
<td>W. Rosenkrantz</td>
<td>Fantasia Ballroom L</td>
</tr>
<tr>
<td>#3</td>
<td>Flea and Tick Control</td>
<td>F. Banovic</td>
<td>Fantasia Ballroom M</td>
</tr>
<tr>
<td>#4</td>
<td>Microbiomes</td>
<td>A. Diesel</td>
<td>Fantasia Ballroom N</td>
</tr>
<tr>
<td>#5</td>
<td>Use of Technicians in a Dermatology Practice</td>
<td>J. Plant</td>
<td>Fantasia Ballroom P</td>
</tr>
<tr>
<td>#6</td>
<td>Journal Club</td>
<td>L. Ferrer</td>
<td>Fantasia Ballroom Q</td>
</tr>
<tr>
<td>#7</td>
<td>Controversies in Immunotherapy</td>
<td>D. DeBoer</td>
<td>Pastoral Room 1</td>
</tr>
</tbody>
</table>

**Disney Animal Kingdom – Hospital Tour (7:15 am – 8:45 am)**  
Disney’s Animal Kingdom

## FRIDAY, APRIL 28, 2017

<table>
<thead>
<tr>
<th>#</th>
<th>Title</th>
<th>Presenter</th>
<th>Room</th>
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<tbody>
<tr>
<td>#8</td>
<td>Perianal and Perineal Dermatitis</td>
<td>K. Doerr</td>
<td>Fantasia Ballroom K</td>
</tr>
<tr>
<td>#9</td>
<td>Behavioral Dermatoses</td>
<td>S. Borns-Weil</td>
<td>Fantasia Ballroom L</td>
</tr>
<tr>
<td>#10</td>
<td>MRS Infection Control</td>
<td>S. Weese</td>
<td>Fantasia Ballroom M</td>
</tr>
<tr>
<td>#11</td>
<td>Otitis</td>
<td>A. Rosenberg</td>
<td>Fantasia Ballroom N</td>
</tr>
<tr>
<td></td>
<td>Supported by Royal Canin</td>
<td></td>
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</tr>
<tr>
<td>#12</td>
<td>Probiotics</td>
<td>R. Mount</td>
<td>Fantasia Ballroom P</td>
</tr>
<tr>
<td>#13</td>
<td>Skin Barrier</td>
<td>J. Pieper</td>
<td>Fantasia Ballroom Q</td>
</tr>
<tr>
<td>#14</td>
<td>Equine Dermatology (ie. Sarcoids)</td>
<td>T. Prange</td>
<td>Pastoral Room 1</td>
</tr>
<tr>
<td>#15</td>
<td>Tech Roundtable - Therapeutic Product Assessment and Comparison for the Treatment of Otitis Externa</td>
<td>C. George</td>
<td>Pastoral Room 2</td>
</tr>
</tbody>
</table>

**Disney Animal Kingdom – Hospital Tour (7:15 am – 8:45 am)**  
Disney’s Animal Kingdom

## SATURDAY, APRIL 29, 2017

<table>
<thead>
<tr>
<th>#16</th>
<th>Tech Roundtable - Demodex</th>
<th>M. Streicher</th>
<th>Pastoral Room 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>#17</td>
<td>ACVD Residents’ Roundtable</td>
<td>Sponsored by Royal Canin</td>
<td>Sorcerer’s Apprentice 3 Ballroom</td>
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<tr>
<td></td>
<td>Supported by Royal Canin</td>
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<tr>
<td>#18</td>
<td>Disney Animal Kingdom – Hospital Tour</td>
<td></td>
<td>Disney’s Animal Kingdom</td>
</tr>
<tr>
<td></td>
<td>(7:15 am – 8:45 am)</td>
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</tbody>
</table>
A FULL LINE OF PRODUCTS THAT MAKES PERFECT SENSE FOR PETS

DVM® dermatology and supplement products blend science-based formulations with quality ingredients to provide a wide range of options for your patients. Oh, the possibilities!

For more information, visit BayerDVM.com, call your local Sales Representative, contact your preferred distributor or call Bayer Customer Service at 1-800-633-3796.
### RESIDENTS’ SHORT COMMUNICATIONS

<table>
<thead>
<tr>
<th>Time</th>
<th>Presentation</th>
<th>Page</th>
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</thead>
<tbody>
<tr>
<td>9:00 am</td>
<td>Simpson: The frequency of urinary tract infection in dogs with allergic dermatitis treated with oclacitinib: a prospective study</td>
<td>25</td>
</tr>
<tr>
<td>9:15 am</td>
<td>Lapraiss: Evaluation of intra-epidermal nerve fibers in the skin of normal and atopic dogs</td>
<td>26</td>
</tr>
<tr>
<td>9:30 am</td>
<td>Lerace: Combined carbon dioxide laser and cryosurgical ablation of rostral nasal septum carcinoma in 10 canine cases</td>
<td>27</td>
</tr>
<tr>
<td>9:45 am</td>
<td>Lane: Multi-drug, extensive drug, methicillin resistance and associated risk factors in feline Staphylococci cultured from 2001-2014</td>
<td>28</td>
</tr>
<tr>
<td>10:00 am</td>
<td>Knight: In vitro comparison of the dermal penetration of three different topical formulations containing lasalocid for meticillin-resistant Staphylococcus pseudintermedius infections</td>
<td>29</td>
</tr>
<tr>
<td>10:15 am</td>
<td>Schnedeker: In vitro bactericidal activity of blue light (465-nm) phototherapy on meticillin-susceptible and meticillin-resistant Staphylococcus pseudintermedius</td>
<td>30</td>
</tr>
<tr>
<td>10:30 am</td>
<td>Mocznarik: Pharmacokinetics and relative oral bioavailability of two oral amoxicillin–clavulanic acid formulations in healthy adult dogs – a preliminary investigation</td>
<td>31</td>
</tr>
<tr>
<td>10:45 am</td>
<td>Hasbach: Pharmacokinetics and relative bioavailability of orally administered innovator-formulated itraconazole capsules and solution in healthy dogs</td>
<td>32</td>
</tr>
<tr>
<td>11:00 am - 11:30 am</td>
<td>BREAK</td>
<td></td>
</tr>
<tr>
<td>11:30 am</td>
<td>Bowden: Canine demodicosis: a retrospective study (2000-2016)</td>
<td>33</td>
</tr>
<tr>
<td>11:45 am</td>
<td>Zewe: Afoxolaner and fluralaner treatment do not impact normal Demodex populations in healthy dogs</td>
<td>34</td>
</tr>
<tr>
<td>12:00 pm</td>
<td>McFadden: A double-blinded, randomized, controlled, cross over evaluation of a zinc methionine supplement as adjunctive treatment for canine atopic dermatitis</td>
<td>35</td>
</tr>
<tr>
<td>12:15 pm</td>
<td>Mochizuki: Pilot evaluation of the efficacy to a topical transient receptor potential M8 (TRPM8) agonist for treatment of pruritic atopic pododermatitis in dogs</td>
<td>36</td>
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<tr>
<td>12:30 pm - 2:00 pm</td>
<td>LUNCH</td>
<td></td>
</tr>
<tr>
<td>2:00 pm</td>
<td>Scholz: Determination of threshold concentrations of plant pollens in intradermal testing using fluorescein in clinically healthy nonallergic cats</td>
<td>37</td>
</tr>
<tr>
<td>2:15 pm</td>
<td>Carnett: Identification of irritant threshold concentrations for percutaneous allergen testing in 20 non-sedated dogs</td>
<td>38</td>
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<tr>
<td>2:30 pm</td>
<td>Foust-Wheatcraft: Determination of irritant threshold concentrations of nine allergens from two different manufacturers in clinically non-allergic dogs</td>
<td>39</td>
</tr>
<tr>
<td>2:45 pm</td>
<td>Schwartz: Dorsal thermal necrosis in dogs: a retrospective analysis of 15 cases (2009-2016)</td>
<td>40</td>
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THURSDAY, APRIL 27, 2017 cont.

CLINICAL SHORT COMMUNICATIONS

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<th>Time</th>
<th>Presenter</th>
<th>Title</th>
<th>Page</th>
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<tbody>
<tr>
<td>3:00 pm</td>
<td>Lam</td>
<td>Evaluation of clinical accuracy of serological and salivary testing for food allergens in asymptomatic dogs</td>
<td>41</td>
</tr>
<tr>
<td>3:15 pm</td>
<td>Vincent</td>
<td>A retrospective study to assess anti-pruritic efficacy of lokivetmab in dogs with canine atopic dermatitis</td>
<td>42</td>
</tr>
<tr>
<td>3:30 pm</td>
<td>Souza</td>
<td>Lokivetmab in the control of pruritus in 135 dogs with allergic dermatitis</td>
<td>43</td>
</tr>
<tr>
<td>3:45 pm</td>
<td>High</td>
<td>A retrospective study comparing the incidence of cutaneous histiocytoma development in atopic dogs treated with oclacitinib and ciclosporin</td>
<td>44</td>
</tr>
<tr>
<td>4:00 pm-4:30 pm</td>
<td></td>
<td>BREAK</td>
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The frequency of urinary tract infection in dogs with allergic dermatitis treated with oclacitinib: a prospective study

A.SIMPSON*, J. SCHISSLER*, and R.A.W. ROSYCHUK*

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

Abstract: Oclacitinib (Apoquel®; Zoetis Inc., Kalamazoo, MI, USA) is a selective Janus kinase inhibitor for the treatment of canine allergic pruritus and atopic dermatitis in dogs at least 12 months of age. Corticosteroids and ciclosporin increase urinary tract infection (UTI) frequency in dogs with inflammatory skin disease. This study prospectively evaluated the frequency of UTI in oclacitinib-treated dogs with allergic dermatitis. Client-owned dogs ≥ 2 years of age with a history of allergic dermatitis without apparent history of urinary tract disease or predisposition to UTI were included. Prior to enrollment, urinalysis and quantitative urine culture were performed after a washout period of at least 14 days from systemic antimicrobials and 28 days for ciclosporin and systemic corticosteroids. Dogs were treated with oclacitinib at labeled dosing for an intended period of 180-230 days with a follow-up urinalysis and urine culture performed regardless of urinary tract signs. Systemic antimicrobials and immune-modulating drugs were not administered during the study. None of the dogs (0/55) in this study developed UTI while receiving oclacitinib based on follow-up urinalysis and urine culture performed during a range of 58-280 days (mean 195 days). Two dogs developed self-limiting abnormal urinary tract signs without urine culture or urinalysis findings consistent with UTI. These findings indicate UTI is not an expected side effect in dogs treated with oclacitinib without a prior history of UTI or predisposing condition during this treatment period. Therefore, routine urine culture is not indicated for such dogs in the absence of abnormal urinalysis or clinical urinary signs.

Source of funding: Zoetis Excellence in Dermatology Research Grant.

Conflict of interest: None declared.
Evaluation of intra-epidermal nerve fibers in the skin of normal and atopic dogs

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† Clinic for Small Animal Internal Medicine Dermatology Unit, Vetsuisse Faculty University of Zurich, Zurich, Switzerland
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§Comparative Medicine Institute, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Abstract: Interest for intra-epidermal nerve fibers (IENFs) is rising in human medicine, as variations in fiber density occur in some diseases and these neurites might contribute to disease pathogenesis. An increase in IENF density is seen in human atopic dermatitis (AD), but information on IENF in atopic dogs is lacking. Our objective was to evaluate and compare the prevalence of IENFs in normal and atopic canine skin. Eight-millimeter punch skin biopsies were taken from six sites of 25 dogs without dermatitis, while canine lesional (23) and nonlesional AD skin (14) biopsies were from previous studies. Thirty micrometer-thick paraffin-embedded sections were stained by indirect immunofluorescence for neuronal beta-3 tubulin. Only sections with detectable dermal nerves (internal positive control) were then screened for IENFs. We found IENFs in all 25 normal nasal planum sections, but in only one biopsy collected from each of the normal canine haired skin (NCHS) sites (axilla, groin, thorax, feet); as there was no significant difference in IENF prevalence between NCHS areas, they were grouped together. The rate of IENF detection was significantly higher in lesional AD (18/23; 78%) than in nonlesional AD skin (4/14; 29%) (one-tailed Fisher’s test, P = 0.004) and NCHS specimens (4/111; 4%) (P < 0.0001). The prevalence of IENF detection in nonlesional AD samples was significantly higher than that in normal canine skin (P = 0.006). In summary, IENFs are detected more commonly in canine AD than in normal haired skin; these results are comparable to those seen for the human disease homologue.

Source of funding: Source of funding: self-funded.

Conflict of interest: None declared.
Abstract: Two-thirds of intranasal neoplasms are caused by carcinomas. Generally, the metastasis rate is low and the goal of therapy is to provide local disease control. Nasal planectomy can provide long term survival, or cure with complete margins (67-100% cure rate). However, cosmetic outcomes of nasal planectomy may be unacceptable. Radiation therapy alone can treat the entire nasal cavity with a median survival time of approximately 12-16 months. The study aim was to evaluate the efficacy of combination carbon dioxide laser surgery and cryosurgery as a palliative treatment modality in dogs with nasal squamous cell carcinomas. Selection criteria were based on the location of the tumor, which was limited to the rostral-most 2.5 cm of the nasal septum, and the owner’s declination of both radiation therapy and nasal planectomy. Ten dogs with nasal squamous cell carcinoma were included in the study, seven neutered males, two spayed females, and one intact male, with a median age of 12.5 years (range 9-15 years). Tumor carbon dioxide laser ablation was followed by cryosurgical ablation of the visible tumor, adjacent and subjacent tissue. Three rapid-freeze-slow-thaw cycles were performed. The treatment was repeated in 8/10 patients with tumor recurrence. Overall median survival time was 189 days with two dogs alive at follow up (median, 163 days). Combination carbon dioxide laser and cryosurgical ablation was a practical and effective alternative to nasal planectomy for rostral septal nasal carcinoma in dogs where nasal planectomy or radiation therapy is objectionable.

Source of funding: Self-funded.

Conflict of interest: None declared.
Multi-drug, extensive drug, meticillin resistance and associated risk factors in feline staphylococci cultured from 2001-2014

M. J. LANE*, A. F. ROY□, M. T. KEARNEY±, C. M. PUCHEU-HASTON*

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Abstract: Data on Staphylococcal resistance patterns in cats are limited. The purpose of this retrospective study was to assess the prevalence of antibiotic resistance in staphylococci isolated from feline patients at the Louisiana Animal Disease Diagnostic Laboratory (LADDL) from 2001-2014. We hypothesized that the relative frequency of meticillin resistant (MR), multi-drug resistant (MDR), and extensively drug resistant (XDR) Staphylococcus isolates significantly increased over the study period. The LADDL database was queried for all feline staphylococcal isolates between 2001-2014. This information was used to determine the prevalence of MR, MDR and XDR in Staphylococcus species isolated from feline patients. Potential risk factors (including administration of antimicrobials and immunosuppressive medications) were also assessed. MR was confirmed in 24.17% (95/393) of the staphylococcal isolates. MDR and XDR were present in 24.94% (98/393) and 3.05% (12/393), respectively. The incidence of MR and combined MDR/XDR were found to increase significantly over the study period (P <0.05). XDR was first noted in 2007. Previous antibiotic administration was associated with a significantly increased risk of MR and MDR (P <0.05); conversely, prior immunosuppressive medication was not (p >0.05). Due to the retrospective nature of the study, it was not possible to confirm the clinical relevance of all of the isolates, and it is possible that this may have impacted our results. Nonetheless, the observed increased prevalence of MR and MDR/XDR, the emergence of XDR during the study period and the increase in MR associated with prior antibiotic use suggest a disturbing trend.

Source of funding: self-funded

Conflict of interest: none declared
In vitro comparison of the dermal penetration of three different topical formulations containing lasalocid for meticillin-resistant Staphylococcus pseudintermedius infections

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†School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia
‡School of Veterinary Science, University of Queensland, Gatton, QLD, Australia
§Luoda Pharma, Caringba, NSW, Australia
¶Centre for Pharmaceutical Innovation and Development, University of South Australia, Adelaide, SA, Australia
**Department of Genetics and Evolution, University of Adelaide, Adelaide, SA, Australia

Abstract: Topical antimicrobials are critical for treatment of meticillin-resistant Staphylococcus pseudintermedius (MRSP) pyoderma due to increasing incidence of resistance to systemic antibiotics. Lasalocid is efficacious against MRSP, thus topical lasalocid may be useful to treat pyodermas due to MRSP. The aim of this study was to determine the effect of various formulations on the penetration and retention of lasalocid applied to canine skin. A solution, lotion and ointment, each containing 2% lasalocid, were applied to ex vivo canine skin. Transdermal penetration was assessed via HPLC of receptor compartment fluid from each Franz-type diffusion cell, collected at time 0, 0.5, 1, 2, 4, 8, 12, and 24 h. HPLC was also used to measure retention of lasalocid in the skin discs at the conclusion of the 24 h period. Three control Franz-cells (one per water bath) containing skin without lasalocid were used. Previous studies confirmed the solubility of lasalocid in the receptor solution. Retention of active ingredient was greatest for the solution (292.75 ± 141.67 µg/cm²), followed by the lotion (143.93 ± 96.38 µg/cm²), then the ointment (39.46 ± 12.31 µg/cm²). The solution method had significantly higher skin retention and proportion of applied dose retained in skin than the lotion and ointment methods (Tukey test, P <0.01). The active ingredient lasalocid was not identified in the receptor fluid in any sample indicating that systemic absorption of the active ingredient in vivo is unlikely. The lasalocid solution resulted in the greatest skin retention and thus may be the most useful in treating MRSP.

Sources of funding: Australian and New Zealand College of Veterinary Scientists, and Luoda Pharma.

Conflicts of interest: Co-author, Stephen Page, works for Luoda Pharma, one of the sources of funding for this study.
In vitro bactericidal activity of a blue-light (465 nm) phototherapeutic device on *Staphylococcus aureus* and *Staphylococcus pseudintermedius*

A. SCHNEDEKER*, L. COLE*, G. LORCH*, S. DIAZ*, J. BONAGURA*, J. DANIELS*

*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp St., Columbus, OH, USA

Abstract: *Staphylococcus* spp. are the most common cause of bacterial skin infections in dogs. Phototherapy is a novel option for treating these infections. The objective of this study was to determine the *in vitro* bactericidal activity of 465 nm blue-light on meticillin-susceptible *Staphylococcus pseudintermedius* (MSSP), meticillin-resistant *Staphylococcus pseudintermedius* (MRSP), and meticillin-resistant *Staphylococcus aureus* (MRSA). We hypothesized that blue-light phototherapy would kill MSSP, MRSP and MRSA *in vitro*. MRSP (ST-71), MRSA (BAA-1680) and an untyped MSSP clinical isolate were serially diluted, and plated in sextuplicate (18 total plates). Treatment plates (three each for MRSP, MRSA, MSSP) were irradiated using a 465 nm blue-light phototherapy device (MR4 ACTIVetPro, MultiRadiance Medical; Solon, OH, USA) at the following cumulative doses: 56.25, 112.5, and 225 J/cm² and incubated overnight at 35°C. Control plates (three each for MRSP, MRSA, MSSP) were not irradiated. Colonies were counted (CC) and aggregate colony areas (ACA) measured using ImageJ software at each dosage. Descriptive statistics were performed and effects of independent variables (treatment, dose) assessed using the Mann-Whitney-Wilcoxon rank-sum and Friedman’s tests in SPSS (v24, IBM). There was a significant decrease at all doses in CC and ACA for MRSA and ACA for MSSP following blue-light phototherapy (P <0.05). Significant treatment or dose differences for MRSP were not identified. These differences might be related to the porphyrin amount in bacteria, as porphyrins are required to absorb blue-light. Further studies are needed to evaluate the relevance of bacterial porphyrin content and efficacy of blue-light phototherapy for treatment of MSSP, MRSP and MRSA.

Source of funding: The MR4 ACTIVetPro device was donated by MultiRadiance Medical. The remainder of the study was self-funded.

Conflict of interest: None declared.
Pharmacokinetics and relative oral bioavailability of two oral amoxicillin–clavulanic acid formulations in healthy adult dogs – a preliminary investigation

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R. GEHRING†# and J. COETZEE†

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§Pharmacology Analytical Support Team (PhAST), College of Veterinary Medicine, Iowa State University, Ames, IA, USA
†Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA
#Institute of Computational Comparative Medicine, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA

Abstract: Clavamox® (Zoetis Inc., Kalamazoo, MI, USA) is a veterinary proprietary amoxicillin (A)–clavulanic acid (CA) formulation labeled for treating canine and feline pyoderma. Similar human generic (HG) A-CA formulations are available, although their use within veterinary medicine lacks supporting evidence. This study aimed to determine the relative oral bioavailability (ROB) of both active ingredients in HG compared to Clavamox®, thus evaluating the potential for HG substitution. Six healthy adult dogs were enrolled, weighing 35-50 kilograms for ease of tablet administration. In this randomized crossover study, dogs received a single oral dose (10-15 mg/kg based on published amoxicillin dosages) of either Clavamox® or a HG formulation (Aurobinda Pharma Ltd., Hyderabad, India) at two time points, following a two-week minimum washout period. Following administration, blood was collected for 24 h at fixed times to measure plasma A-CA concentrations using a liquid chromatography-mass spectrometry method in negative ion detection mode. Non-compartmental pharmacokinetic analysis was performed using Phoenix®WinNonLin® software (Certara USA, Inc., Princeton, NJ, USA). The average ROB of HG compared to Clavamox® was 98.17% for amoxicillin with a 90% confidence interval of 80.68%-114.40%. For clavulanic acid, the average ROB of HG compared to Clavamox® was 152.61% with a 90% confidence interval of 50.46%-189.88%. The rate of absorption was highly variable for both active ingredients and both formulations. There were no statistically significant differences between the pharmacokinetic parameters of either A-CA formulation. Due to unexpected variance, further pharmacokinetic/pharmacodynamic studies and modeling are needed to determine whether these formulations are clinically interchangeable.

Source of funding: This study was funded by the American College of Veterinary Dermatology Resident’s Research Award. Software license for Phoenix® was provided by Certara USA, Inc. (Princeton, NJ, USA) as part of their Centers of Excellence program.

Conflict of interest: None declared.
Pharmacokinetics and relative bioavailability of orally administered innovator-formulated itraconazole capsules and solution in healthy dogs

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*Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA
†Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Abstract: Itraconazole is a commonly used antifungal agent for treatment of systemic and cutaneous mycoses in veterinary medicine. Two oral formulations, capsule and solution, are used interchangeably in dogs. However, marked differences in bioavailability have been reported in humans and cats. As similar investigations have not been performed in dogs, the aim of this study was to determine and compare pharmacokinetics of itraconazole in dogs following administration of equivalent doses of capsules and solution. Eight healthy, adult dogs received itraconazole (Sporanox®; Janssen Pharmaceutica, Olen, Belgium) solution and capsule (approximately 10 mg/kg) PO in randomized crossover design with a 10 day washout period between treatments. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 48 h after itraconazole administration. Plasma drug concentrations were measured using high-pressure liquid chromatography, and concentration data were analyzed via non-compartmental analysis to determine pharmacokinetic parameters. Comparisons of parameters between groups were made using paired student’s t-tests. Dogs reached maximum drug concentrations (Tmax) quicker following solution administration as compared to capsule administration (P = 0.02), and although maximum drug concentrations (Cmax) trended higher following solution administration, this did not reach significance (P = 0.14). Area under the plasma concentration vs. time curves were nearly identical between groups. Contrary to findings reported in other species, overall drug exposures following capsule and solution administration are similar in dogs. Given these results dosage modifications do not appear to be necessary. This highlights the importance of basing treatment recommendations on species-specific pharmacokinetic data.

Source of funding: ACVD/AAVD Resident’s Research Award.

Conflict of interest: None declared.
Canine demodicosis: a retrospective study (2000-2016)

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*Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, Davis, CA USA

Abstract: This retrospective study aimed to determine the occurrence of demodicosis in a veterinary teaching hospital, document breed predispositions, concurrent diseases or underlying immunosuppressive etiologies and frequency of demodicosis recurrence. Inclusion in this study required a diagnosis of demodicosis and records for review. Surveys and telephone calls were used to obtain information about disease recurrence. Breed distributions and concurrent allergic dermatitis (AD) of affected dogs were compared to the hospital population and univariate/multivariate logistic regressions were performed. Between 2000 and 2016, 433 dogs with demodicosis were diagnosed (0.37% of the hospital population) comprised of 225 juvenile- and 139 adult-onset cases and 69 dogs that were between defined ages or had incomplete records. The pit bull terrier group and West Highland white terriers were predisposed to demodicosis. Allergic dermatitis was associated with higher odds (OR=10.11, 95% CI=7.12 to 14.36, P<0.001) of demodicosis compared to dogs without AD. This indicated either that AD or the use of immunosuppressive medications to manage AD could predispose dogs to develop demodicosis. Long term follow up was available for 191 dogs (44.3%). The majority, 167 dogs (87%) were cured of their demodicosis. Relapse (diagnosis of demodicosis within 12 months since resolution) occurred in two juvenile- and 11 adult-onset demodicosis cases, while recurrence (diagnosis of demodicosis > 12 months after initial resolution) occurred in two juvenile- and eight adult-onset demodicosis cases. There were two juvenile- and eight adult-onset cases with recurrence or relapse that required ongoing, long term therapy.

Source of funding: Self-funded.

Conflict of interest: None declared.
Afoxolaner and fluralaner treatment do not impact normal Demodex populations in healthy dogs

C. ZEWE*, L. ALTETŞ, A. LAM*, L. FERRER*

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§Vetgenomics, Parc de Recerca de la Universitat Autònoma de Barcelona, Bellaterra, Spain

Abstract: Oral isoxazoline compounds have been used off-label for treatment of demodicosis, with reported clinical and parasitological success. However, nothing is known regarding the effect of isoxazolines on the commensal Demodex populations in healthy dogs. In humans, Demodex mites have been shown to disappear from the hair follicle by 8 weeks following standard treatment. In this study, we evaluated the response of normal Demodex populations to fluralaner (Bravecto®, Merck Animal Health, Madison, NJ, USA) or afoxolaner (NexGard®, Merial, Duluth, GA, USA), given at the labeled dose, over 90-days. Our hypothesis was that treatment per label instructions would eliminate Demodex mites in the skin of healthy dogs at all time points, as measured by RT-PCR. Twenty dogs with no history of skin disease were divided into two groups of ten. Approximately 50 hairs were plucked from the face, left flank, and right hind paw on day 0 prior to administration of fluralaner or afoxolaner, and then again on days 30 and 90. RT-PCR was performed on the hairs using primers specific for a region of 18S rDNA of Demodex. Dogs in the afoxolaner group received dosing every 30 days; dogs in the fluralaner group were dosed once. Positive PCR was found in 5/20, 3/18, and 6/20 dogs on days 0, 30, and 90, respectively. Two samples were unable to be processed on day 30. The difference in Demodex DNA was not significantly different between timepoints (Chi square, P =0.08). Afoxolaner and fluralaner did not significantly decrease the normal Demodex populations in dogs.

Source of funding: Self-funded. The products used in the study were donated by Merck Animal Health (Madison, NJ) and Merial (Duluth, GA).

Conflict of interest: None declared.

Afoxolaner and fluralaner treatment do not impact normal Demodex populations in healthy dogs

C. ZEWE*, L. ALTETŞ, A. LAM*, L. FERRER*

*Cummings Veterinary Medical Center, Tufts University, North Grafton, MA, USA
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Abstract: Oral isoxazoline compounds have been used off-label for treatment of demodicosis, with reported clinical and parasitological success. However, nothing is known regarding the effect of isoxazolines on the commensal Demodex populations in healthy dogs. In humans, Demodex mites have been shown to disappear from the hair follicle by 8 weeks following standard treatment. In this study, we evaluated the response of normal Demodex populations to fluralaner (Bravecto®, Merck Animal Health, Madison, NJ, USA) or afoxolaner (NexGard®, Merial, Duluth, GA, USA), given at the labeled dose, over 90-days. Our hypothesis was that treatment per label instructions would eliminate Demodex mites in the skin of healthy dogs at all time points, as measured by RT-PCR. Twenty dogs with no history of skin disease were divided into two groups of ten. Approximately 50 hairs were plucked from the face, left flank, and right hind paw on day 0 prior to administration of fluralaner or afoxolaner, and then again on days 30 and 90. RT-PCR was performed on the hairs using primers specific for a region of 18S rDNA of Demodex. Dogs in the afoxolaner group received dosing every 30 days; dogs in the fluralaner group were dosed once. Positive PCR was found in 5/20, 3/18, and 6/20 dogs on days 0, 30, and 90, respectively. Two samples were unable to be processed on day 30. The difference in Demodex DNA was not significantly different between timepoints (Chi square, P =0.08). Afoxolaner and fluralaner did not significantly decrease the normal Demodex populations in dogs.

Source of funding: Self-funded. The products used in the study were donated by Merck Animal Health (Madison, NJ) and Merial (Duluth, GA).

Conflict of interest: None declared.
A double-blinded, randomized, controlled, cross over evaluation of a zinc methionine supplement as adjunctive treatment for canine atopic dermatitis

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†Zinpro Performance Minerals, Eden Prairie, MN, USA

Abstract: Zinc is important for skin health and proper immune system function, with absorption facilitated by essential fatty acids. Potential benefits of zinc supplementation in canine atopic dermatitis (CAD) are unknown. We evaluated a zinc methionine, biotin, essential fatty acid product (TruCare Essentials [TE]; Zinpro, Eden Prairie, MN, USA) compared to a biotin and essential fatty acid product (control; Zinpro, Eden Prairie, MN, USA) in CAD. Twenty-seven client-owned dogs with chronic CAD receiving ciclosporin or glucocorticoids enrolled in a 24-week, randomized, double-blinded study with crossover at week 12 and allergy medication reduction at weeks 8 and 20. Assessments included Canine Atopic Dermatitis Lesion Index (CADLI), pruritus Visual Analog Scale (VAS) and cytologies. In dogs receiving TE and ciclosporin for 8 weeks, 44% (n=7) had significantly decreased CADLI from 11.9 to 6.0 (P=0.0002) with no significant change in pruritus VAS (P =1.0). In dogs receiving TE and glucocorticoids for 8 weeks, 55% (n=6) had significantly decreased CADLI from 10.9 to 5.0 (P=0.0043) and significant reduction in pruritus VAS from 7.4 to 3.2 (P=0.0166). There was no significant difference between TE versus control, 63% versus 37%, respectively, to permit a reduction in allergy medication (± 0.12%, P=0.1027). Two dogs could discontinue glucocorticoids for at least 4 weeks while receiving TE. Seventy-five percent of dogs were treated for superficial skin infections during the study. These results support a potential benefit of adjunctive zinc methionine supplementation in CAD. Dogs receiving glucocorticoids may be more likely to benefit from zinc supplementation.

Source of funding: Zinpro Performance Minerals, Eden Prairie, MN, USA.

Conflicts of interest: D. Tomlinson is an employee of Zinpro Performance Minerals.
Pilot evaluation of the efficacy to a topical transient receptor potential M8 (TRPM8) agonist for treatment of pruritic atopic pododermatitis in dogs

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*Department of Clinical Sciences, College of Veterinary Medicine, NC State University, Raleigh, NC, USA
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Abstract: Atopic dermatitis often requires multimodal treatment, and there is a need to find effective adjunctive therapies without adverse effects. TRPM8 agonists alleviate pruritus by inducing a cooling sensation. Our objective was to evaluate the efficacy of a novel TRPM8 agonist, 1-diisopropylphosphorylnonane (Cryosim-1) to relieve pruritus in dogs with atopic pododermatitis. This prospective, randomized, double-blinded, intra-individual, placebo-controlled study enrolled nine dogs with moderate-to-severe pedal pruritus associated with nonseasonal atopic dermatitis. A 2% Cryosim-1 or vehicle placebo cream was applied to each assigned forepaw twice daily for seven days. At study end, the number of dogs with a pedal pruritus score (pedal PVAS) level of < 2.0 for Cryosim-1 and placebo was 3/9 and 5/9, respectively; the owner’s global assessment of efficacy score of 3 or 4 (good-to-excellent) for Cryosim-1 and placebo was 2/8 and 5/8, respectively – these proportions were not significant between treatment groups (P = 0.17 and 0.31, respectively). Similarly, there was no significant difference between Cryosim-1 and placebo in the median percentage change from baseline (47% versus 75%; P = 0.15), in the number of dogs with a ≥ 50% reduction from baseline pedal PVAS (4/9 versus 5/9; P = 0.50), or in the number of dogs with ≥ 90% PVAS reduction (2/9 versus 2/9; P = 0.72). In this first pilot trial evaluating pruritus reduction with a TRPM8 agonist in atopic dogs, the twice-daily application of a 2% Cryosim-1 cream did not appear to have an antipruritic effect superior to that of its vehicle.

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Conflict of interest: None declared.
Determination of threshold concentrations of plant pollens in intradermal testing using fluorescein in clinically healthy nonallergic cats

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Abstract: Currently the same allergen concentrations for canine intradermal testing (IDT) are recommended for feline IDT. Skin reactions in cats can be subtle and more difficult to interpret than canine reactions. This difference could be due to suboptimal allergen concentrations used for IDT in cats. The aim of this study was to determine the irritant threshold concentration (ITC) of 16 pollen allergens using serial dilutions of allergen. The hypothesis tested was that feline IDT is currently performed using suboptimal allergen concentrations for pollens. Twenty privately owned, clinically non-allergic cats were enrolled in this study. IDT was performed in duplicate using 16 allergens (weed, grass and tree pollen) at a concentration of 8000 PNU/ml. Two blinded investigators independently graded the test reactions using subjective and objective criteria. Intravenous fluorescein was then administered and the test reactions were re-evaluated. IDT was repeated with any allergen that was positive using serial dilutions of allergen at concentrations of 6000 and 4000 PNU/ml. The ITC for 2/16 of the allergens was determined. The ITC of Bermuda grass (Cynodon dactylon) and Peppercorn (Schinus spp.) was determined to be between 6000 and 8000 PNU/ml. The ITC of all other allergens tested in this study was greater than 8000 PNU/ml. This study confirms that suboptimal allergen concentrations are used for feline IDT as the ITC is >8000 PNU/ml for 14/16 grass, weed and tree pollens tested. The ITC of Bermuda grass and Peppercorn was determined to be between 6000 and 8000 PNU/ml.

Source of funding: Self-funded.

Conflict of interest: Craig Griffin received a grant to publish chapters in the publication *Greer Allergy Immunotherapy Compendium*; he participates in a scientific committee for major allergen research sponsored by ALK, a pharmaceutical company that develops and sells allergy immunotherapy products.
Identification of irritant threshold concentrations for percutaneous allergen testing in 20 non-sedated dogs

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Abstract: Percutaneous testing is preferred to intradermal testing in humans for the in vivo identification of allergen hypersensitivity but the methodology has not been well described for use in dogs with atopic dermatitis. The objective of this study was to identify the irritant threshold concentrations (ITC) of eight aeroallergens using a commercially-available human prick test device in normal non-atopic dogs. Percutaneous testing was performed on the medial thighs of 20 non-sedated normal, non-allergic dogs using the GREER® Pick® System (Stallergenes Greer™, Lenoir, NC, USA). Five serial dilutions of glycerinated extracts of *Bromisinermis*, *Sorghum halepense*, *Chenopodium album*, *Ambrosia psilostachya*, *Salix nigra* and *Acer negundo*, as well as four dilutions of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were included. Glycerinated histamine (6 mg/mL) and glycerin/cocas solution were used for the positive and negative controls, respectively. Orthogonal wheal diameters were measured for each test site every 5 min for 25 min. Reactions were considered irritants when the average wheal diameter was equal to or greater than the mean of the positive and negative controls. Reactions to at least one allergen were noted in 5/20 (25%) of dogs. The ITC (≤ 10% of dogs reacting) were 1:20 w/v for *Bromisinermis* and *Salix nigra*, 1:400 w/v for *D. farinae* and 1:200 w/v for *D. pteronyssinus*. The ITC was greater than the most concentrated extract for the other four allergens. This study identified the ITC of four glycerinated allergenic extracts for percutaneous testing with the GREER® Pick® System in non-sedated dogs.

Source of funding: Partially funded by Stallergenes Greer™ and SkinVet® Clinic.

Conflict of interest: None declared.
Determination of irritant threshold concentrations of nine allergens from two different manufacturers in clinically non-allergic dogs

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Abstract: Success of allergen-specific immunotherapy (ASIT) is dependent on the accuracy of allergy testing and optimal testing concentrations to detect allergen specific IgE. The aim of this study was to determine the irritant threshold concentration (ITC) of nine allergens from two different manufacturers. A prospective, blinded study was performed in duplicate. Twenty client owned clinically non-allergic dogs were sedated with dexmedetomidine (Dexdomitor®, Zoetis, Kalamazoo, MI, USA) and had standard intradermal testing performed. Saline and histamine (0.025 mg/mL) were used as negative and positive controls. Alternaria, Bermuda grass, cat dander, cocklebur, Dermatophagoides farinae, lamb’s quarter, mesquite, Timothy grass and white mulberry from two manufacturers (GREER® Laboratories, Lenoir, South Carolina, USA and ALK, Round Rock, TX, USA) were injected intradermally at manufactures recommended testing concentrations and evaluated at 15 and 30 minutes subjectively (0-4) and objectively (horizontal wheal diameter) by two blinded investigators. A higher concentration of each allergen was administered at the same time. A subjective score of 3-4+ by either investigator at either timed reading was considered positive. If both initial and higher concentration reactions were positive, then a series of dilutions were performed. The ITC was defined as the lowest tested concentration that ≥10% of the total animals reacted positive. The following were the results of the ITCs: Alternaria>2,000 PNU/mL, Bermuda <10,000 PNU/mL (ALK) and <6,000 PNU/mL (Greer), cat dander <750 PNU/mL (ALK) and 2,000 PNU/mL (Greer), cocklebur <6,000 PNU/mL, D. farinae<1:10,000 w/v, lamb’s quarter <6,000 PNU/mL, mesquite <500 PNU/mL, Timothy <6,000 PNU/mL and white mulberry <6,000 PNU/mL.

Source of funding: This study was supported by a grant from the American College of Veterinary Dermatology.

Conflict of interest: Antigens from ALK were provided at no charge.
Dorsal thermal necrosis in dogs: a retrospective analysis of 15 cases (2009-2016)

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Abstract: Prolonged sun exposure combined with high ambient temperatures has been recognized as a cause of thermal burns of the dorsal skin of dogs. This type of thermal injury has been termed dorsal thermal necrosis (DTN). The objectives of this retrospective study were to characterize the clinical presentation, histopathological findings, and outcomes of dogs diagnosed with DTN and to identify associated risk factors. Medical records of 15 dogs with dorsal burn injuries, historical protracted sun exposure and histopathological findings consistent with DTN were reviewed. The majority of reported cases (14/15) occurred during warmer months (May-September) and in the southwestern USA. Affected dogs had predominantly dark coat colors and short hair. Five dogs had naturally longer hair coats, but two of these had been recently clipped. Signs consistent with heatstroke were reported two to ten days prior to the development of cutaneous lesions in 4/15 dogs. The most common skin lesions were erythema, ulcerations, alopecia, eschars, and crusts. Histological findings were consistent with other types of thermal burns and included partial to full-thickness necrosis in the majority of cases (13/15). Most dogs were treated with supportive care, including analgesics and antimicrobials. The majority of DTN wounds healed via second intention, although surgery was performed in two dogs. Based on these findings, dorsal thermal necrosis should be considered as a differential for dogs with dorsal cutaneous burns and a history of sun exposure in high external temperatures. Dogs with dark, short hair coats may be at an increased risk.

Source of funding: Self-funded.

Conflict of interest: None declared.
Evaluation of clinical accuracy of serological and salivary testing for food allergens in asymptomatic dogs

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Abstract: Numerous tests purport to measure saliva or serum immunoglobulin levels specific to various foods for evaluation of adverse food reactions (AFR) in companion animals. Despite their widespread use, no validation exists on their utility in diagnosing AFR. The objective of this study was to test dogs without historical or active clinical signs of either dermatologic or gastrointestinal manifestations of AFR with two commonly used commercial serological assays (A and B) and one saliva assay (C). We hypothesized that 1) assays would yield positive results despite lack of clinical disease, and 2) positive results would correlate with prior food exposure. Thorough medical and diet histories were obtained from 30 asymptomatic dogs from the hospital population. The dogs ranged from one to 10 years of age (median = 4 years) and weighed between 2.2 and 50.8 kg (median = 20 kg). Fourteen foods common to all three assays were evaluated. Results were classified into positive or negative responses to each food. All 30 asymptomatic dogs had at least one positive response to a food. One or more dogs tested positive to 14/14 (100%), 12/14 (86%), and 14/14 (100%) foods in assay A, B, and C, respectively. There was no predictable concordance between positive responses and historical food exposure. The results suggest that serologic and saliva test results do not correspond to clinical evidence of AFR and overdiagnosis of AFR is likely if these tests are used in lieu of a strict elimination diet trial.

Source of funding: This study was funded by a grant from Hill's Pet Nutrition, Inc., Topeka, KS, USA.

Conflict of interest: None declared.
A retrospective study to assess anti-pruritic efficacy of lokivetmab in dogs with canine atopic dermatitis

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Abstract: Lokivetmab (CYTOPOINT™; Zoetis, Inc., Kalamazoo, MI, USA) is a caninized monoclonal antibody designed to target and neutralize cytokine IL-31, a T-cell derived cytokine known to induce severe pruritus in dogs, mice, and humans. The purpose of this retrospective study was to evaluate the efficacy of lokivetmab in controlling pruritus in atopic dogs and compare various clinical parameters of dogs that responded to treatment with lokivetmab to those that did not. A review of Tufts University’s medical records between October 2015 and October 2016 identified 138 cases of dogs diagnosed with atopic dermatitis treated with lokivetmab at a dose range of 1.14-4.68mg/kg [median dose 2.56 mg/kg]. Dogs were excluded if other anti-inflammatory or anti-pruritic medications were used during treatment with lokivetmab. Dogs on consistent therapy prior to initiating treatment with lokivetmab were permitted. Pruritus was evaluated by owners using a subjective pruritus score from 0-10. A reduction in pruritus higher than 50% along the 7 days after treatment was considered a positive response. One hundred and one dogs (73%) responded to treatment and 37 (27%) were considered non-responders. No side effects attributed to treatment were recorded. Both groups were statistically identical with regards to mean age (6.45 versus 6.51 years) and sex and breed distribution. Responders received a mean of 4.6 injections and non-responders 1.24. Mean time between injections in the group of responders was 34 days. A decrease in efficacy of treatment was not observed in a group of 32 dogs that received six or more injections.

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Conflict of interest: None declared.
Lokivetmab in the control of pruritus in 135 dogs with allergic dermatitis

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Abstract: Lokivetmab (CytopointTM; Zoetis Inc., Kalamazoo, MI, USA) was administered to 135 allergic dogs from November 2015 through October 2016 at a targeted dose of 2mg/kg, subcutaneously. Following initial administration, client assessed pruritus control was achieved in 116/132 (87.8%). Three dogs were lost to follow-up. Good clinical improvement was noted within 24 hours of the first injection in 38/68 dogs (55.9%), between 1 and 3 days in 27 (39.7%), and ≥ 3 days in 3 (4.4%). Numbers of injections administered were one in 41 dogs, two (n=25), three (n=16), four (n=8), five (n=11), six (n=5), seven (n=7), eight (n=2), nine (n=12), 10 (n=2), 11 (n=2) and 12 (n=4). Frequencies of administration required for pruritus control included every (q) 21 days (n=6), q22-27 days (n=4), q28 days (n=26), q29-34 days (n=6), q35 days (n=4), q42 days (n=6) and q49 days (n=3). In 18/26 dogs, lokivetmab was administered q4 weeks regardless of pruritus status. For 53 dogs, the frequency of required administration varied by 2 weeks in 15 dogs, 3 weeks (n=7), 4 weeks (n=6), 5 weeks (n=3), 6 weeks (n=1), 8 weeks (n=2), 9 weeks (n=1), 13 weeks (n=1), 16 weeks (n=1), 17 weeks (n=1), 20 weeks (n=1), 21 weeks (n=1) and 31 weeks (n=1). Adverse events reported in 11/132 (8.3%) dogs included: lethargy (8), vomiting (2), hyperexcitability (1), painful reaction at the injection site (1) and urinary incontinence (1). Fifty-five (84.6%) of sixty-five dogs that failed oclacitinib therapy at labeled dosages, had a good response to lokivetmab.

Source of funding: Self-funded.

Conflict of interest: None declared.
A retrospective study comparing the incidence of cutaneous histiocytoma development in atopic dogs treated with oclacitinib and ciclosporin

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Abstract: Oclacitinib (Apoquel, Zoetis, Inc, Florham Park, NJ, USA) is a Janus kinase inhibitor licensed for the treatment of allergic dermatitis in dogs. Clinical trials have demonstrated a high margin of safety with few adverse reactions. One of these reactions reported is development of benign skin tumors, especially cutaneous histiocytomas, although a causal relationship has not been established. The objective of this retrospective study was to report and compare the incidence of cutaneous histiocytoma development in confirmed atopic dogs treated with oclacitinib versus ciclosporin (Atopica, Elanco USA Inc., Greenfield, IN, USA). A review of Tufts University’s medical records between 2013 and 2016 identified dogs with a diagnosis of atopic dermatitis treated with oclacitinib (n=533) or ciclosporin (n=654). The signalment, diagnosis, treatment, dose, duration of therapy, location of lesion, and remission information were recorded. There were 14/533 and 4/654 patients who developed histiocytomas while on oclacitinib and ciclosporin, respectively. There was a significantly higher percentage of dogs with histiocytomas on oclacitinib (2.6%) versus ciclosporin (0.6%) (P=0.0041). The mean age of dogs with histiocytomas on oclacitinib (mean=7.0 years) was significantly higher than the dogs on ciclosporin (mean=1.5 years) (P=0.0002). Also, there was a significant difference in duration of treatment between dogs with histiocytomas on oclacitinib (mean=14.8 weeks) versus on ciclosporin (mean=4.8 weeks) (P=0.018). The results of this study demonstrate that histiocytoma development may be higher in patients treated with oclacitinib compared to those treated with ciclosporin. Additional research is needed to determine a causal relationship and pathomechanisms between oclacitinib and cutaneous histiocytomas.

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Conflicts of interest: None declared.
Leishmania spp. in perianal adenoma in a dog: case report

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Abstract: An 11-year-old, intact male mixed breed dog presented with a 1-month history of a 2 cm2 perianal nodule. The nodule had a rubbery consistency and tan coloration. The dog was clinically healthy with moderately enlarged popliteal lymph nodes. Complete blood count and serum chemistry analysis were normal. Cytological examination of the perianal nodule revealed clusters of cells with abundant polygonal eosinophilic cytoplasm, and ovoid nuclei centrally located resembling hepatocytes, along with few lymphocytes. Unexpectedly intramacrophagic bodies compatible with Leishmania amastigotes were observed. Cytology from a popliteal lymph node showed reactive hyperplasia and many intracellular/extracellular amastigotes. Serum protein electrophoresis was within reference range and low antibody levels to Leishmania with indirect immunofluorescence were detected. The nodule was excised. A perianal gland adenoma with amastigotes consistent with Leishmania spp was diagnosed histopathologically with hematoxylin and eosin. Giemsa staining accentuated the amastigotes. Treatment included meglumine antimoniate (Glucantime; Merial Italia Spa, Milano, Italy) administered subcutaneously at 100 mg/kg, once daily for 4 weeks in combination with allopurinol (Allopurinolo Teva; Teva Italia Srl, Milano, Italy) administered orally at 10 mg/kg twice daily for 6 months. At the time of allopurinol discontinuation the dog was clinically normal and had unaltered serum protein electrophoresis. To the authors’ knowledge, the presence of intracellular amastigotes of Leishmania spp. in a canine perianal adenoma has not been described to date. Interestingly, the presence of Leishmania amastigotes in a perianal squamous cell carcinoma in a human immunodeficiency virus infection-infected man has been reported in the literature.

Source of funding: Self-funded.

Conflict of interest: None declared.
Successful treatment of a postsurgical wound-healing disorder in the oral cavity of a Thai water dragon with cold atmospheric pressure argon plasma therapy

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Abstract: A female, seven-year-old Thai water dragon (Physignathuscocincinus, CUvier 1829) was presented with a nodular lump on the left pharyngeal side of the oral cavity, which was excised by an experienced reptile surgeon and histologically diagnosed as a benign salivary gland adenoma. Surgery was performed without any complications. The water dragon re-presented after seven days with an ulcerated, swollen and erythematous lesion in the left oral cavity at the site of excision. During the next three weeks, systemic marbofloxacin (Marbocyl® 2%, Vetoquinol, Magny-Vernois, France) at a dosage of 4 mg/kg/d, daily topical manuka honey (Kruuse Manuka G, 100% Leptospermum scoparium, Albrecht GmbH / Dechra, Aulendorf, Germany), semi-daily topical disinfection with iodine solution (Braunol®, PVP iodine solution, diluted by 1:25, Braun Melsungen AG, Melsungen, Germany) and daily zinc ointment (Lebertran-Zinksalbe vet., Pharmamedico GmbH, Twistringen, Germany) did not lead to significant improvement. Upon presentation, cytology of the swollen, ulcerated mouth lesion revealed a severe neutrophilic inflammation with a moderate number of extracellular, rod-shaped bacteria, which were considered normal oral flora. Cold atmospheric pressure argon plasma therapy (kinPen MED®, Leibniz Institute for Plasma Science and Technology, INP Greifswald neoplas tools GmbH, Greifswald, Germany) was initiated twice weekly for three consecutive weeks together with daily topical disinfection with the iodine solution. Within seven days, marked improvement was noted and after two weeks, re-epithelialization had occurred; complete remission was obtained by day 18. Nonthermal, ionized plasma therapy may offer a viable, painless and well-tolerated treatment option for wound healing disorders in lizards.

Source of funding: The cold-plasma jet device (kinPen MED®) was provided by the Leibniz Institute for Plasma Science and Technology, INP Greifswald neoplas tools GmbH, Greifswald, Germany. neoplas tools GmbH had no influence on the patient selection or treatment.

Conflict of interest: None declared.
Efficacy of oral fluralaner (Bravecto®) for the treatment of generalized demodicosis in dogs from Bangkok, Thailand

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Abstract: Generalized demodicosis in dogs has been considered a challenging skin condition to manage, especially in adult-onset cases, which are often associated with underlying diseases. This study evaluated the efficacy of oral fluralaner (Bravecto®, Merck, Kenilworth, NJ, USA.) at the dose of 25-50 mg/kg every 12 weeks for the treatment of canine generalized demodicosis. Client-owned dogs diagnosed with generalized demodicosis according to published criteria were included. Skin scrapings or trichograms were performed at three to five affected areas to evaluate numbers of mites. Rechecks were performed monthly until parasitological cure, then dogs were followed up in one year. Parasitological cure was considered when the dogs had two negative skin scrapings one month apart. One hundred and fifteen dogs were included in the study, 73 dogs with adult-onset demodicosis and 42 dogs with juvenile-onset demodicosis. Twenty-one dogs were lost to follow up and 27 dogs had one negative skin scraping and did not return thereafter. Sixty-seven dogs (21 with juvenile-onset and 46 with adult-onset) had parasitological cure, which occurred in 2, 3 and 4 months in 63%, 85% and 100% of dogs with adult-onset demodicosis, respectively, and in 2 month and 3 months in 81% and 100% of dogs with juvenile-onset demodicosis, respectively. Underlying causes associated with adult-onset demodicosis included atopic dermatitis, neoplasia, metabolic diseases and idiopathic. No adverse effects were observed in any dogs. In conclusion, fluralaner given at the recommended dose for flea and tick prevention can be considered for the treatment of canine generalized demodicosis.

Source of funding: This study was self-funded.

Conflict of interest: No conflicts of interest have been declared.
Evaluation of positive and negative controls and applicator devices for percutaneous allergy testing in the horse – a pilot study

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Abstract: Equine allergies are common, with identification of causative allergens currently performed via serologic or intradermal allergy testing (IDT). Percutaneous testing (PCT) is the standard technique used to identify allergens in people and correlates better with provocation challenges. The study aim was to evaluate commercially available PCT applicators and compare IDT and PCT responses in 10 healthy adult horses to controls. IDT was performed with 0.0275 mg/mL aqueous histamine and 0.9% phenol buffered saline as the positive and negative control, respectively. PCT was completed utilizing 0.275 and 0.0275 mg/mL aqueous histamine, 6 and 1 mg/mL glycerinated histamine, 0.9% buffered saline, and 50% glycerosaline. Solutions were applied with the GREER® Pick® (GREER®, Lenoir, NC, USA), UniTest® PC (Lincoln Diagnostics, Decatur, IL, USA), and Duotip-Test® II (Lincoln Diagnostics, Decatur, IL, USA). All tests were performed in triplicate and reactions were scored objectively and subjectively at 10, 20 and 30 min. PCT testing with glycerosaline produced significantly smaller reactions than IDT negative controls at all time points and no difference was observed between PCT methods. When compared to the IDT positive control, glycerinated histamine (6mg/mL) was the only PCT solution to produce statistically similar objective results, which occurred with 2/3 devices (UniTest® PC and GREER® Pick®) at 20 min and all three at 30 min. This investigation suggests further studies are warranted utilizing the UniTest® PC or GREER® Pick® system to evaluate PCT in the horse as an allergy testing method using 6mg/mL glycerinated histamine and 50% glycerosaline as positive and negative controls, respectively.

Source of funding: GREER® and Lincoln Diagnostics provided PCT systems. GREER® provided positive and negative control solutions.

Conflict of interest: None declared.
SKIN MICROBIOME – THE LITTLE WE KNOW ABOUT THIS FASCINATING MICROSCOPE WORLD
Sheila Torres, DVM, PhD, DACVD

What is the microbiota or microbiome?
The **microbiota** is the **collection of microorganisms** living in every environmental niche in addition to the body of all multicellular creatures including vertebrates, invertebrates and plants. These communities of microorganisms live in close association with their hosts and include bacteria, archaea, viruses, fungi, and protists. The **microbiome** is defined as the **collection of genes** from these microorganisms. In this manuscript, we will use the word microbiome when referring to either, the collection of microorganisms or their genes.

They are a multitude
It is estimated that for each human cell there are 10 microbial cells and for each human gene there are 100 microbial genes. This accounts for trillions of microorganisms living in and on the human body. It is reasonable to assume that this estimate can be extrapolated to other multicellular organisms. It is easy to understand that this multitude of microbes living in and on organisms through commensalism or mutualism relationship influence their hosts in various ways.

A little on the history of microbe and microbiology
The co-existence of microbes and humans or other hosts, is certainly primordial. In the mid-seventeenth century, a Dutch man named Anton van Leeuwenhoek was the first person to see microbes! Using the microscope he invented, Leeuwenhoek observed these moving creatures (which he called “animalcules”) in various sources of water and in whitish plaques he removed from his teeth. Thus, he was the first person to discover that a community of microbes – the microbiota or microbiome- lives in and on us! More than one-hundred years passed until microbiologists became interested in microbes again with names such as Marcus Plenciz, Louis Pasteur, Robert Koch, Theodor Escherich becoming well-known in the nineteenth century and still to this date. These microbiologists viewed microbes as agents of diseases thus, “villains”. In the 1800s other microbiologists such as Martinus Beijerinck (microbial ecologist), Arthur Isaac Kendall (pioneer in the study of gut bacteria), Elie Metchnikoff (assumed that microbe in sour milk prolonged people’s life), Theodor Rosebury (wrote the book “Microorganisms Indigenous to Man”), believed that microbes lived in environmental niches and in humans and animals bodies in various partnership modes that they called symbiosis. In 1965, a French-borne American microbiologist named Rene Dubos wrote: “Several kinds of microbes play an essential role in the development and physiological activities of normal animals and man”. Thus, the knowledge that a multitude of microbes are an integral and influential part of the world we live in is not that recent.

The technology then and now
Up until the late twentieth century, microbiologists used culture and biochemical tests to isolate, identify and characterize microorganisms, especially bacteria. This technology unquestionably helped advance the field but, unintentionally, favored the microbes that could grow in laboratory conditions which are estimated to correspond to the very small fraction of 1% to 9% of the totality. In the late 1970’s the culture-independent-sequencing-based methods started to replace the traditional culture-based technology. The American microbiologist, Carl Woese was the first to start sequencing the 16S rRNA gene which is ubiquitous to all bacteria. In 1977 using this methodology he published a paper showing that “methanogens” (i.e. microbes that convert carbon dioxide and hydrogen into methane) were not bacteria and named this new prokaryote kingdom as archaea. His seminal work paved the way for other scientists to explore the microbial world comprehensively using the less biased sequencing-based methods which were later named metagenomics (i.e. the genomics of communities). The next-generation sequencing-based methods have indeed revolutionized microbiology and are here to stay!

A brief review on the methods currently available
**Targeted amplicon methods** – in these methods a primer that represents a gene present in all organisms of the same kingdom is used. For example, the conserved 16S ribosomal RNA (rRNA) gene is used to survey bacteria present in various samples and the 18S rRNA gene or the ITS (Internal Transcribed Spacer) gene are used to investigate fungi.
These conserved microbial genes contain hypervariable regions that allow for their identification at genus or species level. This method is still widely used but, it will provide an inventory of specific microbial kingdoms only and not the whole microbial community present in a sample. Moreover, these methods do not provide information on the function of the surveyed microbes or if they are dead or alive.

Whole genome metagenomics (WGM) or whole metagenome shotgun approach – this method allows surveying all microbial communities present in a sample (e.g. bacteria, fungi, viruses etc.) by fragmenting and sequencing DNA present in the sample. It also provides information on potential microbial functions and allows for more accurate identification of the taxa at the species level. This method requires more DNA biomass which can be a challenge if skin and respiratory tract are the desired site. Moreover, the WGM is more expensive than the targeted amplicon approach and the data more laborious to analyze.

Metatranscriptomics (i.e. shotgun sequencing of reverse-transcribed RNA), metaproteomics (i.e. quantification of protein or peptide levels), metabolomics (i.e. investigation of small-molecule metabolites): These methods are not yet widely available but, provide information on the function of the microbial communities.

Challenges in studying the microbiome
One of the challenges faced by microbiome researchers is collecting and extracting enough microbial DNA from samples. This is primarily a problem if low biomass sites such as the skin and respiratory tract are the areas of research interest. However, the biggest challenges are the various factors that impact the microbiome and are difficult if not impossible to control such as genetic, biological, cultural and environmental to name a few. Some examples include: age, gender, race, hygiene habits, life style, immune status, stress, time, diet, cohabitation etc.

The lack of standardization in the methods used to research the microbiome and the potential for contamination of samples or reagents can impact the study outcome and make it challenging to compare study results, to mention the least. Skin can be sampled by swabbing, swabbing and scraping or taking a biopsy; each method will result in slightly different microbial profile. It is important to use the same sample collection method throughout the study. The method used for DNA extraction can also influence the study results. For example, gram-positive bacteria are more difficult to lyse than gram-negative bacteria. If Staphylococcus spp are important components of a community being investigated, it will be important to use measures to disrupt these organisms cell wall including bead beating in addition to detergents or enzymes. Inadequate lysis may misrepresent the taxonomic profile of the microbial community. The 16S rRNA hypervariable region selected will also impact the study outcome. A recent paper showed that the V1-V3 regions should be used instead of the V4 region when surveying bacterial communities of the skin. In this study, the V4 region underrepresented Propionibacterium acne and Staphylococcus aureus present in the samples but the relative abundance of these bacteria using the V1-V3 regions approached the amount present in the mock sample. The authors recommend using the V1-V3 regions when surveying the bacterial skin microbiome. Another important aspect to consider is the potential for contamination especially when studying low-biomass sites such as the skin. Reagents have been shown to be contaminated which can significantly impact the results. In these cases the following is important: (i) include appropriate controls that account for reagent contamination; (ii) purchase high-quality, ultrapure, DNA-free reagents; (iv) treat equipment and reagents with UV; and (v) perform all experiments in a hood. Methods studies have been published more frequently lately and are already guiding investigators on how to perform microbiome research more accurately.

Selected human skin microbiome studies

IN HEALTH

Bacterial microbiome
Skin microenvironment matters:
Initial studies investigating the bacterial microbiota living on various body sites of healthy human skin determined that the communities are quite diverse (expectedly) and four phyla predominate: Actinobacteria, Firmicutes, Proteobacteria and Bacteriodetes. Investigators also found that bacterial communities differ among and within individuals.
In contrast to dogs and cats, the skin of humans can be divided into three microenvironments: sebaceous, moist and dry. Various studies have shown that these microenvironments shape the composition and structure of bacterial communities. The relative abundance of *Propionibacterium* spp. is higher in sebaceous areas whereas *Corynebacterium* spp. and *Staphylococcus* spp. generally predominate in moist microenvironments and beta-proteobacteria more or less dominate dry regions. Moreover, bacterial diversity is higher in dry and lower in sebaceous skin environments but, relatively more stable over time in sebaceous compared to moist and dry sites. Interestingly, studies have shown that the predominant bacterial taxa are much more similar in the same body site of two individuals than between body sites with different microenvironments in the same individual. These studies show the strong influence of skin microenvironment in the composition of bacterial communities.

**Mode of birth delivery matters:**
A study looked at the very first bacterial communities that inhabit the skin of newborns delivered by cesarean or birth canal. Ten mothers and babies were included in the study. Four babies were born via the birth canal and six via cesarean. Samples were collected from the skin, oral mucosa and vagina of the mothers 1 hour before delivery and, from the skin, oral mucosa, nasopharyngeal aspirate and meconium of the babies less than 24 hours after delivery. The authors report two interesting findings: (i) in contrast to their mothers where the bacterial communities were highly different across the sampled body regions, the communities of neonates were very similar across the various sampled habitats independent of the mode of delivery; (ii) neonates born via birth canal acquired bacterial communities resembling their mothers' vagina and neonates born via cesarean acquired communities similar to those found on the mothers' skin surface.

**Age matters:**
A study looked at the skin bacterial communities of 31 healthy infants from 3 to 52 weeks of age. Samples were collected from the forehead, lower volar forearm and buttock and the infants were equally distributed among three age groups (1-3, 4-6 and 7-12 months). *Streptococcus* and *Staphylococcus* species accounted for about 40% of the bacteria in the two youngest age groups (1-3 and 4-6 months) and 23 other genera composed the remaining population. By the age of 7 to 12 months the abundance of *Staphylococcus* and *Streptococcus* reduced and gave space to additional genera increasing the overall diversity of the skin microbiome of this age group. The predominance of *Streptococcus* and *Staphylococcus* species, which are typically found in moist areas of adult skin, can be explained by the relatively better hydrated stratum corneum of infants compared to adults.

Another study looked at the influence of age in the skin microbiota. The authors recruited 28 healthy individuals and using Tanner stages (i.e. standardized measurement of assessing pubertal development based on physiological characteristics), two groups were formed: one group (pre-puberty) had individuals between 2 and 13 years old and the other group (puberty) had individuals between 14 and 40 years old. Samples were collected from the nares, antecubital and popliteal fossae and volar forearm. The phylum Actinobacteria which includes the genus *Corynebacterium* and *Propionibacterium* predominated in the older group (puberty) where Firmicutes that houses *Staphylococcus* and *Streptococcus* were proportionally in much higher amounts in the pre-puberty group. Proteobacteria were also well represented in the pre-puberty group. The predominance of Actinobacteria in the older group could be explained by maturation of sebaceous glands that occurs during puberty.

**The skin microbiome is shared easily:**
A study investigated the effect of co-habitation on the microbiota composition of healthy people and their dogs. The authors included in the study households with various co-habitation scenarios as follows: 8 households with the couple, their children and one or more dogs; 18 households with solely the couple, 17 households with the couple and their children and 17 households with the couple and one or more dogs. The authors analyzed various parameters in the study but, two findings are worth mentioning: (i) the microbiome of couples who cohabit with dogs and/or children is more similar compared to the microbiome of couples who live by themselves; (ii) having a dog added bacterial diversity to the skin of adults. The authors concluded that a shared microbial source (e.g. dogs, each other, home surfaces etc.) may homogenize skin bacterial communities because bacteria are easily transferred to various surfaces. This can also explain the impact of co-habitation in increasing the skin microbiome diversity.

**The microbiome and forensic identification**
Studies have shown that (i) bacteria are easily transferred to environmental surfaces, (ii) bacterial communities of
individual's hands are quite specific (only 13% of bacterial phylotypes on the palm surface are shared between any two individuals) and (iii) palm surface bacterial communities are relatively stable and recover within hours after hand washing. Based on these facts, the authors of a recent study investigated if the owner of a computer keyboard could be identified by the composition of the bacterial microbiome of his/her hands. In other words: can bacteria from the hands of an individual leave a persistent trail in the environment that could be used in forensic identification? Samples were collected from the fingertips of three individuals and their computer keyboards. In addition, samples were collected from 13 additional computer keyboards that the three individuals have never touched. The authors found that bacterial communities on the fingertips and computer keyboard of a given individual were much more similar to each other than to the keyboards of other individuals. In other words, the owners of the three computer keyboards could be identified by matching the microbiota of their hands with the microbiota of the computer keyboards.

**Fungal microbiome**

Various studies have shown that *Malassezia* spp. predominate on the skin of healthy individuals. The first study used the ITS1 gene to investigate the fungal communities living on healthy human skin. The authors included 10 individuals and collected samples from 14 body sites including various core sites, the arms and three foot regions (plantar heel, toenail and toe web). *Malassezia* species were by far the most predominant fungi in all body sites with the exception of the feet which showed in addition to *Malassezia* other fungi genera such as, *Aspergillus*, *Cryptococcus*, *Rhodotorula*, *Epicoccum* among others demonstrating higher diversity compared to the other regions. These results were confirmed by other studies.

**Viral microbiome**

Studies have shown that viruses are also part of the skin microbiota of healthy individuals. Investigators have used the whole genome metagenomics approach which does not survey RNA viruses. The studies have demonstrated considerable viral diversity with multiple polyomaviruses, papillomaviruses and circoviruses being detected on normal-appearing skin.

**IN DISEASE**

**Bacterial microbiome**

**Atopic dermatitis** – An interesting study investigated the bacterial microbiota of patients with atopic dermatitis (i) during periods where the disease was less active and patients did not need therapy, (ii) during disease flare before starting therapy, (iii) during disease flare when therapy was instituted and, (iv) after flare when therapy was discontinued. Samples were also collected from a control group. The authors found a significantly decreased diversity of the bacterial microbiome during disease flare but before starting therapy with a recovery of the diversity during treatment to almost mimic the findings of before and post disease flare and of the control group. The reduced diversity during flare was explained by a relative increase of *Staphylococcus pseudintermedius* and *S. epidermitis* to less extent. Of note, we still do not know if loss of microbiome diversity leads to skin flares or skin flares orchestrate the decreased skin diversity of the microbiome as both are possible scenarios.

**Acne** – An interesting study compared the communities of *Propionibacterium acnes* at the strain level by sampling the pilosebaceous units of the noses of 49 acne patients and 52 healthy individuals. The relative abundance of *P. acnes* was similar in both groups; however, the strain population structures were significantly different in the two cohorts with certain strains being highly associated with acne and other strains were enriched in healthy skin. They speculate that the strains associated with acne could carry virulence factors that play a role in disease pathogenesis and could be target of therapeutic interventions. The authors also emphasize the importance of strain level analysis of the human microbiome to define the role of commensals in health and disease.
Selected canine and feline skin microbiome studies

Bacterial microbiome

IN HEALTH

Dog

Very little is currently known about the skin bacterial microbiome of normal dogs, in fact only two studies have been published to this date. These studies showed that the bacterial communities of healthy canine skin are quite diverse and, similar to humans, the same four phyla predominate: Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. The studies also found that the microbiome varies significantly among different body sites within the same individual dog and same body site across dogs. In contrast to humans where various skin microenvironments appear to shape the composition and structure of the bacterial community, in dogs no clustering of taxa according to body regions was noted likely because the microenvironment of dogs’ hairy skin is more similar. One of the studies also looked at mucosal sites and found that they contain less bacterial species (i.e. less rich) than hairy sites. Similar to people, dogs living in the same household were shown to have more similar microbiota than dogs living in different households in one of the studies. However, there were still significant differences in the microbiota of dogs from the same household. The bacterial taxa with higher relative abundance were different between the studies’ populations with *Ralstonia* being the most abundant genus, followed by *Moraxella* and *Porphyromonas* in one study and *Propionibacterium acnes* being the most abundant, followed by *Corynebacterium* and *Porphyromonas* in the other study. These differences are likely a factor of the variability of the canine skin microbiota, but differences in geographic regions and dogs’ lifestyle should be considered.

IN DISEASE

Dog

One study looked at the bacterial microbiota of six dogs with well-controlled atopic dermatitis and compared that with a population of healthy dogs. They found no significant difference in the most abundant taxa between the groups but, found that the bacterial microbiome of haired skin of the dogs with atopic dermatitis was less rich, i.e. had less bacterial species, compared to normal dogs. Interestingly, in this study the relative abundance of the betaproteobacteria *Ralstonia* spp. was significantly less in dogs with atopic dermatitis compared to controls. In addition, the less abundant taxa (<1%) present in the control dogs were often absent in the dogs with atopic dermatitis. Another study looked at the effect of having a bacterial skin infection in the microbiome of dogs with atopic dermatitis and investigated the impact of antibiotic therapy in the dermatitis and microbiome. They included a control group and collected samples at three time points: (i) during active infection, (ii) during treatment and (iii) post treatment. The authors found a significant decrease in diversity of the microbial community (dysbiosis) during the infection and restoration of the diversity during treatment but, some degree of diversity reduction occurred again post therapy coinciding with infection recrudescence in 5 of 17 dogs. The authors concluded that bacterial dysbiosis may contribute to the clinical signs of atopic dermatitis as CADESI scores and transepidermal water loss improved with resolution of the infection.

Fungal microbiome

IN HEALTH AND DISEASE

Dog

One study surveyed the mycobiota of healthy dogs and dogs with atopic dermatitis. The most abundant fungal genera in both groups were *Cladosporium*, *Alternaria* and *Epicoccum*. The fungal diversity and richness of healthy dogs were generally not significantly different among the body sites with the exception of the nostrils and conjunctiva that had less number of fungal species. However, when the factor “dog” was taken into consideration significant differences in diversity and richness in addition to membership and structure were noted between dogs. Similar results were noted when the most common fungal taxa were evaluated with no significant differences found between body sites from the same dogs but, significant differences were evident between dogs. The results were somewhat different in the dogs with atopic dermatitis where richness and diversity were significantly different among some body sites within the same dog. Similar to healthy dogs, fungal membership and structure were more influenced by the factor
dog than body sites. Overall the skin of allergic dogs had significantly less fungal species (less rich) compared to healthy dogs.

**Cat**

One study investigated the mycobiota of healthy cats and cats with hypersensitivity diseases. The most abundant genera in health and allergic cats were *Cladosporium* and *Alternaria* but *Epicoccum* was over-represented in healthy cats and Sordariomycetes in allergic cats. Interestingly, in contrast to healthy humans where *Malassezia* was found to be the main genus across multiple body sites with the exception of the feet, it was only sequenced in 21% and 30% of the samples in allergic and healthy cats, respectively, and in most samples the relative abundance was less than 1%. There was no significant difference in the microbial community of the various sites investigated within the same cat; however, there were significant differences between cats. The mucosal sites including nostril, reproductive tract and conjunctiva were less rich/diverse (less fungal species) compared to the oral mucosal and all other non-mucosal tested sites. This study showed that the mycobiota of cats is more diverse than that of humans and despite significant difference among cats the fungal community was similar among the various tested body sites in both healthy and allergic cats.

**Selected references**

15. Meason-Smith C et al. What is living on your dog’s skin? Characterization of the canine cutaneous mycobiota and fungal dysbiosis in canine allergic dermatitis. FEMS Microbiology Ecology; 2015;91
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The purpose of this lecture is to review the peripheral and central mechanisms of itch and pain, to describe the many peripheral and central neurotransmitter receptors that are involved in itch (or are expected to be involved in itch), and to review them in terms of their potential as targets for anti-pruritic therapies.

**OBJECTIVE:**
1. To review and compare mechanisms of pain and itch.
2. To review and compare therapeutic strategies for pain and itch.
INTRODUCTION
The World Health Organisation defines allergen specific immunotherapy (ASIT) as the ‘incremental administration
of increasing quantities of an allergen extract to an allergic patient’. The goal of successful therapy is to reduce or
eliminate the clinical signs associated with repeated exposure to causative allergens.

ASIT is the only current treatment for allergy that can modify, or reverse, at least part of the pathogenesis of allergic
dermatitis, both alleviating clinical signs and preventing progression of disease. There are many unresolved questions
about ASIT, especially with regard to defining more clearly its efficacy for dogs and cats. One key question revolves
around differences in atopic dermatitis (AD) and other allergic syndromes such as respiratory disease between
humans and dogs and cats. Most of the evidence for the effectiveness of ASIT in humans is for the management of
allergic rhinoconjunctivitis and asthma and there are many well-controlled studies that demonstrate efficacy, and
critical reviews and meta-analyses that support this conclusion, while the strength of recommendation for use of ASIT
for the management of human AD is weak.

By contrast, the quality of published evidence for efficacy of ASIT in dogs and cats is limited, and at best based on
inconsistent or limited quality patient-oriented evidence with small, uncontrolled trials employing confounding
variables that make interpretation of results difficult. No controlled studies have been performed to determine the
value of ASIT as a modifying treatment for feline allergic skin disease.

Beyond consideration of efficacy, it is clear that ASIT protocols for dogs and cats lack standardization and are subject
to substantial variation. Veterinary clinicians use different allergen dosage regimes with unstandardized allergen
extracts that vary in composition and potency between manufacturers and from batch to batch, using different
schedules of administration and different allergen prescriptions. The allergen concentration, interval between
injections and injection volume are highly variable. There is no single or standardised immunotherapy administration
protocol.

Despite this, ASIT is considered to be a safe and effective treatment option for the management of allergic dermatitis
in cats. Reported success rates in uncontrolled studies vary from 50% to 100% defined by an improvement in clinical
signs and/or a decrease in anti-inflammatory or antipruritic medication use.

MECHANISMS OF ACTION
The mechanisms of action of ASIT are not completely elucidated. Briefly, an early reduction in effector cell activity
(eosinophils, basophils, mast cells) is followed by a long-term immunologic shift from a Th2 cell to a
Th helper 1 (Th1) cell response and development of immunologic tolerance. Allergen-specific IgG concentrations,
particularly IgG4, increase during ASIT, blocking the IgE-mediated reactions by binding to the same epitopes
as allergen specific IgE. Regulatory T-cells (Treg) are activated and increase in number during ASIT and produce
TGF-ß and IL-10. IL-10 levels correlate with clinical efficacy of ASIT in humans. In dogs a significant increase in IgG4
concentrations have been noted within six months of therapy with a decrease in allergen specific IgE after 12 months.
A significant increase of Treg cells and IL-10 within three months of ASIT has been reported in dogs, which correlates
with the clinical response rate. In cats, no studies have been published.

With sublingual administration, there is the additional effect of oromucosal dendritic cells, frequently discussed for
immunotherapy in humans but unexplored in dogs and cats. Oromucosal dendritic cells are responsible for allergen
uptake, processing and presentation to T-lymphocytes. They are abundant in the oral mucosa and have unique
functional characteristics, being the key cell in induction of oral tolerance.

ASIT PROTOCOLS
Current ASIT approaches in dogs and cats focus on either subcutaneous (SCIT) or sublingual (SLIT) administration of
allergens.
**SUBCUTANEOUS IMMUNOTHERAPY (SCIT)**

With regard to SCIT for dogs and cats, two methods have evolved, predominately based on availability of, and regulatory approvals for allergen extracts. All SCIT in North America, Australia and New Zealand and Asia uses aqueous, saline-phenol preserved allergen extracts. In Europe, use of alum-precipitated allergen extracts has been more common. Absorption of the allergen molecules to an aluminium hydroxide adjuvant provides a slower-release formulation, which has the advantage of permitting less frequent injections. However, concern is being increasingly raised regarding the possible adverse effects of chronic aluminium exposure, and the fate of aluminium-based adjuvants widely used in vaccine products is uncertain.

**Conventional induction SCIT** consists of a subcutaneous injection protocol with an induction phase, where allergens are administered over a period of several weeks and a maintenance phase, where injections are typically administered every one to three weeks. Typically, 2- or 3-vial sets of increasing concentration are used, beginning with frequent injections of dilute extract and progressing to less frequent injections of concentrated extract as maintenance treatment.

An on-line survey of veterinary dermatologists reported that 86% (25/29) participants used SCIT for the management of feline allergic dermatitis using either aqueous allergens (72%), alum precipitated (20%) or calcium phosphate bound (8%) allergens. Allergens for ASIT were equally selected either based on intradermal testing, serology or a combination of the two. Depending on the allergen type, injection protocols ranged from once a week to once a month with dose adjustments in volume and interval based on individual patient response. For cats treated with SCIT, 76% of owners injected the allergy vaccines at home whereas 24% were injected by the veterinarian. Side effects were rare and included localised or generalised pruritus or anaphylaxis; local reactions (pain at injection site, swelling, erythema) and vomiting.

**Rush induction with SCIT (rush immunotherapy) [RIT]** consists of a subcutaneous injection protocol with an induction phase, where increasing amounts of allergen are administered every 20 to 30 minutes in a hospital or clinic setting with careful monitoring over several hours until the maintenance dose is reached. The maintenance dose is therefore reached within one day compared to several weeks with conventional induction SCIT. Side effects are rare and similar to those with conventional ASIT: increased pruritus after administration of injections, urticaria and anaphylaxis.

At present, it appears that rush protocols are used by comparatively few veterinary clinicians, though they are the one specific administration schedule that has been examined in dogs and found to be at least equally effective to a conventional injection protocol. A double-blinded study comparing SCIT and RIT with aqueous allergens in dogs resulted in a shorter period until maximal improvement with RIT compared to SCIT.

RIT has a clear advantage of limiting the number of injections that an owner must give at home when initiating ASIT, though safety concerns are potentially greater as well as the cost for a brief hospitalisation under observation. There is one study to date that describes a RIT protocol for a small number of cats. In the on-line survey of 29 veterinary dermatologists, only two individuals were using RIT. The authors experience with RIT will be discussed during the lecture.

**SUBLINGUAL IMMUNOTHERAPY (SLIT)**

SLIT is an effective treatment for humans with a favourable safety profile compared with SCIT and is a popular method of human ASIT in European countries. The lower popularity of SLIT in the United States reflects that registered products have not been available until very recently and, with these, its use is increasing in human medicine. In dogs, use of SLIT has only recently been reported and there are no reported studies in cats.

Anecdotally, benefits have been observed for some cats and SLIT appears to be well tolerated. In the on-line survey of veterinary dermatologists, 25% (6/24) participants were using SLIT. The most common side effect was vomiting; other side effects included increased pruritus and pawing at the mouth. The formulation is palatable and even fastidious cats seem to tolerate the small volume necessary for twice a day administration to the oral mucosa. SLIT can be a useful alternative for dogs and cats and owners adverse to the administration of subcutaneous injections.
**ASIT: HOW CAN WE DO BETTER?**

With such limited evidence of efficacy, the question must be posed as to why are we using ASIT in dogs and cats without more rigorous proof? It has been hypothesised that the high cost and time requirement for performing large, controlled studies of a complex disease that may require a year or more to respond to the intervention may account for this lack of evidence.

Beyond consideration of efficacy, it is clear that ASIT protocols for dogs lack standardization and are subject to substantial variation. It is hoped that veterinary dermatologists will employ a more scientific approach to allergen prescription formulation in the future. With the marked variation among clinicians in how, and how many, extracts of different types are mixed together, it is not surprising that there is such a variable outcome. Should the number of extracts in an allergen prescription be limited from eight to ten to 12 or unlimited? Should protease-containing extracts such as moulds be admixed, or administered only by separate injection? Should the allergen prescription be based upon results of intradermal testing or serologic testing or both? Or made uniform for each dog or cat, based on what allergens are predominant in a region?

A central problem in answering many of these questions is that most studies in dogs and cats evaluating the parameters of successful ASIT have been clinical trials. In conducting such trials, there is significant difficulty in obtaining reproducible, objective data. Objective changes in parameters as biomarkers of successful treatment that could provide useful measures of ASIT success are lacking in dogs and cats.

**ASIT: SUMMARY**

In conclusion, among veterinary dermatologists, SCIT appears to be widely used as a treatment modality for feline allergic dermatitis, even in the absence of rigorous evidence of proof of efficacy. Side effects are rare but can be severe. RIT is not used commonly, but appears to be a safe and practical option for induction of SCIT in cats with allergic skin disease. Cats seem to tolerate oral SLIT even though twice-daily oral application can be a challenge. Studies are needed to elucidate the efficacy and safety of allergen specific immunotherapy in cats.

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FELINE PEMPHIGUS FOLIACEUS

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INTRODUCTION
The pemphigus group includes autoimmune blistering skin disease in which autoantibody-targeted desmosomes lose adhesion and form blisters. Several variants of pemphigus, separated according to clinical signs, the depth of acantholysis and the targeted antigen are recognised in humans and animals. Two pemphigus variants have been described in cats; pemphigus foliaceus (PF) and pemphigus vulgaris (PV).

The target antigen of autoantibodies in human PF is a desmosomal cadherin, desmoglein 1 (Dsg1). Little is known about the pathogenesis of feline PF and the target antigen(s) in cats remains unknown. There is some evidence that cats with pemphigus produce anti-keratinocyte antibodies. Keratinocyte-bound IgG have been detected in a majority of feline PF and PV patients. Detection of circulating autoantibodies by indirect immunofluorescence has been demonstrated in a significant number of cats with PF.

Characteristic signalment and clinical signs based on a literature review of historically published cases will be discussed during the lecture.

SIGNALMENT
The age of onset of feline PF ranges from 3 months to 17 years. A sex predisposition is not reported. A breed predisposition for feline PF is not definitive.

CLINICAL SIGNS
In most cats, lesions initially appear on the face, principally affecting the dorsal muzzle, nasal planum, periorbital skin and pinnae. In these areas, the pattern usually is strikingly bilateral and symmetrical. Lesions have a predilection for involvement of the footpads or ungual folds of claws and can be restricted to this location. The ventrum is commonly affected and periareolar involvement is reported. Of note is that mucosal lesions are not observed.

Skin lesions are typically transient, superficial pustules that evolve rapidly into erosions and yellow crusts with associated scale and exfoliation with alopecia. Pustules are extremely transient, however, and the phenotype is dominated by erosions and yellowish crusts. Some cats with footpad lesions are lame with erythematous swelling at the pad margins with fissures, crusting and hyperkeratosis of pads. The subungual region is frequently involved with erythema, swelling, erosion and crusting paronychia within the ungual fold characterised by green-yellow to cream-coloured, inspissated, thick exudate.

Other clinical signs include pruritus which can be mild to severe in some affected cats and systemic clinical signs of anorexia, depression, fever and weight loss often encountered in cats with widespread lesions.

DIAGNOSIS

CYTOLOGY
Intact non-follicular pustules should be sampled or crusts removed to impress a glass slide onto the moist erosive surface. Cytologic evaluation reveals non-degenerate neutrophils, rarer eosinophils and acantholytic keratinocytes in large clusters or rafts of free-floating rounded keratinocytes. Acantholytic keratinocytes exhibit either microscopic characteristics of normal differentiated spinous or granular layer epithelial cells, or they present with signs of apoptosis with eosinophilic cytoplasm, condensed chromatin or karyorrhexis. Occasionally, neutrophils can be seen in close apposition to detached keratinocytes.

The presence of acantholytic keratinocytes and neutrophils is not specific for PF. Keratinocyte acantholysis has been observed in dogs and cats with bacterial skin infections and Trichophyton fungi induce subcorneal acantholytic neutrophilic pustules.
HISTOPATHOLOGY
Early lesions may appear as vesicles with acantholytic keratinocytes and scarce neutrophils. However, these lesions rapidly evolve into broad, discrete, intragranular or subcorneal pustules with isolated and/or clustered acantholytic keratinocytes evident. In these lesions, neutrophils predominate, but variable numbers of eosinophils may be found. Pustules commonly invade the epithelium and/or the lumen of the follicular infundibulum. In general, the pustules are large and span the length of multiple follicular units, a finding that can differentiate these lesions from those of bacterial folliculitis. Similarly, recornification, defined as newly reformed stratum corneum at the base of neutrophilic pustules, is more suggestive of PF than bacterial folliculitis. Free floating “rafts” of partially adherent acantholytic keratinocytes and adherence of acantholytic keratinocytes to the overlying stratum corneum are also characteristic histopathologic features of PF.

DIRECT IMMUNOFLOUORESCENCE
Direct immunofluorescence (DIF) or immunoperoxidase have been used to detect anti-keratinocyte autoantibodies deposited in vivo in the skin of animals with PF. Skin-fixed intercellular epidermal IgGs are detected in most cats with PF. Direct IF testing of skin biopsy specimens can be negative due to prior glucocorticoid therapy. Furthermore, intercellular epidermal IgG can be detected in biopsy specimens from humans and dogs with other dermatoses reducing the specificity of direct IF testing for the diagnosis of PF.

INDIRECT IMMUNOFLOUORESCENCE
Indirect immunofluorescence (IIF) demonstrates circulating IgG4 and IgG1 anti-keratinocyte autoantibodies in the sera of patients with PF. IIF results vary according to the substrate utilized for autoantibody detection. Using neonatal mouse skin as the substrate, circulating pathogenic anti-keratinocyte IgG4 antibodies have been detected in the majority of dogs with PF. Detection of circulating anti-keratinocyte autoantibodies by IIF has previously yielded only rare positive results in cats and this technique has been considered unreliable; probably due to the type of substrate used to perform the test. The use of feline footpad and buccal mucosa has increased the level of detection of anti-keratinocyte IgG antibodies from sera from cats with PF.

MANAGEMENT
There are no established treatment guidelines for the treatment of feline PF and therefore the choice of treatment is based on published PF cases and other immune mediated diseases. A summary of historical treatment data and outcomes will be discussed during the lecture.

GLUCOCORTICOIDS
An immunosuppressive dose of prednisolone at a dosage of 2 to 6 mg/kg daily has been recommended for the treatment of feline PF. The induction dose should be maintained until the disease is inactive, though alopecia and residual crusts may be present. Following induction, reduction of the dosage to the lowest effective alternate day dosage is required for a maintenance dose. In most reported cases, a monotherapy with glucocorticoids has been sufficient to control clinical signs. In cats that fail to respond to prednisolone, either dexamethasone at a dose of 0.2 to 0.4mg/kg daily or triamcinolone at a dose of 0.6 to 2mg/kg daily may be a useful alternative.

OTHER IMMUNOSUPPRESSIVE DRUGS
If additional immunosuppression is required, chlorambucil at a dose of 0.2mg/kg daily for four consecutive days each week is often the drug of choice and is a safe and reliable adjunctive treatment; complications to treatment are unusual but can include myelosuppression and mild gastrointestinal signs.

Ciclosporin at a dose of 5.5mg/kg q 24hrs can be effective as a monotherapy for induction and/or long term disease management as well as an adjunct therapy. Ciclosporin may provide enhanced glucocorticoid sparing for long term maintenance of feline PF as some cats maintained with ciclosporin can be withdrawn from oral glucocorticoids.

Azathioprine has been used in feline PF but is associated with rapid and severe bone marrow suppression and not recommended for use in cats.

New treatment options, whose efficacy for the management of feline PF awaits investigation include mycophenolate mofetil, leflunomide and methotrexate. Other options for therapy could include plasmapheresis or intravenous immunoglobulin therapy. All these drugs have been used to manage other immune mediated feline diseases and may present a therapeutic option in refractory cases, especially as glucocorticoid-sparing agents.
MONITORING
There are no established guidelines for monitoring cats receiving treatment for PF; the current consensus amongst clinicians is to perform complete blood counts and serum biochemistry with urinalysis one and three months after the onset of induction therapy and every six to 12 months for cats receiving maintenance treatment.

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SETTING ANTIMICROBIAL BREAKPOINTS

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When difficult-to-treat or drug-resistant infections are encountered, culture and antimicrobial susceptibility testing (AST) will provide the best guidance for drug selection. The veterinary clinician should have confidence that if a specimen is submitted to a reputable laboratory that follows the standard methodology of the Clinical and Laboratory Standards Institute (CLSI) (1), the results will be valid and performed according to a public standard that is used throughout the world.

**SUSCEPTIBILITY TESTING REPORTS**

The most important information for the clinician is simply which drugs have an “S” and which ones have an “R”. These results then guide their treatment. What really goes into this interpretation? The standards for interpretation are available from the Clinical and Laboratory Standards Institute – CLSI, (CLSI, 2015, also found at http://www.clsi.org/) (1). Not all laboratories use CLSI standards. It is a voluntary program. However, it is the only global organization that develops susceptibility testing standards for animals. If a laboratory does not adhere to a public standard such as CLSI, breakpoints may vary and interpretation may be inconsistent from laboratory to laboratory, or among different regions of the country.

Microdilution Test for Determination of MIC:

It is becoming more common for laboratories to directly measure the minimum inhibitory concentration (MIC) of an organism with an antimicrobial dilution test. Zone inhibition (also known as the Kirby-Bauer test) also is performed but provides only qualitative information. The MIC dilution test is performed by inoculating the wells of a plate with the bacterial culture and dilutions of antibiotics are arranged across the rows. The test is usually performed in modern laboratories using high-throughput plates, but individual tubes or plates can also be used for dilution tests. Antibiotic drug concentrations are arranged in serial dilutions, with each concentration doubled from lowest to highest in a range. The MIC is not a measure of efficacy, but instead it is simply an in vitro measurement of drug activity and bacterial susceptibility. The lower the MIC value, the more susceptible the isolate is to that drug. The MICs are determined using serial two-fold dilutions (Log 2) of each drug which is added a standardized inoculum media and incubated for a prescribed time. Concentrations are always listed in µg/mL (mg/L). For example, if one were to start at a concentration of 256 µg/ml, the MIC dilution series would be as follows: 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, and 0.06 µg/ml, etc. If, for example, bacterial growth occurs at a dilution of 0.12 µg/ml for a specific drug, but not at 0.25 µg/ml and above, the MIC is determined to be 0.25 µg/ml. The MIC dilution test is only semi-quantitative because there are gaps between each dilution. Realistically, the true MIC lies somewhere between these values, but the MIC is recorded as the lowest value. In some laboratories other methods to measure the MIC are being used such as the E-test® (epsilometer test) distributed by bioMérieux. The E-test is a quantitative technique which measures the MIC by direct measurement of bacterial growth along a concentration gradient of the antibiotic contained in a test strip.

**Why Report Only The MIC?**

The MIC is the Minimal Inhibitory Concentration, which is the lowest concentration that inhibits visible bacterial growth. In surveys, or surveillance data, it is frequently this is expressed as MIC50 or MIC90, which is the MIC that inhibits 50% or 90% of the bacteria, respectively. It is sometimes cited in error that the MIC50 and MIC90 are the average concentrations for 50% and 90% efficacy. These values should not be confused with clinical efficacy (more on that later).

The MBC is the Minimum Bactericidal Concentration, which is the lowest concentration that kills 99.9% of the bacteria (3-Log10 reduction). Standards are not typically available to measure the MBC, the test is more complicated and difficult to perform than the MIC determination. Therefore, the MBC is rarely measured or reported in clinical laboratories.
The MPC is the Mutant Prevention Concentration. This is lowest antibiotic concentration that prevents growth of the least susceptible first-step resistant mutant among a large bacterial population (eg, 10^7 or 10^10 cfu) (2). It also may be defined as the MIC of the most resistant first-step cell present in a bacterial population. The mutant selection window (MSW) is the concentration between the MIC of susceptible organisms, and the MPC. The MPC test is not standardized and is more difficult to perform in a clinical laboratory. Large inoculums are required. The interpretation of the MPC value for clinical dose determinations is difficult and has not been established for veterinary antimicrobials.

**INTERPRETATION OF SUSCEPTIBILITY TESTS**

Resistance and susceptibility are determined by comparing the organism’s MIC to the drug’s breakpoint as established by the CLSI (1). After a laboratory determines an MIC, it will use the CLSI “SIR” interpretive categories and breakpoints in each category (S, susceptible; I, intermediate, or R, resistant). In practice, if the MIC for the bacterial isolate falls in the susceptible category, there is a greater likelihood of successful treatment (cure) than if the isolate were classified as resistant. It does not assure success; drug failure is still possible owing to other drug or patient factors (for example, immune status, immaturity, or severe illness that compromises the action of antibacterial drugs), and interactions. If the MIC is in the resistant category, bacteriologic failure is more likely because of specific resistance mechanisms or inadequate drug concentrations in the patient. However, a patient with a competent immune system may sometimes eradicate an infection even when the isolate is resistant to the drug in the MIC test.

The intermediate category is intended as a buffer zone between susceptible and resistant strains. This category reflects the possibility of error when an isolate has an MIC that borders between susceptible and resistant. If the MIC value is in the intermediate category, therapy with this drug at the usual standard dosage is discouraged because there is a good likelihood that drug concentrations may be inadequate for a cure. However, successful therapy is possible when drug concentrates at certain sites – in urine, or as the result of topical therapy, for example – or at doses higher than the minimum effective dose listed on the label. For example, prescribing guidelines for some antimicrobials allow for an increase in dose when susceptibility testing identifies an organism in the intermediate range of susceptibility. Fluoroquinolone antimicrobials (enrofloxacin, marbofloxacin, orbifloxacin) have been approved with a dose range that allows increases in doses when susceptibility testing identifies an organism in the Intermediate category. In these cases higher drug concentrations make a cure possible if the clinician is able to safely increase the dose above the minimum labeled dose. (For example, in the case of enrofloxacin in dogs, this would be equivalent to a dose of 10 to 20 mg/kg/day, rather than the minimum dose of 5 mg/kg/day.)

MIC data should not be used in isolation, but by coupling the MIC from a laboratory report with CLSI breakpoints and other important information such as the virulence of the bacteria and the pharmacology of the antibiotics being considered, the clinician can make a more informed selection of an antibacterial drug.

**Does the susceptibility test provide tissue-specific interpretation?**

The susceptibility interpretation is based on plasma/serum concentrations. No tissue-specific interpretation can be provided that accounts for differences in drug distribution among tissues. For example, even though it is anticipated that many antibiotics concentrate in the urine, which may be beneficial for treating a urinary tract infection, the susceptibility interpretation is based on achieving adequate concentrations in the blood. There are three exceptions to this in the veterinary document because amoxicillin and amoxicillin-clavulanate interpretations allow for high concentrations in urine and a higher breakpoint is used for urinary tract isolates. Oral cephalosporins also have a higher breakpoint for urinary isolates. For example, a cefazolin or cephexalin “S” breakpoint of ≤ 16 µg/mL is used to predict results for the oral cephalosporin agents when used for therapy of uncomplicated urinary tract infection caused by *E. coli, K. pneumoniae*, and *P. mirabilis*. Cefpodoxime, also may be tested individually because some isolates may be susceptible to these agents while testing resistant to cefazolin or cephexalin. These guidelines apply to uncomplicated urinary tract infections. For complicated, infections (patients with other co-existing disease) one shouldn’t assume that concentrations in urine – even when they are high due to concentration by the nephrons – are sufficient to eradicate infections of the urinary tract. Infections may involve the deeper layers of the mucosa, the renal tissue, or the prostate tissue. In these instances, it is the tissue concentration – which is correlated to the plasma concentration – that will be predictive of a bacteriologic cure (3).
A frequent mistake in MIC interpretation is to compare the MIC with published tissue concentrations that are derived from whole-tissue homogenized samples. Tissue concentration data is sometimes published by pharmaceutical companies in their product information. These concentrations may be misleading because they may either underestimate or overestimate (depending on the drug’s affinity for intracellular sites) the true drug concentration at the site of infection.

In most instances the clinician should not be concerned with the question of whether or not there are tissue-specific susceptibility interpretations. For most tissues, antibiotic drug concentrations in the serum or plasma approximate the drug concentration in the extracellular space (interstitial fluid). This is because there is no barrier that impedes drug diffusion from the vascular compartment to extracellular tissue fluid (4, 5). There is really no such thing as “good penetration” and “poor penetration” when referring to most drugs in most tissues. Pores (fenestrations) or microchannels in the endothelium of capillaries are large enough to allow drug molecules to pass through unless the drug is restricted by protein binding in the blood. Tissues lacking pores or channels may inhibit penetration of some drugs (discussed below).

If adequate drug concentrations can be achieved in plasma, it is unlikely that a barrier in the tissue will prevent drug diffusion to the site of infection as long as the tissue has an adequate blood supply. Clinicians should be concerned when treating tissues that have poor or impaired blood supply. Drug diffusion into an abscess or granulation tissue is sometimes a problem because in these conditions, drug penetration relies on simple diffusion and the site of infection lacks adequate blood supply. In an abscess, there may not be a physical barrier to diffusion – that is, there is no impenetrable membrane – but low drug concentrations are attained in the abscess or drug concentrations are slow to accumulate.

In some tissues a lipid membrane (such as tight junctions on capillaries) presents a barrier to drug diffusion. In these instances, a drug must be sufficiently lipid-soluble, or be actively carried across the membrane in order to reach effective concentrations in tissues. These tissues include: the central nervous system, eye, and prostate. A functional membrane pump (p-glycoprotein) also contributes to the barrier. There also is a barrier between plasma and bronchial epithelium (blood : bronchus barrier). This limits drug concentrations of some drugs in the bronchial secretions and epithelial fluid of the airways. However, this barrier may be compromised during infection (pneumonia).

Is Susceptibility Interpretation by CLSI Specific for Veterinary Species?

Years ago, the veterinary diagnostic laboratories had to rely heavily on the CLSI interpretation from the human standards. There were not enough veterinary-specific interpretive criteria available to establish breakpoints for veterinary drugs and veterinary species. This is now changing. The current edition of the CLSI standard document (1) for veterinary drugs has the tables clearly separated into those drugs with veterinary interpretive criteria, and drugs that still rely on human standards for interpretation. In the last several years, CLSI has tremendously expanded the list of drugs for which there are veterinary-specific breakpoints. For companion animals, veterinary-specific MIC breakpoints have now been established for the four licensed fluoroquinolones (enrofloxacin, difloxacin, marbofloxacin, and orbifloxacin), gentamicin, cefpodoxime proxetil, ampicillin/amoxicillin, amoxicillin-clavulanic acid, first-generation cephalosporins (cephalexin and cefazolin), amikacin, doxycycline, minocycline, piperacillin-tazobactam, and clindamycin. Important changes were also made for the interpretation of Staphylococcus resistance. Until veterinary-specific breakpoints are established for other antibiotics used in companion animals, we will continue to rely on the human breakpoints for drugs such as chloramphenicol, erythromycin, carbapenems (imipenem), some penicillins, sulfonamides, and potentiated sulfonamides. The CLSI committee is working on filling in these gaps. But in the meantime, similarities in pharmacokinetics and pathogen susceptibilities between humans and animals allow for an acceptable approximation to extrapolate human breakpoints to animal situations for many drugs until veterinary-specific standards are available. The human equivalent of the veterinary CLSI standard (M 100) also has made substantial changes in recent years. Because of concerns for mis-identifying extended-spectrum β-lactamase producing Enterobacteriaceae, the cephalosporin breakpoints have been lowered compared to previous criteria (6). Carbapenem breakpoints also have been recently lowered.
HOW ARE BREAKPOINTS DETERMINED?
The paper by Turnidge and Paterson (7) describe in detail the process of setting breakpoints. The CLSI subcommittee for Veterinary Antimicrobial Susceptibility Testing (VAST), uses strict criteria to establish and evaluate breakpoints. Sponsors are required to follow guidelines provided by CLSI and must submit data to support a proposed breakpoint. The data includes pharmacokinetic data in the target species, MIC distributions for the pathogens targeted, clinical data from the drug used under field conditions at the approved dose, and pharmacokinetic-pharmacodynamic (PK-PD) analysis, using Monte Carlo Simulations (8) to show that at the approved dose the drug attains PK-PD targets for the labeled pathogen.

Are These Standards, or Guidelines?
The CLSI is a consensus-driven process and after approval by the subcommittee the standards become public documents. The consensus process involves the development and public open review of documents, revision of documents in response to discussion, and, finally, the acceptance of a document as a consensus standard or guideline. The CLSI documents used for culture and susceptibility testing should be regarded as a public standard, not a guideline.

A Standard is a document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A Standard may, in addition, contain discretionary elements, which are clearly identified. A Guideline is a document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

REFERENCES CITED
Allergen-specific immunotherapy (ASIT) is a familiar treatment for allergic diseases of dogs, cats, and horses, wherein extracts of allergens to which the patient is sensitive are administered, in gradually increasing amounts, to lessen the hypersensitivity state. What’s new in this field, and what can we look forward to in the future?

IS ASIT AN OUTMODED TREATMENT?
With the recent advent of new (and sometimes wondrously effective) drug and biological treatments for allergy, some have questioned if ASIT is as necessary or useful as in the past. The author believes very strongly that ASIT should remain a foundational and important part of a multimodal treatment approach. It is the only treatment for allergy that can modify, or reverse, at least part of the pathogenesis of this condition as we know it – both alleviating clinical signs and preventing progression in the process. This modification is accomplished without the possible long-term adverse effects of a lifetime of drug treatment, with minimal chance for its own adverse effects, and with the potential of long-lasting effectiveness. There are certainly disadvantages to ASIT – including the fact that it takes several months or more to begin working, that it does not always work, and that it may be relatively expensive. Nevertheless, if we are to provide optimal management of this lifelong disease, ASIT still has a primary role.

As far as is known, concurrent treatments with antihistamines, fatty acid supplements, ciclosporin, or low-dose glucocorticoids will not interfere with response to ASIT; preliminary clinical experience suggests the same is true for newer drugs such as oclacitinib, and perhaps for Cytopoint. Such treatments are virtually always necessary as part of the overall treatment plan, to provide immediate and short-term relief while waiting for the ASIT to work. These treatments can be slowly tapered as response to ASIT occurs. Treatment with ASIT is generally considered to be lifelong, though it is possible to attempt discontinuation after 2 to 3 years of injections if the animal has responded very well.

SUBCUTANEOUS INJECTIONS vs. SUBLINGUAL IMMUNOTHERAPY
Current ASIT approaches in canine AD center on either subcutaneous (SCIT) or sublingual (SLIT) administration. There are a great many unknowns about both methods, and elucidating these may facilitate improvements in therapy. To date, there have been no studies in pets that directly compare the results of ASIT with SLIT vs. SCIT; the very limited evidence available suggests both have approximately equal efficacy. In human beings, the choice is still an ongoing discussion.

Sublingual immunotherapy (SLIT) involves administration of allergen extract into the oral cavity, under the tongue, as opposed to by injection. It is commonly used for human allergy in Europe, particularly for atopic rhinitis and asthma. Historically, there are conflicting reports of efficacy, which may be explained in part by the extreme variation in protocols used for dosing, administration, intervals, vehicle, etc. in the different studies reported. Consideration of recent evidence has led authoritative bodies to conclude that, when used correctly, it is clearly efficacious and in fact has a response rate similar to subcutaneous ASIT. Its use in animals is relatively new.

Studies on SLIT and other non-injection methods of ASIT for use in pets are only just being reported. One study in an experimental model of canine AD failed to show evidence for efficacy of orally-administered allergen in laboratory beagles experimentally sensitized to dust mite; however in this study the allergen was fed to the dog rather than applied to the mucosa. Another small open trial of atopic canine clinical patients with dust mite allergy treated with SLIT reported clinical benefit in 80% of dogs, and that clinical benefit was usually accompanied by measurable immunologic changes, including significant increases in allergen-specific IgG and decreases in allergen-specific IgE. Marsella et al. reported some efficacy of SLIT in a laboratory model using sensitized beagle dogs, including significant changes in cytokines such as TGF-beta and IL-10 in treated animals. Finally, a multicenter, uncontrolled open trial of 217 dogs, reported preliminarily by the author, indicated approximately 60% response to SLIT therapy, including approximately 50% of “injection failure” dogs responding. The mechanism(s) by which SLIT works are somewhat different than for SCIT, implying that it may be more or less effective than injections for a given patient.
One big advantage of SLIT is in ease of administration, which may improve compliance. We’ve found that though many owners “don’t mind” giving injections to their pets, most owners clearly don’t relish it, and are delighted to be presented with an alternative to giving injections. Most dogs accept administration easily, even viewing it as a treat, which increases compliance. On the other hand, successful SLIT requires faithful twice-daily administration, and owners with busy travel schedules may find it much more convenient to give an infrequent injection. In human beings, anaphylactic reactions to SLIT are rare to nonexistent, and SLIT can be used in humans with a prior history of reaction to allergy shots. In our experience, the same is true for dogs; we’ve treated numerous patients with SLIT who have had anaphylactic reactions to allergy shots.

CHOOSING ALLERGENS, ALLERGEN ADMIXTURE, AND OPTIMAL DOSING

Protocols for ASIT in pets are completely unstandardized and subject to enormous variation. Different veterinary dermatologists are likely to use different allergen doses, of unstandardized extracts that vary in composition and potency from manufacturer to manufacturer and from batch to batch, using different schedules of administration, differing concurrent treatments, and with different ‘rules’ about how to mix extracts together. With the incredible variation among dermatologists in how, and how many, extracts of different types are mixed together, it is no wonder that patient experience varies. Should the number of extracts in a mixture be limited to eight? Ten? Twelve? Or unlimited? Should protease-containing extracts such as molds be admixed, or administered only by separate injection? Should the mixture be based upon results of intradermal testing? Serologic testing? Both? Or merely made uniform for every patient, based on what allergens are predominant in a region?

On the human side, these debates have not ended, though recent large-scale studies are providing at least some guidance. Evidence is accumulating that in polysensitized humans, treatment with a single, dominant allergen is as effective as multi-allergen ASIT, even though polysensitization is more prevalent.6,7 This “less is more” approach – prevalent in Europe - is unpopular in the United States among physician allergists. The evidence for limiting the number of allergen extracts used in treatment stands in contrast to protocols used by most veterinary dermatologists, and if applicable to dogs, will require a change in our thinking.

Authoritative guidelines specify that mold extracts should not be mixed in the same vial as pollens, as there is clear evidence that pollen allergens will be degraded by mold proteases during storage.8 Despite similar (though less) evidence in dogs,9 many veterinary dermatologists continue to recommend such mixtures. Clearly, there is a need for further experimentation to solve this dilemma, among many others. Because SLIT formulations generally contain glycerin and/or other stabilizers in the vehicle, they protect protein allergens from protease degradation; therefore, SLIT may be preferable to aqueous SLIT if protease-containing extracts are included in the mix.

Most effects of ASIT are thought to be allergen-specific, rather than nonspecific. Thus, accurate testing to identify the offending allergens in each patient is of paramount importance to successful immunotherapy. In particular, the clinician must strive to avoid ‘false positive’ allergy test results, which would result in including an allergen in the patient’s mixture that is not relevant to that individual’s disease.

ASIT IN CATS AND HORSES

Despite substantial clinical need, progress in the world of feline and equine ASIT has been limited. For cats, a big part of the problem is in defining “feline atopic disease” and how cases should be selected for ASIT treatment, and difficulties in interpreting allergy testing results in cats. In horses, some progress has been made in the realm of hypersensitivity to the biting midge Culicoides. One of the problems here is that there are dozens of species of this insect, and each appears to have at least some unique allergenic components. Advances in technology for producing recombinant allergens is producing more promising results, as are trials of intralymphatic administration in horses.10 More intriguing is the recent demonstration that it is possible to express Culicoides major allergens on transgenic grain particles, which when fed to healthy horses create measurable increases in midge-specific IgG.11 Demonstrating efficacy as ASIT in allergic horses is some distance in the future, but the concept is fascinating.

FOR THE FUTURE: WHAT MIGHT WE SEE?

Extract Standardization and Molecular Allergology. For the great majority of environmental allergens, the specific epitopes to which pets are sensitized have not been determined. In the human allergy world, determining this has been a major effort over the past 10+ years, for several reasons. This information permits standardized dosing,
preparation of recombinant allergens, determination of T-cell epitopes, use of peptide immunotherapy, and other advances. New guidelines from regulatory bodies - which increasingly mandate standardization of extracts for human use - have prompted more clinical studies on optimal dose-finding for both safety and efficacy. In veterinary medicine, we don't know the optimal allergen dose for ASIT. Dose-dependency of ASIT efficacy has been long-observed in human trials, and recent studies are increasingly pinpointing these effects using both field studies and animal models.\textsuperscript{12,13} Not only the absolute dose, but dosing intervals have measurable effects on efficacy under some circumstances. Unfortunately, these effects remain unstudied for ASIT in animals.

**Alternate Formulations and Routes.** Canine AD appears to have remarkable similarity to human atopic dermatitis, in that this allows us to examine new research findings in people for ideas about what might be useful in dogs. In addition, murine models of AD being used to reflect the situation in people probably also mirror that in dogs. Potentially important advances in human beings that have yet to be explored in dogs include: use of recombinant major allergens or allergen peptides; enhancing the effect of allergens using adjuvant-like manipulations such as IL-10 inducers; packaging allergen in virus-like particles (VLPs); or in the case of SLIT, mucoadhesive polymers. Co-administration of immunomodulators such as CpG oligodeoxynucleotides or specific monoclonal antibodies might “push” the immune response in the desired, non-allergic direction while reducing inflammatory mediators of active disease, thus allowing ASIT to work more effectively. And last but not least, combining any of the above with new methods of administration such as intralymphatic injection might be tremendously exciting in dogs.

Recombinant allergens, where an entire major allergen molecule such as Der\textsubscript{f} 1 is produced synthetically, have their major advantage in purity and consistency so that dosing can be accomplished very precisely and uniformly every time, in every patient. This principle has been adapted for canine use in Japan with the product “Allermune-HDM,” a purified recombinant Der\textsubscript{f} 2 protein.\textsuperscript{14} Of course, most individuals are sensitized against a number of major allergens of each substance, such that a very large number of recombinant products might need to be manufactured and tested. Peptide immunotherapy carries this principle a step further; major allergens are examined to find specific, even smaller amino acid sequences within the allergen to which T-lymphocytes may react (“T-cell epitopes”), yet are far too small to trigger anaphylaxis. A combination of these peptides can be created that equals the full immunologic potency of the native allergen, without the risk of adverse reaction. Peptide allergen desensitization (PAD) is currently at its most impressive in human cat dander allergy, where initial trials demonstrate a beneficial and long-lasting effect (years) after administering only 4 doses over a few months.\textsuperscript{15,16}

New routes of administration of the desensitizing allergen has amazing potential for future success. Particularly notable in human allergic patients is the recent use of epicutaneous or intralymphatic administration. Epicutaneous immunotherapy can be accomplished with synthetic allergenic molecules that are modified such that they cause no cutaneous or systemic reaction, yet trigger a profound desensitizing effect as demonstrated in murine models.\textsuperscript{17} Clinical trials of intralymphatic ASIT in human allergic disease have shown substantial benefit; a few, very small doses of allergen painlessly injected into lymph nodes can produce a dramatic and long-lasting therapeutic effect with complete safety. Preliminary study in dogs has been reported.\textsuperscript{18,19}

Allergen-specific immunotherapy of food allergy is an exciting topic in human beings, in part due to the social difficulties and potentially lethal consequences of this condition. In the past, ASIT for food allergens such as peanut or shellfish was generally considered too dangerous to even try. This is an area where molecular diagnostics (i.e., identifying the specific epitopes to which the individual patient reacts), modified treatment allergens, and alternate methods of administration have now allowed ASIT to become possible. Large-scale trials of sublingual immunotherapy, in particular, have shown success with peanut allergy.\textsuperscript{20,21} Though food allergy is more easily controllable (thanks to easier dietary restriction) and of less serious consequence in dogs than in man, it will be useful for us to examine the progress that is being made in ASIT of food allergy in people for clues that may help us in better diagnosis and patient management of canine AD.

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WHAT IS DELUSIONAL PARASITOSIS?
Delusional parasitosis (DP) is a psychiatric disorder of humans in which patients have a false and fixed believe that their skin, body and/or environment are infested with parasites or inanimate objects, in a manner which is medically unexplained. Delusional parasitosis is categorized in the Diagnostic and Statistical Manual, 5th Edition, as a delusional disorder of the somatic type, a subgroup of delusional disorders. Most cases are secondary to neurologic or other psychiatric disorders, side effects of medications, substance abuse, or metabolic diseases, while some cases are due to a primary psychosis.

Around turn of century, case reports in European literature described peculiar symptoms in which patients considered themselves infected with parasites, whether they were or not. Ekbom theorized that abnormal sensations and paresthesia lead to delusions that parasites were present. The condition was subsequently referred to as “Ekbom’s syndrome”. Scott noted variables such as mean age of onset age 68, female gender preponderance, average length of symptoms prior to diagnosis of 4 years. More recent surveys of dermatologists and their observations includes Lyell in 1983 which confirmed that patients often brought scrapings of their parasites in matchboxes; the so-called “Matchbox sign.” Patients had seen entomologists and pest-control specialists prior to dermatologists. In Lyell’s sample of 282 patients there were 27 groups of patients, 24 pairs and 3 trios. Folie à deux (2 affected individuals) and folie à trois (three affected individuals) case reports are present in the literature. A more recent variant of this disorder is “Morgellons syndrome”, a disorder involving perceived infestation of fibers or threads and non-healing sores led the Center for Disease Control and Prevention to investigate and identified no common explanation for the symptoms.

WHAT DO WE KNOW ABOUT DELUSIONAL PARASITOSIS IN VETERINARY PRACTICE?
People with DP are likely to ask for help from pest control exterminators, entomologists, microbiologists and physicians/dermatologists, but less likely to seek help from psychiatrists. Affected people may also present their pets for veterinary care as they may believe their pets are infested with parasites (delusional infestation by proxy). Pet owners may believe they are also infested (double delusional infestation) and that their pets might be the source of their infestation. Delusional parasitosis by proxy or a shared delusion involving pets has been poorly investigated, with only a few anecdotal cases reported.

A recent study investigated the frequency of DP among pet owners presenting their animals to veterinary clinics. An e-mail survey was submitted to 32663 veterinary clinicians whom were members of the Veterinary Information Network (VIN), www.vin.com. From 714 responses, there were 724 suspected cases of DP, with 2.3% of the veterinary clinicians having seen at least one case. These findings suggest that DP is more common in veterinary practice compared to previously thought. Most respondents were primary veterinarians from the USA and Canada and most animals were dogs and cats (87%). In this study, a third of the clients reported symptoms not only on their pets but also on themselves and in about half of the clients that reported lesions on themselves their lesions were verified by the veterinarians, although the nature of the lesions could not be verified. Most clients were White women (78%) and most of these women (72%) were aged 30-60 years, with a wide distribution of social class. Most clients reported infestations with ectoparasites or worms.

HOW DOES DELUSIONAL PARASITOSIS IN PET OWNERS AFFECT VETERINARIANS AND THEIR PATIENTS?
Pet owners with DP can potentially hurt their pets in different ways, whether it’s by causing an animal’s skin to become dry and itchy from too frequent bathing, using medications or home remedies (which may include potential harmful substances), by picking often on their pets’ skin in the attempt to remove or catch “bugs”, or even abandoning their pets or requesting euthanasia for a non-existent problem. In the VIN survey report, approximately half of the clients suspected to have DP had treated their pets with pesticides of some sort. Additionally, it can also be very difficult
and frustrating to veterinarians to deal with clients with DP as they are often desperate for help and most of them will reject the absence of parasites in their pets reported by the veterinarians. They avoid psychiatrists and often lose faith in professional medicine. It is not uncommon for pet owners to also have seen multiple veterinarians, which might include specialists such as dermatologists. Veterinarians often do not know what to do and many end up “firing” their clients. Also, there is the concern that veterinarians, especially those unaware of this mental health condition in humans, prescribe unnecessary therapies for the pets, despite lack of evidence of animal disease, as shown in the VIN survey study, where approximately 50% of the clinicians, after several negative diagnostic tests, prescribed some form of therapy. This was often performed “just in case something was missed”, or because “the pet could benefit from deworming or ectoparasiticide prevention anyway”, or because “it would unlikely cause any harm and would satisfy the client”.

**EXAMPLES OF CASES OF CLIENTS WITH SUSPECTED DELUSION PARASITOSIS PRESENTING THEIR PETS TO A VETERINARY DERMATOLOGY REFERRAL PRACTICE**

As a dermatologist at a referral center at the University of Minnesota, Dr. Koch has seen 5 cases of suspected DP within a period of 10 years. Two of these cases are described here.

**Case 1:** A 6 year-old MN DMH and a 13 year-old FS DSH indoor cats were referred because of the complaint of parasites present in the household and affecting the owner and her cats for over 1 year. The owner developed skin lesions and the cats were reported to have dander and head shaking. She attributed the signs to ‘parasites’ that she would see and feel crawling on her own skin. She often tried to remove the parasites from her and from her cats. Her cats were seen by several veterinarians and they were treated with ivermectin injection and flea preventative without any response. The ‘parasites’ were described by the client with multiple detailed descriptions including: “small black objects that move very slowly”, “black spots that disappear” or “translucent mucoid substance on my hair and my cats’ hairs”. The client had exterminator interventions at her household, she burned her furniture, she moved from her house several times and at the time of the visit she was living in a hotel. She had been seen by several dermatologists and she reported to have had skin biopsy with no definitive diagnosis. She brought many samples in plastic bags and she showed her skin lesions caused by the “bites of the parasites”. She said she was referred to a psychiatrist but had declined, stating that she was not crazy and she was clearly desperate for help. The physical exams of the cats were unremarkable, except for very mild scaling. Diagnostic tests including skin scrapings, tape prep, trichogram, fungal culture and examination of the samples brought in many plastic ziploc bags showed no evidence of parasites. Weekly dips with lime sulfur 2% was recommended due the possibility of surface demodicosis or cheyletiellosis, although these conditions were unlikely. Owner was to call with an update in 2 weeks and to schedule an appointment with a dermatologist. Client called many times to continue to report ‘bizarre parasites’ and reported no improvement with the lime sulfur. It was recommended to discontinue lime sulfur and to speak to a social worker, which she did. Permission was asked to speak to her dermatologist. At the last phone call, her cats were at a rescue institution.

**Case 2:** A 9 year-old FS Dachshund was referred for parasites in the skin and eyes. The problem started 8 weeks prior to the visit with occasional licking of paws and abdomen. The owner had treated the dog with baking soda to “kill” the parasites. She reported that four other animals in the household, herself, her husband and her sister were also affected and that they all could see and feel the ‘bugs’ coming out of their skin, nose, eyes and body. She showed pictures and videos of the ‘bugs’, which were not visible to others. She had many skin lesions and she said some of the lesions were caused by ‘crawling parasites’ and others caused by her from itching or trying to remove them. She was very frustrated and stated several times that she was not crazy and seemed desperate for help. Client brought many samples as “proof” including a moldy old piece of chicken with “parasites” and different material she had collected from the animals, herself and the environment in plastic ziploc bags. The physical exam of the dog was unremarkable, except for very mild erythema interdigitally with matted hairs. Diagnostic tests including skin scrapings, skin and ear cytology and examination of the samples brought by client failed to identify parasites. It was suspected that the licking of the paws was due to the matted hairs or less likely allergies, but not associated with parasites. Recommendations included oatmeal baths, oral antihistamine and clipping of the hairs on the paws. Client was assured that there was no evidence of parasites on her dog. We recommended to have the other pets evaluated and for her to consult with a dermatologist. We also suggested her to speak to a social worker, which she declined. She was supposed to call with an update in 2 weeks. Client did not call back or returned for further care.
HOW CAN VETERINARIANS RECOGNIZE A CLIENT THAT MAY BE AFFECTED WITH DELUSIONAL PARASITOSIS?
Clients may present their pets for veterinary care with a fixed, false belief of infestation of their animal, selves, or family member.6-8 They will be concerned about itching and/or lesions on their skin or their animal’s skin, frequently from scratching. Symptoms and severity can vary among individuals, may have been present for months or years and clients may be desperate for help. Since care is frequently sought from primary care providers or dermatologists, they may have been evaluated and found to have no medical explanation for their symptoms. Most will not have consulted with a psychiatrist or psychologist. They may have been treated or treated their pets with anti-parasite creams or other treatments or may have used homemade remedies, which can sometimes be harmful. Clients often bring in materials in plastic bags or small cases believed to be evidence of infestation. In the VIN study,8 2/3 of the clients presented materials that they claimed to contain “parasites”.

A subset of DP patients will provide detailed descriptions or illustrations of aspects of their infestation, including “exhaustive descriptions of the parasite’s appearance, habitat, reproductive cycle, and points of body entry and egress”. In certain cases, exterminators may be involved to treat the home, which poses a potential risk of toxicity.9

SHOULD VETERINARIANS TREAT THE PETS FOR PATHOGENS WITHOUT SYMPTOMS OR MEDICAL EVIDENCE?
In the VIN survey report,5 most clinicians performed various diagnostic tests, although only 25 (3%) of the animals had any identifiable organism, which might or might not have been responsible for the lesions found on the animal. In addition, most pets had no lesions recognized by the veterinarians. However, DP is a diagnosis of exclusion and all reasonable steps must be taken to assess for parasitic infestations and to examine materials which might be brought by clients.

Failure to identify a pathogen does not always completely rule it out. For infestations that are known to be difficult to diagnose with tests such as sarcoptic mange, it may be within the standard of care to prescribe antiparasitic treatment for the animal. However, if the client is repeatedly requesting treatment and the veterinarian suspects there is no medical basis for the concern, the management strategy should shift to reassurance of lack of parasites and enlisting other members of the team, including referrals for support for the client, such as a their physicians. In other words, the veterinarian should not prescribe antiparasitic treatments to the animal with the only goal of reducing distress for the client outside of his/hers reasonable clinical judgment.8 Any treatment recommended for an apparent, but nonexistent, infestation has been reported to reinforce the patient’s delusion and the person’s validation of the infestation.8

HOW TO APPROACH CLIENTS WITH SUSPECTED DP AND THEIR PETS?
The first step is to acknowledge the client’s concerns. It is also very important to obtain a detailed history, perform a detailed physical exam of the pet(s), and attempt to identify the pathogen by performing a thorough clinical inspection of the pet for ectoparasites, such as fleas, and diagnostic tests including skin scrapings, tape prep, trichogram, fungal culture, etc. The samples brought by the clients should also be examined for any relevant parasites. Clients should be evaluated for the presence of lesions or self-trauma, since many parasitic diseases are zoonotic.8 Follow-up visits, if necessary, can provide the opportunity for serial evaluations. Ruling out a true infestation is warranted and can help strengthen the client-veterinarian relationship.8 Veterinarians and their staff should also avoid trying to convince their client of lack of infestation as these individuals can be difficult to convince, and that can frustrate and anger them.

Consider using this highly useful communication technique when communicating with distressed or highly anxious clients, mainly when an interaction with a patient is becoming tense, uncomfortable, emotional, angry, volatile, desperate or crisis. This technique is called “validation” and is a two-step process which involves understanding something legitimate about a client’s experience and actively communicating that understanding.11 If people do not think that they are being heard, they will escalate their emotion (anger, tears, frustration) until they feel they are getting the point across. In other words, clients will calm down when they are heard and understood. Just because you are validating their emotions and/or experience, does not mean that you are agreeing or approving their request or demand or that you like what they are saying. However, try to avoid using the phrase, “I understand how you feel”. Instead, use the following examples to clearly demonstrate that you are hearing what is being said. When possible, try to use the same words that they use, or rephrase what they are saying in an accurate way.
Example: Client: “Doctor, I brought my dog to you because he’s crawling with bugs, can’t you do something? This is taking over my life!”

Veterinarian using validation techniques: “I can’t even begin to imagine how upsetting it must be to feel like you have insects in on your beloved dog. This must be so frustrating and anxiety provoking. I can see why you would be upset” or “At this point we will need to expand the possible causes of these symptoms to develop a plan to evaluate this problem thoroughly and because there are a few steps involved, it seems to you like it will never happen. I see there’s a sense of urgency”.

The veterinarian may suggest the client to speak to a social worker to help reduce their stress and concerns or to consult with their physicians. If the client is describing their own dermatologic symptoms, it may be helpful to refer the client to a human dermatologist, to provide further assessment of this issue which is impacting both the client and the animal. Evaluation by a dermatologist can also provide reassurance to the patient that his or her symptoms are being evaluated seriously and can allow referral to a psychiatrist or psychologist, if appropriate.

It is also important to make sure the clients do not pose any risk to the pet(s), themselves or family members, in which case, proper local authorities or institutions should be contacted.

CONCLUSIONS
Many veterinarians are likely to encounter pet owners with suspected DP, which can be very difficult and frustrating to everyone involved. Veterinarians should make every attempt to rule in/out parasites on the pet(s). With no clinical or diagnostic evidence of parasites, or lack of response to required treatment trials, veterinarians should not share the belief of the infestation with the pet owners and should not prescribe treatment for their patients to simply benefit the owner. It is important to attempt to obtain help for suspected DP clients by consulting with family members or referring them to social workers, their physicians or dermatologists.

REFERENCES
Introduction:
The field of stem cell research, although not new, has been generating considerable interest in veterinary medicine in recent years. Stem cells have been touted as a “cure all” by some, due to their vast anti-inflammatory properties. It is important to understand the fundamentals surrounding their usage as a therapeutic tool in order to fully evaluate their indications and potential in the field of veterinary dermatology.

1. What are stem cells?
Stem cells are undifferentiated cells that are pluripotent, meaning they have the capacity to differentiate into different specialized cell types. Stem cells are found within embryos in the blastocyst phase of development (embryonic stem cells – ESCs), as well as almost all tissues in adult animals (adult stem cells - ASCs). Mesenchymal stem cells (MSCs) are non-haemopoietic cells that reside in, and can be easily harvested from adipose tissue, bone marrow, and the umbilical cord. Proposed characteristics defining MSCs include: i) their ability to adhere to plastics in standard culture conditions, ii) their expression of certain surface cell markers such as CD105, CD 73, and CD 90, and iii) *in vitro* differentiation into osteoblast, chondroblasts, and adipocytes (Harman, 2013). MSCs have generated interest as a therapeutic agent in the field of veterinary medicine because of the numerous purported effects on the immune system, making them an ideal therapeutic agent for the treatment of inflammatory and autoimmune disease.

2. What properties of MSCs make them ideal for use as a therapeutic tool in veterinary dermatology?
Stem cells are capable of “communication” with other cells in their local and more remote environments through the secretion of cell signaling factors and cytokines.

**MSCs are immunomodulatory**
MSCs have the ability to modulate a variety of cell types that play a role in both the adaptive and innate immune response. They inhibit dendritic cell maturation, suppress both CD4+ and CD8+ T-cell proliferation, inhibit the differentiation of B-cells and thus impair B-cell antibody production, and impede activation and expansion of natural killer cells. They have also been shown to promote immune tolerance by enhancing regulatory T-cell populations and associated regulatory cytokines, such as IL-10 (Harman 2013,).

**MSCs enhance wound healing**
MSCs are able to respond to wound healing cytokines and migrate to regions of tissue damage. They secrete a number of angiogenesis-related effector molecules such as hepatocyte growth factor, transforming growth factor-β, vascular endothelial growth factor, basic fibroblast growth factor, and angiopoietin (Harman, 2013). Fibrotic tissue deposition is attenuated through up-regulation of antifibrotic molecules and COX2 and PGE2. It has also been suggested that MSCs have the ability to sense their microenvironment and aid in pathogen clearance during resolution of skin injury (English, 2013).

**Immune privilege**
MSCs do not elicit an inflammatory or immune response when administered intraleSIONally or intravenous to non-self tissues. They are considered to have immune privilege likely because of their low MHC I expression and lack of MHC II expression (Patel *et al*, 2008, Trzil *et al*, 2016), as well as their ability to attenuate T-cell proliferation.

**Homing (chemotaxis)**
MSCs have demonstrated the ability to migrate to a site of injury after intravenous administration. Although their migratory mechanisms have yet to be fully elucidated, it is hypothesized that injured tissues express cell receptors for the recruitment and trafficking of stem cells.
3. How you do obtain stem cells?

Autologous stem cells are obtained through harvesting the patient’s own adipose tissue or bone marrow. Cell collection can be time consuming and requires the patient to have normal bone marrow function (Kim et al, 2013). Allogeneic stem cells are sourced from other healthy animals of the same species, while xenogeneic stem cells are of human origin. The marketing of stem cell products currently falls under the provisions of the Food and Drug Administration. It is recommended that all stem cell products are evaluated for immunogenicity, tumorigenicity, and toxicity prior to therapeutic use in companion animals. However, there are no FDA approved stem cell products at this current time, thus, it is unclear whether these guidelines have been widely employed (Hoffman and Dow, 2015).

MSCs from autologous, allogeneic, and xenogeneic sources have been used to treat a variety of dermatologic conditions such as wounds, canine atopic dermatitis, canine perianal fistula, and pemphigus foliaceus with variable success. Most studies performed in veterinary medicine been case reports, open-label studies, or have lacked a control or placebo group. With the “One Health” initiative gaining popularity, there is an increased interest in the use of companion animals for translational studies in diseases where canines and felines act as a spontaneous model of disease. Given the multipotency and impressive regenerative activity of stem cells, assuredly, we will continue to see a rise in MSC-based research in the future which will broaden our understanding of this innovative therapeutic tool.

References:

**Mechanisms of mesenchymal stromal cell immunomodulation.**

English K1.

**Author information**

**Abstract:** Multipotent mesenchymal stromal cells (MSCs) have generated considerable interest in the fields of regenerative medicine, cell therapy and immune modulation. Over the past 5 years, the initial observations that MSCs could enhance regeneration and modulate immune responses have been significantly advanced and we now have a clearer picture of the effects that MSCs have on the immune system particularly in the context of inflammatory-mediated disorders. A number of mechanisms of action have been reported in MSC immunomodulation, which encompass the secretion of soluble factors, induction of anergy, apoptosis, regulatory T cells and tolerogenic dendritic cells. It is clear that MSCs modulate both innate and adaptive responses and evidence is now emerging that the local microenvironment is key in the activation or licensing of MSCs to become immunosuppressive. More recently, studies have suggested that MSCs have the capacity to sense their environment and have a role in pathogen clearance in conjunction with the resolution of insult or injury. This review focuses on the mechanisms of MSC immunomodulation discussing the multistep process of MSC localisation at sites of inflammation, the cross talk between MSCs and the local microenvironment as well as the subsequent mechanisms of action used to resolve inflammation.
TEN BASIC PRINCIPLES OF SKIN BIOPSY
The ten basic principles of skin biopsy below help to insure quality skin biopsy results. Case specific information guides application and modification of these principles. For example, one should not wait three weeks after treatment withdrawal to biopsy severe disease that is deteriorating rapidly. Safety is always a top consideration.

1. Biopsy early in disease investigation
2. Biopsy before treatment or 3-wks after treatment withdrawal, if stable
3. Treat bacterial skin infections, if any, prior to biopsy
4. Do no scrub or prep the skin prior to biopsy
5. Use appropriate pain management, lidocaine etc.
6. Collect six punch biopsies (eight millimeter in diameter) for histology or wedge biopsies
7. Biopsy primary lesions that are active, newer and nearly fully developed; avoid secondary lesions and inactive lesions
8. Provide history, lesion description, pruritus status, treatment response and differentials
9. Provide digital pictures
10. Work with a dermatopathologist

THE KEY TO HIGH YIELD SKIN BIOPSY RESULTS
The key to high yield skin biopsy results is capturing within the sample the primary morphological features associated with lesion formation and thus disease pathogenesis. This is achieved by selecting the appropriate patient, skin lesion type and specific location within each different skin lesion type to biopsy.

1. Select the Patient: Not every patient is appropriate to biopsy. Select patients where the diseases or disease processes being considered can be differentiated and/or confirmed by a biopsy. Biopsy does not differentiate most causes for pruritic patients, especially when allergies and ectoparasitism are top differentials. Biopsy of the puritic patient is warranted when skin lesions are present that cannot be explained by classical allergic diseases or ectoparasitism, such as mass type lesions. Biopsy does not differentiate most endocrine alopecias, except for some cases of Cushing's disease, but then it is usually no needed to make a diagnosis. Avoid biopsy when only lesions of pyoderma are present and reconsider biopsy after management of infection. Avoid biopsy when active primary lesions are not present to biopsy.

2. Select Skin Lesion Types: It is essential to select active primary skin lesions for biopsy. Primary lesions result from the underlying fundamental pathological process of skin diseases and are therefore most diagnostic. Examples of primary lesions include papules, nodules, plaques, masses, pustules, vesicles, bullae and wheals. Secondary lesions should be avoided whenever possible. Secondary skin lesions evolve from primary lesions, or from the effects of chronic inflammation or self-trauma, and lack diagnostic specificity. Do not biopsy areas of self-trauma, whenever possible. Examples of secondary lesions include excoriations, scar, fissure, lichenification, callus, hyperpigmentation, etc. Some skin lesions develop as primary or secondary lesions and include for example scaling, crusts, erosions, ulcers, alopecia, atrophy and depigmentation (macules or patches). Observation of the temporal development of lesions, clinical context, skin lesion combinations and clinical evidence of self-trauma help to differentiate primary and secondary lesions.

3. Select the Biopsy Site Within Each Skin Lesion Type: Biopsy different primary skin lesion types in different areas to 1) optimize collecting the most diagnostic material and to 2) capture the area of the lesions that helps to explain the pathogenesis (see below). Many biopsies are rendered inadequate because only the margin was sampled. For example, the center is more valuable for alopecia, ischemic dermatopathy and all lesions in the
Biopsy nearly fully developed areas of primary skin lesions as these are usually the most diagnostic; this full development might occur at a margin, a center, an intermediate area or be the whole lesion. The relevant margin of a primary lesion is the active area at the periphery and not necessarily the true morphological margin. For example, erythema and/or hyperpigmentation can be the most peripheral change at the lesion margin but these are not very specific and a biopsy would be better just inside this rim where epidermal atrophy, erosions, and/or smoothing are occurring. Biopsy active areas within individual lesions - clues are adjacent erythema and observation of recent expansion. Avoid healed areas in a lesion, which can be central or peripheral.

**BIOPSY APPROACH TO SKIN LESION TYPES**

**Erosions and ulcers:** Biopsies should include the epidermal margin of active erosions and ulcers to find a cause for epidermal necrosis. Include also the central deep blood vessels to search for vasculitis/vascular thrombosis, especially when ulcers form. Deep wedge biopsies perpendicular to the ulcer margin are ideal. Avoid punch biopsies, if possible because orientation to the ulcer margin is lost. Alternatively, take multiple punch biopsies and alert the pathologist to trim perpendicular to erosion/ulcer margin. It is essential to avoid secondary lesions (those due to self trauma, a mass below, etc), which are very common causes of ulcers. Avoid shallow biopsies in central areas of ulcers – granulation tissue is usually present, it is not specific for any disease and secondary bacterial infection is common. Erosions and ulcers can be secondary to other primary lesions, especially fragile ones, and biopsies should be optimized for these primary skin lesions.

**Draining tracts and tissue tracts:** Collect large wedge biopsies in the center that includes the tissue tract in the middle of the biopsy. It is important to capture the contents of a draining tract encased in the biopsy sample because infectious agents usually are within the tracts. Punch biopsies are too small, fall apart and contents are usually lost. Also, most tissue tracts go deep, into the panniculus, and punch biopsies capture only superficial tissue and do not capture deep tissue areas. Choose a wedge biopsy for tissue tracts and collect more then one, whenever possible. Remember to collect additional samples for culture.

**Macules and patches:** Biopsy depigmented macules and patches at an active margin, especially areas with erythema (active inflammation) and a history of recent depigmentation. Biopsy the margins of depigmented areas with smoothing of the epidermal surface architecture and/or epidermal atrophy. Avoid non-active, static, lesions and secondary areas of depigmentation, such as those due to self-trauma, related to a scar, etc. Remember, depigmentation can remain for life or sometimes partially repigment and history, case monitoring for active change, and a search for erythema are often needed to determine active lesions. Avoid biopsy of hyperpigmented macules and patches because hyperpigmentation is almost always secondary. Before the biopsy of hemorrhage, rule out abnormalities of hemostasis (coagulopathy) in order to avoid complications with biopsy-induced hemorrhage and because there are no diagnostic lesions of hemostatic abnormalities in the dermis. Confirm that erythematous lesions are hemorrhage and not hyperemia by diascopy (see above). Biopsy hemmorhages in the center because red cells radiate from a central injured vessel. For hemorrhage on the sides of animals, biopsy dorsally in large lesions because hemorrhage settles in tissues. Collect deep wedge biopsies for large lesions when large deep vessels are thought to be injured. Collect multiple biopsies (5 to 6 punch biopsies or 2 to 3 wedge biopsies) because vessel lesions are often small and are very hard to capture in a histological section.

**Leukotrichia:** Biopsy the centers of newly/recently developed lesions. Collect multiple (5 to 6), large (8 mm) punch biopsies because it is very hard to capture enough affected hair follicles in histological sections. Avoid secondary leukotrichia related to scars, etc. Avoid lesions that are static for extended periods.

**Crusts:** Because most crusts are secondary, search for more primary lesions in the case to biopsy and optimize the biopsy approach for the primary lesion types discovered. If the patient is stable, possibly wait and biopsy when new primary lesions to develop. Sometimes crusts are primary, or hide more primary lesions below, and should be biopsied directly. During biopsy, keep the crust intact on the surface of the biopsy. Always submit crusts that fall off of a biopsy in the formalin biopsy container. In primary crusting dermatitis cases, collect additional crusts besides those on the biopsy for submission and request evaluation. Crusts often signal secondary bacterial infection, which should be cleared with treatments before biopsy, when ever possible, and then biopsy more primary lesions that remain. Investigate under crusts for evidence of other lesions, like ulcers, pustules, draining tracts, etc. Do not scrub away crusts prior to biopsy.
**Scale:** Collect multiple (5 to 6) punch biopsies from the central most affected areas of scaling.

**Papules, nodules, plaques and masses:** Papules should be centered in the biopsy, usually a punch biopsy is sufficient, and multiple biopsies (5-6) should be collected. Large nodules and plaques are best biopsied with 1 to 2 wedge biopsies at the margin in which 70-80% of the wedge biopsy contains lesional tissue. Avoid secondary ulcers, necrosis and scarring in larger lesions. Large, deep wedge biopsies are needed for ulcerated larger lesions in horses because non-specific proliferative granulation tissue can develop superficially and obscure the underlying, much deeper, cause. Always have a treatment plan for possible equine sarcoid because biopsy will increase aggressive growth behavior. Small nodules are sometimes best handled by complete excision, usually with a wedge biopsy. However, cytology should be performed to address the need for surgical margins and possibility for infectious causes and the need to culture part of the fresh sample prior to fixation in formalin for histology. Cysts are usually surgically excised in their entirety.

**Vesicles, bullae and pustules:** The main goal of the biopsy is to keep the fragile lesion intact. Therefore a careful wedge biopsy is used for larger lesions and delicate application of a punch biopsy is used for small lesions, when the entire lesion can fit into the punch biopsy and not be ruptured. The torsional forces induced by rotating a punch biopsy instrument often shear apart these fragile lesions. Take multiple biopsies. Remember to do cytology of pustules to search for acantholytic keratinocytes and bacteria. Submit pustule contents for bacterial culture. If bacterial infection is confirmed, treat bacterial infections prior to biopsy for underlying disease. Remember to save vesicular fluid contents for viral testing (culture, PCR), usually in large animals, when viral infection is a more likely possible cause.

**Epidermal collarettes:** Biopsy of this lesion is generally avoided because it is one of the characteristic lesions of superficial bacterial skin infection in the dog. Pyoderma should be treated and resolved prior to reconsidering biopsy of any remaining skin lesions. Bacterial culture and sensitivity testing may be warranted, if lesions are not responsive to treatment. Epidermal collarettes are mimicked by dried pustules, and a few other lesion types, that are more diagnostic and these should be sought on the patient. Epidermal collarettes are sometimes mistaken for lesions of “ring worm” that are circular, erythematous and have scaling.

**Lichenification:** Biopsy is to be avoided. Lichenification is secondary, is not specific and is not diagnostic, almost never.

**Atrophy:** When atrophy involves the entire skin thickness (epidermis and dermis), biopsy of the central, most affected, areas is recommended. When epidermal atrophy is a prime concern, multiple biopsies should be from actively expanding margins that are erythematous (inflamed), thin and have evidence of epidermal injury such as surface smoothing, erosions, and depigmentation. The biopsies should include a small portion of the more normal adjacent epidermis. Alopecia can occur from follicular atrophy and the biopsy approach for alopecia is described below. Atrophy in scars is not specific and scars should be avoided.

**Alopecia:** The key feature is to capture enough affected hair follicles. This is difficult because not many hair follicles are present in one histological section and not always is every hair follicle affected. To get enough affected hairs, always biopsy the center of alopecia and collect six biopsies and use eight millimeter punch biopsies. If available, include biopsy of a recently spreading margin, but this means the alopecia at the margin and NOT the area retaining hair near the lesion margin. Mark the line of hair growth on the alopecic skin surface with a permanent marker and biopsy through this line, which will allow for orientation of the biopsy along hair follicles for histology at the pathology lab. In subtle, or unusual cases, biopsy a normally haired area to compare to alopecic skin. This helps to identify hair follicle cycling abnormalities associated with alopecia as well as many subtle changes.

**Mucinosis:** Biopsy the central most affected areas and collect multiple biopsies. Samples can be fragile and care is needed to retain good tissue architecture. Collect mucinotic “vesicles” in the biopsy and this may require use of an excisional biopsy to keep these fragile structures intact.

**Pannicular/subcutaneous lesions:** Always biopsy the center of pannicular and subcutaneous lesions. This is important because it is hard to hit the lesion directly otherwise. The marginal tissue around pannicular lesions is often expanded and made firm by fibrosis, edema, compression, etc. and this area is usually not diagnostically specific. Use
a wedge biopsy for most animals and for large lesions to go deep enough and to collect a large portion of the lesion. Reserve a punch biopsy for very small animals (cats, ferrets, etc) with thin skin and for when deep lesions are small and will fit within the biopsy instrument.
Primary secretory otitis media (PSOM) is a disease that has been described in the Cavalier King Charles spaniel (CKCS). PSOM is a common disease in the CKCS and can be unilateral or bilateral.

**CLINICAL SIGNS:**
Signs suggestive of PSOM include hearing loss, neck scratching, otic pruritus, head shaking, abnormal yawning, head tilt, facial paralysis, or vestibular disturbances; however, none of these signs may be considered pathognomonic for PSOM. Most affected CKCS present with more than one clinical sign. Males and females are equally affected. Age at presentation has been reported to be between 11 months to 12.5 years of age. Some of these clinical signs may lead to the CKCS being suspected to have cervical disease before PSOM was recognized as a clinical entity and there is overlap between clinical signs of PSOM and syringomyelia (SM), another common disease in the CKCS.

**POSSIBLE PATHOGENESIS OF PSOM:**
Currently, the cause of PSOM is unknown but has been speculated to be due to a dysfunction of the middle ear or auditory tube – increased production of mucus in the middle ear or decreased drainage of the middle ear through the auditory tube or both. Auditory tube dysfunction is implicated in the pathogenesis of otitis media with effusion (OME) in humans and in humans may occur secondary to craniofacial abnormalities such as cleft palate. Hayes et al. evaluated the relationship between nasopharyngeal conformation and OME in CKCS (the test brachycephalic group) as compared to boxers (the brachycephalic control group) and cocker spaniel dogs (the mesacephalic control group). Two objective measures of nasopharyngeal conformation were evaluated and included the thickness of the soft palate on a midline sagittal MRI and the cross-sectional area (dorsoventral height and width) of the nasopharynx at the level of the rostral border of the tympanic bulla on a transverse view. Based on the results of their study, there was an association between OME and the brachycephalic conformation; furthermore, specifically for the CKCS breed, those with bilateral OME had a significantly greater thickness of the soft palate and reduced cross-sectional area of the nasopharynx compared to CKCS without OME. The study demonstrates an association between changes in the nasopharyngeal soft tissues and the incidence of OME, but cannot predict the nature of this relationship. These anatomic changes in the nasopharynx may impair auditory tube drainage.

Another proposed etiology of OME in humans is inflammation of the middle ear mucosa, usually due to bacterial infection, leading to auditory tube dysfunction. Positive cultures were obtained in 7 of 13 middle ears of CKCS with PSOM; however, the bacteria identified were only grown in enrichment medium, suggesting normal flora. Cole et al found the effusion sterile in 67% of the middle ears in 41 CKCS with PSOM. In those cultures with positive bacterial growth, thirty-four organisms were cultured, of which 20 (59%) only grew in prereduced thioglycolate broth, again suggesting these organisms were normal flora. Only 2/34 (6%) organisms had growth that one may have considered enough to imply infection. Both organisms were *Staphylococcus pseudintermedius*, which have been cultured from normal canine middle ears. Therefore, bacterial infections are unlikely to be the cause of auditory tube dysfunction leading to PSOM in the CKCS.

**PROGRESSION OF PSOM**
PSOM is an acquired condition and has been found to be progressive. Of 34 CKCS undergoing an initial MRI, 23 had no effusion, 10 had unilateral effusion, and one had bilateral effusion. By the second MRI scan, nine cases had progressed (26.5%); three from unilateral to bilateral effusion, five from no effusion to unilateral effusion and one from no effusion to bilateral effusion; eight that had either bilateral or unilateral effusion remained the same. Therefore, spontaneous resolution of PSOM is unlikely.

**DIAGNOSIS OF PSOM**
Evaluation of a CKCS with suspected PSOM should begin with an otic examination. If a large, bulging pars flaccida is identified, the diagnosis is made, and no other tests are required. However, in many CKCS with PSOM, the pars flaccida is flat, and radiographic imaging (e.g. computed tomography, magnetic resonance imaging) is needed to
confirm the diagnosis as the sensitivity and specificity of other diagnostic tests - pneumotoscopy, tympanometry and bulla ultrasonography - for the diagnosis of PSOM is low. Rarely, the tympanic membrane is already ruptured and the mucus plug can be visualized.

HEARING LOSS AND PSOM
In children, OME can cause impaired language and speech development because of hearing loss, which may lead to learning disabilities. Hearing assessment in the dog is performed utilizing electrodiagnostic testing. The brain responds to sensory stimuli by changes in electrical activity. These changes can be recorded and are referred to as evoked responses. Brain stem auditory evoked responses (BAER) can be used in the dog to help characterize hearing loss. Hearing loss is categorized as either sensorineural or conductive in origin. Sensorineural hearing loss may be due to injury to the cochlear hair cells in the inner ear (sensory) or to the auditory nerve (neural). Conductive hearing loss is due to abnormal propagation or obstruction of sound through the external, middle, and inner ears.

In the study by Harcourt-Brown et al. utilizing BAER testing with subsequent analysis of the latency-intensity function, the authors found that the middle ear effusion in the CKCS was associated with a mean conductive hearing loss of 21 dB nHL. Middle ear effusion was associated with an elevated BAER threshold; however, there was variability between individual cavaliers. Some cavaliers with a middle ear effusion had a normal BAER threshold (< 30 dB nHL) while in others the BAER threshold was markedly elevated (e.g. 100 dB nHL). None of the owners reported any hearing loss in their CKCS in this study. Stern-Bertholtz et. al. reported impaired hearing as a presenting sign of PSOM in 13% of their cases. In a study by Cole et al. thirty-one of 43 (72%) CKCS with PSOM had owner-reported hearing loss; however, only 19 of 31 (61%) regained hearing post myringotomy and middle ear flush, indicating that 29% of those CKCS had sensorineural hearing loss unrelated to PSOM.

TYMPANOSTOMY TUBES AND PSOM
Tympanostomy tubes have been used in human medicine to provide continual tympanic cavity ventilation and pressure equalization for the treatment of OME. Three studies have been published utilizing tympanostomy tubes as an alternative treatment to myringotomy and middle ear flushes in CKCS with OME/PSOM. The insertion of the tympanostomy tube provided relief of the presenting clinical signs; however, return of presenting signs in most cases was due to dislodgement of the tube from the tympanic membrane or plugging of the tube with exudate. Tympanostomy tube insertion may be an alternative to repeated myringotomy and middle ear flushes for the treatment of PSOM, but requires specialized equipment (e.g. operating microscope/video otoscope, tympanostomy tubes) as well as training to perform the procedure.

MANAGEMENT OF PSOM
Currently the most commonly utilized treatment for PSOM involves performing a myringotomy in the caudal-ventral quadrant of the pars tensa with subsequent flushing of the middle ear with sterile saline using a 5 French catheter with the aid of a video otoscope or operating microscope to remove the mucus from the bulla. One can expect to remove up to 1.1 mLs of highly viscous, opaque, mucus from the middle ear cavity by repeatedly flushing the middle ear with the sterile saline. However, the lack of visualization of mucus being expelled from the middle ear during the flush does not indicate that the CKCS does not have PSOM. In order to determine if the middle ear has been evacuated of mucus during the flush, radiographic imaging (e.g. computed tomography scan) should be performed post-flushing. In CKCS with hearing loss, pre-flush air- and bone-conducted BAER testing and post-flush air-conducted BAER testing is recommended to determine the extent of the hearing loss and whether the hearing loss is conductive, due to the accumulation of mucus in the middle ear, or if the hearing loss is sensorineural, and unrelated to the PSOM.

Post-flushing medications dispensed may include a short course of prednisone (0.5 to 1 mg/kg q 24h for seven days, then taper to every other day for two to three weeks) for the swelling and inflammation that may occur post-flushing. Unfortunately, since the cause of PSOM is not known, over time the mucus may recur. Reported treatments which did not appear to prevent the recurrence of the mucus include topical otic glucocorticoids, systemic glucocorticoids, systemic antibiotics, topical otic antibiotics, and mucolytic agents. However, use of the over-the-counter timed-release mucolytic N acetyl cysteine ([NAC] 600 mg q 24h) may help to extend the symptom-free time...
although no prospective studies have been performed to determine the efficacy of this treatment in the management of PSOM.

**BIBLIOGRAPHY:**

General principles for otitis treatment include identification of the causative otic pathogens, identifying and treating the primary cause of the otitis, controlling the predisposing factors, cleaning the ear canals, controlling inflammation, and evaluation for otitis media. Once an otic infection has been diagnosed, treatment may include topical as well as systemic therapy. At each recheck, the patients response to treatment should be monitored, cytology performed, and products changed accordingly. In the majority of the cases of infectious otitis externa, topical antimicrobial therapy alone is sufficient. In those ears with severe infections, or those that have long-standing chronic otitis externa, the addition of a systemic antimicrobial agent may be required to clear the infection that is present in the ear tissue as well as in the lumen of the ear canal. For those dogs with infectious otitis externa and otitis media, both topical and systemic antimicrobial therapy is usually required.

**EAR CLEANING/DRYING AGENTS:**

Once the ear has been cleaned in-hospital, or for routine ear cleaning at home, it is important to dispense a cleaning and drying agent. They are used to clean the ear, as well as keep it dry to discourage the overgrowth of bacterial and yeast organisms. The frequency of their use depends on the chronicity of the otitis externa and the severity of the infection. The goal is to begin treatment with the most frequent application (i.e. daily) and over time, decrease to a maintenance frequency (i.e. weekly). In some cases, daily treatment with an ear cleaning and drying agent (i.e. Epi-Otic Advanced) may be effective for resolution of bacterial and *Malassezia* otic infections.

1. **Douxo Micellar Solution (Sogeval)**
   Phytosphingosine, nonionic surface-acting agent, polidocanol, polysaccharides, light fragrance
2. **Epi-Otic Advanced (Virbac)**
   Salicylic acid 0.2%, disodium EDTA, docusate sodium, PCMX, a monosaccharide complex (l-rhamnose, d-galactose, d-mannose)
3. **Malacetic Otic (Dechra)**
   Acetic acid, boric acid, surfactants
4. **OtoCetic Solution (Vedco)**
   2% boric acid, 2% acetic acid, surfactants
5. **Oti-Clens (Zoetis)**
   Propylene glycol, malic acid, benzoic acid, salicylic acid
6. **OtiRinse (Bayer)**
   Water, benzyl alcohol, propylene glycol, fragrance, dioctyl sodium sulfo succinate, DM-DM hydantoin, glycerin, nonoxynol-12, salicylic acid, benzoic acid, lactic acid, aloe vera.

**TOPICAL GLUCOCORTICOIDs:**

Glucocorticoids are antipruritic, anti-inflammatory, and antiproliferative. During the acute stage of otitis, the ear canal becomes edematous and erythematous. As the inflammation progresses, the dermis becomes infiltrated with a mixed population of cells. Apocrine glands dilate and become hyperplastic, which leads to excessive cerumen production. Therefore, glucocorticoids are beneficial in decreasing the pain, pruritus, stenosis, and edema associated with otitis. In addition, they are effective in decreasing sebaceous and apocrine secretions. They are usually in combination with other agents but may be beneficial when used alone in allergic cases of otitis and some ceruminous otitis cases. It is important to use the lowest potency glucocorticoid at the lowest frequency needed to control the otitis to prevent iatrogenic hyperadrenocorticism.

1. **Cort/Astrin Solution (Vedco)**
   Burow’s solution, 1% hydrocortisone
2. **Synotic (Zoetis)**
   Fluocinolone acetonide in 60% DMSO
TOPOCAL OTIC THERAPY:
In all cases of infectious otitis, specific topical antimicrobial therapy is indicated. The topical otic preparations usually contain various combinations of glucocorticoids, antibiotics, and/or antifungals in a vehicle base. Selection of the active ingredient needed in the product for topical use should be based on cytology. It is important to remember that culture and susceptibility (C/S) results indicate the plasma level of an antimicrobial agent. The advantage of topical therapy is that you can achieve 100 to 1000 times the plasma level of the antimicrobial agent by administering it topically. The patient’s progress while on these medications should be monitored cytologically at each re-evaluation and the topical therapy adjusted accordingly. Remember that any topically administered otic product may cause a topical reaction with the most commonly implicated ingredients being neomycin and propylene glycol; therefore, it is important to be aware of both the active as well as inactive ingredients in the prescribed medications.

None of the commercially available otic topical treatments or the extra-label otic preparations are labeled for use with a non-intact tympanic membrane. However, most all of these products have been used to treat otic infections in dogs with otitis media. Always warn the owner of the possibility of neurological signs of ototoxicity while administering topical medications when the tympanic membrane is not intact. The otic topicals that I will not use in the ear with a non-intact tympanic membrane are those in an ointment or suspension base.

TOPOCAL ANTIMICROBIAL THERAPY:
Topical otic antimicrobials are needed for all cases of infectious otitis. It is important that sufficient volume be used to be able to reach the level of the horizontal ear canal. Most otics are administered once to twice daily – there are two exceptions; one that is administered monthly (Claro) and one that is administered q 45 days (Osurnia).

Topical aminoglycosides such as neomycin and gentamicin have good activity against gram-positive and gram-negative otic pathogens. Gentamicin may be ototoxic and should be used with caution in ears with ruptured tympanic membranes. However, three weeks of twice daily gentamicin sulfate (7 drops of 3 mg/ml) in buffered aqueous solution did not induce vestibular effects or deafness in Greyhounds with either intact or surgically ruptured tympanic membranes.3 Gentamicin and neomycin are available in many combination products, some which contain an antifungal and glucocorticoid.

1. Tresaderm (Merial)
   Neomycin sulfate, dexamethasone and thiabendazole
2. Otomax Ointment (Merck)
   Gentamicin sulfate, betamethasone valerate and clotrimazole
3. Mometamax Suspension (Merck)
   Gentamicin sulfate, mometasone furoate monohydrate and clotrimazole
4. Panolog Ointment (Zoetis)
   Neomycin sulfate, nystatin, thiostrepton, triamcinolone acetonide
5. easOtic Suspension (Virbac)
   Gentamicin sulfate, miconazole, hydrocortisone aceponate

Another aminoglycoside, tobramycin, is available as an ophthalmic suspension and is very effective against Pseudomonas otitis infections.

1. Tobramycin Ophthalmic Solution (generics)
2. Tobramycin

Fluoroquinolones have a broad spectrum of antibacterial activity against gram-negative and gram-positive bacteria.

1. Baytril Otic Emulsion (Bayer)
   Enrofloxacin (0.5%) and silver sulfadiazine (1%)
2. Posatex (Merck)
   Orbifloxacin, posaconazole, mometasone furoate monohydrate

Polymyxin has excellent in vitro activity against Pseudomonas with resistance rarely developing. Inactivated in purulent debris so the ear needs to be kept clean during treatment.

1. Neomycin, polymyxin B, and hydrocortisone (generics)
2. Surolan (ELANCO)
   Miconazole nitrate, polymyxin B sulfate, prednisolone acetate
Florfenicol has been available for a number of years as a fast-acting, long-lasting injectable antibiotic for treatment of bovine respiratory disease. Recently, two new otic medications have been approved for the treatment of bacterial (*Staphylococcus pseudintermedius*) and yeast (due to the addition of terbinafine in the products).

1. **Claro (Bayer)**
   - Florfenicol (15 mg/mL), terbinafine (13.3 mg/mL), mometasone furoate (2 mg/mL), purified water, propylene carbonate, propylene glycol, ethyl alcohol, and polyethylene glycol in a clear liquid solution.

2. **Osurnia (ELANCO)**
   - Florfenicol (10 mg/mL), terbinafine (10 mg/mL), betamethasone acetate (1 mg/mL), propylene carbonate, glycerol, hypromellose, phospholipid, oleic acid and BH in an off-white to slightly yellow translucent gel.

Tris-EDTA is a topical product that enhances the activity of topical antibiotics against otic pathogens by decreasing stability and increasing the permeability of the cell wall of the bacteria.

1. **TrizUltra + Keto (with ketoconazole) (Dechra)**
2. **TrizChlor Flush (with chlorhexidine) (Dechra)**
   - Mal-A-Ket Plus TrizEDTA Flush (with ketoconazole and chlorhexidine) (Dechra)
3. **T8Keto Flush (with ketoconazole) (Bayer)**

Chlorhexidine has broad-spectrum activity against many gram-positive and gram-negative bacteria and fungi; however, *Pseudomonas* may be resistant. Chlorhexidine may be ototoxic and should be used with caution in ears with ruptured tympanic membranes. However, a study done in normal greyhounds with experimentally ruptured tympanic membranes treated twice a day for 21 days with a topical application of 0.2% chlorhexidine failed to show any clinical vestibular or brainstem auditory evoked potential changes.

1. **Malaseb Flush (Bayer)**
   - Chlorhexidine 0.2%, miconazole 0.2%
   - Antifungal agents are used in cases of otitis caused by *Malassezia* or *Candida*.

Ingredients that are active against yeast include nystatin, posaconazole, thiabendazole, terbinafine, miconazole, ketoconazole, and clotrimazole.

1. **Clotrimazole (Otomax, Mometamax)**
2. **Ketoconazole (TrizUltra, T8keto)**
3. **Miconazole (generics, Surolan, easOtic)**
4. **Nystatin (Panolog)**
5. **Terbinafine (Claro, Osurnia)**
6. **Thiabendazole (Tresaderm)**
7. **Posaconazole (Posatex)**

**EXTRA-LABEL TOPICAL OTICS:**

An extra-label topical preparation containing enrofloxacin may be formulated using 1 part of the injectable enrofloxacin (22.7 mg/ml) added to 4 parts of an appropriate vehicle (Cort/Astrin for example). There have been no reports of clinical ototoxicity with this formulation; however, toxicity studies have not been conducted. It appears to be very effective for treatment of *Pseudomonas* otitis infections. For inflamed ears with yeast otitis without a bacterial component, topical antifungal, such as miconazole may be mixed 1:1 with dexamethasone.

**SELECTION OF TOPICAL ANTIMICROBIALS:**

So how would one decide which topical products to choose? Start with the chronicity of the otitis, the results of the otic examination, and otic cytology. Otic preparations that are ointment/suspension-based may not be as effective as those that are lotion, gel, solution or emulsion-based, if the ears are stenotic or hyperplastic, as may be the case in those patients with chronic otitis externa. In addition, those otic products that are reserved as second-line options would be recommended if the otitis is chronic. If the otitis is acute or if the ears are not stenotic or hyperplastic, any of the vehicles (lotion, solution, gel, emulsion, suspension, ointment) would be appropriate. Fluoroquinolones should be reserved for cases of bacterial otitis due to *Pseudomonas* otitis externa or those chronic infections that have not responded to other topical otic antimicrobials.
OVERTREATMENT:
In some instances, too vigorous topical therapy may result in maceration of the lining of the ear canal. Clinically, this appears as a large accumulation of white ceruminous debris in the ear canal. Cytologically, there is no infection, only desquamated epithelial cells. Treatment is directed at discontinuation or reduction of the frequency of topical medications.

SYSTEMIC ANTIMICROBIAL THERAPY:
Systemic antimicrobial therapy for infectious otitis externa and otitis media is controversial. In dogs with end-stage otitis externa and concurrent otitis media, bacterial organisms may be isolated from the exudate in the lumen of the vertical ear canal and middle ear cavity as well as from the tissue from these sites. Therefore, most agree that systemic antibiotics (based on culture and susceptibility testing) are indicated in patients with otitis media, patients with severe proliferative chronic otitis externa, patients with ulcerative otitis externa, patients where inflammatory cells are seen cytologically (indicating deeper skin involvement) and in patients where owners cannot administer topical therapy. The selection of systemic antimicrobial agent must be made based on C/S from the external ear (for otitis externa) and middle ear (for otitis media). However, therapy may be initiated based on cytologic results while awaiting the C/S.

Indications for systemic antifungal agents are similar to those above for bacterial infections and include patients with yeast otitis media, patients with severe yeast otitis externa, or in patients where owners cannot administer topical therapy. However, otic yeast infections require topical therapy in addition to systemic therapy for resolution. Both ketoconazole (5 mg/kg q 24 hr) and itraconazole (Sporanox 5 mg/kg PO q 24 hr or pulse-dosed 2 days on and 5 days off) have been used in dogs.

SYSTEMIC GLUCOCORTICOIDs:
Systemic glucocorticoids are used to decrease stenosis, edema, and hyperplasia of the vertical and horizontal ear canal to allow a complete otic examination as well as allow proper cleaning of the ear. They are also indicated in cases of allergic otitis externa. Initially, 0.5-1 mg/kg SID orally may be needed, followed by a low-dose, alternate day dosing schedule. As with topical glucocorticoids, the lowest dose needed should be administered to prevent the occurrence of side effects. In older patients, or those with concurrent diseases, it may be necessary to perform bloodwork prior to using glucocorticoids.

MAINTENANCE EAR THERAPY:
In some cases of otitis, once the otic medications are discontinued, in time the infection will recur. This is true in cases of chronic recurrent otitis externa where continual inflammation and stenosis have occurred along with increased cerumen production. This may result in an alteration in epidermal migration. In essence, the ear has lost the ability to clean itself. Therefore, ear maintenance therapy should be aimed at keeping the ear clean with a cleaning/drying agent.

Pseudomonas otitis infections may cause ulcerations and subtle changes in the microanatomy of the ear canal. Therefore, dogs that have had a previous Pseudomonas otitis infection require ear maintenance therapy, which would include a cleaning/drying agent as well as a TrisEDTA-containing product. Furthermore, if the Pseudomonas infection does recur, it is usually much more resistant to antimicrobial agents than the original infection, making resolution more difficult and time-consuming.

Dogs which are being worked up for the primary cause of their otitis should also be started on maintenance ear therapy. Underlying allergic diseases, such as atopy or food allergy, are the most common cause of recurrent otitis externa in the dog. Therefore, the maintenance therapy should consist of an ear cleaning/drying agent along with a low-potency steroid for the inflammation and pruritus.

MONITORING:
Re-evaluations include an otoscopic and/or video otoscopic evaluation to monitor response to therapy. Cytology of otic exudate is performed at each re-evaluation, while bacterial C/S is performed if the infection worsens or is non-responsive to therapy and where systemic antimicrobial therapy would be needed. Topical and/or systemic medications are modified based on these results.
If otitis media is present, otoscopic evaluation includes monitoring healing of the tympanic membrane. In some instances, repeat ear flushing under general anesthesia may be required to keep the ear canal clean to monitor healing of the tympanic membrane.

Surgery is necessary if middle ear polyps, neoplasia, foreign bodies, cholesteatoma, or osteomyelitis of the tympanic bulla is present. In addition, if there is inadequate response of the middle ear infection to otic flushing, myringotomy, and medical management, or if the otitis externa and/or otitis media is recurrent, surgical intervention may be necessary for resolution.

REFERENCES:
VETERINARY MEDICINE AT DISNEY

Geoff Pye, BVSc, MSc, DACZM

The application of veterinary medicine, including dermatology, at Disney will be presented. The scope and diversity of our practice and the challenges of such diversity (and how we meet those challenges) will be discussed, with examples of clinical cases

OBJECTIVE
Give insight to the application of veterinary medicine, including dermatology, at Disney
The active ingredients in Malaseb® Shampoo kill susceptible skin pathogens within minutes in *vitra*. A systematic review in 2012 found that there was a sufficient body of "good evidence" to recommend the use of 2% Chlorhexidine in combination with 2% Miconazole against *Malassezia* infections in dogs. With no dyes or added fragrances and available in a variety of sizes, Malaseb® should be your choice for the topical management of susceptible skin pathogens.

For more information, visit BayerDVM.com, call your local Bayer Sales Representative, contact your preferred distributor or call Bayer Customer Service at 1-800-633-3796.
### ABSTRACT SCHEDULE

#### FRIDAY, APRIL 28, 2017

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<td>2:00 pm - 3:00 pm</td>
<td>Advanced Imaging And Video Otoscopy</td>
<td>Mike Canfield, DVM, DACVD Rod Rosychuk, DVM, DACVIM</td>
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Assessing correlation between flea allergy dermatitis assessment scores and CADESI-4 scores in 17 dogs

M. K. LERACE*, M. S. CANFIELD*, F. SUN**, T. KATZ**

*Department of Veterinary Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA

Abstract: Validated severity scales such as Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4 are used to score skin lesions in dogs with atopic dermatitis. A validated scale does not exist to grade skin lesions in dogs with flea allergy dermatitis (FAD) and as such investigators have published FAD studies using various FAD scales or forms of CADESI for patient evaluation. Seventeen dogs were scored using a previously published FAD scoring system as well as CADESI-4. A single investigator performed the assessments at days 0, 30, 60, 90. Statistical analyses were performed to determine if a positive correlation existed between the two scoring methods. Correlations were calculated over all timepoints and at each timepoint. In addition, correlations with the FAD scoring method were calculated for cranial CADESI-4 locations 1-13 (ear and paw) and also for caudal CADESI-4 locations 14-20. Across all days and measurement locations, a positive correlation was found between CADESI-4 and the FAD scoring system (Rho=0.7669; p<0.0001). Correlations tended to be higher when calculations included all days than on any single day. Additionally, correlations tended to be higher for calculations that included all CADESI-4 locations or just the caudal CADESI-4 locations (Rho=0.8008; p<0.0001) than for those that included only the cranial CADESI-4 locations (Rho = 0.6620; p< 0.0001). Based on the tendency towards positive correlation between the FAD assessment used in this study and overall or caudal CADESI-4, one must ask the question if a separate scoring system is necessary for clinical trials for treatment of dogs with FAD.

Source of funding: Grant from Merck Animal Health.

Conflict of interest: M. S. Canfield has consulted for, received grants, and lecturing honorarium from Merck Animal Health.
Validation of a wearable sensor to quantify specific pruritic behaviors (scratching and head shaking) in dogs

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Abstract: Wearable technology is currently used in humans for functions including monitoring glucose levels, heart and respiratory rates and general activity. Wearable technology uses in dogs to date has focused on measurement of overall activity versus rest. Previous attempts to use wearable devices to measure pruritus in dogs have focused on increased overall activity in atopic dogs at times when activity was less expected. The objective of this study was to validate the ability of a high resolution multidimensional wearable sensor (Vetrax®, AGL, Norcross, GA, USA) to quantify specific pruritic behaviors (scratching and head shaking). To establish the difference between behaviors, sensor data and video were collected from 575 dogs. Video annotations specified exact behaviors at specific times in order to compare to sensor data. A computer algorithm was developed to interpret and differentiate between these behaviors. Test subjects were then utilized to test the system’s ability to accurately predict behaviors. Data from pruritic dogs was collected in a clinical examination room setting with both sensor and video collected. The video was then annotated to specify time and duration of specific behaviors. Data from the sensor was analyzed by the computer model facilitated by a second investigator who was blinded to the video and its annotation. Results for prediction of head shaking behavior included sensitivity and specificity of 75.49% and 99.84% respectively. Analysis of scratching produced sensitivity and specificity of 80.01 and 99.80% respectively. False positives were noted in 0.16% of cases for shaking and 0.20% for scratching.

Source of funding: AGL, Norcross GA, USA

Conflict of interest: Dr. Griffies is a member of the AGL Veterinary Advisory board and has received honoraria as a speaker.
Evaluation of reliability and validity of skin barrier function devices (Corneometer®, Colorimeter®, pH-Meter® and Vapometer®) in healthy and atopic dogs

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Abstract: Atopic dermatitis is associated with skin barrier defects. In people, non-invasive techniques are used to quantify the skin barrier functionality. In dogs, trans-epidermal water loss, stratum corneum hydration, and pH have been used to assess skin barrier function. However, no studies have determined the reliability and validity of such methods. Our goal was to assess the reliability and validity of Corneometer®, Colorimeter®, pH-Meter® (all by Courage+Khazaka electronic GmbH, Cologne, Germany) and Vapometer® (Delfin Technologies Ltd, Kuopio, Finland) in healthy and atopic dogs. Fifteen healthy and 15 atopic privately-owned dogs were used. Three repeated measurements using Corneometer®, pH-Meter®, Colorimeter® (erythema and skin absorption with tartrazine) and Vapometer® were obtained from groin, axilla, pinna, and interdigital space by three investigators. Intra- and inter-observer variability were assessed by coefficient of variation, Bland-Altman ratio, Spearman rank coefficients and absolute agreement. A very good reliability and validity was observed for both intra- and inter-observers for all devices except the Vapometer®. The most reliable and precise devise was the pH-Meter® on the pinna (κ=0.93-0.98). The VapoMeter® was the device with the highest intra- and inter-observer variability (κ=0.34-0.68). Atopic dogs had a significantly increased pH (groin: P =0.03; axilla: P =0.02) and erythema (inguinal: P =0.01 and axilla: P =0.02) when compared to healthy dogs. No differences between the two groups were detected using the Corneometer®, Vapometer®, or Colorimeter® (tartrazine absorption). Corneometer®, pH-Meter®, Colorimeter® are precise, reliable devices; their use in skin barrier function in dogs is recommended over the Vapometer®. A larger study is warranted to confirm these results.

Source of funding: University of Florida Foundation research grant.

Conflict of interest: None declared.
Cutaneous microRNA expression analysis by next-generation sequencing in experimentally-sensitized atopic beagles: a preliminary study

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Abstract: Although, canine atopic dermatitis (cAD) is a very common inflammatory skin disease, no many data are available on the genetic characterization of skin lesions in affected dogs. microRNAs have been demonstrated to be a good biomarker in inflammatory and neoplastic disease in people.

Objective: Evaluate the expression of microRNA in the skin of healthy and atopic beagles before and after exposure to Dermatophagoides farinae (DF).

Animals: Four atopic and four healthy, age-matched beagles

Material & Methods: Skin biopsies were taken from healthy beagles and from non-lesional (day 0) and lesional (day 28) skin of atopic beagles. Total RNA was extracted, quantified, and analyzed using miR-seq. Small RNA libraries were constructed and sequenced using HiSeq2500. The microRNA sequences were aligned to CanFam3.1 genome. Differential expressed microRNA (DEmiRNAs) were selected on the basis of the fold-change and statistical significance. Lists were filtered using fold-change ≥1.5 and p≤ 0.05 in T-test as thresholds and Benjamin-Hochberg p-value correction was performed.

Results: A total of 277 microRNA were sequenced. A total of 121 differentially regulated transcripts were seen between non-lesional atopic and healthy skin. Of these 2 were up-regulated and 119 were down-regulated. When lesional skin was compared to healthy skin, 44 transcripts were down-regulated and 1 was up-regulated. However, only two up-regulated transcripts were present in lesional skin when compared to non-lesional skin.

Conclusions: This is the first study in which dysregulation of microRNAs has been associated with lesional and non-lesional cAD. Larger studies are needed to understand the role of microRNA in cAD.

Source of Funding: Self-funded

Conflict of interest: None declared
Comparison of molecular methods in the characterization of Malassezia spp. carriage on the skin of healthy, non-lesional allergic, and atopic lesion induced dogs

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Abstract: The advancement of DNA sequencing technologies has revolutionized the study of skin commensals, how they contribute to health, and their role in disease processes. Recently, next-generation sequencing (NGS) has been applied to estimate the relative abundance of microorganisms on the skin of animals. The purpose of this study is to quantify the colony forming units (CFUs) of Malassezia spp. on skin swabs using real-time quantitative PCR (qPCR), to classify skin-associated Malassezia to the species level using high-throughput phylogenetic analysis of NGS data, and to re-assess the role of Malassezia in allergic dermatitis. To quantify the CFUs of Malassezia from skin swabs, two qPCRs specific for Malassezia and M. pachydermatis were performed on 212 skin swabs, taken from 26 dogs, comprising two previously published NGS studies. Malassezia sequences from one of these NGS studies were speciated by aligning sequences to a published Malassezia database using the bioinformatics software Pplacer. The skin of allergic dogs without lesions had significantly fewer CFUs of Malassezia compared to healthy skin (P<0.05). Atopic dogs exposed to house dust mite showed a significant reduction in the CFUs of Malassezia from pre-exposure to seven days after lesion development (P<0.05). From 37,000 NGS Malassezia sequences from healthy and non-lesional allergic dogs, the top three most abundant species included M. pachydermatis, M. globosa, and M. restricta. Additional molecular methodologies are proving to be useful in the study of skin commensals, and further elucidate how these commensals play a role in allergic skin disease.

Source of funding: Self-funded

Conflict of interest: None declared.
Characterization of the skin microbiome of dogs with strong body odor and the effect of a spot-on product


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Abstract: Next-generation sequencing (NGS) has been used to evaluate the cutaneous microbiota. This study investigated the bacterial and fungal microbiota on the skin of healthy dogs with and without strong body odor and how the use of Dermoscent Essential 6® spot-on (Bayer, Shawnee Mission, KS, USA) influenced body odor and the microbial communities. We hypothesized that dogs with strong body odor would have dysbiosis of their skin microbiota with increased abundances of *Staphylococcus* and *Corynebacterium* spp. Skin swab samples were collected on day 0 and week 4 from two body regions (axilla and dorsum) of eight dogs without, and 16 with strong body odor. Dogs with strong body odor were randomly divided into a placebo or treatment group, and received four weekly topical applications. DNA was extracted and the bacterial 16S rRNA was amplified and sequenced using NGS. Additionally, quantitative PCR for *Malassezia*, *Staphylococcus* spp., and *Staphylococcus pseudintermedius* was performed. Average body odor grading was higher in the strong body odor groups compared to the control group at day 0. Body odor was significantly reduced at week 4 in the treatment group (P <0.05), but not the placebo group. NGS analysis demonstrated significant differences in bacterial communities between study groups (P <0.05). Increased abundances of *Malassezia*, *Staphylococcus* spp., and *Staphylococcus pseudintermedius* in strong odor dogs at day 0 by quantitative PCR (P <0.05) expanded upon NGS findings. Healthy dogs with strong body odor had reduced diversity of their skin microbiota. Further investigations are needed to better identify microorganisms responsible for body odor in dogs.

Source of funding: Laboratoire de Dermo-CosmetiqueAnimale and Merial Veterinary Scholars Program.

Conflict of interest: This study was partially funded by Laboratoire de Dermo-CosmetiqueAnimale.
The effect of breed and environment on the cutaneous microbiota of cats

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Abstract: The feline skin is inhabited by a diverse microbiota. The bacterial skin microbiota varies across different body sites in healthy cats. The fungal microbiota is less specific to body site, but is instead varied between individual cats. Although the microbiota between cats with and without allergic skin diseases has been evaluated, no study to date has evaluated differences in microbiota related to cat breed or housing environment. The objectives of this study were to compare the differences in the bacterial and fungal microbiota between cat breeds and between indoor and outdoor cats. Additionally, we evaluated whether results from a different cohort of cats would correlate with our previous studies. To do this, cats of five breeds (Bengal, Cornish rex, Devon rex, Siberian and sphynx) and indoor and outdoor domestic shorthair cats were swabbed at five sites (axilla, dorsum, ear canal, nostril, and oral). DNA was extracted from the swabs and bacterial and fungal next-generation sequencing was performed. Resulting sequences were processed using QIIME. The results revealed many differences between the cat breeds and indoor and outdoor cats, including higher proportions of Fusarium spp. found in the nostril of outdoor cats when compared to indoor cats (p<0.01), differences in the relative abundance of Malassezia spp. between cat breeds (p=0.043), and differences in the diversity of the bacterial and fungal communities between breeds (p<0.0001, p<0.0001, respectively). Our results indicate the environment that cats live in and the breed of cat affects the microbial communities on the skin.

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Conflict of interest: None declared.
The otic microbiome in canine otitis externa

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Abstract: Otitis externa (OE) in dogs is frequently attributed to Staphylococcus spp., Malassezia pachydermatis, or Pseudomonas aeruginosa. The microbiota in the ear may influence bacterial or fungal overgrowth and infection, but this community is poorly characterized. This study included 30 dogs with OE characterized by clinical examination and cytologic evaluation of ear swabs: 10 with exclusively cocci (≥ 5/high power field [HPF]) on cytology and culture of a pathogenic species of Staphylococcus; 10 with predominantly rods (≥ 1/HPF) on cytology and exclusive culture of P. aeruginosa; and 10 with exclusively yeast that were morphologically compatible with Malassezia spp. (≥ 5/HPF). Ten control dogs free of otic and dermatologic disease were included, with no organisms seen on cytology. Microbiome analysis was performed by sequencing the 16S rRNA gene and the internal transcribed spacer (ITS-1) region for bacterial and fungal community analysis, respectively. Following quality filtering of sequence data, 34 samples (192,4232 16S rRNA gene sequences) were analyzed (10 controls, eight per OE group). Bacterial community analysis showed Staphylococcus to have the greatest relative abundance across all groups and alpha diversity to be lowest in dogs cytologically positive for cocci compared to all groups. Dogs cytologically positive for rods had a greater relative abundance of Pseudomonas compared to other groups, and dogs cytologically positive for yeast or rods had greater relative abundance of Malassezia compared to other groups. Dogs with OE had decreased fungal diversity compared to controls. Multimodal evaluation of the microbiome in disease states provides insight and correlates with clinical practice.

Source of funding: ACVD Resident Research Award/Grant.

Conflict of interest: None declared.
Canine mast cell degranulation induced by a newly identified toxin from Staphylococcus pseudintermedius

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Abstract: No mechanisms have been described in dogs supporting causation and perpetuation of atopic dermatitis by Staphylococcal infection although clinical observations would support such associations. A recent study of delta toxin from Staphylococcus aureus in mice demonstrated a range of effects with the potential to exacerbate and cause atopic dermatitis in humans. Our study identified a homologue of delta toxin from Staphylococcus pseudintermedius. Degranulation of both murine mast cells in vitro and canine mast cells in vivo was demonstrated. Isolates of Staphylococcus pseudintermedius were obtained from four atopic dogs in New Zealand. A purified supernatant of Staphylococcus pseudintermedius cultures was found to degranulate foetal skin-derived murine mast cells in a β-hexosaminidase assay. This compared histamine release induced by supernatant from the test isolates with that of saline and culture medium controls, supernatants from Staphylococcus aureus and a non-delta toxin producing species of Staphylococcus. Further refining of the supernatant allowed the identification of the delta toxin peptide. Intradermal injection of a diluted supernatant from the four isolates into the skin of five mixed age non-atopic dogs was followed by biopsy of each site 15-20 minutes later. Controls were phenol buffered saline and compound 48/80. Histopathological assessment showed significantly greater mast cell degranulation (p<0.01-0.05) at the test supernatant sites. This finding may be relevant to the pathogenesis, diagnosis and treatment of canine atopic dermatitis, otitis and bacterial hypersensitivity.

Source of funding: NZ Companion Animal Health Foundation.

Conflict of interest: None declared.

Animal Ethics Approval: Kaiawhina AEC 013/15.
Abstract: Two pigment types determine the immense variety in color of mammalian hair and skin, and the genetics of their production is conserved among most species. Converting between synthesis of eumelanin, or black/brown pigment, and phaeomelanin, or yellow/red pigment, depends upon the interaction of the Melanocortin 1 receptor (Mc1r) with the Agouti signaling protein (ASIP), as well as CBD103 (a α-defensin protein) in domestic dogs. Activation of Mc1r by α-melanocyte stimulating hormone (α-MSH) or CBD103 despite functional ASIP results in a uniform black coat and is the dominant mode of inheritance. A recessive mode of inheritance occurs via a single nucleotide variant causing an arginine to cysteine substitution at codon 96 of the ASIP, known as non-agouti black, and is reported only in herding breeds. The Schipperke is one breed reported to carry the recessive trait for a uniform black coat and this is the only color coat accepted as breed standard. The ASIP coding sequence of 10 Schipperkes was determined and a missense alteration was identified in exon 4, C427T, that predicts an arginine to cysteine substitution at codon 96. Of the 10 dogs, nine were affected with a disorder causing lightening of the coat to a reddish color progressing to truncal alopecia, and one dog was normal coated. Five dogs were heterozygous for the reported mutation, non-agouti R96C, including the normal coated dog, and four dogs were found to be homozygous for the mutation. Sequencing of additional dogs will better characterize this mutation in the Schipperke breed.

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Conflict of interest: None declared.
IL-31 receptor alpha isoform transcription in normal dogs and atopic dogs with and without allergen challenge

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Abstract: Interleukin-31 (IL-31) has been strongly implicated as an important factor in several pruritic skin diseases. The effects of IL-31 are mediated by a receptor complex, comprising oncostatin M receptor β and the cytokine-specific receptor subunit IL-31 receptor alpha (IL-31RA). In dogs there are currently four IL-31RA mRNA sequences predicted, encoding two protein isoforms: X1 and X2. IL-31RA isoform X2 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 clinically healthy dogs and atopic dogs with or without allergen challenge. Skin samples were taken from seven clinically normal dogs, and from two groups of seven atopic laboratory beagles which were either unchallenged or had been exposed to 14 days of allergen challenge. Real-time PCR assays were designed to amplify mRNAs encoding either IL-31RA X1 or IL-31RA X2. Primer specificity was confirmed by PCR product sequencing. Transcription of both IL-31RA isoforms was significantly lower in the atopic beagles exposed to allergen than in the clinically normal dogs (P<0.05). Transcription of IL-31RA X2 was significantly higher than that of IL-31RA X1 in all three groups (P<0.005). The majority of IL-31RA mRNA transcribed in dogs appears to encode the shorter isoform, which may not bind IL-31. The function of this short IL-31RA isoform remains to be confirmed.

Source of funding: Self-funded.

Conflict of interest: None declared.
Correlation of serum IL-31 with disease severity in atopic dogs
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Abstract: IL-31 is a newly described cytokine that is believed to play an important role in pruritus in atopic dermatitis. IL-31 levels positively correlate with disease severity in people with atopic dermatitis. Currently there is no study that has reported on such correlation in atopic dog. The purpose of the present study was to evaluate the correlation between IL-31 levels before and after allergen challenge in a colony of atopic beagles that had been previously sensitized to house dust mites. A CanineAtopicDermatitis andExtentSeverityIndex (CADESI-03) score was assessed at the same time as the blood draw to measure serum levels of IL-31. An ELISA kit (Neoscientific, Cambridge, MA, USA) labeled to detect canine IL-31 was used. Serum samples from 16 dogs were drawn at baseline and on Day 28 after twice weekly dust mite allergen challenge. Correlation between CADESI-03 scores and serum IL-31 levels was not detected at baseline (Pearson, P =0.3, r =0.006). However, after flare-ups of dermatitis were triggered with allergen exposure, a significant positive correlation was detected between serum IL-31 and CADESI-03 scores (P =0.004, r =0.45) highlighting that individuals with higher IL-31 levels were the ones that developed worse disease when exposed to allergens. These results indirectly support a beneficial effect of therapies aimed at blocking this cytokine in atopic dogs.

Source of funding: Self-funded.

Conflict of interest: None declared.
Abstract: Eustachian tube dysfunction (ETD) is a rare condition described in humans. It is associated with several subtypes and causes. To the best of the authors’ knowledge, this is the first report of ETD in a dog. A 6-year old female spayed dachshund presented for evaluation of chronic left ear otitis. At 6 months of age, the dog started showing sneezing after drinking water which progressed to presence of liquid and food in the ear canal after drinking and eating, a foamy otic material and intermittent episodes of head shaking. Multiple topical and oral therapies were unsuccessful. Video-otoscopic examination of the left ear revealed mild to moderate erythema, mild purulent and frothy discharge, moderate ceruminous gland hyperplasia, mild stenosis and a partial tear at the pars tensa. During otic flushing, saline was draining through the mouth. Left ear cytology showed many neutrophils, few macrophages, many rods and occasional cocci. Culture and susceptibility of the otic exudate from the left ear showed multidrug resistant Pseudomonas aeruginosa and Escherichia coli. Abnormal computerized tomography scan findings were limited to the left ear and included marked lysis of the tympanic bulla, marked dilation of the Eustachian tube and an open communication between the left tympanic bulla and the nasopharynx. Histopathological findings were compatible with chronic otitis externa and otitis media without evidence of foreign body or neoplasia. Total ear canal ablation, bulla osteotomy, Eustachian tube ablation and 2 weeks post-surgery marbofloxacin (Zeniquin; Zoetis Inc., Kalamazoo, MI, USA) at 5 mg/kg orally q24h were curative.

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Conflict of interest: None declared.
Anatomical distribution of aural inflammatory polyps in seven cats

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Abstract: Aural inflammatory polyps (AIP) are non-neoplastic pedunculated lesions in the external ear canal, auditory tube (AT), or tympanic cavity (TC). However precise origin and location of the polyp growth are still unclear. The purpose of this study was to evaluate anatomical distribution of the polyp by clinical features and diagnostic images, especially MRI. Seven cats with AIP were referred to the dermatology service. Domestic shorthair cats (3/7, 42.9%) and Norwegian forest cats (2/7, 28.6%) were over-represented. The most common clinical signs were otorrhoea in six cases (85.7%), head tilt in two (28.6%) and Horner’s syndrome in one (14.3%) case. MRI revealed a mass within the external ear in six (85.7%), the middle ear including the epitympanic recess (ER) in all, the tympanic cavity proper in five (71.4%), and ventral cavity in three (42.9%) of the cases, while no masses extended into the AT. Otic polyps were resected with a per-endoscopic transtympanic traction technique. The remnants of all polyps consistently protruded from the ER and a semiconductor laser was applied to vaporize them. No polyp recurred after the laser therapy in any cases during a median follow-up of 10 months (3-18 months). The polyps in all cases consisted of a core of fibrovascular tissue mixed with small numbers of glandular ducts composed of single-layered, ciliated cuboidal epithelium, which is one of the features of the ER. Based on these findings, it is suggested that AIP arise from the ER and expands into the external ear canal and/or TC.

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Conflict of interest: None declared.
Feline ceruminous cystomatosis in the ears of 25 cats (2014-2016)

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Abstract: Ceruminous cystomatosis is a rare condition involving the ceruminous skin glands that often presents clinically with characteristic bluish-black cystic lesions along the concave pinna, tragus region, and the external ear canal. Literature reports cystomatosis to occur most commonly in middle-aged to older Abyssinian, Himalayan, and Persian cats. This study is a retrospective analysis of 25 cases of cystomatosis (43 ears) with nine cases confirmed via histopathology. Cystomatosis was noted in 12.8% (25/195) of individual cats referred for dermatology consultations with various degrees of otitis and in 0.1% (25/23087) of total cats seen by the hospital from 2014 to 2016. Domestic shorthair cats were most commonly affected (56%, [14/25]). Other affected breeds included: four domestic medium hair cats (16%), four domestic longhair cats (16%), one Persian mix (4%), one Himalayan cat (4%), and one Abyssinian cat (4%). The average age at diagnosis was 10.86 years (range: 3.5-17 years). Cystomatosis was found in six spayed females (24%), one intact male (4%), and 18 castrated males (72%). Two cats progressed from benign cysts to inflamed adenocarcinoma (n=1) or squamous cell carcinoma (n=1). Three cats exhibited clinically similar cystic lesions around the eyes or lips. Feline papillomavirus type 2 DNA was detected in 3/9 cats. In this study, cystomatosis was found to be more prevalent in non-purebred cats and did not appear to be associated with papillomavirus infection or other specific identifiable illnesses. Further studies are needed to investigate the etiology of cystomatosis.

Source of funding: None declared.

Conflict of interest: None declared.
Primary secretory otitis media-like syndrome in a cat

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Abstract: Primary secretory otitis media (PSOM) is a condition described in cavalier King Charles spaniels, characterized by sterile, mucoid bulla effusion. Common clinical signs include head scratching, pain, and vestibular neurologic deficits. Treatment involves flushing the tympanic bulla via myringotomy. A similar condition has not yet been reported in cats. We report a case similar to PSOM in a cat. The case describes a 1-year-old male intact domestic shorthair cat presenting for a suspected polyp in the left ear, observed by the primary veterinarian. Clinical signs included head scratching, sneezing, nasal discharge and a left sided head tilt. Computed tomography revealed bilateral bulla thickening and effusion. A polyp was not observed on video otoscopy, but there was a ceruminous plug overlying a bulging pars flaccida in the left ear, which may be mistaken for a polyp. Both external canals were otherwise unremarkable. Myringotomy was performed bilaterally and a copious amount of thick, grey-white viscous mucoid material similar to that in PSOM was removed by flushing and suctioning in both ears. Culture of the left middle ear was taken at time of myringotomy, which grew Bordetella bronchiseptica. Initial clinical signs were resolved at recheck 2 months later. The primary cause may be auditory tube dysfunction as suggested in PSOM, with Bordetella bronchiseptica as a contaminant. Alternatively, this may be infectious otitis media from Bordetella bronchiseptica, which has not been reported. However, presence of Bordetella bronchiseptica in the middle ear may still suggest abnormal auditory tube conformation.

Source of funding: Self-funded.

Conflict of interest: None declared.
Development of the canine dermatitis quality of life and treatment satisfaction questionnaire: a tool for clinical practice

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Abstract: Characterized by pruritus leading to excoriations and skin lesions, canine atopic dermatitis (AD) can impact the owner and canine’s quality of life (QoL). An owner-completed electronic questionnaire was developed using methodology informed by the FDA questionnaire development guidance to provide a valid measure of canine and owner QoL, facilitating owner-veterinarian communication and assessing treatment benefit beyond clinical parameters specifically in AD. The objective was to evaluate the conceptual comprehensiveness of the questionnaire in assessing canine and owner QoL, and treatment satisfaction (TS). Owners of dogs with veterinarian-confirmed AD and treated with a variety of systemic and topical therapies (n=20) were qualitatively interviewed. Breed and pruritus severity varied. Open-ended questioning was used to explore concepts spontaneously; focused questions were used to assess understanding and relevance of items. Verbatim transcripts were subject to thematic analysis using Atlas.ti and Excel. The most frequently owner-reported signs of AD were itching (n=19;95%), biting/nibbling (n=15;75%), licking (n=15;75%), and sleep disturbances (n=13;65%). All owners reported impacts on emotional wellbeing including empathy (n=14;70%) and sadness (n=12;60%). Impacts on finances (n=19;95%), sleep (n=17;85%) and daily routine (n=17;85%) were discussed, while efficacy (n=17;85%), financial cost (n=15;75%) and mode of administration (n=13;65%) were important TS concepts. Items were well-understood and all concepts in the final questionnaire were relevant to owner experiences. The Canine Dermatitis QoL and TS questionnaire is conceptually comprehensive and suitable for use in clinical practice to enhance owner-veterinarian communication and assessment of treatment benefit.

Source of funding: This work was supported by Zoetis Inc.

Conflict of Interest: Adelphi Values received payment from Zoetis for the conduct of and consulting on this study.
**Diphenhydramine pharmacokinetics and pharmacodynamics after oral and intravenous administration of diphenhydramine and oral administration of dimenhydrinate to healthy dogs**

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**Abstract:** The objective of this study was to determine the pharmacokinetics and pharmacodynamics of diphenhydramine in dogs after administration intravenously (1 mg/kg), orally (5 mg/kg), or administered as dimenhydrinate (orally, 10 mg/kg), which is a combination of 54% diphenhydramine and 46% 8-chlorotheophylline (5 mg/kg diphenhydramine). Each drug was administered to six healthy fasted research mixed-breed dogs in a crossover design. After drug administration, blood samples were collected for pharmacokinetic analysis. Pharmacodynamic response was measured by histamine-mediated cutaneous wheal formation. The mean systemic availability (F) of diphenhydramine was 7.8% and 22.0% after oral administration of diphenhydramine and dimenhydrinate, respectively. The mean maximum (C\text{MAX}) concentration after oral treatment with diphenhydramine and dimenhydrinate was 36 and 124 ng/mL, respectively. The terminal half-life after intravenous and oral diphenhydramine, and oral dimenhydrinate was 1.9, 5.0, and 11.6 hours, respectively. Our results show that there is approximately 3x greater oral absorption and longer half-life of diphenhydramine when administered as the combination product dimenhydrinate. However, there was large variability in our data and one dog was an extreme outlier. We also documented high and sustained plasma concentrations of the major metabolite diphenylmethoxyacetic acid in these dogs (mean half-life 42 and 67 hours). We found high concentrations of 8-chlorotheophylline (13 µg/mL) after administration of dimenhydrinate. Although diphenhydramine plasma concentrations reached concentrations that are considered effective in people (> 25 ng/mL), there was only moderate wheal reduction in our dogs after administration of all treatments and plasma drug concentrations did not correlate with the percentage reduction in histamine-induced wheal formation.

Source of funding: Self-funded.

Conflict of interest: None declared.
Diluted sodium hypochlorite (bleach) in dogs: antiseptic efficacy, local tolerability, and effect on skin barrier lipids and inflammation

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Abstract: Diluted sodium hypochlorite (bleach) represents an inexpensive and widely available topical antiseptic, but there are no tolerability and efficacy data using this antiseptic solution in veterinary dermatology. In the present study, we evaluated the antibacterial effect and tolerability of topical diluted bleach application in healthy dogs. We also assessed its effect on skin barrier lipids and anti-inflammatory properties on canine cultured keratinocytes. The cell viability of primary keratinocytes treated with tap water and diluted hypochlorite at 0.005% and 0.01% reduced the percentage of viable cells by 10%, but this difference was found to not be significant between groups. The exposure of primary keratinocytes to 0.005% diluted hypochlorite significantly reduced the induction of inflammatory genes chemokine ligand-2 (CCL2; at 3 h, p=0.002; at 6 h, p=0.015) and thymus and activation-regulated chemokine (CCL17; at 6 h, p=0.032). There were no changes in skin lipids, in either ceramide or non-ceramide fractions, in stratified epidermal construct cultured for 17 days with 0.05% hypochlorite or tap water control. Topical hypochlorite at 0.05% and tap water applied to both sides of the dorsal thorax of four healthy Maltese-beagle crossbred dogs were well tolerated; there was no development of skin erythema or scaling. Although a marked reduction in bacterial counts was seen within 20 minutes of diluted bleach application compared to the tap water control, this was only marginally significant (p=0.06). The results indicate that topical diluted bleach solution, at 0.05% and 0.005% hypochlorite concentrations, is a well-tolerated antiseptic that also exhibits anti-inflammatory properties.

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Conflict of interest: None declared.
Idiopathic ulcerative dermatitis and response to treatment in two fancy mice

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Abstract: Idiopathic ulcerative dermatitis (UD) is a spontaneous, progressive and debilitating syndrome commonly reported in laboratory C57BL/6 mouse strain. Although the exact etiology of murine UD has not been clearly identified, the disease is multifactorial, including genetic and environmental components. UD diagnosis is done ruling out other causes, such as mycotic, bacterial and parasitic skin infections, and by clinical aspect and by failure to respond to treatments. Two female fancy mice (Mus musculus) presented with chronic, locally extensive, alopecic and erythematous ulcerative skin lesions on the ventral and lateral mandibular areas, ventral chest and axillae, associated with intense pruritus. No infectious agents were detected on skin scrapes, hair and cytological examinations, and on bacterial or fungal cultures. Histopathology revealed diffuse ulceration with serocellular neutrophilic crusts and clusters of cocci within the corneal layer. There was severe locally extensive dermal and adnexal loss with minimal inflammation and severe fibroplasia. Treatment initially included cage and bedding change, topical hydrocortisone, enrofloxacin, amoxicillin/clavulanic acid, meloxicam and essential fatty acid supplement, with no response and rapid progression of UD. Six sessions of laser therapy with toenail trimming were performed under anesthesia with no improvement. Further therapy included: vitamin E, maropitant citrate and topical application of thiabendazole, dexamethasone, neomycin solution. Lesions progressed further and both mice died. Necropsies were not performed. Further studies are needed to clarify the etiology of murine idiopathic UD and establish a more effective treatment.

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Extended low dose dexamethasone suppression (LDDS) test for diagnosis of atypical Cushing’s syndrome in dogs

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Abstract: Recently, dogs with atypical hyperadrenocorticism (AHAC) were shown to have increased serum cortisol concentrations when measured over a 9 h period, suggesting increased cortisol could be responsible for the clinical signs. It is possible that selecting an alternate sampling endpoint in the low dose dexamethasone suppression (LDDS) test would result in a more sensitive screening test for AHAC. The purpose of this study was to evaluate extension of the LDDS test from 8 h to 12 h to detect possible hypercortisolemia associated with AHAC. Six client owned dogs with AHAC and six healthy control dogs were enrolled in the study. Dogs were administered 0.01 mg/kg dexamethasone IV. Serum samples were collected prior to and at 4, 8, 10, and 12 h post-dexamethasone administration. Baseline plasma samples were also collected for endogenous ACTH analysis. Cortisol concentrations of all control dogs and five of six dogs with AHAC suppressed to less than 0.5 µg/dL at all time-points after dexamethasone administration. The cortisol concentration from one dog suppressed to 0.7 µg/dL at 8 h but increased to 1.5 µg/dL at 10 h and 3.7 µg/dL at 12 h post-dexamethasone. Endogenous ACTH concentrations did not significantly differ between the two groups (P >0.2). Based on results of this study use of an extended LDDS test could not differentiate between healthy dogs and dogs with AHAC. Diagnosis of AHAC should continue to be based on prior established criteria until new testing has been identified.

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Prevalence of comorbid immune-mediated disease in dogs with pemphigus foliaceus: a retrospective study

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Abstract: Canine pemphigus foliaceus (PF) shares clinical and pathomechanistic features with human pemphigus foliaceus. Epidemiologic studies on human pemphigus complex patients have demonstrated increased incidence of concurrent auto-immune disease and an association between developing auto-immune disease and degree of relatedness. These findings underline the importance of genetic background in the development of pemphigus and of other autoimmune diseases. The objective of this study was to evaluate the prevalence with which dogs diagnosed with PF at a teaching hospital suffered from comorbid auto-immune diseases. Medical records dating from 2002-2016 were searched for the keyword “pemphigus.” Ninety dogs were diagnosed with PF during this time period. Signalment and incidence of concurrent immune-mediated disease were recorded. Seventeen out of ninety (19%) had concurrent auto-immune disease, a figure similar to that reported in humans with pemphigus (approximately 20%). The following concurrent diseases were observed more than once: immune-mediated polyarthritis (IMPA; 4/17; 23.5%), keratoconjunctivitis sicca (KCS; 4/17; 23.5%), and immune-mediated thrombocytopenia (ITP; 3/17; 17.6%). Three breeds were represented by more than one individual with PF and a concurrent auto-immune disease: Labrador retrievers (3/17; 17.6%), greyhounds (3/17; 17.6%), and Australian shepherds (2/17; 11.7%). These findings suggest that PF, IMPA, KCS, and ITP may share common genetic background and pathomechanisms, and Labrador retrievers, greyhounds, and Australian shepherds may have a genetic predisposition for auto-immunity.

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Feline pemphigus foliaceus in non-specialist veterinary practices: a retrospective analysis

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Abstract: To describe the signalment, clinical signs, and treatment patterns for feline pemphigus foliaceus (PF) cases managed by non-specialist veterinarians, 40 biopsy-confirmed cases from the USA, Canada, United Kingdom, New Zealand, and Australia were provided by members of the Veterinary Information Network (Davis, CA, USA). Median age was 6 years (5 months-18 years), all were neutered males (22/40, 55%) or spayed females (18/40, 45%), and most were domestic shorthairs (25/40, 63%). Systemic signs were common (24/34, 71%) but fever was uncommon (5/34, 15%). Frequently involved sites were the convex pinnae (26/34, 76%), concave pinnae (20/34, 59%), nailbeds (22/34, 65%), and haired facial skin (17/34, 50%). Pruritus occurred in 13/34 (38%) cats. Common lesions were crusts (33/34, 97%), erosions (21/34, 62%), paronychia (21/34, 62%), and alopecia (18/34, 53%). In 33 cases with treatment information, the most common induction therapy was corticosteroid monotherapy (28, 85%) followed by corticosteroid combined with cyclosporine (5/33, 15%). Steroids included oral prednisolone (19), oral triamcinolone (5), and oral prednisone (3). Median prednisolone induction dose was 2.2 mg/kg/day (1-4 mg/kg/day). In 26 cases that achieved initial remission, time to remission was most commonly reported as < 1 month (12, 46%) with the remaining occurring within 1-2 months (6, 23%), 2-3 months (5, 23%), or longer (3, 12%). Relapse occurred in 11 cases, but medication discontinuation was possible in seven cases (18%). Cure with no need for further medication was ultimately reported in 3/33 cases (9%, follow-up time 1-6 years). Death or euthanasia was reported in 3/33 cases (9%).

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Successful treatment and resolution of cutaneous
*Pythium insidiosum* infection in a two year-old dog

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**Abstract:** A two-year old neutered male 25 kg poodle mixed breed dog presented for a 3 month history of progressive tender and exudative nodules on the left lateral thigh. Surgical debridement and tissue biopsies for histopathology and deep tissue cultures were performed, followed by every 3-5 day wound care and bandage changes. Histopathology revealed chronic, multifocal and multinodular pyogranulomatous-suppurative dermatitis and panniculitis, with multifocal ischemic necrosis and positive GMS stain for unevenly septate branching hyphae, consistent with *Pythium/Lagenidium* fragments. Fungal culture and subsequent sequencing revealed *Pythium insidiosum*. Aerobic bacterial tissue culture revealed *Aeromonas caudae* and *Klebsiella oxytoca*. Abdominal ultrasound revealed mild left medial iliac lymphadenopathy. Fine needle aspirate was consistent reactive lymph node and GMS stain negative. PAV-ELISA (Pan American Veterinary Laboratories, Hutto, TX, USA) revealed positive serology for pythiosis. Mycobacterial culture was negative. Thoracic radiographs and ophthalmic examination revealed no abnormalities. Treatment included oral terbinafine (generic preparation; Aurobindo Pharma, Dayton, NJ USA) 750 mg once daily, itraconazole (Sporanox®; Janssen Pharma, Titusville, NJ, USA) 200 mg once daily, pentoxifylline (generic preparation; Teva Pharma, North Wales, PA, USA) 600 mg twice daily and chloramphenicol (Viceton; Bimeda, Le Sueur, MN, USA) 1 gm twice daily, concurrently with the Pythium Immunotherapy (*Pythium insidiosum* Allergenic Extract; Pan American Veterinary Laboratories, Hutto, TX, USA) including three separate, 1.0 mL (200 mcg/mL) subcutaneous injections given on day 1, day 7 and day 21. Clinical signs resolved after 6 months of treatment, with no observation of recurrence in the 8 months to follow treatment discontinuation.

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Signalment
Eight-Year-Old Male/Neutered Newfoundland

Brief History
Five-year history of controlled hypothyroidism and atopy. Recent history of dermal and oral cavity nodules.
Title
Three years of waxing and waning nodules in a Boxer dog

Signalment
9 year old castrated male Boxer dog

History
Between January 2011 and December 2013, this dog had a 3 year history of multiple waxing and waning ulcerated nodules over the trunk and legs. Nodules resolved with scarring, hyperpigmentation and alopecia then recurred at different sites. Nodules were raised, round to oval, and they ranged in size from 2 to 12 cm in diameter. They were predominantly distributed over the dorsal and lateral trunk with fewer on the limbs. Smaller nodules were covered by hair and larger nodules were alopecic and partially to extensively ulcerated. Lesions appeared to worsen with cyclosporine administration.
ERYTHEMA MULTIFORME, STEVENS-JOHNSON SYNDROME AND TOXIC EPIDERMAL NECROLYSIS: COMPARATIVE ASPECTS IN HUMANS AND DOGS

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INTRODUCTION
First described by von Hebra in 1860, erythema multiforme (EM) had long been considered as part of a spectrum of diseases that included Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). The currently accepted clinical classification in humans (Table 1) and dogs (Table 2) defines SJS and TEN as variants of the same disease spectrum that are different from subsets of EM in characteristic clinical appearance and causality. Drugs are thought to induce most cases of SJS/TEN, but infectious triggers dominate in EM, which implicates drug withdrawal as a crucial requirement for the treatment and prognosis of SJS/TEN.

COMPARATIVE ASPECTS OF ERYTHEMA MULTIFORME IN HUMANS AND DOGS

Clinical signs
Erythema multiforme is an acute immune-mediated disorder that affects the skin and/or mucous membranes, including the oral cavity. In humans, typical EM represents a blistering and ulcerative skin disease characterized by target or iris lesions (i.e three different zones of color) distributed symmetrically on the extremities. Atypical EM features widespread, large, round, bullous lesions (i.e atypical targets with two different zones of color) that affect the trunk. Erythema multiforme is divided into minor (EMm) and major (EMM) forms based on mucosal involvement and systemic signs of illness present in the latter. The lesions in typical and atypical EM can be confluent, but they do not lead to large areas of epidermal sloughing as in SJS/TEN. In cases of EM the skin detachment is limited to a few body area percent (1-3%) in EMm (i.e. with acral distribution), but it may be more extensive in atypical EMM with a widespread distribution up to 10% body surface area.

The current description of canine EM in textbooks defines two distinct subgroups: an erosive form characterized by lesions resembling those of the human counterpart and a form described as “old-dog/hyperkeratotic” EM. The proposed clinical classification does not differentiate between typical and atypical targets in dogs; canine EMm and EMM lesions are described as focal to multifocal targets or polycyclic lesions. However, only very rarely are canine EM lesions typical targets; typical targets in humans are defined as individual lesions less than 3 cm in diameter with a regular round shape, well-defined border, and at least three different zones (i.e. two concentric rings around a central disk). Canine EM lesions resemble more atypical EM targets in humans with only two zones of color change and/or a poorly defined border. Several reports in veterinary medicine have described canine EM lesions as erythematous macules with widespread sloughing due to drugs; it is likely that many of these cases represent SJS being published as cases of EMM. A subgroup defined as “old-dog/hyperkeratotic” EM is considered a chronic and persistent idiopathic form of EM that shows marked hyperkeratosis and parakeratosis on microscopic examination, however without any detailed description of larger case series. A careful review of the literature revealed a single case report of “hyperkeratotic” EM: a 7.5-year-old Cocker Spaniel that exhibited generalized, raised annular erythematous plaques with widespread yellow-brown crusts that coalesced in some areas in large patchy lesions. The lesions included hyperpigmented plaques with an erythematous margin and severe adherent crusting. The skin lesions affected the entire body, inner ear pinnae and mucocutaneous junctions (lips, eyelids, anus, and prepuce). Besides sharing only a common tissue reaction pattern, the above described clinical phenotype of “hyperkeratotic” EM does not represent a counterpart to human EM.

Pathogenesis
The clinical behavior of EM in humans is divided into three major subgroups that include classical self-limited EM, recurrent EM (several disease occurrences over a year), and persistent EM characterized by the continuous occurrence of lesions without interruption. The common triggers for the development of self-limited and recurrent EM include viral (e.g herpes simplex virus, influenza or influenza–like) and bacterial infections (up to 70%), medications and...
malignancy; some cases are considered idiopathic as no precipitating factor can be identified.\textsuperscript{10,11} Interestingly, from the recent review of 36 papers (reporting 37 cases) of drug-induced EM in humans, the diagnosis was considered as definite/probable EM in only six cases (16%), possible EM in seven cases (19%) and ‘no case’ in 24 (65%).\textsuperscript{12} The review established that these reports had no information on \textit{in vivo} or \textit{in vitro} drug tests, and some of the cases published as “drug-induced EM” were more likely SJS or SJS/TEN overlap related to anticonvulsants.\textsuperscript{12} The proposed pathomechanism of EM includes autoreactive T-cell generation and activation by antigen (viral, bacterial, drug)-loaded epithelial cells resulting in epidermal damage from lysis of surrounding keratinocytes.\textsuperscript{13} The lesions in herpes simplex virus (HSV)-induced EM are virus free (i.e viral cytopathic changes are not present) but contain HSV DNA fragments, most often comprising sequences that encode and express polymerase gene Pol. Viral protein expression in the skin (notably Pol, rarely thymidine kinase) initiates lesion development through recruitment of a Vβ-restricted population of virus-specific CD4 helper T cells, type 1, that produce interferon (IFN)-γ. This early virus-specific response is followed by an amplified inflammatory cascade, characterized by enhanced cytokine production and the accumulation of T cells that respond to auto-antigens, which are likely released by lysed or apoptotic virus-infected cells.\textsuperscript{13}

Most dogs affected with EM experience a chronic disease course with recurrent episodes or even a persistent phenotype; self-limited canine EM is anecdotaly proposed in textbooks.\textsuperscript{6,7} The most commonly proposed trigger for canine EM are drugs.\textsuperscript{6,7} As for human EM, there are doubts regarding the accuracy of EM diagnosis in several reports of drug-induced EM, as some of these cases may have exhibited SJS or SJS/TEN overlap. In the absence of provocation testing as well as \textit{in vivo} or \textit{in vitro} drug tests, the majority of the published canine cases of drug-induced EM should be considered presumptive at best.\textsuperscript{7} Although considered a form of EM, systemic parvovirus-induced dermatitis in dogs is not analogous to human HSV-EM;\textsuperscript{14,15} the parvovirus produces intranuclear inclusions in lesional keratinocytes indicating a viral replication which is targeted by intraepithelial lymphocytes resulting in lymphocyte satellitosis and keratinocyte apoptosis at all levels of the epidermis. These cases should be better described as cutaneous parvovirosis than true EM.

**Diagnosis**

Histologically, EM in humans is the prototypical cytotoxic interface dermatitis showing transepidermal keratinocyte apoptosis with hydropic changes and dyskeratosis of basal keratinocytes.\textsuperscript{10} Importantly, the diagnosis of EM is mainly based on the history and clinical presentation, as histopathologic features are not pathognomonic for the disease. Depending on the biopsy site and the stage of the clinical disease, full thickness necrosis may be the dominant lesion in a biopsy from the center of a target lesion, whereas interface dermatitis with vacuolar change may be seen at the margin ("zonal changes"). Similarly to humans, the diagnosis of EM in dogs is clinicopathological; two studies evaluating the EM and SJS/TEN spectrum in dogs concluded that veterinary dermatopathologists cannot accurately predict the clinical disease.\textsuperscript{3,16} Historically, the diagnosis of EM in veterinary medicine has been driven largely by the histopathological findings: the microscopic documentation of transepidermal cytotoxic lymphocytic dermatitis with cell death occurring in suprabasilar as well as basal layer of epidermis are considered diagnostic for EM.\textsuperscript{6,7} Unfortunately, the tendency to equate the “tissue reaction pattern” directly to the diagnosis of EM resulted in a heterogeneous group of disorders with diverse clinical manifestations being placed under the same entity, for example “old-dog/hyperkeratotic” EM\textsuperscript{6,7} and proliferative, lymphocytic, infundibular mural folliculitis and dermatitis (PLIMFD) in Labrador Retrievers.\textsuperscript{17}

Erythema multiforme may be difficult to differentiate histologically from other cytotoxic interface dermatoses in humans, particularly acute graft-versus-host disease and some variants of cutaneous lupus erythematosus (CLE). Indeed, occasional foci of grouped suprabasal apoptosis, in conjunction with lymphocytic satellitosisoccurs in several canine CLE variants (e.g exfoliative CLE, vesicular CLE, mucocutaneous CLE and generalized discoid lupus erythematosus).\textsuperscript{18} However, in canine CLE, interface dermatitis and basement membrane thickening are more prominent than suprabasal apoptosis and satellitosis.18 Interestingly, one of the first described canine EM cases in the literature was a 9-year old collie with recurrent annular and polycyclic ulcerative skin lesions that would flare in the summer: this patient likely suffered from vesicular CLE instead.\textsuperscript{19}

**Therapeutic management and prognosis**

The treatment of EM varies according to disease severity and causality; the clinical course of HSV-EM in humans is usually self-limiting, resolving within weeks without significant sequelae.\textsuperscript{19} Any drug suspected to have precipitated
EM should be promptly discontinued. In the severe form of EM, systemic glucocorticoids in conjunction with antiviral therapy (e.g. acyclovir, valacyclovir, famciclovir) are advised depending on the etiology. Some humans with the persistent form of EM have had signs responding to immunosuppressants azathioprine, mycophenolate mofetil or dapsone.10

In animals, there is no obvious counterpart to the HSV-induced cases of EM in people; spontaneous remission is not to be expected. Similarly to humans, if a drug history is suspected, drug withdrawal is mandatory. Canine EM has been reported to respond to immunosuppressive treatment with systemic glucocorticoids, cyclosporine or azathioprine.7

COMPARATIVE ASPECTS OF THE STEVENS-JOHNSON SYNDROME/TOXIC EPIDERMAL NECROLYSIS SPECTRUM IN HUMANS AND DOGS

Clinical signs

Stevens-Johnson syndrome and TEN are rare, predominantly drug-induced, severe cutaneous T-cell mediated immune reactions characterized by widespread sloughing of the epidermis and mucosal epithelium.2 The two terms describe variants of the same disease spectrum, in which SJS is the less extensive (with less than 10% of the body surface affected) and TEN the more widespread form (more than 30% of the body surface is involved).2 Clinical signs of the SJS/TEN spectrum of disease in dogs are homologous to SJS/TEN in humans:2,16 patients exhibit painful, irregular and flat erythematous/purpuric macules and patches that blister into confluent and larger areas of epidermal sloughing. Lesions affect the skin diffusely over the body; mucocutaneous junctions, mucosae (e.g., oral, gastrointestinal, rectal, conjunctival, tracheal), and footpads are frequently involved.3,16 The denuded dermis exudes serum and the lesions can become secondarily infected with crust development and potential sepsis.3,16 In severe cases, necrolysis of the respiratory and gastrointestinal epithelium is accompanied by bronchial obstruction, profuse diarrhea and a variety of systemic complications, including multi-organ failure. The main clinical sign distinguishing TEN from SJS is the amount of body surface area with epidermal detachment, which is defined as any necrotic skin that is already detached (e.g. blisters, erosions) or which is detachable (i.e. areas with a positive pseudo-Nikolskiy sign) at the worst stage of the disease.2 Some humans and dogs with TEN initially exhibit epidermal detachment over less than 10% of their body surface, an extent typical of SJS, but the severity progresses to 30% of the body surface area in a few days, which is more typical of TEN.16 Despite the striking clinical presentation of SJS/TEN, a number of disorders can present with a macular rash, blistering of the skin and mucous membranes in dogs. Some of the clinical differential diagnoses include EMM,2 staphylococcal exfoliative pyoderma resembling staphylococcal scalded skin syndrome,2 burns,16 eosinophilic dermatitis (Well’s like syndrome)20 and toxic shock-like syndrome (TSLS).21 Although flat atypical targets have been described in some patients with TEN, confluent irregular macules with larger areas of epidermal sloughing are not a clinical feature of typical or atypical EMM.23,16 Dogs with eosinophilic dermatitis and TSLS show generalized, sometimes painful, erythematous macules and patches; epidermal detachment (e.g. blisters, erosions) is not present in skin lesions of canine eosinophilic dermatitis20 whereas TSLS lesions21 are characterized by generalized erythema with the subsequent development of coalescing large pustules.

Pathogenesis

Drugs are reported as the leading cause of SJS/TEN, with the risk of a hypersensitivity reaction developing in the first few weeks after drug ingestion.22 A new disease-specific algorithm, the Assessment of Drug Causality in Epidermal Necrolysis (ALDEN), has been recently validated for human patients with SJS/TEN, and it shows superiority to previous algorithms.22 Epidemiologic studies, with the application of the ALDEN, have shown that approximately 70% of SJS and TEN cases in humans are induced by drugs; a growing suspicion exists that some cases may be caused by infections such as Mycoplasma pneumoniae.22 Still, 15% of patients remain for whom a drug causality cannot be implicated; this very small fraction of SJS and TEN cases is considered as idiopathic.22 A recent study evaluated drug presence in food of humans affected by “idiopathic” SJS/TEN; no trace of phenylbutazone and its metabolite were found, however further investigation of other drugs (i.e. oxicams) that can induce SJS/TEN in human food are warranted.23 Strong associations between SJS/TEN and several drugs exists in humans such as sulphonamides, allopurinol, carbamazepine, phenobarbital, nevirapine, lamotrigine, phenytoin, and oxicam-non steroidal anti-inflammatory drugs (NSAIDs).22 In dogs, a strong causal association has been reported for beta-lactam and trimethoprim-potentiated sulfonamide antibiotics, phenobarbital and carprofen.3,16

At the time, the precise molecular and cellular pathogenic mechanisms leading to the development of SJS/TEN are partially understood. The lesions of SJS/TEN are characterized by widespread epithelial keratinocyte apoptosis and
necrosis, a process initiated by drug- or drug/peptide-specific cytotoxic T-lymphocytes (CTL) and/or natural killer (NK)–cells.\textsuperscript{24} Drugs can stimulate the immune system by directly binding to the class I major histocompatibility complex resulting in the clonal expansion of a specific population of CTLs, which infiltrate the skin and secrete soluble proapoptotic factors like granulysin, Fas ligand, perforin and granzymes.\textsuperscript{24} An alternative death pathway, named “programmed necrosis or necroptosis”, has emerged as an important pathway in the pathogenesis of human TEN, as the mechanism of abrupt and rapid TEN lesion progression to full-thickness keratinocyte death cannot be fully explained by an expansion of the apoptotic cell death pathway.\textsuperscript{24,25} Necroptosis is activated in response to death receptor ligands, such as tumor necrosis factor–a (TNF-a) and annexin A1.\textsuperscript{24,25} The programmed necrotic pathway involves the formation of a “necrosome” or a “ripoptosome” complex containing receptor-interacting protein (RIP) kinases 3 (RIPK3) and 1 (RIPK1) with downstream recruitment and phosphorylation of mixed lineage kinase domain-like protein (MLKL).\textsuperscript{24,25} Importantly, the inhibition of necroptosis completely prevented TEN-like lesions in a mouse model, thereby highlighting that RIPK3 could be a potential therapeutic target for the treatment of TEN, as RIPK3 inhibitors, a novel class of kinase inhibitors, are currently under preclinical development.

**Diagnosis**

Although a diagnosis of SJS/TEN is suggested by the history and clinical signs, skin a biopsy is necessary to support the clinical assessment and exclude other blistering dermatoses. A recent study identified histologic variation in the patterns of necrosis and degree of inflammatory cell infiltrate in the skin of dogs with TEN, which illustrates the histologic overlap between EM and SJS/TEN.\textsuperscript{16} This observation is in contrast to previous descriptions of canine TEN, which emphasized full-thickness coagulation necrosis of the epidermis and absent or minimal inflammation as key diagnostic features.\textsuperscript{16} The results of the recent canine TEN studies are consistent with those of TEN in humans, where histopathologic examination confirms but lacks specificity to differentiate EM and SJS/TEN.\textsuperscript{16} Therefore, a pathologist's microscopic interpretation should be restricted to an umbrella diagnosis of an EM-TEN epidermal necrotizing disease, and the further sub-classification of the different entities should depend upon patient history, clinical signs and skin lesion extent.\textsuperscript{16} In suspect TEN cases, clinicians should be encouraged to take multiple biopsies since some skin biopsies in TEN patients may lack epithelium and may not be used for diagnosis. Dermal necrosis is not present in canine TEN skin biopsies despite large ulcer development and bacterial colonization in some cases.\textsuperscript{16} This is important because the exact depth of skin necrosis, when shallow, can be difficult to determine clinically, and histologic examination aids disease classification. There are examples in the literature of cases reported to be TEN, that lacked sufficient clinical criteria for TEN and had evidence of dermal necrosis; these were more likely examples of thermal injury or vascular disease in which dermal necrosis can be a feature.\textsuperscript{16}

**Therapeutic management and prognosis**

Although rare, SJS/TEN is a devastating disease; the mortality for SJS is < 10%, with the figure rising to 40% for TEN. A SJS/TEN-specific severity-of-illness score (SCORTEN) was developed from a logistic regression model to categorize the severity of illness and predict mortality in humans with TEN.\textsuperscript{26} The score uses seven independent prognostic factors that include: age above 40 years, the presence of malignancy, the percentage of epidermal detachment above 10% of body surface area (BSA) at admission, heart rate above 120 per min, serum glucose level above 14 mmol/L (252 mg/dl), serum bicarbonate level below 20 mmol/L (20 mEq/L), and urea nitrogen level above 10 mmol/L (28 mg/dl).\textsuperscript{26} The score's ability to predict outcome and usefulness has been confirmed and the prognostic value of SCORTEN is considered more accurate at day 3 of hospitalization.\textsuperscript{27} The author of a recent review paper,\textsuperscript{7} which compared the etiology, pathogenesis, diagnostic and therapeutic aspects of human and canine TEN, proposed to readily use and adapt the SCORTEN score to canine TEN patients. However, the investigations on mortality of canine SJS/TEN are limited and evidence of analyzing SCORTEN parameters is lacking.

A significant mortality is associated with TEN in humans and dogs, which confirms TEN as one of the few dermatological diseases that constitute an actual medical emergency. Early recognition as well as prompt and appropriate management are detrimental and can be lifesaving. Immediate withdrawal of suspected medication (most commonly beta-lactams, sulfa drugs, NSAIDs) and referral to an emergency center are crucial requirements for improving SJS/TEN prognosis. An extensive epidermal loss results in a massive fluid, electrolyte, and plasma protein losses. Supportive care similar to that for burn patients (aggressive fluid replacement, antimicrobial therapy, wound care, analgesia, and nutritional support) is required. The use of immunosuppressive agents (e.g., glucocorticoids, cyclosporine, azathioprine) has been controversial, but recent evidence shows a possible beneficial role for
cyclosporine in humans during early disease development. Human intravenous immunoglobulin treatment has managed to treat Stevens-Johnson syndrome in two dogs.

Summary

- A pathologist’s microscopic interpretation should be restricted to an umbrella diagnosis of an EM-TEN epidermal necrotizing disease.
- Further sub-classification of the different EM-TEN entities should depend upon patient history, clinical signs and skin lesion extent.
- Drugs are reported as the major leading cause of SJS/TEN in people and animals.
- Clinical phenotype of “old-dog/hyperkeratotic” EM does not represent a counterpart to human EM.
- The causality of canine EM in many cases is unknown and further investigations of etiological causes are warranted.

References


Table 1. Clinical features of erythema multiforme (EM) subgroups, and Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in humans

<table>
<thead>
<tr>
<th>Typical targets</th>
<th>EMm</th>
<th>EMM</th>
<th>SJS</th>
<th>SJS-TEN overlap</th>
<th>TEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macules with/without blisters</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Skin detachment (BSA)</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>10% - 30%</td>
<td>&gt; 30%</td>
</tr>
</tbody>
</table>

EMm: erythema multiforme minor; EMM: erythema multiforme major; BSA: body surface area

Table 2. Clinical features of erythema multiforme (EM) subgroups, and Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in dogs

<table>
<thead>
<tr>
<th>Flat or raised, focal or multifocal, target or polycyclic lesions</th>
<th>EMm</th>
<th>EMM</th>
<th>SJS</th>
<th>SJS-TEN overlap</th>
<th>TEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythematous or purpuric macular eruption (BSA)</td>
<td>&lt; 50</td>
<td>&lt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Mucosae (number)</td>
<td>≤ 1</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
</tr>
<tr>
<td>Skin detachment (BSA)</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>10% - 30%</td>
<td>&gt; 30%</td>
</tr>
</tbody>
</table>

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INTRODUCTION

Human intravenous immunoglobulin (HIVIG) possesses many immunomodulating properties and has been used for the management of immune-mediated conditions in people for decades. Anecdotal reports of successful HIVIG therapy in companion animals have been circulating for decades. Efficacy in human medicine makes HIVIG an attractive choice for the management of immune-mediated disease in veterinary patients, but no consensus exists regarding the indications, dose, or safety of HIVIG infusion in companion animals. Infusion of amino acids to dogs with superficial necrolytic dermatitis (SND) has also been reported, but with minimal evidence as to appropriate dosing and administration, despite anecdotal benefits. The indications, products, doses, and administration recommendations for the infusion of HIVIG and amino acids to veterinary patients with dermatologic disease is outlined below.

HUMAN INTRAVENOUS IMMUNOGLOBULIN (HIVIG)

Immunoglobulin for intravenous administration is prepared from fastidiously purified immunoglobulin collected from pooled human plasma. Donors are screened for infectious diseases and preparations are filtered to ensure no aggregates, kinins, plasmin, or kalikrein activators are present in the finished product. HIVIG formulations contain over 90% biologically active IgG, as well as trace amounts of IgA, IgM, CD4, CD8, and leukocyte antigen molecules. Some commercially available products are adjusted to a low pH to reduce the risk of bacterial colonization and prevent aggregation.

Indications: There are few US Federal Drug Administration (USFDA)-approved uses for HIVIG. Currently, HIVIG is labeled for the treatment of Kawasaki disease, allogenic bone marrow transplantation, chronic lymphocytic leukemia, common variable immunodeficiency, pediatric human immunodeficiency virus, and symptomatic primary immunodeficiency. More than half of all prescribed HIVIG is used to manage off-label conditions such as toxic epidermal necrolysis (TEN), Guillain–Barre syndrome, and necrotizing fasciitis. A multicenter study compared efficacy and risk between label and off-label administration of HIVIG to human patients and found no significant differences in positive outcome or adverse effects. At this time, data regarding HIVIG use in veterinary patients is limited to patients with immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, Evans syndrome, pemphigus foliaceus (PF), sudden acquired retinal degeneration syndrome, myasthenia gravis, cutaneous drug reactions, and other dermatologic diseases.

Conditions such as erythema multiforme (EM), TEN, and Stevens-Johnson syndrome (SJS) cause full thickness epidermal detachment, as well as systemic compromise and are associated with high mortality. Standard immunosuppressive therapy alone is not effective for the management of these syndromes in human or veterinary patients. Although HIVIG is not USFDA-approved for the management of cutaneous immune disease in people, a retrospective study documented an 83% decrease in patient mortality when HIVIG infusion was added to standard treatment. Likewise, a consensus statement recommends HIVIG infusion for human patients with refractory autoimmune blistering cutaneous disease. Currently, no controlled randomized trials have been performed evaluating HIVIG for dermatologic immunopathy, but success in human medicine has generated great interest in the use of HIVIG for skin disease in animals.

Canine Stevens-Johnson syndrome: A case report describes complete resolution of SJS after a single infusion of HIVIG in a dog that developed SJS after exposure to trimethoprim-potentiated sulfadiazine and steadily declined despite standard antimicrobial therapy. Within 12 hours of a HIVIG infusion, visible improvement in dermal lesions was seen and within 7 days all dermal lesions were fully healed. No adverse effects were noted secondary to the HIVIG infusion.

Canine toxic epidermal necrolysis: Another case report describes HIVIG transfusion in two dogs with life-threatening necrotic dermatitis. Each dog was treated with two infusions of HIVIG given 24 hours apart (total dose 2 g/kg), in combination with broad-spectrum antimicrobials, analgesics, and IV fluid therapy. Both dogs experienced significant
improvement in dermal and systemic signs within 72 hours of infusion and no adverse effects were reported. The dogs were followed for 3 years after discharge and no evidence of relapse or delayed reactions were recorded.

**Feline erythema multiforme:** A case report describes the successful treatment of EM in a recently vaccinated kitten with lesions refractory to steroids. The kitten received two HIVIG infusions 24 hours apart; improvement in general health and dermal lesions occurred within 4 days of the infusion and the lesions mostly resolved within 8 days. Adverse effects were not noted and no signs of relapse were present upon recheck 8 weeks later.

**Canine pemphigus foliaceus:** Although PF is the most common canine immune-mediated skin disease and has a high morbidity and mortality rate, there is little research regarding the efficacy of HIVIG for the treatment of PF. A case report documents the successful use of HIVIG to treat PF in a dog, initially in lieu of steroid therapy. Infusions of HIVIG were administered daily for 5 days and an additional HIVIG infusion was given 3 weeks later. Marked improvement was noted during and after the 5-day course of HIVIG, after which time treatment with standard immunosuppressive steroids was started. The dog had a mild relapse 9 weeks after discharge when two additional HIVIG transfusions were given 24 hours apart. The dog was subsequently scheduled for maintenance therapy with HIVIG infusions delivered on weeks 12, 22, 26, and 31. The dog remained asymptomatic for at least 4 months after his final infusion, but was then lost to follow-up. No adverse effects related to the HIVIG infusions were noted, even after multiple infusions.

**Products:** HIVIG preparations are available in lyophilized and liquid forms. Liquid preparations are convenient, but should be refrigerated to discourage bacterial growth. Lyophilized products are reconstituted over 15–20 minutes; a variety of diluents can be used depending on the product, which allows flexibility in determination of the final concentration and osmolality. HIVIG can be administered through a peripheral catheter, but should be given via a dedicated line (i.e., without concurrently administered fluids). During the infusion, the catheter site should be closely monitored for extravasation and swelling. Most manufacturers recommend using an in-line filter during infusion and have varying guidelines for filter size. Some clinicians recommend discontinuation of medications, feedings, and other fluid therapy during HIVIG infusion to decrease the risk of complications, but no consensus opinion exists on this practice. Ultimately, it is best to consult with the manufacturer regarding their specific recommendations.

**Dose and Administration:** The use of HIVIG in companion animals is still in its infancy and veterinary protocols are largely extrapolated from human medicine. Early veterinary investigations utilized doses ranging from 0.5 to 1.5 g/kg, but more recent work has documented doses up to 2.2 g/kg. Veterinary studies have used infusion times of 4–8 hours. In all situations, the infusion should be initiated slowly and gradually increased every 30–60 minutes to a maintenance rate not exceeding 0.8 mL/kg/min. While serial infusions have been used in people for the management of certain immune-mediated conditions, it is unclear if repeated infusions are safe for veterinary patients. While several veterinary reports describe repeated infusions without adverse effects, others report anaphylactic events after serial infusions. Because HIVIG infusion involves introduction of foreign proteins to veterinary patients, repeat infusion should be performed cautiously due to the risk of severe immediate or delayed hypersensitivity reactions. Without question, more research needs to be completed to determine optimal practice guidelines for HIVIG administration in veterinary patients.

**AMINO ACIDS**

All commercially available amino acid formulations for parenteral nutrition provide histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine in amounts varying between 38-57% of the total amino acids, with other amino acids such as alanine, arginine, glycine, proline, serine, and tyrosine comprising 43-62%. Other amino acids (e.g., glutamate, aspartate) are present in some, but not all commercial formulations, and differ by manufacturer.

**Indications:** Parenteral amino acid infusions are often administered for SND, which is also referred to as metabolic epidermal necrosis, necrolytic migratory erythema (NME), or hepatocutaneous syndrome. Associated dermatologic lesions can wax and wane and are often erosive, crusting, and scaling, and distributed symmetrically over the face, distal paws, inguinal area, and areas of constant friction. Hyperkeratosis, fissuring, and ulceration of the footpads can also occur. Systemic signs such as lethargy, anorexia, weight loss, and difficulty walking are also noted by owners. The lesions are commonly associated with an underlying vacuolar hepatopathy; however, other disease processes including glucagonoma of the pancreas, phenobarbital administration, and intestinal disease have been reported to cause similar skin lesions.

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**Pathophysiology:** In people, NME is usually associated with a glucagon-secreting neoplasm. It has been hypothesized that increased portal glucagon levels could result in increased hepatic gluconeogenesis, leading to hypoaminoacidemia due to catabolic effects and hepatic extraction. Because the skin is continuously growing and requires histidine and lysine-rich keratohyalin granules in the stratum granulosum, the epidermis is susceptible to this amino acid deficiency, which can result in skin lesions. Severe hypoaminoacidemia is also documented in veterinary patients with SND, regardless of the associated disease; therefore, it is likely that this metabolic derangement is directly contributing to the cutaneous lesions seen in affected animals. The cause of the hypoaminoacidemia is unclear at this time and cannot be explained by compromised liver function, which is not present in all animals with these skin lesions. However, there might be an unexplained increase in liver catabolism of amino acids, which leads to the severe hypoaminoacidemia and subsequent dermatologic signs.

**Products:** Amino acid solutions are available in different concentrations ranging from 3-15%, but the most commonly used concentration is 8.5-10%. Amino acid solutions are available with or without electrolytes, although solutions without electrolytes are preferred. Mixed amino acid formulations such as 10% Aminosyn® (Abbot Laboratories), 8.5% Travasol® (Baxter Healthcare), and 3% ProcalAmine® (B. Braun Medical) are typically used in veterinary practice and provide both essential and nonessential amino acids.

**Dose and Administration:** Definitive studies on the efficacy of amino acid therapies and recommended doses are lacking. Most reports of using amino acid solutions for dermatologic disease cite a dose of 25 mL/kg over 8-10 hours and repeated every 7-10 days as needed. Others advise repeating the infusion bimonthly, monthly, or when signs recur. When administering amino acids as part of parenteral nutrition, they are typically given at a dose of 4-5 g per 100 kcal resting energy requirements (RER) (dogs) and 6 g per 100 kcal RER (cats). If concurrent liver or kidney failure is present, the daily dose of amino acids should be reduced to 2-3 g per 100 kcal and 3-4 g per 100 kcal in dogs and cats, respectively. The volume administered is then determined by the g of protein per mL of solution (i.e., 10% solution = 0.1 g protein per mL).

Amino acid stability is negatively affected by light; therefore, amino acid solutions must be carefully handled to avoid exposure to light prior to and during administration. Amino acids can be administered through a dedicated peripheral catheter that has been placed using aseptic technique. The sterility of the catheter, intravenous tubing, and amino acid bag must be maintained in order to prevent bacterial or fungal contamination. The line should not be disconnected from the patient unless attaching a new bag (i.e., the patient should be transported with the intravenous lines and amino acid bag attached at all times). The IV catheter through which the amino acids are being delivered should not be used for any other solutions or drugs via the IV tubing or ports and handling of the system should be minimized.

Animals receiving amino acid infusions should be closely monitored for the development of thrombophlebitis (especially with repeated infusions via peripheral veins) or neurologic signs (suggestive of hyperosmolar syndrome). The infusion should be discontinued if adverse side effects are noted. Kidney values and liver function can also be monitored since the presence of kidney or liver dysfunction will affect the dose.

**Combination with Lipid Therapy:** A recent case report described the administration of amino acid infusions (8.5% Travasol®) in combination with intravenous lipids (20% Intralipid®) every 1-6 weeks for 2 years to a dog with necrolytic migratory erythema. The authors believed that the addition of lipid therapy helped to dramatically increase the time between infusions. The benefits of lipid therapy for dermatologic disease are unclear; lipids are rich in linoleic acid, which is integral to ceramide production in the skin. Ceramide allows for intra-keratinocyte adhesion and might help to prevent keratinocyte sloughing.

**REFERENCES**


RESISTANT INFECTIONS
Weese JS
Dept of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON

INTRODUCTION
Antimicrobials revolutionized human and veterinary medicine; however, the parallel emergence and dissemination of antimicrobial resistance continues to compromise these gains. Bacterial pathogens have demonstrated an impressive ability to become resistant to an array of antimicrobials, in a way that threatens to surpass the ability to create new drugs. Resistance of any pathogen to any antimicrobial can be a clinically important event, but certain bacterium-resistance combinations are of particular concern because of the potential for broad impacts on animal (and sometimes human) health.

WHAT DOES RESISTANCE MEAN?
While it may seem overly simplistic, it is useful to consider what ‘antimicrobial resistance’ means from microbiological and clinical standpoints. Resistance simply means that a bacterium has an inherent or acquired ability to evade inhibition or killing by an antimicrobial. This does not necessarily mean there is any difference in ability to cause disease, likelihood of causing severe disease or other clinical factors. Antimicrobial resistance therefore is not, in itself, a virulence factor. Resistance can impact clinical outcome if an effective antimicrobial is not used. However, it is important to note that, beyond the need for a different drug, the overall approach to resistant infections does not differ from infections caused by susceptible pathogens. This highlights the importance of prompt identification of resistant infections, so that proper treatment can be initiated. Similarly, if an antimicrobial susceptible pathogen would be dismissed as irrelevant based on a culture result, the same approach is almost always indicated for a resistant pathogen. Treatment decisions, both whether to treat and what to do in addition to antimicrobials are not inherently different in infections caused by resistant vs susceptible bacteria.

STAPHYLOCOCCI
Staphylococci are classical opportunistic pathogens that are particularly adept at causing skin and skin structure infections (including otitis). From the first emergence of penicillin resistance shortly after the discovery of penicillin to identification of virtually pan resistant strains in humans, staphylococci have created substantial clinical challenges. The emergence of methicillin resistant (MR) staphylococci, particularly S. pseudintermedius (MRSP) and S. aureus (MRSA) as important veterinary pathogens in the 1990s and 2000s perhaps best highlighted concerns about antimicrobial resistance in veterinary dermatology, rapidly changing the field from one where staphylococcal infections were predictably responsive to first line treatment options to a situation where highly drug resistant infections posed substantial challenges. MR staphylococci are of concern for many reasons, including inherent resistance to many commonly used drugs (i.e. beta-lactams), commonness of acquisition of resistance to other antimicrobials, commonness of infections (particularly skin and soft tissue infections) and the potential for zoonotic transmission. These opportunists are widely disseminated in animals, both in clinically inapparent carriers and animals with infections, and MRSP and MRSA can now be considered endemic (if not hyperendemic, in the case of MRSP) in most regions rather than emerging issues. However, continued acquisition of resistance genes can pose new challenges, particularly with MRSP. This Staphylococcus has shown a concerning propensity to rapidly acquire resistance. Interestingly, and rather inexplicably, MRSP isolates from animals tend to be much more resistant than MRSA in humans, despite the fact that MRSA emerged and disseminated decades before MRSP. MRSP isolates are commonly susceptible to a narrow list of drugs such as amikacin and chloramphenicol, with resistance to both of these being in some regions.

The role of staphylococci in dermatological infections is well understood. Increasing use of topical therapies such as topical treatment of superficial folliculitis with chlorhexidine has been a direct response to the increasing incidence of MRSP infections, something that can both be clinically effective and reduce antimicrobial exposure (and corresponding selection pressure). Further evaluation of other local approaches, using properly designed and analysed control trials, are needed to identify other adjunctive or non-antimicrobial approaches.
Treatment of deep infections (e.g. deep pyoderma) may require the use of drugs with undesirable properties (e.g. nephrotoxicity, need for parenteral injection), and concerns about loss of the last few treatment options are real. Other questions pertaining to staphylococci remain, including the potential for emergence of biocide resistance and optimal treatment regimens (drugs, doses, durations) to maximize clinical cure while minimizing resistance. The issues posed by MR staphylococci also highlight the importance of overall patient care and preventive medicine. Since staphylococcal infections are almost always (if not always) secondary to a predisposing factor, identifying and controlling underlying disease whenever possible, is critical for effective treatment and prevention of disease. This is to the point that questions are being posed about the ethical implications of continued prescription of antimicrobials to patients in situations where owners are unwilling to attempt to control underlying disease.

**PSEUDOMONAS**

*Pseudomonas* is a genus of Gram negative bacteria that thrive in moist environments, both on and off the host. As with staphylococci, *Pseudomonas* infections are almost always (if not always) in response to an underlying disease process, with otitis being one of the most common clinical manifestations of *Pseudomonas* infection in small animals. *Pseudomonas* infections can be challenging to treat for many reasons, only some of which relate to antimicrobial resistance. *Pseudomonas* spp are intrinsically resistant to many antimicrobials (e.g. most penicillins and cephalosporins) and often acquire further resistance. Susceptibility to anti-pseudomonal cephalosporins (e.g. ceftazidime) and aminoglycosides remains high, although resistance to either can be found and this may vary geographically. Fluoroquinolone resistance is relatively common, although there can be differences in susceptibility between different fluoroquinolones, with newer fluoroquinolones tending to have greater activity and fluoroquinolones can be effective drugs when the isolate is susceptible *in vitro*. Combination therapy is often used when treating *Pseudomonas* infections in humans, both for synergistic effects and to reduce the likelihood of emergence of resistance. This is less commonly used in animals, probably mainly because of cost rather than lack of need, but should be considered when parenteral treatment is needed.

Topical therapy for superficial folliculitis and otitis externa can be useful to overcome resistance, allow for the use of antimicrobials that are not safe to use systemically (e.g. polymixin B) or avoid the need for systemic drugs. Topical therapy can involve biocides (e.g. chlorhexidine) or antimicrobials (e.g. polymixin). Resistance to both can be present but clinically relevant resistance to the concentrations that can be delivered locally is rare. Adjunctive therapies, such as the use of Tris-EDTA to enhance antibacterial effects may be particularly useful in otitis externa. However, other factors such as the ability of *Pseudomonas* to produce biofilm and the impact of biofilm on antimicrobial susceptibility can hamper treatment.

Another aspect of note about *Pseudomonas* is its ability to survive in water, be resistant to some disinfectants and be a source of hospital- and equipment-associated infections. *Pseudomonas* contamination of canine shampoo has been linked to post-grooming furunculosis and *Pseudomonas* can acquire resistance to biocides to the degree that contamination of disinfectant and patient scrub solutions can occur. Anecdotally, the author have investigated multiple outbreaks of *Pseudomonas* surgical site and hospital-associated skin infections related to likely chlorhexidine resistance and use of chlorhexidine as an environmental disinfectant. Survival of *Pseudomonas* on re-used otoscope tips, despite chemical disinfection, is also a concern.

**ENTEROBACTERIACEAE**

Enterobacteriaceae is a family of Gram negative bacteria that includes animal and human pathogens such as *E. coli*, *Enterobacter*, *Proteus* and *Klebsiella*. While less common than staphylococci, these bacteria can be involved in variety of skin and ear infections, and antimicrobial resistance is of increasing concern. Multidrug resistant Enterobacteriaceae might represent the most dramatic and important shift in resistance patterns in companion animals over the past few years. A few different types of resistance, mediated by a wide (and sometime confusing) collection of resistance genes, can be involved.

Beta-lactamase production is common amongst Enterobacteriaceae and confers resistance to penicillins (e.g. ampicillin, amoxicillin) but not cephalosporins. It can often be controlled through the addition of a beta-lactamase inhibitor (e.g. clavulanic acid). Beta-lactamase producing Enterobacteriaceae are well known and widely disseminated.
Extended spectrum beta-lactamases (ESBLs) are enzymes that confer a broader degree of resistance. ESBLs are effective against extended spectrum cephalosporins (e.g. cefpodoxime, cefovecin, ceftiofur, cefotaxime, ceftazidime) and monobactams (e.g. aztreonam) but are ineffective against cephamycins (e.g. cefoxitin) and carbapenems (e.g. meropenem). They are inhibited by beta-lactamase inhibitors such as clavulanic acid, although clinical response to beta-lactam/beta-lactamase inhibitor combinations can be unpredictable. ESBL-producing bacteria are disseminated widely in humans in a highly diverse population of Enterobacteriaceae, and are significant causes of disease, particularly in hospitals and longterm care facilities. While currently less of a concern than in humans, ESBLs are now being identified in small animal patients, in part as a reflection and consequence of the emergence of ESBLs in humans, as bacteria carrying ESBLs that have been identified in dogs can be the same strains as are found in people.17-19 Exposure through food and food animals (including the environment) or through development of resistance within companion animals also likely occur.

Management of infections caused by ESBL producing bacteria can be complicated because of limited drug options. Antimicrobial options for treatment depend on susceptibility to other antimicrobials as ESBL-producing bacteria have often also acquired resistance to other antimicrobials (e.g. fluoroquinolones).20 Cefoxitin can be an effective option in some situations; however, response to this antimicrobial can be unpredictable. Dosing regimens (route and frequency) are also problematic for outpatients. Beta lactam/beta-lactamase inhibitor (e.g. beta-lactam with clavulanic acid, sulbactam and tazobactam) combinations may be effective, but that depends on the amount of ESBL production, as high production of ESBLs (especially when more than one gene is involved) can overwhelm the beta-lactamase inhibitor. Amikacin and carbapenems are often the main viable choices, both of which can be clinically effective but are often problematic to use.

The similarity of some ESBL genes and ESBL-producing bacteria found in humans and companion animals human-animal transmission may be an important route of infection of veterinary patients, but also indicates that there must be concern about the potential for zoonotic transmission from infected or colonized animals, although the true role of animals in human infections is not known.

Similar to (and sometimes confused with) ESBLs, AmpC (CMY-2) beta-lactamases confer resistance to penicillins and cephalosporins, but in addition they confer resistance to cephamycins such as cefoxitin. They are also resistant to inhibition by beta-lactamase inhibitors. Carbapenems or aminoglycosides tend to be the main options for treatment of veterinary patients.

Carbapenems are uncommonly used in veterinary patients, and mainly in intensive care units (where excessive empirical use is an increasing concern). Emergence of carbapenemase producing Enterobacteriaceae (CPE) is a critically important emerging issue in human healthcare. Carbapenemases are a diverse group of enzymes with variable efficacy against carbapenems (e.g. meropenem) as well as most other beta-lactams. They confer resistance to penicillins, carbapenems, and, depending on the gene that is involved, potentially all cephalosporins, and therefore have a broad range of resistance that is often complemented with acquired resistance to various other drug classes. The effect of beta-lactamase inhibitors is variable and often weak, leading to CPE infections where there may be few viable treatment options. There are only rare reports of CPE in animals; however, anecdotal information suggests that infections are occurring in some regions, particularly in E.coli from urinary tract infections. This may relate to ‘spillover’ from humans, with CPE in animals reflecting movement of CPE into humans into the community, but that has yet to be confirmed.

While CPE constitute a critically important human healthcare issue, they are not the ‘ultimate superbug’. In the past few years, Enterobacteriaceae resistant to almost all, or almost all antimicrobials have been identified, with particular emphasis on the emergence of colistin resistance.22,23 These have also been found in animals, including companion animals,24 and while these highly resistant pathogens are unlikely to become important canine and feline pathogens, the potential role of animals as reservoirs and sources of human infection is of much concern.

From a more common and clinically important standpoint, fluoroquinolone resistance continues to be a problem. Fluoroquinolones can be excellent drugs against Gram negative pathogens but resistance can emerge through various mechanisms, including mutation (e.g. mutations DNA gyrase or topoisomerase IV genes) or acquisition of genes (e.g. those that decrease drug uptake or mediate efflux pumps). Single step mutations may have variable effects
on bacterial MICs, ranging from subtle changes in MIC with continued susceptibility to resistance. Accumulation of multiple mutations is most often associated with clinical resistance, with resistance to one fluoroquinolone typically meaning that resistance is present to all fluoroquinolones (albeit there is some potential variability between drugs, with MICs often being lower to newer fluoroquinolones such as pradofloxacin).

**ENTEROCOCCI**

This genus includes a variety of species of varying clinical relevance, with limited roles in dermatological disease. Most infections are caused by two species, *E. faecium* and *E. faecalis*, both of which can be associated with multidrug resistance. Enterococci are inherently resistant to a variety of drug classes (e.g. penicillin, clindamycin, trimethoprin), some drug classes have poor efficacy in vivo (e.g. cephalosporins) and acquisition of resistance genes is not uncommon. Therefore, they are at best potentially susceptible to only a limited range of 'routine' veterinary drugs, and multidrug resistance is not uncommon. Enterococci resistant to all routinely tested drugs are not unusual and pose challenges.

While enterococci are of limited concern in dermatological disease, at least some consideration is required, because of a few aspects. One is the uncommon presence of these organisms as pathogens. Treatment of these rare infections can be challenging based on the typically limited treatment options. Another consideration is unnecessary treatment of enterococci. As bacteria that are typically of limited virulence and that are commonly found as part of the commensal microbiota, there is the potential for identification of inconsequential enterococci during diagnostic testing. Care must be taken to consider whether enterococci are clinically relevant. Another aspect that cannot be dismissed is the potential for selection of resistant enterococci during treatment of dermatologic infections caused by other pathogens. Enterococci are just one of many potential pathogens present on most animals, and any antimicrobial exposure can potentially influence resistance emergence in the commensal microbiota. The goal of treatment of any infection should be obtaining clinical cure while minimizing the risk of resistance in the target pathogen and commensals. While resistance emergence in the commensal microbiota is not a primary driver in clinical decision-making, prescribers need to consider the potential whole-patient influences of any antimicrobial use. This provides more support to the need for optimizing drug regimens and use of local and/or non-antibacterial approaches to the management of clinical infections.

**OTHERS**

Antimicrobial resistance is not restricted to the above-described groups. Some, such as *Serratia, Acinetobacter* and *Pseudomonas* are commonly multidrug resistant, while resistance is uncommon in others such as streptococci. These are all potential skin and skin structure pathogens, but account for a small minority of infections. While the clinical consequences of resistance in uncommon pathogens such as these is less, virtually any clinically relevant bacterium can be resistant to antimicrobials, and on the individual patient level, that resistance may range from innocuous to life-threatening, largely based on the severity of disease, rapidity of identification of resistance and time to start of appropriate therapy.

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INTRODUCTION
There have been several antimicrobial use consensus statements produced by various groups in the past several years. The consensus guidelines for empirical treatment of common infections in animals can be accomplished by selecting the antimicrobial agent with a high likelihood of success for the suspected clinical infection. Several guidelines have appeared in published proceedings, review papers, consensus documents, and textbooks. Consensus statements have been produced by committees from the International Society for Companion Animal Infectious Diseases (http://www.iscaid.org/), the British Small Animal Veterinary Association (www.BSAVA.com), the AFVAC (French), and guidelines published by specialty journals such as Veterinary Record (1, 2). In addition, national organizations such as the American Veterinary Medical Association (AVMA) have provided recommendations from their Task Force on prudent use of antibiotics (www.AVMA.org). The American College of Veterinary Internal Medicine (ACVIM) also has published guidelines on responsible use of antimicrobials (3).

Although these guidelines vary somewhat in their scope and specific recommendations, the overall theme is consistent. The guidelines promote careful and rational use of antimicrobials in companion animals in order to reduce the risk of resistance and produce a favorable therapeutic outcome. The guidelines promote the use of approved antibiotics and approved drugs for routine infections and discourage the administration of highly active drugs that are ordinarily reserved for drug-resistant infections. Note that these are guidelines, not standards. If these were standards, there is a requirement that the recommendations are followed exactly as listed, without alteration. A guideline allow the user (veterinary clinician) to use as needed, or modified, depending on local, regional, or hospital-specific conditions.

Whether the treatment is for skin infections (pyoderma or bacterial folliculitis) (4), urinary tract infection (5), or respiratory infections (publication pending), the recommendations from these guidelines are to utilize the “first choice” or “first tier” initially for empirical treatment of routine infections. These include the most common drugs usually approved by regulatory authorities for treating common infections. They are listed in these guidelines as a first choice because they are typically active against the wild-type population of bacteria that cause infections in these sites. These guidelines provide recommendations for empirical antibiotic treatment, but do not guarantee a cure. Empirical choices for initial treatment is based on the assumption that the infection is not complicated and the infection is caused by wild-type bacteria. Wild-type strains of bacteria are those that have an absence of acquired and mutational resistance mechanisms. Whereas non-wild-type strains of bacteria are those that have the presence of an acquired or mutational resistance mechanism to the drug in question. Wild-type strains may include bacteria that have inherent resistance to antimicrobials. For example, wild-type anaerobic bacteria are inherently resistant to aminoglycosides by virtue of a lack of an oxygen-dependent drug entry to the bacteria. Gram-negative bacteria of the Enterobacteriaceae and Pseudomonas aeruginosa are inherently resistant to macrolide antibiotics.

Wild-type strains of bacteria may or may not respond clinically to antimicrobial treatment. Likewise, non-wild type strains may or may not respond clinically to antimicrobial treatment. The prediction of whether the bacteria will, or will not respond to treatment has been referred to as the “90/60 rule” (6). The 90/60 rule was derived from the observation that, in general, bacteria treated with antimicrobials to which the strain is susceptible will have a favorable therapeutic response in approximately 90% of the patients. On the other hand, when the bacteria is resistant to the antimicrobial administered, despite the susceptibility result, approximately 60% of patients will respond to therapy. In veterinary medicine, we have no data to confirm or challenge the 90/60 rule. The authors (6) emphasize that these observations apply to immunocompetent patients with infections caused by a single bacteria, when the drug is expected to penetrate to the site of infection adequately. These criteria may not apply to all of their patients and veterinarians must evaluate each patient to determine if there are complicating factors. Many patients have polymicrobial infections treated with more than one antibiotic, have pathologic changes that may affect drug distribution (eg, protein binding changes or altered barrier function), have received oral antibiotics
that are insufficiently absorbed, are immune compromised patients, or have infections at sites that are either poorly
penetrated or diluted, or for which antibiotics are concentrated (for example from topical treatment or by tubular
concentration prior to clearance by the kidneys).

**CONSENSUS STATEMENT RECOMMENDATIONS**

**Skin Infections:**
Susceptibility of the most common isolates has been documented well enough to make recommendations
for empirical antimicrobial drug choices based on past experience, evidence-based studies, and antimicrobial
susceptibility information (1, 2, 4). The guidelines also include agents that drug manufacturers have produced to
treat these common infections encountered in small animals. Most of the medications that can be used for empirical
treatment are approved for use in small animals. For example, recommendations for empirical treatment of pyoderma
cauled by *Staphylococcus pseudintermedius* consists of administration of cephalosporins, amoxicillin-clavulanic acid
(also known as potentiated amoxicillin), clindamycin, or trimethoprim-sulfonamides. If the lesion is amenable to
topical treatment, one can also prescribe shampoos, spray disinfectants, and ointments that contain medications
active against these pathogens. These bacteria are also susceptible to the β-lactam antibiotics cloxacillin, dicloxacillin,
or oxacillin, the lincosamide lincomycin, and the macrolide erythromycin. However these alternatives are rarely used
today because of poor oral absorption, lack of known efficacy, better alternatives within the same class, or lack of
available formulations for animals.

Some skin infections are caused by bite wounds (especially in cats), trauma, abrasions, or trauma. These infections
may be caused by *Pasteurella* species, *Streptococcus* species, and/or *Actnomyces*. These are also typically susceptible to
the bacteria listed above.

**Urinary Tract Infections:**
For urinary tract infections (UTI), empirical treatment assumes that the infection is uncomplicated. In the document
produced by the ISCAID working group (5) uncomplicated UTI was defined as a bacterial infection of the bladder
“in an otherwise healthy individual with normal urinary tract anatomy and function”. In these patients, the urine is
concentrated and normal patient defense mechanisms exist that will assist in eradicating the infection. Ordinarily,
the low pH of the urine, high osmolarity, and presence of salts, urea, and organic acids inhibit bacterial colonization
and growth. Empirical drug selection in these patients can consist of oral treatment with amoxicillin, amoxicillin-
clavulanate, or trimethoprim-sulfonamides. Some guidelines have also considered administration of cephalosporin
antibiotics for these cases in countries in which they are approved by regulatory authorities. In some of the published
guidelines, a long half-life cephalosporin, cefovecin (Convenia) may be allowed if pet owner compliance is a
problem. The guidelines also provide helpful information on diagnosis, duration of treatment, and other aspects of
management.

The success of these empirical choices for initial treatment is based on the assumption that the drugs will concentrate
sufficiently in the urine that organisms that may be ordinarily resistant to systemic concentrations of drugs (eg, plasma
drug concentration) will be suppressed in the high concentrations attained in the urine. The Clinical and Laboratory
Standards Institute (CLSI) allows for higher breakpoints when testing lower urinary tract isolates compared to systemic
infections for amoxicillin, amoxicillin-clavulanate, and oral cephalosporins (7).

**Other Antibiotic Choices**
If the infection is complicated, or if a drug-resistant bacteria is suspected, antibiotics beyond the “first tier” may
be necessary. When empirical treatment fails, or when resistance is suspected, a culture and susceptibility test is
needed to guide therapy. Susceptibility testing should be performed using standards established by the CLSI (7). In
some instances other drugs are considered appropriate first-choice agents. For example if the patient is a male dog
and prostate involvement is suspected, a fluoroquinolone antimicrobial (for example, enrofloxacin, marbofloxacin,
orofloxacin, pradofloxacin) is appropriate for use.

**Antibiotic-Resistant Infections:**
For skin infections, methicillin-resistant *Staphylococcus pseudintermedius*, or other methicillin-resistant *Staphylococcus*
species may be identified. These are often multi-drug resistant strains (8). In these cases either topical treatment – if
the patient is amenable to topical therapy – or other drugs may be considered (9). Before using the alternative drugs
(“second-tier” or “third-tier” agents), it is the veterinarian’s obligation to check with local regulations to determine if the use of these drugs is allowed. Some of the choice that may appear on a susceptibility test are unapproved drugs and restricted by regulatory bodies in some countries.

For urinary tract infections, it is also possible that mecillin-resistant *Staphylococcus* species are identified. Other resistant bacteria are from the Enterobacteriaceae (for example *Escherichia coli*, *Klebsiella* spp., or *Proteus* spp.). Some of these strains may be extended-spectrum β-lactamase (ESBL) producers. For these bacteria, it is important that a susceptibility test is performed (7) to identify the most appropriate treatment. As noted above, it is important when selecting a treatment for these cases to be familiar with local regulations for antibiotic use in companion animals. Some agents identified on the susceptibility test may not be approved in some countries or are not allowed for use.

**OTHER COMPONENTS OF CONSENSUS STATEMENT GUIDELINES**

In addition to providing recommendations for antibiotic selection for common infections, these guidelines also contain information on proper terminology, pathology, diagnostic tests and procedures, and duration of treatment. The guidelines often include antimicrobial stewardship suggestions to prevent unnecessary use of antibiotics in order to reduce the emergence of bacterial resistance.

The consensus statement guidelines also emphasis duration of treatment. Often, veterinarians treat animals for an inappropriately long duration. Shorter courses of treatment may be just as effective as long courses. Short courses of treatment do not increase the risk of resistance emerging. Skin infection may be treated for as little as two weeks in many patients. Urinary tract infections may be treated for 3-5 days, and no longer than 7-10 days.

**REFERENCES CITED**


Video Otoscopy, when compared to conventional otoscopy, allows for the superior visualization of normal and pathologic changes within the ear (vertical and horizontal canals and the tympanic cavity of the middle ear). Video otoscopes provide 25 – 30X magnification and markedly enhanced clarification of images. They are used for both the examination and the performance of various procedures within the ear. There are a number of video otoscopes on the market, and video otoscopy is becoming much more commonly used in both specialty and general practice. Video otoscopic examples of otic pathology are available from a number of resources.

The ear is an anatomically and physiologically complex structure that is difficult to evaluate with radiography. When comparing Computed Tomography (CT) and Magnetic resonance imaging (MRI), CT is noted to provide better detail of bony abnormalities, especially of the middle and inner ear. MRI is superior for demonstrating soft tissue changes and more specific characteristics of soft tissue mass lesions. However, as an alternative, CT does provide a reasonable evaluation of soft tissues. CT is considered the advanced imaging modality of choice for the evaluation of the ear. Most CT studies often provide both a bone and soft tissue image. The bone image is used most frequently. Contrast enhancement can be used to determine the nature and extent of soft tissue changes. It is largely a reflection of vascular supply. Cone Beam CT (CBCT) adds several newer benefits. Its spatial resolution is an improvement compared to most traditional scanners. For example, VetCAT™ (Xoran Technologies LLC) provides resolution as low as 0.1 mm while the field of view is relatively large. The high mobility of the self-shielded low-dose radiation scanner allows for scanning at the point of treatment, without the need to modify the operating room or move the patient. CBCT has now been used for about 15 years in various clinical settings and has become more affordable.

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.

VIDEO OTOSCOPIC: CT COMBINATIONS:
PROLIFERATIVE CHANGES WITHIN THE VERTICAL / HORIZONTAL CANAL

Pathophysiology: Proliferative changes within the canals will consist of variable degrees thickening and folding of the lining of the ear canal and/or fibroproliferative nodules. Both will contribute to variable degrees of stenosis of the canals. Variable amounts of wax/inflammatory debris/hair will accumulate within the canals. Chronic, deep seated inflammation may produce variable degrees of calcification/ossification of the auricular cartilages and peri-auricular soft tissues. Obstruction of the horizontal canal due to proliferative changes may result in waxy/epithelial/exudative debris accumulation that may put pressure on the TM, pushing it back in to the tympanic cavity. If it is pushed significantly in to the middle ear, but remains intact, it has been referred to as a “false” middle ear. The TM may be perforated with the subsequent development of a sterile or infectious otitis media. With otitis media, there may be variable degrees of thickening and fibrosis of the epithelial lining of the middle ear. Epithelial cells (keratin), wax and exudates fill the middle ear.

Cholesteatomas are most commonly noted in severely proliferative ears. They develop when portions of the TM are forced in to (by debris accumulation) or migrate in to the middle ear. This TM tissue essentially becomes an epidermoid cyst wherein the matrix of the cyst is a keratinizing multilayered squamous epithelium producing large amounts of keratin. The cystic content is keratin debris. The matrix rests on the perimatrix, a stroma of varying thickness that is then attached to the bone of the middle ear. The hyperkeratosis and shedding of keratin debris results
in 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.

**Video otoscopic examination (VOE):** VOE shows variable degrees of canal stenosis. In some instances, “folds” of thickened canal wall will “close down” the canal lumen to variable degrees. It may be possible to advance the video otoscope through these folds, then better observe surface changes as the cone is withdrawn from the canal. Variable amounts of wax/inflammatory debris will be present within the canals. These changes often preclude visualization of the TM area. Proliferative nodules will vary significantly with respect to size and breadth of their base. They may contribute to complete stenosis of the canal. Portions of some nodules may have a bluish color, representing the accumulation of secretions in dilated ceruminous glands. With severe proliferative changes, it is often not possible to visualize the tympanic membrane and the horizontal canal adjacent to it. When stenosis is not complete and it is possible to view the TM area (often after a deep ear cleaning to remove debris filling the proximal horizontal canal), changes associated with a “false” middle ear include a horizontal canal that is deeper than normal, a TM that is thicker (myringitis) and more opaque than normal. The lining of the horizontal canal may actually extend in to the middle ear. Support for this phenomena is provided by seeing that portion of the floor of the horizontal canal from which longer hairs are normally noted to grow, extend in to the middle ear.

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.

**CT:** CT will show the vertical and horizontal canal proliferative changes and they would be contrast enhanced. Proliferative changes would narrow the external ear canal. Mineralization/ossification of the auricular cartilages is readily observed. Debris forcing an intact TM in to the middle ear (i.e. “false” middle ear) may be visualized (“finger” sign). If the TM is perforated, the fluid/exudate/wax/epithelial debris seen within the middle ear would be 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. There may be sclerosis and proliferation and potentially lysis (with infection) 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 the past, otolithiasis has been listed as a possible occurrence with chronic otitis media. Ossification was thought to be an end stage of mineralization of dystrophic tissue. However we now know that otoliths (round pieces of bone) are normal structures within the middle ear. They are attached to the free margins of the bulla septum and with otitis media, may break off and “float about” in this inflammatory environment.

The development of an otitis interna might be seen as an obliteration of the fluid-filled spaces of the inner ear. With infection, there may be lysis of the petrous temporal bone in the region of the inner ear. Brainstem involvement may be seen on post-contrast images.

Cholesteatomas are associated with expansion of the tympanic bulla, osteoproliferation, osteolysis and/or osteosclerosis (in decreasing order of incidence). 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 perimatrix.

**NEOPLASIA OF THE CANALS**

**Pathophysiology:** Although a wide variety of neoplasms may be noted within the canals of the ear, the most common would be ceruminous adenomas (more common in dogs than cats) and ceruminous adenocarcinomas (more common in cats than dogs) and squamous cell carcinomas in cats. Exophytic, pedunculated masses are most often adenomas. Broad based, infiltrative, erosive and ulcerated growths tend to be carcinomas. Infiltrative tumors tend to invade the surrounding soft tissue structures, including cartilage. They may extend in to middle ear, causing bony lysis. Masses
that occlude the horizontal canal may result in the accumulation of epithelial debris/wax/exudate that may put pressure on the TM and eventually result in perforation and an otitis media.

*Video otoscopic examination:* Ceruminous gland adenomas tend to be polyloid masses whose surfaces may be smooth to irregular; ulceration is possible. They tend to be narrow based (pedunculated). Adenocarcinomas tend to have irregular surfaces and are more likely to be erosive/ulcerative. They are usually broad based. In both ceruminous adenomas and carcinomas, there are often focal areas that appear blue in color. This blue color is a product of ceruminous secretion accumulation.

CT: Neoplasms appear as soft tissue masses that occlude the canal to various degrees. With malignant tumors, there may be extension from the horizontal canal into the middle ear (soft tissue density). Neoplasms of the canal will enhance with contrast. Enhancement helps to delineate the margins of the tumor. The “mass” effect of having the horizontal canal completely obstructed by a tumor may result in the accumulation of wax/inflammatory/epithelial debris behind the mass and eventual perforation of the TM, with a resultant otitis media and the CT changes associated with this. 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.

**AURAL POLYPS**

*Pathophysiology:* Feline aural polyps arise from the tympanic cavity. If they originate from the area at the entrance to the auditory canal, the polyp tends to grow down the auditory canal to become a posterior pharyngeal polyp. The middle ear fills with variable amounts of inflammatory tissue. Aural polyps generally grow from the more dorsal and central portion of the medial wall of the middle ear. Inflammatory material tends to fill the middle ear and the polyp perforates the tympanic membrane to grow into the horizontal canal. Both aural and nasopharyngeal polyps are only noted in about 5-10% of affected patients.

*Video otoscopic examination:* Aural polyps are seen as smooth, pink masses, often filling the horizontal canal. There is a variable amount of exudate (usually dictated by the amount of secondary infection).

*CT:* 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.

**DILATATION OF THE PARIS FLACCIDA**

*Pathophysiology:* In the dog, otitis media is most commonly seen as an extension from an otitis externa, through a perforated tympanic membrane. In association with the accumulation of exudate/wax/epithelial debris within the middle ear, the pars flaccida may fill with this debris. The pars flaccida itself may also thicken (myringitis). On occasion, the entrance to the pars flaccida may actually become obstructed/fibrosed and this debris will be trapped within the dilated structure and separated from the rest of the middle ear. The dilated pars flaccida may fill the canal lumen to variable degrees, including complete obstruction. Dilatation of the pars flaccida may also be seen with an intact pars tensa. This is most commonly associated with otitis media related to infection ascending the auditory canal or obstruction or dysfunction of the auditory canal, resulting in fluid retention within the middle ear (recalling that mucoid secretions produced by the respiratory epithelium lining the middle ear leave the middle ear through the auditory canal – the natural flushing mechanism of the middle ear).

*Video otoscopic examination:* A dilated, pathologic pars flaccida will usually reveal a smooth surfaced, discolored, rounded structure extending from the dorsal wall of the horizontal canal and occupying various amounts of the canal lumen. It may fill the horizontal canal. Vessels can be seen on its surface.

*CT:* This structure will appear as a smooth, rounded soft tissue mass filling the canal to various degrees (often completely occluding the horizontal canal), just in front of where the TM would be. This does not enhance. There are variably severe concurrent signs of otitis media (see above).
A dilated, mucus filled pars flaccida may be seen with so called “Primary Secretory Otitis Media” in the Cavalier King Charles Spaniel and rarely in other breeds. It has a similar appearance to that outlined above, although it usually is of a lighter color (more similar to normal). On CT the middle ear is filled with a soft tissue density which is actually fluid. There are no bony changes.

**Occlusion of the Canal Due to Trauma and Fibrosis or Congenital Atresia**

*Pathophysiology:* Atresia (absence or abnormal narrowing) of the canal may be seen in either the dog or the cat as congenital or acquired problems. With congenital atresias, the auricular cartilage of the ear may not be formed over variable lengths of the canal. If normal ear canal is noted behind the area of complete atresia, ceruminous/epithelial debris will accumulate, often dilating this portion of the canal and eventually perforating the TM to cause an otitis media. Atresias due to trauma are most typically noted at the junction of the horizontal and vertical canals. They may be very difficult to differentiate from congenital atresias because the patient’s owner may not be aware of the traumatic incident. With traumatic complete atresias, the potential sequel to obstruction of the canal is as noted above for congenital segmental atresias (i.e. debris accumulation behind the “blocked” area). We have seen several individuals who have had partial canal atresias. These are manifest as dramatic narrowing of the canal due to the formation of a ring of fibrosis, with the ring surrounding a canal opening of various size (sometimes very small). The rings of fibrosis are of various thickness. The actual cause of these fibrotic areas is usually not known (trauma?).

*Video Otoscopic Examination:* For cases of both congenital or traumatic atresia, the canal will come to a blind end at the beginning of the area of atresia. This may occur at any depth of the canal. The canal will usually be of a normal diameter until the area of atresia. The blind end will be smooth, often with a normal vascular pattern on its surface. This normal vascular pattern tends to be more common with suspected congenital atresias. In other instances, the more central portion of the blind end may appear fibrotic. This tends to be more typical of traumatic atresias. Partial atresias are seen as a very abrupt, 360 degree narrowing of the canal with a variably sized opening in its center. This opening allows for access to the deeper portion of the canal. The surface of these “walls” varies from smooth and vascular to smooth and fibrotic.

*CT:* With congenital atresia, the affected area of the canal may be seen as a thin “band” of more dense soft tissue. The distal end of the area of atresia (closest to the external ear opening) is an abrupt, smooth, sometimes concave surface. The more normal canal proximal to the area of atresia will be of normal width or dilated and usually filled with material that is soft tissue in appearance (usually a combination of ceruminous debris and variable degrees of wall thickening). There may be changes associated with otitis media, due to material that has “backed up” in to the middle ear. We have seen a case where the area of atresia involved much of the proximal horizontal canal, to the TM. The TM appeared to be intact (no material in the middle ear). Atresias due to trauma really cannot be differentiated from congenital atresias. They may be comparatively shorter segmental areas of atresia. Partial atresias (where the canal narrows very abruptly but leaves a small opening for communication with the more proximal canal behind it) can be seen as a soft tissue density of various thickness (can be very thin) extending from the dorsum to the ventrum of the canal wall.

Knowing the thickness of the fibrotic area can be of help therapeutically in that thin walls can often be broken down/removed to re-establish a more normal canal lumen size.

**References**

Whatever your dog brings home, it shouldn’t be fleas and ticks

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INTRODUCTION
Canine demodicosis is a noncontagious parasitic skin disease caused by different species of Demodex mites (Demodex canis, Demodex injai and Demodex cornei). Most cases of canine demodicosis are caused by Demodex canis. This mite is typically limited to the hair follicle and occasionally the sebaceous gland. In most dogs the number of mites is kept low without clinical disease, but alternations in the immune response can lead to overgrowth of the mites resulting in a number of different clinical presentations. The immune response is complex, probably involving both cellular and humoral mechanisms and requiring the role of co-stimulatory molecules (CD28). It is known that a genetic predisposition for developing canine juvenile generalized demodicosis exists; however, the defect leading to the disease remains unknown. Once the mite proliferation has progressed, dogs show a phenotype that is similar to the T-cell exhaustion characterized by low interleukin-2 production and high interleukin-10 and transforming growth factor-beta production by lymphocytes.1

Demodex injai, the large body species, is larger in all life stages than D canis. This mite tends to reside within the sebaceous glands and is usually associated with dorsal seborrhea. It is more common in the terrier breeds. D cornei usually resides in the superficial layers of the epidermis. It is ~50% shorter than D canis. The clinical lesions of D cornei have a similar appearance to D canis but are generally less severe. PCR techniques that specifically amplified demodex DNA have aided in determining that Demodex canis and Demodex injai are distinctive species and that Demodex cornei is a morphological variant of D. canis, with insufficient genetic difference to separate it from the latter.2

There are a variety of factors that influence the outcome of successful treatment of canine demodicosis.3,4 Many traditional therapies exist for canine demodicosis but what is most exciting is the treatment responses seen with the newer oral isoxazoline molecules that appear to be highly effective and very safe.5-7 The current discussion will be focus on therapy.

CANINE GENERALIZED DEMODICOSIS – OVERVIEW OF TREATMENT
Treatment of canine generalized demodicosis can be challenging. Every case needs to be evaluated on an individual basis and currently there are a wide variety of choices available. Glucocorticoid and other immunosuppressive drug usage must be avoided. Secondary pyoderma usually coexists with canine generalized demodicosis and must be treated concurrently.

Diagnosis remains relatively easy in most cases with simple skin scrapings, trichograms, tape preps and biopsies most commonly used. Cytology is essential in any dog with demodicosis. When a bacterial infection is present, it should be treated accordingly with proper topical and systemic antimicrobial therapy. Ideally, a bacterial culture should be performed if resistance is suspected or in severe cases with deep infection such as a gram-negative cellulitis cases. Topical therapy contributes by removing crusts and debris that may contain mites, exudate and inflammatory mediators. Benzoyl peroxide (2–3%) and chlorhexidine-based shampoos (2–4%) are commonly recommended. They have prolonged antibacterial activity on skin. Benzoyl peroxide has follicular flushing effects and may have miticidal activity on its own. The frequency of topical therapy depends on the case and type of miticidal therapy that is being utilized, but weekly bathing is most commonly recommended. Antimicrobial therapy should be continued beyond clinical and microscopic resolution of the bacterial skin infection.

AMITRAZ BASED PRODUCTS
Amitraz (Mitaban®, Pfizer) is one of the few FDA approved treatments for generalized canine demodicosis. Before amitraz rinses are applied, all medium and long-coated dogs should be clipped and the hair kept short throughout therapy. Clipping plus benzoyl peroxide-containing shampoos afford better penetration of the dipping solution. Mitaban® is diluted per label directions in the USA (0.025% solution) and applied every 2 weeks. A stronger amitraz...
solution (0.050% solution) has been used on a weekly basis routinely in Germany, Australia and elsewhere. Using evidence-based medicine, good evidence is available for the efficacy of amitraz rinses (0.025–0.05% solutions) used every 1 or 2 weeks. Amitraz rinsing should be performed either outdoors or in an open garage. The applier should wear rubber gloves. Amitraz is an MAO inhibitor and should not be used by anyone taking other MAO inhibiting drugs. Continuous rinsing of the entire dog with the dipping solution and soaking of the feet in the solution should continue for 15 minutes. The dog should be kept as dry as possible (avoiding of walking on wet lawns) between rinses. Amitraz rinses should be continued every 2 weeks until multiple negative skin scrapings and hair plucks (no adults, larvae, or eggs) are achieved. After negative scrapings and hair plucks have been achieved, 3 additional rinses should be performed. After 3 additional rinses, reexamination should include 6 to 10 skin scrapings and hair plucks before rinses are terminated. It is not uncommon for 10 to 15 rinses to be required before negative scrapings are achieved. Side effects of amitraz therapy include lethargy, weakness, ataxia, hyperglycemia, polyuria, hypothermia, and bradycardia. Atipamezole (Antisedan) (50 µg/kg, IM) and yohimbine (0.11 mg/kg, IV; 0.25 mg/kg, IM) may be used as a pretreatment in dogs with previous reactions to amitraz or can be used as antidotes for toxicity. Small breed dogs such as Chihuahuas are very sensitive to amitraz and more toxic reactions or even death has been reported. Resistant cases may require off label application of amitraz rinses weekly rather than biweekly. In cases that are controlled but not cured, a maintenance program of every 1 to 2 week rinses may be instituted to keep mite numbers low and clinical signs minimized. Dogs with adult onset demodicosis and concurrent immunosuppressive diseases frequently require extended therapy regimens. Because of the successes seen with many of the other therapies and lower incidence of side effects the author has not used this therapy in years.

MACROCYCLIC LACTONES

Ivermectin: Ivermectin (Ivomec®, Merial) is a commonly used, but non-approved, treatment for generalized canine demodicosis. Ivermectin is given per os at a dosage between 400 and 600 micrograms/kg daily. High dose ivermectin is an efficacious and cost-effective treatment for generalized demodicosis. Using evidence-based medicine, good evidence is available for the efficacy of ivermectin given per os (300-600 micrograms/kg daily). Some practitioner start at even lower dosing, ie 100 micrograms/kg, and increase at weekly intervals until a maintenance dose is reached. Severe adverse reactions to ivermectin are usually limited to dogs that are homozygous for the dangerous mutant ABCB1-1Δ (formerly MDR1) allele leading to neurotoxicity. Collies are particularly sensitive with over 75% of Collies being either carriers or homozygous for the mutation. Information on testing for this mutation can be found at http://www.vetmed.wsu.edu/depts-vcpl/. Other herding breeds such as the Shetland Sheepdog, Australian Shepherd, and Border Collie also may be at increased risk for toxicity. However, idiosyncratic toxicity may be seen in any breed and maybe independent of the ABCB1-1D mutation. Other P-glycoprotein inhibitors, such as ketoconazole or ciclosporin, if given concurrently, increase the likelihood of adverse effects. Supra-pharmaceutical dosing of ivermectin as recommended for demodicosis is contraindicated with concurrent flea control products containing spinosad with reactions of potentiated ivermectin toxicities seen. Similar to the monitoring of efficacy used with amitraz topical therapy, dogs are reevaluated monthly until multiple negative skin scrapings and hair plucks (no adults, larvae, or eggs) are achieved. After negative scrapings and hair plucks have been achieved, therapy is continued for an additional 2 months. Two months beyond the last negative skin scraping and hair pluck, reexamination should include multiple skin scrapings and hair plucks before therapy is terminated. It is not uncommon for 4 to 6 months of therapy to be required before negative scrapings are achieved.

Milbemycin Oxime: Milbemycin oxime (Interceptor®, Novartis) is another commonly used, but non-approved, treatment for generalized canine demodicosis. It is used less frequently than ivermectin, as milbemycin therapy is more expensive. However, milbemycin is a very safe avermectin for the treatment of demodicosis and can be used in herding breeds such as the Collie. Milbemycin also is recommended for small dogs based on cost. Milbemycin is given per os at a dosage of 2 mg/kg daily. Using evidence-based medicine, good evidence is available for the efficacy of milbemycin given per os (2 mg/kg daily). Milbemycin therapy is considerably less toxic than ivermectin therapy. Side effects are rare, but at high dosages may include vomiting, stupor, trembling, and ataxia. Identical to the monitoring of efficacy used with ivermectin oral therapy, dogs are reevaluated monthly until multiple negative skin scrapings and hair plucks (no adults, larvae, or eggs) are achieved. After negative scrapings and hair plucks have been achieved, therapy is continued for an additional 2 months. Two months beyond the last negative skin scraping and hair pluck, reexamination should include multiple skin scrapings and hair plucks before therapy is terminated. It is not uncommon for 4 to 6 months of therapy to be required before negative scrapings are achieved.
**Moxidectin:** Cydectin Injectable (Boehringer Ingelheim Vetmedica, Inc) has been used in a number of studies at doses of 200 to 500 micrograms/ kg/day per os with comparable success to Ivermectin. Adverse effects are similar to those of ivermectin, and some practitioners do a gradual dose increase similar to that described for ivermectin. The most common adverse effects are vomiting and inappetence, but they rarely warrant discontinuation of therapy. Evidence-based medicine indicates good evidence for the efficacy of moxidectin given per os (400 micrograms/kg daily). Imidacloprid and Moxidectin (Advantage Multi®, Advocate®, Bayer) has a label claim for demodicosis at monthly application, however current studies suggest greater efficacy if used on a weekly to bimonthly basis. Despite these studies, the author has not seen similar successes even at weekly applications in his clinical practices.

**Doramectin:** Doramectin is also a macrocyclic lactone that has been reported as a successful treatment for canine demodicosis. It is typically administered at 600 micrograms/ kg SQ weekly. In one of the studies, 232 dogs completed treatment with remission achieved in 94.8% of dogs treated with weekly subcutaneous injections of doramectin. Adverse events were rare with two suspected instances (0.5%) being recorded. The mean duration of treatment was 7.1 weeks.

**ISOXAZOLINE BASED PRODUCTS**

**Afoxolaner (Nexgard® - Merial Animal Health):** Afoxolaner is a member of the isoxazoline family, shown to target a binding site that inhibits insect and acarine ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA), thereby blocking pre- and post-synaptic transfer of chloride ions across cell membranes. Prolonged afoxolaner-induced hyperexcitation results in uncontrolled activity of the central nervous system and death of insects and acarines. The selective toxicity of afoxolaner between insects, acarines and mammals may be inferred by the differential sensitivity of the insects and acarines' GABA receptors versus mammalian GABA receptors. In a variety of studies, Nexgard® demonstrated highly effective flea and tick control. It is a highly palatable beef flavored product that can be given with or without food. Adverse reactions in flea and tick studies are rare, in a US field study; of the 415 administered afoxolaner, vomiting was seen in 17 (4.1%) of the cases. Only 5 experienced anorexia during the study, and two of those dogs experienced anorexia with the first dose but not subsequent doses. A recent published report in eight dogs diagnosed with generalized demodicosis compared the efficacy with a topical combination of imidacloprid/moxidectin (Advocate®, Bayer). Afoxolaner was administered at the recommended dose (at least 2.5 mg/kg) on Days 0, 14, 28 and 56 and the topical combination of imidacloprid/moxidectin was given at the same intervals at the recommended concentration. Clinical examinations and deep skin scrapings were performed every month to evaluate the effect on mite numbers and the resolution of clinical signs. The percentage reductions of mite counts were 99.2%, 99.9% and 100% on Days 28, 56 and 84, respectively, in the afoxolaner-treated group, compared to 89.8%, 85.2% and 86.6% on Days 28, 56 and 84 in the imidacloprid/ moxidectin-treated group. Mite reductions were significantly higher on Days 28, 56 and 84 in the afoxolaner-treated group compared to the imidacloprid/moxidectin-treated group. An unpublished clinical evaluation at the author's practice showed outstanding results with afoxolaner in 102 cases of generalized demodex- 68 were adult onset demodicosis cases. The product was administered at 2.5 mg/kg per os, initially used every 2 weeks in the first 10 cases treated instead of the label interval of every 4 weeks. With the high degree of efficacy seen in the initial 10 cases, the dosage was reduced to monthly in the majority of the remaining cases. Ninety percent of the cases were negative after 2 months of treatment, rarely it took 3 months to achieve negative scrapings. The initial dosing interval was set at 2 weeks because it was felt that the product would need an increased rate of administration to be successful. However, it has proven to be very efficacious and the dose is now administered once monthly at the recommended dose for flea and tick control, which appears to be as effective. The exception to this is one case that was on immunosuppressive therapy that became mite positive when the interval was increased to 4 weeks, but remains mite negative at a 2-week administration. Afoxolaner does not need to be co-administered with food and in the USA afoxolaner can be given to 8-week-old puppies. It is safe for breeding, pregnant and lactating dogs. As the product is an excellent treatment for flea and tick control, most clients elect to maintain ongoing administration for the other parasites.

**Fluralaner Bravecto® - Merck Animal Health:** Fluralaner is also a new molecule in the isoxazoline class that has shown potent acaridical and insecticidal activity through a dual mechanism of binding to neuronal GABA- and glutamate-gated chloride channels in susceptible invertebrates. Numerous studies including field studies in dogs have shown that a single fluralaner dose administered orally provides flea and tick control for twelve weeks. It is
readily absorbed after single-dose oral administration, and has a long elimination half-life, long mean residence time, relatively high apparent volume of distribution, and low clearance. These pharmacokinetic characteristics help to explain the prolonged activity of fluralaner against fleas and ticks on dogs after a single oral dose. For best absorption, bioavailability and efficacy, it should be given with food.28 The safety of this product based on flea and tick studies also appears to be very high with only 4/383 (1.0%) treated dogs having an adverse event and these were exclusively transient gastrointestinal-related events including vomiting and appetite loss. Impressive results have been also seen with Bravecto® for treatment of demodicosis. In one study comparing a single dose of Bravecto® (25mg/kg) to topically applied Advocate® (10 mgimidacloprid/kg body weight and 2.5 mgmoxidectin/kg body weight) at 28-day intervals. Bravecto® was found to be superior in mite reduction and % of mite free dogs. Mites were counted in skin scrapings and demodectic lesions were evaluated on each dog before treatment and at 28-day intervals thereafter over a 12-week study period. Bravecto® reduced mites by 99.8% on Day 28 and by 100% on Days 56 and 84. Mite numbers in the 28-day intervals with Advocate® were reduced by 98.0% on Day 28, by 96.5% on Day 56 and by 94.7% on Day 84. Statistically significantly (P < 0.05) fewer mites were found on Days 56 and 84 on the Bravecto® treated dogs compared to Advocate® treated dogs. The single oral administration of Bravecto® was highly effective against generalized demodicosis, with no mites detectable at 56 and 84 days following treatment. 5 The author has also used Bravecto® successfully for cases of demodicosis. Some researchers are using it at 2-month intervals for perceived increased efficacy, however the author has been impressed with responses at the recommended 3-month interval. The most impressive case series has come from work in Poland from Dr Joanna Karaś-Tečza who has treated 163 dogs with generalized demodicosis including both juvenile and adult onset with Bravecto® (25mg/kg) with an overwhelming 100% success rate with 2 treatments at 3 month intervals. 29 Most interestingly was her ability to treat breeding bitches of 16 different breeds (3 German Shepherd dogs, 3 pugs, 2 Great Danes, 2 Shih Tzus, 2 shibas, 1 malamute, 1 Italian greyhound 1 ridgeback, 1 mix breed) that had produced repeated litters of generalized demodex puppies. In this trial, all bitches were treated with 25 mg/kg fluralaner 10 days prior to scheduled mating and 3 months later with a 2nd dose. Fourteen bitches gave birth to litters that were clinically unaffected by demodicosis, although two puppies from one litter developed localized demodex. 30 Bravecto® is approved for dogs 6 months of age and older and should be given with food for optimal absorption.

**Sarolaner (Simparica, Zoetis Animal Health):** The most recent isoxazoline molecule to come to the market is sarolaner. It has a very similar spectrum of activity, efficacy, pharmacokinetics and safety to the other isoxazoline molecules described above. It was evaluated against *Demodex spp.* in dogs with generalized demodicosis. In the initial publication 16 dogs with clinical signs of generalized demodicosis were randomly assigned to treatment with either sarolaner (2mg/kg) orally on Days 0, 30 and 60, or topical imidacloprid (10mg/kg) plus moxidectin (2.5mg/kg) solution every 7 days from Day 0 to Day 81. For sarolaner-treated dogs, pretreatment mite counts were reduced by 97.1% at 14 days and 99.8% by 29 days after the first dose, with no live mites detected thereafter. Weekly imidacloprid plus moxidectin resulted in 84.4 and 95.6% reduction at these two-time points, respectively, with no mites detected from Day 74 on. All dogs in both groups showed marked improvement in the clinical signs of demodicosis. There were no treatment related adverse events in the study. In this study, sarolaner at an oral dose of 2mg/kg was highly effective in reducing the live mite counts associated with a natural infestation of *Demodex spp.*, with all dogs showing a marked improvement in the clinical signs of generalized demodicosis. 7 It is approved for dogs 6 months of age and older and should be given with food for optimal absorption.

**REFERENCES**


EQUINE PAPILLOMAVIRUSES: PATHOLOGY AND CLINICAL FEATURES

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INTRODUCTION

Eight equine papillomaviruses (PVs) have been discovered to-date and have been associated with four clinical disorders that are relatively distinct. Equine classical papillomas, genital papillomas and aural plaques are three established disorders while EcPV8-associated generalized papillomatosis is a fourth newly described disorder. This presentation will cover the basic biology of papilloma viruses and the clinical presentations and pathology of the four disorders in horses. Equine sarcoids are associated with bovine papillomavirus-1 and -2 infection but this topic will not be addressed.

PAPILLOMAVIRUS BIOLOGY

Papillomavirus Structure: Papillomavirus particles are icosahedral (50-60 nm), non-enveloped, and contain a small, circular (episomal), double-stranded DNA genome of about 8 kb. The viral capsid is formed by pentamers (capsomeres) of the major capsid protein (L1) and by monomers of the minor capsid protein (L2), occurring in a 72:12 ratio. The genome has a long coding region, containing regulatory elements and the origin of replication, as well as eight open reading frames that are divided into early (E) and late (L) coding regions based on the timing of gene expression. The early region encodes for regulatory proteins (E1, E2 and E4) and for transforming proteins (E5, E6 and E7). The late region encodes the major (L1) and minor (L2) capsid proteins.

Infection and life-cycle: Papillomaviruses are host specific and are tropic for skin and mucosal epithelium. The virus requires access to basal keratinocytes to initiate stable infection, usually through minor wounding. To initiate keratinocyte infection, the L1 capsid protein binds extracellular matrix (heparan sulfate proteoglycan) at the basement membrane zone and undergoes a conformational change to expose L2 for enzymatic cleavage by host furan; subsequently, L2 binds the basal cell to initiate cell entry. Infection of basal stem cells is thought to be required for persistent infection by low-risk papillomaviruses but this requirement for high-risk viruses is less clear. Selective mucosal and cutaneous tropisms are attributed to differences in viral particle surface charge (cutaneous Vs mucosal), local furan expression, and viral gene expression (regulatory elements in the long coding region). The identification of papillomavirus in circulating leukocytes adds new complexity to the traditional model of infection.

During initial keratinocyte infection, E1 and E2 viral proteins function in the basal layer to establish viral genomes as low copy number episomes in basal keratinocytes. E2 also tethers viral episomes to chromosomes for partitioning in mitosis to daughter cells, thereby increasing the number of infected cells. Because keratinocytes terminally differentiate after leaving the basal layer, E6 and E7 push suprabasal cells to re-enter the cell cycle to allow viral genome replication and amplification to high numbers in the stratum spinosum and granulosum. High levels of E2 down regulate E6 and E7 to allow terminal keratinocyte differentiation, needed for desquamation, and L1 and L2 capsid protein expression in the stratum granulosum. L1 and L2 package viral DNA and assemble viral particles in the nucleus in the stratum granulosum, where viral inclusion bodies can be seen. During this phase, E4 disrupts cornification by binding intermediate filaments and transglutaminases thereby weakening corneocytes and facilitating viral release after desquamation, which is needed for infection of a new host.

Clinical lesions: Papillomavirus infection results in subclinical infection, papillomas and/or neoplasia, much less often, typically squamous cell carcinoma, but others occur. Subclinical infections can be transient or persistent (latent), and last for months to years, and may or may not be productive. Viral papillomas are proliferative, productive infections that can take different morphological forms (classic exophytic, plaque, endophytic, etc) and have propensities for certain anatomical locations, which is likely important to promote coordinated gene expression. Papillomas can persist for months to years and most are ultimately cleared by the host immune system. In humans, low-risk and high-risk papillomaviruses are recognized based on risk of neoplastic transformation associated with infection. The varied clinical course of infection is attributed to a balance between differences in the papillomavirus genome (low-risk Vs high risk),
host immune response, specific location (cell type) of infection and ability to maintain persistent infection. High-risk viral infections appear to need all of these factors to promote abortive, non-productive infections leading to neoplastic transformation. Immune evasion and persistence allow time for specific anatomical site-related deregulation of viral gene expression and differences in high-risk viral genes, especially E6 and E7, to promote cell proliferation, DNA alterations (via p53, retinoblastoma proteins, etc.) and neoplastic transformation. Low-risk viruses are not completely without risk of cancer but mechanisms are different and may involve synergism with other genotoxic agents, such as UV radiation, to be carcinogenic.

CLINICAL DISORDERS CAUSED EQUINE PAPILLOMAVIRUSES

Equine classical viral papillomas/papillomatosis ("Grass Warts")\textsuperscript{1,5}

**Cause:** Equine caballus papillomavirus-1 (EcPV1).

**Pathology:** **Lesions:** Classical papillomas have exophytic-papillary type (classic) morphology. Fully developed lesions are exophytic masses with a papillary surface morphology and are small, tan-white (depigmented) and sessile or pedunculated. Early and late lesions are smaller, more papular and smooth; late lesions are also often ulcerated and crusted. **Number:** Multiple papillomas typically develop concurrently, which can be very numerous (>100), and qualify as papillomatosis, or can be single or few, which is more common in older horses. **Distribution:** Lesions are usually on the head, especially the muzzle and lips. Eyelids can be affected. Distal limbs are another common location. **Cancer risk:** Neoplastic transformation has not been described.

**Clinical Features:** **Occurrence:** Classical viral papillomas are common. **Signalment:** No breed or sex predilection has been reported. Papillomas occur most often in young horses, less than 3 years of age, but old horses and immune compromised horses develop lesions. Multiple young horses may be affected at one time on a farm and from year to year with new foal crops. **Clinical Significance:** In normal young horses, lesions are considered self-limiting. **Clinical course:** Papillomas usually resolve in 3 to 4 months, but resolution can take 9 months. If lesions persist longer, then assessment for underlying immunosuppression should be considered. Lesions can persist in older horses and may require intervention in some cases.

**Diagnosis:** Lesions are clinically distinct. Large and/or atypical lesions must be differentiated from verrucous variants of equine sarcoid. Histopathology confirms the diagnosis, can differentiate equine sarcoid, but is generally not required.

Equine genital papillomas/papillomatosis and genital squamous cell carcinoma\textsuperscript{1,6-9,12}

**Cause:** Equine caballus papillomavirus-2 (EcPV2). The role of EcPV7 is undermined because the one identified case with a penile mass lacked any clinical or pathology description.

**Pathology:** **Lesions:** Genital papillomas usually have plaque-type morphology, are raised, sometimes only minimally, and are variably pigmented. Early lesions are small pink-white to gray papular masses. Developed lesions vary from discrete, small to large, circular plaques with hypopigmentation to very large lesions that are less discrete with extensive mucosal thickening. The surface can be smooth or very irregular and is occasionally hyperkeratotic with exfoliation and/or horn like growth. Papillary projections and exophytic masses can occur but must be differentiated from squamous cell carcinoma. **Number:** Single papillomas occur but lesions are more often multifocal and these can coalesce. Some horses develop hundreds and this qualifies as genital papillomatosis. **Distribution:** In males, the glans penis is more commonly affected followed by the free part (body) of the penis and the inner prepuceal folds but lesions can extend to outer prepuceal folds and perigenital skin. Single or multifocal lesions are reported in females and affect the vulva, vestibular walls and/or clitoris.

**Cancer risk:** EcPV2 is associated with development of squamous cell carcinoma in-situ and squamous cell carcinoma (SCC). Thus, EcPV2 is thought to cause genital papillomas, some of which, but not all, progress into SCC in-situ and then into overt SCC. Papillomas have a similar distribution as genital SCC in-situ and SCC and can occur concurrently with either neoplasm. Concern develops for SCC in-situ when chronic, persistent papillomavirus plaques developed thickened areas, erosions and/or crusts. Late SCC lesions are more mass-like plaques and nodules in which erosions and ulcers are common. SCC is invasive and metastasis occurs locally in lymphatic vessels, regionally in lymph nodes and ultimately in internal organs. Others, causes for genital SCC in-situ and SCC are proposed, such as solar injury.
Clinical features: Occurrence: Uncommon put true prevalence not known. Lesions go unrecognized without regular examination. Signalment: The mean reported age of genital papillomas ranges from 16-18 years of age and there is no clear breed predilection reported. Male horses are reported more commonly than female horses, and the majority of studies describe geldings. Clinical significance: Lesions range from very small to large and are associated with discomfort and local infection. Transformation to squamous cell carcinoma occurs in some horses, which can lead to radical treatment and/or death. Clinical course: There is limited detailed information on clinical progression of genital papillomas. Papillomas are generally thought to develop slowly and to persistent. Lesions often go unnoticed by owners and the true duration of genital papillomas and even many SCCs is not often know. Individual cases of genital papillomas have been reported to persist for years without significant change or with progression to SCC.

Diagnosis: Diagnosis is complicated by the co-occurrence of SCC in-situ and/or SCC in some patients. Histopathology is an essential early step for diagnosis genital papillomas, SCC in-situ and SCC and for development of a treatment plan. A grading scheme has been reported to assist prognostication.

Aural Plaques (Ear Papillomas, Pinnal Acanthosis, Hyperplastic Aural Dermatitis)\(^1, 10-14\)

Cause: Equine caballus papillomavirus-3, -4, -5 and -6 (EcPV3-6)

Pathology: Lesions: Papillomas have plaque morphology. Individual plaques are pink-white, circular, flat-topped and raised. Lesions start out as 1-5 mm small papules and expand to around 5 to 10 mm, but individual mature lesions rarely reach more than one centimeter in diameter and thus are often not true plaques. Lesions can be smooth but others have mild to marked hyperkeratosis, which can exfoliate and appear to fill the ear. Number: Lesions can be single but multiple lesions are usually present and often coalesce to appear much larger, sometimes covering much of the inner ear pinna. Distribution: Plaques occur on the central concave pinna and usually involve both ears simultaneously but sometimes only one ear is involved. Similar lesions are described less commonly on the ventral abdomen, peri-sheath area and inner thighs, but the specific viral cause has not been determined. Cancer risk: Neoplastic transformation has not been described.

Clinical features: Occurrence: Aural plaques are common. Signalment: Lesions occur in horses of any age and occur without an apparent sex or breed predilection. Horses less than 1 year of age are usually not affected. Clinical course: Aural plaques develop slowly and are reported not to regress, persisting for years. However, non-pigmented, white macules can be found in horses that suggest resolution of these lesions does occur in some horses. Clinical significance: Most cases are asymptomatic, have a benign course and are considered cosmetic complications. Aural sensitivity or pain has been attributed to lesions in some horses, but other causes must be excluded. The onset of lesions in summer months in some locations and the overlap in lesion distribution on the inner pinnal, groin and inner thigh surfaces with black fly (Simulium spp.) feeding sites supports black flies as a possible vector. Viral genome sequencing is needed to determine if the same viruses cause similar plaque-like lesions at different body sites or if additional viruses are involved. Diagnosis: Clinical lesions are distinctive but occasionally hyperkeratotic variants of aural plaques must be differentiated from hyperkeratotic/verrucose variants of equine sarcoid that can occur in the inner pinnal surface and present with unilateral or bilateral ear involvement. Any nodular thickening involving the dermis suggests the presence of another process because aural plaques only expand the epidermis. Histopathology confirms the diagnosis but is not usually needed.

EcPV8-Associated Generalized Papillomatosis\(^2\)

Cause: Equus caballus papillomavirus-8 (EcPV8)

Pathology: Lesions: Papillomas are plaque type, non-pigmented, circular, measuring 0.5 to 1.5 cm and were often very hyperkeratotic. Number: Horses developed dozens to several thousand papillomas that coalesced into large plaques on the ventral trunk, axillary and inguinal areas in severe cases. Distribution: Papillomas were located on the axilla, inguinal and proximal limbs as well as the ventral and lateral neck, thorax and abdomen. Lesions were confluent in ventral areas. Fewer lesions were on the face, ears, distal limbs and genitalia. Cancer risk: Neoplastic transformation has not been described.

Clinical features: Occurrence: EcPV8 associated generalized papillomatosis is considered rare. The true incidence is unknown. Signalment: The three Quarter horses reported were a stallion and two mares, aged 1.0, 1.5 and 10 years old.
at presentation. **Clinical course:** Papillomas nearly regressed after 1.5 years in one horse and regressed only partially after 3 years in another horse. **Clinical significance:** Lesions ranged from dozens to thousands and were disfiguring when numerous, leading to euthanasia in one case. Underlying immune suppression or deficiency was suspected but not identified. Horses did not develop other spontaneous infections and a more restricted innate immunity defect is possible. **Diagnosis:** The diagnosis is based on clinical appearance, papilloma lesion type, distribution, number and histopathology. A Quarter horse breed association is suggested but not established. PCR testing is ultimately needed to identify EcPV8-associated lesions, but commercial testing is not available.

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Diagnosis and Treatment of Equine Sarcoids

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INTRODUCTION

Skin diseases are one of the principal health problems in horses and the skin is the most common location for equine tumors. Equine sarcoids, melanomas and squamous cell carcinomas make up over 95% of equine cutaneous tumors, with the sarcoid being the most common form of skin cancer in Equidae. The most important factor in the development of equine sarcoids appears to be an infection with bovine papillomavirus (BPV) type 1 and/or 2 (and, more recently, type 13). Bovine papillomavirus DNA has been identified in up to 100% of examined sarcoïds, representing the only known cross-species papillomavirus infection in domestic animals. However, it is generally accepted, that a BPV infection alone is not sufficient for neoplastic transformation. Genetic risk factors have been identified: certain equine leukocyte antigen (ELA) haplotypes are associated with increased susceptibility. Quarter Horses, Arabians and Appaloosas appear to be at a greater risk than Thoroughbreds, while Standardbreds are at a lower risk. Finally, sarcoids can develop at the site of any skin trauma (including lacerations, injections, insect bites), especially if the horse has sarcoids in other locations. It appears trauma does not only contribute to the neoplastic transformation, but also to the progression of the disease.

DIAGNOSIS

The list of differential diagnoses for equine sarcoids is relatively long and includes granulation tissue, traumatic lesions, alopecia areata, (fungal) granulomas, papillomas, fibromas/fibrosarcomas, cutaneous lymphomas, habronemiasis, squamous cell carcinomas, peripheral nerve sheath tumors, melanomas and mast cell tumors. The reason for the high number of differentials is the very variable macroscopic appearance of the equine sarcoid, which has led to the creation of a clinical classification system. Sarcoids are classified according to their gross appearance and clinical behavior. The occult or flat sarcoid is the mildest form. It presents as usually slow growing alopecic, sometimes scaly to hyperkeratotic, lesions that are often found in the periocular or perioral aspect of the face, on the neck, medial forearm, or the axilla (“hairless” areas). The verrucose (warty) form has a dry, rough and raised surface with poorly defined margins. These “cauliflower”-like tumors are commonly identified on the head, neck, axilla, groin, sheath as well as the coronary band. Well-circumscribed tumors with a frequently normal surface, although ulceration is possible, are classified as nodular sarcoïds. Type-A1 nodular sarcoïds are well demarcated spherical masses in the subcutaneous tissue that can be moved freely under the skin, while Type-A2 masses are bound to the deeper tissues. In contrast, Type-B nodular sarcoïds cannot be moved independent from the skin, since they involve the epidermis. Nodular sarcoïds are often found on the eyelids, the groin area and the prepuce. The fibroblastic sarcoid has a fleshy appearance with an ulcerated surface that resembles exuberant granulation tissue. Type 1 fibroblastic sarcoïds are pedunculated masses, while Type 2 lesions have a broad base with ill-defined margins. Occult, verrucous and nodular sarcoïds can rapidly progress to this locally aggressive form that can also directly develop in skin wounds. Mixed sarcoïds contain any of the above described types while the malignant (malevolent) form invades along lymphatic vessels and extends to local lymph nodes.

Due to the very variable clinical appearance, histological confirmation might be necessary to confidently diagnose an equid sarcoïd. Because trauma can exacerbate the locally aggressive behavior of this tumor, a biopsy should only be taken if treatment is rapidly initiated after the diagnosis has been confirmed. A complete excisional biopsy should be considered whenever possible.

TREATMENT

The various clinical types of sarcoïds require slightly different treatment regimens and many management protocols have been described – indicating that not a single one is universally effective. Although a horse-owner favorite, “benign neglect” is only suitable for a very small number of cases. Small occult or verrucous sarcoïds that are not exposed to repeated trauma may be monitored, with the understanding that close observation is critical and removal mandatory if signs of deterioration are observed.
Surgical Excision
Although conventional removal can be a fast and effective way for some cases, recurrence rates can be extremely high, with up to 70% of tumors recurring within 6 months. These re-growths also appear to be clinically more aggressive than the original tumor. To maximize the chances of a successful removal, only tumors that have a defined margin and are in a location where at least 12 mm of surrounding, healthy appearing skin can be excised, should be considered for this therapy. Contamination of the wound bed with abnormal cells has to be minimized or (ideally) eliminated and primary closure should be the goal. Superficial lesions (occult, verrucose) and some Type A-1 nodular sarcoids can be suitable candidates for this treatment.

Cryosurgery
In the absence of important underlying anatomical structures (nerves, significant vessels, synovial structures), three freeze-thawing cycles using liquid nitrogen at -196°C can be an effective method in treating superficial lesions. This technique is also commonly used as an adjunct therapy.

Laser Surgery
Success rates following excision with a CO₂, Nd:YAG or diode laser have been reported to be as high as 80%. However, case selection is, as for any treatment of equine sarcoids, critical for the outcome. The laser-induced coagulative necrosis around the margin of the surgery site appears to be a main advantage of this technique.

Intralesional Chemotherapy
Injections of cytotoxic drugs directly into the mass are commonly used to manage equine skin tumors. However, their overall efficacy is limited and the need to for repeated injections over prolonged periods can lead to client frustration and, subsequently, a lack of compliance. Drugs that are frequently used in equine practice include 5-Fluorouracil, Cisplatin and Carboplatin. The use of slow release biodegradable emulsions, sponges and beads is recommended to guarantee longer lasting effects on the tumor tissue. More recently, electrochemotherapy has been shown to be a very effective way of treating localized individual tumors, although the relatively expensive equipment and need for general anesthesia will likely limit its availability to referral practices.

Topical Immunotherapy
The topical application of 5% imiquimod (Aldara™) cream has resulted in excellent outcomes in horses with small sarcoids. The drug is described as an immune response modifier with anti-tumor properties, although its precise mechanism of action remains unclear. Instructions for its use and examples of treatment results can be found on the website of the University of Minnesota (https://www.vetmed.umn.edu/centers-programs/clinical-investigation-center/completed-clinical-studies/veterinarian-instructions-aldara). Other treatments modalities for equine sarcoids are available and regularly used in equine practice, but in many cases, reports about their success rates and potential negative side effects are limited.

Summary
The prognosis after sarcoid treatment should always be considered guarded, because recurrence rates are generally high. Especially the management of tumors located at difficult sites, where complete removal with wide margins is challenging or impossible, can be frustrating. To maximize the chances of a successful outcome, the best possible treatment has to be applied on the first occasion, since recurrent masses tend to be even more likely to return.

5. Broström H, Fahlinbirk E, Dubath ML, et al. Association between equine leucocyte antigens (ELA) and equine sarcoid tumors in the population of Swedish Halfbreds and some of their families. Veterinary Immunology and
Cytokines are soluble factors with critical functions in several biological responses. In particular, they serve as an intracellular communication tool of immune system, and their release and actions help shape the immune response. When cytokines are produced in abnormal amounts, either higher or lower, the homeostasis of the immune system is altered and several pathologies may arise [1]. Cytokines bind to a variety of receptors, but only the so-called type I and type II receptors are relevant for this presentation. Most (but not all) of the interleukins belong to this group of cytokines as well as some hormones and growth factors. Upon binding of the relevant cytokine to its receptors, signaling events that involve several cytosolic substrates are triggered. The activation of the tyrosine kinase of the Janus family (better known as JAKs), is the first critical step. This family is comprised of four molecules, namely JAK1, JAK2, JAK3 and TYK2. JAKs, which work in pairs, become enzymatically active and phosphorylate themselves, the receptor chains, and several other substrates including the Signal Transducers and Activation of Transcription (STATs) family of latent transcription factors which translocate to the nucleus and regulate gene expression [2]. Cytokines act on different cells and likewise, JAKs are expressed in many cell types. On the other hand, JAK3 is selectively expressed in hematopoietic cells and mutation in this kinase results in loss of function and severe combined immunodeficiency in humans [3]. It was therefore hypothesized that blocking the enzymatic activity of JAK3 would also result in immunosuppression. Importantly, gain of function mutations in JAK2 have also been reported in humans and cells expressing the mutant JAK2 proliferate in a cytokine-independent manner [4]. The road to the development of JAK inhibitors was paved by development of inhibitors of other tyrosine kinases. Small molecules such as imatinib, which block the ATP binding activity of tyrosine kinases, were successfully generated and have been employed in the treatment of several malignancies including leukemia, lymphomas and even some solid tumors. First generation JAK inhibitors also compete with ATP by inserting themselves in the binding pocket of the JAKs.

As of today the FDA has approved three JAK inhibitors for clinical use. Ruxolitinib (trade name Jakafi) is a JAK2/JAK1 inhibitor (with some activity on JAK3 and TYK2) currently prescribed for the treatment of intermediate or high-risk myeloproliferative disorders including primary myelofibrosis post-polycythemia vera myelofibrosis and post-essential thrombocythemia and myelofibrosis. Ruxolitinib has also been granted Breakthrough Therapy Designation for the treatment of Graft vs. Host Disease [5].

Tofacitinib (trade name Xeljianz) instead is a JAK3/JAK1 inhibitor (but JAK2 is also affected, albeit to a lesser extent) recently approved for the treatment of rheumatoid arthritis (RA) in patients for which methotrexate therapy was not efficacious. In these cases, tofacitinib is used either as monotherapy or it can be combined with methotrexate or other non-biologic disease-modifying anti-rheumatic drugs [6, 7].

Finally, oclacitinib is a pan-JAK inhibitor approved for canine eczema and atopic dermatitis.

Not yet approved by the FDA (but it will be soon given the excellent results in phase III clinical trials in patients with RA) baricitinib is another JAK1/JAK2 inhibitor that inhibits intracellular signaling of multiple proinflammatory cytokines including IL-6, IL-12, IL-23 and IFN-gamma.

These first-generation JAK inhibitors block the enzymatic activity of all the JAKs with different degrees of specificity. In vitro studies have shown that, in regard to the human kinome, their activity is exquisitely limited to the JAKs [8]. This specificity limits their off-target effects but does not impair their capacity to blunt the effects of multiple cytokines. In fact, in the case of tofacitinib, the capacity to inhibit the actions of several pro-inflammatory cytokines and to act on different immune cells are possibly the reasons why this drug has been so effective in the treatment of RA, a disease which pathophysiology involves the action of several cytokines.

Blocking cytokines such as interleukins, interferons and erythropoietin results in effects on many cell types such as T, B, NK cells and erythrocytes. Administration of a JAK2 inhibitor like ruxolitinib results in anemia and thrombocytopenia...
as expected by the well-known role of JAK2 in erythropoietin and thrombopoietin signal transduction. On the other hand, patients receiving the drug showed clinical improvement in all the myelofbrosis-related symptoms [9].

In the case of tofacitinib, the total number of circulating T cells is not impaired but differentiation of T helper (Th) cells such as Th1, Th2 and Th17 is impaired [10]. Animal studies have also shown a decline in number of circulating NK cells. Patients treated with tofacitinib tend to be more prone to infections, which included opportunistic pathogens and especially herpes zoster. The above-mentioned effect on NK cells does not appear to correlate with increased incidence of tumors, but the long terms effects have not yet been evaluated.

Autoimmune disorders are a classical example of pathologies in which several pro-inflammatory cytokines have been demonstrated to be driver of the disease. Therefore, it is not surprising that several pathologies can potentially be treated with JAK inhibitors. Besides RA (for which tofacitinib is FDA approved) several clinical trials are ongoing with both tofacitinib and baricitinib for pathologies such as inflammatory bowel disease, psoriasis, psoriatic arthritis and atopic dermatitis. It should be noted that for each pathology, specific cytokines and therefore, some specific JAKS are involved. This opens the possibility to use, next-generation, JAK inhibitors which inhibit one JAK but not others. I will briefly discuss these newer, selective JAK inhibitors below.

We have been interested in the possibility of using tofacitinib for the treatment of systemic lupus erythematosus (SLE). In a preclinical study in a mouse model of SLE we showed that daily administration of the JAK inhibitor to the mice resulted in amelioration of the pathology. A phase Ib clinical trial in SLE patients is now ongoing at the NIH.

Several other immune related pathologies have also been shown to be ameliorated by JAK inhibitors although properly designed clinical trial have not been performed. These pathologies include alopecia (both areata and universalsis), vitiligo and myositis. JAK inhibitors are also being tested as immunosuppressants in transplantation and in malignancies such as myeloid leukemias and multiple myeloma.

Moreover, this class of drugs have shown to be life-saving in patients with Mendelian diseases such as patients with mutations in the immunoproteasome subunits or the nucleic acid sensor called STING.

Overall, JAK inhibitors’ side effects partially overlap with what has been observed for some of the biologics utilized in the therapy of autoimmune diseases. Increased lipid levels have been observed in patients receiving JAK inhibitors have also been reported in patients treated with the anti-IL-6R antibody tocilizumab. Interestingly, some of these changes in lipids seems to be potentially beneficial with increases in high density lipoprotein (HDL) and an effect on cholesterol ester catabolism [11]. Similarly, increased creatinine and transaminase levels were detected, but it remains unclear if such alterations or the increase in lipid levels are directly related to JAK enzymatic activity.

Some of the new, “next-generation” inhibitors appear to be more specific. For example, filgotinib is a JAK1 selective inhibitor that is being investigated in RA and has displays results comparable to tofacitinib. Upadacitinib is the first JAK inhibitor that does not compete with ATP but, instead, binds to a non-conserved domain outside the ATP-binding site of JAK1 and is being considered for treatment of RA and atopic dermatitis. Decernotinib is reported to be a JAK3 selective molecule considered for the treatment of RA and Preserving JAK1 and JAK2 signaling, would, in principle, eliminate non-immunologic adverse effects. Tyk2 inhibitors are still in development and no major candidate has emerged so far. Overall, it is still not known whether selectivity will result in increased efficacy or diminished side effects. Nonetheless, we could speculate that these selective inhibitors may be better suited for diseases in which only one or very few cytokines are implicated. The mechanism of delivery (e.g. orally, parenterally, or topically) should need to be considered in regard to their use.

In conclusion, targeting of JAKs was pursued due to their expression pattern and association with several pathologies. The last few years have seen an explosion of data regarding their efficacy for the treatment of autoimmune diseases, and we can now say that inhibition of JAK enzymatic activity has clearly proved successful. Nonetheless, several questions with clinical relevance are still unanswered. We still do not know the best way to achieve proper selectivity for each JAK. In fact, while the latest, next-generation JAK inhibitors appear to be selective in vitro, their selectivity in vivo seems less evident. Furthermore, advantages and disadvantages of selective versus nonselective JAK inhibitors are unclear. So far we have learned that pan-JAK inhibitors are quite effective for disease like RA which involves the
action of several cytokines, but this may not be true for other immune-mediated diseases. Which patients are more likely to benefit from these drugs? What is the ideal dosing regimen? Can we combine JAK inhibitors with other immunomodulatory drugs? Clearly the next few years will be spent trying to answer some these questions.

REFERENCES

OBJECTIVES

1. To understand the different types and functions of JAK.
2. To understand the concept of drug selectivity and how it applies to JAK.
3. To review the safety and efficacy of oclacitinib, the JAK inhibitor approved for use in dogs.
MONOCLONAL ANTIBODIES:  
21ST CENTURY MEDICINE FOR 21ST CENTURY PETS  
Valerie A. Fadok, DVM, PhD, DACVD

OBJECTIVES
1. To understand how monoclonal antibodies are generated and tested.
2. To understand what contributes to the safety and efficacy of monoclonal antibodies.
3. To review the safety and efficacy of lokivetmab and other monoclonal antibodies currently being used or tested in veterinary medicine.
INTRODUCTION
The skin's functions in providing innate protection and maintaining homeostasis along with the systemic factors that can influence its integrity make it a critical sentinel for internal or systemic disease. Some cutaneous changes are so intimately associated with a particular underlying organ dysfunction or disorder that they are immediate visual clues to evaluate for specific diseases. Evaluation for disturbances in hemostasis or vascular integrity is clearly indicated when petechiations and/or ecchymoses are identified on the skin or mucosal surfaces of patients. The color change seen in an animal with icteric mucous membranes is a clear indicator to evaluate for causes of jaundice in that patient. Changes in the appearance of the skin may be markers of pathology occurring in another organ system or they may represent a disease process that is multi-systemic, such as seen with some infectious diseases or in systemic lupus erythematosus. Both the appearance and integrity of the skin are influenced by a number of systemic factors that can be influenced by internal disease. These factors include the nutritional status, hormonal levels and interactions, perfusion and vascular integrity and the overall health and systemic organ function of the individual animal. Consequently changes in the skin can be a critical sentinel for internal disease. The skin is also readily accessible for diagnostic sampling and can in some cases provide the necessary information for making the diagnosis of systemic disease. Recognizing those skin changes that are clinical markers for underlying systemic disease can expedite the diagnosis and timely management of those diseases. In today's lecture we will discuss some of the examples that can be seen when there are underlying hormonal, neoplastic and infectious diseases.

CUTANEOUS CHANGES ASSOCIATED WITH HORMONAL DISTURBANCES
Endocrine diseases provide excellent examples of the connection between disease and the skin. Hypothyroidism, hyperadrenocorticism (HAC), and sex hormone imbalances from testicular neoplasia, ovarian tumors or adrenal tumors can alter the skin's appearance and function. Disturbance in growth hormone and sex hormones other than estrogen will not be discussed.

Thyroid Hormone Disturbances
Thyroid hormones are very important to the skin and promote the initiation of the anagen phase of the hair follicle cycle. Consequently, many hypothyroid dogs have some degree of alopecia. This alopecia is often first noted in areas of wear (neck under collar, dorsal tail, pressure points, lateral trunk). The extent and pattern of alopecia can vary between breeds of dogs and individual animals. For example, Rhodesian Ridgebacks can develop a pronounced striping pattern. The persisting hair coat in hypothyroid dogs is often dry, brittle and can be either dull or faded in color. Failure to regrow hair coat after clipping is sometimes a presenting complaint of hypothyroid dogs. Hypothyroidism results in disturbances in cornification, melanosis, an increase in the number of hair follicles in telogen and accumulation of glycosaminoglycan in the dermis. Clinically this results in alopecia, a dull, dry hair coat, variable hyperpigmentation, scaling, and myxedematous changes. Pinnal margin seborrhea may be seen in some dogs. In hypothyroidism, the normal barrier function of the epidermis is likely impaired and in animal models impaired neutrophil and lymphocyte function have been reported. Consequently recurrent pyoderma and otitis externa often occur. In some cases recurrent or refractory otitis externa or recurrent pyoderma may be the only presenting clinical signs in a dog with hypothyroidism.

Spontaneous hypothyroidism in cats is extremely rare. One reported case had similar clinical signs to dogs but experimentally thyroidectomized cats did not; they reportedly groomed less, developed matting and seborrhea but only focal alopecia on pinnae and pressure points. Hyperthyroid cats can develop matting, seborrhea, increased shedding and over-grooming.
**Cortisol Disturbances:**
Excessive glucocorticoids cause cornification abnormalities, inhibit fibroblast proliferation and consequently collagen production and result in pilosebaceous gland atrophy. Clinically, excessive cortisol (endogenous or exogenous) also results in disturbances in cornification, dermal thinning and delayed wound healing.

1. **Canine Hyperadrenocorticism (HAC)**
   Dogs with HAC or iatrogenic hypercortisolism can develop bilaterally symmetrical alopecia, thin hypotonic skin (with or without striae), increased susceptibility to bruising, easily visible dermal vasculature, phlebectasias (ventrum and medial thighs), comedones, milia, calcinosis cutis and increased susceptibility to recurrent pyoderma and adult onset demodicosis.

2. **Calcinosis Cutis**
   This is a broad term and includes all forms of dystrophic or metastatic calcification of the skin. It is most often used for the dystrophic calcification seen in dogs secondary to hyperadrenocorticism or iatrogenic hyperglucocorticoidism. The chinchilla is the only other species to develop calcinosis cutis with hyperadrenocorticism. In dogs, erythematous papules coalesce into firm, gritty plaques that may ulcerate and develop hemorrhagic crusts. Lesions develop in areas prone to chronic flexure movement and the dorsal cervical, axillary or inguinal areas are common lesional sites. Dystrophic calcification can also involve mucosal membranes and the tongue. Metastatic calcification producing nodular calcium deposits in the skin, especially footpads, has been reported in dogs and cats with chronic renal failure. The author has documented calcinosis cutis lesions in the inguinal region of a dog supplemented chronically with calcitriol post-parathyroidectomy. The mineral present in calcinosis cutis has been shown by infrared spectrometry to be apatite crystals. A recent study showed predisposed breeds to include Labrador retrievers, Rottweilers, boxers and Staffordshire terriers. Lesions of calcinosis cutis typically resolve over time if the underlying metabolic disturbance can be removed. In some cases osseous metaplasia can occur. The resulting osteoma cutis lesions will not regress.

3. **Feline Acquired Skin Fragility**
   Acquired skin fragility in cats is associated with hyperadrenocorticism (often adrenal tumors), iatrogenic hyperglucocorticoidism, or excessive levels of prostaglandin compounds from either adrenal tumors or the iatrogenic effect of administered prostaglandin compounds. Affected cats have extremely thin, fragile skin that easily bruises and can be torn with simple manipulations, often during restraint or handling. There are also rare reports of feline skin fragility being associated with hepatic lipidosis and hepatic neoplasia.

**Estrogen Disturbances: Hyperestrogenism**
Increased estrogen can arise from cystic ovaries, granulosa cell tumors, testicular tumors (Sertoli cell tumors most commonly) or iatrogenically from estrogen supplementation for urinary incontinence or chronic exposure to human topical estrogen products. Estrogen inhibits anagen initiation resulting clinically in alopecia. Hyperpigmentation is often present and can be diffuse or macular. Alopecia often begins in the perineal, caudal thigh, inguinal and flank regions. Identification and correction of the underlying cause of hyperestrogenism may require abdominal ultrasound of ovarian tissue or cryptorchid testes or ultrasound of the testicles. If pathology is identified ovariohysterectomy or castration is indicated. In the already spayed female or neutered male possible exposure to exogenous sources of estrogen (diethylstilbestrol) for urinary incontinence or exposure to human use of estrogen topical therapy needs to be evaluated.

**CUTANEOUS PARANEoplastIC SYNDROMES**
A paraneoplastic syndrome is defined as either a disease or clinical signs or symptoms that develop distant from the site of a tumor, is caused by the presence of the tumor or its metastasis, but is not resulting from the local presence of neoplastic cells. Paraneoplastic syndromes are often mediated by hormones, cytokines or growth factors released by tumors or as an immune response targeted against the tumor. Not all classify the clinical signs associated with an organ that has undergone neoplastic transformation that is producing more of the same substance it normally produces. Consequently, diseases such as hyperadrenocorticism caused by either an adrenal tumor or pituitary tumor is not considered paraneoplastic, although some review papers may cite it as an example. Paraneoplastic skin diseases represent a group of skin disorders that if recognized alert the clinician to underlying internal neoplastic disease. These syndromes are seen most commonly in middle-aged to elderly individuals.
**Testicular Tumors (Hyperestrogenism)**

In male dogs with testicular tumors (with presumed hyperestrogenism) a visually distinctive lesion of linear preputial hyperpigmentation and/or erythema is often seen. Hyperestrogenism can also cause feminization in the male dog and in severe cases bone marrow suppression and aplastic anemia. The alopecia and hyperpigmentation described above under hyperestrogenism also occurs. Intact animals should be neutered. Already neutered animals should be evaluated for possible exogenous sources of estrogen.

**Feline Paraneoplastic Alopecia**

This is a rare, yet uniquely characteristic skin disease that occurs in association with pancreatic adenocarcinoma. Affected cats develop precipitous, ventrally pronounced alopecia in which the skin appears very shiny and smooth but is not fragile. Some cats may also have dry, exfoliative, and shiny footpads often with concentric circular rings of scale. On necropsy exocrine pancreatic adenocarcinoma with hepatic metastases is the most common tumor found but bile duct carcinoma has been reported in two cases. The disease affects older cats and the chief clinical complaint is often the acute and dramatic alopecia that affects the ventral trunk, medial aspects of the limbs and the ventral cervical region but can generalize. Remaining hairs will epilate easily. Secondary *Malassezia* infections are common and may contribute to why some affected cats groom excessively potentially exacerbating the alopecia. Histopathology of a skin biopsy reveals epidermal hyperplasia with marked follicular and adnexal atrophy. Any cat with a tentative diagnosis of paraneoplastic alopecia should undergo an abdominal ultrasound to evaluate for the presence of a pancreatic or hepatic mass. Temporary resolution of the cutaneous disease was reported in a cat after the primary pancreatic tumor was removed; the lesions recurred with the development of metastatic disease.

**Feline Thymoma-Associated Exfoliative Dermatitis**

A rare, exfoliative dermatitis has been described in middle aged to older cats with thymomas. The exact pathogenesis is not known but is thought to be an immunologic etiology potentially T cell mediated. Histologically it is similar to an erythema multiforme or graft versus host type of reaction. Skin lesions tend to begin on the head and pinnae but can quickly generalize to involve the entire cat. Generalized erythema and marked scaling are present. Secondary infections with bacteria and *Malassezia* may develop. Respiratory signs secondary to the cranial mediastinal mass may be present at the time of presentation but in most cases skin changes precede any other systemic signs. Histopathology of representative skin lesions reveals a cell poor, hydropic interface with apoptosis (single cell necrosis) of basal cell keratinocytes. If detected and diagnosed, removal of the thymic tumor will lead to resolution of the dermatologic clinical signs. A recent report describes a group of cats that had clinical and histologic features of this exfoliative dermatitis but had no concurrent thymoma, the cats were managed with immunosuppressive medications.

**CUTANEOUS MANIFESTATIONS OF SYSTEMIC INFECTIOUS DISEASES**

Sometimes the skin provides valuable clues to underlying infectious disease. Skin lesions can develop in association with systemic mycosis, viral diseases, and *Leishmania*. In these diseases the organism can be found within the lesional skin. Infectious diseases can also cause skin lesions as a consequence of the systemic vasculitis or thrombocytopenia that are associated with those infectious diseases as is the case for rickettsial diseases such as Rocky Mountain Spotted Fever, or ehrlichiosis or the protozoal disease canine babesiosis.

**Leishmaniosis**

This protozoal disease has been reported in dogs in the United States that have been imported or spent time in the Mediterranean basin/southern Europe (*Leishmania infantum*) or South America but also from autochthonous foci in many states and 2 provinces in Canada. In North America, foxhounds seem to be predisposed. The first signs of this disease noticed by owners are often skin lesions. Alopecia, erythema, and scaling with ulceration are common lesions involving the pinnae, dorsal muzzle and mucocutaneous junctions. Affected dogs may be systemically unwell with concurrent lymphadenopathy, hyperproteinemia, hyperglobulinemia, nonregenerative anemia, azotemia and proteinuria. Diagnosis is made by demonstration of the organism in aspirates of lymph nodes, bone marrow or splenic aspirates, histology of skin biopsies, isolating organism on culture, or looking for evidence of the infection via immunohistochemistry of tissue samples, serologic tests, or PCR. Recommended therapy is antimonial compounds such as meglumine antimonate (Glucantime) in Europe and sodium stibogluconate (Pentostam) in the United States.
along with oral administration of allopurinol\textsuperscript{16}. Treatment failures and relapses are common. Maintenance therapy with allopurinol (10 mg/kg) decreases parasitemia, maintains treated dogs in an asymptomatic state, and decreases the likelihood of direct or vector transmission.

**Systemic Mycosis**

Many systemic or deep mycoses (blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, aspergillosis) can present with cutaneous lesions. These lesions include papules, nodules, draining tracts and ulceration and typically result from hematogenous dissemination of the fungal organism to the skin. Although rare, direct inoculation of fungal organisms into a cutaneous wound could result in a solitary lesion. Skin lesions are seen most commonly in feline cryptococcal infections and in canine blastomycosis and are reported to occur in approximately 20-40\% of cases with these fungal infections. Typically there are other systemic clinical signs. Nasal aspergillosis can cause depigmentation and ulceration often beneath the nares as a result of a “drainage board” effect from the chronic nasal discharge. Diagnosis of any of the fungal infections is based on demonstration of the organism within biopsied tissue and/or fungal culture. Suspicious cutaneous lesions can provide easy and rapid diagnostic information in the evaluation of animals with systemic mycoses. Appropriate antifungal therapy is chosen based on type of organism and overall health of the animal.

**Systemic Viral Diseases**

A number of viral diseases can in addition to systemic clinical signs also cause cutaneous lesions. These include in dogs canine distemper and pseudo rabies and in cats the retroviruses FELV/FIV, feline herpesvirus, feline calicivirus, and feline coronavirus causing FIP. Both canine and feline papillomavirus cause cutaneous lesions and not systemic disease but they can develop more readily, become more severe or difficult to manage in patients with underlying immunosuppression.

**Canine Distemper Virus**

Distemper virus has long been associated with “hard pad disease”. Hard pad disease represents an uncommon manifestation of canine distemper virus (CDV) infection with a still unknown pathogenesis. Dogs develop excessive keratinous material on the foot pads and nasal planum. Diagnosis is suspected when the cutaneous lesions develop in a dog that shows other systemic signs of CDV (GI, respiratory and neurologic signs). Canine distemper is an epitheliotrophic virus that will target initially the gastrointestinal system, then respiratory tract and then the CNS. Affected pups may also develop impetigo. Diagnosis can be confirmed by immunohistochemical demonstration of the virus within skin biopsies of affected nasal planum or foot pads\textsuperscript{17}.

**Feline Retroviruses**

Opportunistic skin infections, oral ulcerations and gingivitis have been associated with FeLV and FIV. Cutaneous horns can develop on the paw pads of cats with FeLV. In severe cases lameness and discomfort can be marked. Diagnosis is confirmed with a positive FeLV status and skin biopsy. Immunohistochemistry can demonstrate the presence of the virus within a skin biopsy. Cutaneous lymphoma and giant cell dermatosis have also been reported in FeLV positive cats\textsuperscript{18}.

**Feline Herpesvirus**

Feline herpesvirus ulcerative dermatitis typically involves the dorsal muzzle but may extend to involve the nasal planum. Cats do not have to have concurrent ocular or upper respiratory tract signs. Histologically the lesion is a necrotizing, ulcerative dermatitis most often with a concurrent marked eosinophilic inflammation, but the inflammatory pattern may be strongly neutrophilic in some cases. The presence of eosinophilic inflammation and the clinical appearance of the lesions make it difficult to differentiate from mosquito bite hypersensitivity or other feline eosinophilic ulcerative lesions. Unless intranuclear viral inclusions can be identified it is not possible to definitively diagnose the virus as the etiologic agent for the ulcerative dermatitis. Polymerase chain reaction (PCR) has been shown to be a sensitive test to detect the presence of the virus within skin biopsies\textsuperscript{19}. Treatment can include subcutaneous administration of alpha interferon (1,000,000 units/m$^2$, 3 times a week), oral famciclovir (Famvir, Novartis Pharmaceuticals) (60-90 mg/kg)\textsuperscript{20}, and/or lysine.

**CUTANEOUS MANIFESTATIONS OF NUTRITIONAL OR METABOLIC PERTURBATIONS**

The skin can develop lesions secondary to nutritional deficiencies, however this is very uncommon in a patient that has a good appetite and is eating a well-balanced commercial food. Some cutaneous manifestations of nutritional...
deficiencies are recognized in particular breeds suggesting perhaps an alteration in absorption or metabolism while others have been linked to inadequate or unbalanced diets. Superficial necrolytic dermatitis can be a paraneoplastic skin marker if associated with glucagonoma but it is more commonly associated with some yet to be determined alterations in metabolism that causes depletion of amino acids. Underlying disturbances in lipid metabolism can result in the development of cutaneous xanthomas.

**Zinc Responsive Dermatosis**

The skin contains approximately 20% of the total body zinc (Zn) stores and the highest concentrations of Zn are found in the keratinized tissue of the nasal planum, tongue and footpad\textsuperscript{21}. There are a number of recognized syndromes associated with either Zn deficiency or disturbances in Zn assimilation that present with cutaneous signs.

Syndrome I has been identified in Siberian huskies, Alaskan malamutes and occasionally other breeds. Affected dogs typically present with erythema followed by variable alopecia with fine silver scale that becomes adherent or develops into crusting involving the mucocutaneous junctions of the face (peri-ocular, peri-oral), pressure points (elbows, hocks), and footpad margins. Dogs with this disease will manifest signs even on well-balanced diets. Diagnosis is based on signalment, typical cutaneous lesions and histopathology of skin biopsies which shows marked follicular and epidermal parakeratotic hyperkeratosis. Therapy requires Zn supplementation with a recommended dosage of 2-3 mg/kg of elemental Zn in the form of zinc sulfate, zinc gluconate, or zinc methionine. There was not a detected difference between the different Zn salts in one study\textsuperscript{22}. Clinical signs are typically improved within 4-6 weeks.

Syndrome II occurs in rapidly growing puppies that are being fed a poor quality dog food or are being over-supplemented with calcium. These dogs are thought to have a relative Zn deficiency caused by a combination of low Zn intake and calcium or cereal phytate binding of Zn. Affected dogs have generalized crusting plaques with extensive crusting and fissuring of the foot pads. Diagnosis is based on compatible history, clinical signs and histopathology (similar to Syndrome I). Response to Zn supplementation is dramatic but is not needed once dog has reached maturity, unlike most Syndrome I dogs. Many dogs will respond to a higher quality diet.

There has been a report of zinc responsive dermatitis in related Pharaoh hound puppies\textsuperscript{23}. Dogs developed cutaneous lesions including exfoliative, erythematous lesions of the foot pads in the first months of life that histologically were suggestive of an underlying Zn deficiency. Affected puppies also had systemic signs of lethargy, poor growth and mental dullness. Dogs did not respond to oral supplementation and intravenous supplementation with zinc sulfate was required to ameliorate clinical signs.

**Superficial Necrolytic Dermatitis (SND)**

Superficial necrolytic dermatitis has been reported in the dog, cat and black rhinoceroses. SND or also referred to in dogs as hepatocutaneous syndrome (HCS) or canine necrolytic migratory erythema (NME) or metabolic epidermal necrosis (MEN). It is an uncommon skin disease associated with systemic metabolic disease(s). Affected dogs most commonly have a characteristic concurrent hepatopathy, thus the popular use of the term hepatocutaneous syndrome (HCS). As different disease processes (glucagonoma, vacuolar hepatopathy, phenobarbital administration, or intestinal disease) have been reported to cause similar histologic skin lesions it might be more correct to refer to the skin disease as SND or metabolic epidermal necrosis (MEN). The disease is typically diagnosed in older dogs. The mean age of reported cases is 10 years, with a range of 4 to 16 years\textsuperscript{24}. Male dogs comprise 64% of all reported cases\textsuperscript{24}. Shetland sheepdogs, West Highland white terriers, Cocker spaniels and Scottish terriers may have a predisposition to develop HCS as they appear to be over-represented in the literature\textsuperscript{24}.

Footpads develop marked crusting, fissuring and ulcerations. Erythema, crusting, exudation, ulceration and alopecia can also involve the periorical or perioral regions, pressure points on the limbs and scrotum. Secondary cutaneous infections with bacteria, yeast (Malassezia, Candida) or dermatophytes, particularly involving the feet, are often present. Lameness secondary to footpad lesions, inappetance and weight loss can also be associated with SND. Polydipsia and polyuria may be present when there is concurrent diabetes mellitus. Diabetes mellitus has been reported to occur in 25 to 40% of dogs with the hepatic form of SND\textsuperscript{23}.
Diagnosis of SND is based on obtaining skin biopsies with the typical histopathologic changes of a marked parakeratotic epidermis with striking inter- and intracellular edema in the upper epidermis and hyperplastic basal cells, creating the “red, white and blue” lesion that is diagnostic for this disease. Abdominal ultrasound can provide further support for the diagnosis if a characteristic ‘honeycomb’ pattern consisting of variable-sized hypoechoic regions surrounded by hyperechoic borders is documented in the liver. If this ultrasonographic pattern to the liver is not visualized in a dog with a confirmed histologic diagnosis of SND on skin biopsy, evaluation for a possible pancreatic tumor is warranted. Plasma amino acids, if measured, should document a characteristic severe hypoaminoacidemia.

The most effective symptomatic or palliative therapy for dogs with the hepatic form of SND appears to be the administration of intravenous (IV) amino acids. A number of crystalline amino acid solutions are commercially available that vary in their concentration and the inclusion of electrolytes. Although there are minor differences in the amounts of essential and nonessential amino acids between manufacturers there is no data to suggest that one product is more efficacious than another. Solutions without additional electrolytes are preferred. These hypertonic amino acid solutions should ideally be administered via a central vein to diminish the chance of thrombophlebitis. Inducing a hyperosmolar state is possible if administration is too aggressive. Dogs should be monitored for neurologic signs and the infusion discontinued if these occur. If compromised hepatic or renal function is present because of concurrent disease in a geriatric patient, the administration of intravenous amino acids may exacerbate hepatic encephalopathy or augment increases in BUN. Such dogs warrant close monitoring with serial measurements of ammonia, BUN and osmolality during IV amino acid administration. Some dogs show dramatic improvement in attitude with resolution of skin lesions after receiving amino acid infusions. There are no defined protocols for the administration of amino acid infusions in these dogs and repeat infusions are performed bi-monthly, monthly or when clinical signs return.

Oral nutrition should include a high quality protein diet that can be additionally supplemented with an amino acid powder. Unless significant hepatic dysfunction has been documented, suggesting the presence of some other concurrent liver disease and necessitating a low protein diet, most dogs with SND cannot be fed enough protein to overcome the hypoaminoacidemia occurring in SND. Zinc, essential fatty acid supplementation and feeding egg yolks have been recommended in the literature to be beneficial. This might possibly provide micronutrients that may have some as yet unknown role in this disease but in order to maximize additional protein from an egg source, egg whites should be included. Secondary infections should be treated with appropriate antibiotic and antifungal therapy with careful consideration of those drugs that may be hepatotoxic or require hepatic metabolism. Topical therapy with antimicrobial shampoos can also be of benefit in some dogs in helping to manage secondary infections. Therapy with glucocorticoids is not recommended. Although anti-inflammatory therapy for the skin lesions may be helpful to improve comfort, the risk of precipitating or exacerbating diabetes mellitus in these dogs makes the use of glucocorticoids contraindicated. Diabetes mellitus if present requires appropriate management. Surgical removal of a glucagonoma has been reported to result in resolution of lesions in one dog. Serial treatments with octreotide in a dog with glucagonoma associated SND was palliative in one case report.

The prognosis for dogs with SND is generally poor and the majority of dogs have survival times of less than 6 months. However, 20% of dogs in one study were maintained for 12 months or more with oral protein hyperalimentation and periodic parenteral IV amino acid infusions. One dog at UC Davis was managed for close to 2 years with an indwelling catheter and twice weekly amino acid infusions.

**Cutaneous Xanthomas**

Cutaneous xanthomas are rare and occur when there is underlying hereditary defects in lipid metabolism or acquired dyslipoproteinemia secondary to diabetes mellitus, or use of megestrol acetate. These skin lesions result from the accumulation of lipid laden macrophages within the dermis. Feline cutaneous xanthomas may develop in cats with hereditary hyperchylomicronemia, megestrol acetate induced diabetes mellitus or naturally occurring diabetes mellitus. Cutaneous xanthomas have been reported in a dog with diabetes mellitus. Often affected animals are consuming a diet rich in fats or triglycerides at the time they develop lesions.
Clinically, cutaneous xanthomas present as multiple pale yellow to white plaques, papules or nodules with erythematous borders. They are often located on the head, particularly the preauricular area or pinnae. Lesions can develop in paw pads and over bony prominences on limbs. Lesions may bruise readily and larger masses may in rare cases ulcerate and exude inspissated necrotic material. Cats with inherited hyperchylomicronemia may also demonstrate peripheral neurologic signs due to nerve compression from subcutaneous xanthoma formation. Histologic evaluation of skin biopsies reveals large foamy macrophages and giant cells. Serum biochemistry evaluations for diabetes mellitus, hypercholesterolemia and hypertriglyceridemia should be obtained. Feeding of a low fat diet and identification and correction of the underlying disturbance in lipid metabolism is recommended for patients that have had cutaneous xanthomas identified.

**VASCULAR DISEASE**

Vasculitis can occur as a primary disease but is more commonly secondary to some other underlying disease process such as an infectious disease, neoplasia, immune-mediated connective tissue diseases or adverse drug reactions. There are both immunopathogenic and non-immunopathogenic mechanisms that can induce vasculitis. Non-immunopathogenic mechanisms that are involved in vasculitis do so without primary attack on components of the vascular wall. These mechanisms include invasion of the vascular wall with neoplastic cells or microbial agents and influences of burns, trauma, endotoxin or hemodynamic factors on the integrity of the vascular wall. Immunopathogenic mechanisms for vasculitis include in situ formation or deposition of immune complexes, antibodies directed against vascular wall components, anti-neutrophilic antibody-mediated vessel damage, cytotoxic T cells directed against vascular components and cytokine induced mechanisms. Vasculitis can be categorized using a variety of classification schemes that are based on pathologic appearance and the inflammatory infiltrate that is present or by the size and type of vessel involved. Although this classification is useful to the pathologist it does not always correlate with a specific etiology and its clinical usefulness has limitations.

Vasculitis may involve only one organ system such as the skin or may involve multiple organ systems and consequently clinical signs can be variable. Cutaneous vasculitis typically results from small vessel vasculitides with lesions of swelling, erythema, hemorrhagic macules, plaques or bullae. Ischemic necrosis and ulceration are often present in lesional areas often located on extremities or over pressure points. Foot pads if affected often have depressed areas of central pallor.

Perhaps the most important information to ascertain when evaluating a patient with vasculitis is the possibility of an underlying infectious etiology. If infectious vasculitis is not occurring, the clinician needs to evaluate for exogenous or endogenous antigens that may be triggering the disease. In one study greater than 50% of the cases were deemed to be idiopathic. If an underlying antigenic drive cannot be identified, the vasculitis should be described based on pathologic evaluation of vessel type, size, location and inflammatory infiltrate. Histopathologically, vasculitis is often characterized by neutrophilic nuclear debris, so called leukocytoclasia, inflammatory cells within the vessel wall, fibrinoid necrosis of vessels and extravasation of RBCs into the surrounding dermis.

Therapy is dictated by identification of any underlying triggers. Infectious etiologies need to be treated appropriately, possible inciting drugs should be discontinued, identification of any concurrent underlying diseases should be undertaken and immunosuppressive or immunomodulatory therapy may be warranted.

There are breed associated primary vascular syndromes that cause regional vasculitic lesions that are not associated with underlying systemic disease. These include familial cutaneous vasculopathy of German shepherd dogs and proliferative arteritis of the nasal philtrum seen in Saint Bernard dogs. The vasculopathy of greyhounds can be associated with concomitant renal disease.

**AUTOIMMUNE SKIN DISEASES ASSOCIATED WITH SYSTEMIC DISEASE**

Canine autoimmune skin diseases are uncommon skin disorders and are reported to account for less than 2% of all skin diseases seen in small animal practice. They are often clinically impressive and can even be life threatening. Definitive diagnosis requires timely biopsy of appropriate representative skin lesions and cannot be based solely on clinical impression or appearance.
Systemic Lupus Erythematosus (SLE)
SLE is a multi-systemic autoimmune disease. The German shepherd dog is reported to be at increased risk\textsuperscript{32}. Skin disease occurs variably with percentages as high as 40 to 50% of cases of SLE having skin lesions\textsuperscript{32}. Fever, polyarthritis, protein losing nephropathy from glomerulonephritis, anemia, and thrombocytopenia are the more common clinical signs seen with SLE. Organ specific and non-organ specific autoantibodies target a variety of tissue antigens in SLE. Resultant tissue damage occurs when there is immune complex deposition (as occurs in glomerulonephritis) or can occur because of direct cytotoxic effects or cell-mediated immunity.

Cutaneous lesions are variable and can include erythema, scaling, crusting, depigmentation, alopecia and ulcerations. Lesions often involve the face, pinnae and distal extremities. Lesions may be present on mucocutaneous junctions and within the oral cavity. Ulcers and erosions are rarely diagnostic lesions to biopsy as an intact epidermis is needed to make a definitive diagnosis. The histopathologic findings are variable but classic lesions include apoptosis of basal cells and basal cell vacuolation which lead to dermal-epidermal separation and consequent ulceration.

There are published criteria for the diagnosis of SLE in dogs and diagnosis requires the presence of at least 3 or more criteria\textsuperscript{32}. These criteria include identification of immune-mediated disease targeting various organs systems/tissues +/- a positive ANA. Definitive diagnosis requires involvement in at least 2 or more organ systems and a positive ANA. Systemic lupus erythematosus is a progressive disease and evidence of immunologic involvement in multiple organ systems may not always be evident on the initial presentation. A thorough systemic evaluation including a complete blood cell count, serum biochemistry, urinalysis, +/- protein to creatinine ratio, antinuclear antibody (ANA), arthrocentesis and evaluation of joint fluid cytologically may be indicated in patients suspected of having SLE.

Most patients with SLE have an elevated ANA although this may not always be present. Prognosis depends in large part on the organ systems involved. Immunosuppressive therapy with corticosteroids with or without other immunosuppressive drugs (azathioprine (Immunar), chlorambucil (Leukeran), cyclosporine (Atopica)) is utilized.

Erythema Multiforme (EM)
The terminology has been, over the years, confusing in both human and veterinary medicine in regards to EM and Steven-Johnson’s syndrome/toxic epidermal necrolysis (SJS/TEN). It is evident that these should be considered as separate etiologies although in veterinary medicine SJS and EM may not be differentiated based on histopathology alone. Clinical nomenclature categorizes EM based on severity of lesions. In EM minor, characteristic lesions involve only one mucosal surface and affects less than 10% of body surface. EM major has clinically similar lesions with more than one mucosal surface affected, 10-50% of body surface affected and less than 10% epithelial detachment\textsuperscript{33,34}. It has been documented in human beings that the cell-mediated immune response in EM has a Th 1 pattern\textsuperscript{35}. Both EM and SJS/TEN are mediated by cytotoxic lymphocyte responses against keratinocytes altered by infectious agents or drugs. Either direct cytotoxicity against keratinocytes or the effects of soluble mediators such as Fas ligand, granzymes or perforin result in apoptosis (single cell necrosis) of keratinocytes\textsuperscript{34}. The more severe the clinical presentation of EM the more likely it is to be related to adverse drug reaction\textsuperscript{36}. There is a report of EM associated with parvovirus in a dog\textsuperscript{37} and herpesvirus has been implicated in the cat\textsuperscript{38}

Lesions of EM are often pleomorphic with an acute onset of erythematous plaques and macules that can become annular or serpiginous as they coalesce or they may appear targetoid. Progression to ulcerations is common and lesions may become variably crusted. Lesions are often generalized but are most commonly found on the ventrum, axillae, inguinal region, mucocutaneous junctions, oral cavity and pinnae. Biopsies should be obtained from areas of erythema without ulceration or crusting as an intact epidermis is needed for the diagnosis. Histologically, apoptosis with lymphocyte satellitosis is the characteristic histopathologic lesion of EM.

Prognosis for EM depends on the severity of the disease and identification of underlying triggers. In greater than 40% of canine cases, an underlying trigger cannot be found and it is termed idiopathic EM\textsuperscript{35}. Use of immunosuppressive drugs in human medicine is controversial as EM is often induced by herpes simplex virus\textsuperscript{34,35}. In veterinary medicine, EM patients should be evaluated for underlying triggers: drugs, infection or neoplasia. Erythema multiforme minor may resolve on its own but more severe EM cases are often treated with immunosuppressive therapy with
glucocorticoids with or without other corticosteroid sparing immunosuppressive drugs (azathioprine, cyclosporine, mycophenolate, chlorambucil). Severe generalized mucocutaneous EM (EM major) often requires aggressive supportive care in addition to removal of underlying triggers and immunosuppressive therapy. Intravenous immunoglobulin therapy or plasmapheresis, if available, might be useful in cases of SJS/TEN.

**Sterile Nodular Panniculitis**

Sterile nodular panniculitis (SNP) typically presents with ulcerated or draining nodular lesions and/or non-ulcerative subcutaneous nodules. Dogs are often febrile when lesions are present and a peripheral neutrophilia may be present. Lesions are most commonly seen on the trunk but can be present on the head, cervical area, perineum or can be generalized. Diagnosis is made based on appropriate clinical history, compatible histology of deep tissue biopsies with negative special stains and negative cultures for any infectious organisms. One study proposed that SNP was a dermatologic marker for underlying internal disease. Concurrent pancreatic disease (pancreatitis and pancreatic neoplasia) or immune-mediated disease in other organ systems (polyarthritis, SLE, rheumatoid arthritis) have been identified in conjunction with SNP. Sterile nodular panniculitis if associated with pancreatitis often resolves when the underlying pancreatic disease is successfully managed. A recent retrospective study failed to find association with systemic disease except polyarthritis. Often sterile nodular panniculitis requires a tapering course of immunosuppressive therapy, most often glucocorticoids, unless underlying or concurrent diseases make this contraindicated.

**REFERENCES**

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SKIN ANATOMY
The skin of marine mammals reflects an adaptation to life in the aquatic environment. This includes thermoregulation, water density, pressure, energetics, and locomotion. There are distinct differences among the taxa including sirenians (manatees), cetaceans, and pinnipeds. In some species, there is a physiologically active blubber layer composed of adipocytes and vessels.

The epidermis is multilayered stratified squamous epithelium that ranges from a few cell layers in fur seals to >10 layers in walrus, cetaceans, and manatees. Irregular bands of collagen are present within the dermis (fibroadipose).

Blubber is an important indicator of animal health as it is metabolically active. For this reason the thickness can vary based on age, sex, health status, and season. Assessment of blubber thickness at multiple standardized sites is a component of the necropsy.

CETACEANS
Coloration patterns include uniform, spotted, striped, and saddled. The patterns may aid in concealment.

Cetaceans do not have hair or glands except for a few hairs present at birth in the lip region. The epidermis consists of three layers, the stratum externum (stratum corneum), stratum intermedium (stratum spinosum), and stratum basale. Rete ridges are prominent. There are cutaneous ridges which have an unknown function. Keratinization occurs, but is incomplete.

Given their movement in the aquatic environment and friction on the surface, there is rapid turnover of the epidermis which is smooth. Renewal may occur every 2 hours or 12 times a day. To account for this rapid turnover, there are abundant germinal cells.

Blubber is thickest in cetaceans and may represent 80-90% of the integument. The middle and deep layers are the most metabolically active; the superficial layer may have a structural role. Fatty acid composition may also vary with some fatty acids like isovaleric present in animals inhabiting cold water. Depth of blubber is greatest in the mysticetes (baleen whales).

There are individual species variations. Beluga whales undergo a molt. Right whales (North Atlantic and Southern) have thickened epidermal irregular mounded projections (callosities) which may have a tactile function.

PINNIPEDS, URSIDS (POLAR BEAR), AND MUSTELIDS (SEA OTTERS)
Dermal hair follicles are present in moderate numbers. While seals have blubber, sea otters and polar bears do not. Guard hairs and underfur hairs are present. Hormones play a crucial role in the growth of hair including thyroid, adrenal, and gonadal. Other factors influencing hair growth include nutritional and day length. Hairs are arranged in groups of 2 to 4 or in rows in phocids and individually in otarids. Color patterns are observed in pinnipeds with true seals (phocids) exhibiting the a greater variation. The patterns are “disruptive” and are common in pagophilic (ice breeding) seals. Examples include ringed and ribbon seals.

Annual molts occur in phocid seals, sea otters, and beluga whale. Molting occurs in April to June in the northern hemisphere with a pattern of face to flippers, abdomen, back, and remaining body. Molting periods range from about a week to several months.

Vibrissae are stiff and surrounded by three blood sinuses. Three types of facial vibrissae are present: rhinal,
superciliary, and mystacial. Vibrissae play a role as tactile receptors and discrimination of size and shapes of food objects. 9,10

SIRENIANS
Sirens lack adnexal structures, but do have few, scattered follicles. The blubber layer is thinner in sirenians compared to cetaceans. This thin blubber layer accounts for the sensitivity in temperature drops (cold stress syndrome).

DISEASES & SYNDROMES
Ectoparasites/Endoparasites
The external skin may be inhabited by barnacles, diatoms, and whale lice. Whale lice include cyamids, amphipods crustaceans. 11 Cyamids are found on larger cetaceans including right whales, gray whales, and humpback whales. The number of lice may correspond to the health of the animals as large numbers have been observed on right whales that have survived entanglements or ship strikes and may have implications for survivorship. 12 Cyamids may be present on the lip margins, callosities, chest, throat, and rostrum. There are several species which may have host specificity or may be found on multiple cetacean species.

Suckling lice from the family Echinophthiridae are common. Sites of infestation include the eyelids, nostrils, and anus. 13 Echinophthirius horridus serves as transmitter of the heartworm Acanthocheilonema spirocauda. 14 In other seal lice species, Rickettsia 15 and Bartonella henselae 16 have been identified but the impact on the animals is not known.

Barnacles may be observed in large and small cetaceans. Acorn barnacles (Coronula and Cryptolepas), pseudo-stalked (Xenobalanus), and stalked (Conchoderma). There is a localized response which includes epithelial hyperplasia and mild inflammation. 17 Barnacles and algae may be present on manatee skin. 18

Phyllobothrium spp.
Dermal and blubber cestodes are observed in many species of cetaceans and are grossly observable with sectioning of blubber layers. Greater numbers may be observed near the genital regions.

Cookie Cutter Shark Bites
Cookie cutter sharks (Isistius brasiliensis) bite wounds may be observed and result in acute to chronic wounds. 19 The wounds are round to oval and there may be scarring and hyper to hypopigmentation of the skin. The bites extend into the blubber layer.

Crassicauda spp.
Crassicauda spp. are nematodes that are found in the mammary gland in many species of cetaceans. In pygmy sperm whales (Kogia breviceps), they are found in the false gill slit and in the soft tissues of the cervicothoracic region. 20 The host response ranges from mild to granulomatous with fibrosis. Adults and ova can be found. In mysticetes (fin whales) and some odontocetes, Crassicauda spp. are found in the renal vessels and renal parenchyma. 21

Protozoa (Ciliates)
Ciliates are normal flora of the blowhole with Kyaroikeus cetarius a common isolate. However, ciliated-associated dermatitis has been observed on the skin with the organism morphologically similar to K. cetarius. The lesions are ulcerative and ciliates can be found in large numbers. The ciliates are likely secondary invaders to primary disease such as morbillivirus (distemper). 22 Infection has been reported in multiple species including Tursiops truncatus, Stenella attenuata, Delphinus delphis, Lagenodelphis hosei, and Orcinus orca. 22,23

Viral
Poxvirus
In cetaceans, poxvirus from the genus Chordopovinae, are cosmopolitan. 24,25 The skin lesions are referred to as tattoo lesions do to the typically dark discoloration. However, there is variability including grey and yellow stippled lesions. Poxviral infection may be an indicator of population health and response to environmental stressors.
Calicivirus

*Vesivirus* which includes San Miguel Sea Lion Virus vesicular exanthema of swine, and cetacean calicivirus has been found in sea lions and in two Atlantic bottlenose dolphins. The lesions begin as vesicles which ulcerate.

Herpesvirus

Herpesvirus presents as a spectrum of disease ranging from cutaneous to systemic. Cutaneous lesions in cetaceans range from flattened, irregular circular white to gray discoloration with a dark center. Histopathologic changes included necrosis of the epithelium, intranuclear inclusions, and intracellular edema. *Alphaherpesvirus* has been detected in skin lesions from Atlantic bottlenose dolphins and dusky dolphins. Systemic infection may also be associated with the *Alphaherpesvirus*. Genital plaques have been observed in male and female cetaceans. The masses are composed of hyperplastic epithelium. Inclusions are rare or absent. Gamma herpesvirus has been found in some of the plaques.

Papillomavirus

Papillomavirus has been found in captive and wild manatees with raised cutaneous and genital papillomas. TmPV1 is present within the population, but there are other papillomavirus including TmPV2, TmPV3, and TmPV4. The latter two have been isolated from genital lesions.

Confirmed and suspected papilloma virus has been observed infrequently in harbor porpoise and killer whale (captive). Lesions are raised and may be cutaneous or orogenital. In one study, there was a prevalence of greater than 50% and younger males were commonly infected. In Atlantic bottlenose dolphins, papillomavirus has been found and/or suspected as a cause or oral and urogenital papillary masses. In the oral cavity including the tongue, there is possibility of progression to squamous cell carcinoma.

Morbillivirus

Morbillivirus-associated lesions are observed in the external epithelium and mucosal epithelium. Oral lesions are ulcerative and with survivorship progress into regions of hyperplasia (hard-pad like lesions). Systemic lesions are similar to terrestrial species including lymphoid depletion, encephalitis bronchointerstitial pneumonia, and secondary, bacterial, fungal, viral, and protozoal infections.

Bacterial

*Erysipelas rhusiopathiae* can cause dermataological disease and septicemia. Vasculitis leads to dermal infarction and epithelial loss. The pattern is similar to swine with rhomboid areas of discoloration and mild lifting of the epithelium. Since the organism is found in fish, ingestion of contaminated fish or conspecific/interspecific exposure from the teeth of other cetaceans may be the source of infection.

Fungal

*Lacazia loboii* (*Paracoccides brasiliensis*). Lacaziosis (formerly lobomycosis) is a fungal-like zoonotic organism. Initially cases were identified in South America in few species of small cetaceans and few human cases. The organism has been identified in Atlantic bottlenose dolphins in Florida and Texas. Additional cases were found in North Carolina. Lesions present as raised and cauliflower-like. The yeast-like cells form long interconnected chains. Inflammation is granulomatous. Typically, the organism is localized, but there is reported spread to lymph nodes in rare cases. The organism has not been isolated and diagnosis is based on characteristic features and molecular diagnostics. Recent molecular characterization indicates that the organism may be an uncultivated *Paracoccidioides brasiliensis*.

Cryptococcus gatti

Pinnipeds and cetaceans have had *Cryptococcus gatti*. In the pacific NW and NW Canada, there was an epidemic with human, marine mammal, domestic species, and wildlife. Systemic infection was commonly observed. Skin lesions are raised with or without ulceration. Yeast have a prominent clearing with variable inflammation.

Neoplasia

See Viral Section, Papillomavirus
HUMAN INTERACTIONS

Human interactions refer to any anthropogenic activity from use of ballistics to boat trauma to entanglement. Characterization of the wounds is an important component of determining possible causation, determining trends, developing possible regulatory strategies or mitigation, and possible prosecution. Line entanglements range from compressive to cutting with full thickness extension. Entanglement sequelae include loss of mobility, difficulty in feeding, respiratory inhibition, and diminished reproductive activity. Entanglements range from acute to chronic. Responses of the tissue include fibrosis, inflammation, and secondary infection. Marine debris may also lead to entanglement grossly observable changes including sites of stricture.

Dermatopathies of Undetermined Cause

White lesions and blister-like lesions have been described in right whales. The rostrum and nuchal region (behind the blow hole) are common sites. Limited histology has been done and a cause has not been determined.

SAMPLING

Skin is considered an important component of health and population assessment. Skin biopsies are collected from field captures and from stranded cetaceans for genetic analysis and skin lesions are commonly observed. The cause of skin lesions are not always evident and can be multifactorial. Genetic information provides information on the stratification the species (stocks). Skin lesions are recorded and fall under two general categories: human interaction and non-human interaction. Grossly observed changes are recorded and sampling may be done. In addition to samples for histopathology, samples can be frozen (-20, -80) or placed in RNase Later for PCR.

Sampling protocols can be developed and provided to field personnel, but implicit to this is providing a short and straightforward process for sampling with a justification of needs and data sharing. Photos of sampling methods and test kits enhances the process for stranding networks that may have limited experience and resources.

References

(Endnotes)

INTRODUCTION
In the world of veterinary dermatology, it is not often that radiation therapy comes into day to day conversation with owners with regards to therapeutic recommendations. However, there are certain conditions that would well benefit from radiation therapy as a consideration in the treatment plan. We mostly consider this therapeutic option when neoplasia is diagnosed (and then typically get in touch with our veterinary oncology colleagues!), however there is also potential for this treatment modality to be incorporated into therapy for non-neoplastic dermatological conditions. Additionally, with newer and more targeted radiation delivery systems becoming more frequently available for veterinary patients, some diseases that have not had excellent treatment options historically may well now have a viable alternative with reasonable outcomes.

TYPES AND CONSIDERATIONS OF VARIOUS RADIATION THERAPY OPTIONS
Not all types of radiation therapy are created equally…nor are options for radiation delivery units. When one hears “radiation therapy,” external beam therapy is typically what is pictured. However, there are actually three distinct ways in which radiation can be delivered to affected tissue:

1. **External beam radiation therapy** originates from a source located outside the patient. Therapy is administered either via isotope teletherapy units (e.g. in which a radioactive isotope, most commonly cobalt or cesium, is the radiation source) or linear accelerators.

2. **Brachytherapy** involves the placement of small radioactive sources encased in a protective capsule (“seeds”) in or near a target source. The “seeds” may be either temporary or permanent in nature.

3. **Plesiotherapy** or **contact radiotherapy** involves application of a radiation source on the surface or in direct contact with the target tissue. Strontium-90 is a common radioactive source used in plesiotherapy probes.

With regards to the dermatological patient, external beam radiation therapy and plesiotherapy have the most likely opportunities for use. Brachytherapy is used occasionally in veterinary patients, however this method has more benefit against solid tumors likely to be presented to a veterinary oncologist. Plesiotherapy is an intriguing option for small cutaneous masses due to the shallow depth of penetration of the radiation source. It can be used on potentially sensitive tissues including the eye and periocular tissues. When the lesion however is larger or not restricted to the surface of the skin (e.g. extends deeper than 2-3mm), external beam radiation therapy is generally considered.

External beam radiation therapy relies on the delivery of ionizing radiation to the targeted tissue from an external source. There are two main categories of ionizing radiation: charged particle and photon beam. Ionizing radiations are either directly ionizing charged particles (e.g. electrons, protons, alpha particles, etc.) or indirectly ionizing photons (light packets) with short wavelengths and high frequencies (e.g. any X-ray photon in the diagnostic or therapeutic energy range). Biological impact from radiation is the direct result of the ability to either directly or indirectly ionize atoms in the target tissue, thereby leading to cellular damage/alteration and consequently tumor destruction. Electrons are directly ionizing which tend to penetrate less deeply and may be of use to treat superficial lesions, such as those on the skin, while sparing deeper critical tissues. Photons on the other hand are indirectly ionizing and are used to treat deeper tissues in the body or the entire thickness of the body. With development of computer-generated patient specific plans however, photon distributions within the patient can be modified to minimize normal tissue toxicity. Photon beam radiation is the most common type of radiation therapy used in humans and is becoming increasingly more popular in our veterinary patients as facilities become more readily available.¹

There are several types of external beam radiation delivery units currently available in veterinary medicine. Older **cobalt-60 teletherapy** irradiators are still in existence, however are becoming less popular in the United States. These units produce radiation from a constantly emitting radioactive cobalt source which bombards the target...
with gamma-ray photons. The side effects to normal tissue from this older technology are a bit less desirable in many cases, however the units can still perform well for certain clinical presentations. More modern delivery units however aim to minimize the toxicity to normal tissues through the use of more advanced collimation systems and the ability to deliver a more targeted, and patient-specific, treatment plan utilizing computerized treatment planning. Modern linear accelerators use electrical power to produce high-frequency electromagnetic waves to propel electron particles or photons towards the target lesion. C-arm style linear accelerators, for which there are various vendors and products, have the ability to use either electrons or photons to deliver radiation. With these units, the C-arm is either static or rotates around the patient as the radiation plan is being delivered. The treatment plan however may be limited by the number of isocentric setups, field widths, or the ability of the C-arm to only rotate a certain number of degrees if the couch is not completely perpendicular with the delivery unit. Helical tomotherapy on the other hand only uses photons to deliver radiation. This unit delivers radiation in full 360 degrees as the patient is translated through the bore. This unit tends to perform particularly well for larger treatment volumes and although it lacks electron beam capability, it has so many degrees of freedom, it can create exquisite superficial dose distributions while sparing deeper critical tissues. There are currently only two units available in veterinary institutions (University of Wisconsin-Madison, Texas A&M University). More specialized and focused radiation delivery units include CyberKnife® and Gamma Knife®. These units also use photons as their radiation source and are especially good for very small targets. With CyberKnife®, the radiation source is mounted on a robotic arm with the ability to deliver radiation in an almost spherical fashion around the patient. With Gamma Knife®, the patient is positioned under an arc with approximately 200+ individual cobalt sources focused at the target. Both units are specifically geared towards radiosurgery or stereotactic body radiation therapy; they are less beneficial for larger tissue volumes.

SKIN CONSIDERATIONS WITH RADIATION THERAPY

Even with more patient-specific treatment plans and modern radiation therapy delivery units, side effects to normal tissues can still occur. Radiation oncologists generally aim to develop the treatment plan which avoids marked chronic or long-term damage to normal and important structures, however even still, changes to the skin are rather common. Side effects are generally more pronounced with the use of older delivery units and less advanced treatment planning strategies. While chronic damage is minimized with more specific and advanced radiation plans, acute side effects still occur. While these generally resolve rapidly and successfully, they are still concerning to owners and may be a reason for patients to present to other veterinary specialists (e.g. dermatologists) for management recommendations. The progression of cutaneous radiation toxicities in the acute setting includes development of erythema followed by exfoliation and development of scale (dry desquamation), more pronounced erythema, exudative erosion (moist desquamation) and alopecia. Pigmentary changes (either hypo- or hyperpigmentation) to either the skin or hair coat can follow in the post-acute setting. When mucous membranes are involved in the treatment field, mucositis and increased secretions can also develop. If extremities are in the treatment field, nails and paw pads may additionally slough. After the radiation therapy has been completed, late phase toxicities may also develop. In the skin, fibrosis and contraction can occur as well as non-healing ulcers secondary to vascular regression and fistulae. Alopecia and pigment changes may also show up several months after radiation therapy, or may persist from the acute phase reactions.

The most concerning side effect particularly in the early phase is marked erythema and exudative erosions. With these more open wounds, secondary infections can occur adding further risk to the treatment process. Standard management practices tend to focus on prescription of antibiotics for secondary infections along with provision of pain control (tramadol, NSAIDs, etc.)² and basic wound care, however there has been little progress in identifying methods to decrease and/or prevent radiation dermatitis from developing. One study evaluated the use of prednisone in dogs undergoing radiation therapy, however unfortunately this had little effect on the development of radiation toxicity to the skin¹. Various nutriceuticals including turmeric, vitamin E, and fatty acids have been discussed, however the ideal management strategy has yet to be identified. In people, the use of various moisturizing creams has been evaluated in regards to preventing the acute side effects of radiation therapy. One study found a marked improvement with regards to cutaneous toxicities in patients who applied various moisturizing creams containing different topical ingredients for 15 days prior to therapy and one month following therapy cessation⁴. While this may hold promise for translation into our veterinary patients, it may also prove less effective due to the hair coat present in our companion animals. Future studies will need to take this factor into account.
COMMONLY CONSIDERED RT-ABLE DERMATOSES
There are certain types of diseases, primarily neoplastic in nature, where radiation therapy is often considered as part of the treatment recommendations. This may be considered as the sole treatment modality or potentially combined with chemotherapy and/or surgery. With regards to the dermatological patient, conditions where radiation may be commonly considered as part of the treatment plan may include squamous cell carcinomas, mast cell tumors, tumors of the perianal glands (perianal gland adenoma, perianal gland adenocarcinoma), and ceruminous gland tumors (ceruminous gland adenocarcinomas). In the equine patient, sarcoids may also be added to this list.

Depending on the location, species, and size of the tumor, squamous cell carcinomas can have anywhere from a poor to good response to radiation therapy. Smaller lesions may be rather amenable to plesiotherapy with strontium-90 probes. This is used not infrequently in our hospital for equine squamous cell carcinoma lesions particularly around the eyes. With more aggressive and large tumors, radiation therapy may be used in conjunction with surgery either to shrink the tumor pre-operatively or sterilize the bed following surgical removal. This combination may provide more favorable outcomes.

Mast cells tumors tend to be quite radiation-responsive with good to excellent prognosis reported following treatment. Even with lymph node metastasis and higher grade tumors, the treatment outcome can be favorable particularly when radiation is combined with either surgical removal and/or chemotherapy. More modern delivery systems additionally have the ability to treat local lymph nodes when they become involved in the disease presentation.

Tumors involving the perianal glands including perianal gland adenomas and perianal gland adenocarcinomas tend to have a good (adenocarcinoma) to excellent (adenoma) response to radiation therapy. In fact, this may be considered the treatment of choice for the disease given the marked morbidity associated with surgical removal of these tumors. While inflammation and stricture (later phase toxicity) are possible side effects of radiation, this treatment modality tends to preserve function with regards to maintaining fecal continence where surgery may not be quite as successful.

Ceruminous gland adenocarcinomas tend to have a fair to good response to radiation therapy, and may be best combined with surgery (total ear canal ablation) following the radiation treatment course. In one older study, an over 3 year progression-free interval was reported when radiation was utilized with or without surgical removal. Newer technologies may allow this to be extended even further with fewer side effects to normal structures.

Although much success with sarcoild treatment relies on the size of the tumor, radiation can be rather successful for this common equine neoplasm. Of course, the size of the animal and location of the lesion may limit the possibility of utilizing this treatment modality. If external beam radiation therapy is not feasible due to the location (if it can’t fit in the bore, it can’t be treated by an external beam delivery unit), other radiation options may be able to be considered. If the sarcoild is small and rather superficial (e.g. an occult sarcoild or small verrucous sarcoild), plesiotherapy may be possible. This option however is not ideal for larger and/or more invasive lesions due to the only shallow penetration of radiation and size limitations for probe application. Brachytherapy has also been used effectively for these tumors at various body sites in the horse.

LESS COMMONLY CONSIDERED RT-ABLE DERMATOSES
While other therapies are generally considered preferable as first-line treatment options, with regards to the more aggressive and poorly responsive acral lick granuloma, radiation therapy may be a considered option for some patients. As this condition is multifactorial in nature, with influence of behavior in disease progression, radiation therapy may or may not provide a durable response. When a large amount of fibrosis has already developed, radiation may not be quite as successful at reversing these chronic changes. Most of the data however with regards to using radiation therapy for managing acral lick granulomas used older technologies and the most severe cases of the disease. More favorable outcomes may be possible if radiation therapy is brought in earlier in the disease process.

Along the same lines are feline eosinophilic lesions including eosinophilic granulomas and indolent ulcers. While...
these lesions are typically responsive to various forms of medical management (antibiotics, steroids, cyclosporine, allergy management (food, environmental)), they occasionally present a bit more refractory to standard treatment options. Radiation therapy can be beneficial in this sense to help shrink lesions and provide a reasonably durable response. This may be a considered option earlier in the process particularly when other medical therapies are contraindicated (e.g. steroids with concurrent diabetes mellitus).

Novel Uses of Radiation Therapy in the Dermatological Patient

With the development of more modern radiation delivery units, we have the ability to pursue some more novel treatment strategies for certain disease processes. This includes conditions which have historically had rather limited treatment options with often poor outcomes. **Solar-induced squamous cell carcinoma** (and precancerous lesions) is one such condition. This disease tends to affect a large area of the body, often much of the ventral abdomen making surgical intervention not feasible to consider. Medical therapy has not been profoundly successful either. The more modern linear accelerators have the ability to deliver dose just to the surface of the skin using some fairly advanced treatment planning techniques. Helical tomotherapy has proven to be effective at treating this condition with durable response of 2+ years in at least one patient with extensive disease development⁷.

Along the same vein, **cutaneous epitheliotropic lymphoma** can be quite radiation-responsive. Although this has been known for years, treatment for wide-spread variants of the disease has limited the use of radiation therapy as a treatment modality for this condition in veterinary medicine. The more modern delivery units again can deliver dose where it needs to be (e.g. the skin surface) while minimizing toxicity to deeper structures. This has historically been a limiting factor for long-term survival and tolerance. A recent case study reported on successful serial half-body treatment of a dog with extensive cutaneous lymphoma⁸. Another case example highlighted the use of 3D printing technology to enhance dose to the skin surface in a dog with severe disease recalcitrant to numerous other treatment options⁹. The latter was particularly effective at keeping treatment times low for each fraction delivered.

With the wide availability of trilostane, medical therapy for **canine hyperadrenocorticism** is generally reasonably successful with regards to long-term management. However, in some cases, neurologic side effects of a growing pituitary macroadenoma become more problematic and less responsive to successful medical therapy. Radiation therapy has been shown to be rather successful in the setting of a pituitary macroadenoma; the space occupying aspects of the tumor can be controlled for several months to years. The more directed delivery units (Gamma Knife®, CyberKnife®, helical tomotherapy, modern advanced linear accelerator) may perform superiorly in this particular setting.

Although less information is available, particularly in the veterinary literature, there is also the potential to incorporate radiation therapy into a non-neoplastic setting. As with other drugs, radiation has a dose-dependent effect and can act anti-inflammatory as opposed to anti-neoplastic at certain lower doses. This may be beneficial for certain immune-mediated conditions or other inflammatory disease processes. The effect of radiation therapy on chronic proliferative otitis externa is an area of potential application. This may allow for the reversal or arrest of chronic changes which generally require surgical intervention to correct. “End stage ears” may have potential for continued medical management if “anti-inflammatory radiation therapy” proves to be successful. Additionally, with regards to certain immune-mediated diseases, we may find that incorporating radiation therapy into the treatment protocol may decrease the requirement for more immunosuppressive drugs thereby reducing adverse effects of combination therapy. More information however is needed in these areas.

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INTRODUCTION
Veterinarians and dermatologists commonly struggle with strategies to “stay sane” and maintain work-life balance. With a shortage of veterinary dermatologists in many regions of North America, owners might wait months for a specialist appointment addressing their pet’s chronic dermatologic condition. This can place an incredible amount of stress on veterinarians and specialists who often see more than a dozen appointments during a shift, in addition to answering phone calls and emails from clients and/or referring veterinarians. Likewise, the toll that chronic dermatologic disease takes on owners is often underestimated. One study indicated that the quality of life of owners of dogs with dermatologic conditions is highly impacted based on the disease, response to therapy, financial burden, and degree of maintenance therapy required. This can in turn have an impact on veterinarians and dermatologists who might experience compassion fatigue as a result of helping pets and their owners with chronic and sometimes unresolvable dermatologic diseases.

Given all of the difficulties faced by veterinarians and dermatologists on a regular basis, it is important to be able to recognize the signs of burnout and compassion fatigue, as well as put in place practical strategies to foster wellness and resilience. Resilience is a quality that allows a person to be knocked down by life and come back stronger than ever, rather than letting failure take over. Setting boundaries, saying no, and daily debriefing function to expand awareness of a person’s needs, as well as their limited physical and emotional resources. Likewise, they allow life balance including separating work from play and rest.

BURNOUT
Burnout describes the physical and emotional exhaustion that occurs when people have low job satisfaction and feel powerless and overwhelmed at work. This results from a combination of exposure to environmental and internal stressors, as well as inadequate coping and adaptive skills. Some examples of work-related causes include feeling little to no control over work, lack of recognition or rewards for good work, unclear or overly demanding job expectations, doing work that is monotonous or not challenging, and working in a chaotic or high-pressure environment. Lifestyle causes of burnout include working long hours, feeling over-extended or overwhelmed, not getting enough sleep, and lacking a social support system. Personality traits can also contribute to burnout including perfectionism, pessimism, the need to be in control, and being a high achiever.

Signs of burnout include feeling overwhelmed, being unable to meet work demands or deadlines, losing interest or motivation, experiencing reduced productivity, losing energy, and feeling helpless, hopeless, cynical, resentful, or depleted. Consequences of burnout can include drug abuse, excessive sleep, procrastination, avoidance behavior, over- or under-eating, excessive alcohol intake, withdrawing from friends, family, or activities, and angry or emotional outbursts.

COMPASSION FATIGUE
Compassion fatigue results from a caregiver’s unique relationship with sick or dying patients and the empathy felt; it is a profound emotional and physical loss that occurs when caregivers are unable to refuel and regenerate. Whereas burnout can be more easily resolved with changing jobs or adjusting duties at work, compassion fatigue cumulates over time and will follow people no matter what job they have. Compassion fatigue can manifest as negative psychosocial behaviors such as detachment or avoidance, emotional exhaustion, reduced sense of personal accomplishment or meaning in work, mental exhaustion, denial, apathy, or decreased interactions with others (isolation). Physical illness, substance abuse, compulsive behavior, and detrimental workplace conduct including interpersonal conflict or absenteeism can also occur. Moreover, compassion fatigue can establish a negative feedback cycle that can erode a veterinarian’s strong human-animal bond, thus reducing the feelings of compassion and empathy for patients and clients.
BUILDING BETTER BOUNDARIES

Unhealthy boundaries occur when we do not set limits on ourselves or others. Creating healthy boundaries helps to maintain balance, promote resilience, and develop better coping strategies. Some examples of unhealthy boundaries include going against our personal values or rights to please others, letting others direct our life, letting others describe our reality, looking to others to define us, or expecting others to think, feel, and behave the same way we do.

Boundaries are essential to healthy relationships and leading a healthy life, but setting and sustaining boundaries is a skill. Boundary building can be a challenging concept for many people. Having healthy boundaries basically means knowing and understanding what your limits are and not allowing them to be compromised.

- **Name your limits:** In order to set boundaries, you must first identify your physical, emotional, mental, and spiritual limits. In veterinary practice, this also means identifying your moral stressors. Consider what you can tolerate and accept versus what makes you feel uncomfortable or anxious. Identify where you need more space, self-respect, energy, or personal power.

- **Tune into your feelings:** Feelings of discomfort or resentment are “red flags” or cues that you are letting go of your boundaries. Pay close attention to when you lose energy, feel a knot in your stomach, or want to cry. If you notice these feelings coming up for you in certain situations or interactions, ask what it is about the situation, interaction, or expectation that is bothering you. Resentment usually stems from a feeling of being taken advantage of or not feeling appreciated and can be an indication that you are pushing yourself beyond your limits. This can be because of guilt (e.g., wanting to be a good veterinarian) or because someone else is imposing his/her expectations, views, or values upon you (i.e., crossing a boundary).

- **Be direct:** If people have similar communication styles, views, or personalities, they tend to approach each other similarly and maintaining healthy boundaries does not require a direct dialogue. However, when dealing with people who have a different personality or background, you will need to be more direct about your boundaries.

- **Give yourself permission:** Fear, guilt, and self-doubt can inhibit our ability to set boundaries even when we feel drained or taken advantage of. Boundaries are a sign of self-respect, so give yourself the permission to set boundaries and work to preserve them.

- **Practice self-awareness:** Boundaries are all about tuning into your feelings and honoring them. If you notice yourself slipping and not sustaining your boundaries, consider what has changed, what you have control over, and what you can do about it.

- **Consider your past and present:** If you have a history of ignoring your own needs and focusing on others, you have probably previously let yourself become drained emotionally and physically. Consider this when setting boundaries, especially in relationships, and ensure that they are reciprocal.

- **Make self-care a priority:** Give yourself permission to put yourself first, such that your motivation to set boundaries becomes stronger. This also means recognizing and honoring your feelings, which serve as important cues about your wellbeing and what makes you happy. Remember that putting yourself first also gives you the energy to have a more positive outlook and be a better co-worker, friend, partner, etc.

- **Seek support:** If you’re having a difficult time setting boundaries, consider seeking support, whether it’s from a support group, counsellor, therapist, life coach, friend, or mentor. Also consider sharing your boundary-setting goals with friends or family so that you can be held accountable.

- **Be assertive:** It is not enough to create boundaries; we have to abide by them as well. Do not expect others to read your mind and know when they cross a boundary. You must assertively communicate with that person to let them know. There is no need to defend, debate, or over-explain your feelings. Be firm, gracious, and direct, and when faced with resistance, repeat your statement or request. Remember that if you give in, you invite people to ignore your needs.

- **Start small:** As with any new skill, assertive communication of boundaries takes practice. It is best to start with a small boundary that is not threatening or overwhelming you and then slowly increase to more challenging boundaries. Setting boundaries takes courage and practice, but is a skill that anyone can master.
WHEN TO SAY NO

It can be difficult to determine which activities in our lives deserve our time and attention. In order to evaluate opportunities and obligations that come your way, consider the following:

- Focus on what matters most to you; examine your obligations and priorities before making any new commitments. Ask yourself if the new commitment is important to you. If it’s something you feel strongly about then do it, if not, then pass.
- Weigh the yes-to-stress ratio; is what you are considering a short- or long-term commitment? Do not say yes if it will mean months of added stress. Instead, look for other ways to contribute.
- Take guilt out of the equation; do not agree to a request that you would rather decline out of guilt or obligation. Doing so will inevitably lead to additional stress and resentment.
- Sleep on it; before you respond, take a day to think about the request and how it fits into your current commitments. If you cannot sleep on it, at least take the time to think the request through before responding.
- Imagine saying yes and then tune into your feelings; visualize what life will be like if you commit to the request and then become aware of your thoughts and feelings as they arise. If you feel anxious, resentful, or stressed, perhaps you should decline.

HOW TO SAY NO

Saying no is often not as simple as we would like it to be. Here are some simple strategies to help when you need to say no.

- Say no: The word “no” is a complete sentence and has power. Do not be afraid to use it. Be careful about using wimpy substitute phrases, such as “I’m not sure” or “I don’t think I can.” These can be interpreted to mean that you might say yes later.
- Be brief: State your reason for refusing the request, but do not go on about it. Avoid elaborate justifications or explanations.
- Be honest: Do not fabricate reasons to get out of an obligation. The truth is always the best way to turn down a friend, family member, supervisor, or co-worker.
- Be respectful: Good opportunities will arise and it can be tough to turn them down. Complementing the person’s effort while saying that you cannot commit shows that you respect what they are trying to accomplish.
- Be ready to repeat: You might need to refuse a request several times before the other person accepts your response. When that happens, just hit the replay button. Calmly repeat your no, with or without your original rationale, as needed.

OTHER THINGS TO CONSIDER WHEN SAYING NO AT WORK

More than ever, people are expected to do more work in less time. People say yes to requests because they want to be a team player, look eager, or simply be nice. But saying yes all the time can lead to burnout. Saying no can be difficult, but it can also be an asset to fostering resilience and self-care.

- Take time to consider the request; determine how much time you will need to perform the task well and how the request fits into your existing demands. Before you say yes, you want to think clearly about the advantage that doing so does for you.
- Offer an alternative; while saying no, try to help the other person who approached you with the request. Ask if you can do something else to help or offer to comply with the request at a later date.
- Say no in person; email or text messages can be misinterpreted and the willingness that you express through your tone of voice might be missed. To avoid insulting the other person, call them on the phone or schedule a meeting, if possible.
- Avoid details; keep your explanation short and simple. By describing your entire calendar or other commitments, you run the risk of seeming defensive about your choices and the person might question the importance of your other obligations.
- Consider the consequences; weigh the risks and benefits of each refusal, both personally and professionally. If you are an entry-level employee, you might have less leverage when it comes to declining a request. However, saying yes to an opportunity might get in the way of other professional goals.
• Do not respond with self-deprecation; the person making the request might respond with flattery and insist that you oblige the request. Instead, lay out your current assignments or lack of availability as an explanation.
• Ask for help; if needed, explain that you have a real conflict and are trying to resolve it. For example, if a colleague asks you to take “squeeze in” one of their patients for an internal consultation say “I’d love to, but I made a commitment to my family to be home in time for supper each weekday. Can you ask them to drop their dog off tomorrow morning and I can look at him in between appointments instead?” Keep your explanation as simple as possible.

DAILY DEBRIEFING
Personal debriefing is a means of recognizing how an experience was for an individual and aims to help them integrate their experience into their life as a whole, perceive the experience more meaningfully, and bring a sense of closure. It requires personal reflection and can help disengagement from work at the end of a day. Essentially, it gives closure to your work and work relationships, while acknowledging the good work that you have done. Personal debriefing should be performed on daily basis when completing a shift or other work done that day.

STEPS OF DAILY DEBRIEFING
1) Check that tasks are finished and that documentation is completed and deal with any outstanding issues:
   • Complete if essential
   • Delegate
   • Write it down to do it tomorrow
2) Acknowledge the day and recall what went well and what did not:
   • Focus more on the positives and less on the negatives
   • Acknowledge that you did the best you could with the time and resources available to you
3) Handover responsibility for the care of your patients:
   • Be conscious during case transfers that you are not only handing over clinically, but also handing over total responsibility to your colleague(s)
   • Close your computer or paper files with intention
4) Say your goodbyes:
   • This is closure for today on your relationships with patients, clients, and colleagues
5) Debrief and de-role:
   • Talk through any distressing events
   • Arrange for a debrief if needed
   • Take off your ID badge/name tag, scrub top, work shoes, etc. or use other personal rituals to signify when your work is finished
   • Remind yourself you are now out of role
   • Make your journey home a final separation between work life and private life
6) If you are on-call or work from home:
   • Create a specific space for your professional work and try to keep to this space only
   • Develop a ritual that allows you to signify when work is completed
7) Still thinking about work?
   • Particularly if it is something troubling you, write it down and place it in your work bag
   • If it is still on your mind the next day, consider if you need to talk it through or debrief
8) Ensure that no matter what the work you do or where you do it that you never feel alone:
   • Have the names and contact information of trusted co-workers or colleagues that you can call and talk through a situation should the need arise

REFERENCES
WHAT IF DISNEY RAN YOUR VETERINARY PRACTICE? Focusing on Disney-level Guest Experience for Your Clients.

Scott Terrell DVM, DACVP

This presentation will share the philosophy and application of the key components of Disney's guest experience success and those components could apply in the modern veterinary practice environment.

OBJECTIVE

1. Improved customer service and leadership development tools
Melanocyte Biology and Pathology in Animals

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1.0 INTRODUCTION
The biology of each significant step along the pathway from melanocyte development to skin pigmentation will be reviewed as a means to inform the missteps that lead to the related pigmentary disorders in domestic animals. Melanocytic neoplasia will not be addressed.

2.0 MELANOCYTE ORIGIN AND MIGRATION TO THE SKIN
In development, transient neural crest cell (NCCs) delaminate from the neural tube and form migratory, bipotent, melanoblast-schwann cell precursors that express SOX10. Down regulation of FOXD3, expression of PAX3 and ultimately MITF specifies melanoblasts1. Successful migration via dorsal and ventral embryonic routes to skin requires survival, expansion and differentiation of melanoblasts into melanocytes that is dependent on these and other factors, such as KIT, EDNRB, FGF, WNT, P-cadherin, and E-cadherin. Melanoblasts generate melanocytes in the skin, eye, leptomeninges, and ear, while other NCCs contribute to facial connective tissues, heart, enteric nervous system, etc, which explains the syndromic components of several pigmentary neurocrestopathies.

Disorders of melanoblast migration
In humans, mutations in genes affecting embryonic melanoblast migration, proliferation, and survival from the neural crest result in piebaldism (KIT, SCF, SLUG), Waardenburg syndrome (PAX3, SOX10, MITF, SNAI2, EDNRB, EDN3), and Tietz syndrome (MITF)2. Hypopigmentation results from an absence of melanocytes in the skin. Syndromic effects in Waardenburg syndromes result from concurrent deficiencies of NCC contributions to other organs, (eye, ear, intestine, facial bones, muscle, etc). White coat spotting, or patches, in several domestic animals is phenotypically similar to piebaldism in humans, even occurring in similar midline areas such as the forehead, chest and abdomen. Hypopigmented irides and deafness are not features. KIT mutations are most common in human with piebaldism2 and have been identified in dogs3,4, horses5, Birman cats6, cattle7 and pigs8. White coat color in animals occurring with congenital sensorineural deafness (CSD) and/or hypopigmented irides is similar to human Waardenberg syndrome type-2. Gene mutations are described in dogs (MITF, SILV)9,10, cats (KIT)11, and horses (ENDRB)12 but not all animals with mutations in these genes have the ear and eye defects. Involvement of MITF is also similar to Tietz syndrome1. Many genes contribute to CSD in dogs. Waardenburg syndromes type-4 has features of type-2 plus aganglionic megacolon in affected people and this presentation is reported in lethal white foal syndrome12 and lethal white (lamb) syndrome both with mutations in EDNRB13. Compared to humans, the genotype phenotype relationships in animals for coat color are more varied due to intense selective pressure by humans.

3.0 MELANOCYTE STEM CELLS
Melanocyte stem cells of hair follicles are better characterized than those of the epidermis14. Evidence also supports melanocyte stem cells in the dermis and eccrine sweat glands in some species, dermal neural crest stem cell-like cells, and Schwann cell precursors (stem cells) along nerves are sources of melanocytes. Cyclical hair follicle growth and arrest requires a constant source of transiently amplifying melanocytes from stem cells, which share their niche with hair follicle keratinocyte stem cells in the bulge region. Localization and maintenance of melanocyte stem cells to these epithelial niches is thought to be partially dependent on KIT/KIT-Ligand interactions with keratinocytes as well as, cadherins, integrins, and matrix interactions. Additional factors important for melanocyte stem cell interactions in the hair follicle include WNT, B-RAF C-RAF, NOTCH, TGF-B, EDN, NFB, and Collagen-XVII15. Interestingly, melanocyte stem cells can be lured out of the follicle to the epidermis in response to wounding or UVB irradiation and this is dependent on MC1R signaling16.

Disorders of melanocyte stem cells
Hair graying occurs with aging, irradiation and chemotherapy in humans and animals. Inadequate melanin transfer
to the growing hair shaft leads to this graying and is correlated with loss of melanocyte stem cells in hair follicles, possibly from oxidative injury. Melanocyte stem cells activated during follicle cycling, or with injury, fail to repress differentiation, do not return stem cells to a quiescent state and thus deplete stem cell mass. Oxidative injury is thought to play a role in humans. Keratinocyte stem cells support melanocyte stem cells in their shared niche and can affect graying, for example with irradiation. Reversible hair graying occurs with use of receptor tyrosine kinase KIT inhibitors and anecdotal evidence indicates that this phenomenon occurs dogs. In the gray horse phenotype, premature graying is autosomal dominant and is due to a mutation in STX17. Nearly complete loss of hair coat pigmentation occurs by 6 to 8 years of age and cutaneous melanomas occur in 70 to 80% of horses that reach 15 years of age. Progression of graying suggests a defect in hair follicle melanocyte stem cell survival. This mutation is largely responsible for the gray coat phenotype, melanoma formation, vitiligo-like depigmentation, and coat speckling but is modified by other genes, including ASIP.

### 4.0 MELANOSOME FORMATION

Melanin production and storage are the key characteristic features of melanosomes. Melanosomes are formed only by melanocytes and pigment epithelial cells of the eye but are transferred to keratinocytes of the epidermis, mucosae and hair follicles. Melanosomes are lysosome-related organelles (LRO), which include dense granules of platelets, type II pneumocyte lamellar bodies, etc., because they share with lysosomes an acidic pH, certain proteins and a common pathway of organelle biogenesis. This shared biogenesis of different LROs explains the pleiotropy of clinical effects observed in several related pigmentedary disorders. Melanosomes formation involves four distinct morphological stages. Stages-I and –II are melanin free and establish the organelle vesicular structure and internal protein fibrils (premelanosome protein 17 (PMEL), also called Silver (SILV)) that hold melanin and produce the ellipsoid shape. MART-1 and GPNMB also contribute to structure. Acquisition of melanin pigment biosynthetic enzyme machinery starts in stage-II melanosomes and is dependent on organelle cargo sorting complexes. Melanin deposits along, and thickens, luminal fibrils to create stage-III and then obscures fibrils completely to create stage-IV melanosomes.

**Disorders of melanosome formation and cargo sorting**

**Hermansky-Pudlak syndrome** (HPS) is a heterogeneous group of autosomal-recessive disorders in humans that cause cutaneous and ocular hypopigmentation along with variable extra-pigmentary clinical signs. Nine human genes identified involve one of four protein complexes: BLOC-1, BLOC2, BLOC-3 or Adaptor Protein Complex-3 (AP-3), which transport cargo proteins to the developing lysosomes and LROs, including melanosomes. In the nine HPS types, the combination of clinical signs depends on the type of LROs concurrently affected in different cell types, such as platelets, neutrophils, T-cells and type-2 pneumocytes. For example, bleeding occurs in nearly all HPS patients due to platelet dense granule defects. **Gray Collie syndrome** (GC5, also called canine cyclical hematopoiesis) presents with dilute skin and hair coat, cyclical neutropenia, thrombocytopenia, anemia and recurrent infections. A gray or pale-tan planum nasale is a typical. Dilution is due to decreased melanization of melanosomes. GCS resembles HPS type II in humans and is caused by a mutation in AP3B1, which encodes the β-subunit of AP-3. Reversible hair graying occurs with use of receptor tyrosine kinase KIT inhibitors and anecdotal evidence indicates that this phenomenon occurs dogs. In the gray horse phenotype, premature graying is autosomal dominant and is due to a mutation in STX17. Nearly complete loss of hair coat pigmentation occurs by 6 to 8 years of age and cutaneous melanomas occur in 70 to 80% of horses that reach 15 years of age. Progression of graying suggests a defect in hair follicle melanocyte stem cell survival. This mutation is largely responsible for the gray coat phenotype, melanoma formation, vitiligo-like depigmentation, and coat speckling but is modified by other genes, including ASIP.

**Chediak-Higashi syndrome** (CHS) is an autosomal recessive disorder of humans characterized by extensive hypopigmentation of hair, skin and eyes as well as a silvery sheen to the hair. CHS has been linked to mutations in CHS1 (also called canine cyclical hematopoiesis) presents with dilute skin and hair coat, cyclical neutropenia, thrombocytopenia, anemia and recurrent infections. A gray or pale-tan planum nasale is a typical. Dilution is due to decreased melanization of melanosomes. GCS resembles HPS type II in humans and is caused by a mutation in AP3B1, which encodes the β-subunit of AP-3. Chediak-Higashi syndrome in animals has been reported in Herford, Brangus and Japanese Black cattle, Persian cats, beige mice, beige rats, Aleutian mink, blue and silver foxes and a killer whale. Mutations in CHS1 are reported for Japanese Black cattle, beige mice and beige rats. Animals also develop hypopigmentation of the skin, hair coat and eyes and show silver colored hair. Bleeding abnormalities are attributed to platelet dense granule defects and immunodeficiency to multiple effects on leukocyte function. Mutations in SILV cause the merle coat phenotype in dogs associated with CSD and blue irides (see above), equine multiple congenital ocular anomalies in silver and silver dapple horses and contributes to coat color dilution and hypotrichosis in calves (“rat-tail syndrome”) along with to two other loci. Appaloosa spotting and congenital stationary night blindness in horses is attributed to a mutation in TRPM1 that is thought affect melanosome.
5.0 MELANIN SYNTHESIS

In mammalian skin, melanin is a non-protein polymer of modified tyrosine and is present as two main types, brown-black eumelanin and yellow-red pheomelanin. Eumelanin is composed of oligomers of two related indoles, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while pheomelanin is built from benzothiazine units. Tyrosinase (TYR), located in the melanosome membrane, converts L-tyrosine to L-Dopa, along with tyrosine hydroxylase isoform 1 (THI), and subsequently converts L-Dopa to dopaquinone. Copper is an important tyrosinase cofactor. Subsequently, the melanogenesis pathways split into those for eumelanin and pheomelanin. For eumelanin, reactive dopaquinone converts to dopachrome spontaneously. Subsequently, dopachrome is converted to 5,6-dihydroxyindole (DHI) spontaneously and to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) by dopachrome tautomerase (DCT, also called tyrosinase-related protein 2 or TYRP2). DHI, spontaneously, and DHICA, with the help of tyrosine-related protein 1 (TRYP1), combine to form oligomers of eumelanin. If sufficient cysteine is available, dopaquinone is converted to cysteinyl-L-Dopa and additional steps yield pheomelanins. Enzymes specific to pheomelanin production are not well described. Melanosomes with pheomelanin production are unique from those with eumelanin.

Disorders of melanin synthesis

Oculocutaneous albinism (OCA) presents with hypomelanosis of tissues, including the skin, hair and eyes due to a decrease or absence of melanin synthesis in melanosomes. OCA can be accompanied by, but not limited to, delayed visual development, nystagmus, decreased visual acuity and photophobia. Neuronal retinal projections to the visual cortex are altered and nystagmus is a hallmark of nearly all forms of OCA in humans, which in animals, has only been addressed in Siamese cats. OCA in humans is sub-classified into four types depending on clinical signs and the gene mutation involved in melanogenesis. In cats, mutations are reported in TYR that cause complete albinism similar to OCA type-1 and temperature sensitive seal-point variants similar to OCA type-1TS in humans. In dogs, mutations are reported in TYRP1 that cause a brown/chocolate coat consistent with OCA type-3 in humans and in TYRP1 that cause a brown coat, similar to OCA type-3, and in SLC45A2 causing near white, cream coat similar to OCA type-4, but also has a higher risk of eye and skin melanomas. In sheep and cattle, albinism is reported in several breeds but often without genetic investigation. The condition in the Braunveih calves is due to TYR mutation and is similar to OCA type-1 in humans. A mutation in TYRP1 that was strongly associated with the dun (brown) coat color in Dexter Cattle, similar to OCA type-3. In horses, the cream coat color is very pale to nearly white in homozygous animals, occurs with blue eyes, and is attributed to a mutation in SLC45A2 similar to OCA type-4 in humans. In pigs, polymorphisms in the OCA2 gene in a red strain of Iberian pigs possibly contributes to coat color variation, similar to OCA type-2 in humans. Examples of TYR mutations in association with OCA-type phenotypes have been reported in chickens, minks, ferrets, rabbits and gerbils. Dietary amino acid deficiency can cause phenylalanine (Phe) and tyrosine (Tyr) deficiency and both can lead to decreased melanin synthesis and changes in coat color. In the best example, black cats fed Tyr and/or Phe restricted diets develop red-brown hair coats, with decreased melanin in hair shafts, and gave birth to kittens with red discoloration of normally black hair coats. Dietary copper deficiency leads to graying of the hair coat because copper is an essential cofactor for tyrosinase, the rate-limiting enzyme in melanin synthesis. Graying of the pericellular hair coat in black areas gives rise to the common name of "spectacle disease" or "ghost eye". Depletion of copper in puppy dogs caused hair coat graying.

6.0 MELANOSOME TRANSFER TO KERATINOCYTES

Melanosome transfer at dendrites of melanocytes to keratinocytes is referred to as the pigmentedary synapse and different mechanisms are proposed. One epidermal melanocyte is estimated to transfer melanosomes to 36 keratinocytes, which form an epidermal melanin unit, and one follicular melanocyte transfers melanosomes to 5 keratinocytes, the follicular melanin unit. At the melanocyte dendrite tip, three proteins (Rab27a, melanophilin, myosin-5a) facilitate transfer of melanosomes to keratinocytes. Basal keratinocytes accumulate more melanosomes than suprabasal keratinocytes, where most melanosomes are degraded by autophagy prior to cornification. Smaller melanosomes distribute to the perinuclear cap (microparasol), often in membrane bound vesicles, while larger melanosomes distribute widely in the cytoplasm. After endocytosis, smaller melanosomes are bound by dynactin to
dynein motors, for transport along microtubules towards the nucleus, and to microtubules in the perinuclear cap, after arrival.

**Disorders of melanosome transfer**

Three types of Griscelli syndrome (GS) in humans are related to mutations in three genes (MLPH, MYO5A, RAB27a) important for melanosome transfer to keratinocytes. Melanin dispersal to keratinocytes is limited, which leads to color dilution of skin and hair. Silver-gray hairs have pigment clumping and large melanosomes accumulate in the cell bodies of melanocytes. Lavender foal syndrome (LFS, also called coat color dilution lethal) in Arabian horses, characterized by dilute coat color and multiple neurological signs in new born foals, is due to a mutation in MYO5A and is similar to GS type-1 in humans. Dilute coat color in dogs and cats is associated with mutations in MLPH similar to GS type-3 in humans. Color dilution alopecia (color mutant alopecia), possibly related to Black hair follicle dysplasia, is a hereditary conditions associated with coat color dilution in over 15 breeds of dogs and cross breeds. In dogs, mutations MLPH contribute to CDA but additional modifying factors are required.

### 7.0 ACQUIRED PIGMENT ABNORMALITIES

Once the skin and hair are pigmented, acquired pigmentary abnormalities occur from disruption of the epidermal melanin and/or follicular melanin units. Hyopigmentation occurs in diseases that specifically or non-specifically disrupt melanocytes, keratinocytes, or both cell types. For these reasons, acquired depigmentation is very common in veterinary medicine. More specific cell targeting is seen with vitiligo, uveoderatologic syndrome, alopecia areata, and lupus variants. Less specific cell targeting is seen with vascular disease and ischemia, epitheliotrophic lymphoma, autoimmune subepidermal blistering diseases, and others. Simple, generic physiochemical injuries, such as wounds, burns, etc, also disrupt the epidermal and follicular melanin units and lead to depigmentation. Solar and inflammatory stimuli lead to acquired hyperpigmentation and the latter is common in veterinary medicine and non-specific.

### 8.0 REFERENCES


33. SS Schmutz, TG Berryere and AD Goldfinch. TYRP1 and MC1R genotypes and their effects on coat color in dogs. Mammalian Genome 2002; 13: 380-387.


INTRODUCTION
The ISVD listserv is an active discussion forum for all the ISVD members. It contains a variety of interesting cases and the ISVD board felt that, as a unique resource, some of the cases would make interesting presentation for the basis of discussion at our annual ISVD meetings.

Each case data, photographs and its associated on-line comments will be presented with an audience participation through Poll Everywhere.

ISVD would like to thank all members who contribute to the list and to the members who have given us permission to use these cases for discussion.

CASE 1
Sonny is an 8yo MC DSH with a history of an atypical mast cell tumour (diagnosed elsewhere) in the dorsal neck that was excised in March.

Two months later multiple similar masses developed around the periphery of the surgical margins and a solitary mass developed in the flank.

Histologically they are all similar and consist of nodular infiltrates in the deep dermis which consist of a mixed population of cells including lymphocytes (sometimes in aggregates), plasma cells, mast cells and presumed histiocytic cells.

Eosinophils are rare.

The majority of mast cells are poorly granulated and require Giemsa staining to identify. Fites, B and H and GMS special stain are negative.

The unusual feature to me is the plasmacytic infiltrates and relative lack of eosinophils compared to the ones I have seen before in Siamese and the recurrence post-surgery however I see that is described in TLG and Brian and Julies 1986 article.

I am wanting thoughts from ISVD as the resident oncologist is wanting to treat these medically rather than wait for spontaneous resolution which should happen? if it truly is an atypical “histiocytic” mast cell tumour.

Any thoughts as always appreciated.

Cheers, Geoff Dr. Geoff Orbell

CASE 2
Hi folks:
Signalment: 11 yr old Grade equine
Hx: rubbing off mane in focal area. Possible temporal association with deworming.
Histo: minimal perivascular lymphoplasmacytic dermatitis with mild hyperkeratosis and rare abnormal hairs

My questions are:
– Are these hair shafts normal for a gray horse?
– Is the fragmentation purely secondary to trauma from rubbing?
I didn’t see much but wanted to ask about the broken pigmented hairs.

Thanks, David, Dr. David W Gardiner
CASE 3
Hi everybody! I would like your opinions on this unusual case.

Nellie is an 8-month-old DSH cat with a 6 month history of severe progressive generalized (including pawpads) scale and thick crusts, often in linear patterns on trunk.

She had significant pruritus and staphylococcal and Malassezia dermatitis.

She is also of small size for her age and had unusual mentation (dull affect).

The bacterial infection has responded partially to doxycycline and she is acting more normally, but there is persistent yeast infection. Pred helped the pruritus.

The initial biopsies from November 2014 showed moderate hyperplastic exudative hypersensitivity-type dermatitis: marked diffuse acanthosis and parakeratosis with suppurative crusts; many mast cells and eosinophils in the interstitial infiltrate. I am providing clinical photos and photomics from February of this year. These later biopsies show decreased acanthosis with variable hyperkeratosis and parakeratosis. The stratum corneum looks more parakeratotic and/or exfoliative to me than in typical canine ichthyosis. I couldn’t find any vacuolated cells in stratum granulosum. The inflammation is much milder and mostly mononuclear, probably due to appropriate therapy for the pyoderma and yeast infection, although lots of yeast are still present.

I could find only one case of feline ichthyosis in the literature (Credille et al, in Advances in Vet Dermatol, vol 3, 1998), and that case looked much like uncomplicated canine nonepidermolytic ichthyosis.

So is this another case of feline ichthyosis with superimposed secondary inflammation due to staph and Malassezia? There are rare human subtypes of ichthyosis with associated immunocompromise and mental retardation. Has any of you seen anything like that in a cat? Are there any other differentials?

Thanks in advance for your input,
Emily, Dr. Emily Walder

CASE 4
I need help with this case.

4 year-old, castrate mixed dog.

Since 3 months it had a pruritic, ulcerative skin lesion in the internal surface of both (bilateral) pinnae. It has been treated with creams, antibiotics and prednisolone without responds.

Histopathological findings (Surgical biopsy).

There is severe necrosis/ulceration of the epidermis reaching the dermis and a little of the cartilage.

The only alteration that I found is a degeneration of the wall medium size artery (vasculopathy).

So I wonder if this could be an unusual clinic/pathological presentation of Proliferative Thrombovascular Necrosis Pinnae.

Alexis, Dr. Alexis Berrocal, Histopatovet
WHAT IS YOUR DIAGNOSIS?

This is an interactive session with a panel and audience participation through Polleverywhere.

CASE 1
Takafumi Osumi and Koji Nishifugi

Signalment
2 years and 8-month-old Singapura cat.

History and Signs
6-month history of swelling on nasal bridge and upper lip with nasal discharge. The clinical signs included swelling of the bridge of the nose and two small erythematous nodules on upper lip.

CASE 2
Erica Nolan

Signalment
Several Jersey-Holstein cross calves in one barn of approximately 250 to 300lbs.

History and Signs
Calves acutely developed swelling of the distal hind limbs and temperatures of 104F. Within two days, 16 more calves had moderate limb edema and 40% of all calves in the barn had erythema and edema around the dew claws. Affected calves had no lameness, but swollen areas were sore on palpation. The next morning, four animals developed fevers of 104-106F, and few others had mild cough. One animal was sacrificed for a field necropsy during which, only limb edema was observed.

CASE 3
Verena Affolter

Signalment
“Marino”, Dachshund, Neutered male, 2years old:

History and Signs
Owner observed hives on the dogs and treated with diphenhydramine for approximately 3 days with initial improvement. Referring veterinarian started treatment with steroids. As lesions became generalized, Marino was hospitalized and received IVF, steroids, antibiotics (cephalexin, marbofloxacin), famotidine, Cerenia. There was a persistent fever of 105oF. Skin biopsies were performed.

CASE 4
William Craft and Pam Ginn

Signalment
9-year-old neutered male West Highland White Terrier.

History and Signs
Long-term history of atopic dermatitis treated with cyclosporine. Presented with a firm nodule and draining tract on the dorsal neck. Excision and histopathology performed with a diagnosis of folliculitis and furunculosis. Post-operative cefpodoxime. Wound breakdown occurred over the next two weeks. Multifocal nodules and draining tracts with marked cellulitis erupted along dorsolateral neck and trunk and numerous white, semi-firm nodules were on the concave pinnae bilaterally. The dog was febrile, inappetent, and lethargic. The dog was referred to a dermatologist and biopsy samples were collected from the dorsolateral neck and concave right pinna.
CASE 5
Chanran Ganta

**Signalment**
7-year-old female spayed Domestic Long Hair

**History and Signs**
The patient was first presented for a hard lump on the side of the neck that had gotten bigger, and showed signs of weight loss. Another lump appeared on the right side of the top of the shoulder. The cat became febrile and was treated with antibiotics for which the nodules remain unresponsive. On re-examination the cat was presented with generalized lymphadenopathy, unilateral corneal edema and multiple irregularly thickened subcutaneous nodules on the entire body. The cat was then treated with steroids; the subcutaneous nodules have regressed but cat developed mild diarrhea and respiratory distress and febrile.

CASE 6
Jeanine Peters-kennedy

**Signalment**
An adult female Rocky Mountain big horn sheep.

**History and Signs**
The ewe presented with weight loss and muscle atrophy. There was also extensive bilaterally symmetric alopecia, hyperpigmentation, and crusting over the face, top of the head, dorsal trunk, distal limbs, tail and perineum. Crusting and lichenification was particularly severe on the concave surfaces of the pinnae, tail and around the coronary bands and fetlocks. Because of the poor body condition, this animal was euthanized and submitted for a complete necropsy.

CASE 7
David Shearer

**Signalment**
14-month-old female neutered Whippet.

**History and Signs**
The dog had not travelled outside the UK. The vaccinations were up to date. Routine worming with praziquantel. Routine flea/tick control with Advocate (imidocloprid/moxidectin). Frequently swims in lakes and ponds. No other disease history. There was a 10 day history of pyrexia (39-40°C) which had responded to paracetamol, cervico-thoracic spinal pain, lethargy and anorexia. Crusting skin lesions developed initially around the genital areas and resolved before referral. Treatment by referring vet included meloxicam, amoxicillin and paracetamol. Erythematous and bullous lesions progressing to crusts developed around eyes, muzzle and pinnae.
ALOPECIC DISEASES

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GENERAL BREAKDOWN

Alopecia in general is the absence of hair in an area where hair is normally present. This may be either partial or complete hair loss. One of the best ways to divide the causes of alopecia is whether it is inflammatory or non-inflammatory.

INFECTIOUS INFLAMMATORY ALOPECIA

Inflammatory, whether within the hair follicle or surrounding the hair follicle, can cause a loss of hair. This is especially true in the cases of infectious organisms.

Superficial Bacterial Folliculitis

Superficial bacterial folliculitis is commonly caused by Staphylococcus spp. with S. pseudintermedius being the most common. Other bacteria include Streptococcus, Pseudomonas, E. coli, Proteus, and Corynebacterium. Hair may be protruding from the pustule since the inflammation is in the hair follicle. Presentation can be quite variable depending on the breed. Pustules and erythema are quite common in the short-haired breeds with subsequent hyperpigmentation and alopecia. Some long-haired breeds such as Collies and Shetland Sheepdogs commonly develop widespread alopecia with mild erythema and epidermal collarettes on the periphery.

Dermatophytosis

Dermatophytosis is another common cause of folliculitis and subsequent alopecia. It is commonly caused by Microsporum canis, Microsporum gypseum, and Trichophyton mentagrophytes species complex. T. verrucosum is more frequent in cattle, M. nanum in pigs, and T. and M. equinum in horses. Clinical lesions consist of any combination of papules, pustules, focal to widespread alopecia, erythema, scaling, crusting, and nodular lesions (kerions).

Demodicosis

Demodicosis is a common and sometimes serious skin condition in dogs and cats. Demodex canis is the follicular mite in dogs while Demodex injai (long tailed mite) is located in the sebaceous glands and hair follicles of dogs. Demodex cati is the follicular mite in cats while Demodex gatoi (short bodied mite) is found in the stratum corneum in cats and is contagious. Clinical lesions consist of any combination of alopecia with variable erythema, hyperpigmentation, scale, crusts, pustules, erosions, ulcerations, draining tracts, and lichenification.

NON-INFECTIOUS INFLAMMATORY ALOPECIA

Pemphigus complex

The pemphigus complex is characterized by the formation of pustules or vesicles due to acantholysis. Autoantibodies directed at different components of the keratinocyte desmosomes cause the acantholysis. This is identified by acantholytic keratinocytes on cytology and histopathology. Pemphigus foliaceus is the most common of this complex. Pustules are noticeably interfollicular (between the hair follicles), but can include hair follicles as well in some cases. Other clinical lesions include crusts, erosions, and ulcerations which are subsequently followed by alopecia. Lesions commonly are noted on the face, nasal planum, periocular skin, ears, and footpads. Systemic signs can also be present including lethargy, anorexia, depression, fever, and weight loss.

Alopecia areata

This disease has a complex pathogenesis that is believed to be potentially affected by several different factors. Studies have shown that antibodies target the bulbar and inferior part of the hair follicle and potentially the melanocytes in the hair bulb. Clinical lesions present as local or multifocal patches of asymptomatic non-inflammatory alopecia most commonly affecting the head or face. Alopecia is the initial lesion in 92% of the cases.
Sebaceous adenitis
Inflammatory cells are distributed around the adnexa with potentially ultimate loss of the sebaceous glands. While alopecia is not seen in early lesions, it can be noted in chronic lesions. Initial lesions are mild erythema and scaling. Locations typically occur on the dorsum, pinnae, base of the pinnae, tail, and eventually generalized in chronic cases. Some common breeds affected include poodles, Akitas, German shepherd dogs, Samoyeds, vizslas, miniature pinschers, beagles, and dachshunds.

CONGENITAL NON-INFLAMMATORY ALOPECIA
Congenital Alopecia
Animals with congenital alopecia may be born with a decreased amount of hair or it may occur over the next 2-4 weeks after birth. Abnormalities may be restricted to just the hair follicles or additional structures may be affected as well, including epithrichial or atrichial sweat glands, lacrimal glands, sebaceous glands, bronchial glands, claws, or teeth. There is a wide variety of hairless breeds in both dogs and cats such as the Chinese Crested dog and Peruvian Hairless dog. Congenital hypotrichosis has been described in a wide variety of breeds of dogs and cats such as the German shepherd dog to the French bulldog.

Black Hair Follicular Dysplasia
Black hair follicular dysplasia is very characteristic in the fact that the dogs lose their hair only where black hair is located. This has been recognized in a wide variety of purebred dogs and mixed breed dogs. They typically will lose hair about 4 weeks after birth. Macromelanosomes (large melanin granules) are noted in black hair shafts with large bulging of the hairs on trichogram or with biopsy. Near complete alopecia occurs in these dogs by 6-9 months of age.

Color Dilution Alopecia (Color Mutant Alopecia)
Color dilution alopecia is a condition seen in dogs with blue or fawn coat color. It is important to note that some dogs with blue or fawn coat color exhibit hair loss, but this does not occur in all dogs with blue or fawn color. It is most commonly recognized in blue or fawn Doberman pinschers, but has been reported in many other breeds. Alopecia occurs in the region of the coat with blue or fawn color only and spares the other regions. Hair loss occurs initially due to weakening of the hair shafts where macromelanosomes are noted in the hair shafts.

Pattern Baldness
There are 3 different syndromes well recognized in pattern baldness. (1) Pinnal alopecia of the Dachshunds. Hair loss usually starts around 6-9 months of age and progresses to complete alopecia by 8-9 years of age. The underlying skin is usually hyperpigmented. (2) Greyhounds experience hair loss on the caudal thighs typical of endocrine disease. (3) The most common syndrome is hair loss pre- and postauricular regions and along the ventral neck, entire ventrum, and caudomedial thighs primarily in dachshunds. This has also been identified in Boston terriers, Chihuahuas, whippets, Manchester terriers, greyhounds, and Italian greyhounds. This may occur as early as 6 months of age and the hair loss is gradual over the next 12 months, but it is restricted to these areas. All of these syndromes have miniaturized hair follicles and hair shafts with normal adnexal structures when evaluated histologically.

ENDOCRINE NON-INFLAMMATORY ALOPECIA
Hypothyroidism
Hypothyroidism is the most common endocrine disorder of the dog, but it is typically overly misdiagnosed disease. Alopecia is only one of the most common clinical signs seen with hypothyroidism. It is commonly seen in areas of wear, including the bridge of the nose, elbows, entire length of the tail, and trunk. Other clinical signs include: (1) a dull, dry, brittle haircoat; (2) thick, puffy, non-pitting skin (myxedema); (3) variable hyperpigmentation; (4) seborrhea; (5) recurrent skin infections; and (6) lack of pruritus. Alopecia is due to importance of thyroid hormones responsible for initiating anagen, therefore there is a predominant number of hairs in telogen and subsequently lost without a new hair shaft replacing it.

Hyperadrenocorticism
Whether it is naturally occurring or iatrogenic, increased steroids cause a characteristic alopecia. Hair loss is usually symmetric and involves the trunk, sparing the head and distal extremities, but patchy hair loss or involvement of the
flank region or face can occur. Short-coated dogs may have a thinner coat or even a moth-eaten appearance. Other clinical signs include polyuria, polydipsia, decreased hair growth, coat color change, thin skin, hyperpigmentation, seborrhea, comedones, milia, poor wound healing, pyoderma, calcinosis cutis, and striae. Excessive steroids inhibit anagen growth and suppresses the hair growth rate resulting in follicular atrophy.

**Hyperestrogenism**
While this is not common, increased estrogen can cause alopecia typical of other endocrine diseases. Excessive estrogen production can be seen in female dogs with cystic ovaries or rarely with functional ovarian tumors and in male dogs with a functional testicular tumor (most commonly Sertoli cell tumor). This has also been described in dogs which come in contact with estrogen cream used by the owner. Alopecia usually is bilaterally symmetric beginning in the perineal, inguinal, and flank regions. Estrogen inhibits anagen growth and suppresses hair growth rate resulting in follicular atrophy.

**Hair Cycle Arrest (Alopecia X)**
This disease has been well recognized for quite some time in dogs with a double coat and dense under-coat, but not completely understood. Common breeds that are affected include Pomeranians, Alaskan malamutes, chow chows, Samoyeds, toy and miniature poodles, and keeshonds. Hair loss mimics other endocrine diseases with the head and distal extremities spared. Frictional areas such as the collar, tail head region, and caudomedial thighs are usually first affected. The cause of this disease has been hypothesized as an abnormality with adrenal gland steroid hormone intermediates as well as hypercortisolemia.

**Canine Flank Alopecia (Seasonal Flank Alopecia, Cyclic Flank Alopecia, Recurrent Flank Alopecia)**
This is a relatively common disease with unknown etiology. It is typically a bilaterally symmetric localized area of non-inflammatory, non-scarring hair loss over the lateral thorax to lateral lumbar region and occasionally migrating over the dorsum. The area is also typically hyperpigmented. The area may be completely alopecic or it may also just have an annular area of alopecia. A common characteristic of this disease is the cyclic or recurrent nature of this disease. Lesions usually develop between November and April and typically the hair will regrow in spring or summer.

**Telogen and Anagen Effluvium (Defluxion)**
Effluvium means increased hair shaft shedding. Telogen effluvium occurs due to a synchronized transition of all hair follicles to undergo anagen development. This results in a large loss of hair about 1-3 months after the inciting insult. This is usually due to a stressful circumstance such as high fever, pregnancy, shock, severe illness, surgery, drugs (doxorubicin), anesthesia, and nutritional deficiency. Postpartum is one of the most common causes and typically affects the truncal region. Anagen effluvium is much more immediate as hair loss occurs within days of the insult, which can be antimitotic drugs, infectious diseases, endocrine disorders, or metabolic diseases. Anagen effluvium is suspected when a large number of irregular and dysplastic changes of anagen hairs are noted on either trichogram or histopathology.

**Postclipping Alopecia**
A history of shaving the area for a procedure or catheter placement helps significantly. This is seen especially in plush-coated breeds such as the Alaskan malamute, American Eskimo, chow chow, keeshond, Pomeranian, Samoyed, and Siberian huskie. Any breed can realistically be affected. Hair loss is noted in any area shaved with a lack of new hair growth within 3 months. Most of the times, hair coat regrowth will become normal in about 12-24 months. The exact underlying cause is unknown, but it is suspected that the hairs enter telogen and a delay in anagen occurs.

**Traction Alopecia**
Traction alopecia is most commonly seen on the dorsal head of dogs. This has been described in dogs that have had barrettes, rubber bands, or other devices applied too tightly to the hair. This is likely due to ischemic dermatitis. There may be an inflammatory plaque present at the base of the hairs with scale or crusting in acute lesions. If it has been chronic, a scarring alopecia may develop with no regrowth of hair.
TRAUMATIC ALOPECIA
Feline Psychogenic Alopecia
This is a disease that is highly overdiagnosed. It usually presents as alopecia in areas that are easily reached such as the medial aspects of the forelimbs and inner thighs, caudal aspect of the abdomen and inguinal region. The dorsum as a whole is less commonly involved which helps differentiate it from other pruritic conditions. A thorough history, examination, and diagnostic workup must be completed to rule out all other causes of pruritus first. Trichograms of affected hairs can show that the alopecia is considered self-induced rather than spontaneous due to the fractured hair shafts. Hairs should not easily be epilated and will have a mixture of anagen and telogen.

GENERAL FUNCTIONS

EPIDERMIS
The normal animal skin is composed of 4-5 different layers depending on the location. Some refer to it as the living layers (basal layer, stratum spinosum, stratum granulosum, and stratum lucidum) and the dead layer (stratum corneum). The cells originate at the bottom, called the basal layer, and mature while migrating superficially to the stratum corneum. On average, it takes about 22 days for a keratinocyte to go through the full maturation process. This can be shortened with inflammation and some diseases. In general, the epidermis is thin (about 2-3 nucleated cells thick not counting the stratum corneum). The epidermis is thickest on the footpads and nasal planum as it can measure up to 3 times the thickness of the epidermis in haired skin.

Basal Layer (Stratum Basale): As mentioned previously, this is the lowest portion of the epidermis and it sits on the basement membrane zone. The majority of these cells are keratinocytes, which mature and migrate superficially to replenish the epidermal cells above with ultimate shedding as dead cells. These cells are normally columnar to cuboidal shaped with a large nucleus. Other cells present in this layer include stem cells, melanocytes, merkel cells, and Langerhans cells. Desmosomes form a 3-dimensional scaffold connecting the nuclear envelope to the cell membrane by intermediate filaments and also attach adjacent cells to stabilize the epidermis. Components of the desmosomes include 3 major families: (1) plakins (e.g. demsoplakin), (2) armadillo proteins (e.g. plakoglobin and plakophilin), and (3) desmosomal cadherins (e.g. desmogleins and desmocollins). Hemidesmosomes and focal adhesions are present on the deepest border of this layer and are responsible for attaching the basal layer to the basement membrane zone, which keeps the epidermis and dermis attached. Integrins are also a large family of cell surface adhesive receptors important for cell-cell and cell-matrix interactions, and signal transducers.

Spinous Layer (Stratum Spinosum): The daughter cells of the stratum basale form this layer. This layer is relatively thin in haired skin while it may be up to 20 cell layers in thicker areas such as footpads, nasal planum, and mucocutaneous junctions. These cells are nucleated and polyhedral to flattened cuboidal in shape. This layer is called the spinous layer or prickle layer due to intercellular bridges (prickles), which are more prominent in nonhaired skin.

Granular Layer (Stratum Granulosum): This is variably present in haired skin with 1-2 cells thick in areas where it is present. In non-haired skin and the infundibulum of the hair follicle it may be 4-8 cells thick. These cells are flattened and they contain a shrunken nuclei along with large, deeply basophilic keratohyalin granules in their cytoplasm. Keratohyalin granules are synthesized in the stratum granulosum and are composed of filaggrin, keratin filaments, and loricrin. Filaggrin degradation products are important for stratum corneum hydration and also help filter UV radiation. Loricrin is involved in binding keratin filaments together in the corneocyte and anchoring them to the envelope. Ultrastructurally, lamellar granules are present at the cell margins in the granular layer cells.

Clear Layer (Stratum Lucidum): This is a fully keratinized, compact, thin layer of dead cells which is best developed in the footpads; less developed in the nasal planum and absent in all other areas of normal skin.

Horny Layer (Stratum Corneum): This is the most superficial layer, which is composed of terminally differentiated keratinocytes. A common analogy for this layer is the brick (corneocytes) and mortar (lipids) appearance. The corneocytes are flattened, anuclear cells without a true cell membrane and is thicker in lightly haired or glabrous skin. These corneocytes have a well developed cornified envelope (CE) under it plasma membrane which is responsible
for structural support and resists invasion by microorganisms and deleterious environmental agents. Components of
the CE include loricrin, involucrin, filaggrin, elafin, cystatin A, cornifelin, small proline-rich proteins, and late envelope
proteins. Involucrin binds ceramides covalently, forming a backbone for attachment of intracellular lipids. CE is also
present in cells of the inner root sheath and medullar of the hair follicle along with the cuticle of the claw.

BASEMENT MEMBRANE ZONE
This zone is extremely important in (1) anchoring the epidermis to the dermis, (2) maintaining a functional and
proliferative epidermis, (3) maintaining tissue architecture, (4) wound healing, (5) functioning as a barrier, and (6)
regulating nutritional transport between epithelium and connective tissue. This layer has numerous genetic defects
identified and immune-mediated targets which are responsible for several bullous and vesiculopustular diseases. The
four components of the BMZ from superficial to deep are: (1) basal cell plasma membrane, (2) the lamina lucida, (3) the
lamina densa (basal lamina), and (4) the sublamina densa, which includes the anchoring fibrils and dermal microfibril
bundles.

DERMIS
The dermis is extremely important to allow the skin to take the stress of movement and maintain the shape. In thick-
haired skin, the dermis accounts for most of the depth while the epidermis is thin. In very thin skin, the dermis is
comparatively thin. The dermis is composed of fibers, ground substance, and cells along with epidermal appendages,
arrector pili muscles, blood and lymph vessels, and nerves. Major functions of the dermis include (1) the tensile
strength and elasticity of the skin, (2) regulation of cell growth, proliferation, adhesion, migration and differentiation,
(3) modulates wound healing, and (4) structure and function of the epidermis.

Dermal fibers
All of the fibers within the dermis are formed by fibroblasts and include collagenous (collagen), reticular (reticulin),
and elastic (elastin) fibers. Collagen have great tensile strength and are the largest and most numerous fibers within
the dermis (about 90% of all fibers and 80% of all extracellular matrix). There are numerous different types of collagen,
but types I, III, and V predominate in the dermis accounting for approximately 87%, 10%, and 3%, respectively, of
dermal collagen. Type IV (lamina densa) and V (lamina lucida) collagen are found in the BMZ while type VII collagen
is found in the anchoring fibrils of the BMZ. Some collagen abnormalities may result from genetic defects causing
some of the well-recognized diseases. Synthesis can be stimulated and inhibited by a wide range of factors and one
commonly used inhibitor is glucocorticoids. Reticulin are fine, branching structures that are closely associated with
collagen. Elastin are single fine branches which possess great elasticity, but only about 4% of the dermal extracellular
matrix.

Dermal Ground Substance
This is a viscoelastic gel-sol composed of glycosaminoglycans usually linked in vivo to proteins (proteoglycans). It fills
the spaces and surrounds structures of the dermis and also allows nutrients, electrolytes, and cells to pass from the
dermal vessels to the avascular epidermis. Fibronectins are extracellular matrix and body fluid glycoproteins produced
by many cells, including keratinocytes, fibroblasts, endothelial cells, and histiocytes. They are present in the dermis,
especially perivascularly and perineurally, and in the lamina lucida and lamina densa of the BMZ. They modulate
microvascular integrity, vascular permeability, basement membrane assembly, and wound healing. Small amounts
of mucin are present in feline and canine skin, with the exception of the Shar-Pei skin, which has large amounts
throughout the dermis.

Dermal Cellular Elements
There are sparse numbers of cells in the dermis. Fibroblasts and dermal dendrocytes are present throughout.
Melanocytes may be present in the superficial dermis surrounding superficial blood vessels and hair bulbs in dark
skinned animals. Mast cells are commonly located around superficial dermal blood vessels and appendages.

HAIR
Hair serves several functions including serving as a physical barrier against trauma, providing protection from UV
radiation, thermoregulation/insulation, sensation, visual stimulus (piloerection as a warning), repelling water, holding
scents, source of cells for re-epithelialization during wound healing, and in some cases providing camouflage. Primary (outercoat, guard) hairs and secondary (undercoat) hair are medullated in dogs and cats. Hairs are divided into 3 different types based on their appearance including: (1) guard hairs (thickest, straight, evenly tapered to a fine tip), (2) awn hairs (thinner, possessing subapical swelling below the hair tip), and (3) down hairs (thinnest, evenly crimped or undulating). The shape of the hair follicle, which differentiates animals with straight or curly hair, determines the shape of the hair. Puppies do not actually lose their puppy coat, but they gain their adult coat. They have simple hair follicles that produce secondary hairs during the first 12-28 weeks of life.

Hair cycle
In general, there are 4 different phases of the hair cycle: (1) anagen (growth), (2) catagen (regression), (3) telogen (rest), and (4) exogen (shedding). During anagen, the lower portion of the hairs, below the attachment of the arrector pili muscle, will grow deeper through dermis and into the panniculus. It will then regress more superficially for the rest of the phases. Based on the breed, there can be a significant variation in the population of hairs in anagen vs telogen at any time. The hair cycle can be controlled by a wide variety of factors including: photoperiod (predominantly), ambient temperature (lesser extent), nutrition, hormones, general state of health, genetics and intrinsic factors (e.g. growth factors and cytokines). Hair growth is typically maximal in the summer (50% telogen) and minimal in the winter (up to 90% telogen). Artificial light, year round, showed no seasonal change with shedding year round. Protein deficient diets may produce dull, dry, brittle, or thin haircoat, with or without pigmentary disturbances. Hormonal changes can significantly alter the hair cycle as an accelerated growth is noted with thyroid hormones and growth hormones. Glucocorticoids and estrogen inhibit anagen and suppress hair growth rate resulting in follicular atrophy. In regards to hair growth in general, short coats may take up to 3-4 months to regrow after shaving while long coats may take up to 18 months to regrow after shaving.

Hair Follicles
The hair shaft is divided into a medulla (innermost region), cortex (middle layer), and cuticle (outermost layer). The hairs grow at about a 30-60 degree angle to the skin surface directed caudally and ventrally. Dogs and cats have compounded hair follicle arrangement. This includes clusters of 2-5 primary hair follicles surrounded by groups of smaller secondary hairs (5-20 secondary hairs per primary hair). Usually there is a single larger central primary hair. All primary hairs have sebaceous and sweat glands along with an arrector pili muscle while secondary hairs may only be accompanied by sebaceous glands.

The anagen hair follicle is divided into 3 different anatomic segments: (1) infundibulum or pilosebaceous region – which starts at the entrance to the skin surface and goes down to the entrance of the sebaceous duct; (2) isthmus – which starts at the entrance to the sebaceous duct and extends to the attachment of the arrector pili muscle; and (3) inferior segment which starts at the attachment of the arrector pili muscle and extends to the dermal hair papillae. Dogs and cats do NOT have a hair follicle bulge, unlike humans and rodents. This is important because the hair follicle bulge is where stem cells concentrate in humans and rodents. Dogs have stem cells throughout the infundibulum and isthmus region.

The inner root sheath is composed of 3 different layers: from inside to outside (1) inner root sheath cuticle which is a single layer of cells that interlocks with the cells of the hair cuticle; (2) Huxley layer which is 1-3 nucleated cells thick; and (3) Henle's layer which is a single layer of anucleated cells. The inner root sheath keratinizes and disintegrates when it reaches the level of the isthmus. The main function of the inner root sheath is to mold the shape of the hairs, which it accomplishes by hardening prior to the hair. Telogen hairs do not contain an inner root sheath or an inferior segment.

The outer root sheath is thickest near the epidermis and gradually becomes thinner toward the hair bulb. In the inferior segment, the outer root sheath is covered by the inner root sheath and the outer root sheath does not undergo keratinization. At the level of the isthmus the outer root sheath is no longer covered by the inner root sheath and it undergoes trichilemmal keratinization.
Specialized Hairs
There are 2 different specialized types of tactile hairs in mammalian skin: sinus hairs and tylotrich hairs. Sinus hairs (vibrissae, whiskers) are found on the muzzle, lip, eyelid, face, and through, and on the palmar aspect of the carpus of cats (carpal gland, pili carpalis). Pacinian corpuscles are situated close to the sinus hair follicles, which aid in the function as slow-adapting mechanoreceptors. Tylotrich hairs are scattered among ordinary body hairs and are much larger than surrounding follicles. They contain a single hair and a complex of neurovascular tissue that surrounds the follicle at the level of the sebaceous glands. These are believed to function as fast-adapting mechanoreceptors.

ADNEXA
Sebaceous Glands
Sebaceous glands are noted throughout all haired skin in all mammals except whales and porpoises. They are largest and most numerous near mucocutaneous junctions, interdigital spaces, dorsal neck and rump, chin, and dorsal tail (tail gland, supracaudal organ, preen gland). They are not present on the footpads or nasal planum. Secretions function in protection from overwetting, possibly heat insulation, chemical barrier against potential pathogens, and pheromonal properties. The sebum becomes contaminated in the infundibulum with lipase producing bacteria, which results in the production of fatty acids, which are antimicrobial. Sebaceous glands are thought to be under hormonal control as androgens cause hypertrophy and steroids cause involution.

Sweat Glands
Epitrichial sweat glands (apocrine) are generally coiled and saccular and present throughout all haired skin and not present in footpads or nasal planum. These are usually located deeper in the dermis than the sebaceous glands and open into the infundibulum, above the sebaceous gland opening. They are largest where the hair follicle density is lower, so mucocutaneous junctions, interdigital spaces, and over the dorsal neck and rump. The sweat produced by epitrichial sweat glands probably have pheromonal and antimicrobial properties.

Atrichial sweat glands (eccrine) are found only in the footpads and they are small and tightly coiled. They are located deep in the dermis and subcutis of the footpads.

Arrector Pili Muscle
Arrector pili muscle is found in all haired skin and is largest in the dorsal neck and rump. They probably function in thermoregulation and in the emptying of sebaceous glands as well as social signals (piloerection).

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Malassezia dermatitis with a keratin disorder in a green parrot (Araambigua)

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Abstract: Feather-destructive behavior (feather picking) has been reported as a common and often severe problem of captive psittacine birds. Several etiologies have been described including behavioral, ectoparasitism, endoparasitism, nutritional deficiencies, allergic, cutaneous bacterial and fungal diseases, especially Malassezia spp. A female adult green parrot (Araambigua) presented to a rescue center with alopecia on the chest-abdominal area. A cutaneous scrape was negative for parasites and fungi. Over the following nine weeks there was no feather re-growth and no other clinical or behavioral abnormalities. Unexpectedly, the parrot was found dead and then sent to necropsy. The gross examination revealed an alopecic area of nearly 8.0 cm2 located on the chest and abdominal area. Samples of internal organs as well as four skin biopsies were taken and processed routinely for microscopic investigation and stained with H&E, PAS and GMS. The epidermis showed orthokeratotic hyperkeratosis with multifocal invaginations of laminar keratin and intercellular aggregates of numerous oval, unipolar budding yeast microorganisms of 3-4 µm, which stained positive with PAS and GMS. There were also areas of necrosis involving the epidermis and superficial dermis. The dermis showed hyperemia and infundibular keratosis. In the literature there are only few reports of cytological findings of microorganisms resembling Malassezia spp. associated with feather destructive behavior. This is the first documented report revealing Malassezia-like microorganisms with a keratin associated disorder and necrotic epidermitis and dermatitis likely due to the feather picking behavior of this disease.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Aqueous dust mite extracts exhibit protein degradation as a consequence of storage diluent

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Abstract: Dust mites are significant allergens driving canine atopic dermatitis. Current veterinary allergy immunotherapy utilizes extracts stored in aqueous buffers, which are known in human immunotherapy extracts to exhibit protein degradation after manufacturing. In order to characterize dust mite extract stability, experimental aqueous and glycerin Dermatophagoides farinae and Dermatophagoides pteronyssinus extracts were generated at the ALK Allergy Laboratory (Round Rock, TX) using extraction procedures employed at ALK’s commercial production facility (Port Washington, NY, USA). Protein concentration and diversity were determined by Bradford-style and SDS-PAGE/Coomassie assays immediately after extraction and again after 1, 3, 6, 12 and 20 weeks of storage. Reduced protein diversity was evident in aqueous extracts after only 12 weeks of storage at 4°C. Specific allergenic proteins, Der f/p 1 and Der f/p 2, were measured by a validated ELISA assay, and found to be at least three times higher in glycerin than aqueous extracts. To determine if protein degradation impacts IgE binding, extracts were subjected to immunoblotting with dust mite-reactive canine serum. Immunoblots revealed lower reactivity to aqueous extracts than age-matched glycerin extracts for both mites. Furthermore, reactivity to low molecular weight proteins was nearly absent in aqueous extracts. While these results do not indicate how intradermal testing and allergen specific immunotherapy are impacted by protein degradation, our data agree with previous reports that insect extracts degrade rapidly in aqueous compared to glycerin buffers. Furthermore, our research chronicles this process from the point of extraction and shows that protein degradation impacts IgE reactivity to dust mite antigens.


Conflict of interest: Authors are employees of ALK-Abello, Inc.
Correlation between meticillin-resistance and enrofloxacin-resistance in strains of Staphylococcus pseudintermedius from superficial pyoderma and otitis externa in dogs

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Abstract: Antimicrobial resistance is a worldwide concern in human and veterinary medicine. The misuse of antimicrobials is a risk factor for resistance development. The beta lactams and fluoroquinolones, bactericidal drugs, are some of the most frequently used in humans and animals. Bactericidal antibiotics produce large amounts of radical oxygen species by their mechanisms of action, resulting in accelerated mutation rates. Fluoroquinolones are believed to produce higher rates of mutation, thus may able to induce resistance to other antimicrobials. The aim of this study was to evaluate the correlation of enrofloxacin resistance (ER) and meticillin resistance (MR) in Staphylococcus pseudintermedius (SP) strains obtained from superficial pyoderma and otitis externa in dogs. The samples were submitted to disk diffusion test with oxacillin and enrofloxacin per the Clinical and Laboratory Standards Institute recommendations. Of the 104 SP samples, 38 were MR (36.5%), 46 (44.2%) were ER and 33 (31.7%) were both MR and ER. A positive Spearman’s correlation was found between MR and ER (r = 0.64876, P <0.0001). Although it is not possible to infer resistance induction, the results indicate possible MRSP strain selection by fluoroquinolones. The results support the recommendation for rational use of antimicrobials in human and veterinary medicine. Furthermore, one should consider the avoidance of fluoroquinolone use without prior culture and sensitivity testing.

Source of Funding: This work was supported in part by a National Counsel of Technological and Scientific Development, Ministry of Science, Technology and Innovation, Government of Brazil.

Conflict of interest: None declared.
Ceftarolinefosamil resistant *Staphylococcus* spp. in dogs with folliculitis and/or otitis externa

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**Abstract:** Infections caused by meticillin-resistant *Staphylococcus aureus* (MRSA) are a constant concern since this organism is resistant to several classes of antimicrobial agents, including β-lactams. Ceftarolinefosamil, an antimicrobial with anti-MRSA activity, recently approved in the United States, Europe and Brazil for use in humans, has shown a high affinity to the penicillin-binding protein (PBP2a), the altered protein encoded by the *mecA* gene. Reports of ceftarolinefosamil-resistant MRSA are rare in humans and nonexistent in dogs. Furthermore, there are no reports of other staphylococci resistant to ceftaroline. In order to assess the *Staphylococcal* resistance to ceftarolinefosamil, 44 samples from dogs with folliculitis and/or otitis externa due to *Staphylococcus* spp., including meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) and *Staphylococcus schleiferi*, where the *mecA* gene was identified by polymerase chain reaction, were submitted for disk diffusion testing with ceftarolinefosamil, per the Clinical and Laboratory Standards Institute. Fifteen of forty-four samples (34%) were resistant to ceftarolinefosamil. Resistance may be linked to factors other than the *mecA* gene. The high rate of ceftarolinefosamil resistance suggests further epidemiological studies with respect to meticillin-resistant staphylococci in animals are needed.

Source of funding: This work was supported in part by a National Counsel of Technological and Scientific Development, Ministry of Science, Technology and Innovation, Government of Brazil.

Conflict of interest: None declared.
**Susceptibility testing of different *Staphylococcus pseudintermedius* US strains to various antibiotics in superficial pyoderma of dogs**

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**Abstract:** This study assessed in vitro susceptibility of 34 *Staphylococcus pseudintermedius* strains isolated in 2014 and 2015 from 33 dogs with superficial pyoderma, presented at four veterinary establishments from Florida, Missouri, Louisiana and Pennsylvania. Swab samples were collected from affected skin and cultured for pathogen identification by routine bacteriology. Susceptibility testing of *S. pseudintermedius* was performed according to Clinical and Laboratory Standards Institute (CLSI) standards for the following antibiotics: amoxicillin/clavulanic acid (AUG2), ampicillin (AMP), cefovecin (FOV), cefpodoxime (POD), ceftiofur (XNL), cefalexin (LEX), clindamycin (CLI), enrofloxacin (ENRO), gentamicin (GEN), marbofloxacin (MAR), oxacillin (OXA), penicillin (PEN), and trimethoprim/sulfamethoxazole (SXT). Commercially available COMPAN2F panels (Trek Diagnostic Systems, Oakwood, OH, USA) were used for susceptibility testing, except for cefalexin tested using custom panels prepared by Microbial Research Incorporated (Microbial Research Inc., Ft. Collins, CO, USA) with doubling dilution concentrations from 0.06 to 64 µg/mL. Thirty-four *S. pseudintermedius* strains were isolated, identified and susceptibility tested. CLSI recommended breakpoints indicated strain susceptibility for 97.1% of the isolates to AUG2, FOV, MAR, OXA, POD, XNL, ENRO and LEX; for 94.1% to SXT; for 91.2% to CLI, and for 88.2% to GEN. Fewer than 50% of the isolates were susceptible to AMP and PEN. Only 1/34 strains was found to be multi-drug resistant (yet susceptible to SXT and CLI). These data demonstrate the susceptibility of *S. pseudintermedius* to most of the antibiotics in dogs with acute and initial infections. They support the use of cefalexin and amoxicillin/clavulanic acid as effective first line antibiotics in such cases of pyoderma.

Source of funding: Virbac.

Conflict of interest: Two authors are employees of Virbac SAS and one of Virbac Corporate.
Antimicrobial susceptibility of *Staphylococcus pseudintermedius* isolated from canine skin and soft tissue infections from 2011-2015

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Abstract: Since 2011, Zoetis has conducted an ongoing surveillance program to evaluate the in vitro activity of frequently used antimicrobial agents against bacterial pathogens isolated from companion animals seen at primary/general care practices presenting with naturally occurring skin and soft tissue infections. Data were analyzed annually and the first 5 years of susceptibility data against canine *Staphylococcus pseudintermedius* are presented here. Minimal inhibitory concentrations (MIC) for all isolates were determined using a commercially available broth microdilution system (Thermo Fisher Scientific, Oakwood Village, OH, USA) that followed the Clinical and Laboratory Standards Institute standards. The MIC parameters (mode, MIC50, and MIC90) and percent susceptible were determined for 1,441 isolates received from 16 veterinary diagnostic laboratories throughout the United States and Canada. For the beta-lactam antimicrobials, the modes and MIC50 values were stable (≤2 μg/mL); MIC90 values showed no change over time and typically tested at the highest concentration. Meticillin-resistance was detected in this study by testing isolates for the production of the penicillin–binding protein (PBP2a). The prevalence of isolates with PBP2a, while relatively high in this population, showed some variability between years but was generally stable and ranged from 24.8% (64/258 isolates) to 32.6% (61/187 isolates). In this analysis, the MIC parameters showed no change over time for the fluoroquinolone, lincosamide, phenicol, and folate pathway inhibitor antimicrobial classes tested. Over this 5-year period of in vitro testing, there was no change in the overall incidence of resistance of *Staphylococcus pseudintermedius* to any antimicrobial agent tested, including no significant difference in meticillin-resistance.

Source of funding: Self-funded.

Conflict of interest: None declared.
Comparison of the *in vitro* testing of first and third generation cephalosporins used to treat skin and soft tissue infections in companion animals

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Abstract: The Clinical and Laboratory Standards Institute (CLSI) has recommended the *in vitro* testing of cephalothin as a class representative for first generation cephalosporins. This study compares Minimal Inhibitory Concentration (MIC) values for the first generation cephalosporin, cefalexin (Rilexine®, Virbac Animal Health, Fort Worth, TX, USA) with cephalothin and two third generation cephalosporins, cefovecin (Convenia®, Zoetis Inc., Kalamazoo, MI, USA) and cefpodoxime (Simplicef®, Zoetis Inc., Kalamazoo, MI, USA). The MIC values for all strains were tested using a commercially available broth microdilution system that followed the CLSI standards. *Staphylococcus pseudintermedius* isolated from dogs seen at primary/general care practices with naturally occurring skin and soft tissue infections received as part of the Zoetis companion animal surveillance program from 2011-2015 were used. The mode and MIC50 values for 1,441 strains received from 16 veterinary diagnostic laboratories throughout the United States and Canada are presented here. Over the 5 years of testing, the mode and MIC50 values for cefalexin against *Staphylococcus pseudintermedius* were consistent and all tested at 1 μg/mL and 2 μg/mL, respectively. In contrast, cephalothin tested several dilutions lower against *Staphylococcus pseudintermedius* with the mode and MIC50 values all testing at ≤0.12 μg/mL over the same period. The mode and MIC50 values for the third generation cephalosporins, cefovecin and cefpodoxime against *Staphylococcus pseudintermedius* all tested at or within one doubling dilution of 0.25 μg/mL over the 5 years of testing. Data from this study indicate that cephalothin should not be used to predict cefalexin in vitro susceptibility when testing *Staphylococcus pseudintermedius*.

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Conflict of interest: None declared.
Dysbiosis of intestinal microbiota aggravate atopic dermatitis in a mouse model

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Abstract: Environmental factors including intestinal microbiota in early life have been related to the development of atopic dermatitis (AD) in humans. Intestinal microbiota may also be involved in the development of AD in veterinary patients, however, to date there has been little evidence to support this hypothesis. This study aimed to investigate the effects of gut dysbiosis in early life using a mouse model (ovalbumin-induced) of AD. To create gut dysbiosis, antibiotics (a cocktail of four antibiotics) were administered daily with or without oral healthy faeces for 14 days before evaluation. AD phenotypes (clinical score and skin barrier function) and systemic immune response (serum IgE using ELISA) as well as the gut immune response (intestinal innate lymphocytes-3 by flow cytometry) including metabolites were evaluated. Mice who received antibiotics without oral healthy faeces had significantly aggravated phenotypes (clinical score, transepidermal water loss, and histopathology). Total IgE production and skin Th2-cytokines were significantly increased in antibiotic group mice compared to the antibiotic with oral healthy faeces mice. In the gut, interleukin-17 and group 3 innate lymphoid cells were increased and the production of the short chain fatty acids was significantly suppressed by antibiotics in the mice without oral healthy faeces. Intestinal microbiota could play a crucial role in development of AD. Further studies are needed in animals and humans.

Source of funding:
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2. This study was supported by the Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (Grant No. 315016-3-C00).

Conflict of interest: None declared.
Successful management of multifocal pyogranulomas caused by ruptured follicular cysts in a dog

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Abstract: A 3-year-old neutered male Samoyed presented with multifocal nodules on forelimbs. The painful lesions were firm and erythematous. Cytology was not diagnostic and fine needle aspirates for bacterial and fungal cultures were negative. Biopsies were submitted and histopathology revealed diffuse pyogranulomatous inflammation around multifocal follicular cysts. Additionally, captured keratin debris was detected in the pyogranulomatous inflammatory cells. These findings were consistent with multifocal pyogranulomatous dermatitis caused by ruptured follicular cysts. Therapy included oral prednisolone (Prednis Tab®, LLOYD Inc.; Shenandoah, Virginia, USA, 0.5 mg/kg orally twice daily) and cyclosporine (5 mg/kg orally daily, Implanta Cap®, Hanmi Pharm. Co. Ltd, Seoul, Korea) which resulted in marked improvement in 2 weeks. The medications were slowly tapered over 3 months with continued significant improvement of the lesions. This case report describes a dog with multifocal pyogranulomas on forelimbs caused by ruptured follicular cysts that were managed successfully with oral immunosuppressive therapies.

Source of funding:
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2. This study was supported by the Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (Grant No. 315016-3-C00).

Conflict of interest: None declared.
Double blinded, placebo controlled, crossover study to investigate the efficacy of a topical endocannabinoid membrane transporter inhibitor in a colony of atopic beagles

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Abstract: This study tested a topical endocannabinoid membrane transporter inhibitor in gel form compared to placebo (vehicle) for atopic dermatitis (AD) using atopic beagles sensitized to house dust mites (HDM). Nineteen dogs were randomly divided into two groups: one received active ingredient (2mg/cm2) on the inguinal area and the other, vehicle for 21 days. Dogs were challenged epicutaneously with HDM twice weekly for 28 days. Product application started after the third challenge (day 8), twice daily except the day of challenges (once daily, 6 h after challenge). Canine Atopic Dermatitis and Extent Severity Index (CADESI)-03 was assessed before and 6 h after the first challenge of the week. Pruritus was assessed on the same days as CADESI-03. After a 4-week washout, dogs were crossed-over and study repeated. In the vehicle group, on days 15 and 22, CADESI-03 scores in the inguinal area were significantly higher after challenge (mean difference of 16.34 P =0.00887 and 7.42 P =0.048466, respectively). In the treatment group no significant increase was detected after allergen exposure on both days (mean difference of 2.42 P = 0.317661 and 2.58 P =0.319037, respectively). No significant effect of time on pruritus global scores (P =0.0529) was found for treatment group. Significant decrease on pruritic acts both on inguinal area and overall (P =0.048 and P =0.032, respectively) was found in the treatment group. In conclusion, this topical product helped minimize flares after allergen exposure both in terms of skin lesions and pruritus. Further studies are needed to investigate this treatment for canine AD.

Source of funding: This study was funded by Dr. August Wolff GmbH & Co, Germany.

Conflict of interest: None declared.
An evaluation of adherence to medical recommendations in the dermatology service at a small animal teaching hospital


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Abstract: Adherence to medical recommendations is recognized as an important factor in positive patient outcomes. It has been evaluated to a limited extent in veterinary medicine with reported adherence rates anywhere from 30 to 89%. The objective of this evaluation was to examine adherence to written instructions provided to veterinary dermatology clients as part of a quality assurance assessment of our program. Cat and dog owners of returning dermatology patients completed a questionnaire to report how well written recommendations on printed discharge notes were followed. Adherence rates were calculated and contributing factors were evaluated. Adherence was evaluated for 75 patients at 93 different recheck visits to the dermatology service. Where a recheck visit date had been previously recommended, 89% occurred within 1 week of that date. Overall, the average rate of adherence to written instructions was 74%. Adherence rates varied by category of recommendation (p<0.001): diet changes had the highest adherence (100%) while recommendations requiring changes to owner or pet behavior had the lowest (17%). Adherence to medication instructions (the most common category) was 75% overall, but lower for medicated shampoo (62%) and other topicals (58%). Adherence to grooming or cleaning recommendations was 58%. There was some evidence that format of the discharge instructions had an effect on adherence with simplified itemized list having higher adherence than paragraph form (78% versus 71%, p=0.046). Overall, adherence varied based on recommendation category and format. Adherence was higher than previously reported in general practice, which could be attributed to the nature of specialty medicine.

Source of funding: Self-funded.

Conflict of Interest: None declared.
Efficacy and safety of Effipro® duo/plus in cats naturally infested by ticks (European species): a multi-centre, randomised, blinded, positive controlled clinical field study

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Abstract: This study aims to show that a fipronil-pyriproxyfen spot-on formulation (Effipro® duo/plus, Virbac, Carros, France) is as efficacious and safe as a fipronil-S methoprene spot-on formulation (Frontline® Combo, Merial, Lyon, France) to decrease natural tick infestations in cats. A total of 180 privately owned cats in Europe with more than three live and attached (LA) ticks on the day of inclusion (D0) were included and randomized to the Effipro® duo/plus or Frontline® Combo treatment group with a 2:1 ratio. Tick counts were performed on D0, D7 and D14. The percent reduction of the LA ticks compared to the D0 LA tick count number was the primary efficacy criterion (Wilcoxon-Mann-Whitney one-sided test for inferiority). There were 126 cats treated with Effipro® duo/plus and 53 treated with Frontline® Combo. Means (standard deviation) of LA ticks on D0 were of 7.0 (9.1) and 11.2 (26.2) in the respective groups. The following tick species were found: *Ixodes ricinus* (in 79.33% of cats), *Ixodes hexagonus* (19.55%), *Rhipicephalus* spp. (6.70%) and *Dermacentor reticulatus* (2.79%). The percent reduction of LA tick counts was 92.5% and 90.9% on D7 and 90.6% and 83.1% on D14 in cats treated respectively with Effipro® duo/plus or Frontline® Combo (non-inferiority). During the study, 12 adverse events (AE) occurred in eight cats (4% treated with Effipro® duo/plus and 5.7% treated with Frontline® Combo). No mortalities or other severe AE occurred. Effipro® duo/plus is efficacious and safe to treat natural tick infestations (European species) in cats.

Source of funding: Virbac.

Conflict of interest: CN and LM are employees of Virbac. CVS is an employee of Virbac Corp.
The speed of kill of Effitix® when compared to Parastar® plus against artificial infestations of *Ixodes scapularis* and *Amblyomma americanum* ticks on dogs: a randomized, controlled, blinded proof-of-concept study

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**Abstract:** This study evaluated the speed of kill of Effitix® (Virbac, Fort Worth, TX, US) when compared to Parastar® plus (Elanco, Greenfield, IN, US), against *Ixodes scapularis* and *Amblyomma americanum* ticks. Nine healthy adult dogs were randomly allocated to three groups (1 - negative control; 2 - Parastar® plus; 3 - Effitix®) with treatment on D0. Persons assessing viability of ticks were blinded to dog treatment status. Dogs were infested on D7 with 10 ticks of each species per challenge time point (1, 2, 3, 4, 5 or 10 min of exposure). Tick viability was initially assessed immediately after removal post-exposure. All live and moribund ticks were placed in vials and transferred to a controlled environmental chamber. Viability checks were performed again 24 h later. There was no clinically relevant immediate kill of tick when exposed to both treatments for up to 10 min. Effitix® did however have a 90% knock-down effect against *A. americanum* ticks after only 10 minutes exposure (47% with Parastar® plus). When viability was assessed 24 h after exposure to treated dogs, Effitix® showed at least as strong an effect against *A. americanum* ticks and *I. scapularis* respectively (100% at all time points for both tick species) compared to Parastar® Plus (64%, 65%, 71%, 67%, 64%, 73% and 96%, 100%, 79%, 79%, 84%, 86% after 1, 2, 3, 4, 5 or 10 min of exposure), respectively. Effitix® strongly reduced the viability of *I. scapularis* and *A. americanum* ticks after only one minute exposure to the treated dogs.

Source of funding: Virbac.

Conflict of interest: C. Navarro, F. Hurtig, C. Von Simson and C.S. Nicolas received reimbursements, fees, funding or salary from Virbac.
Concurrent ischemic dermatopathy and systemic arteriolosclerosis in a dog

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Abstract: Ischemic dermatopathy may be associated with multiple vasculopathic syndromes. The aim of this presentation is to introduce a canine case with ischemic dermatopathy, in which systemic arteriolosclerosis was confirmed by necropsy. A male castrated mixed breed dog of unknown age presented from an animal shelter with skin lesions consisting of purpura, vesicles, scales, scars and cold skin on the dorsal tail base. The dog had episodes of epileptiform seizures, as well as a positive heartworm test and necrotizing skin lesions in the perianal area that resolved spontaneously. Blood coagulation tests revealed no marked abnormalities. Histopathology of the biopsied skin lesions revealed hyperkeratosis, epidermal necrosis, follicular atrophy, pallor of the dermis with leukocytoclastic vasculitis and thrombosis in small vessels. The dog died suddenly 7 months after the initial visit. Postmortem examination revealed massive hemorrhage in the thoracic mediastinum and abdominal cavity. Petechiae and ecchymoses were recognized in pancreas, kidney and urinary bladder. Histopathological analysis revealed marked vascular intimal and medial hyperplasia in multiple organs including the skin, diaphragm, kidney, urinary bladder, prostate and brain. Arterial thromboses were recognized exclusively in the pulmonary area, but not in other organs including skin. Intralesional dead nematodes were also recognized in the pulmonary lesions. These findings suggest the dog had systemic arteriolosclerosis, and died of severe hemorrhage likely associated with rupture of blood vessels. The relationship between the ischemic dermatopathy and systemic arteriolosclerosis remains unknown; however, this case may have similarities to arteriosclerotic ulcer of Martorell in humans.

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Conflict of interest: None declared.
Auricular chondritis in a dog with severe pain mimicking neurological disease

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Abstract: Auricular chondritis is a rare inflammatory condition in dogs and cats. The etiology and treatment are not always clear due to variability in clinical presentations and low numbers of cases in the literature. A 13 year-old, spayed female Labrador retriever was referred with severe pain localized to the right side of the head with concern of possible otitis media/interna. The dog had minimal improvement on tramadol, carprofen, and amoxicillin clavulanate, which she was receiving at presentation. Physical examination revealed a right head tilt, right-sided miosis with mild ptosis, and severe pain demonstrated by vocalization and aggression upon manipulation of the right ear or head, preventing thorough dermatological and otoscopic examinations. Under general anesthesia, the right pinna was noticeably thickened and erythemic, and otoscopic examination showed clear external ear canals with normal tympanic membranes. Computerized tomography showed no skeletal evidence of nerve entrapment and both middle ear regions were unremarkable; however, the right pinna was markedly thickened. Histopathology of the right pinna revealed marked lymphoplasmacytic and pyogranulomatous inflammation, including multinucleated histiocytic cells, adjacent to and around necrotic cartilage. Treatment with tramadol intermittently for 2 weeks and prednisone (2.2 mg/kg) in a tapering schedule over a two-month period resulted in complete resolution of the pain and swelling of the right pinna. The final diagnoses were 1) idiopathic auricular chondritis and 2) idiopathic Horner’s syndrome. In conclusion, auricular chondritis may present as severe pain mimicking neurologic disease, and in this case, responded completely to glucocorticoid therapy.

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Antimicrobial activity and biofilm inhibition of manuka honey against *Staphylococcus pseudintermedius*, meticillin-resistant *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa*

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Abstract: The antimicrobial effect of manuka honey (MH) has been well established for human cutaneous/wound pathogens, but its activity against veterinary pathogens is poorly studied. The minimum inhibitory concentration (MIC90; lowest concentration that inhibits visible growth in 90% of isolates), minimum biocidal concentration (MBC90; lowest concentration that kills 99.9% of the population in 90% of isolates) and minimum biofilm eradication concentration (MBEC90; minimum concentration that eradicates the biofilm in 90% of isolates) were determined for MH (BotaniVet™ Manuka Honey, Vet Remedy, Allentown, PA, USA) against 15 canine skin/ear isolates from clinical cases (five *Staphylococcus pseudintermedius* (SP), five meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) and five *Pseudomonas aeruginosa* (PA)). MIC90, MBC90 and MBEC90 values were determined using the MBEC™ Assay for High-Throughput Antimicrobial Susceptibility Testing of Biofilms Version 1.0 (Innovotech Inc., Edmonton, AB, Canada) and reported as mean ± standard deviation. MH was tested against all 15 isolates at dilutions of three, six, 12.5, 25, 50 and 100% (volume/volume); tests were run in triplicate. After biofilm formation, plates were incubated with serial dilutions of MH at 37 degrees Celsius for 24h. Optical density was measured with a Cytation™3 Cell Imaging Multi-Mode Reader (BioTek, Winooski, VT, USA). The MIC90, MBC90 and MBEC90 for SP were 12.5±0%, 25±0% and 35±0.1%, respectively. The MIC90, MBC90 and MBEC90 for MRSP were 12.5±0%, 30±11%, and 40±14%, respectively. The MIC90, MBC90 and MBEC90 for PA were 22.5±6%, 45±11%, and 45±11%, respectively. MH has antimicrobial activity and eradicates biofilm formation in canine skin and ear isolates of SP, MRSP and PA.

Source of funding: Self-funded.

Conflict of interest: Dr. Palmeiro has financial investment in Vet Remedy LLC.
Naturally acquired *Sarcoptesscabiei* infestation in a captive southern tamandua (*Tamandua tetradactyla*) and a capybara (*Hydrochoerishydrochaeris*)

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Abstract: A privately-owned 3-year-old, intact female southern tamandua (*Tamandua tetradactyla*) and a 3-year-old, intact female capybara (*Hydrochoerishydrochaeris*) were presented for severe pruritus. Both animals had thickened crusts and excoriations primarily on the ventrum. The animals were housed on shavings in wire-caged indoor enclosures adjacent to each other. Superficial skin scrapings revealed *Sarcoptesscabiei* mites on both animals, which were first identified via microscopic morphologic characteristics before confirmation with genetic sequencing. Pairwise sequence alignment of the partial Cox1 gene of *Sarcoptes* derived from both hosts revealed 100% mutual identity. The animals were treated with selamectin (Revolution, Zoetis Inc., Kalamazoo, Michigan, USA; 9 mg/kg) topically once, and the owner was instructed to repeat treatment in 2 weeks. The tamandua died of unknown causes 3 weeks after initial examination before reevaluation could occur; the body was frozen prior to necropsy preventing definitive diagnosis. There were degenerative kidney changes and blood within the gastrointestinal tract at necropsy. No specific pathological process was identified nor symptoms consistent with selamectin toxicity. Clinical signs and skin lesions in the capybara resolved after two treatments with selamectin. This is the first documented report of sarcoptic mange in a southern tamandua. In addition, this is the first documented case of likely natural transmission of *Sarcoptes* mites between a tamandua and capybara in captivity. Finally, this is the first described use of topical selamectin for the treatment and resolution of sarcoptic mange in a capybara.

Source of Funding: Self-funded.

Conflict of Interest: None declared.
Comparison of immunologic parameters in pit bull dogs with juvenile-onset generalized demodicosis and age-matched healthy pit bull dogs using a commercial cytokine panel (Milliplex) and flow-cytometry


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Abstract: Pit bulls have been documented to develop juvenile-onset generalized demodicosis (JOGD) at a higher prevalence than other breeds. JOGD is thought to occur as a result of immunologic differences relative to unaffected dogs. A cytokine panel has been recently marketed to detect cytokines in low-volume canine serum samples (Milliplex, EMD Millipore Corp., Darmstadt, Germany). The cytokine assays and flow cytometry were used to investigate for differences between seven age-matched normal and seven JOGD pit bulls. Flow cytometry measured the percentage of B cells expressing MHCII or surface bound IgG, percentage of CD4+ T cells expressing MHCII, and percentage of CD8 T cells expressing MHCII or CD11a; surface expression was quantified by calculating the geometric mean fluorescence index. T lymphocyte proliferation utilized the mitogen Concanavalin A and was assessed via flow cytometry. Differences were assessed by Mann Whitney U test (significance P< 0.05). T lymphocyte proliferation in JOGD pit bulls was lower (range = 1.23 – 7.07; median 1.85) than normal pit bulls (1.94 – 2.56; median 2.21). In JOGD pit bulls, the median values for B lymphocyte IgG+, B lymphocyte MHC Class 2 GMFI, CD8 T cell CD11a+ were at least 10% greater than those values from normal pit bulls. Serum concentrations of KC, IL-8, IL-18, and MCP-1 were higher in the pit bulls with JOGD. However, none of the observed differences achieved statistical significance, which may have related to sample size, and so additional cases are being entered.

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Conflict of interest: None declared.
Transcriptomic and proteomic analysis of canine DH-82 monocytes stimulated with canine interleukin-31

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Abstract: Lokivetmab (CYTOPOINT™, Zoetis Inc, Kalamazoo, MI, USA) is a caninized anti-canine interleukin (IL)-31 monoclonal antibody approved in the US to aid in the reduction of clinical signs associated with atopic dermatitis in dogs. Lokivetmab is effective at reducing both pruritus and inflammatory lesions in dogs by neutralizing the effects of IL-31. To better understand the effects of IL-31 on canine monocytes, DH-82 cells were stimulated with canine IL-31 and the transcriptomic and proteomic effects were evaluated at 1, 6 and 24 h post-treatment. Total RNA from six replicates were isolated and gene expression was evaluated using Agilent Technologies Canine v2 microarrays. Differentially expressed genes included the upregulation of both subunits comprising the IL-31 receptor (IL-31Ra – 8.3 fold and oncostatin M receptor - 19.8 fold) at 6 h. Additionally, the upregulation of several pro-inflammatory modulators including IL-8 (10.9 fold), IL-1β (4.9 fold), TNFα (4.6 fold), IL-33 (2.8 fold) and IL-4 receptor alpha (2.1 fold) were observed. To confirm these findings and evaluate expression of genes not present in the microarray, qPCR was performed. These data confirmed both IL-8 and IL-33 upregulation and identified IL-6 and monocyte chemoattractant protein 1 (MCP1) as being upregulated. Interestingly, chemokine/cytokine analysis using culture supernatants identified increased protein expression of IL-8, keratinocyte-derived chemokine-like chemokine, and MCP1 at 6 h post stimulation. Taken together these results demonstrate that monocytes respond to IL-31 stimulation by producing a myriad of pro-inflammatory cytokines and chemokines, and may provide a rationale for the improvement in inflammatory lesions observed following lokivetmab treatment.

Source of funding: This study was initiated and funded by Zoetis, Inc, Florham Park, NJ, USA.

Conflict of interest: All authors are employees of Zoetis, Inc.
Retrospective study of the interval between injections of CYTOPOINT™ in clinical practice

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Abstract: Canine Atopic Dermatitis Immunotherapeutic or CYTOPOINT™ is an injectable anti-cIL-31 monoclonal antibody (Zoetis, Inc. Kalamazoo, MI, USA) that was conditionally licensed for treatment of atopic dermatitis (AD) in dogs in the US during this study. The minimum recommended dose of 2 mg/kg can be administered at monthly intervals, as needed. The study objective was to determine the days between injections when the product was administered in the clinic. Veterinarians, working in general practice or specialty dermatology clinic(s) (n=181), who purchased ≥20 vials of product prior to December 1, 2015 were asked to review their medical records and provide the dates of administration for dogs with AD. Data collection was between December 1, 2015 and March 1, 2016. Veterinarians were asked to record the dates irrespective of the reason for the duration. There was no restriction on administration of concurrent topical or systemic therapies. For dogs that received more than one injection during the study time period, the mean treatment interval was 36.8 days (range, 12-101), 33.5 days (19-79), and 32.5 days (25-56), respectively, for dogs that received two (n=293), three (n=92), or four injections (n=18). Of the dogs that received two injections, 80.2% received the second dose between 28 and 56 days, 7.8% at >56 days, and 11.9% at ß27 days. Dogs in this population averaged 7 years of age, 39 lbs, and were evenly divided between males and females. The mean treatment interval was 36.8 days. The impact of disease seasonality or concurrent therapy was not assessed.

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Conflict of interest: Authors are employed by Zoetis, Inc. None declared by participating veterinarians.
In vitro efficacy of topical piperacillin-tazobactam for treatment of Pseudomonas otitis externa infections in dogs

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Abstract: Pseudomonas spp. have been implicated as common pathogens in otitis externa. Multidrug resistant organisms have also been implicated with increasing frequency in these infections. Use of local, targeted therapies serve to decrease risk of systemic adverse effects, and to optimize the concentration of drug to which susceptible organisms are in contact. Compounded otic preparations enable the use of customized antibiotics which may not be available in commercial formulations. The aim of this study was to evaluate the efficacy of piperacillin-tazobactam (pip/tazo) in vitro against multidrug resistant and susceptible isolates of Pseudomonas obtained from 8 dogs and 1 cat with chronic otitis externa. ATCC 27853 and ATCC 35218 were used as control strains for Pseudomonas aeruginosa and Escherichia coli, respectively. Filter paper disks were impregnated with pip/tazo in 0.9% NaCl solution (200 mg/mL) at day 0, 7, 14, 21, and 28 following initial reconstitution. Aliquots of pip/tazo were frozen on Day 0 and thawed for use on subsequent days. Zones of inhibition for the compounded pip/tazo solution were compared to those of commercially prepared pip/tazo impregnated disks, and to control disks containing only diluent (0.9% NaCl). Nine of nine clinical isolates and two of two control isolates exhibited susceptibility to the compounded pip/tazo dilution at all time points. In-vivo studies are warranted to evaluate clinical efficacy and safety of compounded pip/tazotic solution in cases of Pseudomonas otitis externa.

Source of funding: Self-funded.

Conflict of interest: None declared.
Antimicrobial susceptibility of cefovecin versus Gram-positive and Gram-negative pathogens isolated from patients in Canadian hospitals: CANWARD study 2013 and 2015

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Abstract: We assessed the activity of cefovecin and comparators versus a current collection of organisms obtained from CANWARD, a national, Health Canada endorsed surveillance study assessing pathogens causing infections in Canadian hospitals. In 2013 and 2015, Gram-positive and Gram-negative pathogens were collected from 15 tertiary-care hospitals across Canada. Antimicrobial susceptibility testing with cefovecin (Convenia; Zoetis Inc., Kalamazoo, MI, USA) and various cephalosporin and non-cephalosporin comparators was performed using Clinical and Laboratory Standards Institute broth microdilution methods. Specimen source composition of >4500 isolates was 43.8% blood, 32.7% respiratory, 13.4% urine, 10.2% wound specimens. Patient demographics were: 54.5/45.5% male/female; 13.1/44.4/42.5% patients aged ≤17/18-64/≥65 years; and 38.1/24.9/18.9/18.1% patients located in medical and surgical wards/emergency rooms/ICUs/clinics. The most common pathogens were: E. coli (EC 19.6%), meticillin-susceptible Staphylococcus aureus (MSSA 16.6%), Pseudomonas aeruginosa (PA 8.9%), Streptococcus pneumoniae (SPN 6.3%), Klebsiella pneumoniae (KP 6.1%), Enterococcus spp. (5.5%), meticillin-resistant Staphylococcus aureus (MRSA 4.7%), and Haemophilus influenzae (4.1%). Versus Gram-positive cocci, MSSA and Streptococcus spp., cefovecin demonstrated similar activity (MIC50/MIC90ug/ml) to the first generation cephalosporin cefazolin. Neither cefovecin, nor cefazolin nor any cephalosporin tested was active versus MRSA, Enterococcus faecalis and Enterococcus faecium. Versus Gram-negative bacilli, EC, KP and Proteus mirabilis, cefovecin demonstrated similar activity (MIC50/MIC90ug/ml) to the first generation cephalosporin cefazolin. Versus Serratiamarcescens, Enterobacter cloacae and K. oxytoca, cefovecin was 2-32x more active than cefazolin. Neither cefovecin, nor cefazolin were active versus PA. In conclusion, cefovecin possesses Gram-positive activity similar to first generation cephalosporins and Gram-negative bacillary activity slightly greater than first generation cephalosporins.

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Conflict of interest: None declared.
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Claro® Otic Solution is approved for the treatment of ear infections in dogs caused by susceptible strains of yeast (Malassezia pachydermatis) and bacteria (Staphylococcus pseudintermedius). CAUTION: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian. CONTRAINDICATIONS: Claro® should not be used in dogs known or suspected to be allergic to Claro® or any of its ingredients.

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Otic Solution

Antibacterial, antifungal, and anti-inflammatory

For Otic Use in Dogs Only

The following information is a summary of the complete product information and is not comprehensive. Please refer to the approved product label for complete product information prior to use.

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

PRODUCT DESCRIPTION: CLARO® contains 16.6 mg/mL florfenicol, 14.8 mg/mL terbinafine (equivalent to 16.6 mg/mL terbinafine hydrochloride) and 2.2 mg/mL mometasone furoate. Inactive ingredients include purified water, propylene carbonate, propylene glycol, ethyl alcohol, and polyethylene glycol.

INDICATIONS: CLARO® is indicated for the treatment of otitis externa in dogs associated with susceptible strains of yeast (Malassezia pachydermatis) and bacteria (Staphylococcus pseudintermedius).

DOSAGE AND ADMINISTRATION: CLARO® should be administered by veterinary personnel. Administration is one dose (1 dropperette) per affected ear. The duration of effect should last 30 days. Clean and dry the external ear canal before administering the product. Verify the tympanic membrane is intact prior to administration. Cleaning the ear after dosing may affect product effectiveness. Refer to product label for complete directions for use.

CONTRAINDICATIONS: Do not use in dogs with known tympanic membrane perforation (see PRECAUTIONS).

CLARO® is contraindicated in dogs with known or suspected hypersensitivity to florfenicol, terbinafine hydrochloride, or mometasone furoate, the inactive ingredients listed above, or similar drugs, or any ingredient in these medicines.

WARNINGS: Human Warnings: Not for use in humans. Keep this and all drugs out of reach of children. In case of accidental ingestion by humans, contact a physician immediately. In case of accidental skin contact, wash area thoroughly with water. Avoid contact with eyes. Humans with known hypersensitivity to florfenicol, terbinafine hydrochloride, or mometasone furoate should not handle this product.

PRECAUTIONS:

Do not administer orally.

The use of CLARO® in dogs with perforated tympanic membranes has not been evaluated. The integrity of the tympanic membrane should be confirmed before administering the product. Reevaluate the dog if hearing loss or signs of vestibular dysfunction are observed during treatment.

Use of topical otic corticosteroids has been associated with adrenocortical suppression and iatrogenic hyperadrenocorticism in dogs.

Use with caution in dogs with impaired hepatic function. The safe use of CLARO® in dogs used for breeding purposes, during pregnancy, or in lactating bitches has not been evaluated.

ADVERSE REACTIONS: In a field study conducted in the United States, there were no directly attributable adverse reactions in 146 dogs administered CLARO®. To report suspected adverse drug events and/or obtain a copy of the Safety Data Sheet (SDS) or for technical assistance, contact Bayer HealthCare at 1-800-422-9874. 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.

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