29th PROCEEDINGS
OF THE
NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
Nashville, Tennessee
April 15-18, 2015

Contents

Table of Contents ...............................................................................................................
Registration Hours ..........................................................................................................
Exhibit and Poster Hours .............................................................................................
Meeting Schedule ..........................................................................................................
Roundtable Sessions ....................................................................................................
Abstract Schedule ........................................................................................................

THURSDAY
Residents’ Short Communications and Original Short Communications .............
Scientific Session Presentations ..................................................................................
Concurrent Session Presentations .............................................................................

FRIDAY
Research Short Communications and Clinical Short Communications ..............
Scientific Session Presentations ..................................................................................
Concurrent Session Presentations .............................................................................

SATURDAY
Clinical Short Communications ...................................................................................
Scientific Session Presentations ..................................................................................
Concurrent Session Presentations .............................................................................

Poster Communications ............................................................................................
Sponsors Recognition .................................................................................................
Research Foundation Sponsors ..................................................................................
# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM

## REGISTRATION HOURS

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
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<tbody>
<tr>
<td>Wednesday, April 15</td>
<td>5:00pm - 7:00pm</td>
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<tr>
<td>Thursday, April 16</td>
<td>7:00am - 5:00pm</td>
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<td>Friday, April 17</td>
<td>7:30am - 5:00pm</td>
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<tr>
<td>Saturday, April 18</td>
<td>7:30am - 5:00pm</td>
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## EXHIBIT AND POSTER HOURS

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<tr>
<th>Day</th>
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<tr>
<td>Wednesday, April 15</td>
<td>1:00pm - 4:30pm</td>
<td>(Set Up Only) Tennessee Lobby A</td>
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<tr>
<td>Wednesday, April 15</td>
<td>4:00pm - 7:00pm</td>
<td>(Set Up Only) Tennessee BR D/E</td>
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<tr>
<td>Thursday, April 16</td>
<td>8:30am - 4:00pm</td>
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NORTH AMERICAN VETERINARY DERMATOLOGY FORUM  
Nashville, TN  
Wednesday, April 15, 2015

8:00 am - 4:30 pm  **ACVD Residents' Education Forum**  
*Sponsored by Zoetis*  
Cheekwood G/H

- 8:00 am - 8:30 am  **BREAKFAST**

- 8:30 am - 10:00 am  **Hair Follicle – Dr. Linda Frank**

- 10:00 am - 10:30 am  **BREAK**

- 10:30 am - 12:00 pm  **Basement Membrane Zone – Dr. Petra Bizikova**

- 12:00 pm - 1:00 pm  **LUNCH**

- 1:00 pm - 2:30 pm  **Fleas – Dr. Michael Dryden**

- 2:30 pm - 3:00 pm  **BREAK**

- 3:00 pm - 4:30 pm  **Cutaneous Manifestations/ Systemic Disease – Dr. Catherine Outerbridge**

8:00 am - 5:00 pm  **ACVD Exam Committee/ AOK Committee Meeting**  
Belmont A

9:00 am - 12:00 pm  **NAVDF Organizing Committee Meeting**  
Belmont C

10:15 am - 10:45 am  **BREAK - For Board Meetings**

11:45 am - 12:45 pm  **LUNCH - For Board Meetings**

12:00 pm - 5:00 pm  **ACVD Executive Board Meeting**  
Belmont B

12:00 pm - 5:00 pm  **AAVD Executive Board Meeting**  
Belmont C

1:00 pm - 4:30 pm  **Exhibitor/Poster/ Cyber Café Setup**  
Tennessee Lobby A

3:00 pm - 3:30 pm  **BREAK - For Board Meetings**

5:00 pm - 7:00 pm  **Registration**  
Tennessee Lobby A

5:00 pm - 7:00 pm  **Welcome Reception**  
*Sponsored by Hill’s Pet Nutrition*  
Tennessee Ballroom C

6:00 pm - 8:00 pm  **NAVDF Program Committee Meeting**  
Belmont C

7:00 pm - 9:00 pm  **Resident Presentation Run-throughs**  
Cheekwood G/H

4:00 pm - 7:00 pm  **Exhibitor Setup**  
Tennessee Ballroom D/E
6:00 am - 8:00 pm **Cyber Café**
*Sponsored by Veterinary Information Network*

6:30 am - 8:00 pm **Registration**

7:30 am - 8:45 am **Roundtables**

1. Apoquel – 1 Year Later
   L. McKay
   Belmont A

2. Topical Therapy
   M. Innera
   Belmont B

3. Flea Control: How, What, and When to Choose
   L. Gray
   Belmont C

4. Help: This Pemphigus Foliaceous Won’t Go Into Remission
   J. Seltzer
   Cheekwood B

5. Let’s Talk Claw’s!
   B. Scott
   Cheekwood C

6. Feline Otic Inflammatory Polyps: From Recognition to Resolution
   K. Helton-Rhodes
   Cheekwood F

7. Managing Problems in Feline Derm
   A. Pereira
   Cheekwood G

8. Supplemental Oils (Fish, Flax, Coconut, etc.): A Slippery Solution for Dermatologic Disease
   B. Palmeiro
   Cheekwood H

8:30 am - 4:00 pm **Exhibits & Posters**

9:00 am - 10:30 am **Scientific Session**

1. The Fast Track to Multidrug Resistance
   Dr. Shelley Rankin
   *Sponsored by Elanco*

2. Dead Bug’s Don’t Mutate
   Dr. Shelley Rankin
   *Sponsored by Elanco*
**Thursday, April 16, 2015, continued**

**Concurrent Session**  
President Ballroom A

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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</table>
| 9:00 am - 9:45 am | Biology of The Cat Flea: What’s New in 2015?  
Dr. Michael W. Dryden |
| 9:45 am - 10:30 am | Understanding Speed Of Kill And Flea Adulticides  
Dr. Michael W. Dryden |

**Abstract Session**  
President Ballroom B

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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>9:00 am - 10:30 am</td>
<td>ACVD Residents’ Short Communications</td>
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**Scientific Session**  
Tennessee Ballroom C

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<tr>
<th>Time</th>
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</table>
| 11:00 am - 12:30 pm | Biofilms, Wounds, And Chronic Infections  
Dr. Daniel Wozniak  
_Sponsored by Elanco_ |

**Concurrent Session**  
President Ballroom A

<table>
<thead>
<tr>
<th>Time</th>
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| 11:00 am - 11:45 am | Ticks – Understanding Today, Control For Tomorrow  
Dr. Michael W. Dryden  
_Sponsored by Zoetis_ |
| 11:45 am - 12:30 pm | Tick Borne Diseases in North America: Clinical and Zoonotic Implications  
Dr. Edward B. Breitschwerdt  
_Sponsored by Zoetis_ |

**Abstract Session**  
President Ballroom B

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<tr>
<td>12:30 pm - 2:00 pm</td>
<td>LUNCH On Your Own</td>
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</table>
| 12:30 pm - 2:00 pm | **ACVD Residency Mentors Meeting**  
_Sponsored by Elanco_ |

**Scientific Session**  
Tennessee Ballroom C

<table>
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<tr>
<th>Time</th>
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| 2:00 pm - 2:45 pm | AMP’s Structure & Function  
Dr. Domenico Santoro  
_Sponsored by Elanco_ |
| 2:45 pm - 3:30 pm | AMP’s in Allergy and Infection and Future Potential in Dermatology  
Dr. Domenico Santoro  
_Sponsored by Elanco_ |

**Concurrent Session**  
President Ballroom A

<table>
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<th>Time</th>
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| 2:00 pm - 2:45 pm | Bartonellosis: A One Health Approach to an Emerging Infectious Disease  
Dr. Edward B. Breitschwerdt |
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<tr>
<th>Time</th>
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<th>Location</th>
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</thead>
</table>
| 2:45 pm - 3:30 pm | Papillomavirus Associated Diseases in Horses  
Dr. Christian E. Lange | **Abstract Session**  
Presidential Ballroom B |
| 2:00 pm - 3:15 pm | ACVD Residents’ Short Communications |                              |
| 3:30 pm - 4:00 pm | BREAK/ VISIT EXHIBITS & POSTERS |                              |
| 4:00 pm - 5:00 pm | Salamanders, Skin, And Stem Cells  
Dr. Andrew M. Hoffman  
*Sponsored by Elanco* | **Scientific Session**  
Tennessee Ballroom C |
| 4:00 pm - 5:00 pm | Viruses In Dermatology, A Human – Animal Review  
Dr. Christian E. Lange | **Concurrent Session**  
Presidential Ballroom A |
| 4:00 pm - 5:00 pm | Original Short Communications |                              |
| 5:15 pm - 6:45 pm | **ACVD Diplomates’ Business Meeting** | **Tennessee Ballroom C** |
| 6:00 pm | **ACVD Residents’ Dinner**  
*Sponsored by Dechra Veterinary Products*  
Stop by the Dechra booth for more information | **Honky Tonk Central**  
329 Broadway |
| 6:45 pm | **ACVD Diplomates’ Dinner**  
*Sponsored by Bayer HealthCare LLC Animal Health*  
Boarding Buses at the Presidential Portico | **Country Music Hall of Fame** |
**NORTH AMERICAN VETERINARY DERMATOLOGY FORUM**
Nashville, TN
**Friday, April 17, 2015**

6:00 am - 8:00 pm  **Cyber Café**
*Sponsored by Veterinary Information Network*  
Tennessee Lobby A

7:00 am - 8:30 am  Roundtable Breakfast Buffet (coupon required)  
Magnolia Mezzanine

7:30 am - 5:00 pm  **Registration**  
Tennessee Lobby A

7:30 am - 8:45 am  **Roundtables**

#9  Lesion and Pruritus Severit Scoring for Atopic Dermatitis Patient Management  
J. Plant  
Belmont A

#10  Modifying Allergen Specific Immunotherapy  
C. Kidney  
Belmont B

#11  Shared Perspectives on Oral Immunotherapy  
T. Strauss  
Belmont C

#12  Breaking Through Our Barrier to Epidermal Barrier Function – Practical Applications for Our Patients  
M. Clark  
Cheekwood B

#13  Topical Antibacterial Therapy  
R. Miller  
Cheekwood A

#14  Dermatopathology: It May Be Microscopic, But It’s A Big Deal?  
B. Craft  
Cheekwood F

#15  Microbial Allergies, Dander Allergies  
V. Fadok  
Cheekwood G

#16  BAER Testing  
L. Cole  
Cheekwood H

#17  Technicians Roundtable: Video Otoscopy Procedures  
M. Carlucci  
*Sponsored by Greer*  
Cheekwood C

8:30 am - 4:00 pm  **Exhibits & Posters**  
Tennessee Lobby A &  
Tennessee Ballroom D/E

**Scientific Session**

9:00 am - 9:45 am  Review of Nutrition and Its Role in Skin Barrier Function  
Dr. Cecilia Villaverde  
*Sponsored by Greer*  
Tennessee Ballroom C

9:45 am - 10:30 am  Guide to Home Cooked Diets for Elimination Diet Trials, Pros, Cons and What Mistakes To Avoid  
Dr. Cecilia Villaverde  
*Sponsored by Greer*
Concurrent Session  
Presidential Ballroom A

9:00 am - 9:45 am  Feline Sporotrichosis: Numbers Don’t Lie  
Dr. Alessandra Vieira Pereira
9:45 am - 10:30 am  Cat Chance: Zooming In On Sporo Treatment  
Dr. Alessandra Vieira Pereira

Abstract Session  
Presidential Ballroom B

9:00 am - 9:15 am  ACVD Resident Research Awards  
Sponsored by Bayer HealthCare LLC Animal Health  
ACVD Externship Grants  
Sponsored by Hill’s Pet Nutrition
9:15 am - 10:30 am  Research Short Communications
10:30 am - 11:00 am  BREAK/ VISIT EXHIBITS & POSTERS

Scientific Session  
Tennessee Ballroom C

11:00 am - 11:45 am  Anesthesia For Dermatologists  
Dr. Piedad Natalia Henao Guerrero
11:45 am - 12:30 pm  Anesthesia For Dermatology - Analgesic Options that Dermatologist Could Benefit From  
Dr. Piedad Natalia Henao Guerrero

Concurrent Session  
Presidential Ballroom A

11:00 am - 11:45 am  Unraveling the Mysteries of Hypothyroidism: From Diagnosis to Management  
Dr. Johanna Cooper
11:45 am - 12:30 pm  Immunosuppressive Therapy from an Internist’s Perspective  
Dr. Johanna Cooper

Abstract Session  
Presidential Ballroom B

11:00 am - 12:30 pm  Research Short Communications
12:00 pm - 2:00 pm  ACVD Ethics Committee Meeting  
Belmont A
12:30 pm - 2:00 pm  LUNCH On Your Own
12:30 pm - 2:00 pm  ACVD Residents Lunch Meeting  
Presidential Chamber B
12:30 pm - 2:00 pm  GVDEG Meeting  
Cheekwood F
12:45 pm - 1:45 pm  ACVD Website Committee  
Cheekwood E
Friday, April 17, 2015, continued

**Scientific Session**  
2:00 pm - 2:45 pm  
Mechanisms of Autoimmunity  
Dr. Petra Bizikova

2:45 pm - 3:30 pm  
Update on Selected Autoimmune and Immune-Mediated Skin Diseases  
Dr. Petra Bizikova

**Concurrent Session**  
Presidential Ballroom A

2:00 pm - 2:45 pm  
Creepy Crawlers – Superficial Mites In Veterinary Dermatology  
Dr. Ralf Mueller  
*Sponsored by Greer*

2:45 pm - 3:30 pm  
Demodicosis – What is New?  
Dr. Ralf Mueller  
*Sponsored by Greer*

**Abstract Session**  
Presidential Ballroom B

2:00 pm - 3:30 pm  
Research Short Communications

3:30 pm - 4:00 pm  
BREAK/ VISIT EXHIBITS & POSTERS

**Scientific Session**  
Tennessee Ballroom C

4:00 pm - 5:00 pm  
Feline Immune-Mediated Skin Diseases  
Dr. Petra Bizikova

**Concurrent Session**  
Presidential Ballroom A

4:00 pm - 5:00 pm  
Getting Started in Clinical Research: Issues and Resources  
Dr. Douglas J. DeBoer

**Abstract Session**  
Presidential Ballroom B

4:00 pm - 5:00 pm  
Clinical Short Communications

6:00 pm  
Reception  
*Sponsored by Royal Canin Veterinary Diet*  
Country Music Hall of Fame
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<thead>
<tr>
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<tr>
<td>6:00 am</td>
<td><strong>Cyber Café</strong></td>
<td>Tennessee Lobby A</td>
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<tr>
<td>7:00 am</td>
<td>Roundtable Breakfast Buffet (coupon required)</td>
<td>Magnolia Mezzanine</td>
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<td>7:30 am</td>
<td><strong>Registration</strong></td>
<td>Tennessee Lobby A</td>
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<td>7:30 am</td>
<td><strong>Technicians’ Roundtable</strong></td>
<td>Cheekwood C</td>
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<td>7:30 am</td>
<td><strong>ACVD Residents’ Roundtable</strong></td>
<td>Cheekwood A/B</td>
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<td>7:30 am</td>
<td><strong>ICADA Meeting</strong></td>
<td>Belmont A</td>
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<td>8:30 am</td>
<td><strong>Exhibits/ Posters</strong></td>
<td>Tennessee Lobby A &amp;</td>
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<td>Tennessee Ballroom D/E</td>
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<td>9:00 am</td>
<td><strong>Scientific Session</strong></td>
<td>Tennessee Ballroom C</td>
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<td>9:00 am</td>
<td>Itch Symposia</td>
<td>Presidential Ballroom A</td>
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<td></td>
<td>Pruritus Pathways – Vet Perspective</td>
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<td>Dr. Candace A. Sousa</td>
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<tr>
<td>9:45 am</td>
<td>Itch Symposia</td>
<td>Presidential Ballroom A</td>
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<td>Pruritus – Human Perspective</td>
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<td>Dr. Matthew J. Zirwas</td>
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<tr>
<td>9:00 am</td>
<td><strong>Concurrent Session</strong></td>
<td>Presidential Ballroom B</td>
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<tr>
<td>9:00 am</td>
<td>Otitis: Treating Tough Cases</td>
<td>Presidential Ballroom B</td>
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<td></td>
<td>Dr. Rod Rosychuck</td>
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<td>Dr. Craig E. Griffin</td>
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<tr>
<td>9:45 am</td>
<td>Otitis – Chronic Cases</td>
<td>Presidential Ballroom B</td>
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<td>Dr. Rod Rosychuck</td>
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<td>Dr. Craig E. Griffin</td>
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<td>9:00 am</td>
<td><strong>Technician Session</strong></td>
<td>Presidential Ballroom B</td>
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<td>10:30 am</td>
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**Saturday, April 18, 2015 - continued**

**Scientific Session**

Tennessee Ballroom C

11:00 am - 11:45 am  Itch Symposia  
*The Itchy Miserable Patient: Pruritus*  
Dr. Matthew J. Zirwas

11:45 am - 12:30 pm  Itch Symposia  
*Quality Of Life In Veterinary Dermatology And The Impact Of Pruritus And Allergy On It*  
Dr. Chiara Noli  
*Sponsored by Greer*

**Concurrent Session**

Presidential Ballroom A

11:00 am - 11:45 am  Clinical Immunology For Clinical Dermatologists  
Dr. Cherie Pucheu-Haston

11:45 am - 12:30 pm  Vasculitis And Other Ischemic Dermatopathies  
Dr. Cherie Pucheu-Haston

**Technical Session**

Presidential Ballroom B

11:00 am - 12:30 pm  TBD

12:30 pm - 2:00 pm  LUNCH On Your Own

12:30 pm - 2:00 pm  **AAVD Business Meeting – Lunch**

**Scientific Session – Lunch**

Tennessee Ballroom C

2:00 pm - 2:45 pm  Immunohistochemistry – The Art of Cellular Interrogation Part I  
Dr. Jennifer Ward

2:45 pm - 3:30 pm  Immunohistochemistry – The Art of Cellular Interrogation Part II  
Dr. Jennifer Ward

**Concurrent Session**

Presidential Ballroom A

2:00 pm - 2:45 pm  Cryosurgery Part I: The Fundamentals  
Dr. Michael Canfield

2:45 pm - 3:30 pm  Cryosurgery Part II: The Clinical Applications & Case Reviews  
Dr. Michael Canfield

**Abstract Session**

Presidential Ballroom B

2:00 pm - 3:30 pm  Clinical Short Communications

3:30 pm - 4:00 pm  BREAK/ VISIT EXHIBITS & POSTERS
**Saturday, April 18, 2015 - continued**

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</table>
| 4:00 pm - 5:00 pm | **Scientific Session**  
It’s Not Just Skin Deep: The Relationship Of Cutaneous And Systemic Hypersensitivity Disorders  
Dr. Cherie Pucheu-Haston | Tennessee Ballroom C               |
| 4:00 pm - 5:00 pm | **Concurrent Session**  
MRSA And The Environment: Do I Need To Wash My Hands?  
Dr. Armando E. Hoet | Presidential Ballroom A           |
| 4:00 pm - 5:00 pm | **Abstract Session**  
Clinical Short Communications | Presidential Ballroom B          |
| TBD        | **Reception**  
*Sponsored by Novartis Animal Health US, Inc.* | TBD                           |
# North American Veterinary Dermatology Forum

## Nashville, Tennessee

### Roundtable Sessions 2015

#### Thursday, April 16, 2015

<table>
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<tr>
<th>#</th>
<th>Title</th>
<th>Speaker</th>
<th>Room</th>
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<tbody>
<tr>
<td>#1</td>
<td>Apoquel – 1 Year Later</td>
<td>L. McKay</td>
<td>Belmont A</td>
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<td>K. Helton-Rhodes</td>
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<td>Managing Problems in Feline Derm</td>
<td>A. Pereira</td>
<td>Cheekwood G</td>
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<td>Supplemental Oils (Fish, Flax, Coconut, etc.): A Slippery Solution for Dermatologic Disease</td>
<td>B. Palmeiro</td>
<td>Cheekwood H</td>
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#### Friday, April 17, 2015

| #9  | Lesion and Pruritus Severit Scoring for Atopic Dermatitis Patient Management | J. Plant | Belmont A  |
| #10 | Modifying Allergen Specific Immunotherapy                             | C. Kidney   | Belmont B  |
| #11 | Shared Perspectives on Oral Immunotherapy                            | T. Strauss  | Belmont C  |
| #12 | Breaking Through Our Barrier to Epidermal Barrier Function – Practical Applications for Our Patients | M. Clark | Cheekwood B |
| #13 | Topical Antibacterial Therapy                                        | R. Miller   | Cheekwood A|
| #14 | Dermatopathology: It May Be Microscopic, But It’s A Big Deal?         | B. Craft    | Cheekwood F|
| #15 | Microbial Allergies, Dander Allergies                                | V. Fadok    | Cheekwood G|
| #16 | BAER Testing                                                        | L. Cole     | Cheekwood H|
| #17 | Technicians Roundtable Video Otoscopy Procedures                    | M. Carlucci | Cheekwood C|

*Sponsored by Greer*
Saturday, April 18, 2015

#18  Technicians' Roundtable  
    Sub-Lingual Immunotherapy  
    G. Giguere  
    Sponsored by Greer  
    Cheekwood C

#19  ACVD Residents’ Roundtable  
    Sponsored by CEVA  
    Cheekwood A/B
ABSTRACT PRESENTATIONS

Thursday, April 16

RESIDENTS
9:00 .................................................. Gentry
9:15 .................................................. Bachtel
9:30 .................................................. Falk
9:45 .................................................. Stetina
10:00 .................................................. Goodale
10:30 – 11:00 - BREAK
11:00 .................................................. Tunhikorn
11:15 .................................................. Hnot
11:30 .................................................. Hnot
11:45 .................................................. Gimmmer
12:00 ............................................. Bernardi de Souza
12:15 .................................................. Klinger
12:30 – 2:00 - LUNCH

RESIDENTS
2:00 .................................................. Clear
2:15 .................................................. Hutt
2:30 .................................................. Pieper
2:45 .................................................. Contreary
3:00 .................................................. Layne
3:15 NO PRESENTATION
3:30 – 4:00 - BREAK

ORIGINAL SHORT COMMUNICATIONS
4:00 .................................................. Palmeiro
4:15 .................................................. Mueller
4:30 .................................................. Dunham
4:45 .................................................. Walters

Friday, April 17

RESEARCH SHORT COMMUNICATIONS
9:00 RESIDENT RESEARCH AWARD
9:15 .................................................. Rodrigues-Hoffmann
9:30 .................................................. Smith
9:45 .................................................. Smith
10:00 .................................................. Zewe
10:15 .................................................. Frank
10:30 – 11:00 - BREAK

Saturday, April 18

ACVD CLINICAL SHORT COMMUNICATIONS
2:00 .................................................. DeBoer
2:15 .................................................. Banovic
2:30 .................................................. Pendergraft
2:45 .................................................. Lee
3:00 .................................................. Pereira
3:15 .................................................. Bizikova
3:30-4:00 - BREAK

ACVD CLINICAL SHORT COMMUNICATIONS
4:00 .................................................. Lam
4:15 .................................................. Cain
4:30 .................................................. Noli
4:45 .................................................. Michels

POSTERS
Berger
Falk
Loft
Loft
Martin-Vo
Paulo
Paulo
Plant
Possebom
Udenberg
THURSDAY
RESIDENTS’ SHORT COMMUNICATIONS
AND
ORIGINAL SHORT COMMUNICATIONS
THURSDAY
Comparison of intradermal and percutaneous testing to histamine, saline, and nine allergens in healthy adult cats

Stability and pharmacokinetics of Atopica® capsules stored at -20°C

A retrospective study of the clinical characteristics of doxorubicin-induced alopecia in 28 canine patients

Owner perception of pruritic behaviors in apparently healthy dogs: a survey based approach

Owner perception of the type and frequency of gastrointestinal signs in apparently healthy dogs: a survey based approach

Aspergillus spp. otitis in small animals – a retrospective study of 17 cases

The significance of the numbers of dermal mast cells in the evaluation of skin biopsy specimens from cats with inflammatory dermatoses

Comparison of minocycline and doxycycline susceptibilities of meticillin-resistant Staphylococcus pseudintermedius isolates using current and revised breakpoints

Effect of food on the pharmacokinetics of minocycline in healthy research dogs
11:45  Gimmler  Determining canine skin concentrations of terbinafine for the treatment of *Malassezia* dermatitis

12:00  Bernardi de Souza  Identification of 5α-reductase isoenzymes in canine skin

12:15  Klinger  A placebo-controlled, double-blinded, randomized study evaluating colecalciferol (vitamin D) and paricalcitol in the treatment of canine atopic dermatitis

12:30 – 2:00  LUNCH

2:00  Clear  Investigation of the effects of 30 day administration of oclacitinib (Apoquel®) on intradermal and allergen-specific IgE serology testing in atopic dogs

2:15  Hutt  A survey of the histopathological features of skin from the planum nasale and adjacent skin of dogs unaffected by dermatological or respiratory disease

2:30  Pieper  Coordinate expression of cytokeratins 7 and 14, vimentin, and bcl-2 in canine cutaneous neoplasms

2:45  Contreary  Sterile nodular panniculitis: a retrospective study of 39 dogs

3:00  Layne  Serum *Malassezia*-specific IgE in dogs with recurrent *Malassezia* otitis externa without concurrent skin disease

3:15  NO PRESENTATION

3:30 – 4:00  BREAK
THURSDAY, APRIL 16, 2015

ORIGINAL SHORT COMMUNICATIONS

4:00  Palmeiro  A prospective, randomized, double-blinded, placebo-controlled trial evaluating the effects of a natural triglyceride omega-3 supplement on atopic dermatitis and erythrocyte membrane fatty acid concentrations in dogs

4:15  Mueller  Evaluation of cyclosporine-sparing effects of polyunsaturated fatty acids in the treatment of canine atopic dermatitis

4:30  Dunham  Identification and characterization of ZTS-00103289, a monoclonal antibody neutralizing IL-31-mediated pruritus, in beagle dogs

4:45  Walters  Laboratory dose titration efficacy study of ZTS-00103289, a caninized anti-IL-31 monoclonal antibody, in a canine model of IL-31-induced pruritus
Comparison of intradermal and percutaneous testing to histamine, saline, and nine allergens in healthy adult cats

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Abstract: Intradermal testing with aqueous allergens (IDT) in cats has potential limitations of rapidly fading or weak reactions and has historically been frustrating. A pilot study demonstrated that normal feline patients produce percutaneous histamine wheals statistically equal in size to intradermal wheals using the GREER® Pick® system (GREER®, Lenoir, NC) without reactions to glycerinated saline. The aim of this study was to determine if percutaneously applied allergens would create positive (irritant) reactions in healthy cats. Percutaneous testing (PT) with both glycerinated and aqueous allergens and IDT were compared in triplicate in twelve healthy adult cats. While sedated the left lateral thorax of each cat was clipped and histamine, saline, and nine commonly positive allergens were tested in randomly assigned rows. Results were blindly evaluated both objectively and subjectively at 15, 20, 25 minutes and four hours after testing. Intradermal histamine was significantly larger both subjectively and objectively compared to percutaneous glycerinated (PG) and percutaneous aqueous (PA) allergens at all time points (p<0.001) except at four hours when PG histamine reactions were larger (p <0.001). Both percutaneous methods had significantly smaller saline wheals when compared to intradermal allergy testing (p <0.001). Significantly larger subjective and objective wheal formation was noted for: Dermatophagoides farinae, canine dander, blue grass, kochia, Penicillium, and mosquito via IDT (p <0.001). Overall, PG reactions were significantly larger than PA reactions. Tested PG and PA allergens did not cause irritant reactions at tested concentrations. Kochia, when tested at 1000 PNU/ml via IDT, is suspected to be an irritant.

This study was partially funded by GREER®

Conflict of interest: None declared
Stability and pharmacokinetics of Atopica® capsules stored at -20°C

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Abstract: It has been suggested that placement of ciclosporin (Atopica®: Novartis Animal Health, Greensboro, NC) capsules in a household freezer (approximately -20°C) prior to oral administration reduces the incidence of vomiting in dogs. Although storing Atopica® capsules in a freezer is commonplace, the impact on ciclosporin stability and pharmacokinetics was previously unknown. Ciclosporin concentrations of all available Atopica® capsule strengths (10mg, 25mg, 50mg, and 100mg) were assessed after -20°C storage at five time points (1 hour, 1 day, 7 days, 15 days, and 30 days) and at package insert recommendations (15-25°C). A blinded, randomized cross-over study was also performed to compare blood concentrations of ciclosporin dosed in eight healthy beagle dogs (4.9-5.3 mg/kg per os) after Atopica® storage for 28 days at -20°C versus storage at 15-25°C with a 7 day washout period. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 10 and 24h. Both capsule and blood ciclosporin concentrations were assessed via HPLC-MS/MS. There was no significant difference between ciclosporin concentrations of Atopica® capsules stored at -20°C and those stored at the recommended temperature range (p=0.80). Similarly, in the crossover study, there were no significant differences in pharmacokinetic parameters assessed: area under the curve (p=0.9273), half-life (p=0.71), Cmax (p=0.66), Tmax (p=0.41). Thus, Atopica® capsules can be administered to dogs after storage at -20°C for approximately one month with no significant impact on drug stability or pharmacokinetics.

This study was funded by the Colorado State University College Research Council Foundation and the Center for Companion Animal Studies.

Conflict of interest: None declared
A retrospective study of the clinical characteristics of doxorubicin-induced alopecia in 28 canine patients

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Abstract: Alopecia is a common side effect of chemotherapy in humans, with significant research devoted to understanding the clinical characteristics and mechanism of hair loss. In dogs, chemotherapy-induced alopecia is uncommonly reported, with few accounts on its epidemiology or mechanism of development. The objective of this retrospective study was to describe the epidemiological and clinical characteristics of doxorubicin-induced alopecia (DIA) in canine patients at Tufts University from 2012-2014. Signalment, diagnosis, and treatment protocols were recorded in 150 Oncology patients treated with doxorubicin during this timeframe. Medical records were searched for the keywords “alopecia” and “hypotrichosis.” Dogs were excluded if causal link of hair loss was unclear. DIA was reported in twenty-eight of 150 patients (19%). Two parameters were statistically associated with the development of DIA: coat type and cumulative doxorubicin dose. Dogs that developed DIA received a significantly higher cumulative doxorubicin dose (109.7 vs. 80.4 mg/m², \( p=0.004 \)) than those that did not develop DIA. They were also significantly more likely to have a curly or wire coat type compared to those without DIA (42.9 vs. 5.0%, \( p <0.0001 \)). There was no significant difference in age or sex between dogs that did and did not develop DIA. The odds of developing DIA increased 1.02 times per unit increase in cumulative doxorubicin dose after adjusting for age, sex, weight, and coat type (\( p=0.001 \)). Patterns of DIA recorded included: generalized alopecia (16/28, 57%), facial alopecia (5/28, 18%), truncal alopecia (6/28, 21%), with 3/28 (11%) also suffering whisker loss and 9/28 (33%) developing hyperpigmentation.

Sources of funding: This study was supported by the Companion Animal Health Fund at Tufts Cummings School of Veterinary Medicine

Conflict of Interest: None declared
Owner perception of pruritic behaviors in apparently healthy dogs: a survey based approach

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Abstract: To accurately assess pruritus, it is important to understand normal behaviors that may be interpreted as pruritic. The purpose of this study was to evaluate owners’ perceptions of pruritus in their apparently healthy dogs and determine what factors correlate with these signs. Dogs included had an unremarkable physical exam, no history/current complaint of pruritus, otitis, skin/hair or metabolic/systemic disease, no vomiting and diarrhea for six months, and no medications except flea and heartworm preventative. Thirty-three veterinarians (generalists/specialists) from four countries enrolled 314 dogs (age 12-180 months, mean 57.73 months, median 50.50 months). A pruritus visual analog score (PVAS) was obtained before owners took an online survey. The mean and median PVAS was 0.56/10 and 0/10, respectively (standard deviation 0.98; standard error of the mean 0.06). There were 275 dogs (87.6%) with a PVAS ≤ 1.9. PVAS was negatively correlated with the number of bowel movements/day (p=0.0080, p<0.05 significant), and positively correlated with paw licking/chewing (p<0.0001), facial/muzzle rubbing (p<0.0001), head shaking (p<0.0001), and sneezing (p=0.0177). Licking/chewing paws (p=0.0087), facial/muzzle rubbing (p=0.0201), head shaking (p=0.0255), and sneezing (p=0.0101) were higher in dogs that received treats. Age was positively correlated with facial/muzzle rubbing (p=0.0397) and sneezing (p=0.0189). Number of walks/day was positively correlated with paw licking/chewing (p=0.0254), head shaking (p=0.0036), and sneezing (p=0.0019). Licking/chewing the paws (p=0.0019) was less frequent in dogs receiving probiotics (21/314, 6.7%). Sneezing was significantly higher in dogs that scooted (p=0.0463). The 95% prediction interval for PVAS of apparently healthy dogs fell between 0 and 3.4.

Source of funding: Purina®.

Conflicts of interest: None declared.
Owner perception of the type and frequency of gastrointestinal signs in apparently healthy dogs: a survey based approach

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Abstract: Abnormal gastrointestinal (GI) signs have been linked to several GI and dermatologic disorders in dogs, however established frequencies for GI signs in healthy dogs are inconsistent. The purpose of this study was to evaluate owners’ perceptions of GI signs in their apparently healthy dogs and determine what factors affected these signs. Dogs included were >12 months of age, had an unremarkable physical exam, no history or current complaint of pruritus, otitis, skin/hair or metabolic/systemic disease, no vomiting/diarrhea for six months, and no medications except flea and heartworm preventative. Thirty-three veterinarians (generalists/specialists) from four countries enrolled 314 dogs (age 12-180 months, mean 57.73, median 50.50). A pruritus visual analog score (PVAS) and fecal consistency score (FCS) were obtained and then owners took an online survey. The mean PVAS was 0.56/10. GI signs documented in ≥94.9% of dogs included: ≤3 bowel movements (BM)/day; FCS of 2 or 3/7; daily flatulence; belching and borborygmi ≤2-3/week; coprophagia ≤2-3/month; mucus in the stool, vomiting, regurgitation, black tarry stools, abdominal pain, and tenesmus ≤2-3/yr; and no hematochezia. BM/day were higher on raw diets (p=0.0406, p<0.05 significant) and positively correlated with number of walks/day (p=0.0094). Age was positively correlated with coprophagia (p=0.0458) and borborygmi (p=0.03290). Flatulence was higher in dogs that received treats (p=0.0021) and was less frequent with raw or mixed raw diets (p=0.0406). Chihuahuas had a higher frequency of coprophagia (p<0.01). These values are guidelines to aid in obtaining a GI history and to help veterinarians recognize when further investigation is warranted.

Source of funding: Purina®.

Conflicts of interest: None declared.
Aspergillus spp. otitis in small animals – a retrospective study of 17 cases

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Abstract: Aspergillus spp. are saprophytic opportunistic fungal organisms and are a common cause of otomycosis in humans. Although there have been case reports of Aspergillus otitis externa in dogs, to the authors’ knowledge, this is the first retrospective case series describing Aspergillus otitis in dogs and cats. The study objectives were to characterize signalment, predisposing causes, treatments and outcomes of dogs and cats with Aspergillus otitis. A retrospective review of medical records from 1989-2014 identified eight dogs and nine cats diagnosed with Aspergillus otitis based on culture. All dogs weighed greater than 23 kg. The most common predisposing causes identified were concurrent disease or therapy causing immunosuppression (3/8 dogs, 3/9 cats), historical otic foreign bodies (3/8 dogs) and previous fluoroquinolone usage (topical or systemic) (3/8 dogs, 7/9 cats). Aspergillus otitis was unilateral in all dogs and usually unilateral in cats; two cats had bilateral disease. Concurrent otitis media was identified in three dogs and one cat and suspected in two cats. Aspergillus fumigatus was the most common isolate and was more common in cats (1/8 dogs, 5/9 cats). Aspergillus niger (3/8 dogs) and Aspergillus terreus (3/8 dogs) were most commonly cultured from dogs. Animals received various topical and systemic antifungal medications however otic lavages under anesthesia and/or surgical intervention increased the likelihood of resolution. Aspergillus otitis is uncommon, typically seen as unilateral otitis externa in cats and larger breed dogs and may have an association with immunosuppression, otic foreign bodies and previous fluoroquinolone usage.

Source of funding: None.

Conflict of interest: None declared.
The significance of the numbers of dermal mast cells in the evaluation of skin biopsy specimens from cats with inflammatory dermatoses

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Abstract: The purposes of this retrospective study were to enumerate dermal mast cells (MCs) in normal skin and inflammatory dermatoses of the cat, and to compare the numbers of MCs in allergic dermatoses to those in nonallergic dermatoses. In addition, the number of dermal MCs in normal cat skin was compared between biopsy specimens stained with hematoxylin & eosin (H&E) and toluidine blue (TB). Skin-biopsy specimens from 371 cats with inflammatory dermatoses and 31 cats with normal skin were examined histologically for the presence of MCs in the superficial and deep dermis. For each specimen, MCs were counted in six 400X magnification microscopic fields— one from each end of the specimen and one in the center- in both the superficial and deep dermis. There was no significant difference between the numbers of MCs in the superficial dermis of normal skin compared to inflammatory dermatoses. However, there were significantly more MCs in the deep dermis of inflammatory dermatoses than in normal skin. There was no significant difference in MC numbers in the superficial and deep dermis in allergic versus nonallergic dermatoses. MCs were more numerous in the superficial versus the deep dermis in all three groups. Significantly more MCs were detected in the superficial dermis of normal skin when stained with TB than with H&E. The findings indicate that numbers of dermal MCs cannot be used to distinguish between allergic and nonallergic inflammatory dermatoses of the cat. TB stain detects more MCs in the superficial dermis of normal skin than does H&E.

Source of Funding: Self-funded.

Conflict of Interest: None declared.
Comparison of minocycline and doxycycline susceptibilities of meticillin-resistant *Staphylococcus pseudintermedius* isolates using current and revised breakpoints

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Abstract: Current published Clinical and Laboratory Standards Institute (CLSI) breakpoints to predict minocycline and doxycycline susceptibility of *Staphylococcus pseudintermedius* isolates are not appropriate because they do not meet pharmacokinetic and pharmacodynamic data using a standard dose. New breakpoints have been proposed for minocycline and have been approved for doxycycline. Revised breakpoints are four dilutions lower than current breakpoints, providing a more conservative standard for classification of isolates. Until new CLSI standards are published, many laboratories will continue to use the current tetracycline breakpoints. The objective of this study was to measure minimum inhibitory concentrations (MICs) of minocycline and doxycycline in 100 canine meticillin-resistant *S. pseudintermedius* clinical isolates and compare their susceptibilities to minocycline and doxycycline based on current and revised standards. E-test strips were used to determine MICs. Using the current tetracycline breakpoint of MIC < 4 μg/mL, 76 isolates were susceptible to minocycline and 36 isolates were susceptible to doxycycline, while using the proposed minocycline breakpoint (MIC < 0.25 μg/mL) and approved doxycycline breakpoint (MIC < 0.125 μg/mL), only 31 isolates were susceptible to both minocycline and doxycycline. Thus, use of the current published breakpoints misclassified 45 and 5 of the isolates as susceptible to minocycline and doxycycline, respectively. PCR analysis revealed that 43/45 isolates classified as susceptible to minocycline and 5/5 isolates classified susceptible to doxycycline possessed the tetracycline resistance gene, *tet*(M), known to confer resistance to both drugs. These results underscore the importance of utilizing the proposed minocycline and accepted doxycycline breakpoints in place of current tetracycline breakpoints.

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

Conflict of interest: None declared.
Effect of food on the pharmacokinetics of minocycline in healthy research dogs

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Abstract: The effect of food on minocycline oral absorption in dogs is unknown. The objective of this study was to determine the pharmacokinetics of minocycline after administration of a single oral dose in fed and fasted dogs. Ten research hounds were administered oral minocycline (approximately 5 mg/kg) with and without food, in a crossover study, with a one-week washout between treatments. Blood samples were collected immediately prior to minocycline administration and over 24 hours. Minocycline plasma drug concentrations were measured using high-performance liquid chromatography and were analyzed with compartmental modeling to determine primary pharmacokinetic parameters. Each dog was analyzed independently, followed by calculation of means and variation of the subjects. Wilcoxon signed-rank test (analyzing secondary pharmacokinetic parameters - maximum concentration [C_MAX], area under the concentration-versus-time curve [AUC]) was used to compare the two groups. No significant difference was found between treatments for AUC (P=0.0645), although AUC was consistently higher in nonfed dogs. A significant difference was found for C_MAX (P=0.0059), with nonfed dogs attaining a higher C_MAX. Next, a population pharmacokinetic modeling approach using nonlinear mixed effects modeling (NLME), for primary parameters for the population (volume of distribution, clearance, and microdistribution rates) included in the model as fixed effects and difference between subjects was included as a random effect. The source of variability in the population was determined using covariate analysis. Since feeding was a significant source of variation for the population’s primary pharmacokinetic parameters (P < 0.01) and fasted dogs had higher minocycline concentrations, we recommend administering minocycline without food.

Source of funding: Self-funded

Conflict of interest: None declared
Determining canine skin concentrations of terbinafine for the treatment of Malassezia dermatitis

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Abstract: Terbinafine, an allylamine antifungal, is known to concentrate and persist in human skin. To determine if the same is true in dogs, terbinafine (Liconda, Guadalajara, Spain) was given at 30 mg/kg per os once daily to ten dogs for 21 days. Samples of serum, stratum corneum, and sebum were collected from the thorax and paw on Days 1, 5, 7, 11, 14, 21, 28, and 35. High-performance liquid chromatography was used to determine drug concentrations. Four Malassezia pachydermatis isolates were collected from routinely submitted fungal cultures. Terbinafine minimum inhibitory concentration (MIC) was determined using a broth macrodilution assay. The highest terbinafine MIC of M. pachydermatis was 0.008 mcg/ml. Relevant (mean ± standard deviation) parameters for terbinafine in serum, paw stratum corneum, thorax stratum corneum, and sebum, respectively were: C_max (mcg/mL) 23.59±10.41, 0.31±0.26, 0.30±0.32, and 0.48±0.25; half-life (days) 4.49±2.24, 6.34±5.33, 4.64±3.27, and 5.12±3.33; area under the curve (Day 0-35, day*mcg/mL) 296.78±132.68, 2.59±1.50, 2.46±2.28, and 4.71±3.12; and ratio of Day 21 concentration to MIC (C_{21}:MIC) 1907.10±1248.40, 14.91±19.88, 12.98±7.03, and 18.51±7.66. Terbinafine was above MIC after discontinuation in 89, 67, 44, and 78% of dogs, respectively (after 7 days) and 67, 33, 33, and 44% (after 14 days). These results suggest that terbinafine does not concentrate highly in canine skin compared to serum and that time above MIC is variable after discontinuation. Nonetheless, concentrations were generally above the terbinafine MIC for M. pachydermatis in all samples during dosing, which could explain the efficacy seen when used for the treatment of Malassezia dermatitis.

Source of funding: This study was funded by the American College of Veterinary Dermatology Heska Resident Research Grant and by the American College of Veterinary Internal Medicine Veterinary Pharmacokinetic Research Fund.

Conflict of interest: None declared.
Identification of 5α-reductase isoenzymes in canine skin

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Abstract: Alopecia X in dogs is a noninflammatory alopecia that may be caused by a hormonal dysfunction, similar to androgenic alopecia in men, which is caused by higher levels of dihydrotestosterone (DHT). The 5α-reductase isoenzymes, 5αR1 and 5αR2, and a recently described 5αR3, are responsible for the conversion of testosterone into DHT. However, which 5α-reductases are identified in canine skin has not yet been described. The main objective of this study was to determine the pattern of expression of 5α-reductase genes in canine skin. Skin biopsies were obtained from healthy, intact, young, mature Beagles (3 males, 4 females) at three anatomic sites normally affected by alopecia X (dorsal neck, one caudal thigh and base of tail) and two sites generally unaffected (dorsal head and ventral thorax). Prostate samples (n=3) collected from the 3 males served as positive controls. 5α-reductases mRNA abundance measurement in both tissue types was performed by real-time PCR. In the skin, mRNA encoding 5αR1 and 5αR3 was detected but not 5αR2. There were no significant differences in 5αR1 and 5αR3 mRNA levels between the different anatomic regions, irrespective of gender (p>0.05). Moreover, the mean mRNA abundance in each anatomic region did not differ between males and females (p>0.05). This is the first study to demonstrate the expression of 5α-reductases, including 5αR3, in canine skin. These results may help to elucidate the pathogenesis of alopecia X, and to determine more appropriate treatments for this disorder.

Source of funding: This study was supported by research grants from the Companion animal health funds and the Centenary’s funds of the Faculty of Veterinary Medicine, University of Montreal.

Conflict of interest: None declared.
A placebo-controlled, double-blinded, randomized study evaluating colecalciferol (vitamin D) and paricalcitol in the treatment of canine atopic dermatitis

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Abstract: Canine atopic dermatitis is a common skin disease in small animal practice and is similar clinically and immunologically to its human counterpart. Previous studies evaluating colecalciferol as treatment for human atopic dermatitis showed promising results. In this placebo-controlled, double-blinded, randomized study, 31 dogs with atopic dermatitis received orally either colecalciferol (Vigantol, Merck KGaA, Darmstadt, Germany; 1,500 IU/kg daily, n = 13), the vitamin D receptor analogue paricalcitol (Zemplar, Abbott GmbH, Wiesbaden, Germany; 0.02 mcg/kg daily, n = 8) or placebo (n = 10) for eight weeks. Patients with seasonal signs were only treated during their flare season (if long enough), otherwise they were excluded. Other medications had to be administered unchanged for 12 weeks before and during the study. Blood samples for ionized calcium were obtained on days 0, 7, 14, 28, and 56. A validated canine atopic dermatitis extent and severity index (CADESI-03) and a validated pruritus score were determined on days 0, 28, and 56. After eight weeks of treatment, pruritus in the colecalciferol group was significantly decreased from 6.7 to 3.1 (P < 0.001) compared to 5.4 before and 6.2 after treatment with placebo and 6.3 before and 4.9 after treatment with paricalcitol. Pre- and posttreatment CADESI scores were 37 and 22 for colecalciferol, 43 and 37 for placebo and 33 and 29 for paricalcitol, respectively; these differences were not significant. Adverse effects were only seen in one normocalcemic case with mild polyuria and polydipsia for one day. Vitamin D may have an effect on canine atopic pruritus.

Source of funding: This study was funded by a research grant of the American College of Veterinary Dermatology. The drugs were partially provided by Merck KGaA.

Conflict of interest: None declared.
Investigation of the effects of 30 day administration of oclacitinib (Apoquel®) on intradermal and allergen-specific IgE serology testing in atopic dogs

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Abstract: The use of oclacitinib (Apoquel® Zoetis Inc, Kalamazoo MI, USA) prior to intradermal (IDT) or allergen-specific immunoglobulin E (IgE) serologic testing may help control the exacerbation of pruritus that can occur during the withdrawal of other common anti-pruritic medications known to interfere with testing. The effects of 30 day administration of oclacitinib on immediate phase IDT reactivity and IgE serology were evaluated in 22 dogs with confirmed atopic dermatitis (AD) and initial positive test reactions in a randomized, double-blinded, placebo-controlled study. Eleven dogs were treated with oclacitinib at a dose of 0.4-0.6 mg/kg orally every 12 hours for 14 days, then every 24 hours for 16 days. Eleven dogs were treated in the same fashion with oral placebo. IDT was performed at day 0 and day 30 using a panel of 56 aqueous allergens (Greer Laboratories, Lenoir NC, USA) appropriate to the geographical region. IDT reactivity was assessed both subjectively and objectively. Serum was collected for IgE serology (Heska-ALLERCEPT®, Loveland CO, USA) at day 0 and day 30. Data were evaluated using the D’Agostino & Pearson omnibus normality test and analyzed using multiple t-tests to compare means. Time trends were evaluated by testing for heterogeneity of regression. All analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla CA, USA). At day 30, oclacitinib did not have a statistically significant effect on IDT or IgE serology reactivity. It appears, therefore, that a 30 day withdrawal period from Apoquel® is not necessary prior to intradermal or serologic testing.

Funding was provided by a grant from the American College of Veterinary Dermatology.

Conflict of Interest: None declared
A survey of the histopathological features of skin from the planum nasale and adjacent skin of dogs unaffected by dermatological or respiratory disease

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Abstract: There is a general belief that cells of the immune system are present in larger numbers in the planum nasale and adjacent haired skin in the dog. However, little published information about the normal histopathological appearance of the skin of this area exists. Biopsies from three sites were obtained from the planum nasale and adjacent haired skin of 25 dogs of varying age, breed and sex, with no evidence of dermatological or respiratory disease. Biopsies were analysed to determine and quantify the immune system cells present in the samples. Slides were stained with haematoxylin and eosin, and toluidine blue; immunohistochemical stains for CD3 and CD79a were applied. There was no evidence of lichenoid inflammation. Immune system cells including lymphocytes and plasma cells were either very rare or present in low numbers. The majority of lymphocytes were of T-cell origin, with only infrequent B-cells identified. Biopsies contained scattered melanophages, consistent with pigmentary incontinence, regardless of the presence or absence of inflammatory cells. Mast cells were present in low numbers; within non-haired skin, superficial mast cells showed close association with the epidermis. In conclusion, immune system cells are not present in large numbers in this anatomical location in clinically normal dogs. Inflammatory change noted in biopsies from this area is therefore likely to be of pathological significance. However, pigmentary incontinence appears to be common at this site, in clinically normal dogs without significant inflammatory cell infiltration, and is therefore not necessarily of pathological significance when seen in isolation in this location.

Source of funding: This work was supported in part by a grant from the Dermatology Chapter of the Australian and New Zealand College of Veterinary Scientists.

Conflict of interest: None declared.
Coordinate expression of cytokeratins 7 and 14, vimentin, and bcl-2 in canine cutaneous neoplasms

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Abstract: This retrospective, pilot study was performed to use immunohistochemistry markers to characterize well-differentiated epithelial neoplasms so as to help in diagnosis of poorly differentiated neoplasms with specific immunohistochemical profiles. Canine cutaneous neoplasms were immunohistochemically stained for cytokeratin (CK) 7 and 14, vimentin, and bcl-2. CK7 was positive in anal sac carcinomas, all apocrine tumors and the apocrine cyst in the luminal cells, and the luminal cells of the ceruminous gland carcinomas. All neoplasms were positive for CK14 with the exception of adenocarcinoma of the apocrine glands of the anal sac. Myoepithelial cells were positive for vimentin in the apocrine cyst and all apocrine tumors. Hepatoid gland carcinoma, apocrine ductal adenoma/adenocarcinoma, ceruminous gland carcinoma, and some squamous cell carcinomas were positive for vimentin. Bcl-2 was positive in multiple tumors including anal sac carcinomas, apocrine cyst, apocrine adenocarcinoma, one sebaceous gland carcinoma, sebaceous epithelioma, infundibular keratinizing acanthoma, malignant trichoepithelioma, and the majority of the trichoepitheliomas and squamous cell carcinomas. Although additional studies are warranted, the immunohistochemical evaluation of coordinate expression of CK7, CK14, vimentin, and bcl-2 in canine cutaneous neoplasms using monoclonal antibodies may provide important information that can help to differentiate several neoplasms such as those arising from apocrine glands (including anal sac), hepatoid glands, and several follicular tumors.

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Conflict of interest: None declared
Sterile nodular panniculitis: a retrospective study of 39 dogs

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Abstract: A retrospective study assessing the presence or absence of concurrent systemic disease associated with canine sterile nodular panniculitis (SNP) and documenting breed predispositions was undertaken by a medical records search for dogs diagnosed with SNP at a veterinary teaching hospital, from 1990-2012. Thirty nine dogs met inclusion criteria with compatible skin biopsy findings with negative special stains for infectious organisms. Special stains (Fite’s, Ziehl Neelsen acid fast, Bacille Calmette Guerin for Mycobacterium spp, Gomori methenamine silver and periodic-acid Schiff) were performed in all dogs, some retrospectively, as not all dogs (9/39) had deep tissue cultures performed. Bacterial culture results were negative (24/30) or isolated rare numbers of Staphylococi (6/30). The inflammatory pattern and predominant inflammatory cell types within the panniculus, along with presence or absence of concurrent dermatitis were evaluated. There was no associated histologic pattern with clinical presentation or breed. The breed distribution was compared between dogs with SNP and all other dogs examined at the teaching hospital over the same time period using an exact chi-square test of homogeneity. Australian Shepherd dogs, Brittany Spaniels, Dalmatians, Pomeranians, and Chihuahuas were significantly overrepresented (P<0.05). Thirty two dogs (82.1%) had no concurrent systemic illness at the time of initial diagnosis or during documented follow-up. The concurrent diseases diagnosed in 7 dogs included polyarthritis (4), diabetes mellitus (1) and historical seizures (2). To the authors’ knowledge, this is the first report revealing the above breed predispositions for SNP. In this study, SNP was not typically associated with concurrent systemic illness.

Source of funding: Self-funded

Conflict of interest: None declared
Serum *Malassezia*-specific IgE in dogs with recurrent *Malassezia* otitis externa without concurrent skin disease

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Abstract: IgE-mediated hypersensitivity to *Malassezia pachydermatis* yeast is recognized in some dogs and people with atopic dermatitis (AD). *Malassezia* is also implicated in canine otitis externa (OE); in some dogs, recurrent *Malassezia* otitis occurs in the absence of other skin disease. It is unknown if yeast hypersensitivity contributes to the pathogenesis of recurrent yeast OE. The aim of this study was to determine how frequently *Malassezia*-specific IgE is detected in sera of dogs with recurrent *Malassezia* OE without concurrent skin disease. Serum from 21 dogs with at least three episodes of *Malassezia* OE within 18 months, and no history of other skin disease, were tested for serum allergen-specific IgE against *Malassezia* and 82 common environmental allergens. *Malassezia*-specific IgE was detected in 6/21 (29%) affected dogs. IgE against environmental allergens was more frequently detected than *Malassezia*-specific IgE, ranging from 44% for grass pollens to 89% for tree pollens. These findings suggest that recurrent yeast OE, without other skin disease, may be associated with IgE-mediated hypersensitivity against environmental allergens, and to a lesser extent, against yeast allergens. Allergy may be overlooked as a cause of OE in this patient group because they lack other classical signs of atopic dermatitis. Further studies are warranted to evaluate intradermal test reactivity and allergen-specific immunotherapy in dogs with solely recurrent *Malassezia* OE.

Source of funding: self-funded; ELISA tests were performed gratis by Heska Corporation.

Conflict of interest: D. J. DeBoer is a consultant to Heska Corporation.
A prospective, randomized, double-blinded, placebo-controlled trial evaluating the effects of a natural triglyceride omega-3 supplement on atopic dermatitis and erythrocyte membrane fatty acid concentrations in dogs

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Abstract: The objectives of this study were to evaluate the efficacy of a triglyceride form omega-3 supplement (Canine Omega Benefits (COB): Veterinarian Recommended Nutriceuticals, Plymouth Meeting, PA) for atopic dermatitis (AD) in dogs and to evaluate its effect on canine erythrocyte membrane (EM) fatty acid (FA) concentrations, compared to placebo (mineral oil). Seventy-two dogs with a clinical diagnosis of AD were evaluated by one of two veterinary dermatologists on days 0, 42 and 84; the Canine Atopic Dermatitis Lesion Index (CADLI) was used to score AD lesion severity, and blood was obtained to measure EM FA concentrations. A 10cm validated visual analog scale (VAS) and 0-10 pruritus scale were used by owners to assess pruritus severity. Overall improvement (OI) was assessed via owners/investigators utilizing a VAS. Sixty-eight dogs completed the trial (33 COB, 35 placebo). On days 42 and 84, COB treated dogs had a significant reduction in CADLI scores compared to placebo (p=0.000). On day 84, significantly more COB treated dogs (60%) had a >2cm reduction in pruritus VAS scores compared to placebo (16%) (p<0.05). Owner/investigator OI VAS scores were significantly higher in the COB group compared to placebo on days 42 & 84 (p<0.05). A significant increase in omega-3-index (EPA+DHA/total erythrocyte FA) levels and reductions in Arachidonic Acid:EPA and Omega-6:Omega-3 ratios were found on days 42 and 84 compared to placebo (p=0.000). COB was an effective treatment for the reduction for pruritus and skin lesions associated with AD and improved EM FA concentrations in dogs.

Source of funding: This work was supported by Veterinarian Recommended Nutriceuticals, Plymouth Meeting, PA.

Conflict of interest: At the time of the study, Drs. Palmeiro, Shanley and Mehler were compensated consultants and members of the Veterinarian Recommended Nutriceuticals scientific advisory board.
Evaluation of cyclosporine-sparing effects of polyunsaturated fatty acids in the treatment of canine atopic dermatitis

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Abstract: A randomised, double-blinded, placebo-controlled multicentre trial was conducted in 36 dogs with atopic dermatitis to evaluate the cyclosporine sparing effect of polyunsaturated fatty acids. Dogs were stable on their individual cyclosporine dosage and received either a combined omega-3/omega-6 fatty acid product or placebo orally for 12 weeks. Dogs were examined monthly and the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) was determined by a dermatologist. Pruritus, quality of life, global condition and coat quality were scored by the owner. If the dog’s CADESI-03 and/or pruritus score improved by at least 25% compared to the previous visit, the cyclosporine dosage was decreased by approximately 25%. If the scores deteriorated by at least 25%, the cyclosporine dosage was increased by the same percentage. The median daily cyclosporine dosage per kilogramm body weight decreased in the active group from 3.8 mg to 2.8 mg and in the placebo group from 3.7 mg to 3.4 mg from the beginning to the end of the study. The difference between the two groups was significant (P=0.009). The median pruritus score from inclusion to completion was significantly improved in the active group compared to the placebo group (P=0.04). There was no significant difference in CADESI-03 changes between both groups (P=0.38). The results of this study indicate a cyclosporine-sparing effect of omega-3/omega-6 fatty acids supplementation in dogs with atopic dermatitis.

Source of funding: This study was funded by Novartis Animal Health. The drugs were provided by Novartis Animal Health and WDT Germany.

Conflict of interest: In the past five years, RM and ML have performed studies, lectured and acted as a consultant for a number of companies manufacturing products for canine atopic dermatitis, amongst them Novartis Animal Health. MRM was financially supported by Novartis Animal Health. CL and AR do not report a conflict of interest.
Identification and characterization of ZTS-00103289, a monoclonal antibody neutralizing IL-31-mediated pruritus, in beagle dogs


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Abstract: Interleukin-31 (IL-31) has been shown to induce pruritic behavior in multiple species and its increased expression is associated with the severity of atopic dermatitis in humans. To evaluate the role of IL-31 in canine atopic dermatitis, a caninized monoclonal antibody (mAb), ZTS-00103289, that inhibits IL-31 mediated cell-based signaling was identified and characterized. To further determine whether ZTS-00103289 can inhibit IL-31-induced pruritus in beagle dogs, a single subcutaneous (SC) dose of mAb or placebo was administered to dogs (n=18/group) at a dose of 1 mg/kg and pruritic behavior associated with IL-31 challenge was assessed on days 1, 28 and 56. Results of the analysis of the primary decision variable (day 28 pruritic response) showed the lower 95% confidence limit at Day 28 for the mitigated fraction was 66.7%; thus, the primary decision variable was met. The least-square mean pruritus scores for placebo dogs on days 1, 28 and 56 were 70, 62 and 67 compared to 8, 17 and 53 for treatment dogs, respectively. The mean pruritus scores for treated dogs were significantly lower than for placebo dogs on days 1 and 28 (P<0.0001) but not on day 56 (P=0.1357). These results support the use of 1.0 mg ZTS-00103289/kg, SC to achieve a one month, but not two month, duration of efficacy in this model. Additional studies are necessary to determine whether the efficacy demonstrated in this model is predictive of efficacy in naturally occurring atopic dermatitis with this novel, injectable alternative treatment for atopic dermatitis.

Source of funding: Zoetis, Inc. funded this study.

Conflict of Interest: The authors are employed by Zoetis, Inc.
Laboratory dose titration efficacy study of ZTS-00103289, a caninized anti-IL-31 monoclonal antibody, in a canine model of IL-31-induced pruritus


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Abstract: The IL-31-induced pruritus model, in which the pruritogenic and pro-inflammatory cytokine interleukin-31 (IL-31) is intravenously administered to dogs to induce a short-term pruritic response, was utilized to determine the duration of activity of ZTS-00103289, a caninized anti-IL-31 monoclonal antibody (mAb). A single subcutaneous injection of mAb was administered at 0, 0.125, 0.50, or 2.0 mg/kg to groups of six beagle dogs on day 0. IL-31 challenges were performed on days -7, 1, 7, 14, 28, 42, and 56. Statistically significant reductions in pruritus (p<0.05) were observed from day 1 until day 14, 28, or 42 in the 0.125, 0.50, and 2.0 mg/kg groups, respectively. In addition, serum samples collected on days -1, 1, 5, 7, 8, 14, 28, 42, and 56 were assayed for ‘free’ mAb (not bound to IL-31) and anti-drug antibodies (ADAs) produced by the animal against the mAb. ADA production was minimal and none of the animals developed a persistent ADA response during the study. Pharmacokinetic data calculated from the ‘free’ mAb concentrations showed that ZTS-00103289 had a half-life of 11.1 ± 3.4 days and that increases in drug exposure (Cmax and AUC) were nearly dose proportional. Pharmacokinetic/pharmacodynamic modeling predicted that 95% of animals given a 2 mg/kg dose would be expected to have serum mAb concentrations above the EC50 for 28 days. Together, these results demonstrate a clear relationship between dose and duration of activity and justify additional studies evaluating the efficacy of this antibody therapy for canine atopic dermatitis.

Source of funding: Zoetis Inc. funded this study.

Conflict of Interest: The authors are employed by Zoetis, Inc.
SCIENTIFIC SESSION
PRESENTATIONS
THURSDAY
INTRODUCTION
This presentation will introduce some new research that deals with the response of bacteria that are exposed to subtherapeutic levels of antimicrobials. The results from these studies may have important implications for antibiotic use in veterinary medicine, in particular for combination therapies.

In 2007, Dwyer et al., showed that bacterial DNA gyrase inhibitors, including synthetic quinolone antibiotics, induce a breakdown in iron regulatory dynamics which promote formation of reactive oxygen species (ROS) that contribute to cell death. They suggested that hydroxyl radical formation, using the Fenton reaction, appears to be the most significant contributor to cell death among the ROS formed. The Fenton reaction leads to the formation of hydroxyl radicals through the reduction of hydrogen peroxide by ferrous iron. Dwyer and colleagues proposed that a common mechanism of cell death underlies all bactericidal antibiotics, whereby hydroxyl radicals are formed as a function of metabolism-related NADH depletion, leaching of iron from iron sulfur clusters, and stimulation of the Fenton reaction.

In 2007, Kohanski et al., showed that antibiotics from three major drug classes (fluoroquinolones, beta-lactams and aminoglycosides) stimulate the production of hydroxyl radicals, in both Gram negative and Gram positive bacteria, which ultimately contributed to cell death. The bactericidal antibiotics norfloxacin, ampicillin and kanamycin were shown to stimulate the production of hydroxyl radicals as the end product of an oxidative damage cellular pathway. In contrast, the bacteriostatic drugs erythromycin, spectinomycin, chloramphenicol, tetracycline, and rifamycin did not produce free radicals. The mechanism of hydroxyl radical formation was the end product of an oxidative damage cellular death pathway and stimulation of the Fenton reaction.

Hydroxyl radicals are extremely toxic and will readily damage proteins, membrane lipids and DNA. Following application of bactericidal antibiotics the bacterial SOS response was initiated and Kohanski et al., observed a significant increase in SOS activity upon treatment with norfloxacin. Cumulatively, the hydroxyl radical results suggest that the genetic and biochemical changes that arise following application of lethal doses of bactericidal drugs create a bacterial intracellular environment that promotes the formation of highly deleterious oxidative radical species.

SUBLETHAL LEVELS OF ANTIMICROBIALS
Following the study in 2007, in 2010 Kohanski et al., went on to show that sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. Simply, treatment of bacteria with low concentrations of bactericidal antibiotics can generate multidrug resistance through an increase in the mutation rate that is driven by the formation of ROS. The study looked at the effects of treating an E.coli K12 strain with low concentrations of norfloxacin, ampicillin and kanamycin. All three antibiotics led to a significant increase in the mutation rate (up to 8 fold) compared to controls. The involvement of ROS was confirmed by adding thiourea, a hydroxyl radical scavenger, which reduced the mutation rates to that of untreated cells. Consistent
with the idea that the increased mutagenesis that occurs in response to antibiotic-induced oxidative stress might be involved in the emergence of resistance, they found that treatment with low concentrations of bactericidal antibiotics led to continued sensitivity to the test antibiotic but showed an increased the minimum inhibitory concentrations for a range of other antibiotics. It was also demonstrated that this phenomenon occurred with Staphylococcus aureus (a Gram-positive organism) and with a clinical E. coli isolate. This proposed “reactive resistance” suggest that bactericidal antibiotics can function as active mutagens and may have a role in the emergence of multidrug resistance.

In 2013 Keren et al., 5 challenged the findings above and concluded that, “killing by antibiotics is unrelated to ROS production.” The Keren study re-examined the role of ROS in cell death and found no correlation between an individual cell’s probability of survival in the presence of an antibiotic and its level of ROS. There was essentially no difference in survival of bacteria treated with various antibiotics under aerobic or anaerobic conditions.

RESISTANCE VERSUS PERSISTER TOLERANCE

In 1944, Dr Joseph Bigger, was working in a military hospital in York, experimenting with the recently introduced penicillin. Addition of penicillin to a culture of Staphylococcus resulted in cell lysis. 6 Bigger subcultured this liquid to agar plates and observed bacterial colonies. Upon reinoculation, these colonies grew into a culture that again lysed in the presence of penicillin but formed a small new subpopulation of what Bigger called “persisters” to differentiate them from resistant mutants. The phenomenon, in which a small subpopulation of microbes survives the lethal effects of a drug, is now referred to as “persister.” Persistence is distinct from resistance in that, unlike resistant mutants, persister populations do not expand in the presence of the toxic compound, and population growth resumes only once the drug has been removed. Furthermore, upon retreatment, the subcultured organisms are drug sensitive, suggesting that unlike resistance, persistence is a nonheritable phenotype.

Persisters represent a small subpopulation of cells that spontaneously enter a dormant, non-dividing state. When a population is treated with a bactericidal antibiotic, regular cells die, whereas persisters survive. In order to kill, antibiotics require active targets, which explains persister tolerance. Taking samples and plating them for colony counts over time from a culture treated with antibiotic produces a biphasic pattern, with a distinct plateau of surviving persisters. By contrast, resistance mechanisms prevent antibiotics from binding to their targets. Resistance is measured by observing the ability of cells to grow in the presence of antibiotic. In order to measure resistance, antibiotic is serially diluted in twofold steps in a microtiter plate containing cells and growth medium. After a period of incubation, the lowest concentration of the antibiotic in a well with no growth is recorded and referred to as an MIC.

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Persister eradication is a tough problem. Very few existing antibiotics are active against non-growing stationary cells (primarily fluoroquinolones), and all are ineffective against dormant persisters. Persisters are specialized survivor cells and with multiple mechanisms of formation do not provide a good target that would disable their formation.

A MODEL OF TISB-DEPENDENT PERSISTER FORMATION IN E. COLI.

Fluoroquinolone antibiotics cause DNA damage by converting the DNA gyrase and topoisomerase to endonucleases. This activates the RecA protein, which in turn activates the LexA repressor, causing it to cleave. The canonical SOS response is induced, and repair enzymes that contain lex boxes in their promoter regions are transcribed. The Lex repressor also controls the expression of the TisB toxin, a small cationic membrane-acting agent. Decreases in the proton
motive force and ATP shuts down target functions, including DNA topoisomerase and gyrase, and a dormant persister is formed.

References:

DEAD BUGS DON’T MUTATE

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INTRODUCTION

In 2003, Charles W. Stratton published a “Perspectives” article in the Emerging Infectious Diseases journal with the title I am using for this presentation: “Dead Bugs Don’t Mutate.” It seems so obvious, and yet, because bacterial killing by antibacterial agents is never 100% effective the development of resistant (or persister) subpopulations is common. Therapies that maximize bactericidal effects are important because they reduce the development of bacterial resistance mechanisms.

There are two key themes that we will discuss, the first, as stated below, is that whenever possible bactericidal drugs should be chosen for therapy.

“The urgent need to curtail proliferation of antibacterial resistant bacteria has refocused attention on the proper use of antibacterial agents. That the use of any antibacterial agent, or class of agents, over time will result either in the development of resistance to these agents, or in the emergence of new pathogenic strains that are intrinsically resistant, is now widely accepted. Keeping these phenomena in check requires a comprehensive strategy that includes, whenever possible, the selection of antibacterial agents in dosages sufficient to be bactericidal. A bactericidal effect is desired because, to put it succinctly, dead bugs don’t mutate. In other words, if microbial pathogens causing infection are killed by antimicrobial therapy, rather than inhibited, mutations that might already exist or occur under the selective pressure of the antimicrobial agent are less likely to be promulgated.”

The second theme of interest is the clinical relevance of bacteriostatic versus bactericidal activity.

“All of the effects of antimicrobial agents against microbes, including the delineation of microbial resistance, are based upon the results of in vitro susceptibility testing. Most of these susceptibility tests only measure bacteriostatic activity even though the agent being tested may have bactericidal activity. Thus, the clinical relevance of susceptibility testing itself could be questioned.”

MINIMUM INHIBITORY CONCENTRATION (MIC)

It is critical that we are all clear on the definition of the MIC. Simply, the MIC is defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism. The MIC breakpoints for individual organism-drug combinations have been established in the USA by the Clinical and Laboratory Standards Institute (CLSI) and in Europe by the European Committee for Antimicrobial Susceptibility Testing (EUCAST).

ANTIBIOTIC SUSCEPTIBILITY TESTING 101

The methods routinely available in most microbiology laboratories to establish the in vitro antimicrobial susceptibility of a given bacterial organism are the Kirby-Bauer Disk Diffusion Test (KB), the Etest and the Broth Microdilution Test. KB Disk Diffusion has been the most widely used method in vet medicine. Antimicrobials are incorporated into a small filter paper disk and the disks are placed on a lawn of bacteria on an agar plate and incubated overnight. The disks contain drug concentrations measured in micrograms of drug per ml (ug/ml). The major
disadvantage of KB disk diffusion is that the results are qualitative. This test does not measure the MIC. Results are interpreted for each antimicrobial as Susceptible (S), Intermediate (I) or Resistant (R).

The Etest (also known as the Gradient Diffusion Method) is a rapid and convenient way to determine the MIC of an antimicrobial against a particular organism. The Etest method is a quantitative antimicrobial susceptibility testing method whereby a preformed antimicrobial gradient from a plastic-coated strip diffuses into an agar-based medium inoculated with the test organism. The MIC is read directly from a scale on the top of the strip at a point where an ellipse of organism growth inhibition intercepts the strip.

Broth Microdilution Tests are also now commonly available and the major advantage is the generation of quantitative results in the form of an actual MIC value. Broth microdilution can be performed in a 96-well plate format, which lends itself to automation, plates are commercially prepared, therefore it is less flexible than KB disk diffusion or Etest.

Interpretation of MIC Results
Broth microdilution plates have a range of drug concentrations (in micrograms per ml) in accordance with CLSI breakpoints.

- When growth occurs in all concentrations of the drug tested, the MIC is reported as > the highest concentration on the plate. e.g. ampicillin >16. These are interpreted as RESISTANT
- When no growth occurs in any of the concentrations of the drug tested the MIC is reported as ≤ the lowest concentration in the plate. e.g. gentamicin ≤1. These are interpreted as SUSCEPTIBLE
- An absolute value is shown when the MIC falls within the range of drugs tested on the plate e.g. Penicillin = 8. These are generally susceptible but an interpretation is always given. Some drugs in this group may be INTERMEDIATE.

In summary, the lowest concentration of antimicrobial that inhibits visible bacterial growth is known as the MIC. In most clinical situations, successful in vivo treatment requires that the concentration of an antimicrobial agent achieved at the site of infection should be 1-5 times the MIC.

ANTIMICROBIAL RESISTANCE 101.
Antimicrobial resistance can be inherent or acquired. Intrinsic resistance is natural to all the members of a specific bacterial taxonomic group (genus, species) and results from genetic, structural or biochemical characteristics inherent to the wild-type microorganisms. Intrinsic resistance is important and clinicians should be aware of the most common in order to avoid inappropriate or ineffective therapy. Antimicrobial resistance can also be acquired, often through mutation or genetic exchange. Horizontal gene transfer is any process in which an organism incorporates genetic material from another organism without being the offspring of that organism. By contrast, vertical transfer occurs when an organism receives genetic material from its ancestor, e.g., its parent or a species from which it has evolved. Plasmids, bacteriophage and transposons are key elements of every bacterial cell that play a role in horizontal gene transfer which is a critically important mechanism for the dissemination of antibiotic resistance in bacterial populations.

Plasmids are found in many types of prokaryotic cell, they are small molecules of DNA, distinct from chromosomal DNA, which can replicate autonomously and which are commonly dispensable to the cell. Bacteriophage (or Phage) is defined as any virus that has a bacterial host.
Most – probably all – bacteria can contain plasmids and can be infected by phage. Transposable elements (TE’s) or transposons are “Jumping genes” and they are found in both prokaryotes and eukaryotes. TE’s are normal components of chromosomes, plasmids and phage genomes.

THE MUTANT PREVENTION CONCENTRATION (MPC) AND MUTANT SELECTION WINDOW.

The mutant prevention concentration (MPC) was described by Dong et al. as a novel in vitro measurement of antimicrobial susceptibility, and it takes into account the probability of mutant subpopulations being present in high density bacterial populations. The MPC is the theoretical upper boundary of an antibiotic concentration window in which resistant mutants are selectively amplified. The MPC is the drug concentration that blocks the growth of the least susceptible, single-step mutant. Above this concentration, cell growth requires the presence of two or more resistance mutations. Since two concurrent mutations are expected to arise rarely, few mutants will be selectively amplified when a susceptible population is exposed to drug concentrations that exceed the upper boundary. Thus, resistance is expected to develop rarely when drug concentrations are kept above the MPC. Mutant prevention concentration testing is technically more demanding than MIC testing (>10⁹ cfu/mL versus 10⁵ cfu/mL respectively) and for this reason is unlikely to be available in diagnostic laboratories in the near future.

Antibiotic resistant mutants have been postulated to develop in concentration range (or window) between the lowest drug concentration that blocks the growth of the majority of drug-susceptible cells, and the drug concentration that blocks the growth of the least susceptible, single-step mutant. The lower boundary can be approximated by the minimal inhibitory concentration for half the cells in the population (MIC(50)); however, inhibition of 99% of the cells (MIC(99)) is a more suitable boundary since it is more accurately measured. The upper limit of the window is the drug concentration that blocks the growth of the least susceptible, single-step mutant. Above this concentration, cell growth requires the presence of two or more resistance mutations. Since two concurrent mutations are expected to arise rarely, few mutants will be selectively amplified when a susceptible population is exposed to drug concentrations that exceed the upper boundary. Thus, resistance is expected to develop rarely when drug concentrations are kept above the upper boundary of the mutant selection window (MSW). This expectation led to the upper boundary being designated as the mutant prevention concentration (MPC).

References:

INTRODUCTION:

Antimicrobial peptides (AMPs) are a very diverse group of small proteins characterized by a relatively short amino acid sequence (between 12 and 100 amino acids), positive charge (net charge between +2 to +11), and amphiphilic properties. Only a minority of AMPs have a negative net charge (e.g. dermcidin). Antimicrobial peptides have been isolated from single-celled organisms, invertebrates, plants, amphibians, birds, fish, and mammals. They are synthesized as inactive pro-peptides and activated after been cleaved during or after secretion. Such peptides have been classified based on their chemical structure in seven groups as follow:

1. linear peptides forming amphipathic and hydrophobic helices (e.g. alamethicin, magainin)
2. cyclic peptides (e.g. gramicidin S)
3. small proteins forming β-sheet structures (e.g. defensins, tachyplesin)
4. peptides with unique amino acid compositions (e.g. PR-39, indolicidin, cathelicidins)
5. cyclic peptides containing thio-ether groups (e.g. nisin, lantibiotics)
6. lipopeptides terminating in an amino alcohol (e.g. trichogin, polimixin B)
7. macrocyclic knotted peptides (e.g. circulin A)

Such peptides can be systemically expressed, like in unicellular organisms or as in insects’ hemolymph (e.g. lucifensins) or locally secreted as in vertebrates. In vertebrates, AMPs are mainly expressed in immune and/or epithelial cells most susceptible to infections (e.g. skin and mucosae). Bacteria produce two different kinds of AMPs: lanthionine- (lantibiotics) and non-lanthionine-containing peptides. Their main function is to kill other microorganisms that may compete for the same nutrients in a shared environment. In plants AMPs’ main function is to defend the organism against bacteria and fungi and they are localized in leaves, flowers, seeds, and tubers.

Finally, in vertebrates, AMPs achieve the most complex functionality. They not only protect the vertebrate against microorganisms (e.g. viruses, bacteria, fungi and parasites), but they also actively interact with the adaptive immune system regulating the complex local immune response. In mammals, AMPs are also critical components of the innate local immunity and of the skin barrier. More than 1,500 AMPs have been identified, and of those the most studied are defensins and cathelicidins.

DEFENSINS:

Defensins are cationic, microbicidal, amphipathic, and variably arginine-rich peptides. They contain a minimum of six highly conserved cysteine residues. These residues form three or four pairs of intramolecular disulfide bonds. Determined by the alignment of disulfide bridges and molecular structure, defensins are classified into three subgroups (α, β, and θ). α-defensins, present in people, are characterized by cysteine residues linked in a 1-6, 2-4, 3-5 pattern. β-defensins, present in many mammals including people and dogs, are distinguished by disulfide bonds that link cysteine residues 1-5, 2-4 and 3-6. θ-defensins, identified in rhesus macaque, Old World monkeys, orangutans, and baboons, consist of a circular structure formed by two hemi-α-defensins, each of which contributes three cysteines.

CATHELICIDINS:

Cathelicidins (Cath) are a group of cationic AMPs characterized by the highly conserved N-terminal region known as the “cathelin domain”. Similar to defensins, Cath also contain a variable number of cysteine residues linked by disulfide bonds. Cathelicidins have been identified in many
species with a genetic commonality conserved among them. In humans, there is a single Cath precursor, designated hCAP18. hCAP18 is processed and then released from the carboxyl terminus as an AMP consisting of 37 amino acids beginning with two leucines, designated LL37. hCAP18 is stored in neutrophil granules, NK-cells, epithelial cells and mast cells. Cathelicidins have rapid, potent, and broad-spectrum antimicrobial properties, and they can be further cleaved to form different derivatives with various functions (e.g. RK-31, KS-30 and K20).

ANTIMICROBIAL ACTIVITY:
As mentioned above AMPs have multiple actions in vertebrates. Antimicrobial peptides are able to recognize and attack invading microorganisms in non-specific fashions. In fact, AMPs are able to kill several viruses, bacteria, and fungi. Of these the antifungal activity is the least understood, although it is hypothesized that AMPs may increase the permeability of fungal cell membranes.

As far as their antiviral effect, AMPs may affect both enveloped and non-enveloped RNA and DNA viruses. These may comprise adenovirus, feline calicivirus, and echovirus. The mechanism of action is not completely elucidates, however, the major hypothesis is that AMPs interfere with the viral absorption and entry process. Another possible mechanism is through direct effect on the envelope.

More information is present on their antibacterial properties. Although a high variability in their chemical structures has been revealed, a commonality in their antimicrobial properties is very clear. The complete mechanism of action has not been completely elucidated yet. However, five main models of adhesion, penetration and lysis have been developed: barrel-stave, carpet, detergent, toroidal pore, and aggregate models. The initial step is the adhesion of AMPs on the bacterial surface via electrostatic forces between the positive charged AMPs and the negative charged phosphate groups of either lipopolysaccharide (LPS) and (lipo)teichoic acid (LTA) present on the outer bacterial membrane of Gram-negative and Gram-positive bacteria, respectively. After the adhesion, AMPs form pores, of different size based on the different peptide, leading the lysing of the microorganism. Other AMPs can entry the bacterial membrane creating very small pores, not able to lead to lysis, but allowing the peptide to disrupt critical cellular processes (inhibition of nucleic acid synthesis, protein synthesis, enzymatic activity, and cell wall synthesis) leading to cell death.

ANTIBACTERIAL RESISTANCE:
Nowadays it is very common interact with highly resistant bacteria in practice. In fact, bacteria are able to be selected for evolutionally changes that may disrupt the antibacterial mechanism of action. This is highly evident in the commonly used antibiotics (e.g. methicillin resistant staphylococci). So far antimicrobials like natural AMPs have been shown to have a highly resistant mechanism of action compared with traditional antibiotics. The reason of such unique properties resides in the fact that natural AMPs kill bacterial through interference with basic, critical structures of the microorganism. This phenomenon makes the evolution-driven resistance to AMPs less likely since such changes may lead to bacterial death.

Nevertheless, possible mechanisms of resistance have been hypothesized. Microorganisms may modify their outer cellular surfaces reducing their negative charges leading to a decreased affinity for cationic peptides. This change can be achieved increasing the number of D-alanine in the LTA molecule exposing its positively charged group decreasing the negative charge of these polymers. The D-alanine modification of LTA occurs through the activation of the dlt operon. Another way to reduce the affinity for cationic peptides is the activation of the MprF gene which codes for the introduction of L-lysine into the structure of phosphatidylglycerol, a phospholipid abundantly present in the membrane of Gram-positive bacteria resulting in an increase in positive net charge of the bacterial outer membrane. Other mechanisms of resistance include increased membrane fluidity and increased presence of QacA efflux pumps, a major facilitator superfamily member. Also bacteria such as S. aureus have the ability to increase the production of staphylokinases, able to bind to AMPs and inactivate them.

In Gram-negative bacteria the negative charge is mainly due to the presence of lipid A forming LPS. Similarly to what reported above for Gram-positive bacteria, an increased abundance in
aminoarabinose decreases the negative charge of the bacterial surface decreasing the ability of AMPs to adhere to the cell membrane. This modification is dictated by the activation of pmrE and pmrHIJKL genes.  

Finally, another mechanism of resistance involves biofilms. The production of the cationic polysaccharide intercellular and capsular adhesion molecules prevents the attachment of AMPs to bacterial surfaces.

**IMMUNOLOGICAL ACTIVITY:**

The immunological activity is evident in higher evolved vertebrates. In mammals AMPs represent a critical component of the innate immunity and a pivotal molecule in the orchestration of innate and adaptive immunity. They are able to enhance phagocytosis, stimulate prostaglandin release, neutralize the specific effects of LPS, promote recruitment and accumulation of various immune cells at inflammatory sites (e.g. monocytes and T cells), promote angiogenesis, induce wound repair, chemotact and activate granulocytes, monocyte/macrophages, mast cells, antigen-presenting dendritic cells, and lymphocytes, and influence dendritic cell development with adjuvant and polarizing effects. Also, due to their ability to chemo-attract immune cells and to respond to danger signals by activating the immune system, AMPs have been labeled as “endogenous alarmins”. The immunological properties of AMPs are the subjects of intense research in both human and canine inflammatory and autoimmune diseases such as atopic dermatitis, psoriasis, and periodontal diseases. In particular, in people, an inverse relationship has been observed between defensins, Cath, and anti-inflammatory T helper (Th)2 phenotype molecules (Interleukin [IL]-4 and IL-13) in cell culture system; such trend has not been seen in dogs.

On the opposite, in human keratinocytes the exposure to pro-inflammatory cytokines such as IL-1, IL-17, IL-22 and Tumor Necrosis Factor (TNF)-α, direct bacterial contact, vitamin D, and wound healing have been observed to promote the production of defensins and cathelicidin; such trend has been suggested in dogs.

Finally, AMPs like Cath and defensins have been shown to behave synergistically. This implies a combined role in the orchestration of the host’s innate defense system.

In conclusion, although a high diversity in the structure of natural AMPs have been demonstrated in different species, a commonality in their actions is also very clear. Also, since many AMPs are produced and secreted from the same tissue it is possible to consider the multitude of AMPs as a unique complex of molecules that acts synergistically with the common aim to protect the host and “alert” the local immune response against external and internal dangerous stimuli.

**References**

AMP’S IN ALLERGY AND INFECTION AND FUTURE POTENTIAL IN DERMATOLOGY

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INTRODUCTION:
Antimicrobial peptides (AMPs) have been identified as a major component of the innate immunity in higher vertebrates. In the skin, AMPs are not only representing a critical component of the local innate immunity, but they are also involved in the integrity of the skin barrier and bridging the innate and adaptive immunities. Although, AMPs have been identified in many domestic animals including: dogs, horses, cattle, sheep, goats, pigs, poultry, and cats, little information is available on such peptides in veterinary medicine compared to human medicine.

In human medicine, many studies have shown the myriad of properties of AMPs. In fact, they are involved in enhancing phagocytosis, stimulating prostaglandin release, neutralizing the specific effects of lipopolysaccharide (LPS), promoting recruitment and accumulation of various immune cells at inflammatory sites, promoting angiogenesis, inducing wound repair, chemo-attraction and activation of granulocytes, monocyte/macrophages, mast cells, antigen-presenting dendritic cells, and lymphocytes, and influencing dendritic cell development with adjuvant and polarizing effects. Also an association between AMPs and inflammatory and immune-mediated diseases like atopic dermatitis (AD) and psoriasis have been demonstrated.

In veterinary medicine, very few studies have been focusing on the involvement of AMPs in immune-mediated diseases (e.g. AD) with or without active infection. The majority of the veterinary work on AMPs has been referring to the canine species since the dog has been more commonly used as an animal model for human diseases. In dogs, three major groups of AMPs (β-defensins, cathelicidin, and S100A proteins) have been identified in epithelial tissues, namely skin, testes, and lung. However, the most studied AMPs have been β-defensins (BD) and cathelicidins (Cath).

In the skin, four BD have been identified in people: hBD1, hBD2, hBD3/103A, and hBD4. Of these the most studied has been hBD2 and hBD3/103A. In the canine skin, seven BDs have been identified: cBD1, cBD1-like, cBD2-like/122, cBD3-like, cBD102, cBD103, and cBD127. The cBDs have been compared to the hDBs and an homology varying between 51% and 80% have been confirmed. Among the several BDs, cBD103 and hBD3/103A are the most similar with an homology of 80%. As far as Cath, only one Cath has been identified in both human and canine epithelia. When the cCath was compared to the hCath precursor, hCAP18, a 68% mRNA sequence similarity and 57% protein sequence similarity were identified.

Similarly to human AMPs, the canine AMPs have been tested for their antimicrobial properties, showing very striking similarities confirming a potent antimicrobial activity against numerous pathogens associated with the skin and respiratory tract. Among all the AMPs tested cBD103 and cCath had the most effective antimicrobial properties against bacteria and yeasts.

AMPs, ATOPIC DERMATITIS AND SKIN INFECTIONS:
Current research regarding the role of AMPs has expanded from antimicrobial activity to also include immune-modulating functions and possible involvement in the pathogenesis of AD in both people and dogs. Over the past decade, interest in skin barrier defects related to inflammatory skin diseases, such as AD, has notably increased in both human and veterinary medicine and as components of the skin barrier, AMPs have been widely studied in both people and dogs.

In people, a decreased expression of AMPs in patients with AD compared to patients affected by psoriasis has been observed. It also been observed that atopic patients are more susceptible to skin infections (Staphylococcus aureus) compared to healthy subjects or patients affected by psoriasis and
from this observation a correlation between low AMPs and increase in Staphylococcal infection was postulated.\textsuperscript{15-25} However, this hypothesis has been challenged by more recent studies which have demonstrated that, on the contrary, there is an increased expression of AMPs such as BDs, Cath, psoriasin and ribonuclease\textsuperscript{7} (RNAase\textsuperscript{7}) in atopic human skin when compared to healthy human skin.\textsuperscript{26-28}

Similar to human AMPs, canine AMPs have been localized in tissues that most frequently come in contact with external agents, namely: testicular and mucosal epithelia, skin and blood.\textsuperscript{4,6,7,11-13,29} As today, little information is available regarding the relationship between AMPs and canine AD. One group of investigators compared the expression levels of cBD103 and S100A8 in naturally-affected dogs with chronic AD and in healthy skin. In this study, using antibodies towards the hBD3/103A and mRNA expression for S100A8, the investigators reported a decreased signal of cBD103 and an increase expression of S100A8 in lesional skin compared with non-lesional and healthy skin.\textsuperscript{12} Similarly, Lacto et al. showed a decreased expression of cBD103 mRNA expression in atopic dogs compared to healthy controls.\textsuperscript{30} Another group of investigators\textsuperscript{9} using a similar population were able to show a surprisingly increased levels of cBD103 mRNA in atopic dogs with chronic AD compared to healthy skin. This increase in levels was even more evident when a subgroup of atopic dog with active skin infection was compared to the healthy control population.\textsuperscript{9} The three studies although similar had a very different study design and for this reason a comparison is rather difficult.

Another study\textsuperscript{10} has been performed to assess the mRNA and protein expression of AMPs in a validated canine model of AD.\textsuperscript{31-33} The reason behind the use of such model was dictated by the difficulty to investigate the involvement of AMPs in the pathogenesis of AD in dogs with naturally occurring disease due to the presence of multiple confounding factors (e.g. different breeds, living conditions and diet).\textsuperscript{31-33} In this study,\textsuperscript{10} the investigators were able to evaluate the expression of such peptides in the initial phase of AD in a time controlled fashion. The investigators showed that an increase in the mRNA expression of cBD1-like, cBD3-like, and cCath in the atopic skin compared with the healthy controlled skin before and after acute allergen exposure.\textsuperscript{10} However, a significant increase in protein staining detection was not evident between the two groups suggesting a defect in the translational process. Similar results were evident in a later paper by the same group using a custom-made ELISA on skin extracts from chronic naturally-affected atopic dogs.\textsuperscript{9}

A lack of studies showing a direct correlation between AMPs and skin infection in non-allergic dogs is evident. In fact, the only study that has analyzed the effect of an active infection (bacterial or yeast in origin) on AMPs in atopic dogs showed an increased mRNA expression of cBD103 and a decreased expression of cBD1-like suggesting a different activation of such peptides in course of active skin infection in canine AD.\textsuperscript{9} When the protein expression of cBD3-like and cCath in atopic dogs with or without active skin infection was compared no difference was seen; although a significant difference was shown between atopic skin without skin infection and healthy control skin, suggesting that a different activation of such peptides is seen in AD compared to healthy dogs.\textsuperscript{9}

In conclusion, more studies are needed to better clarify the involvement of AMPs in AD in both people and dogs. In particular, their involvement, if any, in the higher susceptibility to skin infection evident in both people and dogs affected by AD. Also studies on the effect of drugs on AMPs are also needed to better clarify the effects of treatments (topical and systemical) on the secretion of AMP in both species.

**FUTURE POTENTIALS:**

In the past decades the incidence of skin infections due to multidrug resistant microorganisms has significantly increased. Such increase in resistance has not been associated with an increased number of antibiotics to use against such microbes, leading to potentially life-threatening diseases. This risk is even more evident in burn patients in which the control of sepsis is critical to the survival of the patients.\textsuperscript{34} To respond to the necessity of more active antimicrobials with a low risk of resistance, new technologies have been identified and associated with old rediscovered molecules. This is the case of nanosilver molecules proven to be highly effective against multidrug-resistant bacteria, but with side effects inversely proportional to the size of the silver.\textsuperscript{35-37} On the contrary, AMPs, like Cath and BD, proven to be
highly effective against multidrug-resistant microbes, being part of the normal innate immunity may not have any cytotoxic effects making these molecules an attractive alternative to more traditional antibiotics. Also the use of drugs (e.g. plant extracts, or vitamin D) that may increase the action and production of natural AMPs may have important potentials in the treatment of cutaneous infections, but also as treatment of AD since AMPs are actively involved in the local immune response.\textsuperscript{4,5} Furthermore, the evaluation of AMPs in the stratum corneum\textsuperscript{38} or in the serum\textsuperscript{39} of atopic people and dogs, respectively, could be used as non-invasive and more stable marker of skin barrier integrity and severity of AD. Lastly, it is well known that LPS play a pivotal role in the pathophysiology of sepsis due to Gram-negative bacteria leading to endotoxin shock.\textsuperscript{40} In theory molecules present in the bloodstream and able to bind to LPS can neutralize its endotoxin activity and since several cationic AMPs are well known to bind to LPS, they may have a role in treating sepsis suggesting another potential area of development for future use of natural AMP.\textsuperscript{40-42}

References

SALAMANDERS, SKIN, AND STEM CELLS

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SALAMANDERS

Urodeles including salamanders and newts possess a unique talent: they can regenerate body parts to the extent that humans can only dream about. The knowledge of regeneration in lower vertebrates has been very instructive in regenerative medicine and will be highlighted here. Urodeles are a diverse subgroup of amphibians that have evolved complex but accelerated repair mechanisms, presumably as an adaptation to hostile and repeated predator attacks. Fundamentally, urodeles retain many juvenile traits throughout adulthood (‘neoteny’). By remaining juvenile-like well into adulthood (like the teenager that never grows up!) an organism can retain the capacity for growth, self-renewal, and differentiation, features that are required for organ regeneration, survival, and reproductive longevity. Another important, highly evolved feature of urodeles shared with Arthropods and Lizards is autotomy, the strategy to cast off one’s appendage to avoid the death grip of predators (a phenomenon which also includes the defensive abandonment of claws, legs, antennae, or in mammals the skin as in African Spiny Mice[1]. Hence, survival for the urodeles which do not rely on the flashing of sharp teeth, darting with poisonous spines, or fending off with ominous growls, rather autotomy for survival, depends on a good plan to reform body parts, since shedding body parts is not something that can be repeated often. It is largely considered that the following features must be present for successful regeneration of any organ, including skin: (1) limiting the inflammatory response, (2) anti-microbial defenses to minimize infection, (3) mechanisms to avoid scarring at the wound site which are tied into (1), and of course, (4) regeneration of the body part according to the original pattern. Salamanders are the only tetrapods and the only urodeles capable of regenerating an excised limb, a feat which is unimaginable for mammals; hence, salamanders have been well-studied, including genomic and proteomic investigations, and lineage tracing. Understanding how salamanders replace limbs (or lenses, skin, spinal cords, and tails) has led to a new framework with which to understand pathways that could be re-activated in adult mammals, including skin. So how do salamanders replace an excised limb? Principle mechanisms are known to include (1) extreme plasticity of differentiated cells in tissues, (2) positional identity / memory of regenerate, (3) nerve dependence of regeneration, (4) indefinite or strong replication proliferation potential, (5) and species specific proteins (e.g. Prod1) that support positional identity or de-differentiation in tissues. More than one of these salamander mechanisms persists in adult mammals. In skin, plasticity (1), positional identity (2), strong proliferation potential (3) are recognized as important aspects of epidermal and hair follicle regeneration. Indeed positional memory as seen in fibroblasts of salamander blastema[2], has been observed in human fibroblasts[3] suggesting that the stroma of dermal tissues may also retain positional memory contributing to the topography of healing skin. Concerning peripheral innervations (4), development of hair follicles is dependent on innervation in the mammalian fetus, premature regression of hair follicles is regulated by peptides (CGRP, Substance P) released by nerve endings, sonic hedgehog (SHH) is released by nerve endings above the bulge, and epidermal organoid transplants that receive nerve ingress are more likely to form complete units of skin, suggesting that innervations may modulate regeneration and wound healing of the follicular region[4]. Further understanding of nerve-skin interactions may reveal new opportunities to control skin regeneration and repair. Salamander biology pushes us in this direction.

Plasticity of cells is a critical feature supporting regeneration in salamanders. Plasticity is the capacity to transform, including transdifferentiation (changing from one specialized cell to another often involving a lineage change), differentiation (commitment to a less specialized cell type down single or multiple lineages), or even dedifferentiation (becoming less committed, more primitive, even if this is
De-differentiation, in particular, is critical to urodele regeneration, and an element that is largely suppressed in adult mammals. In the case of salamanders, the first step in regeneration involves epithelialization of the wound surface. Basal keratinocytes of the wound demonstrate dedifferentiation (expression of embryonic limb bud gene Sp9) and rapid proliferation, covering the wound[5]; this capacity extends into adulthood, and thus neoteny of the epithelium is a critical aspect of life-long regeneration in salamanders. The wound epithelium cover is absolutely essential to initiate limb regeneration. Further, this wound epithelium must interact with severed nerve endings and associated growth factors (e.g. GDF5, Fgf2, Fgf8 – topical administration causes ectopic limb formation in absence of nerves). The cross-talk between wound epithelium and nerve endings generates specific cues to fibroblasts that reside below the stump (now called the blastema). The fibroblasts are instructed to dedifferentiate (reach a more primitive state), proliferate, and migrate. Primitive genes including Lin28 (which controls Oct4 gene expression) and Sall4 are expressed in these dedifferentiated cells. Thus plasticity, high proliferative capacity, and primitive manifestations, defining traits of stem cells, are exemplified at every step in this model. The dedifferentiated fibroblasts are referred to as mesenchymal stem cells (MSCs). These MSCs are unipotent, thus responsible for a single lineage of the regenerate (bone, cartilage, connective tissue, tendons, etc). They retain positional memory and provide patterning cues to cells that lack positional memory within the regenerate[6, 7].

The question is whether the early process of dedifferentiation occurs in wound healing of mammals, or whether there are only rare, uncommitted cells which proliferate in the absence of dedifferentiating. In general, it is the latter. However, differentiated cells can be experimentally reprogrammed (dedifferentiated) to pluripotency by transduction with embryonic transcription factors (e.g Oct4, Sox2, Klf4, c-Myc). Thus adult mammals do possess the genetic machinery that is necessary for dedifferentiation, but expression of genes encoding dedifferentiation is epigenetically repressed. In the salamander, genes which control the epigenetic state of chromatin are markedly activated during regeneration[8]. In some ‘regenerating’ systems (liver, kidney, lung), partial dedifferentiation has been observed, i.e. stem cells transiently express more primitive markers found on cells during late development[9]. Is it possible to re-activate these primitive pathways, re-establishing dedifferentiation? The answer is yes, according to an elegant study published by Aguirre et al in 2014 [10] based on principles learned from Zebra fish, a species that can regrow their hearts after the apex has been excised. Certain miRNA (let7a/c, miR99/100) were found to be responsible for repressing the dedifferentiation of cardiac progenitor cells in the quiescent (uninjured) heart muscle of Zebra fish. When the heart was injured, these miRNA were turned off (in contrast to mammals which do not turn off these miRNA), allowing dedifferentiation of cardiomyocyte progenitor cells. Interestingly adult mice were found to possess the same repertoire of miRNA, which when inhibited (using miR-antagonists) permitted dedifferentiation and hyperplasia of cardiomyocyte progenitor cells and regeneration of the ischemic heart without scarring. Voila: scar-free regeneration in an adult mammalian heart! The principle of epigenetic reprogramming in vivo to promote dedifferentiation and subsequent regeneration is a new, very exciting approach with great translational potential. The relationship between findings in Zebra fish and salamanders, and the repressed pathways in mammals is astounding. Whether this approach to scar-free wound healing in the skin is developed depends on our knowledge of repressed epigenetic mechanisms which need to be elucidated.

SKIN

Within the skin, the identity and topography of epidermal stem cells are well-described due to pioneering work by Fiona Watt (Kings College, London) and Elaine Fuchs (Rockefeller University)[11-18]. These authors have employed a combination of optical microscopy, transgenics, retroviral and mutation based lineage tracing, high through-put genomic screening, single cell resolution studies, and transplant studies to elucidate stem cells of the interfollicular epidermis and hair follicle in healthy and injured skin[14]. From these studies, it is apparent that skin homeostasis and response to injury involves a complex hierarchy of stem-progenitor-committed cells within the epidermis (basal cells) and hair
follicles (bulge, inner and outer sheath, and dermal papillae cells) [4]. In the interfollicular epidermis, basal cells serve as stem cells, allowing for repair of full thickness injuries. Basal cells (K5^{pos}, K14^{pos}) can reconstitute the entire epidermis (differentiation) and exhibit long-term self-renewal. In response to injury of the skin basal cells delaminate (release from basement membrane) and migrate upwards while differentiating to keratinocytes and cornified epithelium[15]. If mammalian skin possesses stem cells that are perfectly capable to self-renew and differentiate, why does wound healing in the adult mammal proceed with scarring? Fetal and early post-natal mammals can heal skin and heart muscle without scarring, possessing larger quantities of hyaluronic acid in skin. Furthermore, muscle heals without scarring in adult mammals. This appears to relate not to intrinsic properties of stem cells within those organs, but to immunological and epigenetic factors [19]. The release of pro-inflammatory cytokines by macrophages and T cells induce a fibrotic response; in contrast, in muscle, there is a skewing of macrophages toward an anti-inflammatory (M2) phenotype, thus mitigating fibrosis during muscle healing[8]. Interestingly, macrophage depletion in the salamander blastema results in a striking fibrotic stump rather than limb regeneration; therefore, anti-inflammatory macrophages are essential for scar-free healing, and compensatory growth (quasi-regeneration) of many organs (kidney, liver, peripheral nerves) depends on such macrophages. Whether scar-free healing of skin can be achieved by modifying the immunologic response to injury needs to be tested. Stem cells may hold promise in this regard.

STEM CELLS

Based on the above discussion concerning the differences between urodeles or Zebra fish and mammals, it is evident that perfect wound healing requires neotony (juvenile properties in the adult), including control of inflammation (by stromal macrophages), and dedifferentiation of wound epithelium and local fibroblasts to stem cells, which follow a morphologic patterning to restore normal structure and function. Developmental restrictions in mammals pose formidable obstacles to regeneration in adult mammals, because these intrinsic properties are lost with age. Thus, it is imperative to think about ways to induce salamander-like healing by exogenous treatments (gene therapy, in vivo reprogramming, stem cell therapies). This would entail methods to (1) re-activate primitive pathways towards dedifferentiation and re-differentiation/‘redevelopment’, (2) promotion of anti-inflammatory macrophages and thus induce anti-fibrotic pathways, and (4) growth promotion. Mesenchymal stem cells (MSCs) have been exploited for a decade in over 400 clinical trials (www.clinicaltrials.gov), mainly for their immune modulating properties. Interestingly, MSCs secrete a repertoire of paracrine signals that potently reduce T cell proliferation, activation, and cytokotoxic differentiation, increase Tregulatory cells, and repress NK cell maturation, and monocyte cytokine production[20-25]. Thus, MSCs are often viewed upon as a panacea for inflammation in a variety of contexts including auto-immune disease, chronic inflammatory disease, and fibrosis. Included in paracrine signals are diffusible proteins and extracellular vesicles. Much emphasis until recently has been on soluble mediators of immune modulation, including TGFβ1, PGE2, IDO, NO, HLA-G, and IL-10[23]. However, the role for extracellular vesicles shed by MSCs which contain proteins, mRNA, and miRNA is compelling, and increasingly appreciated. Indeed extracellular vesicles can impart a significant immunomodulatory effect on recipient cells (e.g. T cells)[26-29] and due to transfer of miRNA, can induce epigenetic modulation of recipient cell gene expression or translation.

In skin diseases, MSCs therapies have been widely investigated, showing beneficial effects in animal models of Epidermolysis Bullosa[30], full-thickness skin defects[31], repair of skin defects in spina bifida[32], diabetic ulcers[33], atopic dermatitis[34], suppression of hypertrophic scarring[35], photo-aged wrinkles[36], skin flaps necrosis[37], ischemic-reperfusion injury of skin[38] among many others. In psoriasis, endogenous MSCs have markedly reduced ability to suppress lymphocyte proliferation[39] and exhibit Th1-Th17/Th2 imbalance[40], supporting the notion that supplemental MSCs may improve to the immunologic landscape of chronic inflammatory skin diseases. Whether similar defects in MSCs immune homeostatic functions exist in other dermatides is yet to be explored.
A role for MSCs in dermatologic diseases of veterinary patients is well-justified based on animal and laboratory data. Candidate dermatologic diseases for MSCs based therapy might include atopic dermatitis, peri-anal fistulas, pemphigus foliaceous, dermatomyositis, and localized infections which cannot be controlled by anti-microbials. Preliminary data employing MSCs for treatment of peri-anal fistulas in our hospitals will be discussed.

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Our understanding of the on-host and off-host biology of the cat flea changed dramatically in the mid-1980s through the mid-1990s. Many of these basic tenets have not changed but new details have emerged that are having an effect on our understanding of flea population ecology within a home and upon our flea control efforts.

As we examine this new information, it is important to review some important aspects of flea biology. For the past 2 decades *Ctenocephalides felis*, the cat flea, has been considered an obligate parasite that is metabolically and reproductively bound to its host. It was demonstrated that actively reproducing adult *C. felis* develop a rather persistent ectoparasitic relationship with their hosts once reproduction is initiated. *C. felis* that had initiated reproduction died within 1 – 4 days if removed from their host. It was also shown that when grooming activity of cats was restricted 85% of female and 58% of male *C. felis* remained on cats for 50 days. It was conclusive shown that reproduction occurred on the host with eggs falling from the animals pelage into the surrounding premises. Once on a preferred host, cat fleas begin feeding within minutes and start breeding soon after. A female flea can begin laying eggs within 24 to 48 hours of jumping onto a host and in a few days, a female flea can lay 40 to 50 eggs/day. These revelations dramatically changed approaches to managing fleas: by applying products to and on pets, owners and veterinarians attempt to control reproduction and “break the life cycle” rather than focusing on the environment.

While the basic facts as stated above are certainly true, recent investigations have brought more refinement to our understanding of this parasite-host relationship. In 2009 while conducting an in-home field investigation it was surprisingly found that 34.4% of 2,241 fleas (*C. felis*) collected in premise intermittent-light flea traps in 27 homes contained small quantities of blood. Clearly too large a percentage to be accounted for by dog and cat grooming activity. Several factors were postulated to account for this phenomenon such as hyper-excitation of fleas due to application of an insecticide, some of fleas may have fed upon humans in the household and the other possibility is that before establishing themselves as permanent ectoparasites, there may be a period when a percentage of fleas display inter-host movement, which was previously described by Rust (1994). In that study when cats were housed together in a 0.4m² cage for 7 days there was transfer of 8 to 15% of the flea population. Recently a very interesting study was published that may explain the source of the blood contained within the fleas in the premises. In that study cats were infested with *C. felis* and then just 15 minutes later they were co-mingled with non-infested cats. When cats were combed to remove fleas 24 hours later it was found that 20% of the fleas transferred from the infested to the previously non-infested cats. However, when the cats were infested with fleas and held for 48 hours before co-mingling with non-infested cats, only 3.7% of the fleas transferred. In the previous discussed 2009 field study it was found that only 9 of 2,241 (0.4%) fleas collected in the intermittent-light flea traps were considered engorged. Engorgement meaning that the female fleas displayed evidence of prolonged feeding or achieving reproductive status. When results from these different studies are combined it is clear that prior to *C. felis* achieving reproductive status (24-48 hours), a percentage of the fleas can move on and off the host and inter-host movement is occurring. In addition, these data also further establish that once reproduction is initiated movement of fleas on and off the host and inter-host movement is quite limited. The fact that inter-host movement is occurring may have an impact on management of FAD and potential transmission of infectious agents such as *Bartonella sp.*

If those fleas do establish their permanent ectoparasitic relationship and begin laying eggs, those eggs roll off the host, and larvae typically hatch in 3 to 5 days. The larval stage is the most sensitive to environmental extremes. Flea larvae require flea feces for nutrition, protection from direct sunlight,
temperatures in the range of 45°F to 90°F, and relative humidity in the range of 50% to 85%. The rate of flea development depends on the temperature. Development from eggs to adult fleas can take less than 3 weeks at 85°F and can take 7 to 12 weeks at 65°F.

Another little studied but potentially very important aspect of flea development concerns the evaluation of the gender of newly emerged fleas. While most insect species exhibit protandry (males tend to emerge before females), *C. felis* belong to a much smaller group that exhibits protogyny (females tend to develop before males). Protrandry tends to occur in insect species where the females only mate once in their lives. Hence the first male their passes on his genes. We have all experienced walking, jogging or riding into a swarm of gnats. That swarm is composed almost exclusively of males waiting for females to emerge. Protogyny however tends to occur where females mate with multiple males and the last male that mates his sperm is used first (sperm precedence). With *C. felis* the first fleas to emerge from a cohort of eggs are females, followed by both males and females and then lastly almost exclusively males. So does such information have any practical value? It has been demonstrated that if flea reproduction is inhibited by insecticidal or insect growth regulator treatments administered to a pet, then a gender shift in premises flea population takes place overtime from a female dominated or at least gender neutral population towards a more male dominated population. In a 2009 in-home study at the initiation of the study, most fleas in the intermittent-light flea traps were female, representing 57.5% (Day 0) (F:M=1.35:1) of the captured flea population. Within 21 days of treatment the ratio had already shifted to 1:2 (66% males). And by day 60 of the study, the number of unfed female fleas represented only 25% (F:M = 1:3) of the captured flea population, demonstrating that a significant gender shift had occurred during the study period. This information can now be used to evaluate if flea reproduction is still occurring within a home following treatment. If a gender shift fails to occur within 21 to 28 days following treatment then it must be assumed that fleas are still reproducing within the home. Either the treatment has failed or there are untreated flea hosts within the household. Now those hosts may be permanent residents within the home or they may be visitor pets. In another in-home field investigation it was demonstrated that during a 2 month period there were visitor dogs in 16 of 37 (43%) homes on study. These pets must be accounted for or flea control will fail.

It is not just visitor pets that can cause problems in flea control. Other hosts both indoors-and outdoors can be problematic. Common hosts for *C. felis* include cats, dogs, opossums, raccoons, domestic rabbits, and hedgehogs. Squirrels, wild rabbits and birds are poor hosts for *C. felis*.

In a 2013 study conducted in Tampa FL, pet owners were asked if they had observed opossums, raccoons or feral cats in their yards. Of the 37 pet owners, 73%, 49% and 95% reported observing opossums, raccoons or feral cats, respectively in their yards. In North America, feral cats and urban wildlife such as opossums and raccoons can be commonly infested with *C. felis*, which can deposit eggs and contaminate protected outdoor premises locations. Given that such a large percentage of owners' yards are frequented by potentially flea infested animals, it is reasonable to assume that pets are under almost constant infestation pressure. Therefore, a recommendation of year-round and life-long flea control, in such situations seems prudent.

References

Numerous manufacturers of flea products often tout the speed of flea kill of their formulations. So what does speed of kill actually mean and why is it important? Understanding differences in residual flea adulticide flea treatments requires understanding the methods used in flea product evaluations. The important speed of kill evaluations of flea adulticides are “initial speed of kill” and “residual speed of kill”. These evaluations sound similar, but are actually quite different and the results of the different evaluations are extremely important. With “Initial speed of kill” evaluations the aim is to determine how quickly an adulticide kills fleas when it is first administered (topically or orally), and it may or may not be a predictor of the speed at other times during the post-treatment period. In contrast the goal of a “residual speed of kill” study is to determine the rate at which the product kills fleas that jump on the treated pet at some time point after treatment, often days or weeks. Residual speed of kill is likely far more clinically relevant as will be discussed later. In addition, a third type of study that may be combined with a residual speed of kill evaluation is called a “reproductive breakpoint” study. This type of evaluation provides information on the impact of a residual flea control treatment to prevent a flea population from maintaining itself.

“Speed of kill” studies tend to follow the same basic pattern. Efficacy of the product against fleas is calculated by comparing the number of fleas remaining on animals in one or more treatment groups compared with the number of fleas remaining on animals in a negative control group at pre-selected time points. Often a different group of treated and control animals is used for each of the pre-selected time points, but less commonly repeated measurements are made on the same animals over time. The typical “speed of kill” experimental protocol involves the following steps: Control group animals and treatment group(s) animals are initially infested with fleas 1 or 2 days prior to treatment application so that the infestation more closely mimics treatment of naturally infested animals having actively reproducing fleas. Animals (dogs or cats) are then treated on what is usually called “Day 0”.

- Fleas are carefully counted on animals from the treated and control groups at subsequent pre-selected time points (example 4, 8, 12, 24 & 48 hours; more or fewer time points may be chosen). Efficacy is calculated at each time point based on the number of fleas on control animals compared with treated animals. This is called an “Initial Speed of Kill” study and provides efficacy evaluations at specific time points post treatment.

- This is contrasted with a “Residual Speed of kill” study when at selected time-points after treatment the control and treatment group animals are re-infested with new fleas, and the process of counting fleas at each time point is repeated. These post-infestation time points are often 12 and 24 hours or 24 and 48 hours, however earlier time periods are occasionally used.

As an example the residual speed of kill of a monthly flea treatment for dogs could be measured by infesting dogs on days 0, 7, 14, 21 and 28 post-treatment and remove fleas on treated and control dogs at 12 and 24 hours after each infestation. Because speed of kill of residual adulticides slows with time, this portion of the study gives an indication of the time required to kill newly arriving fleas throughout the treatment period. For example, a flea treatment that kills 100% of fleas within 8 hours 7 days post-treatment, typically does not kill newly arriving fleas with the same speed 30 days later.

To get a clearer understanding of the importance of flea adulticide residual speed of kill it is necessary to understand a few facts concerning the reproduction of the cat flea, Ctenocephalides felis. Cat fleas initiate feeding within seconds to minutes of acquiring a host, mate within the first 12 hours and females typically starting to produce eggs at 24 hours post-infestation. These females will continue to produce eggs every day for several months, producing up to 40 eggs/day. Therefore, a topical or systemic flea treatment that has a rapid residual speed of kill, killing or rendering moribund all fleas
within 24 hours of them jumping on the dog or cat can prevent egg production. Preventing flea reproduction then allows the environmental flea population load will steadily decrease. However, this rapid 24 hour kill rate must be maintained at or close to 100% throughout the entire post-treatment period, otherwise fleas will survive and egg laying will start. Historically that was difficult to accomplish and insect growth regulators were often needed to kill the few eggs that were laid by female fleas living longer than 24 hours. If the reproduction inhibition drops below 100% before the 24 hour mark on any day during the treatment interval, then fleas will reproduce and will re-seed the environment with eggs. A topical or systemic flea treatment succeeds or fails based on its ability to control or not control flea reproduction. If fleas can reproduce in spite of the flea treatment, then no amount of treatment will eliminate fleas from the household.

So how do we evaluate a product’s ability to prevent reproduction between treatment applications? That can be accomplished by conducting a “reproductive break point” study. The aim of this type of study is to determine the time point following product administration when the residual speed of kill of the formulation slows sufficiently or ovicidal activity drops below 100%, to allow for viable egg production. In this study design treated and control animals are infested with fleas at specific intervals post-treatment and then 48 to 72 hours after each re-infestation, researchers carefully collect and count any flea eggs falling from the treated and control animals. The eggs are then incubated to determine viability, as indicated by the percent of eggs emerging to adult fleas. The study is designed to identify the approximately time post-treatment when viable flea eggs are first produced from fleas that re-infest treated animals, the “reproductive break point”. When evaluating the residual performance of a flea product, an effective product will have its “reproductive break point” at some time point after the next labeled reapplication interval. If a product is labelled for administration once a month and the reproductive break occurs around day 21, then the product likely will not be effective in eliminating a flea infestation. Considering the results of “residual speed of kill” and “reproductive break point” study together gives the best indication of the real value of a flea treatment. A treatment that can control 100% or close to 100% of flea adults within 24 hours for the full duration of its treatment interval (as shown in a speed of kill study), and that also does not allow a production of viable flea eggs within its treatment interval, can be used to drive fleas in a household into extinction. This is because the treatment will not allow adult fleas to survive long enough to reproduce; and there are no new viable flea eggs being added to the environment.

The use of a flea treatment that drives fleas into extinction within a household is still not quite the end of the story because there is a constant possibility of reintroducing fleas into the household from outdoor flea populations maintained on wildlife or in untreated households. Owners need to routinely reapply an effective the treatment at the recommended retreatment interval, and in most places this needs to be continued year round. A treatment gap will allow fleas to re-seed the environment with viable eggs.

Another important aspect of residual speed of kill is the potential impact it has on mangeing flea allergy dermatitis (FAD). Historically, products containing fipronil, imidacloprid, metaflumizone, selamectin and spinosad had clearly demonstrated their ability had a major impact on reducing the occurrence of FAD. However, the data from the several studies as reviewed by Dryden 2009 demonstrate that these compounds neither stop flea bites nor completely stop flea feeding. These compounds are very likely managing FAD because they decrease in prolonged flea feeding and reduce the amount of salivary protein delivered to the allergic pet and in the long-term reducing flea numbers. It is also worth noting that whether an insecticide works topically or systemically may be irrelevant in the management of fleas or FAD, what is most important is rapid residual speed of kill.

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

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

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

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

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

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

While recent pharmaceutical advances have been made in control of flea reproduction, such advances in the area of tick control are lacking. With the exception of the brown dog tick *Rhipicephalus sanguineus*, our ability to manage tick reproduction is limited, if not almost non-existent. As discussed previously in most flea infestations we have the opportunity to control flea reproduction by either killing fleas before they can reproduce or killing flea eggs. However, it is not just because we have effective...
residual insecticides, insect growth regulators or insect development inhibitors that we are successful, it is also due in large part to the fact we can often target the primary reproductive host, the flea infested dog or cat. And interestingly, failures in flea control often occur when flea infested feral pets or flea infested urban wildlife invade the owners yards.

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

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

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

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

Variation in product efficacy occurs. In two studies conducted at K-State, different results were found when evaluating the efficacy of acaricides against Dermacentor variabilis infestations in dogs from two different regions of the USA. In the first study, the efficacy of imidacloprid–permethrin and fipronil–(s)-methoprene formulations were evaluated against a D. variabilis isolate from California. The 48-h post-infestation efficacy on day 30 post-treatment was 92.0% and 83.2%, respectively, for the imidacloprid–permethrin and fipronil–(s)-methoprene formulations. In the second study, the 48-h post-infestation efficacy on day 30 for the imidacloprid–permethrin and fipronil–(s)-methoprene formulations against a *D. variabilis* isolate from Oklahoma was 17.5% and 75.7% respectively.

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

Recently a new class of insecticide/acaracide has provided the first orally administered approach to tick control. Afoxolaner and fluralaner are members of the isoxazoline class and work by inhibiting insect GABA and Glutamate-gated chloride channels leading to hyper-excitation and death of insects an arachnids.

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

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

Pet owners often view tick infestations of their pets differently than flea infestations. Whether this is due to concerns about tick transmitted diseases or simply a phobia, the presence of a couple of ticks
on the pet often elicits a more pronounced negative reaction than the presence of a couple of fleas. A 95% effective flea product may provide great client satisfaction while a similarly effective tick product may be perceived as a failure. Therefore it is not uncommon that label recommended application of a product does not appear to control the problem. This may be real or perceived, based upon pet owner expectations of product performance. Given pet owner concerns, a need to reduce tick borne disease and lack of 100% efficacy; occasionally additional control measures are needed.12,14 If additional control measures are deemed necessary, pet owners need to be educated as to why additional control measures are necessary and notations made in the pets record before extra label uses are conducted.

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

In some situations, especially in tropical and subtropical regions and in climate controlled kennels brown dog ticks may infest buildings with ticks crawling up walls, curtains and throughout the home or kennel.16,15 In these situations acaracides may need to be sprayed indoors into cracks and crevices, behind and under furniture or cages and along walls and the ceiling. Following application, make sure the acaricide is dry before you allow animals or humans back into the premises to minimize toxicity problems. Finally, restricting pet access from tick-infested environments may be necessary.

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

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TICK BORNE DISEASES IN NORTH AMERICA:
CLINICAL AND ZOONOTIC IMPLICATIONS
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In North America, fleas, mosquitoes and ticks are considered important vectors for a spectrum of infectious agents that can induce disease in dogs. The expanding number of known tick-borne organisms, the broad geographic distribution of many tick species, the ability of tick-borne organisms to induce chronic intravascular infections, and the highly pathogenic potential of some tick-borne organisms makes tick-borne infections the most important subset of canine vector-borne infectious diseases in North America and throughout much of the world. Several factors, including the ongoing Lyme Disease epidemic, suburbanization of tick habitat, the rapid increase in deer numbers as well as other wildlife populations that reside within the peri-domestic environment, the recognition that same species and strains of tick-borne pathogens can induce disease in pets and their owners, and the widespread availability of safe and effective acaricides have all contributed to enhance the awareness of tick-borne infections among both professionals and non-professionals. In conjunction with the above factors, there has also been a concurrent discovery of new tick-borne organisms, for which clinical, epidemiological and pathological data is minimal or lacking, particularly in regard to disease causation in animals. Examples include Borrelia lonestari, Borrelia turicatae, Panola Mountain Ehrlichia, Rickettsia felis, Rickettsia amblyommi and others.

For over two decades, our research group has contributed to the development of diagnostic, therapeutic and preventive strategies for the management of infections caused by tick-transmitted intracellular organisms. As a result of these research efforts, and those of many other investigators throughout the world, we continue to gain an increasingly unique perspective on the clinical and immunopathological consequences of tick-borne infectious diseases. It has been stated that: “Ticks are only interested in nutrition (a blood meal) and sex (i.e. perpetuation of the species).” Although the tick might object to this simplistic view of its complex lifestyle, bacteria, protozoa and viruses have used the predictable behavior of all tick species to facilitate their transmission and therefore the perpetuation of their species. Transmission of a tick-borne organism is most frequently accomplished when the tick obtains a blood meal; however, transmission can occur when the tick is inadvertently ingested by a dog (Hepatozoon canis or Hepatozoon americanum). In those instances, when tick-transmission is the sole means by which an organism such as Ehrlichia canis is transmitted from one infected dog to a previously non-infected dog, and when the dog is the only known reservoir host for E. canis, it becomes obvious that E. canis would evolve to be efficiently transmitted by a tick (R. sanguineus) for which all three tick life cycle stages (larvae, nymph and adult) preferentially involve feeding on dogs. It is equally obvious that E. canis would seek to induce long-lasting infection, accompanied by minimal pathogenicity to the dog (do not destroy the home you live in) and the organism would infect a cell (the monocyte) that would facilitate transfer of E. canis to additional blood seeking ticks. This evolutionary arrangement benefits E. canis, but does not appear to benefit the dog, which can develop disease manifestations ranging from epistaxis to pancytopenia. Although it is difficult to determine the factor(s) that induce disease causation when a dog is infected with a highly adapted vector-borne organism such as E. canis, it is certain that sequential or simultaneous infection with another vector-borne organism can contribute to more severe hematological or immunological aberrations and a more severe course of illness. Tick-borne organisms such as Anaplasma phagocytophilum and R. rickettsii typically induce acute, potentially severe illness, whereas other organisms such as Babesia canis, Babesia gibsoni, Bartonella vinsonii subsp. berkhoffii, Bartonella henselae and E. canis can induce chronic, insidious illnesses, accompanied by longstanding intravascular infections. As described briefly above, specific tick species preferentially transmit different pathogenic organisms, dogs can be sequentially or simultaneously infested with more than one tick
species, and a single tick can transmit more than one organism leading to co-infection. Both the tick species and the organisms that they transmit can vary substantially within and between various geographic regions. For example, infection with R. rickettsii, transmitted by Dermacentor variabilis in the state of North Carolina, occurs much more frequently in the piedmont region (central part of the state) as compared to the eastern coastal plain or the western Appalachian mountain range. All of the above factors make the diagnosis and medical management of tick-borne infectious diseases a complex and challenging task for the practicing veterinarian. Without question, the old adage “An ounce of prevention is worth a pound of cure” is applicable to any discussion of tick-borne infectious diseases. The advent of new, safe and long-lasting acaricides that can repel and kill ticks makes the prevention of tick-borne diseases an important priority for veterinarians and pet owners throughout the world. Based upon experimental infection studies, using tick attachment models, application of acaricide products can decrease the risk of transmission of Borrelia burgdorferi, the cause of canine Lyme borreliosis. Additional studies are needed to define the extent to which commercially available acaricides can prevent infection with various tick-borne organisms in North America.

SPOTTED FEVER GROUP Rickettsiae AND ROCKY MOUNTAIN SPOTTED FEVER

Spotted fever group (SFG) rickettsiae have been described from all continents. In North America, Rickettsia rickettsii is the most important SFG rickettsiae because this tick-transmitted organism can cause serious or fatal illness in dogs and people. The SFG group includes numerous closely related species including R. rickettsii (the type species), R. africae, R. akari, R. australis, R. conorii, R. felis, R. montana, R. parkeri, R. rhipicephali and R. sibirica, although many other SFG rickettsiae have been described. The typhus group rickettsiae, which includes Rickettsia typhi and Rickettsia prowazekii, have not been implicated as a cause of illness in dogs and experimental infection of dogs with typhus group rickettsiae in our laboratory did not result in disease. Throughout various regions of the world, spotted fever group rickettsiae are transmitted by Amblyomma, Dermacentor, Haemaphysalis, Ixodes and Rhipicephalus tick species. Regardless of the strain or species of SFG rickettsiae, these organisms generally induce an acute febrile illness secondary to endothelial cell damage, which results in vasculitis, altered vascular permeability, edema and necrosis. Although it seems likely that other SFG rickettsiae could induce disease in dogs, only R. rickettsii in North America and R. conorii in southern Europe have been documented as canine pathogens. Historically in North America, only Dermacentor variabilis (south and eastern United States), and Dermacentor andersonii (north western US and Canada) were known to transmit R. rickettsii to dogs or human beings. A recent outbreak of RMSF in Arizona was caused by Rhipicephalus sanguineus (The Brown Dog Tick). As discussed above, this tick species prefers to spend all three life cycle stages (larvae, nymph and adult) on a dog. When the environment is infested with large numbers of brown dog ticks or if dogs are removed from a tick infested house, human blood becomes an acceptable, if not an attractive alternative. In this setting, Anaplasma platys, Ehrlichia canis and R. rickettsii can be transmitted to human beings. In the context of morbidity, mortality and severity of disease, RMSF is the most important tick-borne infection of dogs in the United States. Due to variation in the severity and location of vascular lesions among different patients, veterinarians should anticipate a spectrum of disease manifestations following naturally-occurring infection with R. rickettsii. Much of the United States is considered endemic for the ticks (Amblyomma americanum, Dermacentor variabilis, Dermacentor andersonii, and Rhipicephalus sanguineus) that transmit R. rickettsii. Rickettsia rickettsii infection (“Rocky Mountain spotted fever”) also occurs in areas of Central and South America, where several outbreaks of fatal dog and human illness has been reported. Clinical abnormalities associated with RMSF include fever, anorexia, depression, mucopurulent ocular discharge, scleral injection, tachypnea, coughing, vomiting, diarrhea, muscle pain, neutrophilic polyarthritis, and a diverse group of neurologic signs including hyperesthesia, ataxia, vestibular signs, stupor, seizures, and coma. In some dogs weight loss is very severe, considering the short duration of illness. Poorly localizing joint, muscle and/or neurologic pain suggestive of polyarthritis, polymyositis, or meningitis may represent the only or most prominent clinical finding. Retinal hemorrhages are a consistent finding, but may be absent early in the course of the disease. Epistaxis, melena, hematuria, and petechial to ecchymotic hemorrhages
in the skin occur in some dogs, but may not develop unless diagnosis and treatment are delayed for five or more days after the onset of clinical signs. Scrotal edema, hyperemia, hemorrhage, and epididymal pain are frequently observed in male dogs. Signs associated with cardiovascular collapse, oliguric renal failure or brain death can develop in the terminal stages of the disease. Gangrene affecting the distal extremities, scrotum, mammary glands, nose or lips is associated with severe vascular obstruction and can induce substantial tissue loss, necessitating reconstructive surgery. Clinical manifestations in dogs are identical in most instances to manifestations reported in human patients. From a public health perspective, the dog is an environmental sentinel for RMSF and therefore it is important that veterinarians recognize and accurately diagnose RMSF. Diagnostic confirmation of RMSF in a dog allows the veterinarian to discuss the risk of *R. rickettsii* transmission in the peri-domestic surroundings, particularly as this pathogenic rickettsiae is transmitted transovarially among some tick species.

**CANINE FELINE AND HUMAN ANAPASMOSIS AND EHRLICHIOSIS**

*Anaplasma phagocytophilum*, transmitted by *Ixodes scapularis* or *Ixodes pacificus*, causes an acute febrile illness in cats, dogs, horses and humans, which is often accompanied by thrombocytopenia. *Anaplasma platys*, historically only thought to infect dogs, has been associated with infections in cats and humans. At least five *Ehrlichia* spp.; *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and the Panola Mountain *Ehrlichia* are capable of infecting dogs and people. In the United States, both *E. chaffeensis* and *E. ewingii* cause serious disease manifestations in people, including meningoencephalitis, acute renal failure and acute respiratory failure.

There have been considerable advances in defining the efficacy of various antibiotics for treatment of canine ehrlichiosis. It is now clear that 2 weeks of doxycycline is not an effective treatment for *E. canis* infection, whereas 4 weeks of therapy (doxycycline 5mg/kg every 12 hours) eliminates *E. canis* in both naturally and experimentally infected dogs. The prognosis following treatment for anaplasmosis and ehrlichiosis is generally very good. Dramatic clinical improvement usually occurs within 24 to 48 hours after initiation of doxycycline or tetracycline in dogs with acute phase or mild chronic ehrlichiosis; however, periods up to a year may be necessary for complete hematological recovery in chronically infected dogs. The long term prognosis following treatment is much more variable, potentially related to failure to diagnose concurrent infections. Undiagnosed infection with a *Babesia* or *Bartonella* spp. can be misinterpreted as an ineffective therapeutic response when treating ehrlichiosis, as doxycycline is generally an ineffective treatment for babesiosis and bartonellosis. Experimentally, enrofloxacin will suppress the clinical manifestations of *E. canis* infection and may result in hematological improvement, but does not eliminate the infection. Although imidocarb dipropionate has gained clinical acceptance in some endemic regions for treating severe, chronic, or presumed refractory cases of ehrlichiosis, lack of efficacy has been demonstrated in natural and experimentally infections.

**MOLECULAR DIAGNOSTIC TESTING AND VECTOR-BORNE DISEASES**

Because most vector-borne pathogens are difficult, if not impossible to culture from patient samples and because many animals achieve immunological clearance following transmission of the organism, the use of PCR to document active infection prior to or at the time of initiation of therapy or as an aid to document therapeutic elimination of the infection has gained acceptance among veterinary clinicians. PCR testing for *Anaplasma, Babesia, Cytauxzoon* (cats) *Ehrlichia*, hemotropic *Mycoplasma, Leishmania* and *Rickettsia* species is available through the: Vector-borne Diseases Diagnostic Laboratory, NCSU-CVM Rm 462A, 1060 William Moore Dr, Raleigh NC 27607, Phone: 919-513-8279, [www.cvm.ncsu.edu/docs/ticklab.html](http://www.cvm.ncsu.edu/docs/ticklab.html). BAPGM (*Bartonella* alpha *Proteobacteria* growth medium) enrichment culture/PCR for *Bartonella* species and *Anaplasma, Babesia, Ehrlichia*, hemotropic *Mycoplasma* and *Rickettsia* species PCR and human is available from: Galaxy Diagnostics Inc.,7020 Kit Creek Road Suite 130,Durham, NC 27709,[www.galaxydx.com](http://www.galaxydx.com), 919-313-9672
SIMULTANEOUS INFECTION WITH MULTIPLE VECTOR-TRANSMITTED PATHOGENS

Recently, simultaneous infection with more than one tick-borne pathogen has been recognized with increasing frequency in cats, dogs and human patients. Obviously, simultaneous infection with more than one tick-transmitted pathogen has important diagnostic, therapeutic and prognostic implications for the individual patient. For the most part, the pathophysiologic consequences of co-infection in dogs with various combinations of bacteria, rickettsia and protozoa have not been characterized clinically or experimentally. Although retrospective seroepidemiologic studies suggest that dogs may experience simultaneous infection with multiple tick-borne pathogens, microbiologic (culture) or molecular (PCR) evidence of simultaneous infection in dogs is currently limited. In nature, the risk of exposure to ticks, fleas, mosquitoes and biting flies is far greater for dogs than for human beings. In addition, dogs can be infested with hundreds of ticks, and at times infestation may involve different tick species. Therefore, the unknown influences of concurrent infection with multiple tick-borne pathogens, including *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Babesia* and *Bartonella* species, on factors such as pathophysiology, diagnosis, prognosis or therapeutic outcome could be more readily characterized in dogs. Of 27 dogs that were investigated in a kennel due to increased mortality, 25 were seroreactive to an *Ehrlichia* sp., 20 to a *Bartonella* sp., 17 to a *Babesia* sp. and 22 seroconverted to *R. rickettsii* antigen. Based upon PCR analysis, most dogs were co-infected with multiple *Ehrlichia* species, as well as a *Bartonella*, *Babesia* or *Rickettsia* species. Prospective evaluation of sick dogs, managed in our teaching hospital, has yielded molecular evidence of co-infection with multiple tick-transmitted pathogens. Our recent experience indicates that dogs with heavy tick exposure can be infected at a high rate with multiple, potentially zoonotic, tick-borne pathogens. It is imperative that veterinarians recommend and clients use acaracide products routinely and year around to prevent flea and tick-borne infections.

PUBLIC AND OCCUPATIONAL HEALTH CONSIDERATIONS

Due to extensive contact with a spectrum of animal species, veterinary professionals appear to have an occupational risk of infection because of frequent exposure to *Anaplasma*, *Bartonella*, *Ehrlichia*, hemotropic *Mycoplasma* spp. and potentially other tick borne pathogens, therefore these individuals should exercise increased precautions to avoid arthropod bites, arthropod feces (i.e. fleas and lice), animal bites or scratches and direct contact with bodily fluids from sick animals. For example, as *Bartonella* spp. have been isolated from cat, dog or human blood, cerebrospinal fluid, joint fluid, aqueous fluid, seroma fluid and from pleural, pericardial and abdominal effusions, a substantial number of diagnostic biological samples collected on a daily basis in veterinary practices could contain viable vector borne bacteria. The increasing number of defined flea and tick borne pathogens, in conjunction with the high level of bacteremia found in reservoir-adapted hosts, which represent the veterinary patient population, ensures that all veterinary professionals will experience frequent and repeated exposure to animals harboring these bacteria. Therefore, personal protective equipment, frequent hand washing and avoiding cuts and needle sticks have become more important as our knowledge of this genus has improved and various modes of transmission have been defined.

Physicians should be educated as to the large number of tick borne pathogens in nature, the extensive spectrum of animal reservoir hosts, the diversity of confirmed and potential arthropod vectors, current limitations associated with diagnosis and treatment efficacy, and the ecological and evolving medical complexity of these highly evolved intravascular and endotheliotropic bacteria.

References:


BARTONELLOSIS: A ONE HEALTH APPROACH TO AN EMERGING INFECTIOUS DISEASE

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*Bartonella* species are fastidious Gram-negative bacteria that are highly adapted to a mammalian reservoir host and within which the bacteria usually cause a long-lasting intraerythrocytic bacteremia. These facts are of particular importance to veterinarians and physicians, as an increasing number of animal reservoir hosts have been identified for various *Bartonella* species. Among numerous other examples, *Bartonella henselae* has co-evolved with cats, *Bartonella vinsonii* subsp. *berkhoffii* has co-evolved with dogs and wild canines, and *Bartonella bovis* has co-evolved with cattle. Importantly, the list of reservoir-adapted *Bartonella* species, including a large number of rodent species that might serve as “pocket pets”, continues to grow exponentially, as new *Bartonella* spp. are discovered. Prior to 1990, there were only two named *Bartonella* species, whereas there are now at least 32 named and numerous unnamed or candidatus species, based upon deposited Gen Bank sequences or preliminary reports, respectively.

In the natural reservoir host, chronic bacteremia with a *Bartonella* species can frequently be detected by blood culture or PCR in outwardly healthy individuals. In contrast, the diagnostic detection of a *Bartonella* spp. in a non-reservoir adapted host can be extremely difficult. Most, although not all diseases caused by *Bartonella* spp. occur in accidental hosts and these organisms are being increasingly implicated as a cause of zoonotic infections. Until recently, mechanisms that facilitate persistent *Bartonella* bacteremia in mammals were not well understood. Recent reports have identified an intracellular and intra-erythrocytic localization for these bacteria, which represents a unique strategy for bacterial persistence. Non-hemolytic intracellular colonization of erythrocytes and endothelial cells would preserve the organisms for efficient vector transmission, protect *Bartonella* from the host immune response, and potentially contribute to decreased antimicrobial efficacy. Other in vitro studies indicate that *Bartonella* spp. can infect dendritic cells, microglial cells, pericytes, monocytes and CD34+ bone marrow progenitor cells.

Epidemiology

*Bartonella henselae* was initially isolated from an HIV-infected human and subsequently from cats, dogs, horses, marine mammals and other small terrestrial mammals. *Bartonella vinsonii* subsp. *berkhoffii* was initially isolated from a dog with endocarditis in our North Carolina laboratory in 1993 and subsequently from cats, coyotes and human patients. Retrospectively, long-term administration of immunosuppressive doses of corticosteroids for a presumptive diagnosis of systemic lupus erythematosus with cutaneous vasculitis may have facilitated the isolation of the original type strain of *B. vinsonii (berkhoffii)* from this dog that subsequently developed endocarditis. Due to the relatively recent recognition that dogs can be infected with *B. vinsonii (berkhoffii)*, *B. henselae* and potentially other *Bartonella* spp., seroprevalence data is somewhat limited. Seroprevalence was determined in 1,920 sick dogs from North Carolina or surrounding states that were evaluated at a veterinary teaching hospital. Using a reciprocal titer of >32, only 3.6% of sick dogs had antibodies to *B. vinsonii (berkhoffii)*. Risk factors that could be associated with seroreactivity included: heavy tick exposure (Odds ratio 14.2), cattle exposure (OR 9.3), rural vs. urban environment (OR 7.1) and heavy flea exposure (OR 5.6). These data were interpreted to support the possibility that exposure to *B. vinsonii (berkhoffii)* was more likely in dogs in rural environments that were allowed to roam. In addition, these dogs were likely to have a history of heavy tick and flea infestations. Experimental flea transmission of *B. henselae* to dogs has now been
confirmed in the laboratory (Lappin and Breitschwerdt, unpublished data). Also, using sera from dogs experimentally infected with *R. rickettsii* or *Ehrlichia canis*, cross reactivity to *Bartonella* antigens was not detected. However, 36% of serum samples derived from dogs naturally infected with *E. canis* were reactive to *B. vinsonii* antigens. As *E. canis* is transmitted by *Rhipicephalus sanguineous*, this tick may be involved in the transmission of *B. vinsonii*. The possibility of tick transmission was further supported by two additional studies involving dogs infected with one or more *Ehrlichia* spp. from the same geographic region, in which seroreactivity to *B. vinsonii (berkhoffii)* antigens was 30% and 89%, respectively. Seroprevalence, using *B. vinsonii (berkhoffii)* antigens, was 10% (4/40 dogs) in dogs with suspected tick-borne illness from Israel and 36% in dogs with fever and thrombocytopenia from Thailand. Using an ELISA assay, 35% of 869 samples, derived from coyotes in California, contained antibodies to *B. vinsonii (berkhoffii)* antigens. Current data indicates that canine and human exposure to *B. henselae* and *B. vinsonii (berkhoffii)* can be found throughout much of the United States and most tropical and subtropical regions of the world.

Studies from Hawaii, the United Kingdom and Japan identified *B. henselae* seroprevalences of 6.5% (2/31 dogs), 3.0% (3/100 dogs) and 7.7% (4/52). In our laboratory, *B. henselae* is the most common *Bartonella* species found in sick dogs using the BAPGM enrichment blood culture platform. The pathogenicity of all *Bartonella* spp. in dogs is poorly understood; however, like humans, dogs can be infected with numerous *Bartonella* spp. *B. henselae* was amplified and sequenced from the liver of a dog with peliosis hepatitis, a unique pathological lesion also reported in *B. henselae* infected people. *B. henselae* DNA was also amplified from a dog with granulomatous hepatitis, a histopathological lesion reported with some frequency in *B. henselae*-infected children and adults. Similarly, *B. claridgeiae* DNA has been amplified and sequenced from the liver of a Doberman pincher with copper storage disease and from the aortic valve of a dog with vegetative valvular endocarditis. *Bartonella elizabethae*, a species that infects rodents, was found in a dog that had experienced chronic weight loss culminating in sudden unexplained death. Based upon a large seroepidemiological, controlled study from the University of California (Davis), dogs that were seroreactive to either *B. henselae*, *Bartonella claridgeiae* or *B. vinsonii (berkhoffii)* were referred for evaluation of lameness, neutrophilic polyarthritis, nasal discharge, epistaxis and splenomegaly.10

In cats, *B. henselae* and *B. claridgeiae* have been amplified or grown most frequently from cats and the fleas collected from cats. Flea-associated transmission has been well documented amongst cats. In flea endemic areas, *Bartonella* spp. seroprevalence rates in cats can be greater than 90% and bacteremia rates can be greater than 50%. Granulomatous myocarditis has been reported in cats, naturally or experimentally-infected with *B. henselae*. *B. vinsonii (berkhoffii)* caused recurrent osteomyelitis in a cat.3,11

**Pathogenesis**

Although as yet unproven, *B. vinsonii (berkhoffii)* is presumably transmitted to dogs by the bite of an infected tick. Based upon antidotal evidence, dogs may become infected with *B. henselae* by a cat bite or scratch, analogous to cat scratch disease in people. *B. vinsonii* appears to cause chronic intraerythrocytic and endothelial cell infections dogs for extended periods of time, potentially resulting in vasoproliferative pathologies. Similar to other highly adapted intracellular vector-transmitted pathogens, the factors that ultimately result in these bacteria causing disease manifestations are yet to be determined. If similar to babesiosis, another intraerythrocytic pathogen, stress, hard work, parturition, concurrent infection with other organisms or therapeutic immunosuppression may contribute to the development of pathology. Following experimental inoculation of SPF dogs with culture grown *B. vinsonii (berkhoffii)*, there was sustained suppression of peripheral blood CD8+ lymphocytes, accompanied by an altered cell surface phenotype and an increase in CD4+ lymphocytes in the peripheral lymph nodes.11 Therefore, infection with *B. vinsonii (berkhoffii)* appears to induce a degree of chronic immunosuppression that might predispose dogs to other infectious agents, resulting in a wide array of clinical manifestations in
naturally-infected dogs. Less is known about pathogenesis of disease in cats but some mechanisms are likely similar. Cats infected with *Bartonella* spp. are commonly co-infected with hemoplasmas and at times, more than one *Bartonella* sp. However, whether co-infections magnify disease manifestations of either genera is unclear and in most studies co-infections did not appear to potentiate illness. From an evolutionary perspective, it is obvious that vectors, vector-borne organisms, and animal and human hosts have developed a highly adapted form of interaction. In general, vectors need blood for nutrition; bacterial, rickettsial and protozoal organisms need an intracellular environment to survive, and immunologically, most hosts appear to be able to support chronic infection with many vector-borne organisms for months to years without obvious deleterious effects. These factors serve to illustrate the potential difficulty in establishing causation in cats, dogs or people co-infected with multiple tick-transmitted pathogens. Recently, we proposed an addition to Koch’s postulates entitled the Postulate of Comparative Infectious Disease Causation. By satisfying this postulate, *Bartonella* species appear to be able to cause, endocarditis, granulomatous inflammatory diseases, particularly involving heart, liver, lymph nodes, and spleen, persistent intravascular infections and the induction of vasoproliferative tumors.\(^{3,9,12-14}\)

**Clinical findings**

The spectrum of disease associated with *Bartonella* infection in dogs and most other animal species is currently unknown. Endocarditis, has been reported in cats, dogs and humans, infected with a spectrum of *Bartonella* spp. In some dogs, intermittent lameness, bone pain, epistasis or fever of unknown origin can precede the diagnosis of endocarditis for several months, whereas other dogs will present with an acute history of cardiopulmonary decompensation.\(^ {3,9}\) Cardiac arrhythmias secondary to myocarditis can be detected in dogs without echocardiographic evidence of endocarditis. Granulomatous lymphadenitis has been associated with *B. vinsonii (berkhoffii)* and *B. henselae* in dogs. *B. vinsonii (berkhoffii)* and other *Bartonella* species appear to contribute to the development of dermatologic lesions indicative of a cutaneous vasculitis, panniculitis, as well as anterior uveitis, polyarthritis, meningoencephalitis and immune-mediated hemolytic anemia.\(^ {3,10-15}\) Additional research efforts, using carefully designed case controlled studies are necessary to establish the frequency and extent to which *Bartonella* spp. contribute to dermatological, ocular, orthopedic, neurological or hematological abnormalities in dogs (and humans).

Clinically, many disease manifestations have also been attributed to *Bartonella* spp. infections in cats.\(^3\) However, it is very difficult to prove disease associations in cats in the field because of the high prevalence rates in non-clinical carriers. In research cats that are infected by exposure to *C. felis*, fever, endocarditis, and myocarditis are the most common disease manifestations. As discussed for dogs, additional case controlled studies are needed in cats.

**Diagnosis**

Thrombocytopenia, anemia, which frequently can be immune-mediated, and neutropenia or neutrophilic leukocytosis are the hematological abnormalities in dogs that are seroreactive or BAPGM enrichment blood culture/PCR positive.\(^ {12,15}\) Thrombocytopenia is found in approximately half, eosinophilia approximately one third of infected dogs and monocytosis frequently occurs in Bartonella endocarditis. Hematological abnormalities have been rarely reported in cats, but similar to dogs, a subset of Bartonella-infected cats are neutropenic. Serum biochemical abnormalities are usually very mild or nonexistent in both cats and dogs. In cats, *Bartonella* spp. antibodies have correlated with polyclonal hyperglobulinemia and hypoglycemia.\(^ {16}\)

As *B. vinsonii (berkhoffii)* antibodies are infrequently detected (<4%) in sick or healthy (<1%) dog populations in endemic regions, detection of *B. vinsonii (berkhoffii)* antibodies in a sick dog provides strong clinical evidence for prior exposure and potentially active infection. For this reason, treatment of seroreactive dogs or dogs from which any *Bartonella* spp. DNA is detected in blood or tissue samples would be recommended.
Isolation and Molecular Detection of Bartonella species

Because conventional microbiological isolation techniques lack sensitivity, bartonellosis is usually diagnosed by PCR amplification of organism specific DNA sequences and/or through serological testing. Recently, the development of a more sensitive isolation approach, using BAPGM (Bartonella alpha Proteobacteria growth medium) followed by real time PCR has greatly facilitated the molecular detection or isolation of Bartonella species from the blood of sick or healthy animals, including dogs and human beings.5,6,12 Obviously, the relative sensitivity of the diagnostic methods used to detect Bartonella species infection greatly influences an investigator’s ability to establish disease causation or a clinicians ability to initiate appropriate treatment. The use of this optimized microbiological approach has facilitated the recognition of blood-borne Bartonella spp. infections in dogs, horses, human beings and porpoises.17 Diagnostic testing (animals and humans) for Bartonella species (serology, PCR and BAPGM Enrichment Blood Culture/PCR) is available through Galaxy Diagnostics, Inc. (contact@galaxydx.com).

In cats, serology, PCR or culture combined with serology is recommended and can be procured at Galaxy Diagnostics Inc. and Colorado State University (www.dlab.colostate.edu).

Pathologic Findings

In dogs, pathologic findings associated with Bartonella spp. infection include endocarditis, myocarditis, granulomatous lymphadenitis, granulomatous hepatitis, osteomyelitis, bacillary angiomatosis and peliosis hepatitis.3,9,12 Multifocal areas of severe myocardial inflammation can be found in dogs with B. vinsonii (berkhoffii) endocarditis. Although not specific for bartonella infections, organisms can be detected in diseased tissues using silver stains, particularly in acute bartonella infections, such as acute regional lymphadenitis (cat scratch disease). During chronic infections, organisms are often too few in number to be detected in tissues by silver staining, unless a fulminate infection is localized to heart valves. The cardiac abnormalities noted in cats to date are similar to those described for dogs.9 It seems likely that the spleen plays an important immunomodulatory role in controlling persistent Bartonella spp. bacteremia in animals and people.13 The extent to which Bartonella spp. induce splenic pathology deserves additional research consideration.

Therapy

To date, an optimal protocol has not been established for the treatment of bartonella infections in cats, dogs, or people.3,17 Regardless of the antibiotic(s) that is used for treatment, a long duration of antibiotic administration (4-6 weeks) may be necessary to eliminate the infection. Due to the rapid development of resistance, macrolides (azithromycin) I no longer recommend these antibiotics for treating Bartonella infections. Fluoroquinolones in combination with doxycycline are currently being used by the author to treat clinical cases of bartonellosis, while exploring efficacy through experimental infection studies. Doxycycline alone does not appear to eliminate B. vinsonii (berkhoffii), B. henselae or B. claridgeae in cats, dogs or other animal species. Serum antibody titers decrease rapidly (3-6 months) and are generally no longer detectable in dogs that recover following antimicrobial therapy. Therefore, post-treatment serology may be a useful adjunct to BAPGM/PCR to determine if therapeutic elimination of bartonella infections has been achieved. Whether there is clinical benefit to follow serologic or molecular assay results in cats has not been widely studied, but most treated cats do not become seronegative in the short term. However, bacteremia can resolve after treatment or resolve spontaneously in some cats, whereas other cats remain bacteremic despite four to six weeks of antibiotic (documented for several antibiotic regimens) administration, and despite resolution of clinical abnormalities (such as lethargy, inappetance and fever).

Prevention

Although somewhat circumstantial, there is increasing evidence that Bartonella species can be transmitted by fleas and ticks to cats, dogs and human beings.15 Based upon scientific evidence generated during the past several decades, vector-transmitted pathogens can induce clinical manifestations ranging
from acute fatal illness (i.e. Rocky Mountain spotted fever, ehrlichiosis, babesiosis and bartonellosis) to chronic debilitating disease states (ehrlichiosis, babesiosis, borreliosis, and bartonellosis). Therefore, minimizing or eliminating flea and tick exposure is perhaps of greater veterinary and public health importance today, than during any previous time in history. When rigorous flea and tick control measures are instituted, it is highly probable that transmission of *Bartonella* species to pets and their owners will be greatly reduced or eliminated.  

Public and Occupational Health Considerations

There is increasing evidence to support an important role for *Bartonella* species as a cause of a spectrum of disease manifestations in human patients. Due to extensive contact with a variety of animal species, veterinary professionals appear to be at occupational risk for infection because of frequent exposure to *Bartonella* spp., therefore these individuals should exercise increased precautions to avoid arthropod bites, arthropod feces (i.e. fleas and lice), animal bites or scratches and direct contact with bodily fluids from sick animals. As *Bartonella* spp. have been isolated from cat, dog or human blood, cerebrospinal fluid, joint fluid, aqueous fluid, seroma fluid and from pleural, pericardial and abdominal effusions, a substantial number of diagnostic biological samples collected on a daily basis in veterinary practices could contain viable bacteria. The increasing number of defined *Bartonella* spp., in conjunction with the high level of bacteremia found in reservoir-adapted hosts, which represent the veterinary patient population, ensures that all veterinary professionals will experience frequent and repeated exposure to animals harboring these bacteria. Therefore, personal protective equipment, frequent hand washing and avoiding cuts and needle sticks have become more important as our knowledge of this genus has improved and various modes of transmission have been defined. Physicians should be educated as to the large number of *Bartonella* spp. in nature, the extensive spectrum of animal reservoir hosts, and the diversity of confirmed and potential arthropod vectors, current limitations associated with diagnosis and treatment efficacy, and the ecological and the medical complexities induced by these highly evolved intravascular, endotheliotropic bacteria.

References:
PAPILLOMAVIRUS ASSOCIATED DISEASES IN HORSES

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GENERAL OVERVIEW

Papillomaviuses (PVs) are small non enveloped DNA viruses with a tropism for squamous epithelial cells of the skin and of mucosal surfaces. They are pathogens in humans and animals and play a role in the development of benign and malignant neoplasias. They are very diverse and show a high species specificity. More than 150 different human PV types (HPVs) are known and it has been hypothesized that animals may also have their own diverse sets of PVs. So far 13 PVs have been identified in cattle (BPVs), 14 in dogs (CPVs) and 7 in horses (EcPVs) indicating diversity in these species.

The target cells of PV infections are basal squamous epithelial cells of the skin and of mucosal surfaces, as those are the only cells present in the epithelium capable of cell division. It is believed that PVs require sites of small injury to be able gain access to the basal cells. After infection there is an initial phase of genome replication, resulting in a cell containing few to a few hundred copies of the viral genome. After the early events in the PV life cycle each genome is copied on average about once per cell cycle in synchrony with the host genome. This type of maintenance replication is retained in the undifferentiated basal cells. The epithelial cell differentiation process in the infected suprabasal cells triggers various viral mechanisms leading to the production of more copies of the genome as well as late gene expression resulting in the production of viral particles in the superficial cells of the tissue. As PVs are not lytic viruses, the release of infective viruses is probably due to the normal death of at the surface of the epithelia supported however by viral manipulation of the cornified cell envelope. Throughout the PV lifecycle several viral proteins interact with the cellular machinery and are able to keep keratinocytes proliferating even after they lost contact with the basal membrane. The mechanisms by which PVs interact with their host cells differ among PVs but the proteins that interfere with the cellular lifecycle are primarily the early viral products E6 and E7 and, depending on the virus type, also E5.

PV infections, depending on the virus type, can also lead to the formation of different forms of papillomatosis. Besides classical warts those can be flat and plaque like papillomas, inverted ones or fibroblast involving ones (fibropapillomas). A significant amount of PV infections is probably subclinical, as viral DNA is often found in clinically healthy individuals; humans and animals. A classical PV infection results in the development of single or multiple benign proliferations characterized by a hyperplasia of the epidermis and hyperkeratosis. The course of the infection depends on several factors but usually consists of growth and regression phases that are sometimes interspersed by a phase of stagnation. Classical papillomas usually spontaneously resolve without treatment, and the induced immune response protects from re-infections with the same virus type. While the cellular component is responsible for terminating an acute (symptomatic) infection it is the antibodies that protect from re-infections. The current preventative HPV vaccine, which consists of genetically produced empty viral particles (L1 capsid protein), is based on the generation of protective antibodies, and not cellular immunity.

A small percentage of PV infections result in the development of squamous cell carcinomas. Extensive research in humans led to the recognition that PV infections are essential but not sufficient for the development of HPV associated cancers, such as cervical carcinomas. As PVs interfere with elements of the cell cycle regulation, persistent infection is seen as a significant risk factor for the acquisition of further alterations that may lead to a malignant transformation. The development of PV associated cancer or a severe papillomatosis is often associated with additional contributing factors, especially congenital or acquired immunodeficiencies. While smoking and HIV play important roles as additional factors in
humans, contributing factors in animals include primarily genetic defects or the application of immunosuppressants.\textsuperscript{1,13}

CLASSICAL EQUINE PAPILLOMAS

Classical equine papillomatosis is commonly seen in horses worldwide. It typically affects individuals ages 1-3 and does not seem to have any gender or race predilection. Symptoms range from few to more than 100 individual papillomas that can form confluent masses if growing close together.\textsuperscript{15,16} The individual papillomas vary in size and often have a diameter of only a few millimeters but occasionally also up to two centimeters. Lesions start as small smooth papules but with growth over time develop a hyperkeratotic and more irregular surface.\textsuperscript{14,15} Histologically they are characterized by marked epithelial proliferation, hyperkeratosis, parakeratosis, intranuclear inclusion bodies, ballooning degeneration, enlargement and increase of keratohyaline granuals, acantosis, koilocytes, hyperplasia and a decrease of melanin pigments.\textsuperscript{16} The primary and initial sites where the papillomas develop are the muzzle and lips, but the forelegs and chest can be involved as well.\textsuperscript{14,15}

The disease is likely to be transmitted primarily via direct contact from horse to horse. Shared halters, mangers, water dispensers or brushes etc. can act as fomites, considering the papillomaviruses are non enveloped and thus relatively stable in the environment. Flies may possibly act as a vector. Classical papillomatosis is a self-limiting disease in almost all cases. Symptoms peak and regress within a few to several months with a high variability. Although the disease usually does not pose a problem for the horse itself it can interfere with early training and temporarily affect the marketable value. A horse with symptoms of papillomatosis may also be banned from public events.\textsuperscript{14,15}

Classical papillomatosis has probably been well known for centuries, and its transmissibility, viral character and the development of immunity after an infection were shown.\textsuperscript{17,18} Viral particles and viral DNA have been found in samples of classical equine papillomatosis since and a PV was identified, sequenced and postulated as causative agent.\textsuperscript{19-21} Equine papillomavirus type 1 (EcPV1) is the first horse specific PV that was identified. Little is known about the biology of EcPV1 itself, but it is believed that mechanisms in classical papillomas across the species are somewhat similar.

As classical papillomatosis is generally a transient disease, treatment is usually not indicated. Overly long persisting papillomas could however indicate an immunosuppression. Intervention might generally be necessary, if papillomas become infected or physically interfere with eating or with the handling of the horse. Surgical removal, cryotherapy and radiofrequency hyperthermia have been used and are effective in getting rid of individual papillomas.\textsuperscript{14,15} This approach can be curative if only few papillomas are present and all are treated. Whether partial removal of a large papilloma burden has a positive or negative effect on the healing process is controversial.\textsuperscript{22} A variety of medical approaches to the problem have been suggested, but the transient character of papillomatosis makes the interpretation of results difficult. The more recently introduced treatment with 5% imiquimod cream has produced very promising results in various papillomatous lesions in the horse and could be applied, but as a side effect it induces significant local inflammation.\textsuperscript{23} To reduce the chance of spreading the disease, symptomatic horses should be separated and possible fomites disinfected.\textsuperscript{15} A specific prophylactic vaccination would be possible, but due the transient character and the limited economic impact of the disease, no commercial or experimental vaccine is yet available.

AURAL PLACUES

Equine aural plaques describes a form of papillomatosis with a very clear anatomical predilection. It can affect horses of all ages and there seems to be no predisposition of a specific gender or race. The nonpigmented plaques are raised only few millimeters above the surface of the skin, 2-20 mm in diameter and can be singular or multiple with a tendency to merge. Histologically they are characterized by hyperkeratosis, acanthosis, the presence of koilocytes and a decrease of melanin pigments. Equine aural plaques have only been reported from the concave side of the pinna.\textsuperscript{14,15,24,25}

The route of transmission has not yet been determined, however as aural plaques have been reported in association with black flies (Simulium spp) it was suggested that these insects that feed on blood may
act as a vector. While equine aural plaques are usually persistent and show a tendency to expand within
the pinna, they maintain a benign character. The disease can go along with moderate head shaking and
other signs of hypersensitivity about the head and ears, and thus may interfere with the handling as some
horses can become restive as a result. This as well as the cosmetic effect can have an impact on the
horse's marketable value.14,15,24

The etiology of equine aural plaques is not yet fully understood. The histological findings and
electron micrographs indicate a viral character with the possible involvement of an insect vector.24,25
Viral particles and viral DNA have been found in samples of aural plaques and several different PV types
were identified, sequenced and postulated as causative agents. EcPV types 3, 4 and 5 were found in
plaque samples and EcPV6 in the clinically unaffected ear of a horse with aural plaques.7,26,27 Nothing is
known about the biology of these particular EcPVs and little about plaque associated PVs in general.

Aural plaques are usually a persistent or progressive disease that goes along with mild or no
symptoms beyond the plaques themselves. An intervention may be indicated if horses are developing
head shaking or restive behavior. As flies seem to play a role in aural plaques fly protection for example
in the form of masks with ears may be sufficient to prevent head shaking and can also reduce the risk of
transmitting the disease.14,15 Treatment with 5% imiquimod cream has been used successfully in horses
with aural plaques.23 Due to the side effects of this immunomodulatory drug that involve local erythema,
erosions, exudation and crusting this therapy involves the risk that a horse may become head shy. A
specific prophylactic vaccination against the viruses would be possible, but mainly due the benign
character and limited economic impact no commercial or experimental vaccine is available so far.

GENITAL PAPILLOMAS

The term genital papillomatosis primarily describes benign lesions of the external genitalia. As there
is evidence that some of these may progress to squamous cell carcinomas these are sometimes also
referred to under this term. Genital papillomatosis can affect horses of all ages but is more common in
older individuals. Male horses seem to be more prone to develop genital papillomatosis, a race
predilection has not been shown. Symptoms range from few to hundreds of papillomas that can form
confluent masses. The papillomas vary significantly in size and shape. Often they appear in the form of
white or gray plaques but papules or nodules are possible as well. The histopathologic findings are
accordingly diverse but similar to other viral papillomas and usually share at least hyperkeratosis,
acanthosis and koilocytes. The primary sites where the papillomas start to develop are the distal part of
the penis and the vulval lips. But can affect multiple locations along the mucosa and skin of the external
genitalia.14,15,28,29

The disease might be regarded as a sexually transmitted disease; however, as artificial insemination
has become a widely used method and as geldings are affected too, insect vectors could be an alternate
route of infection. Genital papillomatosis is usually persistent and progressive and in later stages
ulceration, secondary infections and problems with urination may arise. Of most concern is the potential
development of squamous cell carcinomas as a late stage form of papillomatosis in some cases.14,15

A viral character of genital papillomas has been suggested as it was considered as a form of classical
equine papillomas. EcPV1 DNA is not found in genital papilloma, but the DNA of three different PVs,
namely EcPV2, EcPV4 and EcPV7 has been found.7,20 While the latter ones have only been found once
EcPV2 has been isolated from all different forms of genital papillomatosis in several countries. In situ
hybridization repeatedly demonstrated viral infections and antibodies in the blood horses support the viral
etiology.28,30,31 EcPV2 DNA has been found in the absence of papillomatous lesions, but with a low
incidence.28,32 The mechanisms of infection and progression have not yet been determined but an effect of
smegma build up was suggested.

Genital papillomas are usually a persistent and progressive disease that initially goes along with mild
or no symptoms. Due to the potential for malignant transformation, early intervention might be necessary
to prevent spread and to preserve the function of the genitalia as much as possible. Surgical interventions
on the affected parts of the penis may be curative14,15,33 A variety of medical approaches to the problem
have been suggested, but no well controlled data exist. Specific prophylactic vaccination would be possible and desirable, but no commercial or experimental vaccine is available yet.

EQUINE SARCOID

Equine sarcoid is by far the most common skin neoplasia in horses worldwide. Horses of all ages can be affected, but the onset of symptoms is often before 7 years of age. There seems to be no gender predilection in horses but certain breeds such as Appaloosas, Arabians, Quarter Horses and thoroughbreds might be at an increased risk. A genetic component associated with the alleles MHC-I A3 and W13 has been found. Clinically the equine sarcoid presents itself in different forms and a clinical classification into occult, verrucous, nodular, fibroblastic, mixed and malignant/malevolent types has been suggested. Horses often have more than one tumor and can have different of these types side by side. The histopathology varies from type to type with fibroblast proliferation being the main denominator. The primary and initial sites where the sarcoids develop are areas of thin skin or previous injuries.

The disease has been suggested to be transmitted from cattle to horses primarily via insect vectors and viral DNA has indeed been found in several species of flies near herds where sarcoids occurred. Direct contact and fomites probably play a role in the transmission from horse to horse. The equine sarcoid is a progressing disease and trauma can lead to more aggressive growth of the tumors. It can be locally invasive but does not metastasize and does not kill the horse, however the location and size of the tumors can cause a variety of problems and often compromise use and value of the animal.

Equine sarcoids have been known for a long time and an involvement of bovine papillomaviruses (BPVs) has been supported by the detection of BPV DNA. While most sarcoids are positive for BPV DNA the incidence in other lesions or normal skin is low. Most cases involve the type BPV1, but BPV2 and BPV13 may account for a number of equine sarcoids. The crossing of a species barrier is an exception among PVs; these particular types also induce fibropapillomas in cattle which is considered their natural host. The biology of BPV1 has been studied in more detail and it was found that it transforms cells differently than HPVs in case of cervical cancer. While BPVs go through their entire lifecycle in bovine tissues, viral particles have never been found in equine sarcoids.

Equine sarcoids are usually persistent and progressive and often require medical intervention. Surgery is commonly used but has a high recurrence rate with more aggressive behavior if large surgical margins are not possible or autoinoculation happens during the operation. Local vaccination with Bacillus Calmette-Guerin shows good results, but the risk of systemic inflammatory side effects requires attention and safety measures. Treatment with 5% imiquimod cream produces good results without systemic side effects but significant local inflammation. Antiviral medications such as Acyclovir have been tested and may be a promising alternative with little side effects for smaller sarcoids. A specific prophylactic vaccine for horses would be desirable and tests with an experimental vaccine showed very promising results, so far no vaccine is available on the market though.

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GENERAL

Viruses are very diverse entities at the edge of life and viral infections likely occur in all organisms. While many viral infections may go unnoticed and without any overt symptoms, viruses are primarily recognized due to their role as actual or potential pathogens of humans and animals. Although viruses are responsible for a variety of important diseases like AIDS, the flu or swine fever their role is often that of one paving a way for secondary bacterial infections by compromising important barrier functions or the immune system itself.¹³

The skin is a primary organ that viruses infect for some viruses such as the papillomaviruses and several types of herpes viruses. The skin is the primary site of initial infection, virus production and viral shedding for both the papillomaviruses and the cutaneous herpesviruses, and there is no significant viral production in other organs.⁴ A dermatological manifestation of a viral infection can on the other hand be part of a systemic viral infection as it is the case in foot-and-mouth disease or smallpox. Here the virus replicates in many organs after an oral or airborne infection. The lesions on the skin are part of a complex of symptoms and facilitate the spreading of the virus; at the same time these dermatological symptoms have a high diagnostic value.² Viruses may also have an indirect influence on the skin by compromising the immune system and thus making the organism in general more susceptible to all kinds of diseases as for example HIV. Despite some differences between human and animal dermatology much has been learned by comparative approaches, especially in terms of infections diseases.¹³ Cross species knowledge can thus be mutually beneficial, not only but particularly in terms of zoonoses.⁵

HERPESVIRUSES

Members of the herpesviridae family are responsible for a various diseases in humans and animals and several affect the skin. Herpesviruses become latent after infection and can be reactivated which happens in phases of short or persisting immunodeficiency.¹ In humans, infection and recurrent outbreaks of labial (HHV-1) or genital (HHV-2) blisters as well as chickenpox and zoster (HHV-3) are short lived in otherwise immunocompetent individuals. Infections with HHV-6 and HVV-7 (roseola infantum) cause a mild rash in infants but despite establishing latency do not cause clinical outbreaks later in life. Some herpesviruses, especially members of the subfamily gamma have the potential to transform infected cells and in humans are associated with Kaposi’s sarcoma (HHV-8), Burkitt’s Lymphoma and nasopharyngeal carcinoma (HHV-4). Kaposi’s sarcoma is very rare in the general population but occurs more frequently in immunosuppressed AIDS patients.¹² Herpes virus infections in animals often cause symptoms in other organ systems, but skin disorders do occur. Infections with feline herpes virus 1 can in rare cases cause an ulcerative dermatitis, with a predelection for the face.³⁶ Herpesvirus infection is also considered responsible for fibropapillomatosis in sea turtles, an emerging and possibly multifactorial disease.⁸⁹ Specific herpesviruses probably exist in many if not all tetrapod species.

Herpesviruses establish lifelong latency by hiding dormant in ganglia where they are invisible to the immune system. Effective medications against herpes viruses that can prevent clinical outbreaks or shorten its course do exist; viral clearance cannot be achieved though. Tumors may additionally be approached with surgery, radiation or chemotherapy.¹² A prophylactic vaccine against the mentioned viruses exists only for chickenpox/zoster.¹⁰ Herpesviruses are generally strictly species specific and thus the zoonotic potential is very low.¹
PAPILLOMAVIRUSES

Papillomaviruses are classical skin pathogens generally induce a variety of benign warts. These often affect young individuals with immune systems naïve to the virus. Exemplary for this are common warts in school-aged children, oral papillomatosis in dogs or classical equine papillomas in horses. The predilection sites for these kinds of warts vary from species to species but generally are sites where contact with others or fomites and minor injuries are common. Single or multiple papillomas at the same time are possible and the typical clinical picture is a scaling nodular, filiform, digitate or cauliflower like papule that only involves the epidermis. The virus is transmitted through direct or indirect contact and relies on small injuries as entry sites. The full virus lifecycle takes part in the epidermis. Most forms of papillomavirus induced lesions are transient, in some cases however papillomavirus induced lesions can progress and develop into squamous cell carcinomas. In those cases cofactors such as inherited or acquired immunosupression, smoking or UV exposure play an important role as well. The number of different papillomaviruses is high and increasing (>250), most of the known ones are human specific types (HPVs). The properties of these viruses differ and thus the predilection sites, route of transmission, clinical picture and prognosis vary for individual papillomavirus types.

Most of the skin lesions induced by papillomavirus infections are benign and self-limited. The immune system is usually able to clear the infection and antibodies protect from re-infections of the same virus type. Several surgical and medical regimes have been established and may be used on a case by case basis in humans. Medications to treat papillomatosis in animals are not as well established yet. Two effective prophylactic vaccines against certain human papillomaviruses have been approved and were introduced within the past decade. These approved vaccines target specific HPVs that cause cervical and other anogenital carcinomas, other HPV types may have a role in the development of certain non melanoma skin cancers. Experimental prophylactic vaccines exist also for some canine papillomaviruses (CPV1 and CPV2) and for the virus inducing most equine sarcoids (BPV1). Papillomaviruses are generally strictly species specific and thus the zoonotic potential is very low.

PARAMYXOVIRUSES

Some members of the paramyxoviridae family induce very distinct skin symptoms although they primarily infect the respiratory system. Measles and a rare chronic form of canine distemper are the most important types in dermatology. The measles virus itself only infects humans but originally derived from the extinct rinderpest virus. In case of measles the characteristic red maculopapular lesions start after an incubation and initial disease period and spread from the head over the entire body. The rash is transient and short lived while the disease can have serious non-dermatologic complications. In dogs that went through an infection with the canine distemper virus, hyperkeratosis of the footpads (hard pad disease) can be a late effect.

Measles virus infections induce easily detectable, self-limited skin lesions as part of a systemic disease for which no specific therapy exists. The maculopapular eruptions spontaneously regress once the immune system overcomes the viral infection. Vaccines against paramyxoviruses induced diseases exist and are commonly used (measles and distemper). Vaccination led to the eradication of the paramyxovirus responsible for rinderpest. Despite the origin of measles virus the anthropozoonotic potential of papamyxoviruses can be regarded as low. The transmission of distemper between animal species however occurs occasionally.

PARVOVIRUSES

Some members of the parvoviridae family are pathogens in humans and animals. The only member that is relevant in dermatology is human parvovirus B19 that causes erythema infectiosum (Fifth disease). This common and usually mild human disease typically affects children and induces a reticulated erythema on cheeks, trunk and distal extremities; most infections are asymptomatic though. The B19 virus induces neutralizing antibodies that protect from re-infections. The virus is transmitted through infectious respiratory secretions. Dermatological complications are rare. Erythema infectiosum is transient and the rash painless. Symptoms regress once the immune system overcomes the
virus. No specific treatment or vaccines exist.\textsuperscript{2,21} Despite the history of the canine parvovirus the overall zoonotic potential of parvoviruses seems to be very low.\textsuperscript{1,22}

**POLIOMAVIRUSES**

Members of the *polyomaviridae* family have been found to infect humans and a variety of animal species. Most polyomavirus infections are latent but asymptomatic. Some polyomaviruses have the potential to transform cells, somewhat similar to papillomaviruses. Some members of this family can infect keratinocytes within the hair follicle, and one human polyomavirus, called merkel cell polyomavirus, is associated with merkel cell carcinoma.\textsuperscript{1,23} This is a rare but fatal cancer that occurs in older individuals or in immunocompromized individuals (patients with chronic lymphocytic leukemia, AIDS, organ recipients). It occurs in UV-exposed areas of the skin.\textsuperscript{1,2} Another polyomavirus induced skin disease in humans is Trichodysplasia spinosum that is characterized by fragile white spinous processes extending from hair follicles instead of hair. It is very rare and only seen in immunocompromized patients.\textsuperscript{1} Hamster polyomavirus and the mouse polyomavirus can induce epitheliomas in the respective species, which appear as uniform nodules arising multicentrically from a proliferation of the hair root epithelium.\textsuperscript{24,25}

Dermatologic symptoms caused by polyomavirus infections are progressive, but there are no specific treatments for polyomavirus infections.\textsuperscript{1} The methods of choice for merkel cell carcinoma are surgery, radiation or chemotherapy. No vaccines exists.\textsuperscript{1,2} Avian polyomaviruses seem to have a low species specificity among birds. There is no indication however that this is true for the mammalian ones, thus the zoonotic potential is very low.\textsuperscript{1}

**POXVIRUSES**

Members of the *poxviridae* family are responsible for a variety of diseases in humans and animals.\textsuperscript{1,2,26} Especially smallpox, as a very serious and often lethal human disease has strongly influenced our view of these viruses and the understanding of viral diseases in general.\textsuperscript{27} Most infections with poxviruses result in a characteristic maculopapular rash and raised fluid filled blisters in later stages. Some pox viruses induce systemic infections with skin pathology (smallpox, sheeppox, goatpox), while others ones primarily induce skin lesions (molluscum contagiosum, cowpox).\textsuperscript{27,28} The virus is transmitted by direct contact or contact with infectious material such as crusts from the lesions. Systemic poxvirus infections involving can ha ve a high mortality, while skin-only forms are transient and cleared by the immune system. The organism produces neutralizing antibodies, which protect from re-infections. Different types of poxviruses exist in a variety of mammalian as well as in avian species.\textsuperscript{1,26,27}

Poxvirus infections induce easily detectable self-limiting skin lesions that can be part of a systemic disease for which no specific therapy exists. Papules and blisters resolve spontaneously once the immune system overcomes the virus but scars may remain. Destructive modalities and medications are sometimes used to hasten resolution in cases of molluscum contagiosum.\textsuperscript{2,27,28} An attenuated live vaccine derived from a cowpox virus (vaccinia) was used to eradicate smallpox and is also effective as a prophylactic vaccine against other related poxviruses.\textsuperscript{29} Poxviruses are zoonotic pathogens. Transmissions from monkeys to humans occur infrequently, primarily in Africa.\textsuperscript{30} More common is the transmission of cowpox viruses to humans and other mammalian species like cats or rodents.\textsuperscript{31} The orf virus, which is a poxvirus of small ruminants can also infect humans and induce orf disease.\textsuperscript{32}

**PICORNAVIRUSES**

Some members of the *picornaviridae* family induce very distinct skin symptoms, which are secondary to a systemic infection. The predilection sites of the relevant viruses are the oral cavity and the distal extremities and are eponymic for “hand, foot and mouth disease” (HFMD) as well as for “foot and mouth disease” (FMD).\textsuperscript{1,2,33} HFMD typically affects young children but adults can be infected as well. Most cases are caused by coxsackievirus A16, some by enterovirus 71 or other types. Individuals develop ulcerations in the oral cavity and erythematous papules on hands and feet that evolve into ovoid, gray vesicles. FMD symptoms are similar and very prone porcine and bovine hosts and very mild in ovine and
caprine hosts. Swine vesicular disease (SVD) is also a picornavirus caused disease with identical symptoms. While HFMD is generally a very mild disease, FMD and SVD result in weight loss, growth depression and reduced milk production in livestock. Mortality even in case of FMD is low, however the economic losses significant and eradication programs were and are in effect in many countries. Picornaviruses are stable in the environment and thus can be transmitted directly or indirectly.

The skin lesions induced by the described picornavirus infections are benign and self-limiting. The immune system is usually able to clear the infection and antibodies protect from re-infections. No specific treatment exists for either disease. A vaccine for FMD is available, however vaccination is not allowed in most western countries in order to maintain the FMD-free status. A vaccine for HFMD targeting enterovirus 71 is in development. No SVD vaccine is on the market. Some picornaviruses are zoonotic pathogens. MKS can infect cows, pigs, goats and sheep, but also a variety of other species including humans in rare cases, but symptoms are very mild in those cases.

TOGAVIRUSES

Several members of the Togaviridae family induce skin rashes that are part of a complex of merely unspecific systemic symptoms. The clinically relevant members of this family, which are rubella virus, chikungunya-virus, o’nyong-nyong-virus, sindbis-virus and Ross river-virus, infect only humans. Rubella is acquired as an airborne pathogen, while many other togaviruses are arthropod-borne pathogens. The symptoms of a rubella infection resemble somewhat those of measles with a rash starting out from the face and spreading over the whole body. Dermatological complications are rare.

Rubella virus infections induce easily noticeable self-limiting skin lesion as part of a mild systemic disease. No specific therapy exists, however the maculopapular eruptions spontaneously regress once the immune system overcomes the virus. A combination vaccine that also protects against measles virus is recommended in wide use. There is no indication that these viruses are pathogenic for animals.

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FRIDAY
RESEARCH SHORT COMMUNICATIONS
AND
CLINICAL SHORT COMMUNICATIONS
FRIDAY
9:00  RESIDENT RESEARCH AWARD PRESENTED

9:15  Rodrigues-Hoffmann  The skin microbiome in an allergen sensitized canine model of atopic dermatitis

9:30  Smith  Fungal microbiome of healthy and allergic canine skin: a next-generation sequencing study

9:45  Smith  A diagnostic tool for the veterinary dermatologist and pathologist: panfungal polymerase chain reaction (PCR) on formalin-fixed paraffin embedded (FFPE) tissues to classify fungal organisms found histologically

10:00  Zewe  Development of a real-time PCR technique to detect *Sarcoptes scabiei* in canine samples

10:15  Frank  Taqman qPCR technique for the diagnosis of dermatophilosis in horses

10:30 – 11:00  BREAK

11:00  Mueller  Can a steam-cleaner reduce the load of bacterial and fungal organisms on veterinary clinic floors?

11:15  Marsella  Effects of PAR2-antagonist on PAR2, TSLP, and epithelial tight junction ZO-1 in canine primary epithelial keratinocytes

11:30  Santoro  Alterations of the filaggrin metabolism is present in atopic skin: a pilot study using a canine model of atopic dermatitis
<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
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<tr>
<td>11:45</td>
<td>White</td>
<td>Single-blinded, randomized, placebo-controlled study on the effects of ciclosporin (CsA) on cutaneous barrier function and immunological response in experimentally-induced atopic beagles</td>
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<td>12:00</td>
<td>Plunkett</td>
<td>Determining potency of allergen extracts used for immunotherapy using major allergen (MA) assays</td>
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<td>12:15</td>
<td>May</td>
<td>Antibacterial effect of acetylcysteine on common canine otitis externa bacterial isolates</td>
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<td>12:30 – 2:00</td>
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<td>Wancura Marcuz</td>
<td>Influence of sexual gender and reproductive status on Fel d 1 concentration in the fur of domestic cats (Felis catus)</td>
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<td>2:15</td>
<td>Wancura Marcuz</td>
<td>Evaluation of the effectiveness of an allergen modulating solution on minimization of Fel d 1 concentration in the fur of cats (Felis catus)</td>
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<td>Devaki de Assuncao</td>
<td>Evaluation of the concentration of allergens from house dust mites in the haircoat of household dogs (Canis lupus familiaris) in Paraná – Brazil</td>
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<td>Park</td>
<td>Clinical features and comparison of treatment efficacy in 289 dogs with atopic dermatitis</td>
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<td>Tater</td>
<td>Patient adherence patterns for veterinary allergen-specific immunotherapy</td>
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<td>3:15</td>
<td>Thomas</td>
<td>Ectoparasites of free-roaming domestic cats (Felis catus) presented to a spay-neuter clinic in Oklahoma, USA</td>
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<td>Petersen</td>
<td>Removal of 17 canine and feline ear masses aided by a holmium:YAG laser</td>
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<td>Kaimio</td>
<td>A comparative study of otic histology, disease history and clinical findings of ear and skin disease in 29 American cocker spaniels</td>
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<td>4:45</td>
<td>Tham</td>
<td>Protozoal nodular dermatitis and panniculitis in a puppy caused by <em>Caryospora bigenetica</em></td>
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The skin microbiome in an allergen sensitized canine model of atopic dermatitis

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Abstract: Human and canine atopic dermatitis (AD) share many similarities, affecting approximately 15% of children, 2% of adults, and 10% of dogs in the United States. This study evaluated changes in the skin microbiome in a canine model of AD, and also compared the skin microbiome between the AD dogs and healthy pet dogs. Eight atopic dogs and eight healthy pet dogs were enrolled in this study. The atopic dogs were sensitized with a suspension of house dust mites and samples were collected prior to and at several timepoints after the challenge. The 16S rRNA gene was amplified and sequenced. The AD dogs displayed differences in bacterial groups on the allergen sensitized skin site, with increases in the proportions of Staphylococcaceae, Streptococcaceae, and decreased proportions of Fusobacteriaceae. The pre-sensitized samples from the AD dogs were significantly different from healthy pet dogs, with increases in Firmicutes and Actinobacteria, and decreases in Gammaproteobacteria. Given the many similarities between human and canine AD, this canine model could be used as a tool to study the role of the skin microbiome during AD flares.

This study was supported by a grant supported by the American College of Veterinary Dermatology.

Conflict of Interest: None declared.
**Fungal microbiome of healthy and allergic canine skin: a next-generation sequencing study**

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**Abstract:** This study utilizes next-generation sequencing to describe the natural fungal microbiome (mycobiome) of canine skin and to identify changes in the mycobiome when allergic skin disease is present. Superficial skin swabs from ten dogs with healthy skin and eight dogs with allergic skin disease were collected from seven haired and three non-haired sites. The DNA was extracted from skin swabs and the Internal Transcribed Spacer (ITS) region was sequenced at MR DNA (Shallowater, TX) on an Illumina MiSeq instrument. The total number of ITS sequences for 144 samples was 5,604,145, with the median number of sequences per sample being 38,680. Sequences were analyzed using the open-source QIIME software. *Cladosporium* and *Alternaria* were the most prevalent fungal genera identified. Other significant genera identified included *Malassezia, Fusarium, Cryptococcus, Claviceps*, and *Wallemia*. In the healthy dogs, the greatest diversity (number of observed species) in fungal genera was found on the dorsal aspect of the nose, while the least diverse sites included the nostril and conjunctiva (p <0.002). Furthermore, when comparing the number of observed species at each site between the healthy and allergic dogs, the fungal genera in allergic dogs was significantly less diverse at three sites (ear, lumbar and nostril) (p<0.05). When performing ANOSIM on the Bray-curritis distance matrix, the composition of fungal communities in healthy dogs was significantly different compared to the allergic dogs (p<0.05). These novel findings infer that, besides the changes previously described by the authors in the bacterial microbiome, the mycobiome is also affected in allergic skin disease.

Source of funding: American Kennel Club Canine Health Foundation ACORN grant; ACORN No. 02111-A: *The skin mycobiome (fungal microbiome) of healthy and allergic dogs*

Conflicts of interest: None declared.
A diagnostic tool for the veterinary dermatologist and pathologist: panfungal polymerase chain reaction (PCR) on formalin-fixed paraffin embedded (FFPE) tissues to classify fungal organisms found histologically


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ABSTRACT: Classification of fungal organisms often poses a problem for pathologists since fungal organisms can have very similar morphologies histologically. Furthermore, fungal culture can take long periods of time, or fresh tissues might not have been collected and submitted for culture. The purpose of this study is to validate the use of panfungal primers via PCR to classify fungal organisms on FFPE tissues. Samples from nineteen cases where fungal organisms were observed histologically were selected and included in this study. These included tissues from canine, feline, equine and bovine with cutaneous, nasal, and pulmonary fungal infections. DNA was extracted and isolated from FFPE tissues using the BiOstic FFPE Tissue DNA isolation kit (MoBio Laboratories, Carlsbad, CA). PCR was performed using ITS3 (5’-GCATCGATGAAGAACGCAGC-3’) and ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) primers targeting the Internal Transcribed Spacer (ITS) region (found in all eukaryotes). Positive bands were sequenced and classified at >97% identity match using the basic local alignment search tool (BLAST) and the NCBI database of ITS sequences. Of the 19 cases, 15 (79%) were PCR positive, nine confirmed the histologic diagnosis to the species level, three cases had an identity match where fungal organisms were seen, but morphology did not allow histologic classification, and three had inconclusive sequencing results. Of these cases, 4 were confirmed with immunohistochemistry and one was confirmed with fungal culture. Further studies are warranted, especially with cases confirmed by fungal culture and other ancillary tests, to continue to validate this protocol.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Development of a real-time PCR technique to detect *Sarcoptes scabiei* in canine samples

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**Abstract:** *Sarcoptes scabiei* infestation in canids remains a diagnostic challenge, given the low sensitivity of superficial skin scrapings. The objective of this project was to develop a highly sensitive new test to diagnose this condition. Our hypothesis is that a real-time PCR test performed on superficial skin scrapings or on paraffin-embedded biopsies will be highly sensitive and specific for *S. scabiei* infestation. Skin scrapings were obtained with a spatula from dogs with a diagnosis of scabies based on clinical signs, positive skin scrapings and response to treatment. The samples were analyzed for the presence of *S. scabiei* mites, mite parts, and eggs. Material from the scrapings was collected with a sterile cotton-tipped applicator, then frozen. DNA extraction was performed using the MoBio Powersoil DNA extraction kit (MoBio Laboratories, Inc., Carlsbad, CA). Additionally, DNA was extracted from skin biopsies of two dogs with a clinical diagnosis of scabies where *Sarcoptes* mites were found histologically. The DNA was extracted from the formalin-fixed and paraffin-embedded samples with the QIAamp® DNA FFPE Tissue Kit (Qiagen, Valencia, CA). Skin scrapings from healthy dogs were used as negative controls. Different sets of primers targeting the mitochondrial r16S DNA of *S. scabiei* were tested. The best results were obtained with the primers F-5’GCTGTTAATAACACTAAGGTAGCG-3’, R-5’TCCCTTCATACAAGTTACCAATT-3’, that amplify a 60bp DNA fragment of DNA. Test runs showed uniform and consistent peaks on known positive skin scrapings and paraffin-embedded biopsy samples. No amplification was observed on negative samples. Agarose gel electrophoresis confirmed a 60bp product specific for *S. scabiei*, as expected.

Source of funding: This study was funded by the Companion Animal Health Fund at the Cummings School of Veterinary Medicine at Tufts University

Conflict of interest: none reported
Abstract: *Dermatophilus congolensis* causes a crusting skin disease in horses. Diagnosis requires the demonstration of organisms from cytology of a crust and can be insensitive, especially in chronic cases. Because the organism is unique among bacteria, molecular diagnostic approaches would allow the identification of this organism from crusts contaminated with mixed bacterial infections. A qPCR probe and primers were designed using Primer3 software targeting a putative mannose-6-phosphate isomerase gene. The design was based on *Dermatophilus congolensis* genomic sequence data available from the Department of Energy Joint Genome Institute (Project ID: 1010677). The qPCR had a limit of detection of approximately 15 cloned DNA copies per sample. Crusts collected from 14 horses with suspected dermatophilosis and 12 horses with “nondermatophilosis-like” single crusts were evaluated by cytology and qPCR. Up to 10 high powered fields were examined and the presence of *Dermatophilus* and other bacterial organisms were recorded. Hairs collected from seven healthy horses were also evaluated by qPCR. Cytology was positive for *Dermatophilus* from nine horses with suspected dermatophilosis, with only rare organisms seen in five. Cytology from all other crusts was negative for *Dermatophilus*. Other bacterial organisms were detected on cytology from 15 crust samples. qPCR for *Dermatophilus* was positive from 11 suspect dermatophilosis crusts; all other samples were negative. Two samples were cytologically negative but qPCR positive for *Dermatophilus*. No samples were cytologically positive but qPCR negative. Results of this study show qPCR may be a more sensitive and easier method than cytology for diagnosing dermatophilosis in horses.

This study was self-funded.

Conflict of interest: None declared.
Can a steam-cleaner reduce the load of bacterial and fungal organisms on veterinary clinic floors?

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Abstract: The purpose of this study was to evaluate bactericidal and fungicidal capacities of a steam-cleaner (AFG 6000, Asum Favorit GmbH, Neusäss, Germany) in an experimental setting. Defined areas (6 x 6 cm) of various floor types (ceramic tiles, polyvinyl chloride, linoleum) were coated with representative organisms and were subsequently cleaned with the steam cleaner for 1 or 3 seconds (s). Suspensions of bacteria in bovine serum albumin (Staphylococcus pseudintermedius (S.p.) or Pseudomonas aeruginosa (P.a.)) in two concentrations (McFarland (McF) 1 and McF 4) and as biofilm structure, as well as Microsporum canis (M.c.) spores were used. To retrieve surviving organisms after steam cleaning, all tiles were rinsed for 15 minutes in physiologic saline and serial dilutions plated on appropriate agars. Colony forming units (CFU) were counted and the mean reduction in CFUs was calculated. S.p. numbers were reduced on all floor types by more than 99.9% (compared to non-steam cleaned controls). For the highest concentration (biofilm created overnight), steam-cleaning for 1 and 3s reduced CFUs by >92%. When P.a. was used, only steam cleaning applied to McF 4 produced significant reductions. Mean reduction on all floor types was 99.97% when applied for 1s. Biofilm created overnight was reduced by >95 % after 1s and 3s steam cleaning. M.c. spores were reduced by > 99.9% after 1 and 3s. The steam-cleaner AFG 6000 reduces bacterial organisms and fungal spores on various floor types and may be useful to limit nosocomial infections in veterinary clinics.

Source of funding: The steam cleaner for this study was provided by Animal Dermatology Deisenhofen.

Conflict of interest: None declared.
Effects of PAR2-antagonist on PAR2, TSLP, and epithelial tight junction ZO-1 in canine primary epithelial keratinocytes

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Abstract: Protease Activated Receptor (PAR)2 plays a crucial role in inflammation and stimulates thymic stromal lymphopoietin (TSLP) promoting T-helper 2 cytokines in allergic diseases. In our previous studies, PAR2 and TSLP did not stain homogeneously in the skin epithelial layer and tight junction zonula occludens 1 (ZO-1) was defective in the experimental canine AD model. This study aimed to investigate effects of a PAR2-antagonist (PAR2-ant) on PAR2, TSLP, and ZO-1 in canine primary epithelial keratinocytes (CPEK). CPEK was cultured with serine protease alone and with PAR2-ant for 1hr, 3hrs, 6hrs, and 24hrs. Slides were stained by immunofluorescence. Four images/slide were included in each time and condition. Intensity of PAR2, TSLP, ZO-1 in the cells were quantified by Image J. Intensity for PAR2 was significantly increased 1 hr later ($P < 0.001$) by serine protease treatment, while ZO-1 was significantly decreased ($P < 0.05$). Then, they gradually returned to baseline. PAR2 and ZO-1 were both undetectable after 24 hrs. TSLP intensity was not significantly changed over time. PAR2-ant significantly suppressed activation of PAR2 and TSLP by serine protease at all times. PAR2-ant significantly increased ZO-1 at 1hr ($P < 0.001$). PAR2-ant also suppressed the expressions of PAR2 and TSLP compared to base line in the slides not treated with protease. These findings suggest that blocking of PAR2 could suppress inflammatory mechanism of TSLP and could preserve the tight junction protein by protease-contained allergens in the dog skin. Further investigation of its action on atopic keratinocytes in vitro and vivo is warranted.

Conflict of interest: None declared.
Alterations of the filaggrin metabolism is present in atopic skin: a pilot study using a canine model of atopic dermatitis

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Abstract: Filaggrin and its metabolites are essential for skin barrier function and hydration of the stratum corneum. Alteration of the filaggrin metabolism could be the basis for an abnormal skin barrier in allergic dogs. The aim of this study was to investigate the expression and distribution of four enzymes involved in filaggrin metabolism (furin, matriptase, caspase-14, and calpain-1) in the skin of atopic and healthy beagles. Skin biopsies were collected from four healthy and four atopic beagles before and after allergen exposure. The dogs were challenged for three consecutive days to mimic an acute exposure, or once weekly to mimic a chronic exposure to allergens. Skin biopsies were taken on day 0, 3 and 10 in the “acute” model and healthy dogs, and on day 0, 14, and 28 in the “chronic” model. Indirect immunofluorescence was used to analyze the distribution and the expression of filaggrin enzymes. Five pictures/section were taken and the intensity analysed tracing the epidermis and using ImageJ on the traced areas. The enzymes’ levels were compared on day 0 among the three groups and over-time in each group. All the enzymes were expressed in all the epidermal layers. A significantly higher expression of calpain-1 [p=0.028 (acute); p=0.048 (chronic)] and matriptase [p=0.016 (acute)] was evident in atopic compared to control dogs on day 0. No differences between groups were seen in furin (p>0.1) or caspase-14 (p=0.079) expression. No over-time differences were seen for any enzyme analyzed. This preliminary data suggest an abnormal catabolism of filaggrin in atopic skin.

Source of funding: This project was self-funded

Conflict of interest: None declared.
Single-blinded, randomized, placebo-controlled study on the effects of ciclosporin (CsA) on cutaneous barrier function and immunological response in experimentally-induced atopic beagles

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Abstract: Ciclosporin (CsA) is a common treatment for canine atopic dermatitis (cAD). The purpose of this study was to describe the clinical and immunological effects of CsA using a canine model of AD. Fourteen beagles were enrolled; seven received CsA orally every 24 hours for 28 days, and seven received placebo. All dogs were stimulated with house dust mite solution one day prior to treatment and once weekly thereafter. Canine atopic dermatitis extent and severity index-03 (CADESI-03) and skin biopsies were performed on day 0, 14, and 28. Quantitative RT-PCR was used to determine levels of cutaneous cytokines and barrier function markers. Indirect immunofluorescence was used to determine protein expression and distribution of nuclear messengers, barrier function markers and thymic stromal lymphopoietin (TSLP). A significant decrease in CADESI-03 occurred for the treatment group compared to placebo (p=0.023) on day 28. On day 14, a significant increase in TSLP protein expression [p=0.019 (placebo); p=0.02 (CsA)] and a significant decrease in TGF-β mRNA [p=0.01 (placebo); p=0.015 (CsA)] was noted. On day 28, a significant increase in cBD103 mRNA [p=0.012 (placebo)], cBD3-like mRNA [p=0.044 (placebo)], filaggrin protein expression [p=0.035 (CsA)], and TSLP protein expression [p=0.0092 (CsA)], and a significant decrease in mRNA of TNF-α [p=0.013 (CsA)], IL-10 [p=0.038 (CsA)], TGF-β [p=0.017 (CsA)], and caspase 14 [p=0.014 (CsA)] was noted. Comparison of the groups revealed no significant effect on skin immunologic milieu or barrier markers despite evident improvement of clinical signs in the treatment group. Larger studies are needed to further investigate the results.

Source of funding: This project was funded by Novartis Animal Health.

Conflict of interest: None declared.
Determining potency of allergen extracts used for immunotherapy using major allergen (MA) assays

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Abstract: Allergen extracts are complex mixtures of proteins that induce immune responses in atopic individuals. These active ingredients are called major allergens when they cause allergic reactions in the majority of subjects. The content of MA in commercial extracts is not determined for most species. The purpose of this study was to develop MA immunoassays and measure multiple lots over ten years to determine the potency and variability of extracts used for immunotherapy. Monoclonal ELISAs for MA were used to monitor the potency of several common allergen extracts used for both human and veterinary immunotherapy. Pollen extracts from grass, birch, English plantain, mugwort, ragweed and olive, and environmental dust mite and cat extracts were studied. Some extracts were further characterized by IgE binding and SDS-PAGE. Standardized grass, mite, cat and ragweed extracts varied about two to three fold in MA content over ten years. These extracts were primarily 50% glycerin and not usually used for animal immunotherapy. Aqueous nonstandardized extracts had higher variability since they were not adjusted for potency and can have MA content ranging from about three to four fold. Grass and mite extracts had significant MA differences between glycerin and aqueous extracts. Group 5 MA in grass pollen and Der 1 in mite extracts were found up to three to ten times more in glycerin than aqueous extracts. The characterization methods demonstrated good stability over the lifetime of the extracts. This study found that MA testing is useful for determining the potency and variability of allergen extracts.

Source of funding: ALK, Inc. Round Rock, Texas

Conflict of Interest: Authors are employees of ALK, Inc.
Antibacterial effect of acetylcysteine on common canine otitis externa bacterial isolates

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Abstract: Otitis externa in dogs can be challenging to manage for many reasons. FDA approved treatments for canine otitis externa are limited in variety, and may contain ototoxic ingredients. In addition, bacterial resistance continues to be a concern, thus it is ideal to avoid exposing potential pathogens to antibiotic treatments if and when possible. Considering these challenges, our group was interested in investigating effective and safe alternatives. The purpose of this study was to determine if acetylcysteine, an otoprotective and non-antibiotic compound, has antibacterial activity in vitro against common bacterial isolates causing otitis externa in dogs. Twenty-two isolates from canine clinical cases were identified and tested, including five *Staphylococcus pseudintermedius*, six *Pseudomonas aeruginosa*, five *Corynebacterium spp.*, and six β-hemolytic *Streptococcus spp.* isolates. Each isolate was grown on blood agar for 24 hours and transferred to Mueller-Hinton broth to achieve a final concentration of $10^5$ CFU/mL. Fifty µl of bacterial suspension was used to inoculate each well. Acetylcysteine was diluted in Mueller-Hinton broth to a starting concentration of 160 mg/ml and serial microdilution assays were performed in triplicate with negative controls for all isolates tested. The acetylcysteine minimum inhibitory concentration (MIC) for all isolates tested ranged from 5-20 mg/ml. MIC for each organism was as follows: *S. pseudintermedius* = 9.7 mg/ml, *P. aeruginosa* = 10.3 mg/ml, *Corynebacterium spp.* = 7.9 mg/ml, and β-hemolytic *Streptococcus spp.* = 8.3 mg/ml. Acetylcysteine actively inhibits clinically relevant and drug-resistant bacteria, and has potential for use as a novel agent for treatment of otitis externa.

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Conflict of interest: None declared.
Influence of sexual gender and reproductive status on Fel d 1 concentration in the fur of domestic cats (*Felis catus*)

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Abstract: The allergens from cats have been identified as extrinsic factors involved in precipitation and exacerbation of rhinitis and allergic asthma in susceptible children and adults, in global indices ranging from 10 to 25%. The major allergen from the epithelium of the cats is Fel d 1, which is responsible for 90% of these allergic reactions. The present study aimed to evaluate the influence of gender and sexual reproductive status on concentrations of Fel d 1 on the fur of cats. Sixty-six healthy cats, 34 males (22 castrated and 12 intact) and 32 females (20 spayed and 12 intact), from households with multiple animals, were evaluated. All samples were individually collected through aspiration with a vacuum cleaner applied for two minutes to the entire body of the cat. The samples were then each sieved, resulting in a fine dust which was used to determine Fel d 1 levels via enzyme-linked immunosorbent assay using anti-Fel d 1 (Indoor Biotechnologies Inc., Charlottesville, VA, USA). The difference between the averages of the different groups was determined by the Student's t-test with p<0.05. The concentration of Fel d 1 in male cats (3.12µg⁻¹±0.40) was higher compared to female cats (2.24µg⁻¹±0.13) (p = 0.04). Fel d 1 concentration was higher in the coat of castrated/spayed cats (3.12µg⁻¹±0.40) when compared to intact cats (1.57µg⁻¹±0.15) (p = 0.01). The Fel d 1 allergen is readily found in the epithelium of cats regardless of their epidemiological characteristics, however is highest in male cats and castrated and spayed cats.

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Conflict of interest: none declared
Evaluation of the effectiveness of an allergen modulating solution on minimization of Fel d 1 concentration in the fur of cats (Felis catus)

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Abstract: The Fel d 1 is the major allergen of the epithelium of cats, believed to originate in sebaceous, anal and salivary glands. The use of modulator solutions have been suggested to reduce the concentration of allergens on cat fur. This study evaluated the effectiveness and residual power of an allergen modulating solution composed of hydrolyzed collagen, allantoin, panthenol and aloe vera gel, in minimizing the concentration of Fel d 1 in the fur of cats. Sixty-five healthy cats were evaluated. The solution was applied to 35 cats (Group 1) and water was applied to 30 cats (Group 2). The allergen modulating solution or water was applied on the coat and let dry naturally. Fur samples were collected before (T0), one hour after, and seven days after the allergen modulating solution or water was applied. All samples collected were individually sieved resulting in a fine dust which was used to determine Fel d 1 levels via enzyme-linked immunosorbent assay using anti-Fel d1 (Indoor Biotechnologies, Charlottesville, VA, USA). All data were analyzed by ANOVA and Bonferroni method, with p<0.05. No statistically significant differences were observed in the average concentration of Fel d 1 in the fur of cats at T0 (2,2623 µg.g⁻¹±1,39477) (p = 0.10), after one hour (2,0499 µg.g⁻¹±0,6035) (p = 0.61) and after seven days (1,5916 µg.g⁻¹±0,086735) (p = 0.28) of the use of the solution compared to the use of water. The allergen modulating solution showed no efficacy in reducing concentration of Fel d 1 in cat fur.

Sources of funding: The study was funded by Araucaria Foundation.

Conflict of interest: None declared
Evaluation of the concentration of allergens from house dust mites in the haircoat of household dogs (*Canis lupus familiaris*) in Paraná – Brazil


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Abstract: Household dogs are continuously in contact with the ecological niches of house dust mites (HDM), and it has been observed that the microclimate between their skin and coat may favor HDM proliferation. The purpose of this study was to evaluate the reservoir potential of Der p 1, Der f 1 and Blo t 5 HDM allergens in the haircoat of household dogs that were regularly groomed and treated with acaricides and pulicides. Forty healthy household dogs, regardless of breed, age and gender, were included in the study. The dogs had been bathed and brushed weekly and preventively medicated with acaricides and pulicides for two months prior to inclusion in the study. Dust samples were collected with a domestic vacuum cleaner, by vacuuming across the length of the dogs’ bodies for two minutes. The samples were collected in separate filters transferred into plastic containers, sealed and kept frozen until ELISA analyses. Among the allergens studied, Der p 1 was the most commonly found (p<0.05). Der p 1 and Blo t 5 were found on 13/40 (32.5%) and 2/40 (5%) of dogs’ haircoats, respectively. Average concentrations of Der p 1 and Blo t 5 were 0.14±0.73μg/g and 0.01±0.07μg/g, respectively. No Der f 1 allergen was found in the haircoat of the studied dogs. These results revealed that HDM allergens, especially Der p 1, can be found on dogs’ haircoats in non-sensitizing concentrations. Household dogs, when bathed, brushed and medicated regularly with acaricides and pulicides, are not a significant source of HDM allergens.

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Conflict of interest: None declared.
Clinical features and comparison of treatment efficacy in 289 dogs with atopic dermatitis

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Abstract: Canine atopic dermatitis (CAD) is a common chronic relapsing allergic skin disease with clinical features varying according to environmental regions. The purpose of this study was to identify risk and flare factors of atopic dogs compared to the general hospital population. Moreover, the efficacy and the complications of the treatments were compared. The medical records of 289 dogs with CAD were retrospectively reviewed (2002-2014). Among 289 atopic dogs, 114 dogs received oral prednisolone (Solondo, Yuhan Medica, Seoul, Korea) and/or ciclosporine (Neoral, Novartis Pharma, Basel, Switzerland) treatment for 3 months or allergen-specific immunotherapy (ASIT) for 6 months. The response to therapy was scored based on Canine Atopic Dermatitis Extent and Severity Index-4 (CADESI-4) and behavior based pruritus scale. Breed predilection for CAD was noted in cocker spaniels, pugs and fox terriers when compared to general hospital population. A high risk for CAD was noted in dogs living predominantly indoors (P<0.001) and when clinical signs started between 1 and 3 years of age (P<0.001). When evaluating infectious and parasitic causes of CAD flares, cocci, Malassezia and Demodex mites were most commonly implicated. Many positive reactions to molds on intradermal allergy testing were associated with more CAD flares. When comparing treatments, three months of prednisolone plus ciclosporine showed marked reduction in CADESI-4 scores, which was comparable to that observed with ASIT at 6 months. Overall, the fewest adverse reactions and lowest relapse rate were observed with ASIT. ASIT may be best for the long term management of CAD.

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Conflict of interest: None declared
Patient adherence patterns for veterinary allergen-specific immunotherapy

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Abstract: Allergen specific immunotherapy (ASIT) adherence is a combination of primary adherence (ordering ASIT after allergy testing) followed by secondary adherence (refilling the ASIT order to continue treatment and evaluate ASIT efficacy). There are limited data on ASIT adherence patterns in veterinary medicine, and especially for veterinary patients treated in general practice with ASIT. To evaluate the extent of ASIT adherence in patients that are prescribed ASIT, an analysis was performed using a database of electronic medical records and invoices aggregated across veterinary practice management software programs (VINx, Davis, CA). A total of 2,811 patients from 43 states met the exposure criteria of environmental allergy testing followed by 24 months of active follow-up time. Serologic allergy testing was performed in 2,644 patients (2,522 dogs, 122 cats), intradermal allergy testing in 146 patients (143 dogs, 3 cats), and both allergy tests in 21 patients (18 dogs, 3 cats). Primary adherence to ASIT was 28.7% for dogs (769/2,683) and 16.4% for cats (21/128), and differed by species (p=0.003). Secondary adherence to ASIT was then measured after initial ASIT formulation, based upon at least one ASIT refill within 12 months of starting ASIT. Secondary adherence to ASIT was 64.4% for dogs (495/769) and 66.7% for cats (14/21), and not different by species (p=0.828). Overall, only 18.1% (509/2,811) of patients both began ASIT and then completed one refill order of ASIT after allergy testing. Our data suggest that overall adherence to ASIT is poor in a database comprised primarily of veterinary general practices.

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Conflict of interest: None declared
Ectoparasites of free-roaming domestic cats (*Felis catus*) presented to a spay-neuter clinic in Oklahoma, USA

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**Abstract:** Though ectoparasites are a common cause of clinical disease, detailed analysis of seasonal variation of the occurrence of ectoparasites on cats is not well characterized. Six hundred eighty nine free-roaming domestic cats that presented to a spay-neuter clinic in Oklahoma were examined for ectoparasites (fleas, ticks, lice, walking dandruff mites, and ear mites) in January–May and September–November 2014. The prevalence of fleas or flea dirt on cats was highest in September (89%, 109 of 122) compared to other months which ranged from 43% (41 of 95) in April to 87% (41 of 47) in January. Over 300 fleas were collected, which were identified as *Ctenocephalides felis*, *Pulex* spp., and *Cediopsylla simplex*. Ticks were found on at least one cat in each month surveyed. Cats sampled in May had the highest prevalence of tick infestation (33%, 22 of 66), followed by April (23%, 24 of 106), September (21%, 26 of 122), and November (20%, 18 of 89). Of more than 285 ticks collected, 71% were *Amblyomma americanum*, followed by *Ixodes scapularis* (23%), *Dermacentor variabilis* (5%), and *Rhipicephalus sanguineus* (1%). All motile life stages of ticks (i.e., adults, nymphs, and larvae) were found on cats. Hair clippings contained *Felicola subrostratus* and *Cheyletiella blakei*. *Otodectes cynotis* was detected in 19% of all cats sampled (89 of 470), ranging from 11% (7 of 64) in May to 23% (23 of 100) in April. Free-ranging cats are likely reservoirs of ectoparasites to client-owned cats, substantiating the need for year-round ectoparasiticide use in Oklahoma.

Source of funding: Self-funded

Conflict of Interest: None declared
Extensive solar induced squamous cell carcinoma/squamous cell carcinoma in situ in a dog treated with helical tomotherapy

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Abstract: An approximately 6 year female intact, lightly pigmented pit bull terrier dog presented for evaluation of chronic skin disease of unknown duration. On examination, multifocal erythematous macules and crusted plaques, some with ulcerated surfaces, were present extending from the ventral thoracic and axillary region into the inguinal fold. Irregular thickenings were palpable along the affected abdominal skin, lateralized to the left. Histopathology confirmed squamous cell carcinoma (SCC)/SCC in situ in all biopsy samples. A helical tomotherapy unit was utilized to deliver ten 2.5 gray (Gy) fractions of radiation therapy (RT), for a total dose of 25Gy. Therapy was administered to the level of the skin daily for two consecutive weeks. Complete blood count (performed every 3-5 days) identified mild leukopenia beginning at the halfway point of RT. Leukopenia persisted for the therapeutic duration without side effects noted; this resolved by one week following RT discontinuation. Serum biochemistry (performed weekly) was unaffected by treatment. Mild moist desquamation was appreciated in the inguinal fold at 10 days post RT; this did not require therapeutic intervention. This resolved completely without additional complications. Repeat biopsy samples at 8 weeks post RT identified only mild solar elastosis without evidence of SCC. This case identifies a well-tolerated treatment option for extensive solar induced dermatoses/neoplasia in the dog where currently therapeutic options are limited. Advances in RT delivery systems allow for minimal cutaneous toxicity even when large body areas are involved. Further investigation and evaluation of the best RT treatment protocols for this condition is warranted.

Conflict of interest: NONE

Source of funding: NONE
Removal of 17 canine and feline ear masses aided by a holmium:YAG laser

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Abstract: Holmium:YAG lasers have been used in urology for treatment of urinary calculi, urethral/ureteral strictures, prostate resection as well as intervertebral disk ablation. To the author’s knowledge use of holmium lasers for treatment of canine and feline ear masses has not been described. Ear masses in seven cats and 10 dogs were treated by surgical debulking and laser ablation from 2007 until 2014. Dog breeds included five cocker spaniels, one springer spaniel, one German shepherd dog, one Labrador retriever and two mixed breed dogs. The seven cats were domestic short-hairs. Ages ranged from three to 16 years. Histopathological examination revealed ceruminous hyperplasia/adenoma (6), sebaceous polyploid hyperplasia (5), chronic proliferative eosinophilic otitis (2), ceruminous adenocarcinoma (1), squamous cell carcinoma (1), apocrine cystomatosis (1), and viral papilloma (1). Ear masses were initially debulked surgically and subsequently ablated with the holmium:YAG laser Odyssey30 (Convergent Technologies, Alameda, CA, USA) during the same anesthetic period, with the fiber inserted through the biopsy channel of the video-otoscope MedRx, (Largo, FL, USA). The only adverse effect of the procedure was deafness in one dog with bilateral disease. Two cats with carcinoma died within a year of the procedure, two dogs with ceruminous hyperplasia subsequently needed a total ear canal ablation. The apocrine cystomatosis recurred within a year. The remaining cases are doing well to date. Use of a holmium:YAG laser to aid in the removal of ear masses in dogs and cats appears to be safe and, in many cases, an effective adjunctive treatment for ear masses.

Source of funding: Self funded

Conflict of interest: non declared
A comparative study of otic histology, disease history and clinical findings of ear and skin disease in 29 American cocker spaniels

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Abstract: Ceruminous gland ectasia and hyperplasia are reported to be breed-related histological features in cocker spaniels with end-stage otitis. To determine associations between aural histological findings and clinical findings of ear and skin disease in American cocker spaniels, 38 client-owned dogs, regardless of otitis history, were enrolled in this open study. Dermatological and video-otoscopic examinations were performed. Ear canal erythema, oedema, ulceration and exudation were scored to distinguish otitis from clinically healthy dogs. One biopsy from each dog was taken from the left vertical ear canal. Diagnostic workup was performed to reveal the primary cause of the ear and/or skin signs. The chi-squared and Fischer’s exact tests were applied to assess statistical associations between aural histological and other findings. Nine biopsies obtained from 4 clinically healthy and 5 dogs with otitis were inadequate leaving 14 clinically healthy and 15 dogs with otitis in the study. Ceruminous gland ectasia and/or hypertrophy were found in 19/29 biopsies. The presence of ceruminous gland changes was associated with clinically determined otitis (p=0.021). Yet 6/14 clinically healthy dogs also had these changes. Dogs with chronic or recurrent ear or skin signs had significantly more ceruminous gland changes (p=0.032). Signalment and clinical or histological findings of the skin were not associated with aural histological findings. In conclusion, the presence of histological changes in the ceruminous glands was associated with clinically determined otitis but not with other dermatologic findings. As clinically healthy dogs also showed changes in the ceruminous glands, these changes may precede otitis in this breed.

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Conflict of interest: None declared.
Protozoal nodular dermatitis and panniculitis in a puppy caused by *Caryospora bigenetica*

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Abstract: *Caryospora bigenetica* is an intracellular coccidian parasite of snakes and raptors (primary hosts), and rodents (secondary host). Experimental infection has been documented in mice and pigs, but natural infection in dogs is rare. To date, there is no report of treatment or outcome in dogs infected with *Caryospora spp*. This paper describes the clinical presentation, histological features, treatment and outcome of a case of cutaneous protozoal nodular dermatitis in a puppy caused by *C. bigenetica*. A 4-month-old, intact female Rottweiler presented with generalized subcutaneous papules and nodules measuring up to 2 cm in diameter. Histopathology showed marked suppurative to pyogranulomatous dermatitis and panniculitis with intralesional protozoal organism of multiple life-cycle stages supportive of *C. bigenetica*, which was confirmed by PCR and DNA sequencing. Treatment with a combination of oral trimethoprim-sulfamethoxazole (TMS), pyrimethamine and high-dose clindamycin (20mg/kg every 12 hours) resulted in resolution of lesions in six weeks. Discontinuation of the treatment two weeks later caused a rapid relapse of skin lesions. Clindamycin and TMS were restarted, and all lesions resolved within three weeks; at which time TMS was discontinued due to side effects. The lesions remained in remission for two months on clindamycin monotherapy before a second relapse of skin lesions occurred. *Caryospora spp* infection is a rare disease in dogs, which presents as a nodular dermatitis and panniculitis with intralesional parasites of different life-cycle stages. Although remission of clinical signs can be achieved with combination therapy of clindamycin and TMS, long-term management is challenging and relapses should be anticipated.

Sources of Funding: This study was self-funded

Conflict of Interest: None declared
SCIENTIFIC SESSION
PRESENTATIONS
FRIDAY
INTRODUCTION

Skin barrier function is important to prevent water loss (inside-outside barrier) and to protect the body from exogenous compounds from the environment (outside-inside barrier)\(^1\). The intercellular lipids from the stratum corneum (SC), especially the ceramides, are considered key to keep this barrier. A defective barrier function might be associated with skin disease, such as atopic dermatitis (AD), in humans and rodent models\(^1\). Preliminary studies in dogs with AD seem to suggest this is also true in this species. One study found microscopic alterations of the SC in atopic dogs compared to healthy controls\(^2\) and another study\(^3\), assessing the barrier function measuring transepidermal water loss (TEWL, defined as the volume of water that passes from inside to outside the body through the upper epidermal layers) was lower in healthy controls compared to dogs with AD. That same study found that non treated AD dogs had a higher TEWL (indicative of a defective barrier function) than treated dogs (with allergen specific immunotherapy and/or cyclosporine), suggesting that skin barrier can be improved with medical treatment and is associated with a good clinical response.

Nutrition plays a role keeping a healthy skin barrier in humans, dogs and cats\(^4\). Whether nutrition modification is capable of improving the skin barrier in veterinary patients with disease is still unknown due to the scarce of research in the area (particularly in cats).

NUTRIENT DEFICIENCIES ASSOCIATED WITH SKIN DISEASE

A variety of nutrient deficiencies result in skin problems\(^5,6\) due to the fact of it being a very large organ with a high turnover rate. See table 1 for a summary. It is estimated that approximately 25 to 30% of the daily protein requirement is destined to skin and coat\(^7\). Hair is basically protein, particularly rich in sulfur amino acids. Protein deficiency is uncommon, and can be found more often in highly demanding stages (like lactation or growth) or in malnourished animals. Essential fatty acids deficiency can also result in skin problems. Its deficiency is rare in commercial diets, but can potentially happen in inadequate storage resulting in fat rancidity. Some low fat diets can be too marginal for individual patients. Trace element deficiencies have been described in diets rich in phytates or other substances that can interfere with trace mineral absorption, but again, this is extremely rare currently, likewise for water soluble vitamins. Lipid soluble vitamins can become deficient in patients with fat malabsorption\(^4\). In all cases, unbalanced home cooked diets can result in all of these deficiencies. In the author’s experience, many inadequately formulated homemade diets are deficient in zinc and linoleic acid. Thus, ensuring that our patients consume a complete and balanced diet is very important to provide the skin with the specific necessary nutrients to keep a healthy skin barrier.

Table 1: nutrient deficiencies associated with skin problems in dogs and cats\(^5,6\)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Dermatological clinical signs</th>
<th>Mechanism of deficiency</th>
<th>Recommended allowance for adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid (C18:3, (\omega_6))</td>
<td>Dogs: coarse, dry hair, desquamation, progressing to greasy, pruritic skin.</td>
<td>Important part of skin ceramides from the intercellular lamellar lipids,</td>
<td>Dogs: 0.36 g/kg(^{0.75})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(arachidonic acid also)</td>
<td>Cats: 0.14 g/kg(^{0.67})</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Cats</th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cats: dry, lusterless hair coat, dandruff</td>
<td>part of the skin water barrier required)</td>
<td>Dogs: 3.28 g/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td>Cats: 4.96 g/kg&lt;sup&gt;0.67&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>Brittle hair, thin hyperpigmented skin</td>
<td>Provider of amino acids for skin proteins</td>
<td>Dogs: 0.12 g/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Cats: crusty material around eyes and nose, rough hair coat with desquamation of the epidermis on the pads with cracking</td>
<td>Part of skin proteins?</td>
<td>Cats: 0.11 g/kg&lt;sup&gt;0.67&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lysine</td>
<td>No signs described in dogs and cats, but yes in birds (graining of feathers) and rats (graying of hair)</td>
<td>Part of skin proteins?</td>
<td>Dogs: 0.11 g/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methionine (and cystine)</td>
<td>Dogs: dermatitis, swelling and reddening of skin, dermatosis of foot pads (lesions become necrotic, hyperkeratotic, ulcerated) Cats: perioral and footpad lesions (similar to overall protein deficiency)</td>
<td>Methionine is a methyl group donor involved in many functions such as cell replication and phospholipid synthesis</td>
<td>Cats: 0.084 g/kg&lt;sup&gt;0.67&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenylalanine (and tyrosine)</td>
<td>Black dogs and cats: reddish skin coat</td>
<td>Tyrosine is precursor of eumelanine, responsible for black coloring of hair</td>
<td>Dogs phenylalanine: 0.15 g/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Copper</td>
<td>Dogs: Loss of hair pigmentation</td>
<td>Cofactor of tyrosinase (necessary for melamin pigment synthesis)</td>
<td>Dogs: 0.2 mg/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc</td>
<td>Dogs: Skin lesions, starting in areas of contact (erithema, alopecia, crusting, hyperkeratosis) Cats: perioral skin lesions compatible with parakeratosis</td>
<td>Cofactor of multiple enzymes, stabilization of DNA and RNA</td>
<td>Dogs: 2 mg/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iodine</td>
<td>Dogs: alopecia, dry sparse hair coat</td>
<td>Cofactor of thyroid hormones</td>
<td>Iodine: 29.6 ug/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Dogs: hyperkeratinization and scaling, alopecia, poor hair coat and increased susceptibility to microbial infections, seborrhea</td>
<td>Regulation of cellular growth and differentiation, important for keratinization</td>
<td>Dogs: 50 retinol equivalents/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Riboflavin (B2)</td>
<td>Dogs: Flaking dermatitis of the abdomen and medial surface of hind leg Cats: periauricular alopecia with epidermal atrophy</td>
<td>Coenzyme (FAD, FMN) important for redox reactions (energy metabolism, glutathione regeneration)</td>
<td>Dogs: 0.171 mg/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Niacin (B3)</td>
<td>Dogs: Ulceration buccal and faringeal mucosa, vermilion bands on lips Cats: red and ulcerated tongue</td>
<td>Coenzyme (NAD, NADP) important for redox functions and fatty acid synthesis</td>
<td>Dogs: 0.57 mg/kg&lt;sup&gt;0.75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Biotin</td>
<td>Dogs (1 report): Scaryf skin Cats: Alopecia, achromotrichia, dermatitis</td>
<td>Cofactor of carboxylases, important for fatty acid synthesis</td>
<td>Dogs: provided by intestinal flora if healthy Cats: provided by intestinal flora if healthy</td>
</tr>
</tbody>
</table>

**NUTRIENTS CONSIDERED IMPORTANT FOR SKIN BARRIER FUNCTION**
Essential fatty acids

Essential fatty acids (EFA) are fatty acids from the ω6 and 3 families (figure 1).

Figure 1: Omega 3 and omega 6 fatty acid biosynthesis (based on NRC data) 5

The essential ω6 fatty acid is linoleic acid (LA, C18:2 w6). LA is important as a precursor of arachidonic acid (C20:4 ω6, precursor of eicosanoids) but has many important functions of its own. One of them is forming part of the ceramides (1 and 9, for example) 8 that form the intercellular lamellar granules at the SC, thus imparting water barrier protection. This may explain the reason why a change to a higher fat diet (usually providing vegetable oils, rich in LA) or corn oil supplementation can result in improved hair coats 5 in dogs. Another ω6, GLA (gamma linolenic acid, C18:3 ω6), although not considered essential in itself, is a precursor of eicosanoids of the 1 series, less proinflammatory than arachidonic acid derived ones, and its use has been proposed as an anti-inflammatory agent in skin disease (by the use of borage or primrose oils). Data about its efficacy is inconclusive 9,10.

All EFA, as polyunsaturated fatty acids, are important for membrane function. However, ω6 fatty acids are more potent than ω3 fatty acids in regards their skin barrier function. Essentiality of ω3 fatty acids has been harder to prove than ω6. DHA (C22:6 ω3), has been shown to be essential in growing puppies for brain and retina development 11 but essentiality in adults is still not demonstrated. EPA (C20:5 ω3), is an important precursor of eicosanoids (from series 3 and 5), which are less proinflammatory than ω6 eicosanoids, which is why they can be of use in skin disease with an inflammatory component 12. EPA and DHA are much more potent than their precursor, ALA (C18:3 ω3) since conversion from ALA to its more bioactive compounds EPA and DHA is low in dogs (10%) and negligible in cats, due to their low levels of delta 6 desaturase.

Topical and oral supplementation with EFA (especially with LA) suggests improvement of ceramide synthesis in dogs, although the studies are still scarce and more data is needed to confirm its effect and decide on an appropriate route, dose and composition of the treatment 13,14.

Water soluble vitamins

Niacin deficiency is very rare, since it can be synthesized from tryptophan by dogs (although not cats) 5. It is part of NAD and NADP. In vitro studies suggest niacin can increase ceramide synthesis 15,16 and reduce TEWL in vivo in humans when applied topically. In vivo data suggests that pantothenic acid may help epidermal barrier function via proliferation and differentiation of keratinocytes 17. Deficiencies of these
vitamins are extremely rare, but the question is if added levels of them in the diet can help improve barrier function and that is still unknown. One study found a positive effect on TEWL of healthy dogs with a diet supplemented with pantothenic acid, niacin, choline, inositol, and histidine. These nutrients showed in vitro stimulation of ceramide synthesis in canine keratinocytes\(^\text{18}\). However, there is very little data on the effects of supplementing these nutrients separately and no data on how does this combination work to improve skin barrier function.

**Fat soluble vitamins: vitamin A, vitamin E, vitamin D**

Vitamin A deficiency results in an altered skin barrier function, due to its importance in maintenance of all epithelia. There is no data to suggest its supplementation can help skin barrier. Vitamin E, an antioxidant, can help protect the lipids of the SC and one study found lower plasma vitamin E levels in dogs with atopy\(^\text{19}\), and this study found that supplementation (8.1 IU/kg q24 hours for 8 weeks) improved the subjective CADESI score. However, there is no research supporting its positive effect specifically on skin barrier. Vitamin D deficiency has been implied in a number of chronic conditions in humans, including allergies. However, a review of the published data did not offer conclusive evidence on improvement of clinical signs or improvement of skin barrier function in humans\(^\text{10}\).

**Other nutritional strategies**

One study with a combination of nutritional supplements (aloe vera, vitamin C, taurin and curcumin) has been tested in vitro to improve water permeability and increase fibroblast migration, but results are not proven in vivo\(^\text{20}\). Topical application of ceramides, although not technically nutrients, have provided encouraging results in dogs\(^\text{71,22}\). Pre (oligosaccharides) and probiotics in humans (and probiotics, Lacobacillus rhamnosus, in dogs) have shown positive results in patients with AD\(^\text{10,23}\), although the mechanism of action is still unclear. One theory is that beneficial bacteria can interact with immune cells in the intestine and act in the skin via cytokine secretion, thus affecting more the immune aspect of the skin problem rather than the structural defect. Another hypothesis is that they can affect filaggrin (an important protein for skin integrity) synthesis although results still do not fully support this\(^\text{24}\).

**REFERENCES**

GUIDE TO HOME COOKED DIETS FOR ELIMINATION DIET TRIALS, PROS, CONS AND WHAT MISTAKES TO AVOID

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INTRODUCTION

Home prepared diets (HPD) are a useful tool for the management of several diseases in dogs and cats, including food allergies. Although there are a variety of commercial options available, they are used as elimination diets and some veterinarians prefer them to the commercial elimination diets. Here we will discuss the pros and cons of elimination HPD.

TYPES OF HPD

HPD can be raw or cooked. The problems associated with raw diets have been described elsewhere\(^1\) so their use should be carefully considered.

HPD can be complete and balanced or not. Complete and balanced diets provide all essential nutrients (around 40-45, depending on the species and the physiological state\(^2\)) whereas unbalanced HPD lack essential nutrients, usually micronutrients like vitamins and minerals but sometimes also essential amino acids and essential fatty acids.

Non complete HPD are basically 2 ingredients (a protein source and a starch source) with the idea of providing only energy and protein requirements during the trial. Complete diets usually require the addition of 1 or more fat sources (depending on the leanness of the meat) and supplements to provide the micronutrients not provided by the basal ingredients.

Most HPD for dogs and cats include an animal protein source (either meat or dairy or eggs) but some canine HPD might be vegetarian, using a legume (such as tofu or beans) as the main protein source. Some feline HPD can be all meat (i.e. no starch source), since they can be more palatable this way.

BALANCED HPD: BRIEF OVERVIEW

To formulate a HPD it is necessary to know 3 things: the energy and nutrient requirements of the animal, the maximum levels of some nutrients, and the nutrient and energy content of the ingredients to be used. For the first two, we have the published information from the NRC (2006)\(^2\) and the industry standards (AAFCO in the USA, FEDIAF in Europe). For the third, we rely on nutrient databases published for humans, such as the USDA one. The diet can be formulated using a simple spreadsheet, but most nutritionist use specific software to balance the 40 plus nutrient required by dogs and cats. To make a balanced diet, we usually require a protein source, a carbohydrate source (this can be omitted, especially in cats, but it will make the diet more expensive), a fat source (or more than one if we want to provide omega 3 fatty acids), and vitamin-mineral supplements (either an all-in-one or a combo of 4-7 supplements intended for humans). The human multivitamins/multiminerals by themselves are not enough to balance a HPD, and many pet products (intended to be used on top of commercial diets) are also insufficient.
WHY HPD? PROS VS COMMERCIAL ELIMINATION DIETS

There are two types of commercial elimination diets: those based on uncommon ingredients and those based on hydrolyzed protein. These diets can work very well, but there are some problems associated with them where a HPD might be beneficial.

Regarding uncommon ingredient diets, the diets made by commercial manufacturers have sometimes been on backorder because these are ingredients where is harder to find a reliable provider and there might be shortage of the protein source. The backorders can be very problematic, especially in animals already diagnosed that are doing well on the elimination diet long term.

The therapeutic uncommon ingredient diets in the US market use duck, rabbit, venison, whitefish, salmon, lamb, kangaroo, and egg as protein sources (by Iams, Hill’s, Royal Canin, and Purina). The most common starch sources are potato for dogs and green peas for cats. If these are NOT novel for a specific patient, either the hydrolyzed diets or a HPD will be the next option.

Another reason for commercial diets’ failure to rule in/our adverse reactions to foods is that it is potentially possible that a patient reacts negatively to a commercial uncommon ingredient diet but that reacts positively to a HPD using the same main ingredients. It has been proposed that the patient could be reacting to the additives in the commercial diet or to neo-antigens formed via Maillard-type reactions (between amino acids and sugars) due to the high temperature processing of commercial pet food, which is considerably higher than home cooking temperatures.

Regarding hydrolyzed protein diets, there are some animals that react to the original protein that will also react to the hydrolyzed protein. The hydrolyzed protein diets in the US market are chicken, soy or feather based. So, in dogs that are sensitive to chicken, the chicken hydrolysate might not be the best choice. Also, these diets are very expensive to produce and some owners might not be able to afford them.

Also, HPD have the great advantage of customization. So, for patients with concurrent diseases such as renal disease or pancreatitis, a HPD can incorporate the nutritional strategies for the concomitant disease while still using adequate ingredients for an elimination trial. This customization is best when the diet is specifically formulated for the patient rather than use a published recipe from books or the internet.

PROBLEMS AND COMMON MISTAKES ASSOCIATED WITH HPD

Problems associated with complete HPD:

- Compliance: due to the cost and the complexity of HPD, there can be compliance issues. These are usually reflected on what can be called “diet drift”, where pet owners change the original recipes for several motives (ingredients not palatable, difficult or expensive to source, etc).
- Price: Pricewise, a HPD can be more expensive or cheaper depending on the ingredients. For example, if the HPD uses a relatively easy to source protein like pork, it could be cheaper than a hydrolyzed diet (especially in small sized animals) but if the owner has to source bison or ostrich, the price of a HPD can be crippling, especially if it is complete and balanced and is going to be fed long term. To this, the time required to prepare the diet also has an indirect cost. Hydrolyzed and uncommon ingredient diets, on the other hand, are one of the most expensive on the market due to cost of prime materials, cost of the hydrolyzation process and the cost to prevent cross contamination, where the diet has to be produced on a dedicated line or after a thorough cleaning of all the pet food producing line. Thus, the financial burden to the owner has to be calculated individually.
- Adequacy: even when formulated by experts, HPD are a bit more risky, nutrition wise, than commercial diets (especially those from reputable manufacturers). Formulation of HPD relies on database information; that is, that the database information will faithfully represent what
ingredient is actually used. Plus, there is no postproduction testing and a HPD cannot be tested for adequacy following AAFCO procedures.

Problems associated with unbalanced HPD:

- All of the above
- Deficiencies or toxicities:
  - Energy: it is common for HPD, if not customized to the patient, to result in undesired weight gain or loss
  - Nutrients: especially micronutrients, although some can also be protein deficient.

Problems of generic recipes: they are not customized, are often written by lay people (or veterinarians without nutrition training), can become outdated (as nutritional knowledge advances), can use difficult to source ingredients, and can be too vague or too complex. There are several papers evaluating published recipes for maintenance, renal disease, and cancer and most of them have been found wanting regarding their nutritional adequacy.

To minimize these issues, it is recommended that a veterinary nutritionist (board certified by ACVN or ECVCN) is consulted for HPD. Web-based sites supervised by ACVN diplomats allow for a semi-customized recipe with a variety of ingredients for a low cost and available immediately. For more complex cases, a fully customized recipe can be obtained from nutrition services, and the wait time and the cost will be higher.

CONCLUSIONS

HPD are a valuable tool in elimination trials since they allow customization (choice of ingredients, protein/fat content, no additives), although they can be expensive and result in compliance problems, and, if not properly formulated, result in weight loss and nutrient deficiencies. Due to the length of the trial, it is recommended that they are complete and balanced, and this is especially important in animals at risk due to elevated demands (growing and reproducing animals). There are a variety of resources to obtain complete and balanced HPD recipes from boarded nutritionists at a reasonable price such as www.balanceit.com and www.petdiets.com and generic book/internet

RESOURCES

Veterinary nutritionists: www.acvn.org

Web based (run by ACVN diplomats): www.balanceit.com; www.petdiets.com

Nutrient content of ingredients: http://ndb.nal.usda.gov/

Nutrient requirements of dogs and cats: http://www.fediaf.org/self-regulation/nutrition/

AAFCO: www.aafco.org

REFERENCES


There are several procedures that dermatologist perform daily that may require either sedation or local/general anesthesia. Several drug characteristics, including no histamine release, reversibility, analgesia, and lack of cardiorespiratory effects, would be ideal for the vast majority of patients. However, available options may have some of these features but not all of them, making the selection judgment difficult. Some of the available options for old drugs, new drugs and some practical regional blocks, are reviewed here and include:

**Alpha-2 agonist:** This group of drugs provides reliable sedation, analgesia, muscle relaxation and anxiolysis, through inhibition of norepinephrine release from presynaptic and postsynaptic receptors situated in the pontine locus coeruleus. The antinociceptive effect appears to be mediated through spinal mechanisms. The main cardiovascular effects include bradycardia, arrhythmias (mainly 1st and 2nd degree atrioventricular block), decreased cardiac output and a biphasic effect in the blood pressure with an initial hypertension from vasoconstriction followed by hypotension from vasodilation. The respiratory effects include mild decrease in respiratory rate and peripheral desaturation, which can give a dark or blue appearance to the mucous membranes. These effects are well tolerated by healthy middle-aged and older dogs but monitoring and support of the cardiorespiratory systems should be provided. More specifically related to dermatology, medetomidine and dexmedetomidine have shown not to affect skin reactivity making them safe when reliable sedation is needed for intradermal testing.

**Propofol 28:** A new presentation of propofol has been available for about 3 years now. PropoFlo 28™ contains 2% benzyl alcohol as a preservative to extend the shelf life of any opened bottle to 28 days, making it very attractive for practitioners to minimize wasting. It is labeled for use in dogs as intravenous anesthetic since there is no risk of toxicity from the benzyl alcohol. The benzyl alcohol uses glucuronidation pathway for metabolism making cats more likely to toxicity, hence it is not labeled for cats. However, to date there are 2 studies looking at the use of propofol 28 in cats and both studies support its use as a single off-label dose for induction of anesthesia. There are no studies looking at constant rate infusions of propofol 28 in cats so for this purpose it is not recommended. Both presentations of propofol can be used for heavy sedation and/or anesthesia of short duration. Due to the potent respiratory depressant effect of the drug and the risk of apnea, oxygen as well as endotracheal tubes should be readily available. The regular formulation of propofol has been shown to decrease the mean wheal size after intradermal injected histamine phosphate, but the clinical implication of this finding appears to be low. Propofol 28 has not been studied as a sedative for intradermal testing, but the presence of benzyl alcohol should be taken into consideration since there are reports, although rare, of hypersensitivity reactions in humans from drugs containing benzyl alcohol as a preservative.

**Alfaxalone (Alfaxan®):** Is a synthetic neurosteroid that acts through binding at the gama-aminobutyric acid subtype A receptor (GABA A) enhancing the effects of the neurotransmitter GABA to cause general anesthesia. Alfaxalone was FDA approved for dogs and cats (class IV controlled substance), and launched last year in the United States, although it has been available in other countries for more than 10 years. This new formulation of Alfaxalone comes in a cyclodextrin vehicle but it does not contain antimicrobial preservative, therefore after opening the bottle it should be used within 6 hours. Different from the previous formulation, which used a derivate of castor oil as solubilizing agent, Alfaxan does not cause histamine release and can be used IV or IM. It causes mild dose-dependent
cardiovascular effects such as hypotension but this may be offset by a transient tachycardia\textsuperscript{11}. Rapid bolus administration can cause decrease in respiratory rate, apnea and hypoxia, therefore oxygen should be readily available as well as an endotracheal tube to control the airway. It is an attractive alternative not only for induction and maintenance of anesthesia\textsuperscript{12-13}, but also to enhance sedation when combined with other sedative drugs and given IM to healthy cats\textsuperscript{14}. In dogs the IM route is not recommended due to the short duration of effect and large volume needed for injection. Poor recoveries have been reported after giving Alfaxalone for induction of anesthesia through the IM route in cats, even after adequate sedation\textsuperscript{15}. The subcutaneous route has been studied in hyperthyroid cats as an alternative route of administration\textsuperscript{16}. Alfaxalone combined with butorphanol provided a reasonable degree of sedation after 30-45 min of administration.

**Regional Blocks**: regional anesthesia can be either used alone, in collaborative patients, or together with sedation for many situations including biopsies, wound cleaning, etc. or can be an alternative for additional analgesia in cases such as self mutilation. Some of the popular techniques for the distal area of the extremities include:

**RUMM block (Radial, Ulnar, Median and Musculocutaneous)**: Structures distal to the elbow can be desensitized using this block. The Radial nerve can be located in the lateral aspect of the forelimb, proximal to the lateral epicondyle of the humerus, between the lateral head of the triceps and the brachialis muscles at the mid-distal humeral level. The brachialis muscle is moved cranially using the operator’s thumb and the needle is inserted caudal to it through the lateral head of the triceps muscle until the caudolateral aspect of the humerus is contacted\textsuperscript{17}. The needle is retracted slightly and local anesthetic can be injected. The ulnar and median nerve are located caudal to the brachial artery and the musculocutaneous nerve cranial to it in the medial aspect of the forelimb. The injection site is proximal to the medial epicondyle of the humerus between the biceps brachialis and the median head of the triceps. Aspiration before injection of the local anesthetic is fundamental to avoid vascular administration. This block can be performed either using these landmarks in a blind technique, using a peripheral nerve locator or with the aid of ultrasound. Lidocaine or bupivacaine can be used, but only bupivacaine has been studied and showed decreased sensation with no motor blockade in dogs for 4-10 hours\textsuperscript{17}.

**Digital nerve block fore limb**: This block is use to desensitize the distal foot and toes by targeting branches of the radial, median and ulnar nerves. A 4-point technique has been described\textsuperscript{18} where local anesthetic is injected subcutaneously 1) dorsomedial just proximal to the carpal joint to block the superficial branches of the radial nerve; 2) dorsolateral to the accessory carpal bone to block the dorsal cutaneous branch of the ulnar nerve; 3) proximal to the accessory carpal bone to block the palmar cutaneous branch of the ulnar nerve; and 4) proximal to the median carpal pad to block the median nerve. Alternatively, a ring block can be performed by administering local anesthetic circumferentially around the limb at the distal level of the radius/ulna, and/or a digital block directly injecting between each toe or at the desired toe only.

**Digital nerve block hind limb**: This block is use to desensitize the distal foot and toes by targeting the superficial branches of the common peroneal and tibial nerves through subcutaneous injection of local anesthetic distal to the tarsal joint on the dorsomedial (peroneal) and ventromedial (tibial) aspects. Alternatively, a ring block can be performed by administering local anesthetic circumferentially around the limb at the distal level of the tibia.

**Tibial-common peroneal-saphenous nerve block**: Blockade of these nerves has been described using bupivacaine (2 mg/kg, 0.5%) and provides desensitization of the femoro-tibial joint and distal pelvic limb\textsuperscript{19}. To block the tibial and common peroneal nerves, on the lateral aspect of the thigh at mid level, the body of the biceps femoris muscle is grasped and lifted, with the thumb of the operator advanced deep and cranially until the femur is reached. A spinal needle is advanced cranially perpendicular to the femur.
until its caudal aspect is contacted, then the needle is withdrawn 1 cm and the local anesthetic is injected along the entrance path as the needle is withdrawn. Aspiration to avoid vascular injection should be performed every time the needle is repositioned. For the saphenous nerve, on the medial aspect of the thigh at the mid level, a needle is introduced between the sartorius and gracilis muscles, and advanced through the superficial fascia overlying the saphenous neurovascular bundle until a distinct ‘pop’ is appreciated and then advanced 2-3 cm more within this fascial plane. Aspiration is performed before injecting the local anesthetic as the needle is withdrawn.

REFERENCES

ANESTHESIA FOR DERMATOLOGY
“Analgesic Options that Dermatologist Could Benefit From”

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Various skin diseases include pain as a symptom, yet it seems that treatment plans rarely include analgesic. A study on needs assessment conducted at the Canadian Dermatology Association Conference in 2012, revealed that 70% of human dermatologists are uncomfortable managing pain and identified lack of training in the area as the number one barrier for using analgesics\(^1\). Hence the importance of reviewing the subject.

Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”\(^2\). It arises from activation of a set of receptors called nociceptors, which are preferentially sensitive to a noxious stimulus or to a stimulus that would become noxious if prolonged. The processes involved in the pain pathway include\(^3\):

a) Transduction: starts at the periphery with stimulation of free A-delta and C nerve endings or nociceptors. These nociceptors translate the mechanical, chemical or thermal energy (noxious stimuli) into electrical impulses.

b) Transmission: after the nociceptors are activated the electrical signals are transmitted initially by small diameter, myelinated A-delta fibers that conduct very fast and are responsible for pricking, sharp pain, and is referred as “first pain”. This pain is usually well localized and only lasts as long as the acute stimulus is activating the A-delta nociceptors. The transmission through smaller, unmyelinated C-fibers is conducted much more slowly, and is referred as “second pain”. A-delta and C fibers are found in skin, peritoneum, pleura, periosteum, subchondral bone, joint capsules, blood vessels, muscles, tendons, fascia, and viscera. The signal reaches the dorsal horn of the spinal cord through afferent fibers of both A-delta and C fibers.

c) Modulation: Consists of the amplification or suppression of the peripheral sensory nerve impulses at the level of the spinal cord. The cell bodies of A-delta and C fibers in the dorsal root ganglia extend axons that synapse with neurons in the dorsal horn within the gray matter. These connections could be with either: Excitatory or inhibitory interneurons; propriospinal neurons involved in segmented reflex activity, which stimulate efferent fibers that cause muscle contraction to generate a simple motor withdrawal response; or projection neurons extending to supraspinal centers (midbrain and cortex) through ascending tracts to synapse with third order neurons located in medulla, pons, midbrain, thalamus and hypothalamus and cerebral cortex.

d) Perception: Consists of the integration, processing and recognition of sensory information. Many parts of the brain are involved in nociception and perception of pain, including the reticular activating system, the thalamus, and the periaqueductal gray matter, but it is at the level of the cerebral cortex that an animal’s anticipation, learning and associations from painful experiences are processed and established.

Analgesia can be achieved by targeting these processes using different therapies. Few of the available options are:
- **Nonsteroidal anti-inflammatory drugs**: they act by inhibiting the enzyme cyclooxygenase (COX), which regulates the synthesis of prostaglandin, thromboxane, and prostacyclin from arachidonic acid. Inhibition of prostaglandin synthesis is key to the anti-inflammatory and analgesic effect. Given that inflammation is a common component of many skin diseases, NSAIDs can be an important alternative in the treatment. However, it is important to remember that they should not be given at the same time as systemic steroid, to avoid increasing the chances of gastrointestinal side effects.

Meloxicam is an NSAID approved for control of pain and inflammation associated with osteoarthritis in dogs. A new presentation of meloxicam is available as transmucosal oral spray (OroCam™). It has shown a 98% bioavailability and its efficacy has been demonstrated in a placebo-control trial in osteoarthritic dogs where the success rate after 28 days of treatment was 73% compared to 43% in the placebo group (Package insert).

Robenacoxib (Onsior®) is another NSAID approved for the control of postoperative pain and inflammation in cats. Its efficacy has been demonstrated in a placebo-control masked trial in client-owned cats, given perioperative 30 minutes before castration with onychectomy and once daily for 2 more days. Success rate was 83.5% compared to 53.8% in the placebo group (Package insert).

- **Opioids**: They act by binding to opioid receptors μ (μ), κ (κ), and δ (δ) at presynaptic and postsynaptic sites in the CNS and outside the CNS in peripheral tissues. Classification of opioids is based on their action at the receptors, whether they fully stimulate the receptor (full agonist), they stimulate the receptor but to a lesser degree (partial agonist), they block the receptor (antagonist), or they have different effect at different receptors (mixed agonist/antagonist). There are many options of opioid available for many types of pain and their selection is based on characteristics such as degree of analgesic effect, duration of action, side effects (vomit, histamine release, dysphoria, etc.).

Buprenorphine is a partial agonist at the μ receptor so it provides analgesia for moderate pain. It has a slow onset of action of about 30 minutes or more even if given intravenously. The most attractive advantage of buprenorphine is its long duration of action. Single doses of the regular 0.3mg/ml presentation given intravenously or intramuscular can last up to 6 – 8 hours. Another FDA approved formulation of 0.18 mg/ml of buprenorphine (Simbadol™) can be given subcutaneously and can last up to 24 hours. Additionally, a non-FDA approved compounded sustained-release polymer system that can release buprenorphine over 3 days is available (Buprenorphine SR™) and has shown to be effective combined with meloxicam managing pain after ovariohysterectomy in beagles5 and cats6. Excessive sedation has been reported in larger dogs.

Methadone is another μ-opioid agonist that has an additional benefit due to its NMDA antagonistic effect, which could be effective for different types of pain including neuropathic pain. It does not cause vomit and there is no histamine release associated with its administration. It is commonly used IV or IM and it is not recommended orally in dogs because its bioavailability is low7. In cats the oral transmucosal route has shown to produce 4 hours or more of antinociception8.

Fentanyl is a synthetic opioid commonly used in dogs and cats for perioperative analgesia. It has a short duration of action that limits its use to constant rate infusion. A new transdermal fentanyl solution (Recuvyra®) has been FDA-approved for dogs only. This is a volatile liquid-based drug delivery technology containing octyl salicylate as a penetration enhancer. It comes in an applicator that allows delivery of small volume to the skin at the dorsal scapular area without the need of hair clipping. Once dried, fentanyl is deposited into the stratum corneum allowing absorption and onset of effect between 2-4 hours and a prolong duration up to 4 days9.
**Local Anesthetics:**

Lidocaine is a very popular local anesthetic that is used for analgesia as well. It can be used for local/regional blocks, intravenously as a constant rate infusion or topical in gel and in a patch presentation. Lidocaine patch are effective at producing site-specific analgesia in dogs and cats with little systemic drug uptake. They can be cut to adjust for a specific area (area needs to be shaved) and to dose accordingly with the patient weight. They can be left in place for 3-5 days but they need to be checked to prevent dislocation or possible ingestion by the patient, which could cause severe toxicity.

**REFERENCES**

The body possesses powerful mechanisms to avoid immune recognition of self-proteins and thus prevent self-damage. Such immune tolerance to self-proteins can be acquired naturally during development or it can be induced experimentally by administration of self-antigens; the latter presents a novel therapeutic approach in several autoimmune diseases in people, including pemphigus and multiple sclerosis.\(^1\)

Central tolerance takes place in the primary lymphoid organs (thymus and bone marrow) before the immature T-cells and B-cells leave to the periphery. Several mechanisms have been identified to mediate central tolerance: i) receptor editing, ii) clonal deletion, iii) anergy, and iv) development of natural regulatory T cells. Self-reactive lymphocytes that escape central tolerance induction must be kept in check by additional measures of peripheral tolerance, which include: i) ignorance, ii) anergy, iii) deletion, and iv) development of inducible regulatory T and B cells. Several examples supporting this intricate network of regulation can be found in the literature. For instance, DSG3, the major autoantigen in human pemphigus vulgaris (PV) is expressed by medullary thymic epithelial cells; cells that are critical for negative selection of autoreactive T cells and development of a central tolerance.\(^2\) Additionally, autoreactive DSG3-specific B, Th1 and Th2 cells have been detected in the periphery in both healthy and affected individuals\(^3\), but, DSG3-specific, IL10-producing regulatory cells called Tr1 have been detected predominantly in healthy individuals, and only in 20% of PV-affected people.\(^4\) These findings imply that active peripheral regulation is critical in the prevention of disease development and might explain why a simple immunization with an autoantigen often fails to produce autoimmunity in experimental animals.\(^5\) Indeed, even if T- and B-cells from such an immunized healthy mouse are transferred into a recipient Rag2\(^{-/-}\) mouse lacking her own lymphocytes, this recipient mouse remains healthy. In contrast, a transfer of T- and B-cells from a mouse lacking DSG3 (DSG3\(^{-/-}\) mouse), a situation in which a tolerance to this self-protein cannot be naturally established, into a recipient Rag2\(^{-/-}\) mouse leads to a production of pathogenic anti-DSG3 antibodies and blister formation. The mechanisms of tolerance are multiple and interlinked.

**PROPOSED MECHANISMS TO EXPLAIN FAILURE OF SELF-TOLERANCE**

Autoimmune diseases are the result of an aberrant immune response against structures of self, or self-antigen. There are many theories attempting to explain the initial steps in the development of autoimmunity. One must keep in mind that genetics as well as environmental factors play critical role in the establishment and maintenance of the autoimmune response.

### 1. OVEREXPRESSION OF SELF-ANTIGEN

Lymphocyte clonal deletion, a part of central tolerance mechanisms, is more likely to affect lymphocyte clones with high receptor affinity to self, while clones with low affinity to self are more likely to escape and reach the periphery. These clones, that escape deletion, remain inactive in the periphery due to their receptor low affinity, which limits their activation, and because the self-antigens are only present in miniscule amounts. This immunological ignorance can be disrupted when larger amounts of self-antigen become released (trauma, impaired clearance, etc) in a supporting cytokine milieu (e.g. inflammation). For example, one of the core hypothesis for lupus pathogenesis involves an impaired clearance of apoptotic cells, leading thus to accumulation of nuclear antigens, which can then be recognized by the
immune system and actively targeted. Additionally, these accumulated self-antigens might become modified during the process of apoptosis and necrosis (methylation, acetylation, etc) forming so called danger signals that promote inflammation. In genetically susceptible individuals and with appropriate environmental triggers (sex hormones, drugs, UV, infection, etc), these accumulated self-antigens and danger signals trigger an autoimmune response resulting in autoantibody production and tissue pathology. Moreover, accumulated dead cells trigger chronic tissue inflammation, which makes the cytokine milieu in the tissue even more supportive for the development of autoimmunity. Interestingly, people suffering with cutaneous lupus exhibit delayed clearance of UV-induced apoptotic cells (up to 72hr) in contrast to healthy individuals (up to 24hr), a phenomenon that could explain the role of UV-light in induction of the disease.

2. EXPOSURE OF CRYPTIC OR SEQUESTRED SELF-ANTIGEN
Proteins in the body are built from amino acids, which are the key components of epitopes recognized by immune cells. Epitopes that are preferentially expressed and recognized are called dominant, while those that are physically hidden by conformation of the molecule, stereochemical alteration or sequestration in immune privileged site are called cryptic. In the thymic selection process, it is clear that immune cells recognizing dominant self-epitopes are deleted and those recognizing cryptic epitopes have a higher chance to escape to the periphery. Similarly, in the periphery, hidden or cryptic epitopes are only minimally presented to immune cells and thus have only limited ability to establish peripheral tolerance. Events leading to the exposure of cryptic epitopes (e.g. trauma, infection) are thought to trigger autoimmunity in susceptible individuals.

Similarly, immune responses to cryptic epitopes play a role in the epitope spreading phenomenon. In this phenomenon, an immune response that had developed against one tissue epitope (for example in an autoimmune reaction started by a different mechanism, etc.) leads to tissue destruction over time, which exposes a second tissue epitope (not cross-reactive with the original triggering epitope) that was originally hidden from an immune surveillance in a healthy tissue. Sympathetic ophthalmia represents a classical example of disease caused by exposure of a sequestered self-antigen in one eye due to an injury, followed by a breakdown of tolerance and evasion of the privileged site of the other, originally uninjured eye.

Three decades of research have confirmed that the hair follicle represents another immune privilege site with features like no MHC class I and II expression by hair follicle cells, reduced presence of antigen presenting cells and presence of special Langerhans cells with limited MHC class II expression and increased expression of pro-tolerogenic molecules including indolamine 2,3-dioxygenase (IDO), IL-10, TGH and MSH. Disruption of this immune privilege and exposure of originally sequestered antigen(s) of the hair follicle is the leading theory of alopecia areata pathogenesis. Loss of immunological tolerance in the follicle is associated with initiation of expression of MHC I molecules, which present originally hidden hair follicle antigen(s) to the immune system.

3. ABERRANT EXPRESSION ON MHC MOLECULES
Expression of MHC class II molecules in a tissue, which usually does not express such molecules, can lead to presentation of self-antigens to autoreactive CD4+ T cells and thus initiate or potentiate an autoimmune response to those self-antigens. Pemphigoid gestationis (PG) is an example of disease in which aberrant expression of MHC class II has been proposed as the initial step in development of autoimmunity. This disease is characterized by urticaria or blister-formation starting usually around the umbilicus of women during the second or third trimester of their pregnancy. The disease shows spontaneous remission with average of 4 and 60 weeks for the blisters and urticarial lesions, respectively. NC16A epitope of collagen XVII has been identified as the major target antigen in this disease, the source of which in PG is the placenta. The cause for production of the antibodies is not fully clear, but several lines of evidence suggest an important role for aberrant MHC class II expression by stromal and trophoblast cells of the placenta.
4. **EPITOPE SPREADING**

Epitope spreading theory, first described by Lehmann *et al.* in 1992, is considered to be an important mechanism contributing to the dynamic and evolving character of autoimmune diseases. The main principle of this theory is that continual tissue damage (e.g. due to infection or already existing autoimmune disease) exposes new epitopes and will cause autoreactive T cells to become activated de novo to these newly uncovered epitopes within the original molecule (intramolecular epitope spreading) or on a new molecule (intermolecular epitope spreading). There are multiple examples of epitope spreading in human as well as veterinary dermatology. In people, for example, intermolecular epitope spreading has been proposed to play a role in the pathogenesis of paraneoplastic pemphigus, in the transition of pemphigus foliaceus to bullous pemphigoid, in development of bullous systemic lupus erythematosus (SLE) from SLE, etc. Although intermolecular epitope spreading has not been well documented in veterinary dermatology, possible examples of this process can be found in the published literature, including mixed subepidermal autoimmune blistering dermatosis in dogs with autoimmunity against laminin 332 and collagen VII, paraneoplastic pemphigus or bullous SLE, etc.

The phenomenon of intramolecular epitope spreading is often discussed in dermatology in conjunction with an entity called endemic pemphigus foliaceus (PF), also called Fogo Selvagem (FS). This disease presents as a superficial blistering skin disease and shares clinical, histological and immunological similarities with the classical PF. The major target antigen is desmoglein-1 and, like in the classical human PF, and possibly in classical canine PF, the pathogenic antibodies are of IgG4 subclass. Intramolecular epitope spreading is one of several key events in the pathogenesis of FS. Studies showed that while healthy inhabitants and FS patients in remission express anti-DSG1 antibodies recognizing the EC5 domain of the protein, patients with active disease produce antibodies against additional epitopes localized in the EC1 or EC2 domains. The pathogenicity of the anti-DSG1 EC1/EC2 antibodies was demonstrated by a blister-formation after a passive transfer to mice, while the anti-DSG1 EC5 antibodies failed to induce blisters.

5. **MOLECULAR MIMICRY**

Although the small number of amino acids used to make proteins allows for numerous combinations, identical sequences (less than 10 amino acids) can be found among self- and nonself-proteins. As a consequence, an immune response mounted against an epitope present in a pathogen (viral, bacterial or parasitic), which is similar or identical to a self-antigen, can trigger immune response not only against a pathogen, but also against self. This phenomenon is known as molecular mimicry and there are numerous examples throughout human and veterinary medicine. Probably the most well known disease with an underlying molecular mimicry mechanism is rheumatic fever, in which the antibody response mounted against a group *A Streptococcus* cross-reacts with self-antigen expressed in the heart, skin, joints and brain. The consequence is clinical disease characterized by polyarthritis, endocarditis, erythema marginatum or subcutaneous nodules, etc.

It has been long hypothesized that molecular mimicry is one of the critical pathomechanisms of FS. Indeed, several lines of evidence support this hypothesis: a strong geographical link, an association with rural life-style, the resolution of clinical signs after leaving the geographical location, a reduction of antibody titer with increasing distance from the reservation, an increased prevalence at the end of the rainy season, an association with other vector-borne infections and exposure to insects. Interestingly, it has been demonstrated that anti-DSG1 IgG response precedes the development of clinical signs and that anti-DSG1 IgG antibodies can be detected in more than half of healthy people living in the Limao Verde reservation. In contrast, the same study showed that only approximately 2% of healthy people from USA and Japan tested positively for anti-DSG1 IgG antibodies. This observation suggested that an environmental trigger present in Limao Verde plays a critical role in the initiation of the disease. In more direct support of this hypothesis, in 2012, Qian and colleagues were able to demonstrate that the salivary gland antigens from *Lutzomyia longipalpis*, specifically the LJM11 salivary protein is recognized by FS antibodies. Moreover, mice immunized with LJM11 generated anti-DSG1 antibodies, thus confirming
that insect salivary antigen delivered into the skin can lead to development of cross-reacting antibodies, which likely occurs in genetically susceptible individuals.22

6. Failute of Central Tolerance
A syndrome called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) represents an example of a failure of central tolerance caused by a defect in the AIRE gene, which is responsible for self-antigen presentation in the thymus and deletion of autoreactive T cells ([OMIM] number 240300). Thus autoreactive T cells, instead of being deleted, enter the periphery, where they cause a multi-organ autoimmunity leading to hypoparathyroidism, hypoadrenocorticism, hypothyroidism, hypogonadism, vitiligo, alopecia areata, hepatitis, etc.

Disruption of central tolerance has been proposed as one of the mechanisms leading to autoimmunity associated with thymoma. Indeed, more than 50% of people with thymic neoplasia were diagnosed with some form of an autoimmune disease 23, and relatively frequent reports of thymic neoplasia-associated autoimmunity can be found also in the veterinary medicine (e.g. paraneoplastic pemphigus and thymic lymphoma, myasthenia gravis and thymoma, erythema multiforme and thymoma, etc).24-26 Hypotheses explaining this increased prevalence of autoimmune diseases include: 1) Theory of immaturity (immature T cells lacking sufficient self-tolerance escape to the periphery); 2) Genetic theory (increased T cell proliferation leads to increased production of autoreactive clones, and/or impaired expression of HLA in neoplastic epithelial cells causes misseducation and possible selection of autoreactive clones).27

Conclusions
One must bear in mind that the etiology of autoimmunity is complex and multifactorial, with a polygenic genetic background interacting with triggering environmental and/or individual factors. Considering this, it becomes clear that initiation of autoimmune disease may involve more than one of the mechanisms described above in conjunction with supportive, proinflammatory cytokine milieu, susceptible genetics and environmental triggers.

References:
WHICH AUTOIMMUNE SUBEPIDERMAL BLISTERING SKIN DISEASE (AISBD) THE DOG HAS?
Since the original report of bullous pemphigoid (BP) in a dog more than 30 years ago, the complexity and heterogeneity of canine AISBDs was expanded to following disease entities: mucous membrane pemphigoid (MMP), epidermolysis bullosa acquisita (EBA), junctional epidermolysis bullosa acquisita (JEBAs), mixed AISBD, type I bullous systemic lupus erythematosus (BSLE-I), linear IgA dermatosis (LAD) and pemphigoid of gestation (PG). Of these AISBDs, MMP and EBA are the most common entities seen in dogs, accounting for about 50% and 25% of all AISBDs, respectively. Vesicles, deep erosions and ulcers and histological demonstration of a dermo-epidermal separation are the hallmarks of AISBDs. Unfortunately, there is no commercially available immunological test to differentiate between them. Therefore, the final most likely diagnosis is obtained by using an analytical thinking, which takes into consideration the unique clinical features of each disease (e.g. breed predisposition, skin lesion type and distribution, presence and type of inflammation histologically), and of “playing the odds”, which takes into consideration the prevalence of each specific disease (see table below). In addition, histological assessment of the degree and type of inflammation accompanying the blister formation and the dermo-epidermal level can further help in predicting the most likely diagnosis.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Rank among canine AISBDs</th>
<th>Predisposed breed(s)</th>
<th>Characteristic clinical signs</th>
<th>Typical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP</td>
<td>#1 (~50% of AISBDs)</td>
<td>GSD</td>
<td>Vesicles, erosions/ulcers and scarring</td>
<td>Mucosae and mucocutaneous junctions predominant</td>
</tr>
<tr>
<td>EBA</td>
<td>#2 (~25% of AISBDs)</td>
<td>Great Dane (young)</td>
<td>Vesicles, erosions/ulcers</td>
<td>Skin predominant, especially friction areas and footpads; + mucosae/mucocutaneous junctions</td>
</tr>
<tr>
<td>BP</td>
<td>#3 (10% of AISBDs)</td>
<td>n.d.</td>
<td>Vesicles, erosions/ulcers</td>
<td>Skin predominant</td>
</tr>
</tbody>
</table>

GSD German shepherd dog; n.d. not determined;

CUTANEOUS LUPUS ERYTHEMATOSUS (CLE)
For a while, discoid lupus erythematosis was the only well characterized cutaneous lupus erythematosus in dogs. Today, canine CLE can be subdivided into four disease entities: i) vesicular cutaneous lupus (VCLE)²³, discoid lupus (DLE), which can be classic facial form⁴ or generalized⁵⁶, exfoliative cutaneous lupus (ECLE)⁷ and a newly described mucocutaneous lupus (MCLE)⁸⁹. Diagnostically useful clinical and histological features as well as the treatments of each form will be discussed in the lecture.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Predisposed breed(s)</th>
<th>Characteristic clinical signs</th>
<th>Typical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLE (classic form)</td>
<td>n.d. (collies?)</td>
<td>Depigmentation, atrophy, erosions</td>
<td>Nasal planum</td>
</tr>
<tr>
<td>DLE (generalized form)</td>
<td>n.d.</td>
<td>Hyperpigmented annular or polycyclic plaques with scaling and erosions</td>
<td>Haired skin (generalized)</td>
</tr>
<tr>
<td>VCLE</td>
<td>collies, shelties</td>
<td>Flaccid vesicles, polycyclic erosions and ulcers</td>
<td>Groin, axillae (glabrous skin)</td>
</tr>
<tr>
<td>------</td>
<td>-------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>ECLE</td>
<td>German shorthaired pointer</td>
<td>Scaling, alopecia, erosions (systemic signs later in life)</td>
<td>Head, face, ears, dorsal back</td>
</tr>
<tr>
<td>MCLE</td>
<td>GSD</td>
<td>Erosions, ulcers</td>
<td>Mucocutaneous junctions and adjacent skin</td>
</tr>
</tbody>
</table>

**THERAPEUTIC EFFECT(S) OF HYDROXYCHLOROQUINE IN LUPUS**

The pathogenesis of lupus is complex and not yet fully uncovered. Genetic and environmental triggers play role. An increased susceptibility to apoptosis and altered clearance of apoptotic cells leads to accumulation of nucleic material, which forms complexes with endogenous DNA-binding proteins. These complexes stimulate plasmacytoid dendritic cells (pDC) through TLR9 and TLR7, which leads to enhanced production of type I IFNs and reduction of Tregs. The overexpression/exposure of a self-antigen (nuclear material) in the supporting proinflammatory cytokine milieu (IFNs, IL-6, TNF-α) allows for activation of autoreactive T cells, which in turn activate B cells to produce autoantibodies. Autoantibodies that cause direct tissue damage, activate complement cascade, form immune complexes and activate other cells through FcαRs (e.g. neutrophils, cytotoxic T cells, NK cells), which releases more nuclear material and perpetuates the autoimmune response.

Hydroxychloroquine (HCQ) is an antimalarial agent with anti-inflammatory properties and it is currently one of the drugs of choice in severe or generalized forms of cutaneous LE in people (an excellent treatment algorithm can be found in the following review paper 11). The efficacy of HCQ in treatment of CLE could be explained by a variety of immunomodulatory effects (see Figure 1). One of the most intriguing mechanisms is the inhibition of TLR7 and TLR9 signaling on pDC with a subsequent disruption of the production of inflammatory cytokines (IFNs, TNF-α, IL-6) and interruption of the downstream steps. In veterinary medicine, HCQ has been used successfully for treatment of exfoliative CLE and generalized discoid LE. In all four dogs treated with HCQ, the agent appeared to be effective to either induce full remission (one case of generalized DLE) or to stop the disease progression (2/3 dogs with exfoliative CLE). The duration of the treatment in the three positively responding cases ranged from 7 to 12 months and the dosage was 5 mg/kg (one dog) or 10 mg/kg (two dogs) once daily. In two dogs receiving the higher dosage, a regular echocardiogram, ophthalmologic examination and blood work did not reveal any abnormalities. The dog treated with the 5 mg/kg dosage exhibited mild retinal degeneration at one year recheck, but this was concluded to be age-related most likely.

*Implications for practice:* Treatment approach for CLE in dogs is dependent on the subtype and its severity. This author’s proposal of a simple treatment algorithm can be seen in a Figure 2. Hydroxychloroquine is a treatment option for cases with generalized disease or for poorly responsive cases. Specifically, HCQ has been shown to be fairly effective for the exfoliative CLE; a disease that has shown relatively poor response to cyclosporine. It is very cheap and relatively safe treatment; however, regular ophthalmological exams are warranted to check for signs of a retinopathy. This side-effect is uncommon and usually dose- and duration of treatment-dependent in people (i.e. cumulative effect of the drug). The onset of action for HCQ is slow; therefore, an additional, fast-acting drug needs to be considered in severe cases to induce faster improvement and/or remission in conjunction with HCQ.
Figure 1: Selected immunomodulatory effects (additional anti-inflammatory effects include inhibition of phospholipase A2 and matrix metalloproteases)

Figure 2: Proposed treatment algorithm for cutaneous lupus erythematosus.

References:


**Conflict of Interest Statement:**
The author does not have any relevant conflict of interest related to this topic.
FELINE IMMUNE-MEDIATED SKIN DISEASES

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INTRODUCTION:
Autoimmunity is a multi-factorial event resulting in the breach of self-tolerance and induction of an immune response against (a) self-antigen(s). Although the traditional classification of autoimmune diseases as either B cell- or T cell-mediated is still used to simplify the complex nature of the processes, it is critical to realize that autoimmunity involves all, innate and adaptive, aspects of the immune response. Furthermore, genetics, environmental triggers like UV-light, drugs and infectious agents are well known factors that can play a role in the initiation or perpetuation of the autoimmune response.

Many feline autoimmune skin diseases recognized over the years represent clinical and/or histological homologues of those seen in humans and dogs. In general, these diseases are very rare, which represents the main impediment for understanding the underlying pathomechanism or for establishing optimized treatment guidelines. Indeed, except a recently published case of a paraneoplastic pemphigus in a cat, there have been no recent updates on autoimmune skin diseases of a cat.

To overcome this lack of information, a translation of knowledge from human and canine disease equivalents is often undertaken. As a result the available treatment recommendations are often empirical, based on a single case report or are extrapolated from human and canine medicine. In general, clinicians prescribing an immunosuppressive treatment should always try to answer several main questions beforehand:
1. What kind of autoimmune skin disease am I dealing with?
2. Is this disease likely antibody-mediated or T-cell mediated?
3. Are there published effective treatment protocols (in cats or other species)?
4. Are there any contradictions for usage of particular drugs in cats? (cats are not small dogs)
5. Are there any contradictions for usage of a particular drug in my patient? (concurrent disease, concurrent medications, etc.)
6. How am I going to monitor for efficacy and potential side-effects?

SKIN DISEASES WITH A DOMINANT ANTIBODY-MEDIATED PATHOMECHANISM
In antibody-mediated diseases where autoantibodies drive the clinical phenotype of the disease, the breach in tolerance will result in the activation of self-reactive T cells that, in turn, will help B cells to produce autoantibodies. Secreted autoantibodies may target specific autoantgens and cause direct tissue damage (e.g. pemphigus, bullous pemphigoid), or they may form immune complexes and thus activate other immune cells and the complement cascade resulting in secondary tissue damage (e.g. SLE, bullous pemphigoid). Activated B cells then further add to the inflammation by producing a myriad of cytokines and, importantly, they internalize self-antigens from damaged tissues and present them back to cognate T cells, thus contributing to an amplification loop of the pathologic immune response.
**FELINE PEMPHIGUS FOLIACEUS**

The pemphigus group includes autoimmune blistering skin diseases in which autoantibody-targeted desmosomes undergo a variety of changes resulting in loss of adhesion and blister formation. Several variants of pemphigus, separated according to the clinical signs, the depth of acantholysis and the targeted antigen(s), have been recognized in humans and animals. Two pemphigus variants are described in cats: a) superficial: pemphigus foliaceus (PF) and b) deep: pemphigus vulgaris (PV). Little is known about the pathogenesis of feline PF, at the time of this writing, the target antigen(s) remain unknown. There is some evidence that cats with pemphigus produce antikeratinocyte autoantibodies. Keratinocyte-bound IgG were detected in a majority of feline PF and PV patients.\(^1\)\(^-\)\(^3\) Detection of circulating autoantibodies by indirect immunofluorescence yielded only rare positive results in the past and this technique had been considered unreliable; this was likely due to the type of substrate used to perform the test. The use of feline footpad and buccal mucosa increased the level of detection significantly. Indeed, seven out of 10 feline PF sera tested in our laboratory contained detectable antikeratinocyte IgG antibodies.

Clinically and histologically, feline PF resembles its canine homologue. Characteristic signalment and clinical signs based on a literature review of historically published cases will be discussed during the lecture. An excellent reference on feline PF can also be found in a review article on superficial pemphigus.\(^3\)

**TREATMENT OF FELINE PEMPHIGUS FOLIACEUS**

Presently, there are no established treatment guidelines for treatment of feline PF and, therefore, the choice of treatment is based on published PF cases (summary of historical treatment data and outcomes will be discussed during the lecture) and other immune-mediated diseases. Traditionally, an immunosuppressive dose of prednisone or prednisolone at dosages 2-6 mg/kg per day was prescribed to treat both PF and PV. In most reported cases, a monotherapy with glucocorticoids was sufficient to control clinical signs.\(^1\)\(^,\)\(^2\) The wide range of the dosages and the observed “resistance” of some cats to glucocorticoids could have been due to the insufficient bioavailability of prednisone in cats. It is, therefore, recommended to use prednisolone instead of prednisone in this species.\(^4\)

If additional immunosuppression is required, chlorambucil is often the drug of choice used in combination with glucocorticoids. Azathioprine had been used in feline PF and also other autoimmune and immune-mediated diseases.\(^5\) Unfortunately, reports of rapid and severe bone marrow suppression, even with every-other-day dosing, have been of concern, and this drug has been abandoned from a wide usage among clinicians. A possible explanation of such high sensitivity of cats to azathioprine could be their low (genetic) activity of enzyme thiopurine methyltransferase responsible for degradation of toxic by-products in cats.\(^6\)

New treatment options, whose efficacy for management of feline autoimmune diseases awaits to be investigated, include mycophenolate mofetil, leflunomide, methotrexate and cyclosporine. All these drugs have been already used to manage other immune-mediated feline diseases and, although not as a first choice drugs, they may present a therapeutic option in refractory cases, especially as glucocorticoid-sparing agents.\(^7\)\(^-\)\(^10\)

**SKIN DISEASES WITH A DOMINANT T-CELL MEDIATED PATHOMECHANISM**

In some autoimmune diseases, self-reactive T cells that lost tolerance are the main effector mechanism responsible for the clinical signs. The tissue damage seen in such diseases is predominantly the result of a direct cytotoxicity of the autoreactive T cells and/or by their soluble
products. Because of the rarity and lack of new information on feline lupus, the presentation will focus on thymoma-associated autoimmunity in cats and the variety of clinical phenotypes this neoplasia can be accompanied by.

**THYMOMA AND AUTOIMMUNITY**

Thymus plays multiple roles in the development and regulation of the adaptive immune system: a) development of immunocompetent T cells, b) differentiation of T cell into subsets, c) establishment of immune tolerance, d) regulation of production of mature T cells in the thymus and at the periphery via hormones. Therefore and not surprisingly, a neoplasia affecting the thymus could disturb this finely tuned process and lead to an immune dysregulation.

In cats, thymomas are an uncommon neoplasia (3 per 1060 cats diagnosed with neoplasia, or 2 per 3200 hospital admitted cats). Although the most common clinical signs are dyspnoea and pleural effusion, numerous reports of autoimmune/immune-mediated conditions associated with thymomas can be found in the literature. The most commonly reported entities are myasthenia gravis and thymoma-associated exfoliative dermatitis (TAED), but a case of immune-mediated granulocytopenia and a case of erythema multiforme have been also described in cats with a thymoma. Similarly, dogs and up to 45% of people with thymomas will be diagnosed with a concurrent autoimmune disease.

It is not fully understood how a thymoma leads to an immune dysregulation, but several hypotheses have been proposed: a) an escape of thymoma-driven thymocytes into the periphery without critical selection and maturation, b) an increased proliferation of thymocytes leading to the expression of aberrant (autoreactive) T cell receptors (TCR), or c) genetic mutations resulting in an impaired expression of HLA class II molecules by epithelial cells in the thymus responsible for T cell education.

**Dermatological presentations observed in cats with thymoma**

<table>
<thead>
<tr>
<th>Thymoma-associated syndrome</th>
<th>Clinical presentation</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exfoliative dermatitis</td>
<td>Erythema, exfoliation and secondary alopecia (generalized, often starting at the head and progressing caudally)</td>
<td>Epidermal hyperkeratosis, interface dermatitis and mural folliculitis; milder transepidermal apoptosis than EM</td>
</tr>
<tr>
<td>Exfoliative dermatitis/Erythema multiforme combination*</td>
<td>Erythema and exfoliation on the head and pinnae, ulceration at the site of imidaclopride application and erythematous papules and “bull’s eye” lesions in the groin and axillae</td>
<td>Keratinocyte apoptosis at all levels, lymphocyte satellitosis and interface dermatitis</td>
</tr>
<tr>
<td>TEN-like*</td>
<td>Large macules progressing into ulcers affecting &gt;70% of the body, oral and mucocutaneous ulceration</td>
<td>Diffuse devitalization of the epidermis, lymphocytic exocytosis and apoptosis at all levels at the periphery, same process affects the hair follicle epithelium</td>
</tr>
<tr>
<td>Paraneoplastic pemphigus19</td>
<td>Maculo-papular rush progressing into deep erosions and/or ulcers (abdomen, ear canals)</td>
<td>Subepidermal clefing with scattered apoptotic keratinocyt throughout the epidermis and interface dermatitis present</td>
</tr>
</tbody>
</table>

* one published case (Godgrey, 1999) and one unpublished; * 2 unpublished case (P. Bizikova)

As seen above, thymoma in cat can be associated with several autoimmune processes, which usually involve both antibody-mediated and cell-mediated cytotoxicity. Treatment should be
aimed at removing the thymoma. In some cases, however, the already started autoimmune response requires an additional immunosuppressive treatment (IVIG in cases like TEN or PNP as an initial treatment could be of benefit, immunosuppressive dose of glucocorticoids with or without concurrent cytotoxic drugs like chlorambucil). In case of complete tumor removal, the immunosuppressive treatment is usually needed only temporarily.

References:
CONCURRENT SESSION
PRESENTATIONS
FRIDAY
SPOROTRICHOSIS: NUMBERS DON’T LIE

Laboratory of Clinical Research on Dermatozoonosis in Domestic Animals, Evandro Chagas Clinical Research Institute/ Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Alessandra V Pereira, MVSc, PhD in progress

EPIDEMIOLOGICAL ASPECTS

Sporotrichosis is a subcutaneous mycosis caused by *Sporothrix schenckii* complex species. The disease occurs worldwide, mainly in tropical and subtropical areas. It is endemic in Latin America, especially in the State of Rio de Janeiro, Brazil, where the first epidemic of zoonotic sporotrichosis was described at the end of the 1990s. It infects a variety of species, including dogs, rodents, parrots, squirrels, horses, birds, cats and humans.

*Sporothrix* can be found in nature, and the classic transmission is related to agricultural work and other activities involving contaminated plants and soil. Such activities may include, farming, wood exploitation and even mining, not to mention occupational or leisure floriculture and gardening. There is also a rare form of transmission and that is by inhalation.

In Brazil, over 4,000 cases of feline sporotrichosis have been diagnosed, since 1998. In other words, the overwhelming majority of the cases of this mycosis have happened in Brazil and in cats, and the classic explanations do not apply down there. Then, our classic transmission of *Sporothrix* is zoonotic.

The cat is the animal species most affected by sporotrichosis and plays an important role in the zoonotic transmission of this disease.

ETIOLOGIC AGENT

*Sporothrix schenckii* complex species comprise a group of thermo-dimorphic pathogenic fungi which is composed by *Sporothrix schenckii sensu stricto, Sporothrix albicans, S. globosa, S. mexicana, S. schenckii luriei and Sporothrix brasiliensis.*

*Sporothrix brasiliensis* is the prevalent etiological agent of feline sporotrichosis and it answers for 96.9% of the cases in Brazil. This fungus has a most virulent profile and that explains the fact that the cases which have been found in Brazil are much more aggressive compared to other reported cases in the world.

SOURCES OF INFECTION AND TRANSMISSION

The agent can be found in nature, especially in soil, rose thorns, sphagnum moss and decomposing wood. However, in the vast majority of brazilian cases, the source of infection is the sick cat.

Cats present a high parasite burden in skin lesions and their natural behavior such as, sharpen their claws in trees, fight for territory and females, facilitates dispersal of the fungus in the environment.

The transmission of *Sporothrix* spp to other cats and to humans occurs via inoculation of yeast cells by means of bites, scratches and contact with skin lesions.

CLINICAL FINDINGS

Sporotrichosis occurs mainly in young adults, intact male cats that roam outdoors. There is no breed predisposition. Clinically, it ranges from a subclinical infection to fatal systemic forms and skin lesions vary from small papules to extensive necrosis areas. However, ulcers and nodules are the most frequent lesions observed in dogs and cats. In cats, they can be found at three or more noncontiguous anatomical sites, usually on the head, especially on the nose, scrotum and legs. Paronychia is also commonly observed and they may end up losing their claws. On the other hand, in dogs lesions are usually limited to their faces. Compared to the cases in cats, human lesions appear more frequently as a cutaneous lymphatic form.
As to extracutaneous signs in cats, respiratory tract signs such as, sneezing, dyspnea, and nasal discharge are more commonly seen and they may precede the dermatological signs.\(^1\) It is important to point out that the occurrence of these signs is associated with treatment failure and death.\(^11,12\)

**LABORATORY DIAGNOSIS**

The gold standard for sporotrichosis detection is fungal culture.\(^1\) The samples are collected by means of sterile swabs and biopsies of lesions. The swabs should be seeded on Sabouraud’s agar and Mycosel agar, where possible, and then sent to the laboratory, at room temperature for mycologic examination.\(^1\)

Molecular techniques, such as polymerase chain reaction (PCR) targeting of the calmodulin gene have been recently adopted as auxiliary tool for the diagnosis.\(^7\) However, it cannot be routinely used except by research institutions, because it is not currently commercially available in Brazil.

Cytopathological and histopathological examinations are very useful tools for obtaining a preliminary diagnosis of sporotrichosis in cats.\(^11\) The sensitivity of cytology using a Romanowsky-type stain is 79%\(^13\) and the sensitivity of histopathology using Grocott methenamine silver (GMS) is 94%.\(^14\) In the case of dogs, though, these kinds of exams are not conclusive, in spite of the fact that there are very rare cases where dog lesions present a high number of yeasts.

Histologically, the lesions of feline sporotrichosis are characterized by a pyogranulomatous inflammatory reaction with a low frequency of well-formed granulomas and high fungal loads.\(^14\) The enzyme-linked immunosorbent assay (ELISA) may be used as a sensitive and specific screening tool for the detection of Sporothrix antibodies in the serum of cats with sporotrichosis.\(^15\)

**DIFFERENTIAL DIAGNOSIS**

The differential diagnosis in cats should include ulcerative and/or nodular diseases.\(^4,12,16\) The most common ones are: neoplasia, especially squamous cell carcinoma; other fungal diseases like cryptococosis and histoplasmosis; allergic skin diseases; bacterial diseases; leishmaniasis; drug reaction.\(^12,16\)

It is important to mention that in endemic areas, first, it is recommended to rule out the sporotrichosis in cases of cats presenting nodules or ulcers.

References:


CAT CHANCE: ZOOMING IN ON SPORO TREATMENT

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Treatment of feline sporotrichosis presents a challenge for many reasons, from simple ones related to cats’ behaviour, like the difficulty of keeping cats confined during treatment and the difficulty of administering oral medication to a lot of them, to more specific reasons like the fact that there are few antifungal agents on the market to treat this disease and these drugs present many adverse effects.1,2

Many owners abandon the treatment when the skin lesions have disappeared and they think the disease is gone, or simply because they think it is too long. Nevertheless, the irregularity of the treatment may lead to the recurrence of the disease, imposing difficulties to the cure process.2

ANTIFUNGAL AGENTS AND OTHER TREATMENT OPTIONS

Itraconazole: It is currently considered the drug of choice for the treatment of feline sporotrichosis. However, it has been used in higher doses because of the difficulty in achieving clinical cure with the doses recommended in the literature (5–10 mg/kg every 24 hours).3 Oral solution is preferred to capsules because it permits more accurate dose measurement, plus it improves absorption and bioavailability4,5, hence, dosages of oral solution are lower (1.25-1.5 mg/Kg/every 24 hours) when compared to those of capsules (8.3 -27.7 mg/kg/every 24 hours).3 Nevertheless, oral solution is not available in some countries. Itraconazole is a lipophilic compound and it is absorbed in very low Ph reason why it must be administered after meals.4 However, an oral suspension containing cyclodextrin has superior bioavailability than oral capsules, consequently, the solution should be given on an empty stomach.4,5

Some drugs like histamine H2-receptor antagonists or proton pump inhibitors must be avoided when using itraconazole because they caused gastric acid suppression and cause significant impairment to absorption.4

As for its adverse clinical effects, some cats may present hiporexia or anorexia, vomiting, apathy, weight loss and increasing enzyme levels.6

Ketoconazole: This azole may be used to treat feline sporotrichosis, but it is not as effective as itraconazole and it is associated with a high occurrence of adverse effects in cats, as it was described in a study with 773 sporotrichosis-infected cats.6

Potassium iodide (KI): It is not considered to be an antifungal agent. The mechanism of the iodide action is still not totally understood, but it seems that it mediates the inflammatory response, enhancing the immune defense system.7 KI can be used in alone or in combination with itraconazole, especially in cats with respiratory signs and nasal mucosa lesions.1 In a study involving 48 cats treated with KI monotherapy, the cure rate was 47.9% and clinical adverse effects were observed in 52.1% of cases.7

Fluconazole: It may be an alternative to itraconazole, because it has better tissue penetrability and cats may tolerate it better.3 However, we need more data. Further clinical trials should be carried out to determine the ideal dosage of this medication for feline sporotrichosis. In addition, fluconazole is much more expensive than itraconazole.

Amphotericin B (AMB): Reports on the administration of AMB for treatment of feline sporotrichosis are scarce.1 Intravenous administration of the drug in cats is limited because of serious adverse effects1. In a study involving 26 cats with residual localized skin lesions refractory to itraconazole, intralesional AMB was used in combination with itraconazole.3 Clinical cure was achieved in 72.7% of the cats.
after administration of that combination once a week, for two or three weeks. Lipid formulations of AMB are reported to be less nephrotoxic than the conventional drug. It was successful used combined with oral itraconazole in a cat with feline sporotrichosis refractory to itraconazole monotherapy.

**Terbinafine:** It has been effective and safe in the treatment of cutaneous sporotrichosis in humans. Nevertheless, results are inconclusive regarding its use in treatment of feline sporotrichosis.

**Voriconazole (VCR):** The efficacy of Voriconazole (VRC) was evaluated in a murine model of disseminated sporotrichosis and the results of this study suggest that VRC might have potential in the treatment of disseminated infection by *S. schenckii* but not by *S. brasiliensis*. However, there have been no study confirming the efficacy of voriconazole in the treatment of feline sporotrichosis. Other options such as cryosurgery has been encouraged as a complementary therapy in cats with persistent and localized skin lesions.

**References:**

ACQUIRED PRIMARY HYPOTHYROIDISM: A CHALLENGING DIAGNOSIS

Cooper JC

CANINE HYPOTHYROIDISM

Canine hypothyroidism is considered a common endocrine disorder in dogs with a reported prevalence varying from 0.2 to 0.8%.\textsuperscript{1,2} It is the result of a deficiency of the active thyroid hormones triiodothyronine (T\textsubscript{3}) and thyroxine (T\textsubscript{4}). The onset of clinical signs of acquired primary hypothyroidism is slow and insidious. Clinical signs may vary with breed and are non specific. Clinicopathologic changes are also non specific and are observed in multiple other disorders. The diagnosis of acquired primary hypothyroidism requires a detailed history including previously administered drugs, a careful physical exam, a minimum database, exclusion of significant non thyroidal illness (NTI) and an appropriate combination of thyroid gland function tests.

PRIMARY ACQUIRED HYPOTHYROIDISM

Hypothyroidism may be congenital or acquired and results from a defect in any part of the hypothalamic-pituitary-thyroid axis. Acquired hypothyroidism may be primary or central. Central hypothyroidism is rare, accounting for less <5% of cases, and arises from a defect in the pituitary (secondary hypothyroidism due to decreased thyroid stimulating hormone (TSH) synthesis) or the hypothalamus (tertiary hypothyroidism due to a thyroid releasing hormone (TRH) deficiency).\textsuperscript{3,4} Primary hypothyroidism, due to an abnormality at the level of the thyroid gland, accounts for the majority of cases of hypothyroidism. Primary hypothyroidism has been attributed to lymphocytic thyroiditis, idiopathic atrophy, neoplastic destruction, iodine deficiency and iatrogenic causes (surgical removal, antithyroid medications, potentiated sulfonamides, and radioactive treatment).\textsuperscript{3} Lymphocytic thyroiditis and idiopathic atrophy occur with equal frequency and account for 95% of cases of acquired primary hypothyroidism.\textsuperscript{5}

Idiopathic atrophy: Idiopathic atrophy is histologically distinct from the changes induced by secondary hypothyroidism and is characterized by a progressive reduction in the size of thyroid follicles and replacement of the degenerating follicles with adipose tissue.\textsuperscript{3} The syndrome is either a primary degenerative disorder or may be the end stage of lymphocytic thyroiditis.\textsuperscript{4}

Lymphocytic thyroiditis: Lymphocytic thyroiditis is histologically characterized by a diffuse infiltrate of lymphocytes, plasma cells and macrophages with progressive destruction of follicles and secondary fibrosis. Clinical signs only occur once 75-80% of the gland is destroyed.\textsuperscript{3} Humoral and cell mediated immunity play a role in the pathogenesis of the disorder. The main targets appear to be the thyroid antigens thyroid peroxidase (TPO) and thyroglobulin (Tg). Initiating factors are poorly understood however documented breed predispositions and the familial nature of the disorder suggests that genetics plays a major role. High risk breeds include English Setter, Golden Retriever, Rhodesian Ridgeback, Cocker Spaniel and Boxers.\textsuperscript{5} A familial association is recognized in Great Danes, colonies of Beagles and Borzoi, Hovawarts and Giant Schnauzers.\textsuperscript{6,7,8,9}

The development of a decreased T\textsubscript{4} and increased serum TSH occurs over a prolonged period of 1-3 years. There is evidence of progression from a Tg autoantibody (AA) positive state to a negative state at the time of functional hypothyroidism.\textsuperscript{10} Four stages have been proposed to describe the progression of lymphocytic thyroiditis. Stage 1 (subclinical thyroiditis) involves positive Tg and thyroid hormone autoantibody tests with normal TSH and T\textsubscript{4} levels. Stage 2 (antibody positive subclinical hypothyroidism) is characterized by a loss of greater than 60-70% of thyroid mass with compensatory increase in TSH resulting in the maintenance of normal T\textsubscript{4} concentrations. Stage 3 (antibody positive overt
hypothyroidism) is characterized by the loss of almost all functional thyroid tissue with decreased T4 and increased TSH. Stage 4 (non inflammatory atrophic hypothyroidism) is characterized by negative Tg antibodies, increased or decreased TSH, and decreased T4 levels.3,4

CLINICAL FEATURES OF HYPOTHYROIDISM

Acquired primary hypothyroidism is most commonly diagnosed in middle age to older dogs. While Golden Retrievers and Dobermans are over represented the disorder is seen in many breeds. There is no obvious association with sex or neuter status.3 Onset is slow and insidious; signs are variable and may differ among breeds. The most consistent signs are those due to decreased cellular metabolism and dermatologic manifestations. Additional clinical signs may affect the cardiovascular system, neuromuscular system, gastrointestinal and reproductive systems.3

Thyroid hormones have diffuse effects on almost every tissue and organ in the body. A deficiency in thyroid hormone results in diffusely decreased cellular metabolism which manifests as lethargy, mental dullness, weight gain, exercise intolerance and possible cold intolerance.4

Dermatologic changes may manifest differently in different breeds due to marked variation in hair cycles and follicular morphology. However classic changes are described by an endocrine alopecia which in the end stages is bilaterally symmetric, truncal and non pruritic.3 Thyroid hormone is necessary to initiate and maintain the anagen or growing phase of the hair cycle. In the absence of the hormone the hair prematurely enters into the telogen phase causing excessive shedding with lack of regrowth.3 Reductions in cutaneous fatty acids and prostaglandin E2 may lead to sebaceous gland atrophy, hyperkeratosis, scale formation, seborrhea sicca and a dry coat. In early stages hair loss may be limited to points of excessive wear or pressure. In the more severe and chronic form of the disease the head and extremities tend to be spared but a diffuse bilaterally symmetric truncal alopecia develops. Variation in dermatologic changes due to breed include; 1) hypertrichosis, 2) primary hair loss with a wooly coat appearance, 3) loss of under coat with retention of primary hairs, and 4) retention of telogen hairs with failure of hair growth with clipping (Beagles).3 Hyperpigmentation may be noted in areas of friction including the axilla and inguinal regions. Accumulation of hygroscopic glycosaminoglycans, hyaluronic acid may occur in the dermis in severe cases resulting in increased water binding and changes in skin thickness with non pitting edema (predominantly eyelids, forehead and lips).3 Additional complicating factors can include superficial pyoderma, adult onset demodicosis and chronic otitis externa possibly related to suppressed immune responses due to depletion of thyroid hormone.3

Clinicopathologic abnormalities: A minimum database is recommended in all cases when evaluating a patient suspected of having hypothyroidism. The minimum database can exclude significant non thyroidal illness, either as a more likely cause of the presenting signs or as an indicator of concurrent disease that may affect interpretation of subsequent thyroid function tests. The most consistent changes seen in advanced disease include an anemia and hypercholesterolemia.1,2

Complete blood count changes include a normocytic, normochromic, non regenerative anemia which is evident in 30-44% of cases.3,4 Anemia is related to reduced erythrocyte production, decreased erythroid progenitor response to erythropoietin and lack of direct effect of thyroid hormone on early hemopoietic pluripotent stem cells. Target cells due to increased erythrocyte membrane cholesterol loading are also frequently seen.3

The most common change on serum biochemistry profile is a fasting hypercholesterolemia which is seen in 75% of cases.1 Synthesis and degradation of lipids are depressed in hypothyroidism but degradation is more suppressed than synthesis, the net effect being accumulation in plasma lipids. It has been suggested that the larger the increase in cholesterol the more likely a diagnosis of hypothyroidism
rather than non thyroidal illness. Additional biochemical changes which may be evident include hypercalcemia, mild increases in AST, ALT, and ALP.

**Diagnostic imaging:** Ultrasound of the thyroid gland is useful for the diagnosis of thyroid neoplasia. It is also an effective tool to discriminate between euthyroid sick and hypothyroid dogs. Thyroid ultrasound has a specificity of 96% in the diagnosis of hypothyroidism using relative thyroid volume and relative cross sectional area. A sensitivity of 98% was reported for the diagnosis of hypothyroidism when combining evaluation of relative thyroid volume and echogenicity relative to the echogenicity of the sternothyroid muscle. Unfortunately early in the course of disease the thyroid glands may appear normal.

Nuclear scintigraphy is regarded as the gold standard method for differentiating between hypothyroid and euthyroid dogs. Adult dogs with primary hypothyroidism have typically low or non-detectable accumulation of radioisotope by the thyroid gland and the thyroid glands may appear smaller than normal. In a study involving 27 dogs, nuclear scintigraphy accurately differentiated 14 dogs with confirmed histologic hypothyroidism from 13 dogs with non thyroidal illness, by percentage of uptake with no overlap. However, in another study some dogs had uptake in an equivocal range. False positive results were seen in thyroiditis (normal or increased uptake in a hypothyroid dog) and increased iodine intake (low uptake in a euthyroid dog). Glucocorticoid administration may suppress uptake into an equivocal range in euthyroid dogs. Conversely in dogs with hypothyroidism secondary to potentiated sulfonamide isotope uptake is typically normal or increased.

**Blood tests of thyroid gland function:** Baseline tests to assess thyroid gland function include measurements of T4, FT4, T3, FT3, rT3, and endogenous TSH concentrations. The utility of each will be discussed.

Baseline serum total thyroxine concentrations (T4) represent the sum of protein bound and free hormone circulating in the blood. Measurement of serum T4 is a good screening test for hypothyroidism. A canine specific radioimmunoassay (RIA) is recommended. T4 is stable however for best accuracy blood samples should be centrifuged and serum decanted into plastic tubes, frozen and sent to the lab on cold packs. Hemolysis and lipemia do not usually affect results when measured by RIA. The only factor that directly interferes with the ability of the assay to measure T4 is the presence of anti-T4 antibodies. Antibodies occur in dogs with lymphocytic thyroiditis and occur in 2% of dogs with clinical signs of hypothyroidism and 15% of hypothyroid dogs. Using most currently available assays anti-T4 antibodies cause a spurious increase in T4. However the probability that this interference will result in a falsely normal T4 concentration appears to be quite low. The sensitivity and specificity of the serum T4 is dependent on the amount of residual thyroid gland function at time of sample, suppressive effects of additional factors including drugs and concurrent non thyroidal illness, and the presence of circulating anti thyroid hormone antibodies. Using a cut point of less than 1.5, serum T4 has a sensitivity of 100% and a specificity of 75% for the diagnosis of hypothyroidism.

Similar to serum T4, serum T3 is typically measured by RIA, is stable in serum, and is affected by similar factors that interfere with T4 measurement. Differences include that anti-T3 antibodies are more common (which can falsely elevate T3), low values are more common in euthyroid dogs (up to 75%) and certain breeds, and it is more severely effected in non thyroidal illness. It has limited diagnostic value as it is neither sensitive nor specific (0.10 and 0.79, respectively) for the diagnosis of hypothyroidism. Its use may be justified in Greyhounds who tend to have low T4 and FT4 concentrations but T3 concentrations within laboratory reference ranges.

Serum FT4 concentration is a measure of free hormone only. Serum FT4 is best measured by an assay using equilibrium dialysis (gold standard) or modified equilibrium dialysis (MED) that has been validated for use in dogs. When dialysis is used interference via antibodies does not occur. It is more
specific than T₄ in the diagnosis of hypothyroidism. It is less affected by NTI however severe illness can result in very low FT4 serum levels and low concentrations have been reported in euthyroid dogs (<10%).¹⁸ Concentrations are low in the majority of hypothyroid dogs but may remain within the low end of the reference range in up to 20% of cases.¹⁴

Assessment of serum FT₃ and rT₃ is not recommended at this time. Clinical benefits of measuring either value have not yet been demonstrated.³

Current assays available for TSH measurement have poor sensitivity. In 20-40% of dogs with hypothyroidism the TSH concentration is within the reference range (sensitivity reported at 63-82%).³ This may be due to persistent stimulation of thyrotrophs via negative feedback leading to TRH receptor desensitization and gradual loss of TSH secretion, TSH being released in a pulsatile fashion, secondary hypothyroidism, suppression of TSH secretion by concurrent disease or drug administration, or an inability of the TSH assay to detect all isoforms.⁴ However, when used in combination with FT₄ and T₄, the TSH assay has a specificity of greater than 90% for a diagnosis of hypothyroidism in dogs when the baseline serum T₄ or FT₄ is concurrently low.¹⁴ High TSH values in the absence of clinical hypothyroidism may indicate recovery from recent NTIS, recent potentiated sulfonamide administration, subclinical hypothyroidism, and breed variation (Bearded collies).¹⁴ Low TSH values may be due to secondary hypothyroidism, pituitary exhaustion, undetectable isomers of TSH, or may reflect non-specific daily fluctuations.¹⁴

Serum autoantibodies: Positive serum thyroglobulin or thyroid hormone antibodies are almost always associated with lymphocytic thyroiditis however provide no information about current thyroid gland function. Therefore, the existence of serum antibodies are not to be used as a sole criteria to establish a diagnosis of hypothyroidism but do suggest ongoing thyroid pathology. Antibodies may be present long before an animal becomes functionally hypothyroid and their existence is not necessarily predictive of eventual hypothyroidism. When thyroglobulin antibodies were monitored for over a year in 171 dogs it was found that 20% developed changes suggestive of declining thyroid function, 5% became overtly hypothyroid, 15% became antibody negative without developing thyroid dysfunction, and 57% maintained antibody positivity.⁴,¹⁰

THYROTROPIN STIMULATION TEST

Evaluation of the responsiveness of the thyroid gland to provocative stimulation (TSH stimulation test) is considered to be the gold standard for definitive diagnosis of thyroid dysfunction. Recent publications have provided a protocol for the use of recombinant human TSH (rhTSH).¹⁸,¹⁹ Given the cost of the product the protocol has established a minimum dose required (dependent on weight) and has proven that the product can be effective even after storage for 4 weeks refrigerated or 12 weeks frozen.¹⁹ This may increase the utility of TSH in the diagnosis of hypothyroidism. Unfortunately equivocal/intermediate results are still apparent using the TSH stimulation test and euthyroid dogs have been shown to have a suppressed response to rhTSH where the severity of illness correlated to the degree of suppression of the serum T₄ concentration.¹⁸,¹⁹

INTERPRETING BLOOD TESTS OF THYROID GLAND FUNCTION

Many factors can lead to an erroneous interpretation of thyroid test results and the misdiagnosis of hypothyroidism in euthyroid dogs. The most common factors are concurrent illness, use of drugs, and random fluctuations in thyroid hormone concentrations. However additional factors include age, breed, athleticism, and diurnal rhythms.

A progressive decline (29-40%) in T₄ has been noted in dogs from middle age to old age.³,⁴ Concurrent with this decline in T₄ there is a higher mean TSH concentration and a blunted T₄ response to
exogenously administered TSH when compared to young animals. It is likely that a total T4 below the reference range is a normal age related change in older dogs secondary to concurrent illness, change in responsiveness to TSH, decreased biological activity of TSH and subclinical thyroid pathology.\(^3\)\(^4\)

Differences in suspected normal reference ranges have been found for various thyroid hormones in Greyhounds, Whippets, Salukis, Shoughis, Basenjis, Borzois, Deerhounds, Wolfhounds, and conditioned Alaskan sled dogs.\(^3\)\(^6\)\(^17\) There is a need for breed specific assays. In breeds known to lie outside the normal reference range the diagnosis of hypothyroidism should rely on history, examination findings, changes on the cbc/biochemistry and multiple tests of thyroid function. In Greyhounds in particular 91% had a total T4 below the non breed specific range.\(^4\)

Random fluctuations occur in baseline serum T4 and T3 in healthy dogs, euthyroid dogs with concurrent illness and hypothyroid dogs.\(^3\) TSH fluctuations also appear to occur during the day in hypothyroid dogs.\(^4\) A diurnal rhythm has also been documented with a possible peak in serum thyroid concentration at mid day.\(^2\)

NTI can result secondary to almost any systemic illness, surgery, trauma, as well as inadequate calorie intake. The pathogenesis is believed to involve decreased TSH secretion, decreased T4 synthesis, and decreased deiodination of T4 to T3.\(^3\) It is believed to be a protective adaptation that reduces cellular metabolism during illness. Disorders frequently associated with NTIS include neoplasia, renal disease, cardiac failure, inflammatory disorders, hepatic disease, and diabetic ketoacidosis. The severity of the illness correlates with the degree of suppression seen in thyroid gland function tests.\(^7\) In a patient with NTI normal results are indicative of euthyroid status but abnormal results do not confirm a diagnosis of hypothyroidism. T4 is most commonly decreased. FT4 is reduced to a lesser extent but can be markedly decreased in cases of severe NTIS. Serum TSH is usually normal but may be increased during recovery from NTIS. TSH response test results will be suppressed. In cases of NTI, treatment should be directed at the underlying illness if possible prior to further assessment for hypothyroidism. If necessary, due to the suspicion that hypothyroidism is contributing to illness, multiple thyroid parameters should be evaluated simultaneously and interpreted in the context of history, examination findings and all other laboratory data. Fortunately simultaneous low T4, low FT4, and high TSH occurred in only 1.8% of 223 dogs with non thyroidal illness and none of 66 dogs in another study.\(^2\)\(^0\)\(^2\)\(^1\)

Any drug, until proven otherwise, should be suspected of influencing thyroid hormone test results, especially if the drug has been shown to alter thyroid hormone concentrations in humans.\(^3\) Drugs proven to effect thyroid hormone test results in dogs include; glucocorticoids (low T4, low FT4 and low T3), phenobarbital (reduced T4 and FT4), sulfonamides (low T4, FT4, T3 and increased TSH), non steroidal anti-inflammatory drugs (NSAIDs) (aspirin decreases T4 and FT4), and clomipramine (decreases T4 and FT4).\(^3\)\(^4\) Ideally, the specific drug should be discontinued, and depending on the dose and duration of the medication, testing should be delayed for a minimum of 4-8 weeks and up to 12 weeks in some cases.\(^3\)

**TREATMENT OF ACQUIRED HYPOTHYROIDISM**

In most cases a diagnosis of hypothyroidism can be made in the face of appropriate clinical signs, lab work findings (anemia and hypercholesterolemia), and a baseline serum T4 of less than 0.5(a normal value establishes euthyroid status in the majority of dogs) or a combination of a low T4, FT4 and high TSH. When faced with discordant results and a high index of clinical suspicion options include; 1) a TSH stimulation test, 2) imaging using nuclear scintigraphy, 3) a treatment trial or 4) repeat testing in 3 months. The option chosen may depend on the severity of clinical signs.

Current treatment recommendations include the use of a non generic veterinary form of levothyroxine sodium at 0.02 mg/kg twice daily (maximum start dose of 0.8 mg twice daily), ideally separate from meals.\(^3\) While a recent study suggests that most dogs can be dosed once daily with L-T4 in
cases of severe clinical signs or a clinical trial, twice daily dosing is recommended initially.\textsuperscript{22} If clinical signs resolve and $T_4$ concentrations are within the therapeutic range the frequency of administration may be reduced to once daily to improve owner compliance. Monitoring of $T_4$ and TSH (in cases where TSH elevation was noted at initial diagnosis) should occur 6-8 weeks after initiating therapy, if signs of thyrotoxicosis, 2-4 weeks after any adjustment to therapy, or when there is a poor response to therapy.

In the case of a clinical trial supplementation should be continued for a minimum of 6-8 weeks. Due to a general increase in metabolic rate with therapy supplementation can temporarily improve clinical signs in a dog without thyroid dysfunction.\textsuperscript{3} If a positive response is noted then either the dog has hypothyroidism or a thyroid responsive disease. Once clinical signs have resolved, supplementation should be stopped, if relapse occurs than the patient likely has hypothyroidism. In the case of a positive response the owner will see an increase in mental alertness in the first week, coat may initially worsen but then improve between 4 and 8 weeks of age, and obesity should start to improve within 8 weeks.\textsuperscript{22}

References


SCABIES

Sarcoptic mange is a contagious disease caused by Sarcoptes scabiei var. canis in dogs and by Notoedres cati in the cat. In our experience it is much more common in dogs. Transmission is usually via direct contact with affected animals, rarely animals can infect themselves from a recently contaminated environment. Clinically nonaffected carrier animals occur. The mite does not survive off the host for very long periods of time. The life cycle is accomplished in approximately 21 days.

Clinically, scabies is characterized by tremendous pruritus. Papules, scales and crusts develop at affected sites, typically the elbows, hocks, face and pinnae in the dog and the face, ears and neck in the cat. Occasionally, nonlesional pruritus is caused by scabies mites (Scabies incognito).

Diagnosis is made by superficial skin scrapings. However, as mites may be difficult to demonstrate, therapeutic trials are commonly used to confirm the diagnosis in animals with negative skin scrapings. Pruritus often increases during the first days of therapy due to the dying mites and concurrent glucocorticoids therapy for the first 3-5 days may be useful.

OTODECTES CYNOTIS (EAR MITES)

These are large, white and freely moving mites with four pairs of legs extending beyond the body margin (except the rudimentary fourth pair of the female). The life cycle lasts 3 weeks. The egg is laid with cement sticking it to the substrate. After 4 days of incubation, a 6-legged larva hatches and feeds actively for 3-10 days, rests a day and hatches to the protonymph (8 legs, last pair very small) and later mouls into the deutonymph. The adult male attaches to the deutonymph end-to-end. If the deutonymph is a female, copulation will take place and the female will become egg-bearing. Females that were not attached, don't lay eggs. They live for 2 months. Transmission occurs via direct and indirect contact, mites can survive for up to 8-12 weeks in the environment. Fleas are thought to be able to transmit mites and eggs that can adhere to the flea.

The mites feed on epidermal debris and tissue fluid from the superficial epidermis. They cause intense irritation and thick reddish brown crusts in the ears of dogs and cats. Mites are commonly found on other areas of the body, especially the neck, rump and tail. The parasites are highly contagious and especially prevalent in the young. Fifty percent or more of all otitides in cats and 10% in dogs are thought to be caused by ear mites. Infestations of cats vary from country to country with values as low as 3.5% in Australia (!) and as high as 75% in the States.

CHEYLETIELLA („WALKING DANDRUFF“)

Cheyletiella are large mites (385mcm) that affect cats (C. blakei), dogs (C. yasguri), rabbits (C. parasitovorax) and humans (transiently affected by C. yasguri or blakei). Host specificity is still a
controversial topic. Four pairs of legs bear combs instead of claws. The most diagnostic feature is the accessory mouthparts or palpi that terminate in prominent hooks. The heart-shaped sensory organ on genu I is diagnostic for *C. yasguri*, the cone shaped for *C. blakei* and the global one for *C. parasitovorax*.

The yellowish adult mites move rapidly in the stratum corneum but do not burrow. They live on tissue fluid piercing the skin periodically. The ova are smaller than louse nits and are attached to hairs by fine fibrillar strands (not cemented firmly to the hairs as nits). They hatch in 4 days. The 6-legged larva moult to the 8-legged nymph I after 7 days, nymph II after 4 1/2 days and adult after 5 days. The mite is an obligate parasite that does not live off the host for longer than 48 hours (except for females which may live for up to 10 days if carefully refrigerated). The mites are highly contagious, especially to young animals.

The course in small animals is chronic, most severe and generalised in 2- to 8-week old puppies. Older individuals may become asymptomatic carriers. Usually scaling is the only change (due to mites and keratin scales) with none to mild pruritus noted. Cats may develop widespread papulocrustous eruptions and severe pruritus in some cases.

Diagnosis is made by tape impressions, superficial scrapings, KOH digestion of debris gathered with a flea comb or faecal flotation samples. Other ectoparasites and seborrhoea are the two major differentials. A hyperplastic, superficial perivascular dermatitis with hyperkeratoses and a variable number of eosinophils is seen on biopsy. Cheyletiellosis is a local disease; it is seen extremely frequently in some areas and very rarely in others.

**DERMANYSUS GALLINAEC (POULTRY MITE)**

The “red mite” (only red when engorged with blood) attacks poultry, wild and cage birds, dogs, cats, cattle, horses and humans. Its size is 1mm, it lives in nests and cracks in cages or houses and lays up to 7 eggs after a meal. These hatch to 6-legged nymphs that do not feed. After 48 hours these moult to 8-legged protonymphs, another 48 hours later to deutonymphs and two days later to adults. The whole life cycle thus ideally takes 7 days but may last up to 5 months.

Most cases in small animals are associated with pets having access to (sometimes old or converted) chicken houses. Thus, taking a good history is essential in diagnosing the disease. Erythema, pruritus and a papulocrustous eruption especially over the back and extremities can be seen, but generalised severe scaling without pruritus was also reported in a dog. Diagnosis may be made by skin scraping. However, the mites tend to live in the environment and feed at night time. Insecticidal dips or sprays will eliminate the mites, but treating the premises is essential to prevent reinfection.

**LYNXACARUS RADOVSKY (CAT FUR MITE)**

These small mites (0.5mm) have flap-like sternal extensions containing the first two legs, which grasp the hair of the host. The mites are not highly contagious and usually there is little itching. They attach to the hair and give a “salt and pepper” appearance to the dull and dirty coat. Hair is easily epilated, the skin is normal or shows a papular eruption. Diagnosis is made by skin scraping or tape impression, the animals are treated with insecticidal dips or sprays.
TROMBICULIDIASIS (CHIGGERS; HARVEST MITES)

Chiggers are scavengers living on decaying vegetable material. They are orange-red, the size of a pin and live about 10 months (females may live longer than a year). The eggs are laid in moist ground and hatch to 6-legged red larvae that are parasitic. They feed on the animal (any large animal, small animal or human may be affected), drop on the ground and become nymphs and finally adults. The entire life cycle is complete in 50-70 days. The bites, usually on ground-skim contact areas like the legs, feet, head, ears and ventrum, produce severe irritation and an intensely pruritic, papulocrustous eruption, but may also produce non-pruritic pustules and crusts with secondary scaling and alopecia in small animals. The organisms adhere tightly to the skin. In humans, intense pruritus on the ankles, legs and belt line is seen, the red mite is frequently scratched off. In sensitised individuals urticarial or granulomatous reactions can occur.

Chiggers are seasonal in summer and autumn. Skin biopsy reveals superficial perivascular dermatitis in which eosinophils are prominent. One or two parasitisidal dips and thiabendazole drops in the ear canals are used for therapy, but patients must be kept from contaminated areas to prevent reinfestation. Corticosteroids for 2-3 days will help relieve the itching.

TREATMENT OF SUPERFICIAL MITES

• Selamectin is a spot on registered for the treatment of scabies and ear mites in many countries. It also has been shown to be effective against cheyletiellosis. I use it every two weeks for three treatments.

• Moxidectin is available as a spot-on registered for the treatment of canine scabies and other superficial mites as well, I use the same protocol of three treatments every two weeks.

• Topical treatments include lime sulfur dips, amitraz, ivermectin and other antiparasitic rinses. They are used weekly for 4 weeks.

• Systemic therapy may be undertaken with ivermectin or milbemycin. The routine protocol for a dog that did not receive ivermectin before, is a slow increase from 50 mcg/kg to 100 mcg/kg to 150 mcg/kg to 300 mcg/kg on subsequent doses every day. The owners get told to monitor the animal carefully during that time for the above mentioned side effects. If any signs of ataxia or tremors occur, administration of the drug must be discontinued immediately. Once the maintenance dose is reached, we continue that dose once weekly for three more weeks in suspected or proven cases with scabies, cheyletiellosis or infestations with Otodectes cynotis. Giving milbemycin oxime at 2 mg/kg twice weekly for 3-4 weeks has also proven a very safe, easy and successful treatment protocol for canine scabies.

• All animals in contact with the patient need to be treated as well!

• Initial deterioration during the first days of treatment may occur and may be treated with glucocorticoids daily for 3-4 days at 1 mg/kg body weight.

• Remission should be achieved within four weeks in most patients, although extended treatment for 8 weeks has been needed in some patients.

• With scabies, it may be useful to administer oral glucocorticoids for the first three days of treatment to decrease the severe pruritus while treating the mites.
DEMODICOSIS – WHAT IS NEW?
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PATHOGENESIS

Demodex canis is an obligate parasite of the dog and low numbers of mites are part of the normal cutaneous fauna. Within the first days after birth, the mites are transmitted from the bitch to the nursing puppies. Transmission of clinical disease from dogs with generalized demodicosis to normal dogs is typically not seen and the disease is not considered contagious. Fusiform eggs of Demodex mites hatch into six-legged larvae, molting into eight-legged nymphs and finally maturing into adults. In the dog, Demodex canis is the most commonly recognized mite. However, a short-bodied and hitherto unnamed mite seems to inhabit the stratum corneum. A long-bodied mite was first reported to cause oily skin and subsequently characterized and named D. injai. It resides in the pilosebaceous unit.

When considering the pathogenesis of demodicosis in dogs, it is important to distinguish between juvenile onset and adult onset disease as well as between localized and generalized forms. The differentiation between localized and generalized disease is sometimes difficult. Dogs exhibiting lesions fitting in neither category need to be decided upon individually. Localised demodicosis usually heals spontaneously. Generalised disease may also spontaneously resolve, but studies to evaluate the approximate rate of self-cure are lacking. With juvenile onset, certain breeds are at risk and cessation of breeding with affected animals reduces if not eliminates juvenile generalized demodicosis from breeding kennels. Other predisposing factors mentioned in the literature include short hair, poor nutrition, stress, oestrus, endoparasites, and debilitating disease. Initially, an immunodeficiency was postulated as a reason for the development of demodicosis. Supporting this pathogenesis, more recent studies show a decreased CD4+ T lymphocyte count, a lower CD4/CD8 ratio, a significantly decreased IL-10 concentration in dogs with recurrent demodicosis, and an increased apoptosis of peripheral blood mononuclear cells. If these changes are a consequence of the proliferation of the Demodex mites or a previous change predisposing to this proliferation is not known at this point. However, a high number of dogs with juvenile demodicosis will be cured and do not develop the disease again.

Drugs or diseases altering the immune response may be the triggers for adult-onset demodicosis. Hypothyroidism, hyperadrenocorticism, leishmaniasis, glucocorticoid therapy, neoplasias or chemotherapy have all been reported. Idiopathic adult-onset demodicosis also exists. In cats, demodicosis is typically due to an underlying systemic disease!

CLINICAL SIGNS

Clinically, canine demodicosis is characterized initially by erythema, papules and comedones, as mites accumulate in the hair follicles. Alopecia and scaling may also be seen. Later on, influx of inflammatory cells may lead to pustule formation. With severe disease, follicles rupture and furunculosis with deep lesions and crusting develops. Lesions can occur anywhere on the body, although the face and feet are most commonly affected. Certain breeds are predisposed for demodicosis, typically short coated breeds such as Chinese Shar Pei, Staffordshire terrier or bullterrier.
DIAGNOSIS

Diagnosis is made by deep skin scrapings or trichogram. A deep skin scraping is one of the most common diagnostic procedures performed in veterinary dermatology. A small area of affected skin (1-2 cm²) is scraped in the direction of hair growth until capillary bleeding is observed. A blade covered with mineral oil should be used. Lesions such as follicular papules or pustules are good sites for scraping. Because Demodex mites live in the hair follicles, it is useful to squeeze the skin prior to and during the scraping in an attempt to push the mites out from the depths of the follicles. Paws and faces are difficult to scrape and skin biopsies may be needed in some of these patients to confirm the diagnosis. Some breeds such as Shar Pei with demodicosis are anecdotally reported to be negative on scrapings and may have to be biopsied for diagnosis. Although Demodex canis is a normal part of cutaneous fauna and thus an occasional mite can be found on skin scrapings of normal dogs, it is extremely rare to see more than one Demodex mite on a dog not affected by demodicosis. If only one mite is found, further scrapings or a biopsy are recommended. When evaluating deep skin scrapings, it is important to assess and to note in the record the site of scraping and the relative numbers of adults, larvae, nymphs and eggs per microscopic field. In subsequent visits, assessment of response to therapy relies on the comparison of such numbers. Scrapings should be repeated at the same sites monthly when monitoring patients with demodicosis. More recently, an acetate tape impression with skin squeezing was found to be more sensitive than deep skin scrapings.

TREATMENT

Localized demodicosis resolves spontaneously in most of the patients. Treatment may be initiated against possible concurrent bacterial infections with benzoyl peroxide or oral antibiotics, but miticidal therapy is usually not necessary and should not be initiated in intact dogs to identify the few patients where the generalised form develops subsequently and neutering is indicated to prevent passing on the predisposition to offspring. The treatment of generalised demodicosis is outlined below. Lime sulfur rinses (2%) weekly for 4-8 weeks are recommended for the treatment of feline demodicosis.

Treatment for generalized demodicosis may include a number of medications, many of which are not registered for that indication anywhere in the world. Amitraz is a miticidal rinse. The adverse reactions associated with amitraz administration or application resemble those induced by alpha 2-adrenergic agonists such as xylazine. These are sedation, bradycardia, hypothermia, hypotension, bloat, polyuria, vomiting and hyperglycemia. Yohimbine at 0.1mg/kg IV antagonises the CNS-depressant and bradycardic effects of amitraz. Amitraz is one of the few drugs registered for the treatment of canine demodicosis in many countries.

Clipping the entire dog is essential to allow better contact of amitraz with the skin. All crusts should be removed (preferably by shampooing with an antibacterial follicular flushing agent such as benzoyl peroxide). The dog has to be dry completely (2-8 hours), before being sponged down with amitraz. The treating person should wear protective gloves and work in a well-ventilated area. Owners with asthma are advised to find somebody else for the rinses. The dog should stand in a tub with its feet in the amitraz solution to allow soaking of the often extensively affected feet. Amitraz causes a transitory sedative effect for 12 to 24 hours. Concentration of the drug and frequency of application influences the response rate. We use a concentration of 600 ppm once to twice weekly. In patients with demodicosis, the procedure should be repeated until 4 weeks after two successive skin scrapings (2-4 weeks apart) fail to reveal live demodectic mites. For pododermatitis and otitis externa, a mixture of 1 ml of amitraz with 30 ml of mineral oil can be used topically on a daily basis. Treated dogs should not get wet or be washed. Last year, a study showed a quicker response, when an inactivated parapoxvirus ovis
preparation was injected initially concurrently to treatment with amitraz. However, the study included only a small number of dogs and further studies confirming those findings are urgently needed.

Ivermectin orally at 300 - 500 mcg/kg daily is used in the treatment of demodicosis with good success. It must not be used in Collies and Old English Sheep dogs, as it commonly causes adverse reactions in these breeds. Ivermectin paralyzes nematodes and arthropods by potentiating gamma-amino butyric acid (GABA) - binding to its receptor and stimulating GABA release. In mammals, GABA is only found in the CNS and ivermectin does not readily cross the blood brain barrier. However, in some breeds adverse reactions can be seen commonly and include ataxia, mydriasis, tremors, stupor, salivation, bradycardia and respiratory arrest. These side effects are seen in Collies at a dose between 100 mcg/kg and 200 mcg/kg due to a genetic defect in the MDR-1 gene. Other breeds may be affected as well showing ataxia and tremors at lower doses. However, there are a number of dogs with adverse effects to ivermectin and an intact MDR-1 gene, alternative mechanisms thus are possible. Thus, the routine protocol for a dog that did not receive ivermectin before, is a slow increase from 50 mcg/kg to 100 mcg/kg to 150 mcg/kg to 300 mcg/kg on subsequent doses every day. The owners get told to monitor the animal carefully during that time for the above mentioned side effects. If any signs of ataxia or tremors occur, administration of the drug must be discontinued immediately. Once the maintenance dose is reached, demodectic patients receive that dose once daily until 4 weeks after the second consecutive negative monthly skin scraping. Note, that with daily dosing the serum level increases for weeks due to the long half life of ivermectin, thus patients need to be monitored for side effects for the first 8 weeks.

Milbemycin oxime is a macrolide antibiotic made from the fermentation of Streptomyces hygroscopicus and registered as a monthly heartworm preventative. It may be used daily at 1-2 mg/kg for the treatment of demodicosis. The advantages of this drug versus the conventional treatment with amitraz include the rare occurrence of side effects and the ease of administration. The treatment is very expensive for larger dogs. Response is comparable to amitraz. Milbemycin is also used until 4 weeks after the second negative skin scraping.

Moxidectin is another milbemycin that was evaluated for the therapy of canine generalized demodicosis. Studies have evaluated moxidectin at 200-400 mcg/kg/day orally, two of which employed the initial gradual dose increase advocated for ivermectin. Reported side effects were ataxia, lethargy, inappetence and vomition. As moxidectin is a macrocyclic lactone and has a similar mode of action to the other drugs in this group, the success rate and rare adverse effects are not surprising. However, more studies with longer follow-up period periods are needed to identify potential benefits and disadvantages of this drug. Moxidectin became recently available as a spot-on formulation approved for the treatment of canine demodicosis. Studies have shown increasing efficacy with more frequent application of the spot-on, but the success rate in dogs seems to be lower than that seen with oral macrocyclic lactones.

Doramectin is the final macrocyclic lactone successfully used for therapy of generalized demodicosis. In one study, 23 dogs were injected weekly with 600 mcg/kg subcutaneously. Ten of the dogs were cured, 7 relapsed after 1-24 months (2 of which responded to repeat doramectin treatment) and 6 were lost to follow-up. None of the animals in this study showed any adverse effects with therapy. Further investigations are needed to evaluate doramectin for the treatment of generalized demodicosis.

A number of other drugs such as levamisole, muramyl dipeptide, vitamin E, lufenuron, homeopathic and herbal preparations, deltamethrin and closantel have been evaluated for the use of generalized demodicosis, but at this point they cannot be recommended based on published reports.

Treatment of the primary disease may result in resolution of the demodicosis. This emphasizes the need for pursuing and treating concurrent diseases in patients with adult-onset demodicosis. The almost invariably existing bacterial secondary infections was treated with antibiotics for many years, more
recent evidence questions the need for oral antibiotics in dogs with demodicosis treated with oral macrocyclic lactones and antibacterial shampoos.35

When dogs do not respond to a given treatment, changing the therapeutic agent leads to remission in 2 of 3 dogs. Similarly, if a relapse occurs after initial remission, a second treatment attempt (either with the same or a different drug) will lead to resolution in approximately 2 of 3 dogs.14

References

GETTING STARTED IN CLINICAL RESEARCH: ISSUES AND RESOURCES

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STARTING OUT: ASKING THE RIGHT QUESTION

Fundamental to designing a good clinical research study is asking the right question. Several “template” methods have been proposed that will help new researchers turn clinical questions into good research questions with testable hypotheses. One common template is the “PICO” method:

Question = Population + Intervention + Comparators + Outcomes

This is sometimes framed as a question with the following structure:
“In [population], does [intervention] as compared to [comparator] result in [outcome]?

**Population:** Define and articulate clearly the population (group) of patients you wish to study. Be as specific as possible. Describe specific inclusion and exclusion criteria, including the ‘gold standard’ criteria for diagnosis. Might your disease vary by age, breed, sex, season? Will you enroll only newly-diagnosed patients, or patients with stable chronic disease? Will you allow patients with disease of all severity – is there a minimum or maximum severity? Will you allow prior treatments – if so, is there a washout period? Will you allow co-morbidities? A guiding principle here is that the more homogeneous your groups, the fewer patients you will need to demonstrate a difference. This has to be balanced with the number of patients you expect will be able to satisfy the entry criteria.

**Intervention:** Prospective studies typically have an intervention that is being investigated. The intervention might be a drug treatment being tested, a new procedure being evaluated, or a new diagnostic test being studied. Typically, these interventions will be evaluated against an established treatment regimen, standard-of-care procedure, or established test. Make sure you specify clearly which concurrent treatments will be allowed or not allowed. Decide if your study needs to be blinded, and if so, the type of blinding that may be necessary.

**Comparators:** The comparators (or “controls”) are the patients you will be comparing to those undergoing the intervention. Controls are usually either a placebo treatment or a standard-of-care treatment, procedure, or test.

**Outcomes:** What will you measure in your patients? It is best to define at least one primary outcome, and possibly one or more secondary outcomes. The primary outcomes chosen should be as objective as possible; this is often difficult in dermatology where we often deal with subjective responses as opposed to blood pressure or serum ALT. When available, use existing, validated scoring systems like the CADESI score or Pruritus Visual Analog Scale; for some disease situations, you may have to devise your own scoring system. When studying a treatment that is an add-on to existing therapy, it may be useful to somehow quantify the existing treatment and how it changes over the course of the intervention. Secondary outcomes can also be included – these may add interest and depth to the study. For example, when studying a new drug for treatment of pemphigus, the primary outcome might be achievement of clinical remission at 60 days. A secondary outcome might be occurrence of neutropenia in the treatment group.

REFINING THE QUESTION

Once you have formulated your initial research question, it may be helpful to assess it for ‘practicality’ using an approach such as “FINER” - and refine the question appropriately.
**Feasible:** Will you have enough patients to enter in the time available? Do you have the required technical expertise? Is the question financially feasible?

**Interesting:** Will answering the question be interesting to you, and will the results be interesting to your colleagues?

**Novel:** Remember that your question doesn’t have to be groundbreaking to be novel. In some cases, your findings will be new. However, you may wish to confirm or refute established findings, or perhaps extend these findings to a different population.

**Ethical:** Will the study meet any objections by the relevant animal care and use review board? Would you volunteer your own pet for the study?

**Relevant:** Imagine the different outcomes of the study. What if the results are ‘negative”? Will each possible outcome help to advance knowledge or change clinical management? Or, will the outcomes suggest possible future research regardless of what they are?

In addition, it is essential that early on in conceiving your study, you do a thorough literature search (see hints below). Carefully examine prior studies related to your proposed question; they may help you define outcome variables, identify design problems, and perform sample size calculations.

**WORRY ABOUT STATISTICS FIRST, NOT LAST**

It’s critically important to make a connection with a statistical consultant at the very beginning of your study. A statistician can help you define appropriate outcome variables, estimate your sample size or power, establish methods for randomization, decide on methods for analysis of data, and a host of other issues – BEFORE you have entered the first patient! Having a clear idea of exactly the data to collect, how to enter it in spreadsheets, which statistical methods to use, which software to use, etc. are all items that should be clear to you “upfront” rather than after you’ve finished your study.

Sample size calculations require at least an estimation of how much difference you expect between your control and intervention. This may come from a study in human beings or another species or a previous publication involving a similar intervention. In some situations, you may not have a clue! This is an indication for a pilot study – literally, a study whose main purpose is to guide you or other investigators as to what to expect in a larger, more controlled study. Pilot studies provide essential information for sample size calculations and basic feasibility, and in addition can provide valuable clues about problems that may occur in a larger study. They are an important part of a complete research effort, but can sometimes be less than satisfying as a standalone project.

**WRITE YOUR ONE-PAGER**

Once you’ve decided on your research question, it’s much easier to specify your methods and design. Here, a one-page study outline is very helpful. Your “one-pager” should encompass the following elements – each should be no more than a few sentences:

1. **Title** – a simple statement
2. **Research question** (written as PICO)
3. **Significance** – why are you proposing this study and why is it important?
4. **Design**
5. **Patients** – describe your population, including brief statements of any relevant inclusion and exclusion criteria.
6. **Outcome measures** – primary and secondary
7. **Statistics** – hypothesis, sample size, power, and methods

The nice thing about a “one-pager” is that each of the elements can next be expanded into sections of a research funding proposal. The proposal will then be useful post-study in analysis, and preparation of the abstract and publication.
RESEARCH RESOURCES AND MISCELLANEOUS HINTS

There are a number of books and websites that are very helpful to consult when designing your research project. Here are a few suggestions:

Don’t rely on Google Scholar or PubMed for your literature search! Many of the worldwide veterinary journals are not indexed in these sites. Use them, definitely, but also be sure to consult a site such as the CAB Abstracts (www.cabi.org). Make friends with a resident in an academic program with access, try your public library, or if you are gutsy, go on the website and request a free trial for your clinic. Typically, reference librarians at your local college health science library will be delighted to help you if you show interest!

Book: Hulley SB, Cummings SR, Browner WS, et al. Designing Clinical Research. Authors for this book vary with the edition. The text has not changed enormously over time – I recommend thrift, and purchasing an older edition on Amazon. Though designed primarily for design of human clinical trials, this book is an easy read and provides lots of guidance about asking good questions and explains statistical analysis in an accessible way.

Book: Holmes M and Cockcroft P. Handbook of Veterinary Clinical Research. This book discusses many of the same elements of the text above, but is specific to veterinary medicine and has a useful and slightly different slant. There is only one edition.

Book: Riegelman RK. Studying a Study and Testing a Test: How to Read the Medical Evidence. Another book with multiple editions, and earlier editions (still very useful) can be purchased on Amazon for a dollar or two. This book is a lengthy but fairly readable treatise on how to critically evaluate a published study. It’s useful to peruse the sections relevant to the project you are considering, if only to be more aware of study design elements that may later prove embarrassing upon publication.

Website: http://www.stat.uiowa.edu/~rlenth/Power/
A useful and entertaining discussion of sample size/power calculations from Prof. Russell Lenth at the University of Iowa, including online calculators.
SATURDAY
NORTH AMERICAN VETERINARY DERMATOLOGY FORUM  
Nashville, TN  
SATURDAY, APRIL 18, 2015  
CLINICAL SHORT COMMUNICATIONS  

2:00  DeBoer  Serum vitamin D concentrations in dogs with atopic dermatitis – a retrospective study (2007-2014)  

2:15  Banovic  Deep pyoderma caused by *Burkholderia cepacia* complex in dogs associated with ciclosporin administration in dogs: a case series  

2:30  Pendergraft  Sterile nodular idiopathic pyogranulomatous panniculitis in a mixed breed dog infected with *Bartonella henselae* San Antonio 2:D  

2:45  Lee  Suspected zinc-responsive dermatosis in nine Boston terrier dogs  

3:00  Pereira  Use of fluconazole monotherapy or in combination with potassium iodide in four cats with sporotrichosis refractory to itraconazole  

3:15  Bizikova  Canine epidermolysis bullosa acquisita: a retrospective study of 20 cases  

3:30-4:00  BREAK  

4:00  Lam  Sterile pustular erythroderma of miniature schnauzers: a retrospective study of seven cases  

4:15  Cain  Clinical and histopathologic features of acute-onset erythroderma in dogs with gastrointestinal illness  

4:30  Noli  Efficacy of a gel compound containing ethanol, guar, triclosan and glycerine (PawCare®, JOKER Technologies, Kerzers, Switzerland) on bacteria and yeast loads in canine pododermatitis  

4:45  Michels  Proof of concept efficacy and safety study of an anti-IL-31 monoclonal antibody for the treatment of atopic dermatitis in client-owned dogs
Serum vitamin D concentrations in dogs with atopic dermatitis – a retrospective study (2007-2014)

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Abstract: Atopic dermatitis (AD), an allergic skin disease affecting both man and dog, can often be linked to multiple genetic and environmental predispositions. Genetic factors generally manifest as skin barrier defects or aberrant immune system regulation. Research in man describes a key role for vitamin D in appropriate immune system function and suggests that low serum vitamin D levels may be associated with certain allergic conditions, including AD. Investigation of this potential relationship in dogs has been limited. Dogs clinically diagnosed with AD were identified retrospectively. Serum samples collected at the time of diagnosis (n=351) were analyzed for 25-hydroxyvitamin D and compared to a group of healthy dogs (n=40). Based on research in man, we hypothesized a lower mean serum vitamin D concentration in dogs suffering from AD vs. healthy dogs; no significant difference was found (P=0.07, t test). Atopic dogs were also subdivided based on demonstration of IgE involvement in their disease (positive vs. negative on intradermal and/or IgE serology test). No significant difference was detected between serum vitamin D levels in the IgE sensitized (n=187) vs. the non-sensitized (n=35) groups (P=0.61, t test). Finally, atopic dogs receiving allergen specific immunotherapy (ASIT) for at least six months were grouped according to response to therapy, and mean pre-treatment serum vitamin D levels were compared between groups; no significant differences were detected (P=0.10, one-way ANOVA). Serum vitamin D levels in atopic dogs (regardless of IgE sensitization status or later response to ASIT) do not appear to differ from those in healthy dogs.

Source of funding: Student financial support provided by Merial and the University of Wisconsin School of Veterinary Medicine. Research funds provided by Zoetis Animal Health.

Conflict of interest: None declared.
Deep pyoderma caused by *Burkholderia cepacia* complex in dogs associated with ciclosporin administration in dogs: a case series

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**Abstract:** Bacteria of the *Burkholderia cepacia* complex (Bcc) are ubiquitous gram-negative bacilli associated with fatal nosocomial infections in humans; multi-antibiotic resistance makes this organism a serious threat in hospital settings. The purpose of this retrospective study is to describe the historical, clinicopathological and treatment characteristics of Bcc-associated deep skin infections in dogs. Electronic medical records searched at three institutions identified six dogs with skin infections in which skin bacterial cultures resulted in pure growth of Bcc. All dogs were receiving oral ciclosporin at the time of skin infection development. All dogs were castrated males and 4 of 6 (67%) were West Highland white terriers. Cutaneous lesions consistent with deep pyoderma were confined mainly to the trunk. In all dogs, skin cytology revealed a strong inflammatory response with moderate to abundant numbers of intracellular (neutrophils and macrophages) and extracellular bacilli. In three dogs, histopathology showed a multifocal, nodular to coalescing pyogranulomatous dermatitis associated with multifocal folliculitis and furunculosis. Tissue Giemsa stains identified numerous Gram-negative rods within macrophages. Antimicrobial susceptibility testing revealed multidrug-resistant Bcc strains with sensitivity to trimethoprim/sulfonamides in all dogs and marbofloxacin, piperacillin and ceftazidime in three dogs. Complete remission was achieved in all dogs using either trimethoprim/sulfonamides, quinolones (marbofloxacin, ciprofloxacin) or doxycycline in conjunction with ciclosporin withdrawal. Clinicians should be aware of the rare potential for Bcc-associated deep skin infections in dogs receiving oral ciclosporin. Owners should be made conscious of the potential transmission risk to humans or other animals.

Conflict of interest: None declared

Sources of funding: Self-funded
Sterile nodular idiopathic pyogranulomatous panniculitis in a mixed breed dog infected with *Bartonella henselae* San Antonio 2:D

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**Abstract:** Canine sterile nodular pyogranulomatous dermatitis/panniculitis (SNDP), is an idiopathic condition. Although recognized for decades, little progress has been made in understanding its etiopathogenesis. We propose a possible case of *Bartonella*-associated SNDP in a 9 year-old male castrated mixed-breed dog presented for pyrexia and subcutaneous nodules June 2011. The patient had a history of seizures (April 2011) controlled with Levetiracetam (Keppra, UCB Pharma, Smyrna, GA). Pyrexia resolved with oral clindamycin (10 mg/kg twice daily) and ciprofloxacin (33 mg/kg once daily) but additional nodules developed. Skin biopsies revealed pyogranulomatous dermatitis and panniculitis. PAS stain, acid-fast stains, aerobic, anaerobic and fungal cultures were negative. Nodules resolved with oral prednisolone, 1.0 mg/kg once daily; relapse occurred upon taper to 0.5 mg/kg/day. Nodules resolved with oral ciclosporin (Atopica, Novartis, Greensboro, NC; 3 mg/kg) and fluconazole (9 mg/kg) once daily. Upon discontinuing ciclosporin and fluconazole, pyogranulomatous submandibular lymphadenopathy, a cervical mass, and diabetes mellitus developed (February 2012). *Bartonella henselae* genotype San Antonio 2:D was amplified from *Bartonella* Alpha Proteobacterium growth medium enrichment cultures of blood, submandibular tissue, and previous paraffin-embedded lesions. Lesions resolved with oral twice-daily doxycycline (4.5 mg/kg) and daily rifampin (4.5 mg/kg), but relapsed following discontinuation. Nodules again resolved with daily ciclosporin and fluconazole. Identification of *Bartonella* and determining its role in inflammatory disease is a diagnostic challenge. We suggest that dogs conventionally diagnosed with SNDP may be infected with *Bartonella*, yet may experience lesion control with immunomodulation. Prospective studies are recommended to investigate the role of *Bartonella* in pyogranulomatous skin disease.
Suspected zinc-responsive dermatosis in nine Boston terrier dogs

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Abstract: Zinc-responsive dermatosis (ZRD) has been documented in several dog breeds but only reported in one Boston terrier dog. Nine Boston terrier dogs with hyperkeratotic lesions localized to the face and pressure points were documented from 2004-2014, and referring veterinarians were contacted for follow-up information. The median age of onset of lesions was 3.5-months-old (range 1-24 months). Symmetrical alopecia with thick scale or hyperkeratotic plaques was noted on the haired skin of the dorsal muzzle (5/9), the margins and concave aspects of the pinnae (8/9), the hocks (2/9), and the elbows (1/9). Skin biopsies revealed severe hyperkeratosis ranging from compact orthokeratosis with interwoven parakeratosis to severe non-spired parakeratosis. Infundibular hyperkeratosis and serum lakes were noted in 5/9 cases. The superficial dermis had a mild to moderate perivascular lymphoplasmacytic infiltrate. One dog had a serum zinc level below reference range prior to zinc supplementation; two dogs had levels assessed due to incomplete response to supplementation, but levels were within reference range. Five dogs were treated with zinc supplementation (sulfate, gluconate, methionine), and four were known to clinically improve or resolve within 1-4 months. Three dogs were treated with salicylic acid 6.6%, and one dog’s lesions were known to improve. One dog was treated with retinoin cream 0.1%, and the lesions improved. The underlying causes and ideal therapies are not definitively known for these lesions, but there are histologic and clinical similarities to ZRD. Prospective studies measuring tissue zinc levels concurrent with clinical response to zinc supplementation would be of interest.

Source of funding: Self-funded.

Conflict of interest: None declared.
Use of fluconazole monotherapy or in combination with potassium iodide in four cats with sporotrichosis refractory to itraconazole

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Abstract: Sporotrichosis is a subcutaneous mycosis with high incidence in Latin America. Clinical signs in cats range from subclinical infection to fatal systemic forms. Itraconazole is effective in many, but not all cases. This report describes treatment with oral fluconazole monotherapy or in combination with potassium iodide in four outdoor cats (2-3.5 years old; 2/4 intact male cats; 2/4 spayed female cats) with sporotrichosis refractory to oral itraconazole. All cats presented with the cutaneous disseminated form; three had concurrent respiratory tract signs. Cytology and histopathology from ulcers revealed pyogranulomatous inflammation with large numbers of Sporothrix yeasts. The diagnosis was confirmed by isolation of Sporothrix schenckii on mycological culture. The cats were treated with oral itraconazole (18.2-25 mg/kg once daily) for 3 months with poor response; two cats developed anorexia from itraconazole. Itraconazole was discontinued and the cats were then treated with oral fluconazole (9.0-12.5 mg/Kg once daily). Clinical cure was noted in 2/4 cats after 4 months of fluconazole monotherapy. The other 2 cats were then treated with fluconazole and oral potassium iodide in capsules (5.0 mg/kg once daily). After 6 months, one cat was clinically normal and the other cat was euthanized at the owner’s request. Antifungal therapy was maintained for 2 months after clinical cure in the remaining 3 cats. No adverse reactions were observed. The cats had no relapse over a follow-up period of 6 months. In conclusion, oral fluconazole monotherapy or in combination with potassium iodide may be beneficial in cats with resistant sporotrichosis.

Source of funding: This study was self funded.

Conflict of interest: None declared.
Canine epidermolysis bullosa acquisita: a retrospective study of 20 cases

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Abstract: Epidermolysis bullosa acquisita (EBA) is a rare autoimmune subepidermal blistering disease of dogs and humans presenting as either inflammatory or non-inflammatory mechanobullous form. Our objectives were to further characterize clinical phenotypes, histopathology, and treatment outcomes of 20 dogs diagnosed with EBA based on a subepidermal blister formation and collagen VII autoreactivity. Most dogs were young (median: 1.2 years) with a male-to-female ratio of 2.3:1. Nine of 20 dogs (45%) developed lesions before one year of age, and 11/20 dogs (55%) were great danes. Tense vesicles and bullae (18/20; 90%) and deep erosions and ulcers (20/20; 100%) were the most common lesions and these affected predominantly the oral cavity (19/20; 95%), pinnae (16/20; 80%), axillae (15/20; 75%) and footpads (14/20; 70%). Histopathology consistently identified neutrophilic perivascular dermatitis (17/17; 100%) without or with (9/17; 53%) eosinophils, which occasionally equaled (2 cases) or outnumbered neutrophils (2 cases). Subepidermal vesicles were either devoid of inflammation or contained neutrophils with or without eosinophils, fibrin and/or hemorrhage. A complete remission of skin lesions was obtained in 14 dogs with a median time of 58 days. Glucocorticoids were used in these dogs either as a monotherapy (3/14; 21%) or in combination with other immunomodulating drugs (11/14; 79%). The median dose of prednisone was 3 mg/kg/day. The remaining six dogs were euthanized. Canine EBA is a rare subepidermal blistering disease with an inflammatory phenotype and a predilection for young great danes and male dogs. The outcome of treatment appears more favorable than assumed previously.

Source of funding: Self-funded

Conflict of interest: None declared
Sterile pustular erythroderma of miniature schnauzers: a retrospective study of seven cases

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Abstract: Sterile pustular erythroderma of miniature schnauzers is a rare and often fatal condition consisting of highly characteristic cutaneous lesions with concurrent systemic disease. The goal of this retrospective study was to compare clinical and pathologic features of this condition in dogs that survived with those that did not. Six cases were diagnosed via histopathologic findings from IDEXX Laboratories (West Sacramento, CA) and one from UC Davis. Data obtained from medical records included signalment, history, clinical signs, clinico- and histopathologic findings, treatments, and outcome. All affected dogs were female miniature schnauzers, from three to 11 years of age. All dogs initially presented with truncal erythema progressing to generalized wheals or papules/pustules. One dog had a recent history of bathing. Systemic signs occurred in five dogs: lethargy (4/5), vomiting (2/5), fever (2/5), while two had no reported concurrent systemic illness. One was an uncontrolled diabetic. A complete blood count and serum biochemical profile were performed in six dogs. Four dogs had a moderate leukocytosis and two had a non-regenerative anemia. Elevations in alanine transferase and alkaline phosphatase were noted in five dogs. Histologic findings were similar in all cases: intraepidermal, panfollicular neutrophilic and eosinophilic pustulation, with adnexal and follicular infiltration. Four dogs were treated with corticosteroids and intravenous fluids, five with systemic antibiotics. Two dogs died from respiratory arrest: one following development of disseminated intravascular coagulation. Four dogs survived, one was lost to follow-up. No historical, clinicopathologic or histologic patterns nor differences in their medical management were identified to provide prognostic markers.

This study was self-funded

Conflict of interest: none declared
Clinical and histopathologic features of acute-onset erythroderma in dogs with gastrointestinal illness

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Abstract: A unique syndrome of acute-onset erythroderma has been previously described in dogs with gastrointestinal illness. Seventeen dogs with acute-onset erythematous macules or generalized erythema were included. Miniature schnauzers (3/17), Labrador retrievers (2/17), and pugs (2/17) were most commonly affected. Gastrointestinal signs, particularly vomiting (16/17; 94%) and hematochezia (10/17; 59%), occurred prior to skin lesions (mean of 4.4 days; range: 1-11 days) in 9/17 dogs (53%); following skin lesions in 3/17 dogs (18%), and concurrent with skin lesions in 5/17 dogs (29%). Mild to moderate hypoalbuminemia was noted for 8/17 dogs (47%). Mild to severe eosinophilic dermatitis and superficial dermal edema, with or without collagen flame figures or fibrinoid vasculitis, were the main histopathologic features. Four dogs (23%) were diagnosed with inflammatory bowel disease via gastrointestinal biopsies; 3/17 (18%) were diagnosed with pancreatitis; and 3/17 (18%) were diagnosed with adverse food reaction. A numeric drug score (+3 to -3) quantified drug exposure for each dog. Drug scores were positive for 3/17 dogs (18%), supportive of causal drug association; negative for 10/17 dogs (59%), inconsistent with causal drug association; and inconclusive for 4/17 dogs (23%). Treatment included drug withdrawal (15/17; 88%), antihistamines (15/17; 88%), corticosteroids (13/17; 76%), and gastroprotectants (12/17; 71%). Gastrointestinal signs resolved in a mean of 4.6 days (range: 1-13 days) and skin lesions resolved in a mean of 19 days (range: 8-30 days) following presentation. In conclusion, this syndrome may be associated with more than one underlying gastrointestinal disorder, and drug exposure may play a role for some dogs.

This study was self-funded.

No conflicts of interest are declared.
Efficacy of a gel compound containing ethanol, guar, triclosan and glycerine (PawCare®, JOKER Technologies, Kerzers, Switzerland) on bacteria and yeast loads in canine pododermatitis

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Abstract: Canine skin is colonized by various microorganisms. Topical treatments may reduce the microbial load on the skin surface thereby preventing infection development. Due to emerging resistance to antimicrobials, alternative strategies focusing on topical therapy are desired. Pawcare® (JOKER Technologies, Kerzers, Switzerland) is a guar/glycerine/triclosan/ethanol gel which mechanically removes dirt, and microorganisms from surfaces and provides mild disinfection. The study aim was to assess efficacy of Pawcare® in decreasing bacterial and yeast loads on the paws of dogs with erythematous, greasy and/or malodorous pododermatitis. Eighteen dogs with at least two affected paws were included. Semiquantitative Malassezia counts were performed on 10 oil-immersion fields (range 0-30) from acetate tapes pressed on the palmar/plantar surface of one paw. Half of the area was sampled before and the other half immediately after the application of Pawcare®. With a similar procedure swab samples were collected from the other paw for bacterial culture, identification and evaluation of colony forming units (CFU) before and immediately after treatment. Statistical evaluation of pre- and post-treatment counts was performed with the Wilcoxon signed rank test. Eight dogs were positive for Malassezia. Mean acetate tape preparation counts decreased significantly from 7.25(+/−7.05) to 3.88(+/−4.19) (p=0.0078). Twenty-one bacterial isolates of eleven different species were cultured in 17 dogs. In seven dogs with initial low (10^1 or 10^2) CFU, post-treatment cultures were sterile, while in cases with higher CFU (10^2-10^6), CFU decreased significantly by an average of 1.4 log counts (pre 3.24+/−1.76, post 1.86+/−1.62)(p=0.0004). Pawcare® significantly decreased microorganism loads in dogs with pododermatitis.

Source of funding: This study was entirely funded by JOKER – Technologies, Switzerland

Conflict of interest: None declared.
Proof of concept efficacy and safety study of an anti-IL-31 monoclonal antibody for the treatment of atopic dermatitis in client-owned dogs


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Abstract: Canine atopic dermatitis is an inflammatory and pruritic allergic skin disease with characteristic clinical features, the most prominent of which is pruritus. In humans, both interleukin (IL)-31 and its receptors are over-expressed in skin with atopic dermatitis lesions. Therapy with monoclonal antibodies has been successfully used clinically in humans for multiple chronic conditions including rheumatoid arthritis, asthma and psoriasis. Anti-IL-31 monoclonal antibodies have been shown to reduce or eliminate the pruritic effects of IL-31 in laboratory dogs. The efficacy and safety of an anti-IL-31 monoclonal antibody (ZTS-00103289) dosed twice subcutaneously at a 14-day interval in dogs with atopic dermatitis were evaluated under field conditions in a masked, placebo-controlled, 42-day study. Owner assessment of pruritus using a visual analog scale (VAS) score and dermatologist assessment of skin lesions scores using the Canine Atopic Dermatitis Extent and Severity Index version 2 (CADESI-02) were measured. Seventy-eight dogs from six veterinary dermatology practices were randomized to one of two treatment groups, ZTS-00103289 or placebo, at a 2:1 ratio. Dogs treated with ZTS-00103289 had a significantly greater reduction from baseline in owner-assessed pruritus VAS than did placebo-treated dogs at all assessments from Days 1-42. There were no serious adverse events reported during the study and no hypersensitivity reactions immediately post dosing. The most frequent adverse events in order of prevalence included vomiting, diarrhea, and lethargy. The results of this study support the effectiveness and safety of ZTS-00103289 for treatment of dogs with atopic dermatitis.

Source of funding: Zoetis, Inc. funded this study.

Conflict of Interest: The authors are employed by Zoetis, Inc.
SCIENTIFIC SESSION
PRESENTATIONS
SATURDAY
Most of the published research into itch discusses what is known in humans. It’s possible that many of the same mechanisms apply to animals, and dogs in particular, but this presentation will focus primarily on what is known in veterinary science.

**WHAT IS PRURITUS?**

Why do dogs scratch? We have all been taught about the causes of itching such as sarcoptic mange, flea allergy dermatitis, atopic dermatitis, *Malassezia* colonization, etc. But why do they scratch, bite and rub to the point where they cause skin disease? And what is the common pathomechanism?

Pruritus is defined as *an unpleasant sensation of the skin, provoking the desire to scratch or rub it*. It is also called itching. We really can’t say that a dog is pruritic, since we don’t know what they’re feeling; We can say, though, that dogs exhibit pruritic behaviors as a sign of many skin diseases.

Itch and pain are similar in that they signal the animal of potentially dangerous stimuli and are associated with protective motor responses. However they differ significantly, as itch-induced stimuli typically elicit scratching or biting to remove the stimulus whereas painful stimuli typically elicit withdrawal of the stimulated body area away from the stimulus and/or other integrated escape or aggressive motor responses.

Acute itch is protective and serves as a warning signal for potential tissue damage caused by an invader such as a parasite. Scratching can in fact suppress the itch sensation, but it often results in damage to the skin. Beyond suppression of the sensation of itch, the scratching dog presumably is also “rewarded” by a feeling of well-being. The response of the central nervous system to scratching depends on the context, as scratching healthy skin may result in pain.

Itch is induced by a variety of stimuli, including mechanical, chemical, thermal, and electrical stimulation of the skin. Both algogens (preferentially eliciting pain) and pruritogens (preferentially eliciting itch) cause either pain or itch, depending on the concentrations of chemicals and the delivery methods.

**HOW DO WE MEASURE PRURITUS IN ANIMALS?**

The owner pruritus Visual Analog Scale (VAS) was validated in 2007 and is a tool that can provide an easy to use, accurate and repeatable method for owners to determine the severity of itching in their dog. It consists of a vertical line anchored by 0 at one end and 10 at the other with word descriptors at equal intervals. Owners mark on the line where they think their dogs’ itch level was for the prior 24 hours and then this mark is measured and given a number between 0 and 10. A later study showed that dogs with no dermatologic disease are scored at less than 2, which correlates to “less than very mild itching” or “itching is not a problem.”
Extremely severe itching. Dog is scratching, chewing, licking almost continuously. Itching practically never stops regardless of what else is happening around the dog.

Severe itching. Prolonged episode of itching when the dog is awake. Itching occurs at night and also when eating, playing, exercising, or when otherwise distracted.

Moderate itching. Regular episodes of itching when the dog is awake. Itching might occur at night and wake the dog. No itching when eating, playing, exercising, or when being distracted.

Mild itching. More frequent episodes of itching. May notice occasional episodes of itching at night. No itching when sleeping, eating, playing, exercising, or when being distracted.

Very mild itching. Occasional episodes of itching. The dog is slightly more itchy than before the problem began.

Normal Dog. Itching is not a problem.

**WHAT MAKES DOGS SCRATCH? WHAT DO WE KNOW ABOUT THE NEUROANATOMY OF ITCH?**

While only pain but not itch occurs in deep tissues (e.g., muscle, joints, or inner organs), both itch and pain are initiated at the surface of the body including the skin and mucosal surfaces. Itch and pain also differ on a few other points. Pain is attenuated by µ-opioids which can elicit or exacerbate itch. Conversely, µ-opioid antagonists suppress itch while sometimes inducing hyperalgesia. And painful counterstimuli (scratch, cold, and heat) inhibit itch. These differences have been used to differentiate between itch and pain in animal models.

After the induction of itch in the skin, specialized afferent nerve fibers are responsible for the transmission of the sensation to the central nervous system. Itch (and pain) is received by unspecialized nonmyelinated free nerve endings located close to the dermo-epidermal junction. It has been demonstrated that removal of the epidermis eliminates the ability to perceive itch. Thus, itch is presumably produced by the activation of subpopulations of sensory fibers located in upper skin layers. Itch appears to be determined by the location of the sensory receptor rather than what activates it.

For years it has been debated whether itch and pain are mediated via distinct pathways, a concept known as specificity theory or labeled-line coding, or if itch is a low-level form of pain on the same sensory continuum, a concept known as the intensity (or frequency) theory. Current evidence supports the concept of specificity or labeled-line coding for distinct sensations of itch and pain.

Itch is transmitted by unmyelinated mechano-insensitive and mechanosensitive afferent C-fibers (~20%) as well as thinly myelinated mechanosensitive Aδ-fiber afferents (~80%). Their cell bodies are located in the dorsal root ganglia (DRG) or the trigeminal ganglia. Unmyelinated C fibers enter the dorsal horn of the grey matter of the spinal cord where they synapse with secondary neurons. These cross over to the contralateral spinothalamic tract and ascend to the thalamus. There, tertiary neurons relay itch to the level of conscious perception in the cerebral cortex. Itch and pain appear to share common ascending sensory pathways such as the spinothalamic tract.

Approximately 5% of the C fibers are histamine-sensitive and respond to pruritogenic and temperature stimuli but not mechanical stimuli. The majority respond to mechanical and heat stimulation but not histamine. These do respond to stimulation by cowhage spicules.

If a limited group of C fibers are stimulated, a limited group of dorsal horn cells are activated and produce itch. The arrival of impulses in large A fibers inhibits the response of spinal cord cells to both A
and C fiber inputs. In the CNS and spinal cord pruritus can be suppressed by painful stimuli. The “gate-control” theory suggests that pruritus is suppressed by stimulation of myelinated A fibers at the spinal level favoring the transmission of the pain sensation. This explains the antipruritic effect of pain induced by scratching.

The pathogenesis of itching involves interplay of the resident cells of the skin, such as keratinocytes, mast cells, lymphocytes and sensory neurons, as well as transient inflammatory cells such as eosinophils. These cells release multiple pruritogenic mediators (e.g. interleukin (IL)-31) which lead to activation of the cutaneous nerves. Specific receptors have been identified on both cutaneous and spinal neurons that are exclusively involved in the transmission of pruritic signals.

Diagram of input to the spinal thalamic tract from cutaneous sensory nerve fibers. Cross-sections of the brain, spinal cord, and skin indicate the origin of sensory input from the skin to the spinal cord. The STT is indicated by the hatched line projecting from the dorsal horn of the spinal cord to the thalamus in the brain. Studies in both humans and experimental animals have demonstrated that both C- and Aδ-fibers transduce itch sensation. DRG, dorsal root ganglion; III, third ventricle. From: Potenzieri C, Undem BJ. Basic mechanisms of itch. Clin Exp Allergy, 2011; 42: 8–19.

WHAT DO WE KNOW ABOUT THE MEDIATORS OF PRURITUS IN ANIMALS?

Histamine

Preformed histamine is present in mast cell granules and, upon degranulation, can be released into the surrounding tissue. Four different histamine receptors have been identified: histamine 1 receptor (H1R), H2R, H3R, and H4R. The H1R is found on smooth muscle and endothelial cells as well as immune cells and it plays a role in the genesis of immediate type hypersensitivity reactions. The H2R plays a role in gastric acid production, whereas the H3R is mainly found in the central nervous system and on peripheral neurons. Interestingly, the H4R is mainly expressed on hematopoetic cells (neutrophils, eosinophils, monocytes, dendritic cells, Langerhans cells, T-lymphocytes, basophils, mast cells), fibroblasts, endocrine cells and neurons, thereby leading to a suspected role in allergy and inflammation. Importantly, histamine-induced pruritus in mice seems to be mediated via the histamine H1 and H4 receptors, while the H3R appears to have a negative regulatory role.3
But histamine-induced pruritus is not the only cause of itching in dogs with allergic skin disease. In dogs with atopic dermatitis, several studies have shown that histamine could be an important factor in the development of lesions and/or pruritus, while other studies have shown that it may not be a major contributor to pruritus. In some studies the histamine level in the skin of dogs with atopic dermatitis (AD) was higher than in normal dogs, but these levels did not correlate with their respective plasma concentrations. In an early study, the total histamine content per isolated skin mast cell was found to be higher in dogs with AD than in control dogs, and mast cells isolated from the skin of dogs with AD released more histamine than those from normal skin when stimulated with mast cell degranulating agents. In experimentally sensitized dogs, the epicutaneous application of house dust mites resulted in a transient rise of dermal histamine.

There are several studies that refute the role of histamine in the pathophysiology of itching in dogs. In one study, the intradermal injection of histamine did not appear to lead to either noticeable pruritus or persisting skin lesions in dogs. In a single survey some clients reported that H1R antihistamines were perceived to be effective as a treatment for pruritus in dogs with AD when used as part of a multi-intervention regimen. Two systematic reviews of randomized controlled trials reported that there was no evidence of antipruritic efficacy of topical or oral H1R antihistamine monotherapy in dogs with AD.

At present in humans we know that H1R antihistamines have the greatest significance in the symptomatic therapy of urticaria, allergic rhinitis and bronchial asthma, while antihistamines at normal dosages have little antipruritic efficacy on chronic pruritus associated with AD. This suggests that histamine may not be a significant mediator of pruritus in people with AD.

**SUBSTANCE P**

It has been shown in rats that the neuropeptide substance P (SP), after release from sensory nerve endings, binds with high affinity to the neurokinin-1 receptor on keratinocytes, endothelial cells and mast cells and produces itch in humans and mice. It can liberate histamine from the mast cells. This attracts proinflammatory cells and degranulates mast cells, with release of pruritogenic and proinflammatory cytokines such as tumor necrosis factor-α or leukotriene B4. In mice, SP elicits scratching through a direct action on primary sensory neurons, as well as by release of NO and leukotriene B4 (LTB4) from keratinocytes, rather than mast cell degranulation. Neurokinin receptors 1 to 3 have been identified on spinal dorsal horn neurons in rats and are involved in the transmission of pruritus, but the role of SP in the pathophysiology of itch in dogs is unclear.

**INTERLEUKINS**

Cytokines are small protein molecules that are secreted by cells and have a specific effect on the interactions between cells, communications between cells or the behavior of cells. These include molecules such as interferons, interleukins and growth factors. Interleukin (IL)-2 is a cytokine that has pruritogenic activity.

In 2004, IL-31 was identified as a T-cell cytokine that caused severe pruritus and cutaneous inflammation resembling AD in a mouse model. It is secreted preferentially, but not exclusively, by T-helper2 lymphocytes after their activation and by skin homing memory T-lymphocytes. It is also secreted by mast cells, monocytes, macrophages and dendritic cells.

IL-31 binds to a heterodimeric receptor consisting of the IL-31 receptor A and the oncostatin-M receptor. In the skin this receptor is found on sensory C-fibers and keratinocytes and in the dorsal spinal ganglia where it probably contributes to the transmission of the pruritic signal. Binding of IL-31 to its receptor activates several signaling pathways, one of them involving Janus kinases (JAK1, JAK2 and TYK2) and downstream STAT (-1,-3,-5) signaling molecules.

The intradermal, subcutaneous or intravenous injection of recombinant canine IL-31 into dogs induced pruritus. That same study showed that IL-31 was not detectable in the serum of nonatopic or laboratory dogs, even after HDM or flea sensitization, but it was found to be elevated in the serum of approximately half of the dogs with spontaneous AD. IL-31-induced itching behavior can be reduced by the administration of corticosteroids (both prednisolone and dexamethasone) and the Janus kinase
(JAK) inhibitor oclacitinib. In the mouse model, IL-31 antagonists were able to prevent pruritus and subsequent scratching.

OTHER MEDIATORS OF ITCH
Platelet activating factor, LTB4, thromboxane A2, 5-hydroxytryptamine (serotonin) and toll-like receptor 7 have also been shown to induce itching in humans and mice, but their role (if any) in companion animals has yet to be determined.

WHAT DO WE KNOW ABOUT TREATING PRURITUS IN ANIMALS?
As our understanding of the pathophysiology of itch and allergic dermatitis in dogs evolves, we are better able to select more effective therapies to treat our patients. There is scientific evidence that treatment with glucocorticosteroids, immunosuppressants such as cyclosporine, a limited number of antihistamines and JAK inhibitors are all effective. Response to treatment also aids in understanding the pathophysiology of itch. Our choice of the “best” therapy for a canine patient balances efficacy, including speed of onset, with safety, ease of administration and cost. In many cases multimodal therapy is the best choice.

References:
THE ITCHY MISERABLE PATIENT

Dr. Matthew J. Zirwas

1. Itchy Patient with a non-specific Rash
   a. Work-up
      i. History, exam
      ii. Consider biopsy, IF, scabies prep, patch test (not TRUE Test), labs
         1. If labs: CBC, IgE, Gliadin abs, BUN/Cr, AST/ALT, HIV, HepC, Stool O&P
   b. Approach to therapy
      i. Pick one thing at a time and treat it, order based on clinical suspicions:
         1. Scabies
         2. Undiagnosed allergic contact dermatitis
         3. Irritant contact dermatitis
         4. Drug Reaction
         5. ID Reaction
         6. Endogenous Unclassified Eczema
         7. Neuropathic itch

2. Itchy Patient with no Rash
   a. Work-up
      i. Similar to patient with a rash, but add a few more labs
         1. TSH, Hgb a1C, Chest X-ray
      ii. Cancer not significantly over-represented
         1. Exceptions:
            a. Bile duct – usually present with jaundice and itch
            b. Hematologic – usually have CBC and/or lymph node findings
   b. Approach to therapy
      i. If localized, try topical therapies first
         1. Capsaicin, caines
      ii. If generalized, or if localized failed
         1. Neuroactive meds
            a. Gabapentin
            b. Sertraline or other SSRIs
            c. Doxepin
            d. Mirtazapine
            e. Butorphanol
            f. Naltrexone
         2. NBUVB
         3. If desperate:
            a. Trial of prednisone
            b. If it works, consider MTX, mycophenolate
               i. If they don’t work, consider chronic prednisone
QUALITY OF LIFE IN VETERINARY DERMATOLOGY

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INTRODUCTION

The expression ‘quality of life’ (QoL) is used to assess general well-being, and is defined as ‘the degree to which an individual enjoys his or her life’. In healthcare, QoL is often evaluated in terms of how it is negatively affected by disease.

QoL evaluation is considered to be one of the main outcomes in human clinical trials and is used frequently in primary care practice. It is only a few years that QoL has been studied and evaluated in small animal medicine. Recently QoL surveys have been developed for various diseases in dogs, such as cardiopathies, spinal cord injuries, osteoarthritis, chronic pain, cancer, kidney disease, and inflammatory bowel disease.

In spite of the obvious fact that itch and pain due to dermatological conditions can have an impact on QoL of affected animals, until 2010 there have only been two studies evaluating QoL in dogs with skin disease. In one of these the dogs represented a control group. In the other study allergic dogs were treated with a 0.025% budesonide leave-on-conditioner and evaluated for lesions, pruritus and quality of life by means of a non-validated scale. Since then, two research groups have developed and validated questionnaires for quality of life assessment in dogs with dermatological conditions, the first being limited to canine atopic dermatitis, and the second one applicable to all skin conditions.

QUESTIONNAIRES VALIDATED FOR CANINE ATOPIC DERMATITIS (CAD)

The main questionnaires on the impact of dermatological diseases on quality of life used in human medicine are the Skindex (and subsequent developments) and the Dermatology Life Quality Index (DLQI) (and subsequent developments). Interestingly, the questionnaire developed by Favrot et al. took the Skindex as an example, while the one developed by Noli et al. was inspired by the DLQI. There are several differences between these two questionnaires. The one developed by Favrot et al. is composed by a number of statements, to which the owner should declare his agreement or disagreement. Evaluation of each item is thus performed by assigning a score to each statement from 0 (strongly disagree) to 4 (strongly agree). The initial version of this questionnaire comprised 16 questions related to the QoL of the owner, and 14 to the one of the dog, followed by 6 related to the treatment of CAD in a subsequent publication. Part of these questions reflected those established and validated for the assessment of QoL of children affected by atopic dermatitis and of their parents. The aim of the other questions was to evaluate the impact of the disease on the overall benefit of pet ownership and some were related to the activities of the dog and its well-being. In this questionnaire the lifestyle of the dog (e.g. indoor vs outdoor, urban vs rural) was also considered, as it has been previously observed that dogs living in a rural or suburban environment have higher QoL, and owners of diseased dogs living mainly indoors may have a lower one due to constant disturbance by their pet’s disease. Favrot also determined that owners with a very close relationship to their dogs considered the impact on their QoL higher.

The questionnaire was evaluated for content validity by asking veterinarians, nurses and pet owners to assess whether the statements were adequate and relevant. Construct validity was evaluated by calculating
the correlation between answers to the "overall assessments questions" and the lesional scores (CADESI-03). Overall assessment questions were two, one aiming at determining the impact of the disease on the owner's QoL and the other on the dog's QoL, and preceded the actual 30+ item questionnaire. Unfortunately no correlation was determined between the whole questionnaire and lesional or pruritus scores. After statistical evaluation of the answers to the single questions and the significance of each for the assessment of QoL, the authors concluded that several questions could be eliminated and propose a shorter 15-item questionnaire for further studies. To the author's knowledge there is no published clinical study yet that used this questionnaire.

The other questionnaire developed by Noli and coworkers comprises questions to which owners can answer "none - a little - quite a bit - very much", with scores ranging respectively from 0 to 3. The content of the questions was derived from detailed preliminary "ethnographic" interviews with owners of dogs affected with severe skin conditions. These were free-form conversations in which owners were encouraged to describe in which way their dog's disease was disturbing their QoL and that of their pets. The main areas identified in which the dog's life could be disturbed by the disease were: sleep, eating, behaviour, play or work, relationship with owners and other dogs, and disturbance caused by administration of therapies. Also, the main areas identified in which the owner's life could be disturbed by their dog's disease were: practical problems (more cleaning, cooking etc.), time loss, psychological aspects (feeling of guilt, frustration, shame etc.), disturbance of the normal family life and of interpersonal relationships within or outside the family, disturbance of sleep, in the relationship with their dog, financial expenditure and reduced dog's working performances. From an initial 19-item version, four irrelevant or redundant questions were removed, and a final 15-item questionnaire was published comprising one question (n.1) on the general severity of the disease, seven questions (n.2-8, named QoL1) dealing with the quality of life of dogs and seven (n.9-15, named QoL2) with that of the owners. This questionnaire is now available as supporting information on the Veterinary Dermatology web page. Criterion-related validity was evaluated by comparing QoL1 and QoL2 scores with CADESI-03 and with pruritus assessed by means of a validated Visual Analogue Scale. Construct validity was determined by calculating the correlation of the owner-perceived general severity (question 1) with QoL1 and QoL2. Furthermore this questionnaire was also tested for repeatability and for sensitivity to capture differences between a healthy and a diseased population and improvement after treatment.

THE IMPACT OF CAD ON QUALITY OF LIFE

As mentioned above, in their validation process both questionnaires developed by Favrot et al. and by Noli et al. were administered to owners of atopic dogs, in which CADESI-03 and VAS pruritus scores were also measured. In the study by Noli et al. correlation between QoL and pruritus scores was good and significant, indicating that pruritus has an important impact on QoL of dogs and their owners. On the other hand correlation was not as good between QoL and CADESI-03, confirming that the sole evaluation of lesional improvement in trials is not sufficient, and that the success of a therapeutical intervention, as experienced by owners and pets, is determined by other factors besides clinical improvement. It is clear that clinical scores alone do not reflect the disease experienced by the patient as they do not take into account any impairment of QoL. To a similar conclusion came Favrot et al., who reported correlations between "overall assessment questions" and CADESI-03, but "never close to full concordance unity, emphasizing that the different parameters measure different facets of the same phenomenon and are consequently all useful to evaluate the severity of the disease."

In the study by Linek and Favrot, 73% of the owners considered that atopic dermatitis had an impact on the QoL of their animals, and the higher the CADESI and the pruritus, the higher was the impact on QoL. The areas of the dogs' lives that were most impaired by atopic dermatitis in the questionnaire by Noli et al. were behavioural or mood changes, playing/working activities and the burden of administering
treatment. In contrast, Linek and Favrot judged sleep disturbance to be an important factor, while playing was not strongly affected. These differences could be determined by the different questionnaire structure or wording, as well as by different life styles in the two study populations, which came from Italy and Germany/Austria, respectively. In both studies, meals were not greatly affected.

In the study by Linek and Favrot, 48% of the owners considered that atopic dermatitis had an impact on their own QoL, and the higher the CADESI and the closer the relationship to the animals, the higher was the impact on QoL. The areas of the owners' life which were most impaired in both studies were increased expenditure, time loss, and emotional and physical distress, while family activities and intra-family relationships were less involved.

THE USE OF QoL QUESTIONNAIRES IN CLINICAL STUDIES

To the author's knowledge, there are only two studies in veterinary medicine using QoL as a parameter for the assessment of a treatment intervention in dogs with atopic dermatitis, one of which is yet unpublished. The first one used an owner-assessed non-validated 10cm long visual analogue scale with descriptors, which ranged from 0 (=distracted, not usual self, uncomfortable, unhappy, not playing or attentive) to 10 (=bright, alert, comfortable, happy, attentive, playful, responsive to family), and evaluated QoL if dogs only. The second one used the validated questionnaire developed by Noli et al., assessing QoL of both dogs and owners in atopic dogs treated with ultramicronized palmytoiletanolamide 10mg/kg once daily PO for 8 weeks. Both studies also evaluated lesions and pruritus pre-and post intervention. Interestingly, in both studies QoL improvement was significant, albeit inferior to that of lesional and pruritus scores, highlighting again the need for QoL assessment as an extra tool for the evaluation of treatment interventions.

In the studies using the questionnaire developed by Noli et al., improvement after treatment of QoL of dogs (QoL1) and that of owners (QoL2) were assessed separately. The first study reported a higher mean improvement for QoL1 compared to QoL2, and analysis of the single questions clearly highlighted that the therapeutical interventions necessary to keep the pet's dermatological conditions under control did impact negatively on the owner's and the dog's QoL.

However, it is worth noting that in this study dogs undergoing any kind of therapy were enrolled, including interventions which are expensive (e.g. ciclosporin) or labour intensive (e.g. shampoo). In the second study, however, dogs received just one or two daily palatable ultramicronized palmytoiletanolamide capsules, which were free of cost for the owner. Interestingly in this study the two QoL subclusters improved to a similar extent in response to treatment, maybe confirming that an inexpensive and not labour intensive treatment for CAD will improve owner's QoL too.

QUALITY OF LIFE ASSESSMENT IN FELINE DERMATOLOGY

There are only a few studies proposing quality of life assessments for degenerative joint disease, cardiopathies, feline infectious peritonitis, diabetes mellitus and chemotherapy for lymphoma in cats, and none of them deals with skin disease.

Our group has developed and is currently validating a questionnaire for the assessment of QoL in cats with skin disease, with a similar format to that developed for dogs, e.g. with one subset for animals and one for their owners. A procedure similar to that of the dogs' questionnaire has been implemented for its development (unpublished data). Twenty long interviews were performed to owners of cats with skin disease, for the identification of areas of the cats' and the owners' life which could be influenced by the pet's skin disease. Interestingly, in the development of this questionnaire items such as physical uneasiness
or discomfort due to the cats' clinical condition were less important for cats' than for dogs' owners (cats obviously have less dramatic skin diseases than dogs). On the other hand, items such as stress due to administration of therapies and to visits to the veterinarian were more important for cats and their owners than for dogs and their owners, highlighting the fact that treating cats is certainly more difficult and a bigger source of psychological stress than treating dogs.

In its validating procedure this preliminary questionnaire has been used in a clinical study assessing the efficacy of liquid ciclosporin in cats with allergic dermatitis. Thirty-two cats were treated with a mean ciclosporin dosage of 7.3 mg/kg/day and followed for three months. Lesional (SCORFAD) improvement was good/excellent in 91.6% of cases, pruritus in 81.5%, albeit quality of life only in 56.25%. Improvement of SCORFAD and pruritus were statistically significant, but not quality of life. A detailed analysis of the two subsets QoL1 and QoL2 have not yet been performed for this study, but it can be anticipated that much of the scores will probably relate to treatment administration.


REFERENCES

IT’S NOT JUST SKIN DEEP: THE RELATIONSHIP OF CUTANEOUS AND SYSTEMIC HYPERSENSITIVITY DISORDERS

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INTRODUCTION:
Atopic dermatitis (AD) is a common inflammatory cutaneous disorder in humans and many veterinary species. In humans, it has been demonstrated that some individuals with atopic dermatitis will go on to develop other forms of hypersensitivity (such as food allergy, asthma or rhinitis) later in life, suggesting an intimate relationship between multiple forms of allergic disease.1,2 The purpose of this presentation is to describe what is known about this phenomenon in humans and to review evidence suggesting the possible interrelatedness of allergic diseases in veterinary species.

THE “ATOPIC MARCH” IN HUMANS:
In humans, atopic dermatitis (or eczema) frequently begins early in life, often before 6 months of age.2,3 This “infantile” phase presents most frequently with a pruritic, erythematous, papular eruption on the face, forehead and scalp, often associated with crusting and exudation.1,4 The extensor surfaces of the extremities are sometimes involved and generalized xerosis may be seen.1,4 In the earliest stages, the condition is not yet associated with the development of antigen-specific IgE.4,5 IgE sensitization will subsequently develop in many of these individuals, and the likelihood of this development is enhanced in the presence of severe eczema.6 Although some patients will have (temporary or permanent) spontaneous resolution of their condition after developing infantile eczema, many patients will develop persistent disease.5,6

A significant subset of children with eczema will go on to also develop clinical hypersensitivity in other organ systems, including rhinitis and asthma.1,2,5 This progression from a purely cutaneous condition to the development of hypersensitivity elsewhere has been called the “atopic march”. The risk of progressing in the “march” is considerably higher for children that have developed allergen specific IgE by 2 to 4 years of age (extrinsic AD).2,7 Furthermore, the presence of severe AD is a major risk factor for the subsequent development of both rhinitis and asthma.1,6

Although not always considered part of the atopic march, food allergy is also often associated with early onset eczema. In fact, IgE sensitization to food allergens often precedes sensitization to aeroallergens.8,9 Indeed, food allergy can serve as an important flare factor for patients with eczema / AD.2,10 Furthermore, early onset eczema and food sensitization is associated with an increased risk for the subsequent development of asthma, at least in a subset of patients.11,12

Possible explanations for the human atopic march: Many possible explanations exist for the development of multiple forms of hypersensitivity. A growing body of evidence suggests that epicutaneous sensitization may play a significant role in the development of not only AD but also of food allergy, allergic asthma and rhinitis. Much of the initial work suggesting this possibility came from experimental studies, in which epicutaneous application of allergens was demonstrated to induce the subsequent development of allergic rhinitis and airway hyper-reactivity as well as elevated circulating allergen specific IgE and IgG1 in mice.13,14 Although similar sensitization experiments cannot be conducted in humans, one study demonstrated that patients with AD and no history of asthma demonstrated both allergen-specific (dust mites) and non-specific (acetylcholine) airway hyper-reactivity.15 Furthermore, children with AD were more likely to develop sensitization to peanuts (especially in association with environmental peanut exposure) than were children without AD.16

The hypothesis that epicutaneous sensitization could be responsible for the subsequent development of sensitization in systems other than the skin has been supported by a number of studies demonstrating a
high degree of correlation between the presence of epidermal barrier defects (especially filaggrin; FLG) and the development of asthma, rhinitis and food allergy. Loss of function mutations may be present in up to 50% of patients with AD.\(^2\) Filaggrin mutations are also associated with an increased risk of developing allergic asthma, but only in patients with concurrent AD.\(^17\) However, FLG defects are associated with an increased severity of asthma even in the absence of AD.\(^18\) Filaggrin defects are associated with increased susceptibility to the development of rhinitis independent of concurrent AD.\(^19\) Furthermore, FLG mutations also increase the risk of food sensitization and clinical food allergy regardless of the concurrent presence of AD.\(^20\) Finally, in infants with eczema and food sensitization, the presence of FLG mutation was found to predict the subsequent development of asthma with a positive predictive value of 100% and a 95% confidence interval of 65.5-100.\(^12\) It is likely that other skin barrier defects may also play a role. Polymorphism of the serine protease inhibitor Kazal-type 5 (SPINK5) gene has been associated with the development of atopic dermatitis, asthma and food allergy, while patients with mutations in corneodesmin may exhibit chronic dermatitis, allergic rhinitis, asthma, food allergy and eosinophilia.\(^21\)

Although the idea of a single “unifying hypothesis” for the development of multi-organ sensitization is very attractive, it must be remembered that not all patients with a documented epidermal barrier defect subsequently develop multiple (or even single) allergic diseases. For that matter, not all patients with AD go on to develop asthma or other forms of extra-cutaneous hypersensitivity, and vice-versa.

**IS THERE EVIDENCE OF MULTI-SYSTEMIC HYPERSENSITIVITY IN ANIMALS?**

**Dogs:** Canine AD has been reported to occur in association with a number of (presumably) allergic phenomena involving body systems other than the skin.

*Food allergy:* Perhaps the most well documented of these associations is that between canine AD and adverse food reactions (AFR). In one retrospective study, 7% of dogs with spontaneous AD were found to also have AFR, and a colony of interrelated Maltese-beagle cross-bred dogs has been demonstrated to be predisposed to the concurrent development of AD and AFR.\(^22,23\) In addition, dogs with nonfood-responsive AD have been demonstrated to demonstrate higher serum food allergen-specific antibodies than normal dogs.\(^24\) There is evidence suggesting that food allergens may act as “flare factors” for some dogs with AD (“food-induced atopic dermatitis”), much as has been demonstrated in humans.\(^25\) In these dogs, exposure to a food to which the patient has been sensitized can produce a clinical flare indistinguishable from that induced by exposure to an environmental allergen. In addition, feeding of some “environmental” allergens (e.g. storage mites) has been associated with clinical symptoms of AD.\(^26\) However, as is the case in humans, canine AFR can also present with signs not typically associated with AD, such as steroid-resistant pruritus and chronic gastrointestinal abnormalities.\(^25\) For this reason, it is not correct to state that canine AFR and food induced atopic dermatitis are necessarily the same entity.

*Conjunctivitis and rhinitis:* Some recent publications on the clinical phenotype of canine AD have included assessment of non-cutaneous signs. The concurrent presence of conjunctivitis in dogs with AD has been reported in 20-60% of dogs, while seasonal rhinitis was reported in 6.7% of dogs.\(^27\)

*Asthma / airway disease:* Dogs are not generally considered to spontaneously display an “asthma-like” phenotype, which is typically clinically characterized by recurring airway hyperactivity, and which may be associated with pulmonary eosinophilia, bronchial smooth muscle hypertrophy and mucus accumulation in the airway lumen. Nonetheless, dogs can develop an airway hyperactivity phenotype following experimental allergen sensitization, and the development of this phenotype may be associated with pruritus.\(^28\) Furthermore, a syndrome of eosinophilic bronchopneumonopathy has been reported in the dog. This condition typically initially presents with coughing, but this may be followed by respiratory difficulty and exercise intolerance.\(^29\) Notably, pruritus has been reported in some of these patients.\(^30\) Although these dogs do not typically demonstrate clinically evident airway hyperreactivity, subclinical to severe bronchoconstriction has been reported.\(^29\) Whether this syndrome represents an alternate variant of
an asthmatic phenotype remains to be determined, and the relationship to other forms of hypersensitivity in the dog is unknown.

**Cats:** Whether or not an IgE-dependent cutaneous hypersensitivity analogous to AD exists in the cat has become the point of some discussion, as the pleomorphic appearances of allergic skin disease in the cat differ so markedly from those seen in the dog and in humans. In addition, there is some question whether IgE plays a relevant role in feline allergic skin disease at all, as allergen specific IgE levels often do not correlate with clinical disease, and cats with apparent “atopic” dermatitis frequently have negative intradermal allergen challenge tests. It is beyond the scope of this presentation to provide a detailed breakdown of the arguments for and against the existence of “feline AD”. However, there is much more agreement that cats appear to suffer from a non-infectious, non-parasitic, non-food induced dermatitis of apparent allergic origin. This syndrome has been given various names, and for the purpose of this presentation I shall use “feline atopic-like dermatitis” (FALD).

**Food allergy:** Very few reports have attempted to document the coexistence of feline AFR with FALD. In one study, 13% of cats with FALD also had AFR, while a second study demonstrated concurrent AFR in only 4.6% cats with FALD. Four studies demonstrated the concurrent presence of FALD in 53%, 19%, 14% and 7.7% of cats with AFR.

**Conjunctivitis, rhinitis and asthma:** Very few reports have attempted to document the coexistence of non-cutaneous signs in conjunction with any form of feline hypersensitivity dermatitis. One study reported the presence of conjunctivitis or respiratory (coughing, sneezing) signs in 8% and 6% of FALD cats and in 5% and 4% of food allergic cats, respectively. A second study demonstrated conjunctivitis or respiratory (coughing, wheezing or asthma) signs in 6.8% and 6.8% of cats with FALD and respiratory signs in 16% of cats with concurrent FALD, flea allergy and AFR. In one study attempting to determine intradermal allergen reactivity in cats with small-airway disease without skin disease, the point was made that it was difficult to find cats that fit the criteria, as many of the cats with airway disease also had skin disease. Unfortunately, no specific numbers were provided. Finally, it is worth mentioning that the role of IgE in the mediation of allergic airway disease in the cat is well documented, unlike that in allergic skin disease.

**Horses:** There is considerable evidence to support the role of IgE in the mediation of allergic skin disease in the horse. Most of this evidence has come from work evaluating insect-bite hypersensitivity. However, IgE may play a role in the pathogenesis of other cutaneous hypersensitivities as well, including urticaria and atopic dermatitis.

**Food allergy:** Cutaneous AFR are very poorly defined entities in the horse. AFR have been reported to induce the development of urticaria in horses, which would suggest the involvement of IgE. However, IgGT has also been demonstrated to be capable of inducing whealing in this species. AD can also manifest as urticaria in horses, but reports demonstrating the concurrent presence of AD and AFR in the horse (whether manifesting as urticarial or otherwise) are lacking.

**Reactive airway disease:** The existence of reactive airway disease similar to asthma is well documented in horse, although there is limited information documenting a true allergen-specific etiology for these conditions. Horses suffer from multiple forms of chronic airway disease, including inflammatory airway disease (IAD) and recurrent airway obstruction (RAO). Of the two forms, there is more evidence to support the possible role of allergen hypersensitivity for RAO, although even this is somewhat controversial. Unfortunately, there is very little information regarding the possible concurrent presence of RAO and allergic skin disease, although one source anecdotally suggests that horses with RAO may exhibit an increased risk of developing insect bite hypersensitivity and urticaria.

**CONCLUSIONS:**

There is ample evidence that humans frequently suffer from hypersensitivity disorders affecting multiple body systems. Furthermore, there is also evidence to suggest that (in many individuals) these concurrent
allergic disorders may in fact have a common initiating or contributing factor in the presence of an impaired epidermal barrier.

There is also evidence to suggest that multi-systemic hypersensitivity disorders may also exist in animals, albeit perhaps at a less frequent rate than in humans. However, two factors are critical to remember. First, for some of the clinical disorders described here definitive evidence of an allergic etiology has not been demonstrated (e.g. eosinophilic bronchopneumonopathy, RAO). Second, the mere co-existence of certain disorders does not necessarily imply a multi-systemic hypersensitivity response for each individual, as documentation of an allergic cause is often not performed (e.g. conjunctivitis, rhinitis).

It is possible that the prevalence of multi-systemic allergy in animals is higher than reported here. It may be that certain manifestations of allergy (i.e., dermatitis) are clinically ignored or not noticed in the presence of more life-threatening phenomena, such as respiratory compromise. A second possibility is that the concurrent disease is noted, but is simply not reported in the literature. A third possibility is that treatment for the more severe condition may mask the presence of a second, milder condition. Finally, it is possible that some patients may exhibit evidence of subclinical hypersensitivity that may go unnoticed unless it is specifically investigated.

In conclusion, evidence suggests that animals may suffer from multi-systemic hypersensitivity disorders much as humans do. However, it is possible that these conditions are underreported in the literature. Ideally, all signs consistent with a possible hypersensitivity disorder should be included in published descriptions of allergic animals, regardless of whether those signs represent the focus of the report. In addition, investigation targeted specifically towards the detection of subclinical hypersensitivity disorders may be warranted in future research studies.

References:


CONCURRENT SESSION
PRESENTATIONS
SATURDAY
CASE 1: Bentley is a 2.5 year old CM Basset Hound

History
1.5 year of a nonseasonal bilateral allergic otitis externa complicated by secondary *Malassezia* infections and more generalized pruritus (relatively mild). The secondary *Malassezia* infections had been treated successfully with topical Otomax® (Merck), but were rapidly recurrent. 5 months prior to presentation to our clinic, Bentley had a “flare” of otitis in the left ear that was initially treated with Otomax® (Merck) and EpiOtic®(Virbac) ear flush. Examination after the Otomax® / Epi Otic® therapy showed a mildly ulcerative otitis with no evidence of organisms on cytologic examination. It was suspected that he might be having a topical reaction to his medications. He was switched to T8 Keto®(DVM) ear flush and topical Baytril Otic®(Bayer) and methylprednisolone and was also started on a restrictive fish and potato diet (Iams FP). This “flare” of otitis seemed to subside, only to recur again in the left ear, 3 months later. Cytologic examination at that time showed “rods”. He was treated with methylprednisolone (.23 mg/kg/day to start), Atopica (0.3 mg/kg/day) , oral ciprofloxacin, T8 Keto ear flush and Baytril Otic®. He failed to respond to this therapy. Two weeks prior to referral to our clinic, he had a deep ear cleaning and the ear was cultured. The tympanum in both ears was not clearly visible, but both were thought to be intact. The culture revealed a *Pseudomonas aeruginosa* sensitive to amikacin, polymixin B, tobramycin and ticarcillin. He was started on a topical tobramycin ophthalmic solution BID. His ears were being flushed with T8 keto® once daily. He failed to be responding to this therapy and was referred.

Physical examination
There was mild to moderate inflammation in the interdigital spaces (dorsal and ventral) of all four feet and mild to moderate inflammation in the axillae bilaterally. The left ear was particularly painful on palpation. Bentley also resisted manipulation of the right ear.

Otoscopic examination
There was mild inflammation at the entrance to the vertical canals of the left and right ears. In the right ear, the vertical and horizontal canals were mildly inflamed and thickened with a mild amount of wax on the walls of the canals. The horizontal canal narrowed by 80% just in front of the tympanum area. Wax appeared to be covering the tympanum. On palpation, the horizontal canals appeared calcified just adjacent to the tympanum area. The canals of the left ear were moderately inflamed and thickened and were covered with light colored exudate. The horizontal canal narrowed by 80% just in front of the tympanum area. Exudate filled the horizontal canal. The tympanum was not visible. On palpation, the horizontal canal also appeared calcified adjacent to the tympanum area (more severe than in the right ear).

Cytologic examination of the ears showed 1 + (0 – 4+) *Malassezia* in the right ear and 2 + “plump”, larger rod shaped bacteria and 2 + neutrophils.

Notes:
CASE 2: Lucy is a 1 year 8 month old Female white and tan Basset Hound

History
One year history chronic otitis worse left side. Suspect atopic or food allergy.
Presented on ZD and the skin and pruritus were doing well with the diet but the ears had continued to have problems. The otitis had been diagnosed as Pseudomonas and this was confirmed on two different prior cultures. The sensitivities had changed and the treatments had been changed based on those results.
Over the prior year multiple treatments for Pseudomonas had been given including:
Systemic therapies: enrofloxacin, orbifloxacin, ciprofloxacin, marbofloxacin, ketoconazole, prednisone
Ear flushes: ears were cleaned multiple times even with sedation or anesthesia.
Topicals: utilized at different times; Triz EDTA, Otomax® (Merck), and in office formulations of drops that contained amikacin, ticarcillin, enrofloxacin in various combinations with dexamethasone and clotrimazole.

Physical examination
There was mild rust staining of the hair on the paws and mild erythema of the ventral paws.

Otoscopic examination
Both ear had erythema though it was very mild in the right ear. The left ear canal was moderately side erythematous with some erosions and ulceration. The right canal had a brown black purulent mucoid exudate.
Cytologic examination of the mucoid exudate revealed rods with neutrophils, many of which were toxic and an occasional mononuclear cell.

Notes:
CASE 3: Casper is an 8 year old CM DSH

History
Casper’s owners had noted periodic head shaking for about 8-12 months (once or twice daily). There was no scratching at the ears. The problem was non seasonal. There were no more generalized signs of pruritus nor obvious skin lesions. There were no respiratory nor gastrointestinal abnormalities. Within the last few weeks, the frequency of head shaking was increasing. On his first Veterinary visit for this problem, a bacterial infection was diagnosed and treated with a topical ointment. The head shaking did decrease, but did not resolve. 3 weeks later the ears were examined while Casper was under general anesthesia for a dental. A possible aural polyp was noted within the left ear. No mention was made of pathology in the right ear. Casper was referred for this problem.

Physical examination
The examination showed no abnormalities other than the ears.

Otoscopic examination
In the left ear, a large accumulation of dark wax (ceruminolith) was filling the horizontal canal, just in front of where the tympanum would be. The rest of the canals looked normal. A very brisk pruritic response (aural-pedal reflex) was noted with swabbing of the ear. In the right ear, the walls of the horizontal canal were covered with a mild to moderate accumulation of light colored wax. The canal walls were mildly thickened. A large accumulation of wax was filling the horizontal canal, covering about 95% of the tympanic membrane. A very brisk aural-pedal reflex (pruritic response) was noted with swabbing of the ear.

Cytologic examination of debris taken from the walls of the horizontal canal just in front of the ceruminoliths in both ears failed to show any organisms. Cytology from the ceruminolith in the left ear showed 1+ (0 – 4+ scale) “rod” shaped bacteria and from the ceruminolith in the right ear, 4+ cocci and diplococcie.

Notes:
OTITIS - CHRONIC CASES

Griffin CE, DVM, DACVD\textsuperscript{1}, Rosychuk RAW, DVM, DACVIM\textsuperscript{2}
\textsuperscript{1}Animal Dermatology Clinic, San Diego, California
\textsuperscript{2}College of Veterinary Medicine, Colorado State University, Ft. Collins, Colorado

CASE 1: Arthur is a 5 year 3month old male neutered black standard poodle

History:
Arthur has struggled with ear infections since his owners bought him at about 3months of age. He has had around 10 “flares” of otitis. He has been treated with Amoxicillin, Tramadol and Posatex® (Merck). The referring veterinarian is concerned about a polyp in his left ear. Arthur is not pruritic, though he does have a littermate that is itchy year round.

Physical Examination:
No skin lesions

Otoscopic examination:
Right ear within normal limits; intact tympanum
Left ear canal is open. Within the distal canal, just in front of where the tympanum would be, the horizontal canal contains purulent debris adhered to the dorsal surface of the horizontal canal.

Flushed the left ear with Douxo Micellar Cleanser® (Soheval) - some purulent exudate removed, revealing a whitish mass that was previously covered by exudate; possibly a bulging pars flaccida?
Assessment: Suspected mass AS and scheduled for general anesthesia, video otoscopy and possible mass removal

Video otoscopic examination and procedures:

Notes:
CASE 2: Jackson is a 7 year old CM Yellow Lab

History:
Jackson has had a recurrent, bilateral otitis and more generalized pruritus problem for the last 4 years. These problems have been seasonal (otitis “flares” a couple times during the spring/summer/fall; more generalized signs of pruritus are relatively mild and primarily directed at the axilla and inguinal regions.). The left ear has generally been more significantly affected. These “flares” of otitis have responded well to topical therapy. The otitis in Jackson’s left ear flared again about 4 months ago (later spring). Cytologic examination at the time revealed *Malassezia*. Topical therapy (Mometamax® Merck; EpiOtic Advanced® Virbac ear flush alternating with TrizUltra + Keto (Dechra) ear flush appears to partially benefit the problem, but as soon as the medications are stopped, signs (head shaking, scratching at the ear) recur. Two weeks ago, an otoscopic examination of the left ear by the referring Veterinarian revealed an apparent mass within the ear.

Physical Examination: Mild inflammation in the axillary regions.

Otoscopic Examination:
Left ear: there was a very mild increase in wax on the walls of the horizontal canal. The canal walls were mildly thickened. A large, smooth surfaced mass was filling the horizontal canal, just in front of where the tympanum would be. A small amount of clear fluid was noted around the mass.
Right ear – normal.

Diagnostics: Cytology from the left ear – 2+ Malassezia (0 – 4+).

Differentials for the “mass” – dilated pars flaccida; ceruminous cyst; fibrous “polyp”; neoplasm

Diagnostic and Therapeutic Plan: General anesthesia; CT scan of bullae; biopsy / excisional biopsy of mass

Notes:
Case 3: Jetice is a 10 year old SF Cocker Spaniel.

History:
Jetice has a history of a bilateral otitis and pedal pruritus that has been present for about 4 years. Her right ear has always been more severely affected. The problem has been non-seasonal but appears to be worse in the summer. The more generalized pruritus has been treated with a series of antihistamines. Pedal pruritus appears to be somewhat better on chlorpheniramine, but this does not appear to have benefited the ears. The ears have been treated with Mometamax® (Merck; gentamicin, clotrimazole, mometasone) for “flares” of otitis. Treatment durations have been anywhere from 3 to 10 days. For many of these “flares”, the owner has made the decision as to when to start the medication and how long to treat the ears. The Mometamax® does appear to benefit the problem significantly. 3 weeks prior to referral, Jetice was examined for yet another “flare” of otitis associated with head shaking and scratching at primarily the right ear. Jetice’s veterinarian was concerned that there might be a “mass” in the right ear. Jetice was started on topical Gentizol® (VetOne; gentamicin, betamethasone, clotrimazole) and referred. Throughout all the above, Jetice appears to hear well.

Physical Examination:
Alopecia and mild to moderate inflammation of the ventral and dorsal interdigital spaces of all four feet. Right ear: moderate inflammation and moderate to severe thickening of the proximal ½ of the medial pinna. Moderate inflammation and severe stenosis of the entrance to the vertical canal (unable to pass the video otoscope in to the vertical canal). Small amount of light, tan colored exudate in the entrance to the vertical canal. The vertical and horizontal canals were not collapsible with pressure and felt thickened and calcified/ossified, especially in the area of the junction of the vertical and horizontal canals and throughout the horizontal canal (to the skull).

Left ear: mild inflammation and thickening at the entrance to the vertical canal. Horizontal and vertical canals were mildly inflamed and thickened. The surface of the canals were mildly roughened. There was no significant amount of wax on the walls of the canals. 50% of the tympanum was covered by light yellow wax.

Diagnostics:
Cytology :
   Right ear - 2+ short rods, 2+ diplococcic (0 – 4+); gram stain : short gram positive rods
   Left ear - no organisms
   Left front and right hind dorsal interdigital spaces – occasional Malassezia / OIF
   Left front and right hind ventral interdigital spaces – occasional Malassezia / OIF

Notes:
INTRODUCTION:
A significant proportion of cases seen by the dermatology practitioner involve derangements of the immune response. This lecture is not intended to provide a detailed discussion of all aspects of immunology, but rather aims to provide a brief overview of two major aspects of the cutaneous immune response: the role of the innate immune system on the developing immune response and the role of different T cell functional phenotypes in mediating cutaneous immunity.

CUTANEOUS INNATE IMMUNITY AND THE ROAD TO ADAPTIVE RESPONSES:
A growing body of evidence suggests that the cells and non-cellular components of the innate immune system play active and critical roles in the initiation and development of a productive immune response. These cells interact with microbes, environmental agents and ultimately with each other to not only mediate local immunity but to activate and shape the subsequent adaptive immune response. We will first discuss the mechanisms by which these cells sense and are activated by threats in their environment. We will then discuss the specific responses generated by these threats. Finally we will discuss the interactions of these cells with lymphocytes and their role in influencing the developing immune response.

Target recognition by the cutaneous innate immune system: Unlike lymphocytes, cells of the innate immune system are not capable of recognizing specific antigens. Instead, these cells recognize and are activated by exposure to common molecular structures and substances. These “pathogen associated molecular patterns” (PAMPs) correspond to molecules or structures that are highly conserved, generally because they represent structures that are critical for the survival of the pathogen. Furthermore, these structures are NOT found in normal host tissues. Examples of PAMPS include lipopolysaccharides from gram negative bacteria, peptidoglycan from gram positive bacteria, fungal cell wall carbohydrates and viral nucleic acids. PAMPs bind pattern recognition receptors (PRRs) on the host, resulting in cellular activation.

The most studied of the pattern recognition receptors are the Toll like receptors (TLRs). These receptors are expressed as homodimers or heterodimers. They are expressed on cell membranes—both on the outside of the cell and inside the cell on endosomes. There are 13 receptors known to date, but not all receptors have been identified in all species. The best known of the TLRs is TLR4, which recognizes lipopolysaccharide. However, other PAMPs recognized by TLRs include peptidoglycan (TLR2), viral double-stranded RNA (TLR3), flagellin (TLR5), viral single stranded RNA (TLR7, TLR8) and microbial CpG DNA (TLR9). Other examples of PRR include the cytoplasmic nucleotide binding oligomerization (NOD) like receptors, (which are expressed in the cytoplasm and recognize intracytoplasmic bacteria) and C-type lectins (which are expressed on the cell surface and bind to carbohydrates on microbial cell walls).

However, the stimulus activating innate immune cells need not necessarily be microbial in origin, as these cells may also be activated by exposure to damage associated molecular patterns (DAMPs, aka alarmins). These are substances that are produced by the host’s own cells. They may be actively secreted by cutaneous “sentinel cells” or released by damaged cells. DAMPs recruit and activate inflammatory cells, but may also have independent antimicrobial functions. Examples of DAMPs are defensins and cathelicidins. These small cationic peptides are produced near body surfaces, including the
epidermis. In addition to their role as DAMPs, they can induce lethal disruption of microbial membranes. Other DAMPs include fibrinogen and heat shock proteins, which may activate TLRs.

Still other factors may also result in the activation of innate immune cells. Many cells (including keratinocytes, macrophages, mast cells, dendritic cells and eosinophils) express protease activated receptors (PARs). These surface-expressed G-protein coupled receptors are cleaved upon exposure to serine or cysteine proteases, which may be expressed by microbes (e.g. to facilitate invasion of epithelial structures). However, proteases can also be found in a number of non-microbial sources, such as house dust mites, insects and some types of pollen. Regardless of the source, these proteases can produce rapid activation of cells expressing PARs. In addition, proteolytic cleavage of certain cell surface molecules has the potential to further enhance and shape a developing immune response. In this manner, exposure to non-pathogen environmental agents has the potential to produce and influence the development of an active inflammatory response.

Cells of the cutaneous innate immune system:

**Keratinocytes:** Although not typically thought of as part of the immune system, keratinocytes play both passive (physical barrier formation) and active roles in the developing immune response. Their external location frequently makes them the first cells to come in contact with microbes, allergens or irritants. Keratinocytes are known to express a number of PRRs, among them several TLRs and PAR-2. Binding / cleavage of these receptors causes activation of the keratinocytes, inducing the production of and release of a number of cytokines. The exact cytokines released depends in part upon the nature of the insult. For example, cleavage of PAR-2 resulted in the release of granulocyte-macrophage colony stimulating factor, interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-a) from a canine keratinocyte cell line. In contrast, activation of TLR3 and TLR9 on human keratinocytes induced production of the chemokines CXCL9 and CXCL10, which are associated with the generation of Th1 immune responses. Other keratinocyte-derived factors include thymic stromal lymphopoietin (TSLP), which may play a critical role in the induction of Th2 responses and the T cell chemotactic factors thymus and activation regulated chemokine (TARC), CCL27 and CCL28.

**Dendritic cells and Langerhans cells:** Both dermal dendritic cells (DC) and epidermal Langerhans cells (LC) play a critical role in the development and shaping of the cutaneous immune response. They are uniquely placed to sample both exogenous and endogenous antigens, and the conditions under which they encounter antigen largely determines their future response. Antigen encounter in the absence of inflammatory stimuli (cytokines, PAMPs or DAMPs) is generally not sufficient to induce DC/LC activation and migration. In contrast, antigen encounter with co-exposure to inflammatory cytokines, proteases or PAMPs/DAMPs will induce the activation of the cell. This is associated with increased surface expression of antigen-major histocompatibility complex (MHC) as well as the expression of numerous costimulatory molecules such as CD80, CD86 and CD40. As for keratinocytes, the exact nature of the activating stimulus will affect the subsequent DC response. Exposure to microbial and allergen-derived proteases can decrease DC production of a in the presence of lipopolysaccharide induces increased expression of a number of cytokines (IL-1b, IL-10, IL-12p40, IL-13 and TNFa), the net effects of which favored the ultimate development of a Th1 response. DC responses may also be influenced by the cytokine milieu in which they are activated. Activation in the presence of keratinocyte-derived TSLP induces the development of DC capable of potent induction of Th2 responses.

**Mast cells:** Mast cells are best known for their role in the effector phase of IgE-dependent Type I hypersensitivity responses. However, mast cells may also play a role in the induction of an immune response as well. Like keratinocytes, mast cells express several TLRs as well as PARs. Proteases have been demonstrated to produce IgE-independent degranulation of mast cells in mice and humans, while epicutaneous application of proteolytic house dust mite extract induced degranulation in allergen-naïve dogs. Mast cells can release not only vasoactive substances such as histamine and prostaglandins,
but also may produce cytokines such as TNF-a, IL-4, IL-15 and IL-13.\textsuperscript{1-3} These cytokines may help to create a milieu highly conducive to the development of allergic sensitization.

**Granulocytes:** The role of granulocytes in the cutaneous immune response is more likely to be as effectors than as initiators. In normal skin, very few neutrophils, eosinophils or basophils are present.\textsuperscript{1-3} However, the activation of dermal “sentinel cells” (such as DC, macrophages and mast cells) causes the rapid recruitment of granulocytes (especially neutrophils) via the induction of vasodilation, vascular leakage and the elaboration of neutrophil chemoattractants such as IL-8.\textsuperscript{1-3}

**The role of the innate immune system in shaping the adaptive response--first impressions matter:**

As stated above, the circumstances under which an antigen presenting cell (APC) initially encounters an antigen will determine whether or not that cell becomes activated. APCs are constantly sampling their environment, even at rest, and most of what they will encounter will be innocuous “self” antigens. These antigens will be processed and presented on the cell surface, but they will not induce activation of these cells.\textsuperscript{1-3} As a result, the APC will not upregulate the expression of the critical costimulatory molecules CD40, CD80 and CD86, and it will not release inflammatory cytokines. If this APC subsequently encounters a T cell that recognizes one of these antigens, antigen presentation will still occur. However, in the absence of costimulation and inflammatory cytokines, the result is the generation of a non-reactive (tolerant or anergic) T cell phenotype.\textsuperscript{1-3}

In contrast, if the APC encounters the antigen in the presence of inflammatory stimuli, it becomes activated, upregulates costimulatory molecules and starts to express inflammatory cytokines (the exact nature of which will depend upon the circumstances under which activation occurred, as stated above).\textsuperscript{1-3} These activated APC are also induced to migrate to the local lymphoid tissue, where they are likely to encounter and present antigen to T cells. Antigen presentation in the context of costimulation and in the presence of inflammatory cytokines will trigger T cell activation. Furthermore, the cytokines to which the T cell is exposed at this point will largely determine the subsequent functional phenotype that it assumes.

**ADAPTIVE IMMUNE RESPONSES--DEVELOPMENT AND FUNCTION OF CD4 T CELL PHENOTYPES:**

T cells may be divided into CD8- and CD4-expressing cells. CD4 cells typically function by directing the activities of other cell types. These cells include other lymphocytes (CD8 cells, NK cells, B cells and even other CD4 cells) or cells of the innate immune system (particularly macrophages and neutrophils). This direction may be direct (involves cell to cell interactions) or indirect (via the production of cytokines and chemokines), and may result in increase, decrease or alteration of function of the target cells. CD4 cells can be subdivided by their functional phenotypes, which are indicated using “Th” designations.

**Th1** cells are generated by exposure to the cytokines IL-12, IL-18 and interferon-gamma (IFN-g) during their activation.\textsuperscript{1-3} Their main function is to stimulate cell mediated immunity to increase killing of intracellular pathogens (e.g., *Salmonella*, viruses). This is produced by the elaboration of IL-2 and IFN-g. IL-2 is critical for the survival, proliferation and differentiation of both CD4 and CD8 T cells as well as NK and B cells. IFN-g enhances the cytotoxicity of CD8 and NK cells, while simultaneously facilitating the activation of B cells and their differentiation into antibody producing plasma cells. In particular, IFN-g promotes the differentiation of B cells into the production of certain IgG subclasses and decreases the production of IgE.

Th1 cells can also increase macrophage activation during direct cell to cell contact via CD40 / CD40 ligand interactions.\textsuperscript{1-3} Together with IFN-g, this interaction enhances macrophage killing via enhanced lysosome-phagosome fusion, increased antimicrobial peptide production, increased costimulatory molecule expression and enhanced cytokine / chemokine production.
**Th2** cells are generated by exposure to the cytokine IL-4 during their activation.\(^1\)\(^-\)\(^3\) Their main function is to provide protection against parasites, and enhance humoral immunity via the production of antibodies, although they may also play a role in the development of hypersensitivity. They release several cytokines and chemokines, notably IL-4, IL-13, IL-5, IL-10, eotaxin and TARC. IL-4 induces the differentiation of B cells into antibody producing plasma cells. In particular, IL-4 promotes the production of IgE, but also the production of IgA and some subclasses of IgG. Both IL-4 and IL-13 stimulate the recruitment of inflammatory cells and activate mast cells. IL-5 and Th2 chemokines attract and activate eosinophils as well as other T cells. Finally, IL-10 inhibits the polarization of naïve T cells towards a Th1 phenotype.

**Th17** cells are generated under a variety of conditions, often during exposure to transforming growth factor beta (TGF-b), IL-6, IL-23 and IL-21.\(^1\)\(^-\)\(^3\) Their main function is to increase the killing of extracellular bacteria (e.g. *Staphylococcus*) and fungi by stimulating neutrophilic inflammation. These cells are often the first functional T cell subset to differentiate. The major cytokine that they produce is IL-17, which stimulates the production of chemokines and other cytokines, which stimulate the recruitment and activation of neutrophils and to a lesser extent macrophages and other inflammatory cells. It also increases the production of antimicrobial peptides. Th17 cells also produce IL-22 and IL-21. Overexpression of the Th17 phenotype has been associated with the development of a number of inflammatory conditions, including psoriasis.

**Treg.** There are actually several forms of regulatory T cells (Treg).\(^1\)\(^-\)\(^3\) Most self-reactive T cells are actively deleted during T cell maturation in the thymus, although some cells with the potential to react to “self” antigens may escape deletion. However, this usually isn’t a problem. As mentioned before, APC that encounter antigens (even “self” antigens) normally present them to T cells in the absence of costimulation and inflammation. The result is that the potentially auto-reactive T cell becomes unresponsive, or anergic. Similar mechanisms are responsible for the production of tolerance to orally administered antigens. In addition, T cells that are activated in the presence of “immunomodulatory” cytokines (typically IL-10 or TGF-b) do not differentiate into inflammatory effector cells. Instead they may develop an immunomodulatory or regulatory phenotype. These cells typically express CD4, CD25 (part of the IL-2 receptor; also expressed on recently activated T cells) and the regulatory component FoxP3. They may induce antigen-specific or antigen non-specific tolerance by the production of soluble suppressive cytokines, such as IL-10 and TGF-b. Together, these cytokines decrease the activation of numerous cell types. In addition, the elaboration of these cytokines during the activation of nearby naïve T cells may induce the development of tolerance in those cells as well.

**CONCLUSIONS:**

In conclusion, new work suggests that the interplay between the cells of the innate and adaptive immune systems is much more complicated than previously suspected. We now know that innate immune cells are not merely effectors but instead are actively involved in the development and shaping of the subsequent immune response. This shaping is largely determined by the circumstances under which the innate cells encounter antigen. These circumstances will influence the immediate responses of APCs (activation vs not; cytokine producing vs not, etc.), which in turn will largely determine the entire future of the adaptive immune response.
INTRODUCTION:
Vascular inflammation and/or insufficiency is a common cause of dermatologic disease in humans and animals. Disruption of the cutaneous vasculature may be associated with a number of local or systemic disorders, including infection, inflammation, neoplasia, hypersensitivity, immune mediated disease, photodermatitis and idiopathic phenomena. Regardless of the underlying cause, the resulting vascular compromise impedes tissue oxygenation, causing cutaneous atrophy and potentially overt necrosis. Proper management of these phenomena requires a thorough understanding of potential etiologies and appropriate diagnostic measures.

VASCULITIS:
The term “vasculitis” is used broadly to refer to conditions in which the blood vessel walls are or appear to be the target of an inflammatory response. This is considered to be a cutaneous reaction pattern, rather than a specific diagnosis.

Causes of vasculitis: A variety of infectious, inflammatory and immune-mediated phenomena have been associated with the development of vasculitis (Table 1).

Immunopathogenesis: The immunopathogenesis of vasculitis can often be explained by application of the four Gell and Coombs categories, and it is likely that multiple mechanisms may contribute to the development of disease in individual patients. Type I hypersensitivity reactions are rare causes of chronic vasculitis in animals, although IgE-mediated mast cell degranulation may be associated with the wheal reaction associated with early urticarial vasculitis. The development of anti-neutrophil cytoplasmic antibodies (ANCA) represents a form of Type II hypersensitivity. These antibodies bind to neutrophil granules (including myeloperoxidase and serine protease 3) and trigger the release of toxic mediators, resulting in the direct and indirect (via complement activation) damage of endothelial walls. These antibodies have been reported to play a role in some forms of vasculitis, especially in humans. These include Wegener’s granulomatosis, Churg-Strauss syndrome and polyarteritis nodosa. Anti-nuclear cytoplasmic antibodies have also been demonstrated in dogs, although association with vasculitis has not yet been reported.

Perhaps the most relevant pathophysiologic mechanism in the development of vasculitis is immune complex formation/deposition (Type III hypersensitivity). Large antigen-antibody complexes deposit in blood vessels, resulting in the activation of complement and endothelial damage. This in turn results in the recruitment of neutrophils and further cell damage.

Clinical appearance: The clinical appearance of cutaneous vasculitis is dependent upon several factors including inciting cause, the size of involved vessels, severity, etc. Mildly affected animals may present with only alopecia (secondary to ischemic follicular atrophy), recurrent whealing or edema. More severe cases may be associated with crusting, erosion, ulceration or frank necrosis, especially on areas
with poor collateral circulation (such as the pinnae and tail tip). These lesions often heal with scarring. Footpads may be affected. In particular, digital pads may develop circular “punched-out” areas of erosion or ulceration. Another common syndrome is palpable purpura, in which raised indurated areas of erythema or violaceous change are seen. These areas may coalesce to form plaques.

Patients with vasculitis may or may not demonstrate systemic signs at the time of presentation. Pain, pruritus or obvious involvement of non-cutaneous organ systems may or may not be noted. Concurrent organ involvement may include kidneys (glomerulonephritis), mesothelium (pleuritic, pericarditis, peritonitis), the gastrointestinal tract, muscle (myositis), joints (polyarthropathy), heart, lungs and CNS. Depending upon the degree of associated endothelial damage, patients may be in a hypercoagulable state secondary to widespread platelet and clotting factor activation.

Specific clinical vasculitis syndromes:

Rabies Vaccine associated vasculitis: This syndrome may be seen either as a local or a more generalized phenomenon. The lesions are frequently located at the injection site or ventral to it. Lesions often appear initially as dermal induration, which may be followed weeks to sometimes a few months later by focal alopecia and cutaneous atrophy. Atrophic lesions often feel somewhat “adhered” to underlying tissue. Myositis or muscle atrophy may also be seen. A causative link to vaccination has been made in several cases in which viral antigen has been detected in the lesions. Repeat vaccinations may be associated with the development of similar lesions, but at least one anecdotal case exists of serious systemic disease in a dog that had previously only had a local reaction.

Vaccine induced vasculitis: These lesions may or may not occur at the site of vaccination or at locations distant from it. Distant lesions typically affect areas with poor collateral circulation, such as tail tips, paw pads, pinnae, planum nasale. Areas prone to trauma may also be affected, such as the face and over bony prominences. Mild lesions may present with focal or multifocal alopecia. More severe lesions may be associated with varying degrees of crusting, erosion and ulceration. “Punched out” lesions on the digital pads are particularly suggestive. In severe cases, sloughing of one or multiple nails may be seen, in addition to mucosal and mucocutaneous junction erosion. Small breed dogs appear to be predisposed to this phenomenon, but it can be seen in any dog.

Fulminant multisystemic vasculitis associated with human serum albumin (HSA) administration: Several cases have been reported of fulminant multisystemic vasculitis following a single administration of human serum albumin. In one report, 6 of 6 healthy dogs administered HSA developed delayed adverse reactions between 5 and 13 days following administration. These included lameness, edema of the face and limbs, “cutaneous lesions indicative of vasculitis”, ecchymoses, vomiting and bloody diarrhea and atrophy of the temporalis and masseter muscles. One of these dogs had also experienced an immediate hypersensitivity event during administration. Four of the dogs were severely affected enough to require hospitalization. In two dogs, symptoms progressed to include oliguric renal failure and pulmonary edema, resulting in death. A similar case has been seen by the author.

Purpura hemorrhagica: This condition typically occurs 2-4 weeks following infection with *Streptococcus equi* or *Clostridium perfringens*, although other etiologies (vaccination with Streptococcal M protein, respiratory infections, open wounds) have also been associated with its development. The condition presents as the development of subcutaneous edema of the limbs, ventrum and occasionally the face. Affected horses may demonstrate petechiation of mucous membranes. Severely affected animals may develop fever and multisystemic vasculitis progressing to infarction of skin, muscle and gut.

Facial vasculitis / arteritis: This condition has been reported in a number of breeds, but is particularly prevalent in Scottish terriers and Saint Bernards. In Scottish terriers, the condition presents in young adulthood as ulceration and necrosis of the philtrim and nasal cartilage, although severe cases may demonstrate necrosis of the cranial muzzle. Patients are painful and systemically ill. In Saint Bernards, the condition typically affects 3-6 year old dogs. Affected animals display a “v” shaped necrosis of the nasal planum. This condition may be associated with mild or profuse arterial bleeding.
Cutaneous and renal vasculopathy: This condition has been reported most commonly in racing greyhounds fed raw or undercooked beef, but can potentially be seen in non-racing greyhounds or other breeds, including Great Danes. A verotoxin produced by E. coli has been proposed as an inciting cause, although experimental inoculation of verotoxin producing bacteria has failed to replicate the disease. The condition presents initially as multifocal areas of erythematous swelling that may or may not drain a serosanguineous fluid. Lesions often evolve into ulcers and pitting edema. The ventral abdomen and extremities are most commonly affected while the head and dorsum are usually spared. Affected animals are often febrile and lethargic and may or may not have gastrointestinal involvement or some degree of renal failure.

Vasculitis associated with systemic lupus erythematosus (SLE): Systemic lupus erythematosus is frequently associated with multisystemic vascular disease secondary to immune complex deposition. Although animals are frequently systemically ill by the time of appearance of cutaneous lesions, the author has recently seen a case in which cutaneous lesions preceded overt systemic illness by several months.

OTHER ISCHEMIC DERMATOPATHIES

Familial canine dermatomyositis: This condition is seen most commonly in Collies and Shetland Sheepdogs but may appear in other breeds as well. The condition usually appears prior to 6 months of age, but occasionally does not appear until adulthood. Affected animals develop erythema, alopecia and crusting on areas prone to trauma (especially the face and pinnae) and on pressure points. Later lesions may be associated with erosions and ulcerations, which heal by scarring. Lesion severity may vary greatly between individuals. Affected animals may have significant clinical myopathy (often presenting as muscle weakness or atrophy of the muscles of mastication). Alternately, animals may demonstrate electromyographic abnormalities in the absence of clinical myositis.

Proliferative thrombovascular necrosis of the pinnae: Dachshunds and Rhodesian ridgebacks appear to be predisposed to this condition, although other breeds may also be affected. Affected pinnae demonstrate varying degrees of swelling, crusting, scaling, fissuring and bleeding. In extreme cases, overt progressive necrosis of the pinnae may be seen. Individual cases have been reported in association with drug (fenbendazole) or vaccine administration, or food hypersensitivity.

DIAGNOSIS:
The presence of crusting, ulcerative to erosive lesions on the extremities and pinnae is strongly suggestive (but not diagnostic for) of the presence of a vasculopathy, especially when combined with punched-out lesions of the footpads. However, lesions demonstrating erythema or violaceous change may be examined by diascopy. Failure to blanch with direct pressure suggests extravascular hemorrhage. It is important to note that this result, while suggestive of vascular compromise, may also be seen in other conditions (e.g., coagulopathies).

Skin biopsy is the definitive diagnostic tool to demonstrate the presence of a vasculitis. Samples should ideally be taken from active edges of lesions, as these are the most likely to contain active vascular inflammation. Biopsies taken from more chronic lesions may only contain findings suggestive of prior ischemia, such as atrophy of the follicles and adnexae, “smudging” of dermal collagen and occasionally vacuolar basal cell change resulting in dermal-epidermal clefting. Biopsies obtained of vaccine associated vasculitis may occasionally demonstrate deep dermal to subcutaneous inflammation associated with amorphous debris (presumed vaccine adjuvant).

Since vasculitis is considered to be a cutaneous reaction pattern rather than a specific diagnosis, any diagnostic approach must include an attempt to identify an underlying cause. A thorough questioning of the owner to identify any drug administration (including vaccinations, flea and heartworm prevention) should be performed and any suspect drugs discontinued immediately. A minimum data base (CBC, chemistry profile, urinalysis) may be useful to help detect systemic involvement and possibly demonstrate infectious diseases (e.g., Babesia, Histoplasma). Serum titers for tick-borne diseases are indicated in
endemic areas. Tissue or blood cultures may be appropriate to identify primary infections or secondary invaders. If systemic involvement is demonstrated, an anti-nuclear antibody test may help to identify SLE. In severe cases, coagulation panels and D-dimer assays may detect the presence of hypercoagulability and provide prognostic value.

**THERAPY:**
Effective therapy of vasculopathic disease involves the identification and elimination of underlying disease, whenever possible. If the condition is thought to be vaccine-associated, it may be prudent to avoid further vaccination in that animal, where laws permit this option. If re-vaccination is unavoidable, eliminating optional vaccines and minimizing the number of vaccines given at any one time may be advisable.

Symptomatic management of vasculopathy generally involves a combination of anti-inflammatory medications (reviewed in Innera 2013 and Morris 2013). Pentoxifylline is often useful as a sole (for mild disease) or adjunct therapy because of its several anti-inflammatory and hemorrhheologic effects. A wide range of doses have been suggested for this drug (15-30mg/kg, BID to TID), with 20mg/kg TID being the author’s preference. Other therapies used for less severe cases of vasculitis include Vitamin E and the combination of tetracycline (or doxycycline) and niacinamide.

More severe cases may benefit from systemic glucocorticoids at high anti-inflammatory to immunosuppressive dosages. Prolonged administration of high doses may be counterproductive (via worsening of cutaneous and muscular atrophy) and should be avoided when possible, but intermittent “pulse” administration may be useful for treatment of flares. Potent topical glucocorticoids may be used as adjunctive treatment for individual refractory lesions, although overusage may result in significant atrophy. Alternately, focal lesions may respond to topical tacrolimus applied once daily to every other day. In persistent, chronic or refractory cases, adjunctive immunosuppressive therapy may be required. These drugs may include cyclosporine, azathioprine, chlorambucil, dapsone, and sulfasalazine.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Bacterial / Fungal</th>
<th>Viral</th>
<th>Rickettsial / protozoal</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines</td>
<td>S. equi</td>
<td>FIP</td>
<td>RMSF</td>
<td>Paraneoplastic</td>
</tr>
<tr>
<td>Allergen immunotherapy</td>
<td>C. perfringens</td>
<td>Circovirus (pigs, dogs?)</td>
<td>Ehrlichia</td>
<td>Adverse food reaction</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>S. aureus</td>
<td>Equine viral arteritis</td>
<td>Anaplasma</td>
<td>Insect bite</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>S. (pseud)intermedius</td>
<td>Malignant catarrhal fever</td>
<td>Borrelia</td>
<td>SLE, DLE and rheumatoid arthritis</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>E. coli verotoxin</td>
<td>Toxoplasma</td>
<td>Genetic / familial</td>
<td></td>
</tr>
<tr>
<td>Carbamazone</td>
<td>Bartonella</td>
<td>Babesia</td>
<td>Photoaggravated/solar</td>
<td></td>
</tr>
<tr>
<td>Meloxicam</td>
<td>A. baumanii</td>
<td></td>
<td>Atopic dermatitis</td>
<td></td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>Histoplasma</td>
<td></td>
<td>Atherosclerosis and hypercholesterolemia</td>
<td></td>
</tr>
<tr>
<td>Cefalexin</td>
<td></td>
<td></td>
<td>Idiopathic</td>
<td></td>
</tr>
<tr>
<td>+/- Firocoxib, Deracoxib</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Numerous others</td>
<td></td>
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</tbody>
</table>
References:


CRYOSURGERY PART I: THE FUNDAMENTALS

Mike Canfield DVM, DACVD
Animal Dermatology South, 7741 Congress Street, New Port Richey, FL, 34653

HISTORY:
Cryosurgery is a surgical technique utilized to destroy undesirable tissues. Cryosurgery is relatively inexpensive, easy, and suitable for an outpatient practice. Therapeutic destruction of tissue by freezing began in England in 1845-1851.1-2 Liquid nitrogen use was thought to have occurred in 1948 when Allington recognized that its properties were similar to those of liquid air and oxygen.3

Cryosurgery is the most commonly performed dermatologic procedure in human medicine.4 Veterinary cryosurgery has celebrated its fourth decade yet the number of dermatologists offering this form of therapy appears quite limited. Some of the earlier veterinary works describe cryosurgical treatment of eyelid lesions, pannus, and glaucoma in dogs, and, sarcoïds, squamous cell carcinoma, and orthopedic conditions in horses.5 Cryosurgeons often hope that tumors frozen in situ will retain antigenicity or even enhanced antigenicity.

INDICATIONS:
Cryosurgery is suitable for many benign, some premalignant, and malignant conditions.6

CONTRAINDICATIONS:
Conditions where the patient has an abnormal reaction to cold such as, cryoglobulinemia and cryofibrinogenemia, are considered contraindications for cryosurgery.7

ADVANTAGES:
Cryosurgery has the potential to be performed without general anesthesia or sedation. It is a quick and easy procedure once the surgeon is adequately trained. After the initial investment of equipment is made, the supply cost is low, which makes it profitable. Sutures are not necessary.6-7

DISADVANTAGES:
Liquid nitrogen must be delivered and stored. Histopathology of the tissue may not be submitted. Hypopigmentation, leukotrichia, and lack of hair regrowth are possible cosmetic drawbacks for some clients. Although not as concerning of an issue for benign lesions, surgical margins cannot be accurately determined because deeper levels of tumor invasion are possibly left in situ.6-7

SIDE EFFECTS OF CRYOSURGERY:
- Burning and pain
- Scarring
- Headache
- Hypopigmentation or hyperpigmentation
- Neural damage
- Cartilaginous necrosis

CRYOGENS:

<table>
<thead>
<tr>
<th>Table 1 Principle Cryogens in Veterinary Cryosurgery</th>
</tr>
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<tbody>
<tr>
<td>Agent</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Liquid nitrogen</td>
</tr>
<tr>
<td>Nitrous oxide</td>
</tr>
</tbody>
</table>
CRYOBIOLOGY:
Cryobiology deals with the physical effects of low temperatures and the changing temperatures in living tissues. The tissue response to low temperature varies with the intensity of the cold induced. The effects of cryogenic injury can be divided into immediate and delayed effects. Immediate destruction results from mechanical damage due to ice formation. The delayed effects relate to vascular stasis, ischemia, and cell death.

Rapid freezing induces extracellular and intracellular ice formation, as there is insufficient time to allow water movement from the intracellular compartment to the extracellular compartment. Intracellular ice formation is thought to be lethal for the cell.

Slow thawing allows for recrystallization of the intracellular ice crystals, resulting in additional damage. Following the slow thaw process there is a brief period of vasodilation and increased vascular permeability. Capillary obstruction and vascular stasis occurs, resulting in tissue ischemia and death of the cell.8-9

CRYOIMMUNOLOGY:
Cryoablation can be immunostimulatory or immunosuppressive depending upon the mechanism of cell death that is induced. Thrombosis and ischemia occur within the cryoablated region, which results in necrosis. Pro-inflammatory cytokines and heat shock proteins are released from necrotic cells and activate the innate immune system. Along the periphery, many of the cells will undergo apoptosis, as the temperature is not cold enough to cause necrosis. The periphery communicates with viable tissue and wound repair begins as inflammatory cells and new blood vessels infiltrate the injured tissue. Apoptosis does not stimulate an immune response, as intracellular contents are not expelled from apoptotic cells.

Necrosis and apoptosis play a role in tumor cell death after cryoablation, thus an immune response may be elicited or suppressed, respectively. The amount of necrosis versus the amount of apoptosis varies upon the rate of freezing, number of freeze-thaw cycles, and size of the cryolesion.

Although there has been a renewed interest in cryoimmunology, there are many unanswered questions that will require additional research.10

References:
CRYOSURGERY PART II: THE CLINICAL APPLICATIONS & CASE REVIEWS

Mike Canfield DVM, DACVD
Animal Dermatology South, 7741 Congress Street, New Port Richey, FL, 34653

EQUIPMENT:
Due to the increased utility of liquid nitrogen as a cryogen and limited time, the focus will be on the application and use of liquid nitrogen based systems. Other equipment may have overlap in utility.

DEWAR:
Liquid nitrogen is stored in a Dewar. Dewars vary in their volume and storage time. The liquid nitrogen may be withdrawn using a ladle, valve system, or a withdrawal tube. The withdrawal tube is the most simple and efficient method. The lid or top does not form a seal, which is very important when the Dewar is moved from place to place. The storage time may vary significantly between Dewars so ask the manufacturer.

CRYOGUN:
The cryogun serves as a short term liquid nitrogen reservoir and user actuated application device. Various spray tips, probes, and closed cones are available. There are cryoguns, which incorporate video capture and surface temperature monitoring.

SPRAY TIPS:
Spray tips typically have a single orifice where liquid nitrogen spray is produced. In general, there is a proportional increase in the volume of liquid nitrogen spray produced as the diameter of the spray tip orifice increases.

CLOSED PROBES
Closed probes have solid ends and liquid nitrogen does not have direct contact with the patient. There is a tube attached to the probe to vent the excess nitrogen from the system.

CLOSED CONES:
Closed cones have a cup shape with an opening which is applied over the lesion. It is sealed against the skin and liquid nitrogen is deposited into the cone. Excess liquid nitrogen escapes through the attached tube.1-2

CRYOSURGERY PRINCIPLES

PREOPERATIVE HISTORY AND CONSIDERATIONS:
The type of lesion should be known. Once cryosurgery is considered as an acceptable form of treatment for the lesion; the surgeon should establish which technique is safe, efficient, and appropriate.3

TREATMENT CONSIDERATIONS:

FREEZE TIME:
The duration of cooling is based on the lesion. A benign lesion will require a short freeze time whereas a malignancy will require a longer freeze time. As the lesion size increases, the freeze time increases proportionately. Once the lesion is frozen, it should be allowed to thaw spontaneously. The thaw cycle will typically be two to three times longer than the freeze cycle.

LATERAL SPREAD OF FREEZE:
Lateral spread of freeze refers to the visible spread of the ice ball during the freeze cycle. The freeze cycle should be performed in a manner where the tissue is frozen as rapidly as safely feasible. The ice ball should be increased in size beyond the lesion. However, the distance beyond the lesion will vary.
based on the nature of the lesion. A benign lesion may only require two to three millimeters of lateral spread beyond the lesion. However, premalignant or malignant lesions require the surgeon to achieve additional lateral spread of the freeze, sometimes exceeding 5mm, or more, beyond the diameter of the lesion.

FREEZE-THAW CYCLE:

The freeze should occur as rapidly as safely possible. The thaw should be gradual and without assistance. As a general rule about 75% of the palpable ice ball dies. Although the freeze portion of the freeze-thaw cycle is important, the veterinary surgeon should bear in mind the importance of the thaw. Repetition of the freeze-thaw cycle is based upon the lesion type. For example, a skin tag typically requires only a single freeze-thaw cycle, whereas a malignant lesion may require three or more freeze-thaw cycles.

TEMPERATURE AT THE BASE OF THE TISSUE:

Two basic methods of tissue temperature monitoring are commonly utilized. Visualization with palpation is the predominant form of monitoring tissue temperature. A digital and visual assessment of the ice ball is made recognizing that only about 75% of the ice ball will slough. Thermocouples or pyrometers and infrared thermometers are more accurate means to monitor temperature. Thermocouple needles are placed at the peripheral margin and deep margin of the lesion.2

Table 1 Cryosurgical Events1

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Biological Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>+11 to +3</td>
<td>65% of capillaries and 35 to 40% of arterioles and venules develop thrombosis</td>
</tr>
<tr>
<td>-0.6</td>
<td>Freezing begins to occur in tissue</td>
</tr>
<tr>
<td>-4 to -7</td>
<td>Melanocytes die</td>
</tr>
<tr>
<td>-15 to -20</td>
<td>100% of blood vessels develop thrombosis</td>
</tr>
<tr>
<td>-20</td>
<td>Cells in sebaceous glands and hair follicles die</td>
</tr>
<tr>
<td>-21.8</td>
<td>Ice crystals form in the tissue</td>
</tr>
<tr>
<td>-20 to -30</td>
<td>Keratinocytes and malignant cells die</td>
</tr>
<tr>
<td>-30 to -35</td>
<td>Fibroblasts die</td>
</tr>
<tr>
<td>-50 to -60</td>
<td>All cells die including cartilage cells</td>
</tr>
</tbody>
</table>

CRYOSURGERY METHODS

LEARNING THE TECHNIQUES:

Prior to undertaking any form of cryosurgery, one should understand basic cryobiology, equipment, the various techniques for its use, and a thorough understanding of the condition being treated.

Table 2 Cell Sensitivity to Cryosurgery1,2

<table>
<thead>
<tr>
<th>Cell/Lesion Type (human)</th>
<th>Temperature for Destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanocyte</td>
<td>-5°C</td>
</tr>
<tr>
<td>Keratinocyte</td>
<td>-25°C</td>
</tr>
<tr>
<td>Benign lesion</td>
<td>-20 to -25°C</td>
</tr>
<tr>
<td>Malignant lesion</td>
<td>-50 to -60°C</td>
</tr>
</tbody>
</table>

DESCRIPTION OF TECHNIQUES

COTTON-TIPPED APPLICATOR TECHNIQUE:

Cotton-tipped applicators in a Styrofoam cup with liquid nitrogen can be utilized for some benign and premalignant lesions. The cotton-tipped applicator is removed from the liquid nitrogen and applied to
the lesion. The applicator size should be adjusted to match the diameter of the lesion. Temperatures achieved are not likely to get below -20°C\textsuperscript{1-2-4-5}.

**CRYOGUNS:**

Cryoguns are more efficient and effective. A cryogun is essentially a reservoir with a vented lid, fitted with a trigger actuated valve. Various accessories may be attached to the end of the cryogun. Once filled, the cryogun can be utilized to treat multiple lesions.\textsuperscript{1-2-4-5}

**OPEN SPRAY:**

The cryosurgical unit is utilized with variously sized spray tips held approximately one to two centimeters from the treatment site. Intermittent spray is applied to the lesion until the appropriate sized ice ball and temperature are achieved. This technique may result in excess collateral damage to normal tissue if the surgeon is less skilled.\textsuperscript{1-2-4-5}

**CONFINED SPRAY:**

A modification of the open spray technique wherein a cone is placed over the lesion and the liquid nitrogen spray is directed into the cone and onto the lesion which confines the spray more precisely. The duration of freeze time is the same as required by the open spray technique. The surgeon may at times be able to hasten the rate of freeze by applying pressure between the cone and the underlying skin to decrease vascular perfusion to the treatment area.\textsuperscript{1-2-4-5}

**CLOSED CONE:**

This technique involves the use of a closed cone attached to the cryosurgical unit. The closed cone diameter should be approximately the same diameter of the lesion. Upon actuation of the cryosurgical device’s trigger, liquid nitrogen enters the cone which is sealed against the skin. The pressure escapes the closed cone via a silicone rubber tube into the environment. The surgeon must be cognizant as to where this tube is directed as liquid nitrogen will escape from the end of the tube, possibly injuring the patient, surgeon, or nurse. An advantage of the closed cone method is that the freeze time is approximately half that of the spray technique.\textsuperscript{1-2-4-5}

**CLOSED PROBE:**

The closed probes have solid ends and as is the case with closed cones, there is a silicon tube which allows the excess pressure to escape. Closed probes are ideal in regions where more precise control is required. Closed probe diameter should closely match the lesion diameter and the surgeon must recognize that the freeze time will exceed that of other techniques. A benefit of this technique is that compressive force may be applied to the lesion.\textsuperscript{1-2-4-5}

**SPECIAL CIRCUMSTANCES:**

When proliferative or bulky lesions are to be treated, debulking with scalpel, scissors, laser, curette, or radiosurgery is necessary. Debubling is performed to improve visibility, uniform ice ball formation, and to improve access to the lesion.\textsuperscript{4}

Certain locations may require the injection of lidocaine subcutaneously to lift the lesion up and away from bone or superficial nerves. This application is particularly useful when treating digital or pinnal lesions.\textsuperscript{2}

**AFTER CARE:**

Typically, benign neglect is appropriate for postoperative cryosurgical sites. The owner's expectations of normal should be established. The lesion will develop swelling, erythema, and blistering. Then, changes will progress with the formation of an eschar, which subsequently sloughs over the following one to three weeks. Antibiotics are generally not indicated.\textsuperscript{3-6}

**APPLICATIONS:**

Cryosurgery may be used for benign skin tumors such as sebaceous adenomas and perianal adenomas, low-grade tumors of the oral cavity, and tumors of the eyelid including squamous cell carcinomas. It may be valuable for inoperable tumors or for palliative therapy.\textsuperscript{3-6}
LESIONS THAT SHOULD NOT BE FROZEN:
Osteosarcoma of long bones, circumferential freezing of any body orifice, mast cell tumors greater than one centimeter, or large aggressive tumors should not be treated with cryosurgery.¹

CONCLUSION:
Cryosurgery is nothing new, maybe it is new to you and if it is, I hope that I have provided you the basic understanding and encouragement to explore its possibilities for your patients.

References:
UNDERSTANDING THE EPIDEMIOLOGY OF ENVIRONMENTAL
METHICILLIN-RESISTANT STAPHYLOCOCCI IN A
DERMATOLOGICAL SERVICE.

Hoet AE1,2, van Balen JC1

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2. Division of Epidemiology, College of Public Health, The Ohio State University, 1920 Coffey Rd,
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Methicillin-resistant Staphylococci (MRS) are emerging disease-causing agents most commonly associated with severe skin, ear, and wound infections in dogs. Among them, methicillin-resistant *S. aureus* (MRSA) is considered an important zoonotic pathogen, and methicillin-resistant *S. pseudintermedius* (MRSP) has become a multidrug resistant agent. Although MRSA and MRSP infections are consistently recognized today, no long-term epidemiological studies analyzing these pathogens in veterinary settings have been performed. Over a 7-year period, our team has followed the prevalence of these pathogens on environmental contact surfaces to try to understand their epidemiology and ecology in a small animal veterinary hospital, including the Dermatology service. The main focus of this talk would be to share the epidemiological data generated until now in regards to these pathogens with increase detail on MRSA due to its occupational safety and public health importance.

BACKGROUND

*S. pseudintermedius* is part of the normal flora present in dogs and cats, and it has been isolated from several anatomical locations like the nares, mouth, groin and perianal area of healthy animals 4,6,13-16. On the other hand, *S. aureus* is not considered a common bacterium colonizing dogs, but still can be isolated from the same anatomical locations highlighted above. Since *S. pseudintermedius* was first described over 30 years ago 17, the majority of the isolates were susceptible to beta-lactam antibiotics 6. However, during the last decade, an increasing trend has been documented with isolates resistant not only to beta-lactams but to other classes of antimicrobials as well 1,2,6,18,19.

Different animal species and humans can be colonized (presence of the bacterium with no clinical sings or implications), infected (causing disease and clinical manifestations) or contaminated (presence of the bacteria in skin and/or nose but it is easily washed off) with MRS. The MRSP prevalence has been reported to range between 0 – 4.5% depending on the populations of healthy dogs sampled (community dogs vs. admitted dogs in veterinary hospitals), and from 0 – 7% in dogs with skin health problems 1. Nevertheless, in some populations the prevalence of MRSP can be as high as 30-90% 9,20. Most importantly, MRSP is known to be an opportunistic pathogen capable of causing major health problems for dogs, especially severe skin and ear infections 1-8. Evidence of this was revealed in a study performed in China, which reported that 12.7% of dogs with pyoderma were positive for MRSP 21. Other studies have found the presence of this pathogen not only in skin clinical specimens, but also producing illnesses in the urogenital tract, auditory channel, gastrointestinal tract, as well as producing systemic infections, among others pathologies 8. Likewise, the prevalence of MRSA in dogs depends very heavily on the population studied and their geographical location. In any case, their prevalence has been reported from 0 to 9%, with a 5.7% observed in incoming patients visiting our tertiary health care hospital.

Methicillin-resistant Staphylococci are considered opportunistic pathogens capable of causing major health problems and severe infections that are empirically hard to treat. While bacterial infections can occur in any breed, certain characteristics predispose an individual to the development of skin ailments. Such is the case of breeds with skin folds (e.g. Pugs, English Bulldog and Pekingese), dogs with pressure calluses (often seen in short-coated breeds), thick-coated longhaired breeds which are prone to pyotraumatic dermatitis
(e.g. Golden Retrievers, Saint Bernard), and German Shepherds (known to be prone to a specific form of pyoderma).

During the clinical management of MRS infections, the possibility of treatment failure is heightened due to the increased prevalence of multidrug resistant (MDR). This multidrug resistance manifested by MRS is explained by the fact that the mobile genetic element that confers the resistance to beta-lactams and cephalosporins (the mecA gene) is capable of acquiring other genes that provides resistance to other antimicrobial drugs. In Europe and North America, high percentages of resistance to erythromycin, clindamycin, trimethoprim, ciprofloxacin, streptomycin and kanamycin have been described ². An example of the negative effect of MDR MRS infections was described in a study in which over 45% of dogs empirically treated for MRSP pyoderma were still infected or carrying the pathogen after antimicrobial therapy was applied, indicating a clear failure of the treatment plan ¹⁰. This increasing trend of resistance against the most commonly used antimicrobials generates concern and must be considered when selecting treatments against MRS infections.

MRS have also been reported as a probable cause of nosocomial infections in veterinary hospitals, especially in cases in which the patient underwent a surgical procedure and/or were hospitalized ¹¹,¹². Considering the close contact between patients, personnel, and the environment in these settings, cross-contamination and circulation of MRS is likely to occur among them within the hospital. This represents a major challenge for the control and prevention of MRS in veterinary hospitals from two different perspectives. First, contaminated contact surfaces within the hospital denote an increased risk for patients to develop nosocomial infections during their stay, especially in the case of MRSP. Second, colonized and/or infected patients may introduce MDR MRS into these settings with each visit, contaminating the environment and exposing other patients. Additionally, occupational exposure of the hospital personnel is also a matter of concern, especially since some of these pathogens (e.g. MRSA) are zoonotically transmitted.

Considering that MRS infections typically require longer treatments involving several rounds of antimicrobials, it is safe to assume that the financial burden incurred by pet owners or the affected individual will be of greater impact. However, some consequences cannot be measured. Such is the case with the emotional impact that these infections have on owners, family members and/or the individual. This in turn highlights the importance of improving our understanding of the nature, pathogenic potential, and evolution of infectious agents like MRS in our veterinary clinics.

Even though MRS carriage and infections have been described within canine populations in various countries ¹,⁶,²², presently no longitudinal studies have been performed with regards to the distribution and evolution of MRS over time in a veterinary hospital, especially in a dermatological clinical service. Comparisons have been made between results reported from different clinical studies ²,²³. However, since the occurrence of MRS can be affected by the study’s population, design, and geographical location, associations can only be made up to a certain point. The elucidation of this information will help in the control and prevention of this significant opportunistic and nosocomial pathogen responsible for a variety of ailments affecting the canine population.

MRS SURVEILLANCE.

In 2007, every month during one year, an active surveillance was performed at The Ohio State University – Veterinary Medical Center (OSU-VMC) companion animal hospital where environmental contact surfaces and incoming canines from Community Practice, Dermatology, Intensive Care Unit (ICU) and Surgery were screened for the presence of MRS ²⁴. A total of 435 dogs were screened from all four services and 11.7% (51/435) and 5.7% (25/435) of them were positive for MRSP and MRSA respectively. At least 7.8% (4/51) and 16% (4/25) of MRSP and MRSA positive canines were considered healthy dogs with no medical issues either upon arrival or departure from the hospital, demonstrating that healthy dogs can also carry
MRS. In addition, it was observed that 10.7% (61/569) and 13.5% (77/569) of the environmental surfaces from all four targeted hospital services were contaminated with MRSP and MRSA respectively 24.

In particular, 145 dogs were sampled from the Dermatology service and 13.1% (19/145) were positive for MRSP and 3.4% (5/145) for MRSA 25. Moreover, compared to the other three services, Dermatology had the highest number of MRSP positive dogs (37.3%, 19/51). This bacterium was isolated from 36.8% (7/19) of the dog’s noses, 47.4% (9/19) of the perianal area and 42.1% (8/19) of their ears. From the MRSP positive dogs detected in Dermatology, 68.4% (13/19) had skin lesions at the time of sampling, and 61.5% (8/13) of these lesions were positive for MRSP. From the MRSA positive dogs detected in the same service, 80.0% (4/5) had skin lesions at the time of sampling, and 50.0% (2/4) of these lesions were positive for MRSA.

Regarding the environmental contamination, 134 total contact surfaces were sampled in the Dermatology service, and it was observed that 13.4% (18/134) of them were contaminated with MRSP. In addition, of the 61 MRSP positive surfaces found throughout the hospital, 29.5% (18/61) was represented by surfaces present in Dermatology, prevalence that was matched by Surgery (29.5%, 18/61) but was significantly different from Community Practice (16.4%, 10/61) and ICU (14.8%, 9/61). The monthly MRSP prevalence in Dermatology fluctuated from 0% to 46.2% over the 12 months, where the top three contact surfaces contaminated were: examination lamps (33.3%, 4/12), computers (33.3%, 4/12) and examination floors (16.7%, 4/24).

In regards to MRSA environmental contamination, 6.7% (9/134) of the contact surfaces sampled in Dermatology were contaminated with MRSA, which was significantly lower compared to Community Practice (21.3%, 13/61), ICU (13.4%, 13/97) and Surgery (11.1%, 27/243). During the year, the monthly MRSA environmental prevalence in Dermatology ranged from 0% to 18.2%. Of the twelve contact surfaces sampled in Dermatology, only two were positive for MRSA: floors (33.35, 8/24) and paper towels/alcohol gels dispensers (8.3%, 1/12).

Since 2009, after the one year surveillance study to establish the MRS contamination baseline, the same environmental surfaces at the hospital have been sampled quarterly, with MRS prevalence in the Dermatology service ranging from 0.0 to 50.0% over the years. This clearly shows that this group of pathogens is present and widely distributed throughout several contact surfaces of the Dermatology ward on a regular basis, which is not surprising due to the type of clients they work on a routine basis.

Parallel to the active surveillance, between 2007 and 2013, over 500 MRS isolates were obtained from canine clinical cases at the OSU-VMC, most of them related to infections present in skin/mucosa, the ears and wound/incisions; and at least 80% of them are from dermatological cases 26. Although the majority of them have not been fully characterized and studied due to the lack of external funding sources, MRS clinical isolates obtained passively between February 2007 and December 2010, showed high MDR. Besides the expected resistance to beta-lactams, over 50% of these isolates were resistant to lincosamides, fluoroquinolones and trimethoprim sulfamethoxazole. In the case of MRSP, the majority of these isolates carried SCCmec types V (VII), II-III and IV; in contrast, the majority of the MRSA isolates were SCCmec type II. The PFGE band pattern analysis showed that the MRSP isolates were highly diverse overall but with genotypic similarities, which in the case of MRSA it was very little diversity observed with major cluster grouping the majority of the strains.

Currently, hundreds MRS isolates from the environment and incoming canines obtained from the hospital active surveillance have been banked. Unfortunately, because of lack of funding sources to specifically support the study of MRS, further phenotypic and genotypic (molecular) characterization of these isolates has not been extensively performed. Without this information, it is nearly impossible to properly understand the circulation, maintenance, transmission, and pathogenicity potential of MRS (or any other microorganism), preventing us from fully understanding its epidemiology and limiting our efforts to prevent and control it.
Finally, a short description of disinfection procedures used in the different surfaces that we have observed to be frequently contaminated with Staphylococci is provided in a recently published book chapter 27.

In conclusion, our surveillance has confirmed that MRS are frequently found in two (animal and environment) of the three (animal, environment and human) components involved in the transmission and dissemination of MRS in veterinary hospitals. It is very important to fully characterized all these isolates, so we can understand their evolution and ecology (i.e. distribution, maintenance and movement), including their pathogenicity potential, possible epidemiological origin and relatedness. This information is essential to adjust current antimicrobial protocols and stewardship practices designed to treat MRS infections, to improve cleaning and disinfection protocols, as well as decrease and prevent nosocomial and occupational transmission of these pathogens in veterinary settings.

REFERENCES

POSTER
COMMUNICATIONS
Berger  Pseudorabies virus in a dog subsequent to hunting wild hogs in the Southeast United States

Falk  Use of ciclosporin-modified (Atopica®) to treat atopic dermatitis in a miniature donkey

Loft  Feline idiopathic ulcerative dermatosis treated successfully with oclacitinib

Loft  Meticillin-resistant Staphylococcus organisms at a tertiary referral veterinary hospital in New England (2011-2014)

Martin-Vo  *In vitro* antimicrobial activity of a product range containing plant antimicrobials against *Staphylococcus pseudintermedius* and *Malassezia pachydermatis*

Paulo  Evaluation of the effectiveness of an allergen modulating solution on minimization of Can f 1 concentration in the haircoat of dogs (*Canis lupus familiaris*)

Paulo  Influence of coat length, gender and reproductive status on the concentration Can f 1 in coat of dogs (*Canis lupus familiaris*)

Plant  Validation of a smartphone app (Itchology®) for scoring owner-assessed pruritus severity in dogs

Possebom  Sebaceous adenitis in a cat

Udenberg  Efficacy of 0.011% topical hypochlorous acid for the treatment of canine superficial pyoderma
Pseudorabies virus in a dog subsequent to hunting wild hogs in the Southeast United States

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Abstract: A 6-year-old intact male Plott hound residing in Pine Hill, Alabama was presented for acute periocular swelling and intense pruritus, with a history of hunting wild hogs. Physical examination revealed marked right-sided periocular swelling, facial alopecia, erythema, and excoriations. Initial treatment consisted of symptomatic and supportive care, however, the patient’s condition deteriorated quickly and sedation was utilized in an attempt to control his maniacal facial pruritus. Despite therapeutic interventions, the patient progressed to a comatose state and passed away within 48 hours of initial presentation. The dog was necropsied and sections of brain, liver, spleen and tonsil were submitted for further evaluation. Histopathologic examination revealed moderate lymphoplasmacytic encephalitis with numerous intranuclear eosinophilic inclusions, multifocal gliosis and neuron necrosis. The brain tissue was positive for pseudorabies virus (PRV) by real-time PCR with a cycle threshold value of 31. PRV was further isolated from the brain tissue in porcine kidney-15 cells as confirmed by immunofluorescence staining. Swine are the only natural host of PRV, although the virus may affect other wild and domestic animals including dogs. PRV was eradicated from U.S. commercial swine operations in 2004 but remains in some localized feral swine populations. This report highlights transmission of PRV from feral swine to a dog subsequent to exposure during hunting. Occurrences similar to the one reported here are likely under-reported due to the acute progression and fatal nature of the disease and may be encountered in a larger geographic range as PRV positive feral swine populations expand.

Source of funding: None

Conflict of interest: None declared
Use of ciclosporin-modified (Atopica®) to treat atopic dermatitis in a miniature donkey

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Abstract: Equine atopic dermatitis (EAD) is characterized by urticaria and/or pruritus, associated with the development of IgE antibodies to environmental allergens. Treatments for EAD include antihistamines, glucocorticoids, and allergen-specific immunotherapy. Here, we report a case of EAD that was successfully managed with ciclosporin-modified (Atopica®: Novartis Animal Health, Greensboro, NC). A six-year old intact female miniature donkey presented with a one week history of severe, refractory pruritus and urticaria progressing to ulcerative lesions on the face, trunk, and legs due to self-trauma. Historical seasonal urticaria was reported. Skin scrapings were negative for parasites. Impression smears revealed a neutrophilic exudate, with numerous cocci. Serum chemistry panel and complete blood count were unremarkable. Histopathology showed a marked, diffuse, ulcerative eosinophilic dermatitis with neutrophilic exocytosis and crusting with cocci. History, clinical presentation, and histopathology supported a diagnosis of EAD with secondary bacterial overgrowth. Initial improvement was seen with ceftiofur (Naxcel®: Zoetis, Florham Park, NJ) 2.2 mg/kg/12h intravenously, dexamethasone 0.05 mg/kg/24h intravenously and doxepin 0.6 mg/kg/12h per os. While the bacterial overgrowth resolved following completion of the ceftiofur, pruritus and self-trauma continued. Lack of complete resolution resulted in discontinuation of other medications; ciclosporin-modified 5 mg/kg/24h per os was initiated as sole therapy. Treatment was well tolerated. Clinical improvement was seen after two weeks, with complete remission after four weeks. Ciclosporin-modified was tapered to 5 mg/kg/48h and then to every 72h. The mare is currently maintained solely on ciclosporin-modified seven months later. This case report suggests that ciclosporin-modified may be a viable option for treating EAD.

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Conflict of Interest: None declared.
Feline idiopathic ulcerative dermatosis treated successfully with oclacitinib

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Abstract: Oclacitinib (Apoquel®, Zoetis, Florham Park, NJ) inhibits pruritogenic and pro-inflammatory cytokines involved in atopic dermatitis via inhibition of Janus kinase enzymes (JAK), especially JAK1 and JAK3. Feline idiopathic ulcerative dermatosis (FeIUD) is an uncommon, frustrating, automutilation condition of unknown etiology. The authors report a case of FeIUD that positively responded to oclacitinib and remained controlled. A 6.5 year old, 6.5 kg spayed female indoor domestic shorthair cat presented with a 3.5 year history of a non-seasonal automutilation ulcerative lesion over the right scapular area. Over a 2-year period of time, fungal infection was ruled out and the cat was treated for secondary bacterial infections, empirically treated for endo- and ecto-parasites, and completed multiple 12 week food elimination diet trials without significant improvement. Intradermal and serology testing for 100+ Northeastern allergens were negative. Surgical excision and histopathology of the non-healing wound revealed chronic ulcerative dermatitis; no infectious agents were observed including on special stains (PAS and GMS). The lesion recurred despite surgical excision. Symptomatic therapy with compounded oral gabapentin or gabapentin plus oral butorphanol resulted in no improvement. Pentoxifylline, tramadol and various oral steroids reduced the automutilation and resulted in only transient partial control. No improvement was noted with oral cyclosporine-modified (Atopica®, Novartis Animal Health, Greensboro, NC), anafranil, chlorambucil or amitriptyline. Complete clinical resolution of the FeIUD was gradually achieved after 4-6 weeks of oclacitinib (1-1.5mg/kg q24h); the patient remains free of signs of FeIUD for at least 2 months. Oclacitinib may be considered a treatment option for cats with FeIUD.

Source of funding: self-funded

Conflicts of interest: none
Meticillin-resistant Staphylococcus organisms at a tertiary referral veterinary hospital in New England (2011-2014)

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Abstract: Staphylococci are a leading cause of nosocomial and community acquired infections worldwide. *Meticillin-resistant Staphylococcus aureus* (MRSA) is of increasing public health significance, whereas *Staphylococcus pseudintermedius* (MRSP) and *Staphylococcus schleiferi* (MRSS) are more significant in veterinary medicine. This retrospective study aims to show the incidence of meticillin-resistant Staphylococcus positive (MRS+) cultures at Angell Animal Medical Center (AAMC) laboratory. The inclusion criteria for this study were limited to samples submitted by AAMC veterinarians from January 2011 through November 2014 within the general hospital population (n=101231). Cultures were performed according to national laboratory standard guidelines; MRSA isolates were further identified using oxoid penicillin binding protein latex agglutination testing. MRSS, MRSP or MRSA were isolated in 130 samples. A total of 118 dogs and nine cats had positive cultures (incidence 0.13%, no statistical difference between years p≤0.05). Sterilized animals accounted for 85% of the MRS+ patients consistent with the general hospital population. Spaniels, pugs and retrievers were the most prevalent groups of breeds, but also represent the most common breeds seen at AAMC. Of the 130 MRS+ cultures, MRSA, MRSS, MRSP and other MRS+ species were isolated in 6.92% (n=9), 37.7% (n=49), 50% (n=65) and 5.28% (n=7), respectively. Both MRSP and MRSS were more commonly cultured than MRSA, which is consistent with findings in previous literature. The low overall incidence of MRS+ is likely due the strict inclusion criteria; further studies to investigate risk factors among the different specialty services is warranted to encourage awareness and devise treatment strategies of these organisms.

Conflict: Self-funded
In vitro antimicrobial activity of a product range containing plant antimicrobials against Staphylococcus pseudintermedius and Malassezia pachydermatis

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Abstract: The aim of this study was to evaluate the antimicrobial potential of 3 commercial preparations (PYOclean Oto, PYOclean wipes and PYOclean Shampoo, Dermoscent®/LDCA, France) containing essential oils and specific plant extracts against Staphylococcus pseudintermedius (CIP 108864T) and Malassezia pachydermatis (IP 1649.86). The Minimum Inhibitory Concentrations (MIC) were determined by using the microbroth dilution technique and the Minimum Bactericidal or Fungicidal Concentrations (MBC or MFC) were obtained through subculturing on agar media by using a Denley multipoint inoculator. Positive controls containing the same tryptone salt dilution of each microorganism culture and growth medium and negative controls containing growth medium and the test product were included. S. pseudintermedius was cultured aerobically on Muller Hinton broth or agar at 37°C and M. pachydermatis anaerobically on modified Dixon broth or agar at 32.5°C. For S. pseudintermedius, the MIC was the 1/1024 dilution for the 3 products and the MBC was the 1/1024 dilution for PYOclean Oto and PYOclean Shampoo and 1/512 for PYOclean wipes. The MIC for M. pachydermatis were the 1/4 dilution of the product for PYOclean Oto, 1/128 for PYOclean Shampoo and 1/16 for PYOclean wipes. The MFC for M. pachydermatis were equal to the MIC for PYOclean wipes and PYOclean Shampoo and ½ dilution for PYOclean Oto. These preliminary in vitro results suggest that the test products could be useful in association with conventional antimicrobial therapy for the treatment of pyoderma, Malassezia dermatitis or otitis externa in dogs. Clinical trials are necessary to confirm the obtained outcomes.

Source of funding: LDCA, Castres, France

Conflict of interest: C. Martin Vo is employed by LDCA
Evaluation of the effectiveness of an allergen modulating solution on minimization of Can f 1 concentration in the haircoat of dogs (Canis lupus familiaris)

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Abstract: Can f 1 is the major allergen from dog epithelium with adhesive properties and is easily found in the environment. This study aimed to evaluate the effectiveness of a solution of allantoin, panthenol and aloe vera gel in a collagen base, to minimize the Can f 1 concentration in the haircoat of dogs. Eighty healthy dogs, greater than one year of age, were enrolled. The modulatory solution was applied to 40 dogs (Group 1) and water was applied to 40 dogs (Group 2). Samples from the haircoat were collected through vacuuming, before (T0) and then one hour and one week after applying the modulatory solution or water to Group 1 and Group 2 dogs, respectively. Can f 1 levels from each sample were analyzed by enzyme-linked immunosorbent assay using anti-Can f 1 (Indoor Biotechnologies, Inc, Charlottesville, VA, USA). All data were analyzed by ANOVA and Bonferroni method, with significance level of 5% (p ≤ 0.05). There was no significant reduction in the mean concentration of Can f 1 in the haircoat of dogs at one hour (1.14 μg.g⁻¹) (p ≥ 0.05) and one week (1.24 μg.g⁻¹) (p ≥ 0.05) after solution application. In conclusion, the solution evaluated in this study did not reduce the concentration of Can f 1 in the coat of the dogs.

Conflict of interest: none

Source of funding: Araucária Foundation of the state of Paraná
Influence of coat length, gender and reproductive status on the concentration Can f 1 in coat of dogs (Canis lupus familiaris)

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Abstract: The allergens from cats and dogs have been implicated as extrinsic factors involved in sensitization, precipitation and exacerbation of allergic rhinitis and asthma in susceptible children and adults, at rates ranging 10-25%. The major allergen from the epithelium of dogs is Can f 1. This study aimed to evaluate the frequency and the average concentration of Can f 1 in the coat of dogs, and to determine if there is an influence of coat length, gender and reproductive state on concentrations of this allergen. Eighty healthy dogs, 40 males (20 castrated and 20 intact males) and 40 females (20 spayed and 20 intact females), greater than one year of age, of any breed, and ectoparasite free, were enrolled. For sample collection, Dustream filters (Indoor Biotechnologies, Charlottesville, VA, USA) were used. Can f 1 levels were determined by enzyme-linked immunosorbent assay. Anti-Can f 1 (Indoor Biotechnologies, Charlottesville, VA, USA) was used for quantification. All data were analyzed by t-student method, with p ≤ 0.05. There was no significant difference between females (1.34 ±0.11 μg.g-1) and males (1.15 ±0.11 μg.g-1)(p ≥ 0.05), and there was no significant difference between females (1.28 ±0.73μg.g-1) and castrated males (1.22 ±0.61μg.g-1), and females (1,36 ± 0.08 μg.g-1) and intact males (1,11± 0,09 μg.g-1) (p≥ 0.05). There was also no significant difference between long coated (1.28 ±0.62μg.g-1) and short coated dogs (1.33 ±0.42 μg.g-1). In conclusion, there was no significant difference in Can f 1 concentration with respect to gender, reproductive status and coat length in dogs.

Conflict of interest: none

Source of funding: Araucária Foundation of the state of Paraná
Validation of a smartphone app (Itchology®) for scoring owner-assessed pruritus severity in dogs

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Abstract: Veterinarians rely on pet owners to describe their dogs’ pruritus severity when taking a dermatological history. A smartphone app (Itchology®: Pet Health Apps, Lake Oswego, OR) was developed to facilitate daily pruritus scoring and graphing by pet owners. With a vertical slider, the user selects a numerical value in one-tenth increments (0.0-10.0) while scrolling through six corresponding behavior descriptions. The objective of this study was to evaluate the agreement between pruritus severity recorded with the Itchology app (ItchologyPS) and the previously validated canine pruritus severity scale (CPSS). The pruritus severity of 50 dogs presented consecutively to a veterinary dermatology referral practice was scored by consenting pet owners with Itchology (v0.1, iPhone® 5s) and the CPSS. Both scorings were performed in random order at the beginning of a consultation, and then repeated after 15-20 min. Agreement between ItchologyPS and CPSS was evaluated with the Bland-Altman method. Reproducibility was analyzed with a variance components analysis. Comparing owners’ scores for ItchologyPS to CPSS, the bias was 0.1 points and the precision 0.8 points. The intra-class correlation coefficients were 0.82 and 0.88 for ItchologyPS and CPSS, respectively. Taken together, these results support the Itchology app as a valid and reproducible tool for owner scoring of dogs’ pruritus severity.

This study was self-funded.

Conflict of interest: J. Plant is the President of Pet Health Apps, Inc.
Sebaceous adenitis in a cat

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Abstract: Sebaceous adenitis (SA) is an inflammatory, dyskeratotic, and chronic disorder, characterized by the degeneration and post-inflammatory atrophy of sebaceous glands, which rarely affects cats. The dermatological signs commonly found include non-pruritic scaling, crusting and alopecia in regions of the face, neck and trunk. This report describes sebaceous adenitis in a twelve year old, spayed female domestic shorthair cat who presented with a one month history of dyskeratosis, xerosis, comedones, follicular casting, chronic otitis, progressive hypotrichosis, alopecia and moderate to intense pruritus of the dorsal thorax, limbs and face. Trichogram, fungal culture, sticky-tape cytologies, skin scrapings, parasitologic assessments of cerumen were negatives. No improvement was noted after feeding a novel protein elimination diet trial for 10 weeks. Histopathological examination revealed hair follicles in all stages of development, with hyperkeratosis and cystic dilation of some hairs and the complete absence of sebaceous glands. A mild inflammatory infiltrate composed of lymphocytes, histiocytes and neutrophils was noted in the periadnexal region. Based on these findings, a diagnosis of SA was made. The cat was treated with weekly Epi-Soothe® shampoo and Allerderm spot-on® (both Virbac. Fort Worth, Texas, USA) and ciclosporin (Sandimmun Neoral®, Novartis AG, Basileia, Suíça) (5 mg/kg/once daily per os). After two months of treatment, the cat was clinically normal, without otitis, alopecia and pruritus; the SA remained in remission for one year; the patient was then lost to follow up. Although rare, SA should be a differential diagnosis in pruritic cats with clinical and histological changes similar to the cat described herein.

Source of funding: Self-funded.

Conflict of interest: None declared.
Efficacy of 0.011% topical hypochlorous acid for the treatment of canine superficial pyoderma

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Abstract: Canine superficial pyoderma due to methicillin resistant Staphylococcus pseudintermedius is an increasing problem. Topical therapy is often sought as an alternative treatment. Hypochlorous acid (HOCl) is a reactive oxygen species produced during oxidative burst within neutrophils. A non-irritating, pH neutral, 0.011% HOCl solution (Vetericyn VF, Innovacyn, Rialto, CA) has previously demonstrated in vitro antimicrobial activity. This activity has been attributed to an irreversible reaction between HOCl, sulfur and heme-containing membrane enzymes as well as bacterial structural proteins. The objective of this randomized, double blinded, placebo controlled study was to evaluate the efficacy of HOCl in the treatment of canine superficial pyoderma. Nineteen client-owned dogs with superficial pyoderma were enrolled based on lesion score (from 1-64) and polymorphonuclear leukocytes (PMNs) and intracellular cocci (ICC) demonstrated on cytology. Patients were sprayed with saline (11/19) or HOCl (8/19), dosed at 0.09mL/cm² of lesion twice daily for 4 weeks. Regression analysis calculated the baseline-adjusted outcome differences between saline and HOCl groups regarding lesion score, PMN count, and ICC count (p<0.05). Adjusted differences between saline and HOCl groups (95% confidence intervals) were: lesion score 1.6 (-2.9, 6.1), PMN count 1.7 (-3.6, 7.0), and ICC count 0.8 (-1.9, 3.6). Eight dogs (4/11 saline, 4/8 HOCl) were withdrawn from the study due to lack of improvement. A statistically significant difference was not detected between groups for any parameter. Larger studies are required to draw definitive conclusions about the efficacy of HOCl in treating canine superficial pyoderma.

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Conflict of interest: 0.011% HOCl solution was donated by the manufacturer
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