# 28th PROCEEDINGS
# OF THE
# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
## Phoenix, Arizona
## April 9-12, 2014

Contents Page

# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
## Phoenix, AZ

**REGISTRATION HOURS**

| | | |
|---|---|---|
| Wednesday, April 9 | ..... | 5:00pm - 7:00pm |
| Thursday, April 10 | ..... | 7:00am - 5:00pm |
| Friday, April 11 | ..... | 7:30am - 5:00pm |
| Saturday, April 12 | ..... | 7:30am - 5:00pm |

**EXHIBIT AND POSTER HOURS**

| | | |
|---|---|---|
| Wednesday, April 9 | ..... | 3:00pm - 7:00pm (Set Up Only) |
| Thursday, April 10 | ..... | 8:30am - 4:00pm |
| Friday, April 11 | ..... | 8:30am - 4:00pm |
| Saturday, April 12 | ..... | 8:30am - 4:00pm |

# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
## Phoenix, AZ
## Wednesday, April 9, 2014

| | | | | |
|---|---|---|---|---|
| 8:00 am | - | 4:30 pm | **ACVD Residents' Education Forum**<br>*Sponsored by Zoetis* | Paradise Valley Room |
| | | 8:00 am | - 8:30 am BREAKFAST | |
| | | 8:30 am | - 10:00 am Cytokines - Dr. John Angus | |
| | | 10:00 am | - 10:30 am BREAK | |
| | | 10:30 am | - 12:00 pm Wound Healing - Dr. Colleen Mendelsohn | |
| | | 12:00 pm | - 1:00 pm LUNCH | |
| | | 1:00 pm | - 2:30 pm Exotic Medicine - Dr. Stephen White | |
| | | 2:30 pm | - 3:00 pm BREAK | |
| | | 3:00 pm | - 4:30 pm Histopathology Review - Dr. Emily Walder | |
| 8:00 am | - | 5:00 pm | **ACVD Exam Committee Meeting** | North Mountain Room |
| 8:00 am | - | 8:00 pm | **Cyber Café**<br>*Sponsored by Veterinary Information Network* | Cave Creek Room<br>(3rd Floor) |
| 8:00 am | - | 11:00 pm | **WAVD Administrative Committee** | Alhambra Room |
| 9:00 am | - | 12:00 pm | **NAVDF Organizing Committee Meeting** | South Mountain Room |
| 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** | Estrella Room |
| 12:00 pm | - | 5:00 pm | **AAVD Executive Board Meeting** | South Mountain Room |
| 2:00 pm | - | 5:00 pm | **ACVD AOK Committee Meeting** | Ahwatukee B |
| 3:00 pm | - | 7:00 pm | **Exhibitor and Poster Setup** | Phoenix Ballroom C |
| 3:00 pm | - | 3:30 pm | BREAK - For Board Meetings | |
| 5:00 pm | - | 7:00 pm | **Registration** | 3rd Floor Prefunction |
| 5:00 pm | - | 7:00 pm | **Welcome Reception**<br>*Sponsored by Hill's Pet Nutrition* | Phoenix Ballroom D/E |
| 6:00 pm | - | 8:00 pm | **NAVDF Program Committee Meeting** | South Mountain Room |

# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
## Phoenix, AZ
## Thursday, April 10, 2014

| | | | | |
|---|---|---|---|---|
| 6:00 am | - | 8:00 pm | **Cyber Café**<br>*Sponsored by Veterinary Information Network* | Cave Creek Room<br>(3rd Floor) |
| 7:00 am | - | 5:00 pm | **Registration** | 3rd Floor Prefunction |
| 6:30 am | - | 8:00 am | Roundtable Breakfast Buffet (coupon required) | Encanto Room |
| 7:30 am | - | 8:45 am | **ADVT Committee Meeting** (Committee members only) | Alhambra Room |
| 7:30 am | - | 8:45 am | **Roundtables** | |
| | | | #1 Client Compliance<br>J. Angus | Ahwatukee A |
| | | | #2 SLIT- A Game Changer?<br>D. Logas | Ahwatukee B |
| | | | #3 Pyoderma 2014<br>R. Garfield | Laveen A |
| | | | #4 Exotic Dermatology- Stylish or Frustrating?<br>A. Martin | Laveen B |
| | | | #5 Otic Gemish- For or Against?<br>M. Hall | South Mountain |
| | | | #6 Are there ABCs for ALD?<br>T. Tapp | Maryvale A |
| | | | #7 Pododermatitis<br>K. Irwin | Maryvale B |
| | | | #8 The Next Generation of Antifungals<br>B. Sakai | Estrella |
| 8:30 am | - | 4:00 pm | **Exhibits and Poster Sessions** | Phoenix Ballroom C |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 9:00 am | - | 10:30 am | ACVD Residents' Short Communications<br>*Sponsored by Elanco* | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 9:00 am | - | 9:45 am | Feline Dermatophytosis: Decontamination - The Evidence Shows It's Not as Hard as We Think<br>Dr. Karen A. Moriello | |
| 9:45 am | - | 10:30 am | Feline Dermatophytosis: Treatment- Getting to "Cured" with Less Smell and Mess<br>Dr. Karen A. Moriello | |
| 10:30 am | - | 11:00 am | BREAK | |

# Thursday, April 10, 2014, continued

| | | | | |
|---|---|---|---|---|
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 11:00 am | - | 12:00 pm | ACVD Residents' Short Communication<br>*Sponsored by Elanco* | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 11:00 am | - | 11:45 am | What to do When Feeling Blue: Understanding Feline Allergic Asthma<br>Dr. Carol Norris Reinero | |
| 11:45 am | - | 12:30 pm | What to do When Feeling Blue: Novel Treatments of Feline Allergic Asthma (ASIT and Beyond)<br>Dr. Carol Norris Reinero | |
| 12:30 pm | - | 2:00 pm | LUNCH On Your Own | |
| 12:30 pm | - | 2:00 pm | **ACVD Residency Mentors Meeting**<br>*Sponsored by Elanco* | Ahwatukee Room |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 2:00 pm | - | 3:30 pm | Research Short Communications<br>*Sponsored by Elanco* | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 2:00 pm | - | 2:45 pm | Meditations on Feline Allergic Dermatitis for the Practical-Minded Clinician<br>Dr. Jill L. Abraham<br>*Sponsored by Zoetis* | |
| 2:45 pm | - | 3:30 pm | Leishmaniasis Part 1 – The Challenge of the Diagnosis<br>Dr. Lluis Ferrer<br>*Sponsored by Zoetis* | |
| 3:30 pm | - | 4:00 pm | BREAK | |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 4:00 pm | - | 5:00 pm | Research Short Communications<br>*Sponsored by Elanco* | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 4:00 pm | - | 5:00 pm | Leishmaniasis Part 2 - New Tools for the Treatment and Prevention<br>Dr. Lluis Ferrer<br>*Sponsored by Zoetis* | |
| 5:15 pm | - | 6:45 pm | **ACVD Diplomates' Business Meeting** | Phoenix Ballroom D/E |
| 6:30 pm | | | **ACVD Residents' Dinner**<br>*Sponsored by Dechra Veterinary Products* | TBD |

## Thursday, April 10, 2014, continued

| | | | |
|---|---|---|---|
| 7:00 pm | | **ACVD Diplomates' Dinner**<br>*Sponsored by Bayer HealthCare LLC Animal Health* | Heard Museum<br>2301 North Central Avenue |
| 7:00 pm | - 12:00 am | **Reception**<br>**"Lucky Strike"**<br>*Sponsored by Virbac Animal Health* | Lucky Strike – 50 W. Jefferson Street |

# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
## Phoenix, AZ
## Friday, April 11, 2014

| | | | | | |
|---|---|---|---|---|---|
| 6:00 am | - | 8:00 pm | **Cyber Café**<br>*Sponsored by Veterinary Information Network* | | Cave Creek Room |
| 6:30 am | - | 8:00 am | Roundtable Breakfast Buffet (coupon required) | | Encanto Room |
| 7:30 am | - | 5:00 pm | **Registration** | | 3rd Floor Prefunction |
| 7:30 am | - | 8:45 am | **Roundtables** | | |
| | | | #9 | Dogma Dispelled & Other Pet Peeves in Dermatology<br>D. Vaughn | Ahwatukee A |
| | | | #10 | Developing & Enhancing Referral Relationships<br>J. Griffies | Ahwatukee B |
| | | | #11 | How do You Practice Infection Control in Your Clinic?<br>J. Pendergraft | Laveen A |
| | | | #12 | Rush or Mosey?-ASIT Protocols<br>D. Crow | Laveen B |
| | | | #13 | Equine ASIT<br>C. Rees | South Mountain |
| | | | #14 | Pruritus ani<br>S. Barlett | Maryvale A |
| | | | #15 | Itch/Lick or Obsession/Lick Cycle?<br>S. Weisglass | Maryvale B |
| | | | #16 | Technician Roundtable: Autoimmune Dermatoses<br>L. Reuter | Estrella |
| 8:30 am | - | 4:00 pm | **Exhibits and Poster Sessions** | | Phoenix Ballroom C |
| | | | **Scientific Session** | | Phoenix Ballroom D/E |
| 9:00 am | - | 10:30 am | Research Short Communications | | |
| | | | **Concurrent Session** | | Phoenix Ballroom A/B |
| 9:00 am | - | 9:45 am | Introduction to Social Media for Veterinary Specialty Practices<br>Lauren Spencer | | |
| 9:45 am | - | 10:30 am | Social Media Best Practice for Veterinary Specialty Practices<br>Lauren Spencer | | |
| 10:00 am | - | 12:00 pm | **WCVD8 EOC Meeting** | | Ahwatukee B Room |
| 10:30 am | - | 11:00 am | BREAK | | |

# Friday, April 11, 2014, continued

| | | | | |
|---|---|---|---|---|
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 11:00 am | - | 11:45 am | Aerobiology – Part I<br>Dr. Richard W. Weber | |
| 11:45 am | - | 12:30 pm | Aerobiology – Part II<br>Dr. Richard W. Weber | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 11:00 am | - | 11:45 am | Non-inflammatory Alopecia – It's Not Always Hormonal!<br>Dr. Linda A. Frank | |
| 11:45 am | - | 12:30 pm | What's So Atypical About Cushing's Syndrome?<br>Dr. Linda A. Frank | |
| 12:00 pm | - | 2:00 pm | **ACVD Ethics Committee Meeting** | Arcadia Room |
| 12:00 pm | - | 2:00 pm | **ICADA Meeting** | Ahwatukee A Room |
| 12:30 pm | - | 2:00 pm | LUNCH On Your Own | |
| 12:30 pm | - | 2:00 pm | **Canadian Academy of Veterinary Dermatology Committee Meeting** | South Mountain Room |
| 12:45 pm | - | 1:45 pm | **ACVD Website Committee Meeting** | Camelback B |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 2:00 pm | - | 2:45 pm | Solar Dermatosis in Companion Animals<br>Dr. Anthea Schick, Dr. Alexander Werner,<br>Dr. Kimberly S. Coyner | |
| 2:45 pm | - | 3:30 pm | Solar Dermatoses Of Humans: Selected Examples<br>Dr. Robert J. Pariser | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 2:00 pm | - | 2:45 pm | Ophthalmic Manifestations of Cutaneous Disease – Part I<br>Dr. J. Phillip Pickett | |
| 2:45 pm | - | 3:30 pm | Ophthalmic Manifestations of Cutaneous Disease – Part II<br>Dr. J. Phillip Pickett | |
| | | | **Technician Session** | Maryvale Room |
| 2:00 pm | - | 3:30 pm | Dermatophytosis: Trouble Shooting Diagnostics, Clinic Decontamination and Client Education<br>Dr. Karen Moriello | |
| 3:30 pm | - | 4:00 pm | BREAK | |

# Friday, April 11, 2014, continued

| | | | | |
|---|---|---|---|---|
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 4:00 pm | - | 4:45 pm | Skin Drug Allergy – Part I<br>Dr. Sidonie Lavergne | |
| 4:45 pm | - | 5:30 pm | Skin Drug Allergy – Part II<br>Dr. Sidonie Lavergne | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 4:00 pm | - | 4:45 pm | Our Patients Lick The Floor And .... Their Butt (The Role Of The Environment In Disease Transmission)<br>Dr. Jeff Bender | |
| 4:45 pm | - | 5:30 pm | Dermatology-Related Public Health Case Discussions<br>Dr. Jeff Bender | |
| | | | **Technician Session** | Maryvale Room |
| 4:00 pm | - | 4:45 pm | Dermatophytosis: Trouble Shooting Diagnostics, Clinic Decontamination and Client Education<br>Dr. Karen Moriello | |
| 6:30 pm | | | **Reception**<br>*Sponsored by Royal Canin Veterinary Diet* | Corona Ranch |

# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
## Phoenix, AZ
## Saturday, April 12, 2014

| | | | | |
|---|---|---|---|---|
| 6:00 am | - | 4:00 pm | **Cyber Café**<br>*Sponsored by Veterinary Information Network* | Cave Creek Room |
| 6:30 am | - | 8:00 am | Roundtable Breakfast Buffet (coupon required) | Prefunction outside South Mountain Room |
| 7:30 am | - | 5:00 pm | **Registration** | 3rd Floor Prefunction |
| 7:30 am | - | 8:45 am | **Technicians' Roundtable**<br>Dermatophytes<br>S. Grable | Estrella Room |
| 7:30 am | - | 8:45 am | **ACVD Residents' Roundtable** | Ahwatukee Room |
| 8:30 am | - | 4:00 pm | **Exhibits and Poster Sessions** | Phoenix Ballroom C |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 9:00 am | - | 10:30 am | Clinical Short Communications | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 9:00 am | - | 9:45 am | Tough Case Relay 1<br>Dr. Dana A. Liska & Dr. Valerie Fadok | |
| 9:45 am | - | 10:30 am | Tough Case Relay 2<br>Dr. Dana A. Liska & Dr. Valerie Fadok | |
| | | | **ISVD** | Encanto Room |
| 9:00 am | - | 9:45 am | Dermatopathology : Mystery Slides – Part I<br>Dr. Judith Nimmo | |
| 9:45 am | - | 10:30 am | Dermatopathology : Mystery Slides – Part II<br>Dr. Judith Nimmo | |
| 10: 30 am | - | 10:45 am | **ACVD Resident Research Awards**<br>*Sponsored by Bayer HealthCare LLC Animal Health* | Phoenix Ballroom D/E |
| | | | **ACVD Externship Grants**<br>*Sponsored by Hill's Pet Nutrition* | Phoenix Ballroom D/E |
| 10:30 am | - | 11:00 am | BREAK | |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 11:00 am | - | 12:30 pm | Clinical Short Communications | |

# Saturday, April 12, 2014 - continued

| | | | | |
|---|---|---|---|---|
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 11:00 am | - | 11:45 am | Steroids & Laminitis – Is the Hoof More Than Thick Skin?<br>Dr. Harold Schott II | |
| 11:45 am | - | 12:30 pm | Steroids & Laminitis – What is the Evidence?<br>Dr. Harold Schott II | |
| | | | **ISVD** | Encanto Room |
| 11:00 am | - | 12:30 pm | Correlation Of Histopathology And Clinical Presentation<br>Dr. Verena K. Affolter | |
| 12:30 pm | - | 2:00 pm | LUNCH On Your Own | |
| 12:30 pm | - | 2:00 pm | **AAVD Business Meeting – Lunch** | Phoenix Ballroom D/E |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 2:00 pm | - | 2:45 pm | Methicillin-Resistant Staphylococcal Infections<br>Dr. Meghan F. Davis | |
| 2:45 pm | - | 3:30 pm | Eqidemiology of MRS – Panel Discussion<br>Dr. Meghan F. Davis & Dr. Dan O. Morris | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 2:00 pm | - | 2:45 pm | Pathophysiologic Intersection of Pain and Itch<br>Dr. John H. Rossmeisl, Jr. | |
| 2:45 pm | - | 3:30 pm | Neuropathic Pain and Itch: Clinical Conundrums<br>Dr. John H. Rossmeisl, Jr. | |
| | | | **ISVD** | Encanto Room |
| 2:00 pm | - | 3:30 pm | Interface Dermatitis: An Immunologist's Point of View<br>Dr. Valerie Fadok | |
| 3:30 pm | - | 4:00 pm | BREAK | |
| | | | **Scientific Session** | Phoenix Ballroom D/E |
| 4:00 pm | - | 4:45 pm | Alternative Therapies in the Treatment of Human AD<br>Dr. Peter Lio | |
| 4:45 pm | - | 5:30 pm | The Bleeding, Crusting, and Oozing Edge: Updates in Human AD<br>Dr. Peter Lio | |
| | | | **Concurrent Session** | Phoenix Ballroom A/B |
| 4:00 pm | - | 4:45 pm | Radiotherapy for Neoplastic and Non-Neoplastic Disease<br>Dr. Michael A. Deveau | |
| 4:45 pm | - | 5:30 pm | Disorders of Pigmentation<br>Dr. Karen L. Campbell | |

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|---|---|---|---|---|
| | | | **ISVD** | Encanto Room |
| 4:00 pm | - | 4:45 pm | Autosomal Recessive Congenital Ichthyosis in American Bulldogs is Associated with Decreased Expression of ICHTHYIN (NIPAL4)<br>Dr. Elizabeth Mauldin | |
| 4:45 pm | - | 5:00 pm | Canine Epidermal Neural Crest Stem Cells – Characterization, Isolation and Expansion<br>Dr. Barbara Gericota | |
| 5:00 pm | - | 5:30 pm | Skin Microbiome<br>Dr. Aline Rodrigues Hoffmann | |
| TBD | | | **Reception**<br>*Sponsored by Novartis Animal Health US, Inc.* | TBD |

# NORTH AMERICAN VETERINARY DERMATOLOGY FORUM
# Phoenix, AZ

## Roundtable Sessions 2014

**Thursday, April 10, 2014**

| | | |
|---|---|---|
| #1 | **Client Compliance**<br>J. Angus | Ahwatukee A |
| #2 | **SLIT- A Game Changer?**<br>D. Logas | Ahwatukee B |
| #3 | **Pyoderma 2014**<br>R. Garfield | Laveen A |
| #4 | **Exotic Dermatology- Stylish or Frustrating?**<br>A. Martin | Laveen B |
| #5 | **Otic Gemish- For or Against?**<br>M. Hall | South Mountain |
| #6 | **Are there ABCs for ALD?**<br>T. Tapp | Maryvale A |
| #7 | **Pododermatitis**<br>K. Irwin | Maryvale B |
| #8 | **The Next Generation of Antifungals**<br>B. Sakai | Estrella |

**Friday, April 11, 2014**

| | | |
|---|---|---|
| #9 | **Dogma Dispelled & Other Pet Peeves in Dermatology**<br>D. Vaughn | Ahwatukee A |
| #10 | **Developing & Enhancing Referral Relationships**<br>J. Griffies | Ahwatukee B |
| #11 | **How do You Practice Infection Control in Your Clinic?**<br>J. Pendergraft | Laveen A |
| #12 | **Rush or Mosey?-ASIT Protocols**<br>D. Crow | Laveen B |
| #13 | **Equine ASIT**<br>C. Rees | South Mountain |
| #14 | **Pruritus ani**<br>S. Barlett | Maryvale A |
| #15 | **Itch/Lick or Obsession/Lick Cycle?**<br>S. Weisglass | Maryvale B |
| #16 | **Technician Roundtable: Autoimmune Dermatoses**<br>L. Reuter | Estrella |

**Saturday, April 12, 2014**

| | | |
|---|---|---|
| #17 | **Technicians' Roundtable**<br>**Dermatophytes**<br>S. Grable | Estrella Room |
| #18 | **Residents' Roundtable** | Ahwatukee Room |

# ABSTRACT PRESENTATIONS

## Thursday, April 10

**RESIDENTS**
9:00 D. Genovese – Sphynx cat
9:15 D. Kunder - MRSS
9:30 J. Short - CHPC
9:45 H. Roberts – Equine mites
10:00 H. Roberts – Equine mites IDAT
10:15 T. Udenberg – Quantitative cytology
BREAK

**RESIDENTS**
11:00 A. Stich – Laser pedal pruritus
11:15 A. Foster – Middle ear effusion
11:30 H. Edginton – Lymphocytes dogs/alpacas
11:45 E. Maina – Anal pruritus
12:00 None
12:15 None
LUNCH

**RESEARCH SHORT COMMUNICATIONS**
2:00 D. Santoro – Peptides
2:15 H. Kim – PAR2 & TSLP
2:30 R. Marsella – Caspase 14
2:45 R. Marsella – ZO-1 and Occludin
3:00 N. Sanchez – SC lipids
3:15 K. Iyori – Cefpodoxime

**RESEARCH SHORT COMMUNICATIONS**
4:00 K. Rook – T cell lymphoma
4:15 P. Little – Apoquel vs Pred
4:30 P. Little – Apoquel vs CsA
4:45 C. Lange – Papillomas

## Friday, April 11

**RESEARCH SHORT COMMUNICATIONS**
9:00 L. Ferrer – Cutaneous microbione
9:15 C. Hwang – Staph pyoderma
9:30 A. Detwiler – MRSP
9:45 A. Brazil – MRSA homes
10:00 M. Davis – MRSA pet's
10:15 M. Davis – MRSA owner decolonization
BREAK

## Saturday, April 12

**CLINICAL SHORT COMMUNICATIONS**
9:00 L. Gotthelf – TRI-726
9:15 C. Griffin – ASIT AE's
9:30 M. Rossi - MCLE
9:45 C. Cain – Dorsal furunculosis
10:00 C. Cain – Perianal fistulas
10:15 L. Ferrer – Perianal fistulas - stem cells

RESIDENT RESEARCH AWARDS 10:30-10:45

BREAK 10:45-11:00

**CLINICAL SHORT COMMUNICATIONS**
11:00 L. Frank – Cortisol Cushings
11:15 A. Diesel – Corynebacterium pseudoTB
11:30 A. Diesel – Prototheca
11:45 A. Gonzales – Pregabalin
12:00 R. Frank – Apoquel/Mastocytosis - cat
12:15 C. Navarro – Rilexine
LUNCH

## POSTERS

W. Collard – PK/RD oclacitinib
W. Craft – Plasma cell pododermatitis
A. Lam – IDAT/SAT agreement
E. Layne – Impression smear vs tape
K. Loft – Apocrine cysts - ears
C. Navarro – EasOtic

# THURSDAY

# ACVD RESIDENTS' SHORT COMMUNICATIONS THURSDAY

**NORTH AMERICAN VETERINARY DERMATOLOGY FORUM**
**Phoenix, AZ**

**THURSDAY, APRIL 10, 2014**

**ACVD RESIDENTS' SHORT COMMUNICATIONS**

| | | |
|---|---|---|
| 9:00 | Genovese | Histologic characterization of Sphynx cat skin |
| 9:15 | Kunder | Periodic selection of meticillin-resistant *Staphylococcus schleiferi* in the United States |
| 9:30 | Short | Adverse events associated with chloramphenicol use in dogs: a retrospective study (2007-2013) |
| 9:45 | Roberts | Establishment of the mite fauna from an equine stable in the Midwest of the United States |
| 10:00 | Roberts | Seasonal equine intradermal test threshold concentrations and allergenspecific serum IgE levels for mite allergens |
| 10:15 | Udenberg | Reproducibility of a quantitative cutaneous cytology technique |
| 10:30 - 11:00 | | BREAK |
| 11:00 | Stich | Clinical efficacy of low level laser therapy (LLLT) on localized CADESI-03 and PVAS scores in dogs with pedal pruritus from atopic dermatitis |
| 11:15 | Foster | Incidence of sub-clinical middle ear effusion in dogs diagnosed during routine computed tomography |
| 11:30 | Edginton | Resident lymphocytes in the dermis of normal dorsolateral thoracic skin of dogs and alpacas |
| 11:45 | Maina | Anal pruritus in dogs with skin disease |
| | | LUNCH on Your Own |

**Histologic characterization of Sphynx cat skin**

D. GENOVESE*, T. JOHNSON†, K. LAMB‡, D. GRAM§

*Animal Allergy and Dermatology, Virginia Beach, VA, USA
†IDEXX Laboratories, Inc., Westbrook, ME, USA
‡Lamb Consulting, West Saint Paul, MN, USA
§University of Florida College of Veterinary Medicine, Gainesville, FL, USA

**Abstract:** The alopecic Sphynx cat has a mutation in *KRT71*, which is expressed in the inner root sheath (IRS). Hair shaft and follicular abnormalities, including those of the IRS, have been demonstrated in murine *KRT71* mutants. The aim of this study was to characterize Sphynx cat skin histologically. Biopsy samples were collected from the thorax of 14 Sphynx cats and three normally coated control cats. Mean follicular density was similar for Sphynx and control cats (13.13/mm and 14.97/mm, respectively). Dermatoscopic images from Sphynx cats revealed similar coat density compared to six additional normally coated control cats. In Sphynx cats, follicles were often misshapen, slightly decreased in size, and the IRS demonstrated non-uniform thickness, large eosinophilic clumps, and foci that lacked staining. Infundibular hyperkeratosis and dilation were also common in Sphynx cats. Most Sphynx follicles to which a hair cycle stage could be assigned were in anagen. Anagen bulbs in Sphynx cats were occasionally dysplastic and reduced in diameter ($p < 0.0001$) compared to control cats (Least Squares (LS) means of 58.87 μm [SE ± 1.50] and 74.58 μm [SE ± 2.84], respectively). Hair shafts were present in most Sphynx follicles, but often misshapen and of reduced diameter ($p < 0.0001$) compared to control cats (LS means of 10.56 μm [SE ± 0.31] and 15.94 μm [SE ± 0.49], respectively). Few medullated hairs were noted in Sphynx cats. These findings demonstrate that Sphynx cats are affected by follicular dysplasia with abnormal shaft production and have histologic changes similar to murine *KRT71* mutants.

Source of funding: Self-funded

Conflicts of Interest: None declared

**Periodic selection of meticillin-resistant *Staphylococcus schleiferi* in the United States**

D. KUNDER*, C. CAIN*, S. C. RANKIN§

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**Abstract:** *Staphylococcus schleiferi* is a known pathogen that causes canine skin and ear infections. The aim of this study was to determine the molecular epidemiology and antimicrobial susceptibility of clinical veterinary isolates from different geographic regions in the United States. It was hypothesized that *S. schleiferi* would maintain genotypic homogeneity across the different geographic regions and that meticillin-resistant (MR) isolates of *S. schleiferi* would predominate. Isolates were identified as *S. schleiferi* by a commercial microbiology identification system and confirmed by *nuc* gene PCR. Antibiotic susceptibility data were collected and PBP2a latex agglutination testing was performed on MR-isolates. Pulsed-field gel electrophoresis (PFGE) was performed and clonal clusters were identified with a Dice coefficient similarity of > 80%. There were 116 isolates from the mid-Atlantic region and 101 from across the United States. Of these 217 isolates, 209 (96%) were obtained from cutaneous sites. Of the mid-Atlantic isolates, 62% (72/116) were MR and 16% (18/116) were multi-drug resistant (MDR). Of the isolates from the other geographic regions, 73% (74/101) were MR and 24% (24/101) were MDR. All MR isolates were positive by PBP2a latex agglutination. PFGE identified 155 individual pulsed- field profiles in two major clonal clusters (CCs). These two clusters were geographically heterogeneous. CC 2, which was previously shown to contain both meticillin-susceptible and resistant isolates in a 50:50 ratio, is now dominated by MR-isolates (86%). This study demonstrates the dissemination of a successful MR clone of *S. schleiferi* across the United States and the further evolution of multi-drug resistant strains.

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

**Adverse events associated with chloramphenicol use in dogs: a retrospective study (2007-2013)**

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**Abstract:** Chloramphenicol is a broad-spectrum antibiotic that has seen an increase in use with the emergence of meticillin-resistant staphylococcal infections. Because of toxicities in humans, namely aplastic anemia, the drug's use has been limited. In dogs, gastrointestinal signs are the more common adverse events described, and bone marrow suppression is possible. The aim of this study was to evaluate the adverse events associated with chloramphenicol in dogs from one dermatology practice from January 2007 to June 2013. The database was searched for all dogs prescribed chloramphenicol during the time period. Age, weight of the dogs, dose, and duration of treatment were recorded as well as any adverse events that occurred during treatment. A total of 105 cases were evaluated. The mean age, weight, dose, and duration of treatment were 7.6 years, 27 kg, 46.8mg/kg, and 50 days, respectively. Thirty-nine out of 105 dogs experienced at least one adverse event while on the medication. The most commonly noted adverse events were gastrointestinal in nature (46%) and hindlimb weakness (36%). The mean weight for dogs with hindlimb weakness was 35.3 kg, which was statistically significant. Age, dose and duration of treatment were not significant in relation to adverse events. Resolution of adverse events was documented in 54% of the cases after chloramphenicol was discontinued. Meticillin-resistant *Staphylococcus pseudintermedius* on bacterial culture was listed as the reason for chloramphenicol use in 76% of the cases. Based on this information, further prospective studies are recommended to evaluate the reproducibility of this report.

Source of funding: Self-funded

Conflict of interest: None declared

# Establishment of the mite fauna from an equine stable in the Midwest of the United States

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**Abstract:** The presence of house dust mites (HDMs) and storage mites (SMs) in the human environment has been established worldwide and both contribute to allergic disease in some individuals. *Dermatophagoides, Acarus, Tyrophagus* and *Lepidoglyphus* mite genera contribute to the pathogenesis of atopic disease. The mite stable fauna for the Midwestern region of the United States is currently unknown. The primary objective of this study was to characterize the mite fauna of a stable in this region across three seasons. Specialized mite traps and modified flotation methods were used to collect mites in spring, late summer and winter from nine locations on one farm. Selected locations for mite collection represented the three different stabling environments used, bedding types, feed materials or combinations thereof. A single-baited mite trap was placed at each of the locations for a four-day period (96 h), while 200 g of material was gathered from each site on the fourth day for flotation. An acarologist morphologically identified and quantified the species of HDMs and SMs collected. At least one mite from all four genera specific to this study was identified. *Tyrophagus* mites were the most prevalent with *Dermatophagoides* mites being the
least numerous. Collectively, *Oribatida*, *Cheyletus*, *Glycyphagus* and *Tarsonemidae* represented the majority of the genera detected. These results establish that horses stabled in the Midwestern United States are exposed to a diverse Acari population. Provocation and allergy testing of allergic horses with specific mite allergens would be necessary to determine the significance of these mites in relation to disease.

Funding: This study was supported by the Ohio State University Equine Intramural Funds, and VCA/Antech.

Conflicts of interest: none declared.

# Seasonal equine intradermal test threshold concentrations and allergen-specific serum IgE levels for mite allergens

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**Abstract:** House dust mite (HDM) and storage mite (SM) equine intradermal test (IDT) threshold concentrations (TCs) for the United States Midwest region are unknown. The objectives of this study were to determine IDT TCs for HDM and SM species and to quantify mite-specific IgE concentrations in thirty-eight clinically normal horses over two seasons. Subjective measurements of IDT reactions were used to determine the TCs. The reactions were scored using a scale of 0 to 4+. Allergen testing concentrations ranged from 1:320,000-1:5,000 w/v. Threshold concentrations were defined as the highest concentration of a mite allergen where ≤10% of horses had a positive subjective reaction (≥2+) at 15 min. Analysis of equine serum-specific IgE was performed using commercially available allergen-specific IgE ELISA test. Subjectively determined TCs were: 1:80,000 w/v for *Dermatophagoides farinae* in both seasons, 1:80,000 w/v in spring and 1:160,000 w/v in late summer for *Dermatophagoides pteronyssinus*, 1:40,000 w/v in spring and 1:20,000 w/v in late summer for *Acarus siro*, 1:20,000 w/v for *Lepidoglyphus destructor* in both seasons, and 1:20,000 w/v in spring and 1:10,000 w/v in late summer for *Tyrophagus putrescentiae*. In both seasons, at least one horse had a positive serum IgE result for each HDM or SM evaluated. Negative serum IgE concentrations for all mite species were present in 55% of horses in spring and 66% in late summer. The determined TCs from our study differ from published recommendations for equine HDM and SM IDT dilution concentrations, suggesting the need to consider seasonal and regional influences on IDT TCs.

Funding: This study was supported by the Ohio State University Equine Intramural Funds, VCA/Antech and HESKA®.

Conflicts of interest: none declared.

**Reproducibility of a quantitative cutaneous cytology technique**

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**Abstract:** Cutaneous cytology is a valuable tool for diagnosis and monitoring of canine superficial pyoderma. Current published techniques are semiquantitative. The aim of this study was to evaluate the reproducibility of a quantitative methodology. Impression smears were collected from five normal dogs and 20 dogs with clinical and cytological evidence of superficial pyoderma. Four investigators evaluated 10 oil immersion fields (OIF; 1000x) on each of the 25 slides selecting fields based on inflammatory cells, nuclear streaming, keratinocytes under 10x. Investigators repeated blinded evaluations of all slides two or three separate times. For each OIF, polymorphonuclear leukocytes (PMNs), intracellular (IC) cocci, extracellular (EC) cocci, IC rods, EC rods and yeast were quantified. Nuclear streaming was scored as present or absent. For each parameter within reader and between reader agreements were expressed by the intraclass correlation (ICC) value (<0.20 poor, 0.21 -0.40 fair, 0.41–0.60 moderate, 0.61–0.80 good, and 0.81–1.00 excellent) or the similarly interpreted kappa statistic (□). Reproducible parameters included: nuclear streaming (ICC=0.68), PMNs (ICC=0.58), EC cocci (ICC=0.64), IC cocci (ICC=0.32). ICC values for EC rods (0.16), IC rods (0.02), yeast (0.06) were poor as there was low prevalence due to the inclusion criteria. When qualified as present or absent, within reader □ for IC cocci was 0.71. The technique demonstrated 93% sensitivity in identifying dogs with superficial pyoderma. Specificity of 51% was attributed to EC bacterial counts and nuclear streaming observed in some fields on normal dog slides. None of the slides from normal dogs contained PMNs or IC cocci.

This study was part of a grant proposal funded by the ACVD.

Conflict of interest: none declared

# Clinical efficacy of low level laser therapy (LLLT) on localized CADESI-03 and PVAS scores in dogs with pedal pruritus from atopic dermatitis

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**Abstract:** Canine atopic dermatitis is a genetically-predisposed inflammatory and pruritic skin disease often requiring multimodal treatment. Low level laser therapy (LLLT) utilizes low-level energy to alter cellular function through photobiomodulation. LLLT has been proposed to have anti-inflammatory effects in various diseases. The purpose of this study was to evaluate the effect of LLLT on localized scoring utilizing the canine atopic dermatitis extent and severity index scoring system (CADESI-03) and owner pruritic visual analog scale (PVAS) compared to treatment with a placebo laser. Thirty client-owned dogs with symmetrical atopic pododermatitis had one paw treated with LLLT and one paw treated with a placebo laser (both front or hind paws) in a double-blinded fashion. Treatments were administered identically during each session at 4 joules/$cm^2$ (area from carpus/tarsus to distal aspect of digit 3) three times per week for the first two weeks and two times per week for the second two weeks. Scores were assessed for each paw at weeks 0, 2, 4 and 5. There were no significant differences in CADESI or PVAS scores between LLLT and placebo between weeks 0 and 5 ($p=0.0804$ and 0.5017 respectively). However, CADESI and PVAS scores significantly decreased from week 0 at weeks 2, 4 and 5 in both LLLT and placebo groups ($p<0.0001$ for all). There were no side effects noted with either treatment. These results do not differentiate between placebo and therapeutic effects of LLLT. Further studies including a true negative control group are needed to evaluate this form of therapy.

This study was supported by the Novartis ACVD Resident's Research Award and laser equipment was donated by Companion Therapy Laser, LiteCure LLC.

Conflicts of interest: None declared

# Incidence of sub-clinical middle ear effusion in dogs diagnosed during routine computed tomography

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**Abstract**: The objective of this study was to determine the incidence of middle ear effusion in dogs presenting for head and cervical spine imaging via computed tomography during a two year period. Fourteen dogs were referred for trauma, 136 dogs for assessment of neoplasia, 57 dogs for infectious or inflammatory disease, and 21 dogs for primary otic disease. A board-certified veterinary radiologist independently reviewed studies from 228 dogs. Radiologic evidence of middle ear effusion was identified in 23 dogs. Myringotomies were performed in seven dogs, total ear canal ablation with bulla osteotomies were performed in five dogs and one dog had a bulla osteotomy performed concurrent with soft palate repair. Eight of 13 cultures yielded positive growth. Negative cultures were obtained in three dogs with sterile otitis media with effusion, one dog with soft palate hypoplasia and one dog with ceruminous gland adenocarcinoma. Fourteen of 23 dogs with middle ear effusion had chronic history of, physical examination findings consistent with and/or radiologic evidence of otitis externa; three of 14 dogs were diagnosed with sterile otitis media with effusion via myringotomy and culture, despite having concurrent otitis externa. Of the remaining nine dogs, five had nasosinal disease, two had soft palate hypoplasia, one had effusion associated with trauma, and one had idiopathic persistent middle ear effusion. Statistically significant associations for middle ear effusion were present in dogs with otitis externa and soft palate hypoplasia. Neoplasia, trauma and inflammatory/infectious disease did not significantly increase the incidence of middle ear effusion.

This study was self-funded.

Conflict of interest: None declared.

# Resident lymphocytes in the dermis of normal dorsolateral thoracic skin of dogs and alpacas

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**Abstract**: Small numbers of resident T-lymphocytes are present in the dermis of normal skin of humans, cattle, and sheep. One six mm biopsy specimen from normal skin of the dorsolateral thorax from 26 dogs was examined histologically and immunohistochemically for the presence of CD3+ cells (T-lymphoctyes) and Pax5+ cells (B- lymphocytes) in the superficial and deep dermis. All examinations were negative. It appears that lymphocytes rarely occur, or occur in very small numbers, in the superficial and deep dermis of normal dog skin. Dermal lymphocytes should, therefore, be considered potentially abnormal in canine skin-biopsy specimens. In a separate study, one six mm biopsy specimen from normal skin of the dorsolateral thorax from 31 alpacas was examined histologically and immunohistochemically for the presence of CD3+ and CD79a+ cells (B- lymphocytes) in the superficial and deep dermis. Resident CD3+ cells were found in the superficial and deep dermis- primarily in a perivascular location- in 31/31 samples (100%) and 27/31 samples (87%), respectively. Resident CD79+ cells were also found in the superficial and deep dermis also in a perivascular location- in 21/31 samples (68%) and 19/31 samples (61%), respectively. The total number of superficial and deep perivascular CD3+ cells per specimen (one to 106 [median 22] and zero to 41 [median five], respectively) was greater than that for CD79a+ cells (zero to 39 [median one] and zero to 17 [median one], respectively. Lymphocytes in these perivascular locations in skin-biopsy specimens from alpacas without obvious features of inflammation must be cautiously interpreted.

Funding: Self-funded

Conflict of interest: None declared

# Anal pruritus in dogs with skin disease

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**Abstract:** Perianal/perineal pruritus was reported in dogs with anal sac disease (ASD) but not in healthy dogs. Perianal/perineal pruritus is anecdotally described as typical of allergic dogs. The aims of this prospective study were to investigate the association between anal pruritus and atopic dermatitis (AD) and adverse food reaction (AFR) in dogs, and to identify non-allergic pruritic skin diseases possibly associated with this clinical sign. For this purpose, 250 privately owned dogs with skin disease but without ASD, were included. Presence/absence and behaviours related to anal pruritus, macroscopic and cytological evaluation of perianal/perineal skin surface and presence/absence and macroscopic aspect of the anal sac content were assessed. Chi-square and Fisher exact test were performed to assess associations between anal pruritus frequency and the different clinical diagnoses and parameters. If all dermatological diseases were considered, prevalence of anal pruritus in dogs with AD and/or ARF was significantly higher than in dogs with other dermatological diseases ($p<0.0001$). No other disease was significantly associated with anal pruritus. No statistically significant difference in prevalence of anal pruritus was noted in dogs with AFR compared to those with AD. If only pruritic diseases were considered, prevalence of anal pruritus was higher in dogs with AD and ARF than in those with other non-allergic pruritic skin diseases, although the difference did not reach significance. Anal pruritus was significantly associated with alopecia, erythema, excoriations, lichenification and hyperpigmentation of the perineal area, but not with presence or numbers of bacteria or yeast, or with anal sac content.

**Sources of Funding:** This study was self-funded.

**Conflict of Interest:** No conflicts of interest have been declared.

# RESEARCH SHORT COMMUNICATIONS THURSDAY

**NORTH AMERICAN VETERINARY DERMATOLOGY FORUM**
**Phoenix, AZ**

**THURSDAY, APRIL 10, 2014**

**ACVD RESEARCH SHORT COMMUNICATIONS**

| | | |
|---|---|---|
| 2:00 | Santoro | Evaluation of antimicrobial peptides and cytokine production in primary keratinocyte cell culture from healthy and atopic beagles |
| 2:15 | Kim | First report of staining pattern and distribution of PAR2 and TSLP in an experimental model of atopic dermatitis |
| 2:30 | Marsella | Investigation of caspase-14 expression in canine atopic dermatitis |
| 2:45 | Marsella | Decreased expression of ZO-1 and occluding in an experimental model for atopic dermatitis: a role for tight junctions |
| 3:00 | Sanchez | Stratum corneum lipid comparison between atopic and control dogs |
| 3:15 | Iyori | Evaluation of cefpodoxime disk diffusion breakpoint for *Staphylococcus Pseudintermedius* and clinical outcome of cefpodoxime-treated canine superficial puoderma |
| 3:30 - 4:00 | | BREAK |
| 4:00 | Rook | Cytokine profiles in the diseased skin and blood of canine patients diagnosed with cutaneous epitheliotropic T-cell lymphoma, a pilot study |
| 4:15 | Little | Efficacy and safety of oclacitinib (Apoquel: Zoetis, Florham Park, NJ, USA) compared to prednisolone (Delta-Cortef: Zoetis, Sydney, MSW, Australia) for the control of pruritus and clinical signs associated with allergic dermatitis in client-owned dogs |
| 4:30 | Little | Efficacy and safety of oclacitinib (Apoquel: Zoetis, Florham Park, NJ, USA) compared to ciclosprin (Atopica: Novartis Animal Health, Sydney, NSW, Australia) for the control of atopic dermatitis in client-owned dogs |
| 4:45 | Lange | Canine papillomatosis: Antibodies peak around clinical regression |

# Evaluation of antimicrobial peptides and cytokine production in primary keratinocyte cell culture from healthy and atopic beagles

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**Abstract:** Antimicrobial peptides (AMPs) are small proteins present in epithelial tissues. The most studied AMPs are β-defensins (BDs) and cathelicidins (Cath). The purpose of this study was to evaluate/compare cBD3-like, cCath, and cytokine production in cultured keratinocytes harvested from healthy and atopic beagles. Keratinocytes were collected from seven atopic and five healthy age-matched beagles. Second passage keratinocytes were used. The keratinocytes were stimulated with several immune-stimulants for 24 hours. Supernatant was collected and the presence of AMPs and cytokines measured. P values ≤0.05 were considered significant. A significantly higher production of cBD3-like was present at baseline in the culture supernatant of "atopic" canine keratinocytes compared with the supernatants of keratinocytes from healthy dogs (p=0.05). This production did not increase in keratinocytes from atopic dogs after stimulation with the immune-stimulants tested. After stimulation with IL-17 and lipopolysaccharide, keratinocytes from healthy dogs produced more cBD3-like than those from atopic dogs (p=0.0035 and p=0.035, respectively). A significant increase in cCath was present in both groups after stimulation. Keratinocytes from atopic dogs produced higher amount of IL-8 and keratinocyte-derived chemokine- like than those from healthy dogs. After stimulation with house dust mite extract, the IL- 8 expression further increased while INF-γ production decreased. These results may suggest a state of over-stimulation of keratinocytes in atopic dogs that may lead to a failure to respond to immunological stimuli with increased production of AMPs. More studies are needed to better understand if this over-stimulation is associated with signaling pathway alterations in canine atopic keratinocytes.

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

Conflict of interest: none declared.

# First report of staining pattern and distribution of PAR2 and TSLP in an experimental model of atopic dermatitis

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**Abstract:** Protease Activated Receptor (PAR)2 plays a crucial role in inflammation and skin barrier. PAR2 activation stimulates thymic stromal lymphopoietin (TSLP) promoting T-helper 2 cytokines. In humans with atopic dermatitis (AD) increased expression of PAR2 and TSLP is reported, but no description of cutaneous staining pattern and distribution exists. This study aimed to compare the pattern of staining of PAR2 and TSLP between normal and atopic Beagle dogs using an experimental model of AD. Eight atopic and five normal dogs were challenged for three days with house dust mites. Biopsies were taken before, during, and after allergen challenge (days 0, 3 and 10). Sections were stained by immunofluorescence. Six images/section were randomized and blindly scored subjectively by four investigators for intensity, pattern, and distribution. Epidermis was traced and intensity of PAR2 and TSLP were quantified. For intensity, ANOVA found no significant effect of group, time, and group x time interaction in objective and subjective scores. Atopic samples showed significant patchy staining compared to controls for TSLP, at all times and PAR2 at day 0 and 3. For distribution, in atopic samples TSLP was strongly expressed and significantly different in the stratum basale compared to controls, at all times and it showed significant increase between day 0 and 3 (atopic) and day 0 and 10 (normal). PAR-2 was expressed in the stratum spinosum and granulosum in both atopic and normal samples. Significant differences found in distribution and pattern in atopics warrant further investigation on underlying mechanisms and impact on skin barrier.

This study was supported by the Blanche Saunders Dermatology and a grant of the Laboratory Korea Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A110868).

Conflict of interest: none declared.

**Investigation of caspase-14 expression in canine atopic dermatitis**

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**Abstract:** Impaired skin barrier plays a role in atopic dermatitis (AD) in both humans and dogs. Caspase-14 is a protease important for filaggrin processing and it has been shown to be decreased in humans with AD. An experimental model for AD has been identified in a colony of atopic Beagles. The aim of the present study was to investigate caspase-14 expression in normal and atopic Beagles. Biopsies from eight atopic and six normal Beagle dogs were stained by immunofluorescence using a polyclonal antibody specific for caspase-14. Six images/section were randomized and blindly scored subjectively by five investigators (based on intensity, pattern, distribution, and location) and objectively by tracing the epidermis using Image J to quantify the extent of immunofluorescence for caspase-14 in the epidermis (cell total cell fluorescence/area). The data were analyzed for normality and tested with unpaired Student t-test. A P of 0.05 was considered significant. Caspase-14 expression was decreased in atopic skin compared to normal control skin both quantitatively ($p < 0.0001$) and qualitatively ($p < 0.0061$). For the subjective evaluation the total agreement among investigators was 0.9264, and the consistency was 0.9352. It is concluded that caspase-14 expression is decreased in this canine model of AD similarly to what has been reported in humans, highlighting the relevance of a defect in filaggrin metabolism in AD.

This study was self-funded.

Conflicts: none to declare.

# Decreased expression of ZO-1 and occludin in an experimental model for atopic dermatitis: a role for tight junctions

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**Abstract:** Impaired skin barrier plays a role in atopic dermatitis (AD). Tight junctions (TJ) are important for skin barrier and could be relevant in modulating allergen penetration in AD. TJ are composed of many proteins including transmenbrane (occludin, claudins), and scaffolding proteins (zonula occludens, ZO-1]. To our knowledge, there is no published study on measurement of intensity of occludin and ZO-1 expression in AD. This study aimed to accomplish this using an experimental model. Biopsies from seven atopic (non lesional skin) and 6 normal Beagle dogs were stained by immunohistochemistry. Intensity was evaluated both objectively and subjectively. Six images/section were randomized and blindly scored subjectively by five investigators for intensity. Epidermis was traced and intensity was quantified by Image J. T test was used to compare intensity measurements between normal and atopic dogs. Intensity of ZO-1 was significantly decreased in atopics both objectively (P=0.0052) and subjectively (P=0.0017). Intensity of occludin was significantly decreased in atopics subjectively (P=0.0274) but not objectively. Intensity of claudin was not significantly different between groups by either quantification. Since these findings were present in non-lesional atopic skin prior to allergen exposure it is possible these changes may play a role in allergen penetration. Future studies will need to address the role of inflammation on the expression of TJ proteins to investigate whether these changes are secondary or primary.

This study was self-funded.

Conflicts: none to declare.

**Stratum corneum lipid comparison between atopic and control dogs**

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**Abstract:** Stratum corneum lipid composition may be altered in atopic dogs. With this aim, stratum corneum lipids of normal control dogs and atopic dogs were compared using ultra-performance liquid chromatography/time-of-flight tandem mass spectrometry (UPLC/TOF-MS). Client-owned dogs [21 atopic dogs (12 French bulldogs; 9 Labrador retrievers) and 22 control dogs (11 French bulldogs; 11 Labrador retrievers)] were sampled by tape-striping (Scotch Magic, 3M; St. Paul, MN) on non-lesional inguinal skin. Stratum corneum lipids were identified by UPLC/TOF-MS. Average chain length of ceramides was calculated. Values of $P<0.05$ were considered significant. Comparing atopic versus control dogs, an increase in N-acyl-sphingosines (CerNS) and α-hydroxy-acyl-sphingosines (CerAS) was observed, with a fold change of 1.44 (P=0.04) and 3.29 (P=0.03) respectively. Average chain length of N-acyl-6 hydroxy- sphingosine (CerNH) and CerNS decreased in atopic versus controls (CerNH: 44.5 versus 44.7, P = 0.002; CerNS: 44.2 versus 44.4, P=0.03). Contrarily the average chain length of α-hydroxy-acyl-phytosphingosine (CerAP) increased in atopic versus control dogs (37.3 versus 36.7; P=0.006). Free carboceric acid (C27) decreased in atopic versus control dogs, with a fold change of 0.73 (P=0.04). Atopic French bulldogs showed greater free fatty acids and diacylglycerides than atopic Labrador retrievers, with a fold change of 1.46 (P=0.05) and 3.14 (P=0.02), respectively. Stratum corneum free fatty acid (C14, C15, C17, C16 and C16:1) contents differed by breed, being higher in French bulldogs than Labrador retrievers (P=0.05). In conclusion, we found stratum corneum lipid differences in non-lesional skin from atopic and control dogs and between French bulldogs and Labrador retrievers.

Funding: Funded by Affinity Petcare.

Disclosure of Interest: N. Sanchez & C. Torre work for Affinity Petcare.

**Evaluation of cefpodoxime disk diffusion breakpoint for *Staphylococcus pseudintermedius* and clinical outcome of cefpodoxime-treated canine superficial pyoderma**

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**Abstract:** Cefpodoxime (Simplicef®, Zoetis) is a third-generation cephalosporin antibiotic used for treatment of pyoderma in dogs. The accuracy of cefpodoxime disk diffusion breakpoint (≤17 mm) to detect methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has not been evaluated. The objective of this study was to estimate the cefpodoxime disk diffusion breakpoint in predicting MRSP, and to determine the usefulness of the estimated breakpoint in prediction of clinical outcome of canine superficial pyoderma. A total of 221 strains of *S. pseudintermedius* were subjected to cefpodoxime- and oxacillin-disk diffusion tests. There was a significant linear correlation in a zone diameter of growth inhibition between cefpodoxime and oxacillin ($r = 0.938$, $P<0.01$). Zone diameters of cefpodoxime disk diffusion test in oxacillin-resistant strains were significantly smaller than those in oxacillin-susceptible strains ($P<0.01$). Receiver operating characteristic analysis revealed that a zone diameter of 24 mm in cefpodoxime disk diffusion test resulted in high sensitivity (94.6%) and specificity (94.6%) for detection of oxacillin-resistant strains. When the cefpodoxime susceptibilities were determined by the novel breakpoint, no difference was observed in the improvement rates of the clinical score of canine superficial pyoderma after treatment with cefpodoxime between dogs carrying resistant strains (mean:64.6±31.2%, n=6) and dogs carrying susceptible strains (mean:83.9±13.7%, n=4), ($P>0.05$). These findings indicate that the cefpodoxime disk diffusion diameter of 24 mm is valuable as an *in vitro* breakpoint in predicting MRSP. Further studies will be needed to estimate the better clinical breakpoint of cefpodoxime disk diffusion test in prediction of clinical outcome of canine superficial pyoderma.

Funding: Zoetis Japan Inc.

Conflicts of interest – none declared.

# Cytokine profiles in the diseased skin and blood of canine patients diagnosed with cutaneous epitheliotropic T-cell lymphoma, a pilot study

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**Abstract:** Cutaneous epitheliotropic T-cell lymphoma (CTCL) is a rare and often fatal disease of older dogs typically demonstrating a CD8+ gamma/delta T-cell phenotype. In an effort to characterize the immunologic milieu, a pilot study was initiated examining skin biopsies and peripheral blood from four dogs with CTCL, one non-epitheliotropic lymphoma and three normal dogs. Histopathology, immunohistochemistry for cell typing, and qPCR on extracted mRNA for interleukin (IL-)-4, IL-5, IL-13, IL-17, IL-31, TNF-alpha, and interferon-gamma were performed. Dogs with CTCL had increased mRNA for IFN-gamma, TNF-alpha, IL-4, IL-5 in four of four, IL-13 in two of four, and IL- 17 in three of four in affected skin samples compared to skin samples from healthy control dogs. Peripheral blood samples had less IL-4, IL-13, and interferon-gamma mRNA production and variable IL-5, TNF-alpha, and IL-17 compared to normal dogs. The non-epitheliotropic skin had detectable TNF-alpha and IL-5 only. Interleukin-31 mRNA was not detected in any samples. Skin samples stained positively in 2 of 2 patients studied for CD8 and 2 of 3 patients studied for gamma/delta T-cells, respectively. In this small study, our findings demonstrate a pleomorphic cytokine environment within the skin with contributions from not only Th1, but also Th17 and Th2 immunopathology.

Funding: ACVD Resident Research Award.

Conflicts of Interest: None declared.

# Efficacy and safety of oclacitinib (Apoquel: Zoetis, Florham Park, NJ, USA) compared to prednisolone (Delta-Cortef: Zoetis, Sydney, NSW, Australia) for the control of pruritus and clinical signs associated with allergic dermatitis in client-owned dogs

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**Abstract:** A total of 123 dogs, at 12 Australian veterinary clinics, with moderate to severe pruritus (owner-assessed) and a presumptive diagnosis of allergic dermatitis were enrolled in a double-blind 28-day study. Enrolled animals were randomized in a 1:1 ratio to receive either oclacitinib (0.4-0.6 mg/kg orally twice daily for 14 days, then once daily) or prednisolone (0.5-1.0 mg/kg orally once daily for 6 days, then every other day). Enhanced 10 cm visual analog scales (VAS) were used by owners to assess pruritus (day 0, 4 h, and days 1, 6, 14, 28), and by veterinarians to assess dermatitis (days 0, 6, 14, 28). Pre-treatment mean VAS scores were similar in both groups (pruritus 7.2-7.3 cm, moderate to severe pruritus; dermatitis 4.9 cm, moderately severe dermatitis). Both treatments produced a rapid reduction in pruritus, with mean percentage reductions of 31.1% and 28.1% at 4 h post treatment, and 41.3% and 43.1% on day 1, for oclacitinib and prednisolone, respectively. There were no significant differences between the treatments for pruritus or dermatitis score reductions, except on day 14 when oclacitinib was significantly better than prednisolone (67.5% and 52.2% for pruritus, p=0.0193; 71.0% and 53.7% for dermatitis, p=0.0252, respectively). At study completion, mean pruritus VAS scores were 3.3 cm (mild to very mild pruritus) for both treatments. Overall the reported number of adverse events was similar in both groups. Serum alkaline phosphatase increased above the reference range in 16.0% and 4.3% of dogs in the prednisolone and oclacitinib groups, respectively.

This study was self-funded by Zoetis Inc (formerly Pfizer Animal Health).

Conflict of Interest: All authors are current or former employees of Zoetis Inc.

# Efficacy and safety of oclacitinib (Apoquel: Zoetis, Florham Park, NJ, USA) compared to ciclosporin (Atopica: Novartis Animal Health, Sydney, NSW, Australia) for the control of atopic dermatitis in client-owned dogs

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**Abstract:** Seven Australian-registered veterinary dermatologists enrolled a total of 226 dogs with a history of chronic non-seasonal atopic dermatitis in an 84-day study. Enrolled animals were randomised in a 1:1 ratio to receive either oclacitinib (0.4-0.6 mg/kg orally twice daily for 14 days, then once daily) or ciclosporin (3.1-6.7 mg/kg orally once daily). Assessments of pruritus (owner) and dermatitis (veterinarian) were made on days 0, 14, 28, 56 and 84; pruritus was also assessed on days 1, 2 and 7. Pruritus was scored using an enhanced 10 cm visual analog scale (VAS), and dermatitis by the canine atopic dermatitis extent and severity index (CADESI-02). Pre-treatment mean pruritus VAS scores were similar for both groups (7.4 cm, moderate to severe pruritus). Oclacitinib was rapidly effective in reducing pruritus; the mean percentage reduction from baseline was significantly higher than for ciclosporin on days 1, 2, 7, 14 ($p<0.0001$) and 28 ($p=0.0057$). At study completion, mean pruritus VAS scores were below 3 cm (mild to very mild pruritus) for both treatments. The mean percentage reduction from baseline in CADESI-02 was significantly higher in the oclacitinib group on day 14 (58.7% compared to 43.0%, $p<0.0001$). Overall, more adverse events were reported in the ciclosporin group, mainly due to gastrointestinal signs; vomiting and diarrhea occurred in more dogs receiving ciclosporin (43.8% and 15.2%, respectively) than those receiving oclacitinib (14.0% and 3.5%, respectively). Mild reductions in mean white cell count (within the reference range) occurred in both groups between days 0 and 84.

This study was self-funded by Zoetis Inc (formerly Pfizer Animal Health).

Conflicts of Interest: All authors are current or former employees of Zoetis Inc.

# Canine papillomatosis: Antibodies peak around clinical regression

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**Abstract:** Most forms of canine papillomatosis are believed to be caused by or involve papillomavirus infections. Fifteen different canine papillomavirus types (CPVs) have been discovered to date. CPV1 is the causative agent of most oral and several forms of cutaneous papillomas. Experimental antibody induction against this virus had been shown, however reactions to natural infections have not been examined yet. Forty dogs showing different degrees of classical clinical oral or cutaneous papillomatosis were included as one single study group. Tissue and serum samples were taken upon initial presentation; serum samples were taken again upon and 3 months after clinical resolution. None of the dogs underwent antiviral therapy. The tissue samples were tested by PCR to detect CPV DNA while the serum samples were tested using a specific ELISA for antibodies against CPV1. All tissue samples were positive for CPV1 DNA, and 87.5% of all serum samples contained measurable antibody levels against the virus (cut off value 0.3). The average optical density measured in the ELISA was 0.7 before, 1.65 upon (+/- 1 month) and 0.83 after clinical recovery. Although antibodies are probably not playing a role in the resolution of papillomavius infections, they can serve as good indicators for past infections. The data support the hypothesis that CPV1 is the most abundant virus in canine papillomatosis. The data also show that the healing process of such lesions in general correlates with a strong antibody response and that the antibody titers peaked around the time of clinical recovery.

This study was self funded.

# CONCURRENT SESSION PRESENTATIONS THURSDAY

# FELINE DERMATOPHYTOSIS
# Decontamination- It's Not as Hard We Thought

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**BUSY? READ THIS**

Bottom Line: If you can wash it, it can be decontaminated. Confinement of the pet to an easily cleaned room makes cleaning easier. Confinement and cleaning will speed time to cure as it prevents false positive fungal culture results. The most important part of decontamination is the "hard clean": mechanical removal of debris followed by washing of the surface with a surface safe detergent until visibly clean. Disinfectants are only needed to kill spores remaining after the hard clean (often the hard clean alone will remove all of the infective spores). A 10 minute contact time is recommended. If there are only one or two infected pets AND OWNERS ARE USING A TOPICAL ANTIFUNGAL ON THE PET(S), owners can do a hard clean/disinfectant application once or twice a week provided they do adequate mechanical removal of debris followed by a one-step cleaner with antifungal efficacy against *Trichophyton mentagrophytes* on the label. There are many alternatives to bleach, accelerated hydrogen products are a good option. Dry cleaning will kill spores on 'dry clean only clothes'. Don't overstuff the laundry tub, use the longest cycle possible, hot or cold water and washing twice will decontaminate laundry. Pet dishes can be decontaminated by simple washing in hot water and dish soap.

**Introduction**

Dermatophytosis is a superficial fungal skin disease of keratinized tissue and infections are most commonly encountered in cats. In cats, the primary pathogen is *Microsporum canis*; however other pathogens have been associated with disease, e.g. *Trichophyton spp*., *M. persicolar.* The disease is self-limiting and non-life threatening, but it is a known low level zoonotic disease. Treatment is desirable because it shortens the course of the disease and thereby minimizes the risk of the disease being transmitted to susceptible people or other animals. Treatment involves the use of topical and systemic antifungals, some reasonable confinement of the pet, cleaning of the environment where the pet has been confined and monitoring of the pet until mycological cure. Mycological cure is determined by repeat negative fungal cultures and contaminated environments can result in false positive cultures confounding the determination of cure.

**Why environmental decontamination? What is the risk of environment to human transmission?**

There are two equally important reasons to emphasize decontamination of the environment. The first, and most important, is because environmental contamination will result in false positive fungal cultures. This is particularly important when trying to determine treatment endpoint. Lack of appropriate cleaning and disinfection can potentially lead to unnecessarily long treatment and confinement of cats. Unnecessary treatment increases the potential for adverse drug effects. **Prolonged confinement of cat is problematic for all owners and excessive confinement of kittens interferes with appropriate socialization and family bonding.** The second reason is that the disease evokes a negative response in owners disproportionate to the pathogenesis of the disease. In pet owners, this response is almost always associated with misconceptions about the environmental contamination. The biggest concern is contracting the disease from causal contact with the environment. However, confirmed case reports of

transmission of the disease from a contaminated environment to a person in the *absence of contact* with an animal are rare.

**Don't Fear the Spores: What to Emphasize To Clients**

- Dermatophyte spores are a naturally dormant stage of the organism, not unlike many other microbes already in their home.
- Dermatophyte spores do not multiply in the environment; they cannot as there is no growth medium for them. Dermatophyte spores do not invade the home. Pet owners do not make any distinction between 'fungus' and 'mold' and assume that environmental contamination with dermatophyte spores is similar to mildew, black mold, or mold due to water damage. This misconception alone causes huge anxiety among pet owners and if not immediately addressed leads to unnecessary and excessive cleaning and use of disinfectants (e.g. daily washing of walls and use of undiluted sodium hypochlorite). It is not uncommon to hear owners state "you mean it's in my HOUSE?!"
- Although there are studies that have shown that spores can remain viable for 18 to 24 months, the vast majority of spores are not viable for this long and start to die within 3 to 6 months.
- Spores are less viable in humidity as it causes them to sporulate and in the absence of proper growth medium, they die shortly afterward.
- Naturally infective material is trapped by furnace filters and is not being circulated via heating ducts. *Microsporum canis* causes skin infections, not respiratory infections.
- Spores are easily removed from the environment.

**When to Culture the Environment**

A common question is "when to culture the environment?" The most important question answered by environmental fungal cultures is not "are there spores present?" but rather "is cleaning effective?" Fungal cultures of the environment are only recommended when there is strong suspicion that lack of cure may be due to environmental contamination. However the following may be more helpful: 1) review cleaning protocols with the client, 2) have them intensively clean a room the cat has not been 3) apply a topical antifungal rinse to the cat and move it to the just cleaned room, 4) hard clean the room(s) where the cat has been, 5) reculture the cat in 72 hours. If the problem is fomite contamination the cat will be culture negative. If there are infection sites present, the hair coat will be reseeded with fungal spores at the end of 72 hours. If a client needs to know that a room is decontaminated, only two fungal cultures per room are needed. One is taken from the floor area and the second is obtained from surfaces where the cat has been observed to go. These are best obtained with Swiffer cloths; most laboratories are not familiar with inoculation of these samples and these should be done in house.

**Selecting a Disinfectant**

*What to Read:* The first is the *disinfectant product label* and note the active ingredients, precautionary statements, what the disease organisms are controlled, and recommended use. Some products can have multiple uses, e.g. cleaning vs disinfection and require different contact times. The second is the *Safety Data Sheet*. For most products in the United States this known as the Materials Safety Data Sheet (MSDS) but in 2013 a requirement was made to for these sheets to comply with the Globally Harmonized System for Chemical Information and Labelling or "GHS". These are particularly helpful because key information on hazards, first aid measures, flammability, how to handle spills, storage, exposure safety, pH, toxicity, and incompatibly with other compounds is available.

"Hard cleaning": The term hard cleaning is often found on disinfectant labels. This term refers to the gross removal of organic material or other debris via sweeping or vacuuming followed by washing of the surface with a detergent until it is visibly clean.

"One step" Cleaners: The term 'one step' is often found on many disinfectant labels in both the home and institutional market and is very confusing. Technically 'one step' cleaners are those that can clean a lightly soiled surface and sanitize it in one wipe. Test requirements require showing 99% efficacy within 5 minutes against selective bacteria (e.g. *Staphylococcus*), not fungal spores. Careful reading of products labelled as 'one step cleaners' will reveal a statement that organic material needs to be removed (i.e., hard cleaning) before application of the product. Many clients will assume this literally means, "It's all I have to do". **One step cleaners can be used <u>between</u> hard cleanings, but not in place of it**.

**Effective Disinfectants**

There are many effective antifungal disinfectants, however several are worth special mention because of their wide spread use in homes or in veterinary clinics. The following compounds were tested in suspension studies with increasing challenges of spores 1:10, 1:5 or 1:1. Neat plates had confluent growth of *M. canis* or *Trichophyton* and a compound was considered efficacious at 10< spores per plate.

<u>Sodium hypochlorite:</u> Sodium hypochlorite at 1:10 and 1:32 is consistently antifungal even after short contact times, however it can fail if out of date or the dilution is not freshly prepared. Reasons not to use sodium hypochlorite include, but are not limited to: lack of detergency, potential to react with other chemicals to create toxic gases, unpleasant odor, damage to hard surfaces, discoloration of fibers and colored surfaces, damage to floor finishes, rapid loss of efficacy once diluted.

<u>Enilconazole</u>: It is available as concentrated spray or as a fogger. It is widely available in many countries and in the United States is available as Clinafarm® spray or Fogger, (Eli Lilly and Company). A major obstacle to more widespread use in the United States is that it is not available in reasonably priced small quantities. A 10 minute contact time is recommended even though enilconazole was antifungal at shorter contact times.

<u>Accelerated Hydrogen Peroxide:</u> Accelerated hydrogen peroxide (AHP) is a proprietary compound that is increasingly available worldwide. What makes this product different than over the counter hydrogen peroxide is that it contains surfactants (wetting agents) and chelating agents that help to reduce metal content and/or hardness of water. This product has been tested using isolated infective spore suspensions of both *Trichophyton* and *M. canis* and is an effective disinfectant. A 10 minute contact time is recommended even thought AHP was antifungal at shorter contact times. Of important note is that the Materials Safety Data Sheet states that it should not be mixed with concentrated sodium hypochlorite product. If recommending this to clients it is important to make this clear.

<u>Potassium peroxymonosulfate</u>: This is the main component of Trifectant® (Vetoquinol,) and has broad spectrum antibacterial and antiviral properties. In recent studies this product was found to be antifungal against both *M. canis* and *Trichophtyon spp* when applied liberally and with a minimum contact time of 10 minutes. More recent studies found a 2% solution to be more effective than 1% against dermatophtyes.

<u>Over the Counter Products:</u> A recent study investigated the efficacy of ready to use over the counter products as alternatives to sodium hypochlorite. The criteria for selection were easy access by the consumer, preferably ready to use formulation, and label claim as antifungal against *T. mentragrophytes.* Active ingredients included sodium hypochlorite, quaternary ammonium, lactic acid, accelerated hydrogen peroxide, and an ethoxylated alcohol mixture.

**Specific Decontamination Recommendations**

**Hard Surface Decontamination**: A hard surface is any non-porous surface. Cleaning recommendations: 1) Remove debris, ESPECIALLY hair! 2) Wash with soap and water until visibly clean, 3) **Rinse with water- this is important as many detergents inactivate disinfectants,** 4) After a hard clean, apply a disinfectant, ready to use products are recommended for clients, 5) Frequency depends upon the number of cats present (1-2x a week), 6) between cleanings remove debris and use 'one step cleaners' and dusting.

**Wood Floors, Specialty Floors, Specialty counter tops:** These surfaces can be easily decontaminated by removal of debris from the surface and repeated washing. The 3M easy Trapper (3M Company) is recommended as these are "sticky" Swiffer like surfaces. Floors are ideally cleaned with a detergent using a flat mop (3M Flat Mop). These may need to be repeated several times. Most wood floors can be washed with Murphy's Wood Oil Soap or similar.

**Walls, Windows, Ledges:** Unless there are large numbers of infected cats housed in a room, it is not necessary to hard clean these areas. If cat hair is visible, clean by using Swiffer Dusters or an equivalent to remove any dust or spider webs. Spores tend to be located on areas where the cat goes.

**Soft Surfaces/Laundry**: In general, if it can be washed-it can be decontaminated. Studies using experimentally exposed linen, towels, and denim and field studies revealed that washing alone was adequate. With the exception of items heavily contaminated by hair, one washing was sufficient. Cleaning recommendations for laundry items: 1) change blankets and bedding daily, 2) store exposed laundry in a plastic bag until washed and keep it separate from other laundry, 3) hot or cold water can be used , 4) bleach is optional but if used 1 cup per laundry tub, 4) do not over stuff the laundry tub-allow for adequate agitation, 5) use the longest wash cycle possible, 6) always use the high water level to enhance agitation 6) although one is adequate, two washings are recommended large amounts of hair are visible, 7) wash pet laundry at the end of the day, 8) mechanically clean out the laundry and lint trap, 9) spray laundry tub with disinfectant after use and then run a load of water only. Any item that needs line drying should be washed twice, especially if it has a tight weave.

**Dry Clean Only**: Fabrics that were dry clean only were decontaminated via routine dry cleaning. Hair had been removed using a lint roller. Dry cleaners handle all clothing as potentially 'contaminated' and wear gloves when receiving the items; even so it is helpful to tell the merchant that the clothes were soiled by animals.

**Carpets:** In general, the recommendation is to make every effort to keep infected cats/kittens off of carpeting. This can be decontaminated but it was the most difficult to do. Routine vacuuming of carpets should be done to remove gross debris and cat hair; however it will not decontaminate carpets. Spores will adhere to the carpet fibers and will settle into the carpet nap. Steam cleaning of intentionally contaminated carpets removed >95% of spores 48 hours post cleaning. Routine washing of carpets with a carpet shampooer did not have the same effect; however when carpets were thoroughly wetted and washed the carpets were culture negative at 7 to 10 days. It is hypothesized that the water soaking caused dormant spores to sporulate and in the absence of a suitable growth medium, the spores died. Carpets can also be chemically decontaminated as follows: 1) vacuum carpets to remove gross debris, 2) thoroughly spray the carpet with a disinfectant (accelerated hydrogen peroxide carpet cleaner, Trifectant, Simple

Green, any quaternary ammonium compound), 3) using a carpet shampooer or deck brush, scrub the carpet and allow the disinfectant to remain in contact with the surface for no less than 10 minutes, 4) using a carpet scrubber wash the carpet, 5) using a carpet shampooer use only water to rinse the carpet until the surface does not foam. In many instances, 48 hours after washing there may be a "bloom" on the surface of the carpet due to sporulation of spores. It is recommended to keep children off of carpets until they are dry. This bloom coupled with the rough surface of the carpet may represent an exposure risk.

**Bowls, Litterboxes, Pet Carriers:** These can be easily decontaminated by using hot water and dish soap or a detergent. Wash twice and rinse thoroughly.

**WHAT MIGHT YOU DISPOSE OF?**

**Cat Trees:** If this is a popular place for the cat to spend time and it is heavily contaminated by hair, it maybe easiest to discard the cat tree. However if the tree is new and there has been limited exposure to the infected cat, the carpeted areas cat tree can be decontaminated.

**Pet Sweaters, etc:** Unless the item is a family pet "heirloom sweater", it is best to discard this item. This is worn closely to the body and will become readily contaminated even in the most limited of infections.

**Non Metal Collars:** These can be decontaminated by washing but many animals tend to have lesions in close proximity to the collars and spores and hairs are trapped in these items. It is easiest to discard these.

*References are available from the author upon request.*

# NOTES

# FELINE DERMATOPHYTOSIS
# TREATMENT "GETTING TO CURED WITH LESS SMELL AND MESS"

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## PATHOGENS OF IMPORTANCE

Feline dermatophytosis is one of two common fungal skin diseases of cats, the other being *Malassezia* overgrowth. The most commonly isolated pathogen from cats is *Microsporum canis*, although infections with *M. persicolor*, *M. gypseum*, and *Trichophyton spp* have been reported[1,2]. In the author's experience in the United States with shelter cats, *Trichophyton spp* infections present in one of two ways: focal lesions on the ears or muzzle of outdoor cats often with known exposure to large animals or widespread generalized lesions in severely debilitated kittens again having a known exposure to large animals. From a treatment perspective, infection with *M. canis* is the most challenging to treat. *Microsporum canis* is not part of the normal fungal flora of cats and isolation on fungal culture is compatible with true infection, fomite carriage from a contaminated environment or object, or cross contamination of the fungal culture plate. In the case of the latter, it is the author's experience that the number of colony forming units per plate (cfu) is low (1-3), however this is a complication wrinkle best avoided.

## UPDATE ON PATHOGENESIS AND COMMENTS ON TRANSMISSION

Recent information on the pathogenesis of *M. canis* has emerged from work done using a reconstructed interfollicular feline epidermis model of infection.[3] Using this model, adherence assays showed that arthroconidia of *M. canis* start adhering within 2 hours and increasing up to 6 hours post inoculation.[4] The penetration of the stratum corneum starts with the emergence of germ tubes from arthroconidia. As hyphae develop, arthroconidia are formed.[5,6] In these models, infection was documented via histological examination and fungal culture within 5 to 7 days. From a clinical perspective, it becomes important to remember that the time from exposure to clinically obvious lesions is from 2 to 4 weeks. *However, emerging information on how quickly spores can adhere and germinate emphasizes two important points relative to treatment. The first is that there can be infection sites capable of shedding spores long before clinical lesions develop. And second, prevention of contact with infective spores is an important disease control point. In order for a true infection site to develop, viable spores must contact the skin surface and defeat host protective mechanisms (sebum, normal flora, grooming, and innate immunity). Topical therapy is an important barrier.*

Infective spores are present in the hair follicle and on the hairs. Contact with infective spores in the hair follicle is physically unlikely therefore infection transmission occurs from contact with spores on the hair coat. As infected hairs emerge, cuffs of spores are present on the hair shaft. The disease is primarily transmitted from cats to other animals and people via direct contact. Studies in children experimentally exposed to infective *M. canis* hairs revealed that contact alone with the skin did not create an infection. However when hairs were placed on the skin and the immediate site gently traumatized, infection occurred.[7] *From a treatment perspective, this means that any type of micro trauma (e.g. fleas, mites, aggressive grooming or clipping of the hair coat) to the skin of cat exposed to a critical mass of infective spores is a risk factor for infection.*

Transmission to people primarily occurs via direct contact with an infected cat. Although it is widely stated that one of the major reasons for decontamination of the environment is to prevent transmission of the disease to other susceptible animals and people, strong evidence is lacking that this is a major risk

factor for contracting disease. In experimental infection studies and natural exposure experiments cats in heavily contaminated environments did not develop lesions and under natural exposure, the development of the disease closely mirrored the social structure of the group of cats (DeBoer and Moriello, University of Wisconsin studies). Cats that were most social developed lesions first (particularly in head butting areas) and those that were least social were the last to develop lesions. If published case reports reflect occurrence, it is difficult to find compelling case reports that describe a person contracting *M. canis* solely from the environment in the complete absence of exposure to an infected animal. There is one case report in the English language that describes a child contracting the infection from the interior of a car.[8] Readers should not infer that the author is implying this does not or cannot happen, but rather it is less of a risk factor than commonly thought. *From a clinical perspective, management of environmental contamination is necessary primarily to prevent fomite carriage and false positive fungal cultures and secondly to minimize the risk of transmission even if it is low.*

**RE-THINKING CLINICAL PRESESENTATIONS RELATIVE TO TREATMENT DECISIONS**

The development of clinical signs is directly related to pathogenesis-invasion of the hair follicle and again greatly influenced by the cat's immune system and overall health. Further complicating any treatment decision is the effect of any prior treatment. *Treatment and monitoring decisions are optimally based upon a "global assessment" of the cat taking into account the amount of physiological stress. In this treatment scheme, there are three clinical presentations of feline dermatophytosis. Treatment needs to be tailored to the patient.*

- Simple infection: This group consists of otherwise healthy cats or kittens with confirmed infections. Lesions are obvious but limited in extent. Provided the cat/kitten remains healthy, these cats will respond well to almost any therapy protocol. Since dermatophytosis is a self-limiting disease it is many kittens/cats that have simple infections cure without any medical intervention.

- Complicated infection: This group consists of cats with wide spread lesions, inflammatory lesions, long-haired/matted hair coats, other illnesses (most notably upper respiratory infections), a history of prior treatment, surrender for "resistant dermatophytosis", and/or are semi-feral or feral cats. (This group also includes the "geriatric house cat" that contracts *M. canis* from the new kitten that was recently adopted to "keep him active" or eventually replace him/her.) In many cases, clipping of the hair coat reveals the true extent of the lesions. These cats are more complicated to treat because of the extent of their lesions and/or because of other health factors. These cats are complicated to treat because antifungal therapy must be coordinated with treatment for other pre-existing diseases.

- Lesions Free but Culture Positive Group: : This group of cats may consist of cats mechanically carrying spores on their hair coat (i.e. "dust mops") or cats with very early lesions that are not easily seen but mature enough to be shedding arthrospores. Colony forming counts on fungal culture coupled with a re-examination under both white light and a Wood's lamp are helpful differentiating fomite carriers from cats with early lesions. The major risk these cats pose is contamination of the environment and spread of the disease to other cats. In field work with shelters that do routine screening at the time of admission, these cats with rare exception consistently divided into one of two groups.[9]

    - The fomite carrier: At the time of admission, these cats had negative Wood's lamp examinations and no lesions. At the time of re-examination 7 to 14 days later, cats still had negative Wood's lamp examinations, no lesions, and repeat cultures were negative. These cats were considered fomite carriers and once placed in a clean environment removed spores via grooming. (Admittedly it is possible that some of the cats could have had subclinical lesions that cured

between the two culture points; however cats were carefully examined with Wood's lamps prior at the time of admission making this unlikely. See "Wood's Lamps below)

- Infected cats: These were cats that did not have skin lesions under white light (room light or high beam) or any detected by Wood's lamps at the time of admission. At the time of re-examination 7 to 14 days later these cats were lesional, Wood's lamp positive and repeat culture positive. Lesions were small and typically located near or in the ears, on the muzzle, between the digits, on the tail or under the axilla.

When confronted by one of these situations, it is important to remember that fungal culture results reflect the status of the cat's fungal flora at the time of examination and not on the day the results are reported. The fungal culture results itself may be helpful in differentiating the two groups. Cats with true infections have >10 cfu/plate and often too many to count cfu/plate. Cats with fomite exposure tend to have few cfu/plate (often 5 or less). If culture results are merely reported as "positive or negative" this information is lost and hence is one of the reasons that the author is strong advocate for in house cultures. The ideal approach is to treat the cat as "truly infected" and have the owner represent the cat for examination as the last appointment of day. The cat should be examined under white light and with a Wood's lamp as this will identify lesions often missed by room light examination. If the cat has skin lesions, repeat the fungal culture and treat as truly infected pending results. If the cat does not have skin lesions, re-culture and recommend a whole body treatment with an antifungal rinse or shampoo (see below). In the shelter, these cats were considered fomite carriers and moved through to adoption.

**WOOD'S LAMPS**

This author's current opinion based upon a decade of field work with shelters and data collection on lesion status, Wood's lamp status and fungal culture results, is that Wood's lamp examinations are valuable. All cats should be examined with a Wood's lamp if presented for first examinations, pre-surgery if kittens and for skin diseases.

- In cats, this is a time and cost effective procedure. The major problems with use is not taking enough time to examine the cat, not being trained to recognize Wood's positive hairs and or having an inferior Wood's lamp.
- Battery operated Wood's lamps are inadequate. The author recommends Burton Ultraviolet UV Wood's Exam Light (Burton). This lamp has magnification and greatly enhances visualization of glowing hairs.
- Wood's lamps do not need 5 minutes to warm up; the lamps are functional as soon as they are turned on. "Delayed fluorescence" is due to slow eye adaption of the examiner to the dark.
- It is often said that less than 50% of strains do not fluoresce. This comment was extracted from the human literature and perpetuated into veterinary literature. *We do not know this information and scientific data on strains and fluorescence is unknown.* In the author's experience *M. canis* commonly glows.
- Crusts do not fluoresce, it is important to lift crusts and examine hairs beneath as often glowing hairs are found here. Many cats deemed "Wood's negative" were found to have Wood's lamp positive hairs under crusts.
- Cats with no visible lesions on gross examination will often have fluorescing hairs. Wood's lamp examinations will often reveal sites of infection not visible in white light.
- "Dust mop cats" will not fluoresce because there is not active infection; spores are being carried mechanically.
- Wood's lamp examinations are valuable during monitoring of treatment, especially if the cat is not curing.

- **Bottom line: Wood's lamp positive hairs examined by direct examination confirm an infection, and Wood's lamp examination can find sites of infection that are not visible on gross clinical examination.**

**CCATS PLAN TREATMENT**

**Confinement of the cat/kitten to an easily cleaned room:** Confinement needs to be reasonable and appropriate for the pet. Kittens should not be left alone in a home unsupervised and older cats may not move around much and may have other diseases that require intense monitoring. Some older cats will not eat or be easily medicated if not in close contact with the owner. This shortens treatment time because it makes cleaning easier and minimized the spread of infective material into the home. Treatment needs to be as short as possible because many infected animals are new family members and need to be socialized.

**Cleaning:** Cleaning shortens total treatment time because it helps prevent false positive fungal cultures.

**Assessment:** Assessment refers to monitoring of treatment. **The recommendation is to treat cats until they have two negative fungal cultures**. The global cost of treatment needs to be considered when determining how the cat/kitten will be monitored. This global cost includes time and money spent on: systemic drugs, topical rinses and time to apply them/dry the cat/clean up after the treatment, money spent on buying cleaning supplies and time spent doing extra cleaning if what is requires is above what the client normally does, issues and time spent on confining the cat. The latter is an overlooked cost as this may be an emotional cost. It could also include money and time spent interacting with the kitten/cat and then washing clothes specifically identified for this purpose. If children are involved, this magnifies the problem as children are more likely to contract dermatophytosis and most likely to circumvent any 'confinement' measures. The author recommends weekly fungal cultures, especially if children are in the home. The goal of treatment is to shorten the course of the disease as rapidly as possible. *If the preference is to start cultures at X weeks after starting therapy, an important question to consider is "What am I asking the client to do while awaiting fungal culture results?"*

**Topical Therapy:** In a series of in vitro experiments and limited field studies the author has investigated the antifungal efficacy newer topical shampoos and rinse formulations. Water, enilconazole and lime sulphur were used as untreated and treatment controls. Testing included suspension tests with spore to treatment ratios of 1:10, 1:5 and 1:1 at 10 minutes of contact time. In addition, toothbrushes from infected cats and confirmed to be culture positive were exposed to topical alternatives for 1, 5 and 10 minutes.

- Whole body rinses: enilconazole (1:100), lime sulphur (1:16), accelerated hydrogen peroxide (Pure Oxygen Derma Wash Concentrate 1:20 or 1:40). Twice week application is recommended.
- Climbazole Mousse: 0.5% climbazole mouse used as directed by the manufacturer. This may be an option for cats with simple infections and/or older cats that have contracted the disease from exposure to a kitten and it is limited in scope.
- Shampoo Alternatives: It is recommended to comb the hair coat to remove any easily displaced hairs, use products with 1-2% ketoconazole, miconazole, or 0.5% climbazole at 1:10 or greater. Use no less than 2x a week, preferably more frequent. A minimum of a 3 minute contact time is recommended. Another option is to shampoo the cats, rinse with water and then apply accelerated hydrogen peroxide rinse.
- Difficult to Treat Areas: Spot therapy as sole therapy is not recommended; however persistent culture positive status can occur if infected hairs are not treated in 'hard to treat areas', e.g. ears.

Adjuvant focal therapy includes: 0.5% climbazole, terbinafine 1% (human product), thiabendazole in a commercial preparation, or any of the following compounds at ≥1% - clotrimazole, ketoconazole, miconazole[10,11]. It is important to consider the application method and the risk of owner exposure.

**Clipping of the hair coat-Pick your battles!**

- Clipping infected hairs grossly debulks the amount of infective material
- Clipping needs to be done carefully to avoid traumatizing the skin; electric clippers can cause thermal burns.
- Ideal in long haired cats, but sedation may be necessary.
- Scissor clipping is the recommended method to clip cats. Children's round tipped metal scissors are ideal. They are inexpensive so they can be discarded and are blunted so there is minimal risk of injury.
- *May not be needed in most cases if through application/drenching of topical solution can be applied.*

**S**ystemic Therapy: The most commonly used drugs are fluconazole, itraconazole, and terbinafine. Recently terbinafine was shown to have residual activity in the hair[12] and to be effective in a 21 days continuous treatment protocol with adjuvant topical therapy.[13] Systemic therapy pharmacokinetic studies are performed in healthy adult cats. Pharmacokinetics will be different in cats with complicated infections, adjust treatment to fit the patient.

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# NOTES

# WHAT TO DO WHEN FEELING BLUE: UNDERSTANDING FELINE ALLERGIC ASTHMA

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## INTRODUCTION

Asthma is a common and serious bronchopulmonary disorder in cats which has been associated with substantial morbidity and occasional mortality. It is believed that feline asthma is allergic in etiology, that is, it is a type I hypersensitivity reaction against aeroallergens.[1] Once cats with the appropriate genetic influences and environmental background become sensitized to an allergen, they are susceptible to a variety of pathologic consequences upon repeated exposure to that same allergen. The hallmark features of naturally occurring asthma include eosinophilic airway inflammation, mucus hypersecretion, bronchoconstriction (both in response to allergenic and non-allergenic stimuli), and airway remodeling (permanent architectural changes in the lungs). Collectively, these pathologic changes lead to clinical signs of cough, wheeze and expiratory respiratory distress. Because there are other disorders which may mimic the clinical signs and some of the pathologic changes seen with feline allergic asthma, it is important that the diagnostic plan be able to cover testing for those disorders. With the ultimate goal to be able to specifically treat (or perhaps cure!) asthma, it is critical that there is confidence in this diagnosis.

## IMMUNOPATHOLOGY OF ALLERGIC ASTHMA

A brief review of the immunology of allergic asthma is helpful in the understanding of why these pathological changes occur. It is also important in helping evaluate where novel treatments might make the largest impact. Genetic and environmental influences affect development of asthma. In susceptible individuals, when an allergen is inhaled into the airways, it can be picked up by local antigen presenting cells (usually dendritic cells). Non-allergic individuals fail to mount an immune response to allergens, recognizing them as non-threatening. In allergic individuals what should be benign inhaled substances elicit a harmful response. Dendritic cells take in the allergen and process it, so that a portion can be presented on the surface of the cell in conjunction with the major histocompatibility complex (MCH) II. Local naïve T helper 0 (Th0) cells recognize the allergen in conjunction with MHC II and are stimulated to become Th2 cells, which produce a variety of cytokines that will polarize the immune response to an allergic phenotype. Thus, the Th2 cell is considered the major cell which orchestrates the subsequent inflammatory response. The cytokines produced by Th 2 cells can induce the maturation, differentiation and survival of eosinophils, and stimulate B cells to produce antibodies of the IgE class. IgE antibodies that are secreted become bound to mast cells just below the surface of the airways. Upon reexposure to the allergen, two IgE antibodies are crosslinked, send an intracellular message and trigger mast cell degranulation locally in the lung. A variety of preformed mediators within mast cell granules are immediately released causing bronchoconstriction, increases in vascular permeability and inflammatory cell influx. Other eicosanoids and cytokines are elaborated by the mast cells, additional cells infiltrate into the airways and a second wave of bronchoconstriction (“late phase response”) can occur hours to a couple days later.

**HOW IS ASTHMA DIAGNOSED?**

Diagnosis of feline asthma generally starts by evaluating historical and clinical signs. Cough (often confused by owners as "hacking up a hairball"), wheeze and episodic expiratory respiratory distress are compatible with the diagnosis of asthma. Exercise intolerance is less frequently recognized because of the often sedentary lifestyle of some cats. Physical examination may reveal and easily elicited cough on tracheal palpation, wheezing (audible with or without a stethoscope), tachypnea, or respiratory distress with a pronounced "expiratory push". Cats presenting with any of these historical or clinical signs are candidates for thoracic radiography. Thoracic radiography can help rule out cardiac, pulmonary parenchymal or pleural cavity disease which may cause cough or respiratory distress. If radiographs are suggestive of one of these disorders, other more targeted diagnostics can be considered.

Thoracic radiographs in asthmatic cats may be normal; or they may show some of the following abnormalities: bronchial or bronchointerstitial patterns, hyperinflation, and lung lobe collapse (most frequently the right middle lung lobe). In many cases, they are surprisingly non-specific. It is important to remember there are other airway-oriented diseases which may mimic signs of feline asthma, including chronic bronchitis, lung worms, migrating ascarids and heartworm associated respiratory disease (HARD). This is particularly critical prior to recommending therapy which specifically targets the allergic inflammatory cascade. Hyperinflation which is at least in part reversible with a bronchodilator (visualized radiographically) is supportive of a diagnosis of asthma; no change in hyperinflation might be more consistent with end stage chronic bronchitis/emphysema. Enlargement of pulmonary arteries is a fairly consistent change with HARD but not asthma.

At this point, other ancillary diagnostics to rule out the aforementioned differentials can be performed including a complete blood count looking for peripheral eosinophilia (present in about 20% of the cases of asthma and with many of the parasitic diseases), fecal flotation and baermann testing or a course of an antihelmintic to rule out lungworms and ascarids, and a heartworm antibody and/or antigen test. Multiple baermann tests may be needed for definitive diagnosis of lungworm infection since larvae are intermittently coughed up and swallowed to appear in the feces. There are currently no clinically available tests to assess for pre-patent ascarid infections (ie stage of migration through the lung prior to establishment in the small intestine with shedding of eggs in the feces). Heartworm antibody and antigen testing are insensitive and while positive tests in light of appropriate clinical signs and pathologic findings would support a diagnosis of HARD, negative results would not definitively rule HARD out. Regular use of selamectin can prevent HARD; it can also be used to treat ascarids and potentially lungworm infection.

Ultimately bronchoalveolar lavage fluid should be collected for cytology and culture; the latter to rule out a secondary bacterial infection which may be exacerbating clinical signs. Lavage fluid can be collected by a blind technique via an endotracheal tube, [2] or via bronchoscopy. Classic cytologic findings with feline asthma include an increase percentage and number of eosinophils, although mixed inflammation (especially with neutrophils) is fairly common. A positive culture must be interpreted in conjunction with cytology. That is, a positive culture in the absence of septic, suppurative inflammation on cytologic examination more likely reflects transient colonization of the lower airways or contamination. Secondary infections in feline asthma are uncommon.

Because asthma is postulated to be allergic in nature, allergen-specific IgE testing can be performed. This can be done either by intradermal skin testing which evaluates IgE bound to mast cells in the dermis, or by serum testing which evaluates circulating IgE. Care must be taken in selecting the laboratory for serum testing, as there are no regulatory bodies ensuring accurate results, and widely discrepant results between laboratories have been found. [3] False positive and false negative test results

are not uncommon. Concurrent use of glucocorticoids has been shown to diminish skin test positivity to allergens in experimentally asthmatic cats, but a 2 week withdrawl was adequate to restore skin reactivity in this study. [4] In contrast, glucocorticoids do not appear to impact serum allergen-specific IgE testing. The value of running skin and serum allergen-specific IgE tests is to try to determine which allergens are implicated in disease. This is important in trying to make recommendations for allergen avoidance. Additionally, in the future, allergen-specific immunotherapy[5-7] may become a viable option for pet cats with this disease and knowing which allergens to use in the allergy shots is important. Other diagnostic tests that are not routinely available but may provide additional evidence of airway hyperresponsiveness or remodeling changes, respectively, include pulmonary function testing and high resolution computed tomography.

Additional research is being performed to find "biomarkers" of disease which might be of use in the diagnosis, disease stratification, assessment of response to therapy, or prognosis. There are a limited amount of species-specific reagents which can be used in cats and some assays are not sensitive enough to detect very small quantities of relevant mediators. For example, in one study, attempts to assess differences in cytokines between cats with asthma and chronic bronchitis were not fruitful. [8] In this study bronchoalveolar lavage fluid was used to detect these cytokines, and collection of BALF is not easily applicable for repeat collection (e.g., to monitor efficacy of therapy). Thus, less invasive diagnostics that still reflect the local microenvironment should be performed. More recently, there has been work to investigate the utility of exhaled breath condensate from cats. This can be collected non-invasively with cats spontaneously breathing and moving in an airtight box, with an outlet that can cool and trap expired gases. The exhaled breath condensate is more dilute than BALF, making highly sensitive diagnostics a requirement. Pilot work is being performed to evaluate exhaled breath condensate using nuclear magnetic spectroscopy to screen for unique biochemical fingerprints that could correlate with a diagnosis of asthma, or a flare up of inflammation. The hope is that eventually this work can be translated to a bed-side test.

**SUMMARY**

A hypersensitivity to aeroallergens has been implicated in the pathogenesis of feline asthma. With an increasing emphasis for targeted drug therapy (i.e., medications which alter specific aspects of the inflammatory cascade) in place of broad "immunosuppression", it is critical there is confidence in the diagnosis of feline allergic asthma. As many other airway-oriented diseases have similar clinical and radiographic features of allergic asthma, additional testing is necessary to reach a definitive diagnosis.

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# NOTES

# WHAT TO DO WHEN FEELING BLUE: NOVEL TREATMENTS FOR FELINE ALLERGIC ASTHMA (ASIT AND BEYOND)

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## CURRENT STANDARD OF THERAPY FOR FELINE ASTHMA

There is no cure for feline asthma at this time. There are three major treatment recommendations for cats with asthma used in practice: environmental modulation, bronchodilators, and glucocorticoids. These treatment options focus on decreasing environmental triggers of allergic symptoms, reducing bronchoconstriction and blunting airway inflammation. The latter two treatments are unfortunately working after the allergic cascade has already been well established and are only palliative.

It would be ideal to remove the clinically relevant allergen(s) from the cats' environment, but in practice, that can rarely be done either because the allergen(s) are ubiquitous, or because they are not accurately identified. It is more realistic to modify the environment so the cat has minimal contact with non-allergenic stimuli that could trigger airflow limitation in a non-specific manner, such as powders, dusts (kitty litter), aerosols, and smoke.

Bronchodilators are crucial for the emergency management of cats presenting with bronchoconstriction. They are not recommended as monotherapy for management of chronic asthma since they do nothing to control (often subclinical) inflammation that can progress to irreversible architectural remodeling changes in the lung. Commonly used classes of bronchodilators include the methylxanthines and the beta 2 agonists. Oral or injectable forms (in conjunction with corticosteroids) work best for chronic therapy, and inhalant albuterol works well for acute "rescue" therapy. Albuterol is a short acting beta-2 agonist which is very commonly prescribed. There is evidence in humans with asthma, and more recently in cats, that frequent use of inhalant racemic albuterol may paradoxically contribute to airway inflammation. [1]

Glucocorticoids are considered the mainstay of therapy for asthma as they break the cycle of inflammation. Steroids may be given orally, by injection, or as inhalant therapy. The author prefers use of the oral form initially, and if effective, a switch can be made to the inhalant form while gradually tapering off the oral form. The inhalant forms have been shown to decrease airway inflammation in asthmatic cats, and have fewer systemic endocrine and immunologic side effects than the oral form. [2,3] There has been work in an experimental model that reduction of eosinophilic inflammation can be achieved with a lower dose of inhaled steroids—in other words, giving more is not always better as the effects of the inhalant steroids may plateau. [4] The inhalant form is not recommended for emergency management of this disease as it takes several days to have an effect. In emergency situations, injectable steroids such as dexamethasone (but not repositol depomedrol) are preferred.

## OTHER THERAPEUTICS

It would seem rational to design therapy which could block the effects of the allergic asthmatic cascade. Antagonists of inflammatory mediators and smooth muscle constriction, modulators of T lymphocyte activity, cytokine inhibitors, antibody blockade (especially IgE), and small molecule inhibitors (e.g., tyrosine kinases) and are thought to be reasonable targets. Other "immunomodulators" may work on multiple aspects of the allergic inflammatory cascade (e.g., allergen-specific immunotherapy, stem cells). Not all of these have been evaluated in cats, and the therapies mentioned below that have been tested have been done so in experimental models of feline allergic asthma.

**Miscellaneous drugs that blockade inflammatory mediators:** It is appealing to specifically block the physiologic function of mediators released after mast cell degranulation, as there are many drugs available to do this (e.g., antihistaminics, antiserotonergics, leukotriene receptor antagonists). Cetirizine,

a 2nd generation selective histamine receptor 1 antagonist that has effects both dependent and independent of histamine antagonism, was also evaluated for suppression of eosinophilic airway inflammation in experimentally asthmatic cats, with no significant beneficial anti-inflammatory or immunological effects. [5] Serotonin is known to cause bronchoconstriction in cats, and cyproheptadine, a serotonin antagonist, was shown in asthmatic cats to block airway smooth muscle constriction *ex vivo*. [6] Additionally, the cysteinyl leukotrienes are implicated as important mediators in human asthma, which is why leukotriene receptor antagonists (eg, zafirlukast (Accolate) or montelukast (Singulair)) help a subpopulation of human asthmatics. However, in experimental studies, neither cyproheptadine nor zafirlukast had an effect on decreasing eosinophilic airway inflammation in asthmatic cats. [2] As the pharmacokinetics of cyproheptadine suggest that in some cats a higher dose (8 mg twice daily instead of the standard 2 mg twice daily) may be needed, the higher dose of cyproheptadine was also tested in experimentally asthmatic cats. The results of this study again failed to show benefit of cyproheptadine on eosinophilic airway inflammation. [5]

**Allergen-specific immunotherapy**: As mentioned previously, the current strategy for treating asthma focuses on relatively late events in the allergic inflammatory cascade to minimize the effects of inflammation and reverse bronchoconstriction once they have been triggered. To cure asthma, strategies should target early events in the allergic cascade. For example, allergen avoidance has the potential to be curative. Unfortunately, there are practical difficulties in both identifying all clinically relevant allergens in cats as well as completely eliminating them from the environment (many are ubiquitous). However, there is another treatment that has potential to cure asthma: allergen-specific immunotherapy (ASIT). In humans, the only potential curative treatment for allergy is ASIT, also commonly called allergy shots. While it is used most commonly for allergic rhinitis and anaphylaxis, it has been successfully used for allergic asthma. The remainder of the talk will discuss advances in ASIT as a potential curative treatment for feline asthma.

The first step in assessing ASIT was to develop an abbreviated protocol called rush immunotherapy (RIT) to be able to evaluate if the treatment was working within a shorter time period (conventional ASIT takes months to years to work). RIT involves rapid loading of serially increasing doses of allergen to which a patient has been sensitized. The first protocol tested in experimentally asthmatic cats involved a combination of intranodal and subcutaneous allergen injection. [7] While there were significant reductions in airway eosinophilia, there were also (some severe) side effects. This was the first promising step in documenting ASIT could effectively blunt eosinophilic airway inflammation but safety needed to be improved.

Since asthma is triggered by mucosally delivered allergens, and since there were studies in humans suggesting benefit to mucosally delivered ASIT, a comparison of the safety and efficacy of mucosal versus subcutaneous RIT protocols was warranted. This study, in experimentally asthmatic cats, involved randomizing cats to receive either subcutaneous (SC) or intranasal (IN) RIT, followed by weekly maintenance injections for six months. [8] Endpoints included clinical respiratory scores after allergen challenge, bronchoalveolar lavage fluid eosinophil percentage (% BALF eosinophils), and cytokine concentrations in BALF. Additionally adverse events during the RIT administration were recorded. Results of the study showed that clinical respiratory scores after allergen challenge were significantly lower by month 6 of the study compared with baseline in both SC and IN RIT groups. The % BALF eosinophils significantly decreased in both groups (overall average at baseline was 62 ± 12% versus at month 6, 14 ± 6%); importantly ten of twelve cats had % BALF eosinophils return to the reference range for healthy cats (ie, <17%) by the end of the study—suggestive of a cure! Restoration of the abnormal immune response from a predominantly T helper 2 cell driven process (predominantly IL-4) to T helper 1 cell immunity (predominantly Ifn-γ) was shown, as the BALF IL-4: Ifn-γ ratio went from an average of 2.4 to 1.0 between the start and end of the study. The bottom line of the study was that while either protocol was associated with decreased eosinophilic airway inflammation and could be used, the subcutaneous protocol subjectively demonstrated more consistent resolution of clinical signs of bronchospasm after aerosol challenge with allergen and was slightly preferred.

Although in this experimental model, there was tremendous promise for ASIT, it must be remembered that a major hurdle for translational application of ASIT in pet cats is the accurate identification of allergens to which a patient has been sensitized. Recall that it is straightforward to assess ASIT in experimental asthma because the sensitizing allergens are known, but in pet cats allergenic triggers may be intermittent (for example, seasonal) or their identification may be impaired by concurrent therapy with glucocorticoids. As an additional point of confusion, identification of allergen-specific IgE does not necessarily imply that allergen is the driving force behind the type 1 hypersensitivity in the lower respiratory tract; IgE can be produced but might not trigger mast cell degranulation. To try to answer the question of which test might be best in cats to identify aeroallergen sensitization, intradermal skin testing (IDST) and serum allergen-specific IgE determination using an FcεRα -based ELISA were compared using experimentally asthmatic cats sensitized with either bermuda grass allergen (BGA) or house dust mite allergen (HDMA). [9] The sensitivity of the IDST was greater than serum allergen-specific IgE determination, meaning that it might serve as a better screening test. However, both tests were specific, meaning that either can be used to guide selection of allergens for ASIT. A second part of this study compared two different commercial laboratories that perform allergen-specific IgE determination in cats, one using the FcεRα -based ELISA and the other using an enzymoimmunometric assay. The enzymoimmunometric assay produced unreliable results, including failure to detect BGA-specific IgE as well as inappropriate identification of allergens to which the cats had not been sensitized even in samples in which IgE had selectively been destroyed by heat inactivation. It is critical that future studies be performed to more rigorously evaluate the accuracy of diagnostic laboratories offering feline allergen-specific IgE testing if ASIT is to become a viable treatment option for pet cats with allergic asthma.

Because there is likely to be some difficulty in appropriately identifying sensitizing allergens in all cats with allergic asthma, the question arises as to whether inadvertent "inappropriate" selection of allergens would confer cross-protection against the clinically relevant allergens. This was addressed in an additional study in which cats were sensitized with one allergen and administered a different allergen for RIT. [10] Results showed that an irrelevant allergen could also decrease eosinophilic airway inflammation, but that the underlying immunologic mechanism was different than in cats sensitized and treated using the same allergen that also had reduction in eosinophilic airway inflammation.

Before recommending ASIT to pet cats, one important issue is what to do about concurrent glucocorticoid therapy--that is, pet cats are symptomatic and need treatment during the time that allergens are identified and ASIT is administered and glucocorticoids suppress the immune system. A subsequent study evaluated RIT concurrent with either oral or inhaled steroids. [11] Importantly, RIT was found not to be effective when given concurrently with oral steroids. Thus inhalant steroids should be administered to control airway inflammation yet allow beneficial immunomodulation by ASIT.

**Allogeneic adipose-derived mesenchymal stem cells (aMSCs):** Mesenchymal stem cells have emerged as novel immunotherapeutic options for a variety of inflammatory lung diseases, including asthma. Their beneficial role is likely mediated through paracrine effects which modulate the immune system and not via incorporating into the tissue and providing cells for regeneration. Their effects are likely dependent on multiple factors, including but not limited to the stage of asthma (acute versus chronic), number of MSCs/infusion, timing of administration, and number of doses. Our laboratory has evaluated administration of allogeneic adipose derived aMSCs in an acute model of feline allergic asthma (which would mimic early onset disease prior to permanent remodeling changes in the lung). [12] Cats were given aMSCs after experimental asthma induction using Bermuda grass allergen and followed longitudinally. Results suggested this administration of aMSC cells is safe, even with repeated dosing. There was a modest reduction in airway eosinophilia and some aspects of the immune response were altered in the early phases of this project. Cats were followed for 9 months total, with ventilator-acquired pulmonary mechanics and computed tomography being employed at latter time points to evaluate airway hyperresponsiveness and airway remodeling, respectively. A subsequent study evaluated effects of aMSCs in chronic established allergic asthma. Results (as yet unpublished) will be shared.

**Tyrosine kinase inhibitors**: Inhibition of tyrosine kinases, a group of proteins which regulate cell survival, growth and differentiation, has more recently been of interest for treatment of asthmatic patients. [13] Tyrosine kinases are broadly classified either as receptor tyrosine kinases (RTKs) by way of involvement in transmembrane signaling or non-receptor tyrosine kinases (non-RTKs) which are intracellular enzymes involved in other cell signaling pathways. Tyrosine kinase inhibitors are small molecule inhibitors which block the ATP binding sites of kinases. In asthma, the RTK c-KIT receptor has been associated with proliferation and degranulation of mast cells and eosinophils in humans and mice and seems to be a logical target for therapeutic intervention. There are a number of commercially available receptor tyrosine kinase inhibitors that have been used in cats, generally for cancer therapy. Results of one study in an experimental model of feline asthma documented efficacy in reduction of airway inflammation and improvement of pulmonary mechanics. [14] Toxicity of these small molecule inhibitors is a concern and the side effects must be carefully weighed against the potential benefits on airway inflammation and airflow limitation. In the non-RTK class, one TKI (a Janus Kinase or JAK inhibitor) has been shown to be safe and effective for reduction of airway inflammation in experimental feline asthma. [15]

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# NOTES

# MEDITATIONS ON FELINE ALLERGIC DERMATITIS FOR THE PRACTICAL-MINDED CLINICIAN

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## INTRODUCTION

While skin allergies in cats are often encountered, accurate diagnosis and successful management can be difficult to accomplish. This lecture will commence with a review some of the unique cutaneous manifestations of allergic skin disease in cats, and will follow with a discussion on the main types of allergic dermatoses encountered in cats. Given time restrictions, the presentation will be limited to a brief review of currently available diagnostic and treatment options, and will offer an update on some newer diagnostic techniques and therapeutic options that either have become available or may be on the horizon for use in feline dermatology.

## CUTANEOUS REACTIONS PATTERNS

There are several cutaneous reactions patterns that are unique to cats. These cutaneous lesions are often manifestations of either parasitic infestation or allergic dermatitis. The reactions patterns include miliary dermatitis, self-induced alopecia, lesions of the eosinophilic granuloma complex, and pruritus/dermatitis of the head and neck.

## SPECIFIC ALLERGIC DERMATOSES

The most common allergic dermatoses encountered in cats are flea bite hypersensitivity, environmental allergies, and cutaneous adverse reactions to foods. One publication reports the incidence of flea, food, and environmental allergies in cats with miliary dermatitis, eosinophilic granulomas, or self-induced alopecia as 70%, 17%, and 13%, respectively.[1] True prevalence rates are difficult to establish given variations between studies and the fact that an individual cat may be afflicted with more than one hypersensitivity disorder.

## FLEA BITE HYPERSENSITIVITY

There have been advances in the research of the cat flea biology and life cycle, and this information helps guide more targeted and effective therapies. There are various newer flea preventative and treatment products available for cats, some of which will be reviewed in the lecture.

## CUTANEOUS ADVERSE REACTIONS TO FOOD

### Incidence, food allergens, clinical features

The exact incidence of food allergies in cats is unknown, and reports in the veterinary literature vary widely. Several studies report the incidence between 1-6% of all feline dermatoses.[2,3,4] Food allergy may account for up to11% of cases of miliary dermatitis.[4] In one prospective study, 17% of cats with chronic pruritus, chronic vomiting, chronic diarrhea, or concomitant pruritus and vomiting and/or diarrhea were diagnosed with food allergy.[5]

In cats, the most commonly reported food allergens are beef, dairy products, and fish. These proteins account for nearly 90% of the cases documented in 10 different studies.[6] Chicken, lamb, pork, rabbit, egg, corn, clam juice, and wheat have also been implicated as food allergens in cats.[5-8] In a study of cats with chronic gastrointestinal symptoms, 50% of cats with food allergy were allergic to more than one ingredient.[9]

The most common clinical sign is pruritus, which is typically non-seasonal. Other signs include the various cutaneous reaction patterns, and much less commonly chin acne, vasculitis, angioedema, urticaria,

and plasma cell pododermatitis have been associated with food allergies cats.[10,11] While there are no clinical signs that are pathognomonic for food allergies in cats, cats confirmed to have food allergies often have pruritus and lesions affecting the head, face, ears, and neck.[12,13] Gastrointestinal signs may occur concurrently with dermatologic signs in 10-23% of cats with food allergies.[2,5]

**Diagnosis**

Serum testing, intradermal testing, salivary allergen-specific antibody testing, and gastroscopy testing have all been shown to be inaccurate in making the diagnosis of food allergies in cats.[4,9] The "gold standard" of diagnosis is to feed a strict elimination diet, achieve significant improvement or resolution of clinical signs, and then demonstrate worsening or relapse of signs upon diet challenge with the foods previously fed. The success of this test relies greatly on diet selection, duration of the diet trial, and owner compliance. As there is nothing inherently hypoallergenic about any particular protein,[8] it is necessary to obtain a thorough dietary history in order to select the best elimination diet for an individual cat. There are three categories of elimination diets: home-cooked novel protein diets, commercial novel protein diets, and hydrolyzed diets.

**Elimination diet trial**

Examples of "novel" proteins include venison, duck, lamb, rabbit, goat, lobster, kangaroo, pork, and ostrich, and potentially fish for cats that have not previously eaten fish.[8,10,14] Several commercial prescription novel protein diets are available for cats: Hill's® Prescription Diet® offers d/d® Feline Skin Support Duck & Green Pea Formula and d/d® Venison & Green Pea Formula, and Royal Canin® Veterinary Diet offers Hypo Selected Protein PD (pea and duck), PV (pea and venison), and PR (pea and rabbit). There are now numerous over-the-counter (OTC) "limited" ingredient or "novel" protein diets available, but given the potential for protein contamination OTC diets are not recommended for use as test diets. Home-cooked diets are preferred because the clinician and the owner have complete control over the ingredients, additives and preservatives are eliminated, the diet can be more specific tailored to the patient, and a small percentage of food allergic cats may not improve on a commercial diet. But many home-cooked diet recipes have been found to be nutritionally inadequate.[15] Therefore, it is advisable to have a nutritionally balanced recipe formulated by a board certified veterinary nutritionist, especially if the diet will be fed for more than three weeks or if the cat is not fully mature or has concurrent medical concerns. While in-person access to veterinary nutritionists is limited, balanced recipes in veterinary textbooks and internet-based nutrition consultation services are currently available.

Rayne Clinical Nutrition is a relatively new company that produces limited whole ingredient diets that undergo less processing than other commercial diets. Novel protein diets offered by Rayne Clinical Nutrition include kangaroo, rabbit, pork, and cod, mixed with either squash, potato, or chickpea, depending on the protein.

Currently there are three hydrolyzed diets available for cats: Hill's® Prescription Diet® z/d® Low Sensitivity and z/d® ULTRA, Royal Canin® Hypoallergenic HP, and Purina Veterinary Diets® HA Hypoallergenic® Feline Formula. Hill's® Prescription Diet® z/d® ULTRA is a canned formula, while the others are available as dry kibble only. Studies have demonstrated that up to 50% of dogs hypersensitive to a protein may experience worsening of their clinical signs when fed a hydrolyzed diet derived from that protein.[16] This emphasizes the importance of obtaining a thorough dietary history prior to selecting a specific elimination diet for an individual patient.

## FELINE ATOPIC DERMATITIS

**Reviews and updates on allergy testing**

Once the clinical diagnosis of environmental allergies has been made, testing may be performed to either identify allergens that can be avoided, or select allergens to include in allergen-specific immunotherapy. Current options for testing include intradermal and serum allergy testing. A study published in 2011

demonstrated no difference between healthy and atopic cats in terms of the proportion of cats that tested positive with serum allergy testing.[17] Another study from 1993 comparing the results of intradermal tests to a commercially available ELISA serology test in atopic cats concluded that the ELISA was not a useful diagnostic test.[18] Interpreting intradermal test reactions can be difficult in cats, as reactions may be weak and transient, even to histamine solutions. Some practitioners inject fluorescein dye intravenously at the start of the intradermal test, and then evaluate the reactions utilizing a Wood's lamp. Despite the challenges encountered with testing, response to immunotherapy using allergens selected with these testing methods can be acceptable. But given the limitations of currently available testing methods, a more accurate and easier to interpret testing method for cats would prove valuable.

The percutaneous testing method, also referred to as "prick testing," introduces allergens into the top-most layers of the skin via the use of a special applicator to scratch or prick the skin. In people, the results of percutaneous tests are thought to correlate more accurately with clinical allergy than the results of intradermal tests.[19] In dogs, the results of intradermal tests were found to be more easily interpretable than the results of percutaneous tests.[20] In a pilot study by Rossi *et al*., percutaneous testing was performed in ten client-owned clinically healthy cats. The objectives of the study were to compare the skin test responses to percutaneous and intradermal testing of control solutions, and to evaluate the efficacy of two different percutaneous test applicators (Greer Pick, Greer Laboratories, Lenoir NC, and Duotip-Test II, Lincoln Diagnostics, Decatur IL). The authors found that percutaneous reactions to histamine were easier to read and better demarcated than intradermal injection reactions, and that positive reactions to histamine solutions using the Greer Pick were superior to those from the Duotip-Test II.[21] Further research is needed, but the results of this study are promising, as better environmental allergy testing methods may lead to better management of our allergic feline patients.

**Treatment options**

Other than a few exceptions, treatment options for environmental allergies in cats have not changed much over the past years. Treatments including antihistamines, essential fatty acids, glucocorticoids, and cyclosporine (Atopica®) have been available for several years. Topical treatments can be helpful in some cats, especially in light of research identifying epidermal barrier dysfunction as playing a key role in the development of atopic dermatitis in people and in dogs. Somewhat newer options include topically applied ceramides and fatty acids such as Allerderm® Spot On or Dermoscent® Essential 6 Spot-on Skin Care.

**Allergen specific immunotherapy**

Allergen-specific immunotherapy is the treatment of choice of most dermatologists for the long-term management of atopic dermatitis in cats. Response rates in the literature vary from 45-100% in open studies,[22] but in personal communication many dermatologists report success rates between 70-80%. Subcutaneous injections are the most common method of delivery of allergens to cats for immunotherapy. While many cats are tolerant of subcutaneous injections, some are not, some may have reactions to the injections (even with very diluted concentrations of allergens), or some cat owners may not be able to administer the injections. There now exists an alternative method to deliver allergy serum, sublingual immunotherapy (SLIT).

While SLIT has been in use in people for over 50 years, this method of allergy serum delivery has only been introduced recently into the field of veterinary dermatology. Given the various immunologic differences between the oral mucosa and the subcutaneous region, there may be a difference in patient response to SLIT versus subcutaneous delivery of allergens. Dr. DeBoer has demonstrated that 50% of dogs that were considered deemed to have "failed" injectable immunotherapy for various reasons responded very well to SLIT. To the author's knowledge there have not been any published studies evaluating the use of SLIT in cats. But anecdotal evidence suggests that cats can respond very well to SLIT, so this is a wonderful addition to our allergy treatment options. One main limitation to the use of

SLIT in cats is the need for consistent and frequent dosing. Most SLIT protocols require twice daily administration for the duration of treatment, which may be life-long.

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# NOTES

# LEISHMANIASIS I: THE CHALLENGE OF THE DIAGNOSIS

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## THE DIAGNOSIS OF CANINE LEISHMANIOSIS IN FIVE MINUTES

The diagnosis of canine leishmaniasis requires an integrated approach that includes careful documentation of the clinical history (living in an endemic area o visiting an endemic area in the past –even remote-; breed), a thorough physical examination, general clinic-pathologic tests (CBC, biochemical profile, urinalysis, urine protein/creatinine ratio and serum electrophoresis) and specific diagnostic tests.

Since high serological titres are closely associated with clinical disease and they are uncommonly detected in clinically healthy carriers of *Leishmania*, quantitative serology would be the first recommended specific assay for the disease. [1]

Table 1 presents clinical signs that develop dogs with leishmaniasis. [2] It is evident the marked pleomorphism of the disease. Some dogs when brought to the veterinarian present only one clinical sign (lameness, epistaxis) meanwhile others are severely affected and show a much richer clinical picture. Any dog with one of these clinical signs, living in an endemic area of leishmaniasis or that has visited an endemic area in the past, should be tested for the disease. The diagnostic approach according to the Leishvet Group Consensus is shown in Figure 1.

## PROBLEM 1: A SEROPOSTIVE DOG OR A PCR + DOG WITHOUT CLINICAL SIGNS IS A DOG WITH CLINICAL LEISHMANIASIS?

When approaching the diagnosis of leishmaniasis it is important to distinguish the *diagnosis of the infection* from the *diagnosis of clinical canine leishmaniasis*. Infected dogs that control the disease with an efficient immune response (in fact, the majority of dogs in endemic areas) do not show clinical signs, have low anti-*Leishmania* antibody titres and low parasitic loads when investigated with a RT-PCR. There are also some immunologic tests to detect these animals (IST with leishmanin, IL-10 , IFN-γ), although they are not available for the practitioner. This situation is becoming even more common now, after the launching of a vaccine in Europe (vaccinated dogs become low seropositive).

In most cases the clinician is interested in the diagnosis of the disease, and not in the detection of infected but healthy dogs. There are however several purposes for which diagnosis of *L. infantum* infection is carried out in clinically healthy dogs [who have no clinical signs and clinic-pathological abnormalities], for instance: (a) screening healthy dogs living or travelling in endemic areas; (b) select blood donors; (c) epidemiologic studies.

In any case, a dog that doesn't present clinical signs but is seropositive or PCR+ is not a case of clinical leishmaniasis. It should be considered only an infected dog. However, we know that this dog could be at an early stage of an active infection leading to clinical leishmaniasis (and develop clinical signs in the near future) and we also know that several circumstances can change the immune control of the infection (drug treatments, co-infections, age…). We have to do regular follow ups recheck to monitor these patients. Our suggestion would be:

(1) *Clinically healthy and PCR+ dogs*: perform quantitative serology. If negative, no additional tests are needed. If positive, proceed as below (2).

(2) *Clinically healthy but seropositive*: If the titre is high a CBC, biochemical profile and urinalysis are recommended. High titres (in general) are associated with clinical disease and it is expected that the majority of these dogs develop the disease shortly. With medium or low titres repeating serology is recommended (after 3, 6, 12, 24 months). The treatment of these dogs is highly controverted. Most authors do not recommend starting any treatment, unless the titres are really high. Recent publications suggest that the use of the immune-potentiating drug domperidone is the most adequate treatment for these dogs, together with the regular follow-up.

**PROBLEM 2: LEISHMANIASIS OR IMMUNEMEDIATED DISEASE (DLE, SEBACEOUS ADENITIS) IN AN INFECTED DOG?**

Leishmaniasis can mimic many diseases (discoid lupus erythematosus, pemphigus foliaceus, idiopathic sebaceous adenitis, zinc responsive dermatosis, systemic lupus erythematosus,..). In some cases the clinical signs are identical and the clinic-pathologic changes also similar. If an infected dog develops one of these diseases, then the diagnosis becomes challenging. Is this a dog with clinical leishmaniasis or a dog infected with *Leishmania* that now has developed a discoid lupus erythematosus?

Two diseases than frequently are difficult to diagnose are DLE and idiopathic sebaceous adenitis. The clinical signs are very similar to those of canine leishmaniasis and also the clinic-pathologic changes can be similar (*but not the treatment!*). If the dog has a high titre of anti-*Leishmania* antibodies, the diagnosis is straightforward. However, if the titre is low or only a +PCR result is available, the diagnosis is more challenging. In these cases, the question that has to be answered is: Are these lesions caused by the *Leishmania* infection or not? A skin biopsy and a histopathologic study, including immunohistochemistry for *Leishmania*, is the best way to solve this conundrum. The histopathologic changes in leishmaniosis are characteristic and the presence of *Leishmania* amastigotes in the lesions is conclusive.

**PROBLEM 3: A DOG WITH A DIAGNOSIS OF LEISHMANIOSIS AND CURRENTLY ON TREATMENT DEVELOPS NEW SKIN LESIONS. IS THIS CONSEQUENCE OF THE DISEASE, OF A CONCOMITANT DISEASE OR OF AN ADVERSE DRUG REACTION?**

This is also a common diagnostic dilemma. A dog with a firm diagnosis of leishmaniosis is treated and is responding well. After a few months (probably still on treatment with allopurinol) develops new skin lesions. Is this a consequence of the disease, of a concomitant disease or of an adverse drug reaction? [3]

A skin biopsy is usually necessary to identify the origin of the new lesions. However, there are a few findings and diagnostic test that can help to recognize a flare or a relapse of leishmaniasis:

1. Flares of the disease usually are accompanied by an increase in γ–globulines, in total serum proteins and in the titre of anti-*Leishmania* antibodies.
2. C-reactive protein has been associated with active flares of the disease (high values are reached a few days after the flare).

3. The parasite load in RT-PCR (bone marrow) usually is high during flares of the disease. [4]

## PROBLEM 4: A VACCINATED DOG DEVELOPS CLINICAL SIGNS COMPATIBLE WITH THE DISEASE; THE DOG IS TESTED AND IS SEROPOSITIVE. IS THIS A CASE OF VACCINE FAILURE OR A DIFFERENT DISEASE IN A VACCINATED AND SEROPOSITIVE DOG?

This is a relatively new challenge that has appeared in the last two years after the launching of the vaccine in Europe. Vaccinated dogs become seropositive (apparently with low titres, at least during the first year) and therefore serology is not as useful in the diagnosis as in non-vaccinated dogs. It is important to keep in mind that the vaccine doesn't protect 100% of dogs (in the field trials over 7% of vaccinated dogs develop clinical leishmaniasis). The company that launched the vaccine (Virbac) has developed a test that does not detect vaccine antibodies (Speed Leish K). If the test is positive, there is infection. This can help to distinguish vaccine failures (vaccinated dogs with leishmaniasis) from vaccinated dogs with other diseases. Table 2 also offers other diagnostic clues.

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Table 1. Common clinical signs in canine leishmaniasis (Ferrer, 2013). [2]

- *General signs:* lethargy, decreased or increased appetite, loss of body weight (cachexia and muscle atrophy in advanced cases), generalized lymphadenomegaly, splenomegaly, polyuria and polydipsia, vomiting and diarrhea.
- *Cutaneous:* non-pruritic exfoliative dermatitis with or without alopecia, erosive-ulcerative dermatitis mostly in mucocutaneous junctions, nodular or papular dermatitis, pustular dermatitis.
- *Ocular:* keratoconjunctivitis, blepharitis, anterior uveitis/endophthalmitis
- *Other less common signs:* lameness (erosive or non-erosive polyarthritis, osteomyelitis), epistaxis, mucosal lesions (oral, genital), myositis and polymyositis, atrophic masticatory myositis, cutaneous and systemic vasculitis

Figure 1. Diagnostic approach for sick dogs suspected of suffering from leishmaniosis in endemic areas (Solano-Gallego *et al*, 2011). [1]

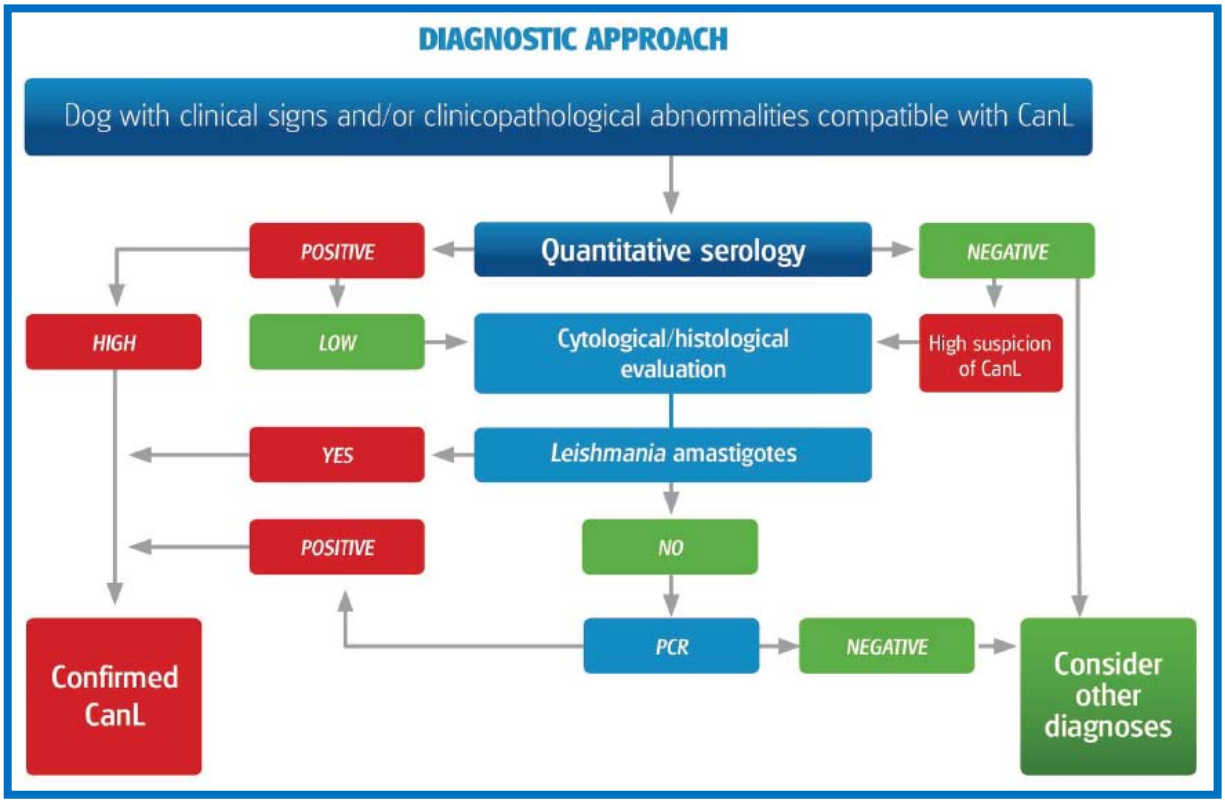

Table 2. Diagnosis of the vaccinated dog with clinical signs compatible with leishmaniasis.

| | Leishmaniosis in vaccinated dog (vaccine failure) | Different disease in vaccinated dog |
|---|---|---|
| **Qualitative serology (rapid in-clinic tests)** | Positive | Positive |
| **Speed Leish K (Kinesins)** | Positive | Negative |
| **Quantitative serology** | Positive (variable depending on severity) | Positive, low o medium titers |
| **Serum protein electrophoresis** | Typical of leishmaniosis | Normal or typical of the disease |
| **Q-PCR** | Positive, usually medium or high values (>100 parasites/ mL bone marrow) | Negative or low values |
| **Skin histopathology and immunohistochemistry** | Granulomatous inflammation / amastigotes | Depending on disease |

# NOTES

# LEISHMANIASIS II:
# NEW TOOLS FOR THE TREATMENT AND PREVENTION

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## THE TREATMENT OF CANINE LEISHMANIASIS IN TEN MINUTES

Dogs are considered having leishmaniasis when they present clinical signs or clinic-pathologic changes consequence of the *Leishmania* infection or of the immune response against the infection. These dogs, together with characteristic clinical signs and characteristic clinic-pathologic changes, present high anti-*Leishmania* antibody titres and high parasitic loads (qPCR on bone marrow or blood). A clinical staging system, designed to help in the selection of the most suitable therapy for each patient and also to establish a prognosis, it has been proposed by the LeishVet Group. [1] The proposed system includes four clinical stages, based on clinical signs, clinic-pathological abnormalities and serological status (see Table 1).

In *stage I* probably the best option currently is the use of domperidone. This immune-potentiating medication has demonstrated efficacy in some trials to prevent the progression from infection without clinical signs to infection and to control mild cases. It is recommended at the dose of 0.5 mg/kg/day, for one month. This treatment is repeated every four months (one month on and three off treatment).

In *stages II* and *III* the treatment is based on the use of anti-parasitic (leishmaniostatic or leishmanicidal) drugs. Pentavalent antimonials (as meglumine antimoniate) remain the drugs of choice for the treatment of leishmaniasis, as they usually induce clinical remission and a marked reduction on the parasitic burden. However, they  they do not prevent relapses and for this reason they are usually combined with allopurinol. A controlled clinical trial demonstrated a similar efficacy of meglumine antimoniate (100 mg/kg/q24h; 4 weeks) and miltefosine (2 mg/kg/q24h; 4 weeks), together with allopurinol (10 mg/kh/q 12h). [2]

Other drugs, such aminosidine, metronidazol, furazolidone, marbofloxacine, perifosine, OIPC, have been also evaluated, either alone or combined, with results clearly below the two drugs of reference. At present time there is no evidences to recommend the use of any of these drugs for the treatment of canine leishmaniosis.

After 4 weeks of treatment a recheck is done and if the clinical signs are markedly improved, the meglumine antimoniate is withdrawn and the patient remains on allopurinol for at least 6 months. Allopurinol can be discontinued when the combination of the following criteria is made: (1) Presence of complete physical and clinic-pathological recovery evaluated by a thorough physical examination, CBC, full biochemistry panel and urinalysis. (2) Marked decrease of antibody levels. In addition, allopurinol might be discontinued if it is not possible to control or decrease the xanthinuria with low purine diets or by reducing the drug's dosage, to avoid the risk of urolithiasis. [3]

In *stage IV* the treatment is directed to the control of the kidney disease. IRIS guidelines have to be followed (http://www.iris-kidney.com/guidelines/en/treatment_recommendations.shtml). Allopurinol can be used in early stages.

Clients should be informed that canine leishmaniasis is a chronic disease that requires a lengthy treatment and lifelong follow-up. [4] Patients should be evaluated after 1, 3, and 6 months of treatment and then twice a year for life. Evaluation should include a thorough physical examination, CBC, biochemistry, urinalysis, and serology. Real-time PCR can be useful to identify a relapse (high parasitic load in sample). [5, 6]

## IMMUNE-POTENTIATING / IMMUNE-MODULATING THERAPY

Clinical leishmaniasis is both an infection and an immunodeficiency. Dogs with clinical leishmaniasis develop a type of immune response that is unable to control the progression of the infection and the development of lesions and clinical signs. Sick animals show signs of immuno-deficiency (T-cell exhaustion) [6] and immunopathologic abnormalities. Numerous attempts have been made to help the immune system to control the infection (immunotherapy), including:

(1) Non-specific immune potentiating drugs. Domperidone is the best example in this group. The drug is currently marketed in some European countries to prevent leishmaniosis and to treat mild/early disease. Domperidone is a prolactinogogue drug that induces an increase of the T cell responses and of the phagocytic function of macrophages and neutrophils. Controlled trials have demonstrated that is a safe and effective alternative for the treatment of early/mild cases and seropositive animals. A recent trial also suggested that P-MAPA (a protein aggregate magnesium-ammonium phospholinoleate-palmitoleate anhydride immuno-modulator) has potential as an immunotherapeutic drug in leishmaniasis, since it assists in reestablishing partial immunocompetence of infected dogs. TLR activators (imidazoquinolines: imiquimod, resiquimod) are clearly helpful in cutaneous leishmaniosis and are promising in visceral leishmaniosis.

(2) Cytokines. There are a few experimental trials using γ-IFN, IL-12, anti-IL-10 and an IL-10 receptor antagonist, but the results have been only partially satisfactory or inconsistent. These treatments are very expensive and are not available for use in clinical cases. Only canine γ-IFN has been marketed so far (in Japan).

(3) Vaccines. There are several studies and trials demonstrating that vaccines can be used as therapeutic drugs in dogs with clinical leishmaniasis. [8] In a clinical trial in Brazil the Leishmune® vaccine reduced the clinical signs and the parasite load, modulating the outcome of the infection and the dog's potential infectivity to phlebotomines. In another trial, the subunit vaccine Leish-111f + MPL-SE was effective in the treatment of dogs with mild disease but not of dogs with severe clinical leishmaniasis. In Spain, an autologous (auto) - vaccine prepared with parasites isolated/cultivated from the ill dog is marketed.

Immunotherapy in canine leishmaniasis is certainly challenging, but has clearly some advantages that make it very attractive. Less and milder side effects than traditional chemotherapy, absence of resistance and the possibility of using it in combined protocols together with parasiticidal drugs, are some of them.

## THE PREVENTION OF CANINE LEISHMANIASIS

The prevention of leishmaniasis should follow an integrated approach to reduce the risk of the infection/disease development, the parasite transmission and the geographic spread. [1, 6, 9] The measures will be different for dogs living in endemic areas as compared to non-endemic areas.

*Dogs living in endemic areas:*

1. Interventions that reduce sand fly bites: (i) keeping the dog indoors during the sand fly season from dusk to dawn; (ii) reducing the microhabitats favourable to sand flies in the vicinity of the house or in locations where the dog spends time; (iii) using domestic insecticides.

2. Use of insecticides with rapid and residual activity is essential to protect the dog. Various insecticide formulations have been evaluated under laboratory and field conditions with excellent results showing reduction of parasite transmission in dogs. For protection of dogs it is crucial to provide owner education on the maintenance of appropriate insecticide throughout the period of sand fly activity.

    a. Spot-on formulations:

        i. Permethrin/imidacloprid combinations provide repellent (anti-feeding) activity against Phlebotomines for three weeks. It is recommended to repeat administration every 3 weeks in dogs.

    b. Collars:

        i. Deltamethrin-impregnated collars prevent phlebotomine feeding for a period of 5 – 6 months.

        ii. Flumethrin impregnated collars has shown encouraging results in the field. At present, the use of these collars for preventing sand fly bites is considered off-label.

3. In addition, the vaccination could be considered in healthy dogs. In Europe only one vaccine (excreted-secreted purified *L. infantum* antigen – Virbac - CanisLeish) is currently licensed. The decision should be based upon the following characteristics of the dog: age, breed, life style and use, habitat, reproductive status. The individual benefit/risk for each dog will determine if vaccine use is appropriate. If vaccine use is elected, quantitative serology (IFAT, ELISA) must be negative. A negative qualitative rapid test requires confirmation by a quantitative method (risk of false negative results).

4. In addition to insecticide use and if vaccination is not indicated, the use of immunomodulatory drugs is an option. Domperidone is the only drug currently licensed for this indication.

*Dogs travelling to endemic areas:*

a. Dogs travelling for less than 3 weeks, use of topical insecticide spot-on formulation should be applied at least two days before travelling/exposure.

b. For longer or more frequent trips preventative measures should be the same as for dogs living in endemic areas.

c. Imported dogs from endemic areas should be tested for this infection prior to breeding.

It is important to underline that all infected dogs (healthy or sick) should be treated with insecticides continuously to avoid parasite transmission. Recent data clearly indicates that vertical transmission occurs. In non-endemic areas (US) vertical transmission probably plays a major role in the spreading of infection/disease in dogs. In non-endemic areas it is recommended to breed only non-infected dogs.

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Table 1. Clinical staging of canine leishmaniasis (Solano-Gallego et al 2011). [1]

| Clinical stage | Serology | Clinical signs | Clinico-pathologic changes | Treatment | Prognosis |
|---|---|---|---|---|---|
| Stage I<br>Mild disease | Negative or low positive | Mild clinical signs | Absence of changes<br>No renal changes | Scientific neglect<br>Allopurinol | Good |
| Stage II<br>Moderate disease | Positive (low, medium or high) | Classical clinical signs: Lympadenopathy, skin lesions | Mild anemia, HyperGGlob, Hypo-Ab<br>Sub-stages<br>a) Normal renal profile<br>b) Creatinine <1.4 mg/dl y UPC = 0,5 – 1 | Allopurinol + Glucantime | Good to guarded |
| Stage III<br>Severe disease | Positive (low, medium or high) | Clinical signs immune-mediated (vasculitis, uveitis,..) | Renal disease IRIS I (UPC > 1) o stage II (creat 1.4 – 2 mg/dl) | Allopurinol + Glucantime<br>Follow IRIS guidelines | Guarded to poor |
| Stage IV<br>Very severe disease | Positive (usually medium or high) | Nephrotic syndrome or severe renal disease | IRIS stages III (creat > 2.5 mg/dl) and IV (UPC> 5) | Follow IRIS guidelines<br>Allopurinol | Poor |

# NOTES

# FRIDAY

# RESEARCH SHORT COMMUNICATIONS FRIDAY

**NORTH AMERICAN VETERINARY DERMATOLOGY FORUM**
**Phoenix, AZ**

**FRIDAY, APRIL 11, 2014**

**ACVD RESEARCH SHORT COMMUNICATIONS**

| | | |
|---|---|---|
| 9:00 | Ferrer | Profiling of the canine cutaneous microbiome by high-throughput amplicon sequencing |
| 9:15 | Hwang | Characterization of *Staphylococcus pseudintermedius* isolates from canine pyoderma and their interaction with canine keratinocyte cell line |
| 9:30 | Detwiler | Surveillance of meticillin-resistant *Staphylococcus pseudintermedius'* prevalence at colonizing sites of the veterinary dermatology patient and as a surface contaminant in a veterinary teaching hospital |
| 9:45 | Brazil | Anatomical patterns of colonization of pets with staphylococcal species in homes of people with methicillin-resistant Staphylococcus aureus (MRSA) skin or soft tissue infection (SSTI) |
| 10:00 | Davis | Dermatologic disease in pets residing with MRSA-infected owners |
| 10:15 | Davis | Effect of owner decolonization treatment on MRSA carriage in pets within homes exposed to a person with MRSA SSTI |
| 10:30 - 11:00 | | BREAK |

# Profiling of the canine cutaneous microbiome by high-throughput amplicon sequencing

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**Abstract:** Preliminary analysis of the canine cutaneous microbiome was undertaken. The main goals of this study were (1) to develop and standardize a methodology to characterize the canine cutaneous microbiome, (2) to identify the main groups of bacteria populating different regions of the healthy canine skin, (3) to standardize the methodology to assess changes in the microbiome in response to disease and to treatments. Four healthy dogs were swabbed in three different skin sites (perioral, abdominal, perianal). Hairs were also plucked from the same skin sites. Samples obtained from swabbing human skin and soil samples were used as positive controls. Blank swabs were used as negative controls. DNA was extracted from all samples using the Mo-Bio PowerSoil DNA Isolation Kit (MO-BIO Laboratories, Carlsbad, CA). PCR amplicons targeting the V1 - V2 region of 16S rRNA gene were sequenced in an Illumina MiSeq instrument. Principal Coordinate Analysis was used to visualize phylogenetic distances between bacterial populations from different body sites. Compared to control samples extracted from soil and from human skin, canine skin microbiome profiles formed a tight cluster. When the controls were omitted from the analysis, samples from the perioral, abdominal and perianal sites formed distinct clusters. Analyzed separately, dog skin and hair samples also clustered separately. A taxonomic analysis revealed the presence of >200 genera of bacteria inhabiting the canine skin surface. Taxonomic analyses are being performed to identify bacterial taxa which differ significantly among sites. The study will be extended to compare the skin microbiome of dogs and humans.

Funding: This study was supported by a departmental research grant, Tufts University

Conflict of Interest: None declared.

**Characterization of *Staphylococcus pseudintermedius* isolates from canine pyoderma and their interaction with canine keratinocyte cell line**

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**Abstract:** *Staphylococcus pseudintermedius* is the most common pathogen of canine skin infections. However, direct stimulation of canine keratinocytes by *S. pseudintermedius* has not been well investigated. The aim of this study was to investigate the characteristics of canine pathogen *S. pseudintermedius* and the interaction of *S. pseudintermedius* with canine keratinocyte cell line (CPEK). Nineteen *S. pseudintermedius* isolated from dogs with pyoderma were analyzed for antimicrobial resistance profile, the presence of virulence factors, and biofilm formation. The ability of these isolates to induce adherence, invasion, and immune responses in CPEK was also examined. *S. pseudintermedius* isolates showed multidrug resistance (three or more antimicrobial classes) and high prevalence of virulence factor genes (leukocidines, hemolysins, and enterotoxins). Biofilm-forming ability of *S. pseudintermedius* was found in 57.9% of isolates, displaying varying degrees of biofilm formation in a microtiter plate assay. All the isolates induced highly variable host cell responses, including cell adhesion, invasion, and production of proinflammatory cytokines such as IL-6 and TNF-α. The present results demonstrated that clinical *S. pseudintermedius* isolates can interact with canine keratinocytes, consistent with clinical manifestations of disease, although the detailed mechanisms remain unknown. These findings may provide not only useful information for the establishment of skin infections by *S. pseudintermedius* but new approaches for preventive or therapeutic strategies.

Source of funding: This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A1A2004).

Conflict of interest: None declared.

# Surveillance of meticillin-resistant *Staphylococcus pseudintermedius'* prevalence at colonizing sites of the veterinary dermatology patient and as a surface contaminant in a veterinary teaching hospital

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**Abstract:** The objective of this study was to survey the prevalence of meticillin-resistant staphylococcus (MRS), specifically meticillin-resistant *Staphylococcus pseudintermedius* (MRSP), in the dermatology environment and canine dermatology population. The first part of the study included a survey of the environment commonly associated with dermatology patients. The cultures were obtained using a Swiffer® collection method, sampling twenty indirect, direct, and negative control sites, for 60 total environmental sites. The second part of this survey study involved culturing the first 70 new referral or recheck canine dermatology patients, with client consent and no exclusions. Patients were cultured using saline moistened, mini-tip culturettes at the nares, oral mucosa and perineum. Including four saline controls, 214 total cultures were submitted. The environmental and canine cultures were isolated with mannitol salt agar and mannitol salt agar with oxacillin, with susceptibilities also performed. The environmental MRS isolates, including MRSP and the meticillin-resistant *Staphyloccocus schleiferi* subspecies, were dru-typed (direct repeat unit). Of 23 positive environmental cultures for MRS, 87% (20/23) were MRSP. MRS was isolated at 15% (3/20) negative control sites, 40% (8/20) direct sites, and 60% (12/20) indirect sites. Of the 70 patients, 42 (60%) presented with active or resolving bacterial pyoderma. MRS and MRSP were isolated in 21.5% (51/214) and 16.4% (35/214) of the canine patients, respectively. Meticillin-resistant *Staphylococcus aureus* and meticillin-resistant *Staphylococcus schleiferi v. coagulans* were isolated from 2.3% (5/214) and 4% (8/214) of the canine patients, respectively. Dru-typing identified seven MRS dru-types, three of which were newly added into the worldwide database.

This study was funded through Heska Corporation and an Endowed Research grant through Michigan State University College of Veterinary Medicine.

Conflicts of interest: None declared.

# Anatomical patterns of colonization of pets with staphylococcal species in homes of people with methicillin-resistant Staphylococcus aureus (MRSA) skin or soft tissue infection (SSTI)

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**Abstract:** Household environments are a potential exchange point for community- associated methicillin-resistant Staphylococcus aureus (CA-MRSA) and other coagulase-positive staphylococci (CPS). While sampling of the nares is the established protocol for MRSA screening in humans, standardized protocols for screening pets have not yet been established. Determining optimal sites for sampling multiple species of staphylococci simultaneously has merit, given that pets may carry veterinary pathogens S. pseudintermedius and S. schleiferi in addition to S. aureus. Survey data and multiple swabs (nasal, oral, inguinal, and rectal) were collected from 179 pets (dogs, cats, reptiles, birds, fish and pocket pets) living in households with an MRSA-infected person. CPS was identified in 74% of pets sampled. The overall prevalence of MRSA and methicillin-susceptible S. pseudintermedius was 8% and 18% respectively. One dog was positive for methicillin-resistant S. pseudintermedius. Overall, mammals had nine times ($p<0.001$) increased odds of carrying CPS than reptiles, fish, and birds. Within mammals, dogs had a four times ($p=0.015$) increased odds of carrying CPS than cats and other mammals. The mouth was the most sensitive (78% sensitivity) anatomical site for recovery of CPS. The most sensitive (92% sensitivity) combination of two anatomical sites was mouth-nares. These results support that S. pseudintermedius is more prevalent in pets than S. aureus, and suggest that the mouth is the most sensitive site to sample for recovery of CPS.

Sources of project funding were the American College of Veterinary Dermatology, the Morris Animal Foundation and Johns Hopkins Center for a Livable Future. This work was supported in part by a Commonwealth Universal Research Enhancement Program grant from the Pennsylvania State Department of Health (to E.L.).

Conflict of interest: None declared.

**Dermatologic disease in pets residing with MRSA-infected owners**

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**Abstract:** *Staphylococcus* species, including *S. pseudintermedius* (MRSP/MSSP) and occasionally methicillin-resistant *S. aureus* (MRSA), are leading causes of pet dermatologic disease. To identify risk of dermatologic disease in MRSA-exposed pets, 184 pets in 95 homes of people diagnosed with a MRSA infection were evaluated. Of these 184 pets, 113 (61%) were re-sampled three months later. Skin lesions and pet bedding were sampled when present. Staphylococcal species and methicillin-resistance were tested by PCR. Clonality of isolates was determined by pulsed-field gel electrophoresis. Owners reported that only 30% of pets had veterinary contact in the prior year. Dermatologic lesions were identified in 12 dogs, a cat, a snake and a bird at either or both visits. Of these 15 animals, nine dogs (75%) had MSSP-positive lesions. Pet beds were present in nine (60%) of these households. Overall, nine (13%) of 72 dogs had a MSSP-associated skin lesion during at least one visit, and three (8%) of 38 dogs sampled twice had persistent MSSP lesions. Mixed infections were present in two dogs: one with MSSA and one with MRSA. Another dog developed a MRSA surgical-site infection between the baseline and follow-up visits. The bedding of this dog was MRSA-positive at baseline and clonal (≥85% related) to the subsequent skin infection isolate. The MSSP isolate from pet bedding was clonal to the MSSP derived from the respective pet's lesion in five (71%) of seven households. These results confirm that MSSP pyoderma is common in dogs in the community. Pet beds are a reservoir of *Staphylococcus* species.

Sources of project funding were the American College of Veterinary Dermatology, the Morris Animal Foundation and Johns Hopkins Center for a Livable Future. This work was supported in part by a Commonwealth Universal Research Enhancement Program grant from the Pennsylvania State Department of Health (to E.L.).

Conflict of interest: None declared.

**Effect of owner decolonization treatment on MRSA carriage in pets within homes exposed to a person with MRSA SSTI**

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**Abstract:** Households are a transmission point for methicillin-resistant *Staphylococcus aureus* (MRSA). Owners likely serve as a major source of *S. aureus* exposure for pets. To evaluate whether treating people in MRSA-exposed households reduces pet carriage, all pets present in 95 homes of patients diagnosed with a community-acquired MRSA infection were sampled. Environmental surfaces were sampled (refrigerator, towel, faucet, TV, +/- pillow and shelf). Sixty-five percent of households were randomized to chlorhexidine baths and nasal mupirocin; pets were not treated. Pet and environmental samples were repeated three months later. After culture, isolates were determined to be MRSA via PCR. Risk associations for MRSA positivity were tested using adjusted logistic regression models controlling for household clustering. At baseline, human MRSA prevalence was 22% (77/355) and pet prevalence was 7% (13/179 pets). At follow-up, MRSA prevalence was 21% (53/247) people and 5% (7/130) pets, with 3% (3/113) of pets positive at both visits. Overall, 63% of homes at baseline and 50% at follow-up had at least one environmental site MRSA positive. Recent cleaning lowered rates of surface contamination, but reductions were not significant. Decolonization reduced MRSA carriage rates in the treated people and home environments compared to households not treated, but reductions were not significant. Risk of MRSA carriage in pets at follow-up was associated with baseline MRSA status (p=0.005), human carriage rates (p=0.002), household environmental contamination (p=0.10), and household randomization to decolonization (p=0.33). This study showed that randomization to decolonization treatment in people did not reduce the risk of MRSA carriage in pets.

Primary sources of project funding were Morris Animal Foundation and Johns Hopkins Center for a Livable Future. The American College of Veterinary Dermatology funded other aspects of this project. This work was supported in part by a Commonwealth Universal Research Enhancement Program grant from the Pennsylvania State Department of Health (to E.L.).

Conflict of interest: None declared.

# SCIENTIFIC SESSION PRESENTATIONS FRIDAY

# DORSAL THERMAL NECROSIS

Schick, AE

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## ETIOLOGY AND PATHOGENESIS

The critical functions from the cornified envelope, the epidermis, dermis and appendages can be lost with a significant burn[1]. Most burns in veterinary patients are caused by heat from fires, hot liquids, heating pads, driers and hot metals like wood stoves[2]. Of these, most of us are familiar with the dramatic and often extensive burns caused by heating pads, which can cause full thickness burns of large areas of the skin, often on the dorsum. Sustained exposure to sunlight combined with high ambient temperatures can cause burns that are very similar in appearance to heating pad burns. This type of burn has been reported in dogs and has been called 'dorsal thermal necrosis'[3]. As opposed to most other types of sun damage, which tend to affect light-colored animals or areas of sparse hair, dark haired dogs or those with patches of dark hair are affected[4]. The following dog breeds have been reported with dorsal thermal necrosis: Dalmations, Shar Pei, Rottweiler and Dachshund as well as mixed breed dogs with dark hair[3-4]. Black skin absorbs approximately 45% more solar radiation that white skin[4], so it is likely that these dogs are absorbing more damaging UV and visible radiation. Thermal burns in people are rare and associated with reduced perception of pain[4]. Many cases of dorsal thermal necrosis occur in dogs that have been accidentally left outside at high temperatures or in dogs that are taken on long walks/hikes during the summer. It is probable that these dogs could feel the heat and likely pain during the sun exposure but were unable or not allowed to move away from the heat.

## CLINICAL FEATURES

There is often a delay of several days to a week or more from the time of injury to presentation. Some dogs are presented to veterinarians immediately after the heat exposure for heat related problems like fever, lethargy, and dehydration. They have no overt clinical cutaneous lesions and are often treated with supportive care. The owners of some dogs presented to our clinic for dorsal thermal necrosis report that their dogs seemed to resent petting of their backs soon after exposure with increasing discomfort with time. Cutaneous signs include well-demarcated erythema to erosion and ulceration with deeper burns. The affected area is irregular but affecting the dorsal midline although one of our cases was more affected on one side presumably due to the angle of the sun during a long summer hike. In full-thickness burns, eschar will form and the skin will slough. Secondary infection, with purulent exudate and crust formation is usually present, especially with full-thickness burns.

## DIAGNOSIS

History of heat exposure is most helpful to diagnose dorsal thermal necrosis. Lesions confined to the dorsum with irregular well-demarcated borders strengthen suspicion of this disease. Cytology and possibly cultures for secondary infection management are important. Biopsies should be taken away from eschars, ideally at areas of erythema and skin thickening without ulceration[3]. Histopathology is consistent with full thickness thermal burn[4].

## CLINICAL MANAGEMENT

Control of secondary infections, pain management and wound care are essential in cases of dorsal thermal necrosis. Surgical removal of affected skin (once the affected area has declared itself) can speed healing. In cases with large surface area affected, sometimes multiple surgeries are required involving skin grafts and stretching techniques. For smaller areas or for owners where surgery is not an option, wound management with frequent rechecks can lead to a good outcome.

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# NOTES

# DIAGNOSIS AND MANAGEMENT OF SOLAR DERMATITIS IN COMPANION ANIMALS

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## PATHOGENESIS

The solar spectrum is comprised of approximately 40% visible light rays (400-700nm), 50% infrared light rays (700-20,000nm) and approximately 9% ultraviolet (UV) light rays (100-400nm)[1]. UVA rays (320-400nm) penetrate more deeply into the skin than UVB, and are associated with photosensitivity reactions[2,3]. Prolonged exposure to shorter wavelength UVB solar rays (290-320nm) causes phototoxicity (sunburn), directly damaging keratinocytes and causing superficial skin blood vessel dilation and leakage. Sun damage of epithelial structures subsequently leads to increases in inflammatory cytokines, prostaglandins and leukotrienes as well as toxic oxygen intermediates that perpetuate and amplify tissue injury by depleting antioxidants, recruiting inflammatory cells, and damaging connective tissue [2, 4-6]. Ultraviolet radiation can alter the skin immune system by decreasing the number of epidermal Langerhans cells [2,11]. Prolonged and repeated sun damage leads to keratinocyte proliferation, mutagenesis, atypia and premalignant actinic keratoses, which can progress to invasive squamous cell carcinoma [2]. Natural barriers to UV light damage include the stratum corneum, melanin, blood, and carotenes [2]. Melanin present in pigmented skin absorbs UV rays, which in most cases helps prevent deeper UV light penetration and actinic damage[1].

## CLINICAL SIGNS

In cats, solar dermatitis most commonly affects non-pigmented skin on the ear pinnae, nose, and eyelids. Early lesions present with non-pruritic erythema and fine scaling which progress to skin peeling and crusting; discomfort/scratching of the pinnae may occur as lesions progress, and pinnal margins may be slightly curled or take on a scalloped appearance[2,11]. Actinic keratosis or squamous cell carcinoma in situ can develop and appear as crusted non-healing lesions with slight erosion [11].

In dogs, solar dermatitis most commonly affects the white-haired and nonpigmented skin of short-coated breeds such as pit bull and Staffordshire bull terriers, bull terriers, boxers, Dalmatians, American bulldogs and whippets, but any dog with white or lightly pigmented hair and skin is at risk. Sun damage usually occurs on nonpigmented thinly haired areas such as the flank, inguinal and axillary areas, and the dorsal nose, but it can occur on the dorsal and lateral trunk and lateral legs as well as other areas [2]. In dogs that prefer to lie on one side of their body, lesions may be worse on the more chronically sun-exposed side.

The duration and intensity of sun exposure influences the degree of skin damage. The initial signs of actinic damage are erythematous scaly lesions, which may be tender. With repeated sun exposure, actinic folliculitis, follicular cyst formation, and dermal fibrosis occur [8]. In dogs with pigmented areas on their skin, there is often sharp demarcation between areas of normal skin with protective pigment and damaged nonpigmented skin[2]. With chronic sun exposure, damaged areas become thickened and scarred with comedones, erosions, ulcers, crusts, and draining tracts.[2] Secondary bacterial pyoderma is common.[9] Actinic or solar keratoses can occur and appear as non-healing erythematous, scaly to crusty macules and plaques which represent focal areas of abnormal keratinocyte proliferation/ differentiation which with time may progress to invasive squamous cell carcinoma [19,20]. With chronic solar damage, sun-induced skin tumors such as squamous cell carcinoma, hemangioma, and cutaneous hemangiosarcoma may occur. Although the dogs may lick affected areas, the pruritus associated with solar dermatitis is usually otherwise minimal, unlike dogs with allergic dermatitis. However, some dogs can have concurrent allergies and solar dermatitis.

## DIAGNOSIS

Diagnosing solar dermatitis involves consideration of a patient's signalment and clinical signs, and ruling out other causes for scaly erythematous dermatitis or folliculitis (*e.g.* bacterial, *Demodex* species, and dermatophyte infections). Ultimately, skin biopsy and histopathology are used to diagnose solar dermatitis and solar-induced neoplasia. Prior to biopsy, systemic antibiotics may be indicated for 2-3 weeks to ensure that secondary infection does not alter histopathologic interpretation. Additionally, clearance of secondary skin infection also increases the likelihood of correctly selecting solar-induced rather than infection-induced skin lesions for biopsy[10]. Biopsy can be performed by administering lidocaine locally and obtaining multiple skin punch or excisional biopsy samples of different lesions.

Since some of the histologic changes can be seen with other conditions such as bacterial folliculitis, the keys to a definitive histopathologic diagnosis are to include a complete history with the biopsy submission form, including signalment, degree of sun exposure, distribution of lesions, clinical description of lesions, response or lack of response to prior therapies, and current medications (including glucocorticoids) that could affect the histologic findings. Requesting a full histological description and interpretation by a veterinary dermatopathologist are recommended.

In early cases of solar dermatitis, histologic examination shows variable degrees of perivascular dermatitis, folliculitis, and dermal fibrosis, increased collagen accumulation or collagen damage. Epidermal hyperplasia with intraepidermal edema is seen, and vacuolated (sunburn cells) and apoptotic keratinocytes may be seen [2,6]. Solar elastosis (linear bands of degenerated basophilic elastin accumulation arranged parallel to the skin surface) may also be present. In chronic cases, histologic examination may show follicular cysts, pyogranulomatous inflammation, and precancerous actinic keratosis or squamous cell carcinoma.[2,9,10]

## TREATMENT FOR SOLAR DERMATITIS AND ACTINIC KERATOSIS

***Sun avoidance****:* The main treatment recommendation for solar dermatitis is restricting sun exposure by keeping the dog indoors during the day, especially between 9 a.m. and 3 p.m., which is considered the most intense UV radiation time[2]. Reflected sunlight (i.e. white concrete sidewalks or dog run flooring) must also be avoided [2]. If some sun exposure is unavoidable, then frequent (twice daily [11] ) topical application of a waterproof, high SPF sunscreen (SPF 30 products absorb more than 96% of UVB rays [12]) labeled as safe for babies and that protects against UVA and UVB should be used [2]. Sunscreen should be applied 10-15 minutes before sun exposure, and sunblock products which contain zinc should not be used in cats, and in dogs should be used very cautiously and only in areas inaccessible to the dog's tongue, as zinc toxicity can occur if the dog licks the product off [2]. Tattooing is ineffective, as the tattoo ink is deposited in the dermis, which does not protect the epidermis; additionally, colorants absorb or reflect visible light but have no protection against ultraviolet rays [20]. Having the dog wear a T shirt may be helpful to decrease sun exposure, however, it is often impossible to cover all at-risk areas of the skin.[2] A dog sun suit is available at www.designerdogwear.com, or talented clients may be able to sew a sunsuit for their pets using sun-blocking fabric available for people.

***Acute therapy***: To treat mild acute cases of solar dermatitis, topical application of a steroid (ie. 1% hydrocortisone applied daily to BID for a week), or a 5-7 day course of oral anti-inflammatory prednisone may be helpful to reduce erythema and discomfort [11].

***Non-medical options***: In cats with pinnal lesions, cosmetic amputation of pinnal margins may be necessary if progressive actinic lesions develop, but aggressive sun protection is required to prevent development of new lesions[2]. Depending on lesion location, actinic keratoses can also be potentially treated with surgery, cryotherapy, photodynamic therapy or laser ablation [2,11,19,20].

***Systemic therapies***: Carotenoids - To decrease sun damage, it has been reported in dogs that beta-carotene (30 mg orally BID for 30 days, then 30 mg/day for life) in combination with anti-inflammatory doses of oral glucocorticoids could be effective in early cases.[2,13] In cats, B-carotene and canthaxanthin (25mg doses of active carotenoids) have been used orally with mixed success [2, 7].

Retinoids and Vitamin A - Skin damage may also be reduced by administering oral retinoids such as acitretin 0.5 – 1mg/kg PO q 24 hours [2]. Potential side effects of retinoids include keratoconjunctivitis

sicca, mucocutaneous lesions, vomiting, diarrhea, musculoskeletal abnormalities, triglyceride elevations hepatotoxicity and teratogenicity [2,11]. Positive clinical response to retinoids should be noted within 4-6 weeks, and then frequency of medication can be reduced to an alternate day basis [11]. In limited human studies, oral Vitamin A 25,000IU/day given to people with actinic keratoses caused a 32% risk reduction in squamous cell carcinoma development; doses as high as 50,000-75000IU/day showed increased efficacy [22]. Due to expense of retinoids and difficulty in obtaining them for veterinary use, oral Vitamin A has been used anecdotally for canine solar dermatitis, however controlled clinical trials and published studies elucidating doses or side effects are not available. The suggested dosage of oral Vitamin A for dogs is 800-1000 IU/kg PO daily for 3 months, then the frequency is reduced to three times weekly [20].

Non-steroidal anti-inflammatories - Cyclooxygenase-2 (COX-2) overexpression and a potentially contributory role in epidermal carcinogenesis have been demonstrated in human actinic keratoses and cutaneous squamous cell carcinoma. Expression of COX-2 was studied in 18 cases of feline and canine actinic keratoses, and 36 cases of squamous cell carcinomas. COX-2 immunoreactivity was detected in all feline and canine squamous cell carcinoma and in 3/9 canine and 4/9 feline cases of actinic keratoses [23]. This suggests that COX-2 inhibition may be a helpful therapeutic option. In a case report of 5 dogs with chronic solar dermatitis, daily oral administration of firocoxib, a COX-2 selective inhibitor (5mg/kg/day; Previcox, Merial), caused clinical and histopathological improvement (reduced dyskeratosis) at 2 and 6 months follow up, as well as decreased COX-2 expression in post treatment skin biopsies [24].

***Topical therapies****:* Imiquimod - Actinic keratosis in people may be helped by using a topical immunomodulator such as imiquimod (Aldara—3M Pharmaceuticals). The mechanism of action of imiquimod involves induction of local antitumor immune responses by stimulation of lymphocytes, dendritic cells and macrophages [15-18]. Studies in dogs are lacking and in dogs with more extensive lesions of solar dermatitis, expense of Aldara may preclude its use. Imiquimod was used in one case of pinnal actinic keratosis and squamous cell carcinoma in a cat treated three times weekly for 82 days; the lesions resolved clinically and did not recur in 5 months of clinical follow up, although post treatment biopsies were not performed. Side effects observed included erythema, crusting, alopecia and mild discomfort [21].

Diclofenac gel – In humans with actinic keratoses, topical application of 3% diclofenac gel (Solaraze, PharmaDerm, part of Fougera Pharmaceuticals Inc., Melville, NY) has moderate efficacy with low morbidity. In vehicle-controlled studies, human patients treated for a mean of 60-90 days had complete resolution of 50-70% of treated lesions, compared to 20-44% of patients using the control vehicle (2.5% hyaluronic gel). [19] In a more recent study comparing efficacy of imiquimod to diclofenac for the treatment of actinic keratoses in people, 61 patients with actinic keratoses were randomized into three treatment groups to receive topical treatment with either diclofenac gel (twice daily for 12 weeks), imiquimod (twice per week for 16 weeks) or a control base cream (twice daily for 12 weeks), and were evaluated monthly for 6 months. Complete lesion clearance rates for diclofenac, imiquimod and the base cream at the end of the treatment and at the end of the total follow-up period were 19.1%, 20% and 0%, and 14.3%, 45% and 0%, respectively. Although diclofenac and imiquimod each had considerable efficacy in the treatment of actinic keratoses, the efficacy of diclofenac seemed to decrease after cessation of treatment [25]. Diclofenac gel has not been critically evaluated for the treatment of solar dermatitis in companion animals, but there are anecdotal reports of its use as being effective and well tolerated. [20]. It is now available as an economical equine liposomal formulation (Surpass 1% diclofenac sodium, Boehringer Ingelheim Vetmedica, Inc.St. Joseph, MO), and may be a potential topical treatment option for lesions which are too extensive for imiquimod.

**SOLAR INDUCED NEOPLASIA**

Three types of neoplasia in dogs and cats are associated with chronic solar damage to the skin. These are hemangioma, hemangiosarcoma, and squamous cell carcinoma. Each of these tumors may arise without chronic solar exposure. However, evidence of ultraviolet light radiation damage is often seen in tissues surrounding these tumors, especially when they are found in skin areas with high sun exposure. Interestingly, basal cell carcinoma and melanoma, that along with squamous cell carcinoma represent the most common sun-induced neoplasias of human skin, do not appear to be frequently induced by sun

damage in the dog and cat. Indeed in the dog, cat, and horse, these latter tumors often arise in sun-protected and/or haired areas of skin.

**HEMANGIOMA**

Several forms of hemangioma (HA) are reported, and HA is more common than hemangiosarcoma (HSA) in the dog, but less common in the cat. There is a strong correlation between chronic solar damage and the development of dermal HA in dogs[26]. As compared with non sun-induced HA, in which lesions are usually solitary, sun-induced HAs are often multiple and develop on the glabrous and sun-exposed, lightly-pigmented skin of the ventral abdomen and inguinum, medial thighs, and axillae. Whippets, Beagles, Basset Hounds, American Bull Terriers, and Dalmatians are predisposed.

Tumors initially appear as punctate discolorations on the skin, and then enlarge to form firm or fluctuant, red to purple dermal plaques to nodules varying in size from 0.5 to 4 cm. These tumors rarely metastasize, and morbidity is associated with excessive hemorrhage and secondary infection.

**HEMANGIOSARCOMA**

Hemangiosarcoma (HSA) caused by chronic sun damage may represent malignant transformation within sun-induced HA rather then *de novo* development of tumor. Thus, they most often occur as multiple growths on the ventral abdomen and axillae of dogs and the head and pinnae of white cats. These tumors are discrete or poorly-circumscribed, dark red to purple, fluctuant masses less than 2 cm in diameter. Similar to sun-induced HA, they rarely metastasize, and morbidity is associated with excessive hemorrhage and secondary infection.

**SQUAMOUS CELL CARCINOMA**

Squamous cell carcinoma (SCC) is an uncommon cutaneous malignancy in dogs and cats. In humans, more than 80% of cutaneous squamous cell carcinomas arise from or are associated with actinic keratosis, and some authors prefer the term "actinic carcinoma *in situ*" denoting similar pathology but without dermal invasion by neoplastic cells. SCC is also noted to develop in areas of chronic inflammation, injury, and viral infection. Sun-induced SCCs occur most frequently on the glabrous skin of the axillae and ventral abdomen in dogs, and on the non-pigmented pinnae, eyelid margins, and nasal planum of cats. Sun-induced SCC may be multiple and adjacent to evidence of actinic damage. Initial lesions may appear as persistent crusts and scabs. Tumors are slow-growing and often appear proliferative or crateriform. Older lesions are easily traumatized and ulcerated, leading to persistent serum drainage and/or hemorrhage. In dogs, secondary infection is common causing pain and further exudation.

**TREATMENT FOR SOLAR INDUCED NEOPLASIA**

Distinguishing between sun-induced tumors requires biopsy and histopathological examination of tissues, with suspicion heightened by the appearance of multiple lesions in areas of sun-exposure. Regularly-scheduled examinations for tumor development – similar to melanoma-screening in humans – is helpful for early detection of tumor development and therefore easier subsequent excision.

As mentioned earlier for actinic keratosis, individual tumors may potentially be treated with surgery, cryotherapy, photodynamic therapy (PDT) or laser ablation. Solar-induced hemangiomas may not require excision if they remain small. Radical surgical resection of squamous cell carcinoma and hemangiosarcoma should be attempted when possible. Pinnectomy and nosectomy in cats is an effective treatment, particularly when tumor margins are apparent. Reports of the success of cryotherapy, radiotherapy, and PDT vary widely[27-29], although most studies agree that these modalities should be restricted for treatment of smaller and more superficial lesions. As mentioned earlier, treatment of individual SCC *in situ* lesions with imiquimod has shown efficacy.

**CONCLUSIONS**

It must be kept in mind and communicated to owners that oral and topical medications cannot take the place of sun avoidance in treating and preventing solar dermatitis. Additionally, even with future sun

avoidance, prior skin damage can still progress to skin neoplasia months or years after exposure. Once skin neoplasia has occurred, aggressive surgical resection and/or laser therapy should be performed. Ultimately, the best treatment for canine solar dermatitis is prevention by educating owners of at-risk dogs about the need for sun avoidance starting at a young age.

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# NOTES

# SOLAR DERMATOSES OF HUMANS: SELECTED EXAMPLES

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*Mad dogs and Englishmen go out in the midday sun.*
-- Noel Coward

## NEOPLASMS

Humans' relatively long lifespan, limited hair coverage, and outdoor activities predispose to sunlight-induced skin pathologies. Sunlight contains ample ultraviolet radiation in the UVB spectrum (290-320 nm), which is most efficient at producing carcinogenic genetic damage.[1] Additional genetic damage can result from UVA (320-400 nm) radiation via production of highly reactive chemical intermediates.[2, 3]

**Basal Cell Carcinoma:** Basal cell carcinoma (BCC) is the most common malignancy of humans with lightly pigmented skin.[4] In the US there are well over one million new cases yearly, and perhaps several million (BCC's typically are not documented in cancer registries). Incidence estimates vary from 146/100,000 (Minnesota) to 726/100,000 (Australia).[5] It is estimated that for a Caucasian living in North America the lifetime risk of developing BCC is 30%.[4] These tumors generally grow slowly and can destroy adjacent structures, but they rarely metastasize. The less than 0.1% of cases which do metastasize tend to involve the lung, bone, and lymph nodes.[6] BCC's occur most frequently in chroically sun-exposed anatomic sites. Of the many clinicopathologic variants the most common is the nodular/cystic type, which presents as a slowly growing, friable, translucent lesion with telangiectasia and often central crusting. The sclerosing or morphea-like type tends to consist of a subtle, firm, ill-defined plaque. Pigmented BCC's tend to show dark flecks at the periphery of the lesions, but can resemble melanocytic nevi or melanomas. The superficial, multifocal variant, which can easily be mistaken for an inflammatory lesion, tends to occur on the trunk, often without regard to sun exposure.[7]

**Actinic keratosis:** Actinic (solar) keratoses (AK) are hyperkeratotic, non-indurated, flat or elevated lesions which arise on chronically sun-exposed skin, mostly in fair-skinned individuals. They are defined pathologically by the presence of atypical keratinocytes predominantly in the basal layer. Early lesions can be quite subtle, consisting of only mild erythema and minimal hyperkeratosis, best identified by palpation. More advanced lesions tend to show more prominent hyperkeratosis, sometimes reaching the level of cutaneous horn. Lesions may be isolated or confluent. In a region in which a population of lightly pigmented individuals inhabits an area of intense solar irradiation, namely Australia, the incidence of AK is 40%-60% in those over age 40.[8]

Actinic keratoses have traditionally been viewed as pre-malignant lesions, but their actual behavior is somewhat uncertain. A large, prospective Australian study found that over a 5-year period of observation the likelihood of an AK giving rise to a squamous cell carcinoma (SCC) was less than 1/1000 per year.[9] Other studies have estimated this rate of occurrence to range from 0.025% to 16% per year.[10]

**Squamous cell carcinoma:** SCC is the second most common malignancy arising in the skin,[11] but it accounts for the majority of deaths attributable to non-melanoma skin cancers; approximately 3000 deaths occur yearly in the US from SCC.[12] Most SCC's arise in sun-damaged skin, sometimes evolving from AK's. These lesions have a very low risk of metastasis,

but those arising in sun-protected regions (particular perimucosal sites), in old burn scars, ulcers, or in areas of chronic inflammation or radiodermatitis can behave more aggressively. Immune deficiency from medications or disease (e.g. xeroderma pigmentosum) portends worse prognosis.[13] Human papillomavirus infection is increasingly implicated in the pathogenesis of some SCC's.[14]

**Melanoma:** Melanoma is the third most common skin cancer in Caucasians, but by far the most lethal. The American Cancer Society estimates that 76,690 new melanomas will be diagnosed in Americans in 2013 and 9480 will die from this tumor. There has been a three-fold increase in melanoma incidence in U.S. Caucasians over the last 20 years; it is estimated that invasive melanoma will develop in 1 of 57 individuals over their lifetime (1 of 33 if in-situ melanomas are included).[15] Similar data are reported internationally. The highest incidence of melanoma is reported in Australia and New Zealand (37.7/100,000 in men and 29.4/100,000 in women compared to 6.4/100,000 and 11.7/100,000, respectively, in North America).[16] It is notable that despite this marked increase in incidence, the death rate from melanoma has remained fairly stable. There is controversy as to the reasons for this discrepancy, but possibilities include a change in the biologic behavior of melanomas, a trend toward early diagnosis with subsequent higher cure rates, increased diagnostic scrutiny, and gradual changes in the histopathologic criteria for diagnosis, with the net effect of classifying as malignant some lesions with little or no potential to kill and which may have formerly been classified as benign.[17] These possibilities are not mutually exclusive.

Human primary cutaneous melanomas have traditionally been classified into types, namely superficial spreading, nodular, acral lentiginous, lentigo maligna, and mucosal. There is some evidence that there may be pathophysiologic differences underlying these types. Lentigo maligna melanomas tend to occur in chronically sun-exposed skin, often in conjunction with actinic keratoses, whereas superficial spreading melanoma of the trunk is more often seen in the context of an above average number of melanocytic nevi in the absence of overt sun damage.[18] Melanomas in sun-damaged skin as well as mucosal and acral lesions tend to show KIT mutations, whereas those in covered areas tend to have BRAF mutations.[19] Recognition of melanoma has traditionally rested on irregularity and asymmetry in growing pigmented lesions. Several clinical guidelines for screening have been published.[20-24] Particular scrutiny is advised for changing pigmented lesions in individuals over 50 years of age[25] and for lesions which are different in form from the overall pattern of one's benign nevi.[26]

## DEGENERATIVE CHANGES

**Photoaging:** This term has been accepted to encompass the visible signs of "weathering" on human skin. It includes wrinkling, actinic comedones, solar purpura, and telangiectasia. The histopathologic correlates of photoaging include epidermal atrophy, elastosis, vasodilation, and erythrocyte extravasation. Tissue laxity and sagging are more likely related to chronologic aging.[27] It has been estimated that $291 billion will be spent on "anti-aging" interventions by 2015.[28]

## INFLAMMATORY DERMATOSES

**Lupus erythematosus:** The cutaneous manifestations of human systemic lupus erythematosus (SLE) are generally grouped into three categories, namely acute, subacute, and chronic. The acute type shows a typical photosensitivity distribution of an erythematous, edematous eruption, most commonly on the malar areas of the face. Rarely lesions are vesicobullous. It generally occurs in context with systemic illness, polyserositis, and visceral disease. The typical serologic markers of SLE are generally present.[29] Subacute cutaneous lupus erythematosus (SCLE) presents as a photosensitive eruption of inflammatory, non-scarring lesions which can be annular, papular, or psoriasiform. If present, systemic manifestations tend to be milder than those in acute

SLE. Patients generally show anti-Ro (SS-A) antibodies and sometimes anti-La (SS-B). Anti-nuclear antibody (ANA) is sometimes positive.[30] Transplacental transfer of autoantibodies may result in neonatal LE in which skin rash and sometimes heart block can be seen.[31] Chronic cutaneous LE occurs in three variants: discoid (DLE), tumid LE, and lupus panniculitis. DLE typically presents as persistent, scarring plaques with acrhomic centers and hyperpigmented peripheries and varying degrees of erythema. Lesions are mostly located on the face and scalp, but can occur in other light-exposed sites. In hair-bearing areas permanent alopecia can result. Most, but not all, individuals with DLE do not have signs of systemic disease.[32] Tumid lupus presents with erythematous, indurated, boggy plaques which resolve without scarring. Lupus panniculitis presents as painful, deep-seated inflammatory nodules and plaques which may ulcerate and heal with lipoatrophy.[33]

**Dermatomyositis:** Dermatomyositis (DM) can occur with or without muscle involvement. The cutaneous signs consist of purplish discoloration of the facial skin in a partially photodistributed pattern. Eyelid involvement, which is characteristic, is not typically seen in other light-induced dermatoses. Gottron's papules over the extensors of the hands and periungual erythema and cuticle changes are also typically seen. Areas of poikiloderma may develop. Extracutaneous involvement may present as proximal muscle weakness, dysphagia, or arthritis. An increased incidence of visceral malignancy requires vigilance in patient management.[34]

**Photo-drug eruptions:** Photosensitivity from medications can be based on phototoxicity or photosensitivity. The former is considered to be non-allergic, can occur on first exposure, and can occur in a high proportion of individuals exposed to a sufficient quantity of the drug. The latter is allergic in nature, should occur only on second or subsequent exposures, and requires only a small amount of the offending agent. In clinical practice it is often difficult to separate out these two possibilities. Sunlight-induced cutaneous drug reactions can be acute or chronic and can assume a variety of morphologies, but their distribution tends to consistently involve the upper torso and dorsum of the arms. Strangely, the face is sometimes spared. Although many medications can result in photo-distributed rashes, some are more common offenders.[35]

**Pemphigus erythematosus:** Pemphigus erythematosus and pemphigus foliaceus are clinical variants of superficial pemphigus, in which the stratum corneum and the most superficial viable keratinocytes separate from the remaining epidermis by acantholysis due to damage from autoantibodies directed against desmoglein-1. The clinical and laboratory findings can be considered to be an overlap between pemphigus and LE. The clinical expression is that of an erosive dermatitis of the sun-exposed areas. Clinical features of visceral LE may be present. Intact blisters are sometimes present. IgG and C3 are deposited in the superficial epidermis; the ANA is usually positive.[36]

**Porphyria cutanea tarda:** PCT is a hepatic porphyria in which overproduction of mostly uroporphyrins results in endogenous photosensitivity. It may be caused by deficiency of uroporphyrin decarboxylase, an enzyme in the heme synthesis pathway, and may be triggered by hepatic disease, such as viral or alcoholic hepatitis. Affected individuals present with skin fragility and blistering of the sun-exposed areas, chiefly involving the dorsum of the hands and arms, resulting in scarring, milia, and pigment changes. In women excessive facial hair may be seen.[37]

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# NOTES

# CUTANEOUS DRUG ALLERGIC REACTIONS – PART I

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**Cutaneous Drug Hypersensitivity Reactions - Introduction**

There are 2 types of adverse drug reactions (ADR). The first type is referred to as "type A" or "dose-dependent" reactions, and the second type as "type B" or "idiosyncratic" reactions.

Type A reactions are dose-dependent; they are the direct consequence of a pharmacological, chemical, physical property of the drug or one of its metabolites. Type A reactions are common, and represent around 70% of all ADRs. Type A reactions rarely involve the skin, with the exception of a few chemotherapeutic agents.

Type B reactions are not directly related to any of the drug properties, and are therefore not predictable. When they are immune-mediated, they are referred to as "drug allergy" or "drug hypersensitivity. In human medicine, the skin is the most common target of these drug hypersensitivity reactions, and it appears to be one of the most common targeted organs in veterinary patients as well. However the reason for this remains unknown since most drugs are administered orally or by injection rather than transdermally. The two lectures will focus on these cutaneous drug allergic reactions, especially delayed ones.

**Cutaneous Drug Hypersensitivity Reactions – Clinical signs**

Clinical signs and skin lesions associated with drug hypersensitivity vary widely from one patient to another.

- Immediate clinical signs: urticarial or angioedema
- Delayed type clinical signs: lupus & lupoid reactions; pemphigoid reactions, or pemphigus foliaceus-like drug reactions; cutaneous vasculitis; fixed drug eruption; erythema multiforme; maculopapular eruptions & maculopapular exanthema
- Pruritus: not all cutaneous drug reactions are itchy, but when they are, the pruritus intensity is said to be quite extreme by human patients.

It is important to note that the nomenclature in dermatology sometimes differs between human and veterinary medicine.

**Cutaneous Drug Hypersensitivity Reactions - Incidence**

Adverse drug reactions (ADR) in general occur commonly in both human and veterinary medicine. In human medicine, ADRs account for up around 20% of all hospitalized cases and around 10% of the general population; and their management is thought to cost >$140 billion every year in the US alone. These ADRs can be very serious, and are considered to be the $4^{th}$ to $6^{th}$ cause of death in human hospitals.

In veterinary medicine, ADRs also occur fairly commonly, but their true prevalence is much more challenging to determine, especially when it comes to drug allergic reactions. This is likely due to lack of reporting or misdiagnosis. There is an increasing number of small animals having access to veterinary care, and sometimes at a level close to what can be seen in human medicine. It is therefore likely that cases of drug hypersensitivity will increase drastically in the near future.

It is important to remember that beyond their overall frequency and morbidity/mortality risk, drug hypersensitivity reactions have numerous negative consequences. In any case, they will prevent the patient from ever receiving the culprit drug again. These reactions, like other ADRs. can break the owner's confident in his/her veterinarian. Finally, a significant risk for drug hypersensitivity or cases during clinical trials can prevent needed drugs from reaching the market or lead to the withdrawal of an important medicine.

**Cutaneous Drug Hypersensitivity Reactions - Categories**

Different types of hypersensitivity reactions

- Type I = IgE-mediated; e.g. anaphylaxis, urticaria, angioedema
- Type II = IgG-mediated (complement-mediated lysis); e.g. drug-induced pemphigus
- Type III = IgG-mediated (immune complex deposition); e.g. cutaneous vasculitis
- Type IV = cytotoxic T cell-mediated; e.g. toxic epidermal necrolysis

Type I are referred to as "immediate" reactions; they indeed happen within 24h of exposure (usually much less than that). Other drug hypersensitivity reactions occur after at least 5 days of drug exposure, unless the patient is being re-exposed to them; in that case, clinical signs can start within a few days or even a few hours. Types II, III and IV are therefore referred to as "delayed" reactions.

It is important to note that the Gell & Coombs classification of hypersensitivity does not seem to apply very well to cases of drug allergic reactions. Indeed, immune markers of different types of hypersensitivity have been detected within the same patients.

Cutaneous drug hypersensitivity reactions versus drug-induced autoimmune cutaneous reactions

Most cutaneous drug reactions are drug-specific hypersensitivity reactions where the immune system appears to target the drug itself (see pathogenesis section for details). However, a few reactions seem to be induced/triggered by the exposure to the drug, but the immune response targets specifically a self-protein rather than the drug, leading to a clinical picture of lupus or pemphigus.

**Cutaneous Drug Hypersensitivity Reactions - Pathogenesis**

The field of immediate drug hypersensitivity is relatively well advanced when it comes to understanding of the mechanisms behind the elicitation of IgE-mediated lesions. However, like for any other drug allergy, the reason why the skin can be targeted from a non-cutaneous exposure remains a mystery. Much less is known about delayed drug hypersensitivity, especially in veterinary patients.

Different theories in the field of drug allergy

Regardless of the hypersensitivity type and its specific clinical signs, we know little to nothing about the events associated with the sensitization phase against the drug. Theories trying to address these mysterious pathogenic events:

*Hapten Hypothesis*

- Immunogen = drug covalently bound to a protein
- Some drugs are protein reactive (e.g. penicillins) but most drugs require bioactivation.

*"Danger" Theory*

- The immune system cares more about "danger" than non-self.
- "Danger" could be necrotic cell debris, oxidative stress, inflammation…

*Pharmacological Interaction concept*

- The drug itself non-covalently interacts with the MHC II receptor and the T cell receptor like most drugs would its pharmacological cellular target.
- This reversible drug-immune receptor interaction leads to immune activation and lymphocyte proliferation.

*Viral reactivation*

- There is some data in human medicine suggesting that some drug hypersensitivity reaction might be linked to the reactivation of a latent viral infection.

Note that these theoretical scenarios are not mutually exclusive.

Pathogenic components in drug allergy

In true anaphylaxis, the drug induces mast cell degranulation via drug-specific IgE antibodies; this is an immune-mediated reaction (Type B ADR). Urticaria and angioedema are drug-induced immune-mediated skin reactions, but they are IgE-mediated (Type I hypersensitivity).

Delayed drug-induced skin reactions (Type II, II or IV) are IgG- or T cell- mediated. Anti-drug as well anti-tissue antibodies have been detected in both humans and dogs with a history of drug

hypersensitivity. Drug-specific lymphocytes have been isolated from the blood and the skin from humans with a drug allergy history. There are many published studies investigating these cells *in vitro* in human medicine. Drug-specific lymphocytes have been recently detected in a few dogs with severe skin drug reactions.

Risk factors of drug allergy

Drug hypersensitivity is a multi-factorial entity. In some human patients, there is some evidence for a genetic predisposition, with certain MHC II allele being associated with a higher risk of developing a delayed hypersensitivity reaction against certain drugs. In human medicine, there is also some evidence that drug allergic reactions have a higher incidence in patients with chronic conditions such as HIV, cystic fibrosis, or an autoimmune disease. To date, there is no corresponding information in veterinary medicine.

Why the skin?

Presently, there is no explanation for why a drug hypersensitivity reaction would target the skin rather than another organ after being administered systemically. Reactive metabolites thought to initiate drug hypersensitivity could end up in the skin via 2 pathways: the parent drug was metabolized in the liver and its metabolites reached the skin via the blood; skin cells bioactivated the parent drug themselves. After clinical sign onset, drug-specific lymphocytes have been isolated fro skin lesions in human patients. However, the exact cellular and molecular events leading from the initial immune sensitization phase to the clinical signs is still a mystery.

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# NOTES

# CUTANEOUS DRUG ALLERGIC REACTIONS – PART II

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**Cutaneous Drug Hypersensitivity Reactions - Diagnosis**

Challenges

The diagnosis of a Type B ADR heavily depends on the clinician's awareness about ADRs in general. ADRs are usually not a specific component of a veterinarian's training; they are often quickly mentioned at the end of each pharmacology lecture. Furthermore, ADRs adopt a multitude of presentations; they can indeed target any organ and mimic non drug-related diseases, rendering their diagnosis especially challenging at time. Even skin reactions can adopt miscellaneous presentations.

Clinical approach

In addition to awareness, the diagnosis of drug hypersensitivity reactions requires to take a thorough history and to conduct a careful clinical exam. Even with obvious cutaneous reactions, it is recommended to add some blood clinical tests because cutaneous drug reactions can be associated with less obvious but as or more dangerous problems, such as hepatitis or blood dyscrasia.

Which drug did it?

When a diagnosis of cutaneous drug reaction is considered, identifying the culprit can also be difficult with patients receiving several drugs at the same time. The clinician's awareness of the risk for an ADR whenever a drug is prescribed needs to be associated with a thorough investigation of past and present drug exposures.

ADR algorithms

The diagnosis of ADR can be facilitated by algorithms that have been developed for human medicine, but translate very well to veterinary patients: e.g. RUCAM; Naranjo; or Austin Bradford-Hill. This approach is very useful with Type B reactions such as skin drug reactions that are idiosyncratic, not dose-dependent, and not related to the drug pharmacology.

Skin tests

Skin tests have been developed to in human medicine to confirm the diagnosis of hypersensitivity to certain drugs. Their sensitivity and specificity is subject to controversy in human patients with drug allergy. None of them has been validated for drug hypersensitivity reactions in veterinary medicine. In addition,

Specific lab tests

There are no diagnostic tests fully validated for drug hypersensitivity reactions. However, several academic researchers can apply some of their research tools for diagnosis purposes. This can help the clinician confirm a drug allergic reaction and determine which drug was responsible when the patient was on multiple drugs at the time of the reaction. Such tests could detect and quantify anti-drug antibodies (IgE or IgG) and anti-tissue antibodies (IgG). Other tests look for circulating drug-specific lymphocytes (e..g lymphocyte transformation test, LTT).

Others

Note that in cases of Type B reactions, which are not dose-dependent, therapeutic drug monitoring (TDM) is usually not useful. In the field of drug hypersensitivity specifically, there are only a few routinely used pharmacogenomic test in human medicine (e.g. abacavir; carbamazepine), and none in veterinary medicine.

**Cutaneous Drug Hypersensitivity Reactions - Management**

#1 = Drug discontinuation

Any suspicion of an immune-mediated drug reaction should lead to the immediate drug discontinuation. Indeed, even a minute amount of the culprit drug could entertain the immune reaction and worsen clinical signs.

#2 = Management of clinical signs themselves

The clinical signs need to be addressed according to their nature as if they were not drug-associated. Type I reactions can be treated with antihistamines. Anti-H1 drugs are the common sense option, however, some data in human medicine suggests that combining anti-H1 and anti-H2 drugs (antacids) might provide better control of the clinical signs. Anaphylactic reactions might require short-acting corticosteroids, and sometimes more extensive management.

Anti-histamines can also be useful with delayed skin reactions associated with significant skin inflammation and/or pruritus. Cutaneous reactions where the skin integrity is maintained are probably best left alone. Mild skin lesions or very localize deep lesions, local treatment is usually sufficient, and the more gentle formulations should be favored. When skin lesions are widely spread (>25% body surface) and/or very severe, a more aggressive strategy is probably required, and patients should be approached as severely burnt cases. Thus, SJS and TEN human patients are often referred to burn units.

Immunosuppressive therapy or not?

There is little EBM on immunosuppressive therapy in human cases of *delayed* drug, and only anecdotal evidence for veterinary patients. In human medicine, corticosteroids do not seem to make a significant difference in the recovery of delayed drug hypersensitivity; there is less evidence available for agents such as cyclosporine. The decision of using them or not to date is left to the discretion of the clinician. However, there is increasing evidence that aggressive immunosuppressive therapy can lead to fatal systemic fungal infections.

Antimicrobial coverage or not?

Unless the skin integrity is not affected or the lesions are very localized, it is important to implement a careful antimicrobial therapy. Indeed the skin then becomes a high-risk route of entry for various pathogens. It is advised to start the animal on both antibacterial and antifungal therapy, and a systemic route should be favored. If the clinician decided to put the patient on immunosuppressive therapy, a systemic wide-spectrum antimicrobial prophylaxis becomes imperative.

Prognosis

Many skin drug reactions have a very good prognosis, especially when the diagnosis happens early on and leads to the immediate discontinuation of the culprit medication. However, it is important to remember that certain cutaneous drug reactions carry a very high mortality rate, even in human medicine. This, Steven-Johnson Syndrome and toxic epidermal necrolysis are still associated with 30-50% fatality rate in modern human medicine. This rate seems much higher in veterinary medicine where practical, financial, and ethical implications come into play.

**Cutaneous Drug Hypersensitivity Reactions - "The Aftermath"**

Alternative to the culprit drug

After diagnosing a drug hypersensitivity reaction, the clinician will sometimes have to identify an alternative drug if the original patient's disease continues to require a pharmacological treatment. This can be easier said than done. Note that the clinician will have to make sure that there is no risk for immune cross-reactivity between the culprit drug and its replacement (e.g. between penicillins).

Recording the reaction

The confirmed or suspected drug allergic reactions MUST be added to the patient's medical record and the owner MUST be made aware of the drug or drug family that the animal should not be exposed to again.

Reporting the reaction

ADRs should be reported TO THE DRUG COMPANY itself. There, the clinician will be able to talk directly to someone who handles calls about this drug daily who will therefore be able to give advice on management. Pharma companies will detect a pattern of drug hypersensitivity with a drug that was recently launched on the market. They are then legally obligated to report these reactions back to the FDA-CVM who will receive well-organized and up-to-date reports.

Samples for research

Finally, it is imperative that private practitioners and academic clinicians collaborate with researchers who investigate idiosyncratic drug reactions. Indeed, the limited access to patient samples has dramatically slowed down advances in this field. Working with a researcher will help improve our understanding of the pathogenesis of drug allergic reactions.

**Cutaneous Drug Reactions - References**


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# NOTES

# CONCURRENT SESSION PRESENTATIONS FRIDAY

# NON-INFLAMMATORY ALOPECIA: IT'S NOT ALWAYS HORMONAL

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## OVERVIEW

There are many causes of alopecia. These can be divided into two basic categories: traumatic and nontraumatic. Traumatic alopecia is often associated with pruritus in which the dog is scratching the hair out. Common causes of traumatic alopecia include allergies, parasites, pruritic pyoderma, and *Malassezia* dermatitis. Clinically, traumatic alopecias are usually characterized by broken hairs. If the hair is not traumatically being removed, then it could be falling out. Nontraumatic-induced alopecias can be further divided into inflammatory and non-inflammatory causes. Typical inflammatory causes of alopecia include bacterial folliculitis, dermatophytosis, and demodicosis. Less common but important considerations include sebaceous adenitis and vasculitis. Clinically, alopecias associated with inflammation usually present as patchy alopecias. Hair may also fall out when there is no associated dermal inflammation. Non-inflammatory alopecias are usually diffuse and symmetrical and may be associated with either abnormal hair growth or hair cycle abnormalities.

The hair cycle consists of the resting telogen phase, the active growth or anagen phase, and the catagen phase which is the involution from anagen to telogen. Shedding, or exogen, is separate from the hair cycle. Both telogen and anagen predominant cycles have been described.[1] People, poodles, and likely dogs that need regular clipping, have anagen predominant cycles. Most other dogs have telogen predominant cycles.

## CONDITIONS ASSOCIATED WITH ABNORMAL HAIR GROWTH

Follicular dysplasias are abnormalities in the development of the hair. Clinical signs are, therefore, apparent during anagen. Hairs are fragile and malformed. Follicular dysplasias are rare and are more often described in mice and large animals. Examples of dysplasias in small animal medicine include certain alopecic breeds such as the Chinese crested dog, Mexican hairless dog, and the Sphinx cat.[2]

Congenital hypotrichosis may be included in this category but the condition is not well characterized. Usually dogs are either born without normal pelage or experience non-color linked hair loss within the first 6 months of life. Additional ectodermal defects may also be noted.

Color dilution alopecia is associated with the coat color dilution gene. It involves a defect in melanosome migration and/or transfer; therefore, the defect occurs during the anagen phase of the hair cycle. This condition is seen most commonly in breeds with blue or fawn colorations. The alopecia usually starts gradually in the first 3 years of life. The clinical signs are color-restricted and consist of alopecia, seborrhea, and pyoderma. Often lesions on the trunk are most severe.[2] Diagnosis involves microscopic examination of hair (trichogram) which may reveal abnormal clumping of melanin within the hair shafts resulting in distortion of the hair cortex. Histopathology is diagnostic and reveals large melanin clumps in the hair shaft and bulb and perifollicular melanosis. Treatment is symptomatic. There are some anecdotal reports of partial response to retinoids and melatonin.

Black hair follicular dysplasia also appears to involve defective melanosome migration and/or transfer.[4] It is often noticed in younger dogs than those with color dilution alopecia. Clinical signs and histopathology are similar to those described for color dilution alopecia; however, trichograms are not useful. The most severely affected regions are usually the head, pinnae, neck and back.[2]

## CONDITIONS ASSOCIATED WITH HAIR CYCLE ABNORMALITIES

Alopecias associated with hair cycle abnormalities seem to occur because the hairs remain in telogen and fail to re-enter anagen. Three basic morphologic features of hair cycle abnormalities are miniaturization of hairs and adnexa, increased number of telogen hairs and increased number of hair follicles without hairs.[1] The hair cycle is influenced by genetics, intrinsic and extrinsic factors.[5]

There are many diseases that appear to be associated with hair cycle abnormalities. These include

endocrine dermatoses, alopecia X, patterned alopecia of various breeds, cyclic flank alopecia, post-clipping alopecia, and anagen/telogen defluxion. The clinical signs we associate with endocrine dermatoses may also be seen with many of the other non-inflammatory alopecias associated with hair cycle arrest. In addition, many of the histological characteristics that we associate with endocrine dermatoses may also be seen with other hair cycle abnormalities.[6] It is important to realize that, with few exceptions, histopathology cannot differentiate endocrine diseases from other causes of non-inflammatory alopecia.

**Endocrinopathies:** Canine hypothyroidism occurs most commonly in medium to large purebred middle-aged dogs. Clinical signs can be quite vague and include mental dullness, lethargy, exercise intolerance, obesity without polyphagia, and cold intolerance. The dermatologic signs can also be quite vague. Hairs may be dull, dry and brittle, associated with the lack of hair cycling, and may fail to regrow following clipping. Hypothyroidism is not commonly associated with the classic bilaterally symmetrical alopecia that we attribute to other endocrine diseases.[7] Instead, alopecia tends to occur in areas of wear such as the elbows, hips, thighs, collar area, and bridge of the nose. The tail is often alopecic ("rat tail"). Some dogs may have a preferential loss of guard hairs resulting in the appearance of a "puppy coat". Owners may notice a lack of the yearly shed and a lightening of the hairs since they are not being shed and replaced. Hyperpigmentation of the skin may be noticed in alopecic areas such as the bridge of the nose. The skin may demonstrate an increased thickness referred to as myxedema which clinically may result in a "tragic facial expression." Recurrent infections of the skin and/or ears are commonly seen in hypothyroid dogs and are sometimes the only clinical signs observed. Histopathologic findings that may support a diagnosis of hypothyroidism include mucinosis and acanthosis instead of an atrophic epidermis. Diagnosis of hypothyroidism should include compatible clinical signs, low to low-normal concentrations of total T4 or free T4, ideally with concurrent elevations in TSH, and finally clinical signs should resolve with thyroid supplementation.

Canine hyperadrenocorticism occurs most commonly in middle-aged to older dogs, with small breeds predisposed. The hypercortisolemia can be pituitary-dependent, adrenal-dependent, or iatrogenic. Clinical signs may include polyuria, polydipsia, polyphagia, recurrent urinary tract infections, weakness, muscle atrophy, and pendulous abdomen. Dermatologic signs include bilaterally symmetrical alopecia sparing the head and distal extremities, seborrhea, comedones, and milia. Atrophy of the skin may be noted resulting in easy visualization of the dermal vessels. Recurrent skin infections are common and may be the only clinical sign initially. Adult onset demodicosis is also commonly associated with hypercortisolemia. Poor wound healing and bruising from minimal trauma due to increased blood vessel fragility may occur. The presence of dystrophic mineralization (calcinosis cutis) on physical examination or on histopathology should lead one to strongly suspect hypercortisolemia. Diagnosis of hyperadrenocorticism is not always easy and usually requires more than one test, which may include ACTH stimulation test, low dose dexamethasone suppression, and ultrasonography.

Hyperestrogenism may occur in association with cystic ovaries, testicular tumors, or from exogenous estrogen supplementation. Estrogen is a known inhibitor of anagen initiation; therefore, bilaterally symmetrical alopecia sparing the head and distal extremities is a common clinical presentation of hyperestrogenism. Other dermatological signs include comedones, seborrhea, hyperpigmentation, and recurrent infections. Hyperestrogenism also may result in enlargement of the nipples and vulva, irregular heat cycles in females, and feminization of males. In males, linear preputial dermatosis, characterized by a narrow strip of hyperpigmentation and scale along the ventral midline from the prepuce towards the scrotum, is highly suggestive of hyperestrogenism. Macular melanosis (hyperpigmented macules on the ventrum and perineum) has also been associated with hyperestrogenism.

Hyperandrogenism from testicular (or adrenal?) tumors is rarely associated with alopecia. This condition is primarily seen in intact males and results in hyperplasia of the circumanal glands and tail glands. Seborrhea oleosa may also be present.

**Alopecia X:** This condition has gone by many names including adult-onset hyposomatotropism, growth hormone-responsive alopecia, pseudo-Cushings disease, castration-responsive alopecia, biopsy-responsive alopecia, and, more recently, adrenal hyperplasia-like syndrome.[8] This is a common condition seen in Poodles

and the Nordic and "plush-coated" breeds. The pathomechanism of the alopecia is not known but appears to involve a defect in anagen initiation or maintenance. The alopecia develops in adult dogs between 1 and 10 years of age and occurs equally in males and females regardless of their neuter status. The alopecia may first appear as a primary loss of guard hairs progressing to complete alopecia of the neck, tail, perineal region, caudal thighs, and ultimately the trunk. The alopecia is bilaterally symmetrical and spares the head and distal extremities. In addition to hair loss, the skin may become intensely hyperpigmented, especially in the alopecic areas. Systemic illness is not associated with this disease. Diagnosis of alopecia X is reached by ruling out endocrine dermatoses and other causes of non-inflammatory alopecia. While abnormalities of adrenal steroid intermediates and sex hormones may be noted in some affected individuals, not all have this abnormality.[9] Because abnormal adrenal steroid intermediates are unlikely the cause of the hair cycle arrest, testing for these is seldom indicated. Histopathology can be used to confirm that the alopecia is non-inflammatory and help rule out inflammatory causes of alopecia such as sebaceous adenitis, which can also be seen in similar breeds. There is no one guaranteed treatment for this condition and hair growth following treatment may not be permanent. Neutering of intact dogs often results in hair regrowth and is the first treatment recommendation. Melatonin results in hair regrowth in approximately 40% of dogs.[9] The mechanism is unknown but may involve alterations of sex hormones or blocking estrogen receptors on the follicle. Because melatonin treatment appears to be very safe, it is usually recommended as a first line treatment after neutering. Melatonin can cause insulin resistance; therefore, it is contraindicated in dogs with diabetes. It may also cause sedation. When the alopecia was thought to be a growth hormone-related condition, treatment with growth hormone was recommended and often successful. Unfortunately, growth hormone is not readily available and administration can result in diabetes. Because synthetic progestins can induce growth hormone secretion in the mammary gland of dogs,[10] a recent study investigated the safety and efficacy of a synthetic progestin for treatment of alopecia X. Treatment with medroxyprogesterone acetate resulted in partial hair regrowth in three and complete hair regrowth in one of eight Pomeranian dogs with alopecia X, with no adverse effects noted.[11] Based on work relating this disease to an adrenal steroid hormone imbalance, treatments with mitotane and trilostane have resulted in hair regrowth in a number of dogs with alopecia X.[9,12,13] These treatments are also not without risk.[14] In addition, testosterone administration has resulted in hair regrowth in some dogs but can cause cholangiohepatitis and aggressive behavior.

**Patterned alopecia:** Different breeds appear to have alopecic conditions that are termed patterned alopecia. In general, the alopecia usually starts at a young age and progresses over time. Dachshunds often develop alopecia of their pinna. With time the ears may take on a leathery feel. Dachshunds and Boston terriers may develop alopecia of their ventral abdomen and perineal region. Portuguese water dogs, American water spaniels, and Irish water spaniels develop an alopecia that is unique to these breeds.[15] Alopecia of the caudal thighs of greyhounds is also recognized. The cause of these alopecic conditions is not known and is likely not the same for each breed. Because of this, there is no one treatment recommendation; however, there are some anecdotal reports of response to melatonin.

**Cyclic flank alopecia:** This is a seasonal truncal alopecia that tends to occur during periods of shortened day length, resolving within several months.[16] The alopecia does not always occur annually and may be more prolonged or possibly permanent in some dogs. The pathomechanism of the alopecia is unknown. Breeds most frequently affected include Airedales, Boxers, and English bulldogs but it has also been recognized in French bulldogs, American Staffordshire terriers, and other breeds. Dogs present with a symmetrical alopecia affecting the flanks which can progress to involve the thoracolumbar region. The skin is often hyperpigmented. Affected dogs are usually young to middle-aged adults. Both intact and neutered animals of either sex may develop the alopecia. Interestingly, occasionally the alopecia is associated with hyperpigmentation of the bridge of the nose. Histopathology is reported to be diagnostic with the presence of follicular keratosis, deformed follicles, and melanization of sebaceous glands and ducts. The deformed follicles represent telogen follicles and can be seen in other telogenizing conditions. Treatment with melatonin may shorten the duration of the alopecia and help prevent recurrence in some cases.

**Post-clipping alopecia**: Prolonged alopecia following clipping occasionally occurs.[17] Post-clipping alopecia may result from hair follicles entering an arrested stage. This may be as a result of vascular perfusion changes in response to cutaneous temperature changes. In our experience it is common at sites prepared for surgery or epidurals. Possibly the vigorous scrubbing of the site or the injected anesthetic leads to hair cycle arrest. Post-clipping alopecia may also be caused by clipping the coat when the hair cycle is in a prolonged telogen phase ("follicular hibernation"). This latter condition is seen more commonly in Nordic and "plush-coated" breeds such as those described under alopecia X. The hair usually grows back within one year, especially after experiencing a heavy shed. Biopsy may document a telogen arrest (all hairs in telogen) or possibly an abundance of anagen hairs indicating that the coat is about to return to normal. A dog with post-clipping alopecia could also have an endocrine disease or another non-inflammatory alopecic condition (such as alopecia X) that becomes exposed when the dog is clipped. The other condition is not yet clinically evident but the hairs are no longer cycling; thus, clipping simply uncovers the problem sooner.

## CONCLUSIONS

Both endocrine and non-endocrine dermatoses may cause non-inflammatory alopecia. In some instances the breed, signalment and clinical presentation will help differentiate one from the other. In many cases, however, it is necessary to rule out the endocrine differentials and inflammatory causes of alopecia to allow one to diagnose some of the other non-inflammatory causes.

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# NOTES

# WHAT'S SO ATYPICAL ABOUT CUSHING'S SYNDROME?

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## INTRODUCTION

Hyperadrenocorticism (HAC), or Cushing's syndrome, is one of the most common endocrinopathies of older dogs. Diagnosis is based on compatible history and physical findings coupled with demonstration of either hypercortisolemia or decreased sensitivity of the hypothalamic-pituitary adrenal axis to negative glucocorticoid feedback.[1] Common tests for screening dogs for HAC include the ACTH stimulation test and the low-dose dexamethasone suppression (LDDS) test. Both tests have good sensitivity and specificity but diagnostic accuracy is not 100%, especially when dogs with non-adrenal disease are included in the study population.[2,3]

The term atypical Cushing's syndrome (AHAC) has been used to describe dogs with clinical signs of hypercortisolemia in which standard screening tests for Cushing's syndrome are within normal limits.[1] Alterations in concentrations of adrenocortical precursor hormones, before and after ACTH stimulation, have been used to diagnose dogs with AHAC.[4-6] The questions remain – what actually is AHAC and what is the best way to diagnose this condition?

## EITIOPATHOGENESIS

The etiopathogenesis of AHAC remains unknown. It has been proposed that the condition is the result of elevations of adrenal sex hormones and steroid hormone intermediates. Can elevated concentrations of precursor hormones explain the clinical signs associated with AHAC such as PU/PD, elevations in ALP, and vacuolar hepatopathy? These clinical signs are known to be associated with excess cortisol administration, but are also seen in dogs with AHAC. Progestins are known to cross-react with the cortisol receptor, potentially resulting in its activation.[7] Dogs and cats with progesterone-secreting adrenal tumors can present with clinical signs of hypercortisolemia including polydipsia, polyuria, polyphagia, thin skin, alopecia, and diabetes mellitus.[4,8-10] However, 73% of healthy dogs with alopecia in which both Cushing's syndrome and hypothyroidism had been ruled out had at least one baseline or post-ACTH stimulation adrenal sex hormone or steroid hormone intermediate greater than the reference interval.[11] With the exception of alopecia, these dogs did not have clinical signs of hyperadrenocorticism. Dogs with nonadrenal neoplasia and no evidence of hyperadrenocorticism may also have increased concentrations of 17-hydroxyprogesterone (17 OHP) post-ACTH stimulation, similar to changes seen for cortisol concentration.[12] Additionally, healthy intact female dogs have increased concentrations of progesterone and 17 OHP for prolonged periods of time during estrus, diestrus, and pregnancy without development of clinical signs of Cushing's syndrome.[13] Finally, corticosteroid-induced ALP expression is upregulated in hepatocytes by cortisol, but not 17 OHP, progesterone, estradiol or androstenedione administration.[14] Results of these studies suggest that excess adrenal sex hormone concentrations are unlikely to be the cause of the clinical and clinicopathologic findings in dogs with AHAC.

Because adrenal sex hormones precede cortisol production in the cortisol pathway, dogs with hypercortisolemia also have elevations in adrenal sex hormones.[15] Some dogs diagnosed with AHAC progress to overt Cushing's disease over time.[15] Clinical signs in some dogs with AHAC, therefore, could be due to hypercortisolemia, but current testing methods are not sensitive enough to diagnose the condition. Based on these findings, some endocrinologists have recommended that AHAC be renamed occult hyperadrenocorticism.[1]

A recent unpublished study showed the sum and mean cortisol concentrations over a 9 hour period in dogs with AHAC to be significantly increased when compared to control dogs but less than those of dogs diagnosed with PDH. These results support the hypothesis that dogs with AHAC have increased cortisol concentrations. In this study dogs with AHAC also had significantly larger adrenal gland measurements than control dogs, but adrenal gland size did not differ for dogs with AHAC and PDH. Interestingly, dogs with AHAC were statistically older than dogs with PDH, suggesting that there could be a different

pathomechanisms of AHAC that is yet to be identified.

**DIAGNOSIS**

Because of limitations in the diagnostic accuracy of all currently available screening tests for hyperadrenocorticism, results of both the ACTH stimulation test and the LDDS test should be confirmed to be within reference intervals prior to evaluation for AHAC.[1]

Currently, diagnosis of AHAC is made based on presence of increased concentrations of adrenal sex hormones (17 OHP, progesterone, estradiol, and androstenedione) pre- and post-ACTH stimulation.[1] Because these hormones are precursors to cortisol, they can also be increased in dogs with Cushing's syndrome [15-17] and up to 26% of dogs with non-adrenal illness.[17] Therefore, evaluation of adrenal sex hormones for diagnosis of AHAC is questionable.

Recommendations for dogs with suspect AHAC based on this new research include first performing both LDDS and ACTH tests. Adrenal ultrasound should be considered to rule out an adrenal tumor, even with normal cortisol concentrations if clinical signs suggest Cushing's syndrome. If no adrenal tumor is detected, consider retesting in a few months. Increased adrenal sex hormones and steroid hormone intermediates post ACTH-stimulation have been documented prior to development of hypercortisolemia in at least one dog.[15]

**TREATMENT**

Ideally, treatment should wait for definitive diagnosis of HAC. If clinical signs are severe with negative testing, consider treatment with trilostane as for HAC.

Melatonin has been suggested as a treatment of AHAC. Melatonin is a neurohormone produced in all vertebrate species by the pineal gland in response to darkness and decreasing day length.[18-20] Melatonin therefore controls the circadian and seasonal reproductive and hair growth cycles. In a previous study, melatonin treatment for 28 days resulted in decreases in estradiol, testosterone and DHEAS in intact female dogs, and decreases in estradiol and 17-OHP in intact male dogs.[21] However, when neutered dogs were evaluated, no significant decreases in hormone concentrations were detected after 4 months of melatonin treatment.[22] This difference between the two studies may be because melatonin's primary influence on sex hormones is via gonadotropin secretion and their effect on gonadal production of sex hormones. Melatonin may also inhibit ACTH-stimulated cortisol production by the adrenal glands; however, this is controversial. While melatonin receptors have recently been identified in primate adrenal cortices,[23] studies in rats, horses and humans in which melatonin and cortisol were compared under various natural and experimental conditions failed to show an association between the two hormones.[24-26] In a previous study, cortisol concentrations in dogs after 4 months of melatonin were similar to their pre-treatment values.[22]

Flaxseed has also been suggested as a treatment of AHAC because of its ability to alter estrogen metabolism. The relationship of hyperestrogenism and AHAC is questionable. With the exception of truncal alopecia, increased estradiol concentrations are not typically associated with clinical signs attributed to hypercortisolemia, such as polyuria, polydipsia, polyphagia, muscle wasting and elevated liver enzymes.[27-30] In addition, hyperestrogenism is associated with certain clinical signs highly characteristic of the hormone, such as gynecomastia, swelling of the vulva in females, and preputial dermatitis in males.[27,30,31] Lastly, estradiol concentrations have been shown to be quite variable in individual dogs. [32] Therefore, the significance of increased estradiol concentrations is unknown in cases of suspect atypical Cushing's syndrome.

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# NOTES

# OPHTHALMIC MANIFESTATIONS OF CUTANEOUS DISEASES

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## OCULAR DISORDERS ASSOCIATED WITH THE COLOR DILUTE GENOME

Of general interest to the dermatologist are the ocular diseases associated with dilute coat color genetics in dogs, cats, and horses. In Bull Terriers, Whippets, Boston Terriers, and Dalmatians, the S-gene (white spotting gene) determines body pigmentation pattern. The S-gene (also known as the microphthalmia-associated transcription factor) also codes for ocular anomalies (blue eyes, multiple ocular defects such as small eyes, cataracts, and posterior segment defects) as well as sensorineural deafness associated with cochleosaccular malformation/degeneration. Not all white blue-eyed dogs have additional ocular anomalies, but many are deaf due to this genetic disorder. The M- or merle-gene has both a dominant (M) and recessive (m) allele and is associated with deafness and ocular anomalies. In Collies, Shelties, Great Danes, and Long-haired Dachshunds, heterozygote ([Mm]-merle colored) animals are predisposed to sensorineural deafness and blue "normal" eyes. Dogs with dominant merle allele (MM) genotype typically are deaf and blind due to microphthalmia and other associated congenital anomalies. In the past 10 years, this has especially become common in the Miniature LH Dachshund. In the Australian Shepherd, the mode of inheritance for ocular lesions in merle dogs (Merle ocular dysgenesis) is due to a recessive trait with incomplete penetrance. Puppies from two "blue merle" parents ("double blue merle" offspring) are many times afflicted with multiple ocular defects that may or may not be blinding as well as sensorineural deafness.

In the cat, blue irides, deafness, and white coat occur as a dominant condition with incomplete penetrance for deafness, incomplete dominance in the production of blue irides, and complete penetrance for white fur. Although similar to Waardenburg's syndrome in man, the disorder in man has other facial defects not seen in the cat. Chediak-Higashi syndrome is an autosomal recessive disorder seen in Persian cats characterized by partial oculocutaneous albinism, bleeding disorders, and increased susceptibility to infection due to neutropenia. Afflicted cats have pale, yellow-green irides, reduced fundic pigmentation, and congenital cataracts as well as a pale hair coat color.

In the horse, multiple congenital ocular anomalies of the Rocky Mountain Horse, Kentucky Mountain Saddle Horse, Shetland Pony, Deutsches Classic Pony, Comtois, and Icelandic pony are seen due to the *Silver* mutation (*PMEL 17* mutation) that codes for the reticulated "chocolate" coat color with the flaxen mane and tail. Homozygote *PMEL 17* horses typically have megalocornea with myopia ("near sighted"), iris abnormalities, cataracts, ciliary body cysts, and potential retinal detachments. Heterozygote *PMEL 17* horses have thin walled ciliary body cysts, and non-carriers of the *PMEL 17 Silver* mutation genome of these breeds have normal eyes.

## KERATOCONJUNCTIVITIS SICCA AND DERMATOLOGIC DISEASE

Keratoconjunctivitis sicca (KCS or "dry eye") is a common ophthalmic disorder that may be associated with dermatologic disease. The primary abnormality with KCS is the lack of production of aqueous tear from the main lacrimal/3$^{rd}$ eyelid glands resulting in xerophthalmia and corneal/conjunctival disease. Cavalier King Charles Spaniels may be afflicted with a congenital KCS and ichthyosiform dermatosis ("curly coat disorder") where the cause of the KCS is not well understood. Medical attempts at stimulating lacrimation (topical use of cyclosporine, tacrolimus, or pilocarpine or oral use of pilocarpine) in these patients are fruitless, and lifelong topical supplementation with artificial tears is necessary to provide comfort/prevent blindness.

The most common cause of decreased aqueous tear production in the dog is immune-mediated lacrimoadenitis and subsequent lacrimal/3rd eyelid gland atrophy. Affected lacrimal glands from immune-mediated KCS cases have a mononuclear cell infiltrate dominated inflammatory response with subsequent acinar cell/gland atrophy. This inflammation may be specific to this tissue, and is more commonly seen in breeds such as the Cavalier King Charles Spaniel, Cocker Spaniel, English Bulldog, Lhasa Apso, Shi Tzu, and West Highland White Terrier. This lacrimal adenitis may also seen be in conjunction with other immune-mediated diseases (food/environmental allergies, systemic lupus). The calcineurin/T-helper cell inhibitors cyclosporine (Optimmune™, 0.2% and compound pharmacy formulations, usually 1-2% in oil base) and tacrolimus (compound pharmacy formulations, 0.01-0.03% in an aqueous base) prevent intracellular interlukin-2 production and suppress T-helper cell activation and glandular inflammation to allow for normal production of aqueous tear.

Another less common cause of KCS is due to a lack of parasympathetic neurologic stimulus via the facial nerve (CN VII). Lack of parasympathetic innervation (neurotropic KCS) may also be accompanied by dry mucous membranes on the ipsilateral side as the nerve deficit. A common cause of this is otitis media/interna. Inflammatory or surgical trauma to the facial nerve branches as they pass through the middle/internal ear can lead to aqueous tear production deficits as well as post-ganglionic Horner's syndrome (lack of sympathetic innervation to the eye and periocular structures). For treatment of neurogenic KCS, the parasympathomimetic agent pilocarpine can be administered orally (1 drop of the 2% ophthalmic solution per 10 kg BW twice daily with increasing dosage until the patient either develops adverse systemic side effects or the tear production increases to an acceptable level) or topically (dilute ophthalmic pilocarpine in saline based artificial tears to a 0.125-0.25% concentration, applied three times per day). Adverse side effects of oral administration include salivation, diarrhea, vomiting, and bradycardia; adverse side effects of topical administration include immediate irritation, conjunctivitis, and miosis.

One of the least common causes of KCS is drug induced lacrimal gland degeneration. Systemically administered sulfonamides (sulfadiazine, trimethoprim-sulfonamide combinations as well as phenazopyridine and sulfasalazine) causes lacrimal/3rd eyelid gland atrophy/fibrosis by an unknown mechanism. Prior to the 1980's, TMS products were extensively used long-term for the treatment of bacterial dermatitis and genito-urinary tract bacterial infections. Papers incriminating TMS and similar products with iatrogenic KCS led to discontinuation of this practice in the canine species. Small (less than 12 kg BW) dogs are more susceptible to the toxic effects of TMS, possibly due to a tendency to overdose small patients. If TMS must be used, weekly or more often monitoring of the Schirmer tear test is warranted. If the STT value drops, most dogs will recover with discontinuation of the drug. If, however, total discontinuation of tear production occurs while on TMS products, recovery to normal tear production is unlikely.

## OCULAR-DERMATOLOGIC DISEASE OF THE EYELIDS

The eyelids are made up of haired skin, mucous membranes, mucocutaneous junctions, and sebaceous glands. It is not uncommon for bacterial, fungal, viral, parasitic, neoplastic, and immune-mediated diseases of the skin to involve the eyelids/periocular skin.

Bacterial blepharitis (many times *Staphylococcal spp.)* is many times seen in those dogs with coexisting staphylococcal pyoderma. Swollen lids, alopecia, ulcerations, dried crusts, intense pruritus with self-trauma, and swollen Meibomian glands are common. Cytology/culturing of ulcerated skin lesions, pustules, and expressed secretions from inflamed Meibomian glands along with coexisting generalized skin lesions confirms the clinical diagnosis. Along with long term appropriate systemic anti-bacterials, supportive care such as meticulous periocular hygiene, warm/cold moist compresses, judicious use of systemic corticosteroids and ocular antibiotic-corticosteroids, and Elizabethan collars to limit self-trauma will aid in resolution of clinical signs. Some ophthalmologists in the past have routinely used staphage lysate preparations and

autogenous staph bacterins, but this author has no experience with these treatment modalities. Dogs with repeated bouts of Meibomianitis may improve with tetracycline therapy as an immunomodulating agent or an agent to modify sebum content in these sebaceous glands.

Nasal fold pyoderma is commonly seen in brachycephalic breeds of dogs due to excessive moisture providing an excellent growth environment for bacteria and yeast down in those dark, skanky recesses where the sun don't shine and the wind don't blow. A common cause of increased moisture is epiphora either due to increased tear production (frictional irritation to the corneal surface from distichia, ectopic cilia, conjunctivitis, and chalazia/Meibomianitis) or inappropriate nasolacrimal duct drainage of tears (impatent palpebral punctae, medial trichiasis acting as a wick to misdirect tear flow, medial entropion, lower lid ectropion, and/or short noses with pinched ducts). As an ophthalmologist, I try to address tear production/drainage in an attempt to dry up the moisture source. When proper drainage cannot be accomplished (a common occurrence in these brachycephalics as well as in mesocephalic dogs with "poodle epiphora"), supportive care is indicated. Oral tetracycline was used for years to help reduce the tear staining associated with "poodle epiphora". Today, tylosin (Angels' Eyes) is used for tear staining due to "poodle epiphora" and in some cases this will help nasal fold pyoderma as well. Overgrowth of bacteria and yeast can be lessened by keeping the hair around and in the nasal folds cut short and by swabbing with either dilute bleach (1 tablespoon/gallon of water or stronger) or dilute Betadine solution (0.5-1% in water) as a disinfectant/astringent down in those folds with a Q-tip. Chlorhexidine wipes also help reduce bacterial growth and are good astringents, but chlorhexidine is epithelial toxic to the cornea, so care much be taken using them periocularly.

Specific dermatophyte infections (*Microsporum spp., Trichophyton spp.*) may be seen as alopecia and skin hyperemia of the lids. This is most commonly seen in dogs and occasionally in foals. Unlike the canine bacterial blepharitis cases, pruritus and self-trauma are seldom seen with these fungal infections. Treatment similar to that for dermatophyte infection elsewhere on the body is appropriate with the exception that Betadine scrub and miconazole lotion should not be gotten on the conjunctival or corneal surfaces (Betadine solution and miconazole creams are safe to use around and even on the ocular surface).

Feline herpes virus surface ocular disorders (neonatal ophthalmia, symblepharon, conjunctivitis, ulcerative keratitis/dendritic ulceration, corneal stromal keratitis, eosinophilic keratoconjunctivitis, corneal sequestrum) are some of the most commonly seen feline ophthalmic diseases presented to veterinary ophthalmologists. On occasion, I have seen cats with ophthalmic manifestations that also have facial dermatitis characterized by ulceration, eosinophilic inflammatory cell infiltrate, and apparently intense pruritus with self-trauma. The armamentarium for treating ocular herpes disorders includes topical medications (idoxuridine, trifluridine, vidarabine, cidofovir, alpha interferon 2a) and systemic medications (famcyclovir, l-lysine, alpha interferon 2a). When faced with cats that have chronic herpetic eye disease and accompanying facial dermatitis, I feel compelled to evaluate the patient for underlying immunosuppressive viral infections (FeLV and/or FIV). Although the FeLV-FIV status of the patient will seldom change my course of therapy, knowledge of this status will allow me to better counsel the client concerning possible long-term outcome and the need for long-term therapy. Of the cats where I thought our medical intervention was worthwhile, the cats were treated with a poly-pharmaceutical approach (the old "both barrels of the shotgun" approach). The optimal dose for oral famcyclovir in the cat is 40mg/kg bid or tid. Other systemic antivirals are either ineffective in the cat due to poor blood/tissue levels or are extremely hepatotoxic. Oral alpha interferon 2a is administered as 1000 units in 1 ml volume daily, 7 days on, seven days off. I have kept cats with chronic recurrent herpetic eye disease or that were co-infected with FeLV or FIV on this regimen for years with no untoward effects. L-Lysine at a dose of 500 mg/day has been shown to reduce virus levels and virus shedding in experimentally inoculated cats and can be given indefinitely. Topical therapy by veterinary ophthalmologists has gravitated towards use of 0.5-1.0% cidofovir solutions twice daily. The prolonged therapeutic level in the tear film of cidofovir and its active

metabolites (as well as its acceptance/comfort level by the patient) makes the bid therapy much more palatable for owners than the q2-4h dosage schedule for the other commonly used topical anti-virals. An idoxuridine ophthalmic ointment is available through compounding pharmacies as well as generic commercially available vidarabine ointments for topical application on skin lesions.

In the canine patient, periocular sarcoptic mange and demodex infestations are occasionally primarily seen by ophthalmologists. Periocular sarcoptic mange infestations usually have other cutaneous manifestations or intense pruritus, alopecia, and crusts/scaling and are usually diagnosed via clinical signs and skin scrapings. Application of "old school" dips to the periocular areas of the face should be meticulous to minimize corneal/conjunctival toxic effects. Topical selemectin use is preferred over dips concerning ocular safety. Focal periocular demodex infestations may be seen as areas of alopecia with mild erythema. Unlike sarcoptic mange infestations, these areas are usually not pruritic. This author has seen palpebral alopecia in older adult dogs that are being treated with topical cyclosporine or tacrolimus containing products for KCS. Some ophthalmologists feel that the localized decreased cell mediated immunity due to topical application of these agents allows for the localized growth of the demodex mites in the hair follicles with resultant alopecia. Most of these infestations do not require therapy, but if needed, topical avermectin and milbemycin products may be used. The antiquated use of focal topical application of the organophosphate fenthion is no longer recommended due to toxicity potential.

In the horse, periocular habronema blepharitis is rarely seen due to the common use of avermectin compounds in routine equine deworming protocols. The granulomatous, ulcerative lesions may be seen on the face, periocularly, and on the ventral abdomen. These lesions are seen during the warm weather months in tropical and temperate climates. The nematodes *Habronema* and *Draschia* are normally found in the stomachs of horses. Larvae are passed in the feces and houseflies and stable flies act as intermediate hosts. Larvae are usually deposited near the mouth, are licked from the lips, pass to the stomach, and the life cycle is completed. Larvae deposited near the eyelids or in wounds may grow and result in the granulomatous lesions. Cytology of scrapings from these ulcerative lesions usually reveals large numbers of eosinophils, typical of parasitic infestations. Another characteristic of habronemiasis are the presence of calcified bodies ("sulfur granules") that gives these lesions their characteristic "gritty" feel. Therapy involves treatment with systemic ivermectin. Debulkment of lesions and intralesional injections of corticosteroids may be necessary to speed resolution and minimize self-trauma. Benign, firm nodules may be found along lid margins during the winter months in horses that have healed without medical therapy or animals that have been medically treated but the "sulfur granules" were not excised. These rounded, firm bodies represent the calcified remains of the "sulfur granules" and are usually encased in a fibrous coat of scar tissue. They do not usually cause any inflammation or self-trauma, as is the case with the active inflammatory lesions.

Eyelid neoplasia seldom presents to the dermatologist; usually these cases are referred to an ophthalmologist. For the sake of completeness, some relatively common eyelid tumors that may also have dermatological involvement are listed here so that the dermatologist may understand the mentality of an ophthalmologist when it comes to treating these tumors.

A disorder well recognized in the Bernese Mountain Dog is histiocytosis. This disorder is familial, affects males more than females, and is also seen in Rottweilers, Golden, Flat-Coated, and Labrador Retrievers. Two forms of this disorder are recognized, systemic histiocytosis (SH, a non-neoplastic form considered by some to be a disseminated form of cutaneous histiocytosis) and malignant histiocytosis or histiocytic sarcoma (HS). This disorder was commonly seen in the 1980's and 90's, but due to its genetic predisposition, breeders have almost eliminated the disorder in most of the predisposed breeds. Initial lesions of both diseases may include cutaneous nodules, plaques, and depigmenting crust lesions of the face, especially of the mucocutaneous junctions of the eyelids, nares, and mouth. Compared to the relatively "benign" reactive

cutaneous histiocytosis seen in many breeds, both SH and HS usually have ocular involvement manifested as nodules of the conjunctiva, sclera, extraocular muscles, orbit, and ciliary body with anterior uveitis, choroiditis, glaucoma, and retinal detachments being frequently seen. Unlike cutaneous histiocytosis, SH and HS will usually have localized lymphadenopathy and multiple organ involvement. Systemic histiocytosis may somewhat be controlled with immunosuppressive levels of corticosteroids and azathioprine, cyclosporine A, and/or leflunomide. Histiocytic sarcoma is usually a rapidly progressive disorder with usually fatal sequela.

Mast cell tumors are occasionally seen of the eyelid skin in dogs and cats. These masses may be focal, multifocal, or may be seen in conjunction with multifocal involvement elsewhere on the body skin. These masses can appear as small, firm, superficial skin masses, soft, more wide based masses, or as ulcerated erythematous alopecic areas of intense urticaria and pruritus , and are usually seen in older animals. Fine needle aspirate with cytology or biopsy are usually diagnostic. Biologically, most of these masses are rather benign; most histologically grade as low-grade (grade I or grade II tumors). Surgical excision is the treatment of choice for these masses, but size and location may make excision with wide margins and subsequent reconstruction to a functional eyelid difficult. In cats and dogs alike, excision with "dirty margins" many times still results in resolution with no local or metastatic recurrence. Because of the deleterious effects (KCS, keratitis, cataract formation, retinal degeneration) of periocular megavoltage radiation therapy, few ophthalmologists recommend post-excisional radiation therapy. Many ophthalmologists will treat with systemic or intralesional corticosteroids following excision without wide, "clean" margins with good long-term results. For years some ophthalmologists have treated large, broad based, eyelid mast cell tumors in old cats with intralesional sterile water or repositol corticosteroids. Many ophthalmologists with varying periods of post-therapeutic follow-up have anecdotally described shrinkage of the mass with no apparent local or distal metastasis.

Eyelid melanomas in the dog are of two variations. Flat, broad based skin masses or small, pedunculated masses of the external lid tend to be easily excised or debulked and treated with cryotherapy with success. Melanomas that arise from the eyelid margins or palpebral conjunctiva or that extend into the palpebral conjunctiva are usually more aggressive, more difficult to resolve with surgical therapy, and are more likely to have regional lymphatic metastasis. They are, however, usually less malignant/aggressive than melanomas of the oral cavity or distal extremities.

In the cat, eyelid melanomas are extremely rare, usually involve the palpebral or third eyelid conjunctiva, and are usually highly malignant with local recurrence/distal metastasis being commonplace. Some recommend orbital exenteration in an attempt to save the lives of afflicted animals.

In the horse, eyelid melanomas may be seen on the palpebral/third eyelid conjunctiva, eyelid margins, or as subcutaneous masses within the external eyelid stroma. Usually seen in older, grey colored animals, these masses are typically benign and surgical excision is curative. Ancillary treatments following surgical debulkment include cryosurgery, $CO_2$ laser therapy, and photodynamic therapy with generally good results. Many of these animals also have other cutaneous melanomas at sites distant to the eyelid masses. No studies have been published indicating effectiveness of oral cimetidine therapy as has been proposed for other cutaneous melanomas.

Ocular squamous cell carcinomas (OSCC) are seldom seen in the dog. Most OSCC originate at the corneoscleral limbus and are seen in those animals that are also afflicted with KCS. Eyelid margin OSCC in the dog is extremely rare. Surgical excision with or without ancillary treatment such as $CO_2$ laser therapy or cryosurgery is usually curative.

In the cat, small eyelid skin or eyelid margin OSCC masses are easily treated with excision and cryosurgery with good results. Unfortunately, many cats are not referred until the mass has invaded the eyelid skin or the palpebral/third eyelid conjunctiva. In this author's hands, these

more invasive cases have uniformly ended up with local metastatic disease that has resulted in euthanasia despite aggressive therapy such as orbital exenteration, cryosurgery, and/or local beta irradiation.

In the horse, OSCC is commonly seen in lightly pigmented horses of an older age. The Appaloosa, Paint, Pony of the Americas, Tennessee walking horse, Belgian, and Haflinger breeds are over represented in this author's hospital population. OSCC in the horse may be seen originating from the eyelid skin, eyelid margins, third eyelid/bulbar conjunctiva, or corneoscleral limbus. Most eyelid skin/margin lesions begin as ulcerated lesions that progressively enlarge and eventually invade the eyelid stroma. On occasion, a solid, non-ulcerated subcutaneous mass may be the earliest form of presentation. Scrapings/FNA and cytology may be diagnostic, but ulcerated lesions may be heavily contaminated with bacteria leading to an erroneous diagnosis. Biopsy with histopathologic evaluation is usually definitive. Although complete surgical excision may be curative, OSCC in the horse tends to be very locally invasive, especially within the eyelid stroma. Numerous ancillary surgical techniques (liquid nitrogen cryosurgery, $CO_2$ laser therapy, photodynamic therapy, brachytherapy, and radiofrequency hyperthermia) have been used in conjunction with surgical excision through the years with varying results. Intralesional chemotherapy (cisplatin in oil, cisplatin slow release beads, 5-flurouracil injections, and immunotherapy with BCG injections) either as the only therapy or in conjunction with surgical excision has had mixed results concerning efficacy. Local metastasis to regional lymph nodes is more commonly seen with eyelid OSCC than with OSCC of the globe itself, and recurrence at the same or other distant sites is commonplace.

Equine sarcoid is a common clinical entity of the eyelid (second only to OSCC in occurrence). Bovine papilloma virus infection has been shown to be an underlying cause of equine sarcoid, but a genetic predisposition has also been proposed. Sarcoids are typically seen in young horses, a distinct difference from the occurrence of OSCC. Five broad types of sarcoids are classified (occult, verrucose, nodular (A and B), fibroblastic (A and B), and mixed), and most periocular sarcoids are nodular, fibroblastic, or mixed. Surgical biopsy is usually necessary for definitive diagnosis, but surgical manipulation of sarcoids usually stimulates the mass to grow and spread, so additional therapy should be planned either in conjunction with biopsy or soon thereafter. Because of location, complete surgical excision of a periocular sarcoid is rarely feasible. Ancillary treatment in conjunction with surgical excision includes cryosurgery, radiofrequency hyperthermia, and $CO_2$ laser therapy. In the hands of this author, none of these treatment modalities has been effective either because the mass recurred or scar tissue formation following surgery left a poorly functioning lid. Local immunotherapy (repeated injections of the immune stimulant BCG) has been quite successful in this author's hands. Intralesional chemotherapy (intralesional injections of 5-FU, cisplatin powder emulsified in oil, or especially the implantation of cisplatin slow release beads) has been relatively effective as reported in the literature. Brachytherapy (radioactive iridium or gold intra-lesionally implanted in flexible straws) as well as debulkment and photodynamic therapy have also been reported to be effective. This author has no personal experience with topical application of imiquimod, but in the hands of some, this appears to be an effective therapy.

"Immune-mediated" diseases involving the eyelids are relatively common in the dog and can be occasionally seen in cats and horses as well. The pemphigus complex of diseases, discoid and systemic lupus erythematosus, and juvenile pyoderma/cellulitis ("puppy strangles") many times have eyelid skin/margin involvement with their systemic/dermatologic manifestations. Eyelid/conjunctival disease may be seen in conjunction with other systemic/dermatologic disease following allergic reaction to drugs, foods, inhaled substances, and insect/spider bites/stings. Many of these disorders are initially seen by the veterinary ophthalmologist but are best eventually being dealt with by dermatologists/internists who see these conditions more commonly and are more experienced with therapy for these patients.

Canine pemphigus foliaceus (PF) is one of the more common immune-mediated dermatoses affecting the eyelids. Mucocutaneous junction/lid margin lesions are rare, a differentiating feature from pemphigus vulgaris (PV). Much more rare in presentation and much more severe, PV typically presents with mucocutaneous junction ulcerations of the eyelids, nares, mouth, anus, vulva, and prepuce. In the cat, PF is much more common than PV, and it is similar to the presentation of canine PF (bilaterally symmetrical crusts with less pustules on the skin of the eyelids, pinna, and face with claw fold paronychia). In the horse, PF is occasionally seen as vesicles, erosions, scaling, and crusting of the skin of the face, lids, limbs, and ventrum. Systemic corticosteroids are the mainstay of medical therapy for these conditions with use of azathioprine, chlorambucil, and cyclosporine A being used in addition to corticosteroids in resistant cases and in cases where corticosteroid therapy is problematic. Long-term prognosis for PV is poor, with euthanasia being a common choice by owners.

Discoid lupus erythematosus (DLE, also called cutaneous lupus erythematosus) is much more common than systemic lupus erythematosus (SLE) in the dog, and neither disorder is commonly seen in the cat. DLE typically is seen as depigmentation, erythema, and scaling of the nose and nasal planum and eventual erosions, ulcerations, and crusting of the nose. Left untreated, extension of these lesions to the eyelids and ears is seen. SLE is a multisystem disease with dermatological manifestations being seen only about 50% of the time. Interestingly, over the past 25 years at our hospital, a number of the dogs presented with SLE have been treated for immune-mediated KCS prior to diagnosis of SLE. Polyarthritis, FUO, anemia with thrombocytopenia, proteinuria with glomerulonephritis and potential renal failure, are all systemic signs of SLE in the dog. In the eye, anterior uveitis is occasionally seen with more common choroidal/retinal vasculitis being seen in dogs and especially in man. Systemic corticosteroids are the mainstay therapy for SLE with additional drugs such as azathioprine, chlorambucil, and even vincristine being used. Topical corticosteroids and avoidance of sunlight may lead to remission in DLE cases. Long-term prognosis for SLE cases is guarded.

Juvenile pyoderma/cellulitis ("puppy strangles") is a disorder usually seen in 2-4 month old puppies as an initial swelling of the eyelids, lips, and muzzle. Submandibular lymphadenopathy is commonly seen, and left untreated, progression to fistulated, draining lesions with crusts may be seen on the face, lid margins, and pinna, along with panniculitis and otitis externa. Cytologic examination of papulopustular lesions reveals pyogranulomatous inflammation with rare bacteria. Culture and sensitivity usually shows these lesions to be sterile for bacterial growth. Since this disorder progresses rapidly with a potential for severe scarring, aggressive immunosuppressive doses of corticosteroids should be initiated early with weaning of the dosage only beginning once the disorder is controlled. Some recommend broad-spectrum antibiotics in conjunction with the systemic corticosteroid use, but this is only necessary in cases where secondary bacterial infection is present.

Dogs with atopy, food allergies, and flea allergies may also have conjunctivitis with eyelid/facial urticaria and pruritus. Allergic conjunctivitis is much more commonly seen in man than in animals, but can be seen in our canine patients. In persistent cases of steroid responsive conjunctivitis, food trials/allergy testing with desensitization following rule out of primary ophthalmic disease (lash anomalies, bacterial infection, eyelid conformational disorders) may be indicated. In the cat, seasonal occurrences of eosinophilic keratoconjunctivitis may indicate underlying allergic phenomena. In the horse, eosinophilic keratoconjunctivitis is an ocular disorder seen as seasonal conjunctivitis/eosinophilic plaque formation on the corneal/conjunctival surfaces with superficial corneal ulcerations that are difficult to resolve. Decreased tear production is also seen in some cases that have palpable enlargement of the main lacrimal glands. Many of these horses also have seasonal allergies with pruritus, urticaria, and hives as well as the ophthalmic manifestations. Allergy testing and avoidance/desensitization has been beneficial in some of our hospital's cases in an attempt to prevent yearly recurrence of this painful, frustrating disease.

Vaccine/drug reactions and adverse response to insect stings/bites commonly present with wheals, facial swelling, pruritus, urticaria, and palpebral chemosis. These symptoms are more likely to be seen by the general practitioner or an emergency facility clinician. There are a few topically applied ophthalmic medications that with chronic use can cause blepharitis with lid swelling, erythema, alopecia, and pruritus being the presenting clinical signs. Chronic topical application of aminoglycoside (neomycin, gentamicin, tobramycin) antibiotic containing solutions, the anti-glaucoma medication dorzolamide, atropine, and corn oil based, compounding pharmacy made cyclosporine A are well recognized by veterinary ophthalmologists for causing "contact allergies" in dogs. Certain ophthalmic drop preservatives are also offenders (therefore the large human market for "preservative free" moisturizing drops for contact lens wearers and dry eye patients), and this can be problematic for dogs on chronic topical medications.

## CORNEOSCLERAL DISORDERS WITH A DERMATOLOGY CONNECTION

Besides the disorders described previously under eyelid diseases and conjunctival diseases, there are few diseases of the cornea and sclera that have a direct connection to skin disease. There are a few disorders, however, where systemic disease that may be addressed by a dermatologist may present with ocular signs involving the cornea and sclera.

Corneal lipid degeneration in the dog is seen clinically as a white spot or arc shaped opacity of the cornea. There are many underlying causes (inheritance, previous corneal wounds/surgeries, chronic use of topical corticosteroids, and systemic lipid metabolism defects) of corneal lipid deposition. Hypothyroidism and Cushing's syndrome are two lipid metabolism defects that may cause corneal lipid degeneration and skin/hair coat disease. Testing for thyroid dysfunction and/or hyperadrenocorticism and appropriate treatment of these disorders may resolve corneal lipid deposition or reduce its progression in predisposed dogs.

Nodular granulomatous episclerokeratitis (NGE) is a sterile granulomatous disorder of the cornea, episcleral, third eyelid, and lids of collies and "shepherd type" dogs. "Collie granuloma" may also be seen of the nose, ears, and general skin as a sterile subcutaneous nodular granulomatous disorder that is histologically indistinguishable from the microscopic disease of the eyes. In the past, these dogs were treated with topical and systemic corticosteroids with mixed results. Azathioprine therapy (beginning at 2.2 mg/kg/day with slow weaning) has been used in the past with good results. By slow titration of the dosage, some dogs could be reduced to a dosage as low as 0.5 mg/kg/72 hours with control of the condition. As is the case with other sterile skin granulomatous disorders in man and dogs, oral tetracycline and niacinamide may be used with good results in some of these NGE cases.

Scleritis usually presents in dogs as raised, pink/tan subconjunctival sector lesions caudal to the limbus. Localized corneal edema/infiltrate may also be seen. Ocular pain, anterior uveitis, and posterior segment disease (choroiditis +/- retinal detachment) may also be noted. In some cases, focal nodular lesions from the face/muzzle may appear histologically similar to biopsy specimens of sclera (dense infiltration of lymphocytes and plasma cells with no sign of malignancy or infection). The American Cocker Spaniel seems to be predisposed to this disorder, and many of the cases we see in our hospital also have history of "skin allergies" and/or KCS. Testing for systemic collagen diseases (canine rheumatoid factor, anti-DNA- antibody, LE cell identification) is seldom fruitful (unlike the case with necrotizing scleritis in man). Medical therapy is in the form of topical corticosteroids and/or immunosuppressive doses of corticosteroids. Some patients with slow weaning can eventually be gotten off systemic steroids with no ill effects.

**DISEASE OF THE INTERNAL EYE WITH A DERMATOLOGY CONNECTION**

The classic derm/ophtho intraocular disease is uveodermatologic syndrome or VKH-like syndrome in the dog. This inflammatory disorder is seen as a panuveitis with potential ulcerative lesions of the facial skin with poliosis and vitaligo. One hypothesis for the disease is an immune-mediated destruction of melanocytes. It has been suggested that the skin disorder is due to a Th1-mediated inflammatory response while the ocular signs are due to a Th2-mediated inflammatory response. This may be why some dogs present with the ocular disease only, occasionally some dogs will present with the skin disease only, and most dogs will present with one disorder prior to onset of the other. Ocular signs initially present as blepharospasm, conjunctival, and episcleral injection due to anterior uveitis. Blindness due to choroiditis and secondary retinal detachment may be the initial client complaint without signs of ocular discomfort. Uncontrolled, secondary glaucoma and cataract are common sequela to the intraocular inflammation. Even with control of intraocular inflammation, depigmentation of the irides and choroid/tapetum is frequently seen. This disorder was originally described in man as uveitis, vitaligo, poliosis, hearing difficulty, and meningitis. The ocular and skin manifestations are the only disorders described in the dog, therefore the name "VKH-like syndrome". The Hormel Sinclair strain of pigs and a strain of quail are the only other animal species described to have this disease. The Akita was the first breed to be described, but other predisposed breeds may include Australian Shepherds, Samoyeds, Huskies, Dachshunds, Chows, Shetland Sheep Dogs, Beagles, and others. Age of onset is usually 2-6 years of age. Due to the probability of permanent blindness due to secondary glaucoma, cataract, or irreparable retinal detachment, aggressive medical therapy in the form of systemic immunosuppressive corticosteroids is recommended following diagnosis. Supportive topical medical therapy (topical steroids and anti-glaucoma medications) may help prevent/control sight threatening glaucoma while intraocular inflammation is being settled down. The serous/exudative retinal detachments seen in some cases may resolve with vigorous medical therapy, and blind dogs may regain vision. Long-term medical therapy is required, and some dogs may be controlled with systemic azathioprine or cyclosporine A to reduce the untoward effects of chronic corticosteroid therapy.

Canine distemper virus occasionally has dermatologic manifestations ("hard pad"), but almost always has ocular manifestations as well as neurologic and gastrointestinal signs. Initially, conjunctivitis and decreased aqueous tear film is seen in almost all dogs. Most dogs will have some manifestation of retinitis and optic neuritis that may cause obvious vision impairment. Chorioretinal scarring ("gold medallions" in the tapetal fundus and multifocal areas of choroidal depigmentation in the non-tapetal fundus) is commonly seen following recovery of the acute disease.

Systemic blastomycosis is an infectious fungal disease seen in dogs from endemic areas. Because of the multisystem nature of this infectious disease, clinical signs involving the skin, eyes, bones, lungs, lymph nodes, prostate, brain, and testicles may be seen. Skin lesions may include draining or non-draining pyogranulomatous lesions of the subcutaneous tissues. Localized or multifocal lymphadenopathy and draining pyogranulomatous lymphadenitis is also seen with frequency. Ocular disease may include anterior uveitis with secondary glaucoma, exudative choroiditis with retinal detachments, endophthalmitis, and blindness. Although most cases have bilateral ocular involvement, unilateral eye disease is not uncommon. Fever, pneumonia, osteomyelitis, and meningioencephalitis are also seen in many acute cases. Diagnosis is usually obtained by identifying the yeast form of the organism from draining skin wounds, FNA of lymph nodes, or vitreous centesis. The organism is usually present in such large numbers in infected tissues that identification of the organisms from tissue aspirated is not difficult. Thoracic radiography with identification of perihilar lymphadenopathy and "cotton wool spots" or "snow storm" pulmonary infiltrate is indicative of blastomycosis. In our hospital, agar gel immunodiffusion (AGID) testing for antibodies sometimes lags behind the clinical

disease. Blastomycosis antigen testing in urine has been found to be a more reliable diagnostic test for disease than the AGID test, and can be used to monitor treatment efficacy. Treatment is expensive since most patients are larger breed, outside and hunting dogs and involves oral itraconazole for long periods of time. Dogs with endophthalmitis and blindness should have their eyes enucleated for pain relief and to reduce the possibility of the eye being a site for organisms remaining to reproduce following discontinuation of medical therapy. Intact males should also be castrated as the testicles may act as a site for organisms to reside, as is the case of blind, painful eyes.

Lymphosarcoma is the most common metastatic neoplasia to ocular tissues. Anterior uveitis, hyphema, hypopyon, secondary glaucoma, chorioretinitis, retinal hemorrhages, and retinal detachments are common ocular manifestations of lymphosarcoma. Dogs with intraocular disease due to lymphosarcoma usually have advanced disease and have shorter survival periods than do dogs with focal disease or no ocular disease. Besides normal chemotherapy for the primary disease, topical corticosteroids to control anterior segment inflammation and anti-glaucoma therapy (topical beta blockers and carbonic anhydrase inhibitors) for those dogs having secondary glaucoma are commonly used supportive therapy.

Ivermectin toxicity in dogs commonly shows signs of blindness with or without pupillary light deficits as well as well as depression or coma, muscle tremors, hyper- or hypothermia, and other ophthalmic reflex (menace, strabismus) defects. It has been known for years that Collies and other breeds (Australian Shepherds, English Shepherds, Longhaired Whippets, Old English Sheepdogs, Shelties, and Silken Windhounds) have a genetic defect for the multiple drug resistance gene (MDR-1 gene) that makes these breeds sensitive to ivermectin toxicity. Accidental over exposure to ivermectin (licking/eating residual cattle or horse dewormer) or intentional high dose ivermectin therapy for external parasites may lead to ophthalmic and neurologic clinical signs in these and other breeds. On clinical examination, ophthalmic signs may include optic neuritis and retinal edema/folds as well as pupillary light reflect abnormalities. Discontinuation of ivermectin treatment and supportive care usually results in restoration of vision and neurologic status in 2-10 days.

**FOR ADDITIONAL IN FORMATION, YOU MAY WISH TO READ:**

Cullen CL, Webb AA. Ocular Manifestations of Systemic Disease, Part 1: The Dog, in Veterinary Ophthalmology, 5th Edition. Oxford, UK: Wiley-Blackwell; 2013, 1897-1977

Cullen CL, Webb AA. Ocular Manifestations of Systemic Disease, Part 2: The Cat, in Veterinary Ophthalmology, 5th Edition. Oxford, UK: Wiley-Blackwell; 2013, 1978-2036

Cullen CL, Webb AA. Ocular Manifestations of Systemic Disease, Part 2: The Horse, in Veterinary Ophthalmology, 5th Edition. Oxford, UK: Wiley-Blackwell; 2013, 2037-2070

# NOTES

# OUR PATIENTS LICK THE FLOOR…AND THEIR BUTT – ROLE OF THE ENVIRONMENT AND CHALLENGES IN INFECTION CONTROL

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**INTRODUCTION:**

It is estimated that 1 out of 20 hospitalized patients develops a healthcare-associated infection (HAI) in the U.S.[1] This translates to 1.7 million HAI resulting in 99,000 deaths. In a review of the annual estimates of methicillin-resistant *Staphylococcus aureus* (MRSA) in the U.S. there were an estimated 250,000 hospital discharges, 94,000 new invasive infections, resulting in 19,000 deaths. This is a clear example of the magnitude of the potential problem of HAI in U.S. hospitals. The key pathogens for these infections include MRSA, vancomycin-resistant enterococcus (VRE), *Clostridium difficile*, *Acinetobacter*, Norovirus, and mostly recently carbapenem-resistant *Enterobactericeae* (CRE). Many of these same pathogens are found in veterinary teaching hospitals, but estimates for HAI are not readily available. The following discussion will focus on the role of the environment in transmission of healthcare associated infections in the veterinary setting and measures to evaluate cleaning and hygiene measures on the hospital environment.

**ROLE OF THE ENVIRONMENT:**

Weinstein et. al. estimated the source of HAI among ICU patients. They attributed endogenous flora to account for 40 to 60% of infections, cross-infection from hands of hospital personnel for 20 to 40%, antibiotic driven changes in flora for 20 to 25%, and other factors like environmental contamination for 20%. There is strong evidence supporting the role of the environment for disease transmission. This includes documentation that surfaces in the room of a colonized/infected patient are frequently contaminated, that pathogens are capable of surviving on hospital surfaces and equipment for long periods of time, that healthcare personnel hands and gloves are readily contaminated after being in contact with a contaminated environment, that a person admitted to a room previously occupied by a patient colonized/infected have an increased likelihood of being colonized/infected, and that terminal cleaning does lead to decreased rates of infection.[3] In a review of recent documented MRSA events in veterinary settings it was noted that environmental contamination ranged from 1 to 12%.[4-7]

| **Organism** | **Duration of Persistence (range)** |
|---|---|
| Norovirus | 8 hr – 7 days |
| Influenza virus | 1 – 2 days |
| *Clostridium difficile (spores)* | 5 months |
| *Chlamydia psittaci* | 15 days |
| *E. coli* | 1.5 hours – 16 months |
| *Klebsiella* | 2 hours - > 30 months |
| *Pseudomonas aeruginosa* | 6 hours – 16 months |
| *Salmonella spp.* | 1 day – years |
| *Staphylococcus aureus* | 7 days – 7 months |

Many of these organisms can persist in the environment (Table 1). Survival and persistence depends upon ambient temperature, presence of organic matter, surface type, and hygiene practices.[8]

**METHODS TO IMPROVE CLEANING:**

Several studies have documented that <50% of hospital rooms are adequately cleaned and disinfected.[9,10] This begs the question about how well do we clean and disinfect our veterinary facility surfaces. There are a number of methods used to improve cleaning and disinfection; these include staff education, use of checklists, and hygiene assessment tools. There are also newer technologies such as "no touch" methods of surface disinfection.[3] These include UV light and hydrogen peroxide mists. Also, self-disinfecting surfaces with impregnated heavy metals (i.e. Copper and Silver) or germicides (i.e. Triclosan) are increasingly being used.

**University of Minnesota – Case Studies**

Historically, environmental cultures are the main means to assess environmental cleanliness. Cultures can be definitive if looking for a specific pathogen (e.g. Salmonella) but can be costly and time consuming. In food and hospital areas, the use of luminometers or fluorescent dyes are being used to assess environmental contamination. At the University of Minnesota, we have tried to utilize these tools to assess our approaches to environmental hygiene.

In 2004, our infection control group noted an increase in multidrug resistant (MDR) *E. coli* among patient in our ICU. Subsequent investigation noted that this clonal group of *E. coli* was observed in larger, heavier dogs with frequent human contact (e.g. nursing care). A subsequent environmental assessment found evidence of the same *E. coli* clone on several human contact surfaces such as infusion pump face plates and animal holding areas. This cluster required a temporary closure of the ICU, in-service training on hand-hygiene, and continued pathogen surveillance. This represented one of the first times where environmental contamination was taken seriously in our teaching facility with attempts to measure our cleaning and hygiene methods.

To assess our environmental cleaning, we conducted several studies to look at contamination levels in critical and busy areas of our teaching hospital (treatment room, ICU). Our first attempt was to measure the correlation of luminometer values (relative light units - rlu) to culture results. Other attempts included assessing hourly and weekly luminometer values in busy areas of the hospital including the treatment room floor where many patients are held for collection of diagnostic samples. A subset study was done to assess keyboard contamination with *Staphylococcus* spp. and luminometer values.[11]

Similarly, our infection control staff noted an increase in *E. coli* contamination of urine samples submitted for culture. Hygiene assessment with fluorescent dyes was used to assess the cleanliness in the areas used to collect samples. These tools have been critical for our staff and student education. The impact of these techniques have facilitated changes in hospital policy and improved our environmental hygiene.

**SUMMARY**

Hospital surfaces are frequently contaminated from our patients and staff. The contaminated environment provides numerous opportunities for person-to-animal or animal-to-person transmission of potential pathogens. Good surface cleaning and environmental monitoring needs to be incorporated in the veterinary setting to reduce HAI.

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Key Words: Environment, Healthcare-associated infections, MRSA, multidrug resistant (MDR) organisms

# NOTES

# TECHNICIANS' SESSION PRESENTATIONS FRIDAY

# DERMATOPHYTOSIS
# TROUBLE SHOOTING DIAGNOSTIC TESTS

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Dermatophytosis is superficial fungal skin infection of the skin, hair, and nail. It is most common in young animals, particularly kittens. In house diagnostic testing is strongly encouraged for both the diagnosis and monitoring of pets. The most commonly used diagnostic tests are the physical examination, examination under a strong beam light, Wood's lamp examination-YES! This is a valuable tool, direct examination of hairs and spores, and toothbrush fungal culture. Toothbrush fungal cultures are strongly recommended for monitoring of cats/kittens under treatment.

## IN HOUSE SUPPLIES-SPECIALTY ITEMS

**Wood's Lamp:** Battery operated Wood's lamps or black lights are not adequate. A medical grade diagnostic Wood's lamp is needed a long wave light. The two types recommended are: UVL-21(http://www.minresco.com/uvlamps/handlamp.htm) or Burton Ultraviolet UV Wood's Exam Light (Burton). This is my favourite and preferred lamp as it has replaceable bulbs and magnification.

**Clearing Agents/Direct Examination**: Mineral oil! KOH is not necessary.

**Microscopic Stains:** Lacto phenol cotton blue is the preferred stain, however new methylene blue can be used. Lactophenol cotton blue stain is preferred because this stain is rapidly absorbed by hyphae and macro and micro conidia making them easier to see. The dropper packs (50/pac) are ideal for practices as one dropper can be used several times and it does not dispense an excessive amount of stain. This can be purchased on Amazon.com.

**Toothbrushes:** Individually wrapped toothbrushes can be purchased from Overstock.com. They are sold in cases of 144 and range from $6.00 to $10.00 per case depending upon the quantity they have to sell.

**Fungal culture plates:** Avoid diagnostic mediums sold in jars, that have small surfaces, or are not easily inoculated. The preferred plates are sold at Hardy Diagnostics: Sab-Duet (DTM/Mycobiotic plates) or Derm-Duet. The latter may be more useful in practice because the plates are individually packaged and will be less likely to become contaminated.

## PRACTICAL TIPS FOR TROUBLE SHOOTING SPECIFIC TESTS

### Wood's lamp

- Plug in Wood's lamps are "ready to go" and do not need time to warm up. The most important thing is to be able to darken the room.
- Fluorescence may appear to increase with exposure to the Wood's lamp; this may simply be due to light adaptation of the viewer's eyes.
- In cats, this is a time and cost effective procedure. The major problem with use is not taking enough time to examine the cat and/or not being trained to recognize Wood's positive hairs. These hairs are bright apple green and it is the shaft that glows.
- It is often said that less than 50% of strains do not fluoresce. This comment was extracted from the human literature and perpetuated into veterinary literature. We do not know this information and scientific data on strains and fluorescence is unknown.
- Crusts do not fluoresce, important to lift crusts and examine hairs beneath. VERY commonly glowing hairs are seen here.

- Cats with no visible lesions on gross examination will often have fluorescing hairs, infection can be confirmed via direct examination
- "Dust mop cats will not fluoresce-likely the source of "less than 50% of strains glow"
- Field and experimental studies on treatment protocols documented that a decrease in Wood's positive hairs correlated with response to treatment
- Wood's lamp examinations are valuable during monitoring of treatment, especially if the cat is not curing
- **Bottom line: Wood's lamp positive hairs examined by direct examination confirm an infection, and Wood's lamp examination can find sites of infection that are not visible on gross clinical examination.**

*Practice Tip: Keep a "positive control slide". This can be used to train new staff or it can be used if the viewer is "not sure". This is easily made by either sandwiching glowing hairs between two glass slides and taping the ends closed OR by pressing CLEAR scotch tape to the skin where there are strongly fluorescing hairs. Stick this on a glass slide. Fluorescence will persist for years! Also, glowing hairs are commonly seen in the bristles of infected cats. Collect some to practice with.*

**Direct Examination of Hairs**
- Technique is not difficult to learn.
- Time and cost effective only with Wood's lamp positive hairs
- Pluck hairs in the direction of growth using fine forceps
- Place in a drop of mineral oil and coverslip.
- Abnormal hairs are clearly visible: they are paler, wider, and the arthrospores are more refractile.
- Use the Wood's lamp to find or identify infected hairs on the microscope.

*Practice Tip: Use a digital camera to take "reference photos" at 4x, 10x and 40x.*

**In House Fungal Cultures**

**<u>Fungal Culture Medium</u>**

- Criteria for selection: most volume of medium for the money, how easily the plate can be inoculated, how easy it is to sample the plate, does the plate have a red indicator, shelf life, and cost
- Avoid glass jars because they can be difficult to inoculate and sample, if sealed too tightly can have increased growth of contaminant bacteria and fungi
- Avoid mini culture plates as there is too little surface and volume of medium for veterinary patients. These were designed for use in human medicine.
- Small medium plates will often turn red very rapidly making it difficult to monitor colonies.
- Use flat culture plates with phenol red colour indicator
- Plates that require refrigeration are at risk for getting ruined if they are accidently frozen, keep the plates in the door or vegetable crisper

**<u>Diagnostic Sampling</u>**
- Samples can be obtained using the toothbrush fungal culture technique or by plucking individual hairs. Plucking hairs is only recommended if hairs are glowing. In general toothbrush sampling is preferred. Individually plucked hairs may need to be cultured as part of monitoring. Toward the end of treatment, only the tips of hairs may glow and not all of these are culture positive.
- DO NOT USE GAUZE as this is not reliable. It does not get deep enough in the hair coat and you may get false negative cultures.
- Toothbrush cultures are recommended in cats and kittens. Toothbrush an area for 20 swipes; hairs should be visible.
- Sample the body and the LESION LAST.

- DO NOT remove hairs from the toothbrush for culture unless you are specifically culturing a glowing hair. Random sampling is not reliable.

**Inoculation Tips**

- Toothbrush Fungal Cultures: Gently stab bristles in the plate STARTING at the center of the plate and moving out to the edges OR if using split plates start at the top and move down. If there is yellow medium on the bristles, you have pressed hard enough. Do not press too hard or the medium will be pulled off the plate.
- Plucked hairs: Press firmly onto the surface of the medium but do not embed; use sterile cotton swabs to press to surface if necessary. Be sure to disinfect and sterilize the forceps.

*Practice Tip: Inoculate plates upside down over a moisten paper towel. This will minimize contaminates from getting on the plate and the wet paper towel will minimize possible spread of infective spores that could result in false positive test results for another patient.*

**Optimizing Incubation**

- Label plate! Use a consistent system or a sticker.
- Seal Cultures in Self Sealing Sandwich Bags: This will increase humidity and minimize premature dehydrate of the plates. It will prevent the plates from cross contamination and from contamination by media mites. It increases biohazard safety in small in house laboratories.
- Incubate fungal culture plate medium side UP. If moisture accumulates it will deposit on the lid and not on the plate minimizing overgrowth of bacteria and fungi.
- Incubate Plates at 75 to 80°F. This will increase growth and sporulation. This is warmer than room temperature, especially in the summer if air conditioning is used.
- Place a small inexpensive digital fish tank thermometer near the plates to monitor temperature. Place thermometer in a plastic bag so it does not become contaminated.

*Practice Tips: Fungal cultures can be incubated in small clear plastic shoe box. This will contain specimens and often be adequate for incubation at optimum temperatures. It is also easily decontaminated. If room temperature is still too cold a "mini incubator" can be made using a "PlayMate" cooler and a fish tank water heater. Remove drain from side of cooler and place fish tank incubator into the hole. OR a small glass terrarium can be used with a reptile floor heater stuck to the bottom.*

**Optimizing Monitoring of In-House Fungal Cultures**

- Examine plates daily. Print off a copy of the calendar month and tape it to the top of the container with the fungal culture plates. Place your initials in the box when this task is done. Use this sheet to alert other technicians and staff doctors to fungal culture plates that are suspect.
- Minimize opening of plates until it is optimum to sample a colony. Growth can easily be detected by holding the plate up to the light.
- *M. canis* rarely needs more than 14 days to grow. Plates with no growth should be held for 21 days as *Trichophyton spp* can take up to 21 days.

**Dermatophyte Test Medium**

- Originally developed for use in Vietnam to help rapidly identify dermatophytosis in soldiers. The goal was to develop a fungal culture medium that could be used as a visual "positive" or "negative" tool. It was very rapidly shown that DTM was helpful in decreasing the growth of contaminants but false positives were common in people. Shortly afterward this was shown in animals.
- DTM Medium is a basic fungal culture medium (Sabouraud's medium) which contains antimicrobials to inhibit/slow the growth of contaminant bacteria and fungi. It also contains a colour indicator that is pH sensitive. The pH of the media is 5.5 (straw yellow). Pathogens turn the medium alkaline (red) as they grow. So do many contaminants.
- The benefit of DTM is lost once the plate fully changes color.

- Target colonies to examine in animals that have <u>not been treated</u> are those that are pale in color and have a red color change around the medium as they grow. There are three commonly encountered problems that you need to be aware of. The first is that the gross and microscopic morphology of *Microsporum canis* may be very different than what is depicted in text books. This is because the phenol red affects fungal growth. The second is that if the toothbrush is from heavily infected cat, large numbers of spores may be deposited on the plate. The spores are struggling to grow and will not readily sporulate. The plate will appear 'swarmed' with a dense growth. In these cases, there may be lots of growth and a delayed color change. The third is that post treatment fungal cultures typically show both abnormal gross and microscopic growth. If serial weekly or biweekly cultures are being done you will soon learn to recognize the gross and microscopic morphology.

**<u>Rules of Thumb for DTM</u>**

- *Red Does NOT MEAN I am POSITIVE. Red means LOOK AT ME.*
- *Skip me if I am heavily pigmented, I am not a pathogen. I am a contaminant.*
- *Skip me if I am heavily pigmented even if I develop a red ring of colour around me as I grow. I am a contaminant.*
- *Skip me if I am pale or buff and do not have a red ring of colour developing around me as I grow. I am a contaminant.*
- *DO NOT SKIP ME if I am pale/buff and a red ring of colour is developing around me as I grow. I may be a pathogen. Examine me microscopically.*
- *Sometimes I look suspicious, circle me and watch me daily.*

**<u>Microscopic Examination of Colonies</u>**

- Microscopic examination of representative colonies is needed to positively identify dermatophytes.
- It is however very important to recognize the gross morphology of pre-treatment and post treatment colonies.
- The number of colonies and the amount of time spent performing microscopic examinations is markedly decreased by focusing on only highly suspect colonies.
- Highly suspect colonies are grossly pale or buff and have a red colour change developing around them as they grow.
- Truly infected cats tend to have mono-cultures of just the pathogen, but not always.
- Microscopic examination of colonies is done by gently pressing the stickly side of CLEAR scotch tape to the most representative colony and placing that over a drop of lactophenol cotton blue. It is very helpful to wait 15-20 minutes before examining the sample. The stain will be absorbed and make identification easier.
- Examine the colony at 4x, 10x and then 40x.
- *Microsporum gypseum:* It grows much faster than *M. canis* and produces lots of macroconidia. The macroconidia are so plentiful it is often hard to see hyphae. Key features are: much less variation in size, thin walls, large number of macrocondida, rarely see budding off of hyphae, much more consistent number of segments.
- *Microsporum canis:* It does not produce large numbers of macroconidia, although some strains can. Early growth is typically characterized by lots of segmented hyphae and early budding characteristic of M. canis may present as thick 'fingers' at the end. The number of segments is HIGHLY variable. The most consistent feature is the thick walls. Other 'classic' features include a hooked knob at the end and rough walls.

**<u>Post Treatment Colonies</u>**

- Post treatment cultures can be typical of *M. canis* or atypical.
- Atypical findings of *M. canis* post treatment samples include: delayed growth of colonies and abnormal gross morphology. In addition, it is typical of post treatment colonies to not produce characteristic macrocondidia. All that may be seen are the "fingers" or thickening at the ends of the hyphae. This is occurring because the antifungal treatment is affecting the organism's growth.

- It is also common for there to be 'classic' starburst growth but delayed color change on the plate.

**Using Colony Forming Units (CFU) to Monitor Therapy**

Typically, culture results are interpreted as positive or negative. Based on a screening and treating program established for cats in shelters, this provides an inadequate amount of information to the clinician. Tracking the number of CFUs helps monitor an animal's response to treatment. As treatment progresses and the animal responds, the number of CFUs per plate will decrease. The following are guidelines for using this information.

- Use a culture plate system with an adequate surface area that allows for counting of individual colonies. Standard Petri dish fungal culture plates or dual compartment plates are adequate.
- Inoculate the entire surface of the plate and incubate it at 25° C to 30° C. Hold plates from animals receiving treatment for at least 21 days.
- Develop a shorthand system for recording growth on fungal culture plates and a record sheet he following shorthand system is used: NG (no growth), C (contaminants), HC (heavy contaminants), or the pathogen listed (P).
- A semi quantitative system that translates this into a "severity score" that is recorded on the laboratory data sheet can be used.
    - Pathogen score 1 (1 to 4 CFUs/plate) or P1
    - Pathogen score 2 (5 to 9 CFUs/plate) or P2
    - Pathogen score 3 (>10 to too many to count) or P3
- This system makes it easy to monitor culture results and provides a visual record of the pet's response to treatment. In most cases, animals with severe infections will have a starting culture score of P3. As treatment progresses, the P score becomes lower. Cured animals have cultures with no growth or just contaminant growth on culture.
- The scoring system is also very helpful in identifying pets undergoing treatment that are exposed to fomite contamination. These animals commonly will have cultures fluctuating from negative to P1. When this pattern is seen, the owner can be instructed to improve hygiene in the home; as fomite contamination is removed, the fungal cultures become negative. In addition to identification of fomite exposure, this system also rapidly alerts the clinician to animals that are failing therapy or are relapsing for one reason or another. Lack of response to therapy will be evident by a persistently high P score. Relapses will be represented by a sudden increase in the P score.
- Weekly fungal cultures speed detection of cure. The cat in the example below had its first negative culture at week three (12/23) and second at 12/30.

| ID number | Are results final? | Results | Date collected | Date inoculated | Week 1 | Week 2 | Week 3 | P score | Woods? |
|---|---|---|---|---|---|---|---|---|---|
| **Claws (1st exam)** | **Y** | ***M. canis*** | **12/2** | **12/2** | **suspect** | ***M.canis*** | ***M.canis*** | **3** | **+** |
| Claws | Y | *M.canis* | 12/9 | 12/9 | ng | *M.canis* | *M. canis* | 3 | |
| Claws | Y | *M.canis* | 12/16 | 12/16 | ng | *M.canis* | *M.canis* | 2 | |
| Claws | Y | neg | 12/23 | 12/23 | ng | ng | ng | 0 | |
| Claws | N | neg | 12/30 | 12/30 | ng | ng | ng | 0 | |
| Claws | N | neg | 1/6 | 1/6 | ng | ng | | | |

*References are available from the author upon request*

# NOTES

# DERMATOPHYTOSIS
# IN CLINIC DECONTAMINATION

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*Note: The reader is referred to the "Feline Dermatophytosis: Decontamination-It's not as hard as we thought" for specific information on decontamination.*

## VETERINARY CLINICS-WHAT IS THE RISK?

Dermatophytic fungi have been isolated from the floors of veterinary clinics[1], however the true risk of contamination was found to be very low in a one year surveillance of a veterinary medical teaching hospital.[2] On random days, 10 sites with high cat traffic were sampled once weekly for one year. The dermatology examination room was culture positive 10/23 times and all of the other sites in the hospital combined were positive only 13/23 times and all had two or less cfu/plate. Positive culture results in the dermatology examination room always followed known or highly suspect infected cats. When there was known exposure, additional cultures were performed to see if the staff and students were 'tracking' *M. canis* throughout the hospital. This did not occur either because of containment practices by the dermatology service and/or cleaning practices in the hospital. During the one year time, routine cleaning consisted of rooms were continually dry mopped to remove hair and debris and only wet mopped to remove bodily fluids. In the evenings, the floors were swept, mopped and disinfected with a quaternary ammonium based product. A common concern among staff members is whether or not exposure to an infected cat at work is a risk for their pets at home. In another field study, environmental cultures from 16 staff members working with infected cats under treatment were collected weekly for three weeks to look for fomite carriage on street clothes and/or transfer of spores to their home or pets (Moriello 2004 unpublished field data). Staff wore smocks, coveralls, or protective gowns during the day. Contamination with *M. canis* of street clothes, home environments or pets was not found. Based upon findings in these two studies, routine cleaning of floor throughout the day and daily disinfection is adequate control for unpredicted clinic exposure. The wearing of laboratory coats or smocks that are changed daily or sooner if soiled is protective against mechanical fomite carriage off site.

## CATS UNDER TREATMENT

When cats under treatment are being re-examined, the client should come at the end of the day. The cat should be in a carrier and the carrier covered by a towel. Ideally, cover the table with a large towel or blanket and keep the examination of the cat limited to that surface. Once the examination is done, the towel/blanket can be wrapped with the contaminated material in the middle and transported to the laundry in a plastic bag. Contaminated towels should be washed separately.

## WHAT TO DO IN THE CASE OF UNEXPECTED EXPOSURE

In the event that exposure is discovered during an examination, every effort should be made to keep the cat confined to one examination room. The cleaning for a known dermatophyte exposure in the clinic is no different than what should be done for any other infectious disease (e.g. respiratory virus, parvovirus).

- If the cat arrived without a carrier, send the cat home in one if available.
- Immediately spray any surfaces contacted by the cat with a disinfectant to keep hair from leaving the room.
- Staff members should immediately change laboratory coats and smocks and place them in plastic bags. This is done to minimize the accidental spread of spores in the clinic and to prevent fomite contamination of other pets.

- The first major priority is mechanical removal of debris. This is best done with a Swiffer or vacuum cleaner. If the vacuum cleaner is portable from room to room it will need to be cleaned, hence preference for Swiffers or equivalent. An excellent general cleaning product to have in the clinic is 3M Easy Trapper. This material is similar to a Swiffer except that it is 'sticky'. Debris otherwise not collected by Swiffers sticks to this material.
- Mechanical removal is followed by washing exposed surfaces with detergent and soap. In general, this means the table, counters, floors and client seating areas. Flat mops are especially helpful for this. Mechanical cleaning alone can decontaminate a room. The hard cleaning is done until the surface appears clean.
- The final step is application of a disinfectant. Accelerated hydrogen peroxide (Accel) used at 1:16 is recommended. This is odourless and the product is not irritating to staff or animals or corrosive.
- If there is concern that this is not enough, simply simply re-clean the room. Discretion need to be used in public areas of the clinic where the owner and cat may have been. Dust mopping the floors with the Easy Trapper Duster followed by a flat mop cleaning will not be distressing to the other clients in the waiting room.

**IN PATIENTS**

If a dermatophyte infection is discovered in a cat that needs hospitalization, move it to an isolation ward or as far away as possible from other cats, but close enough for appropriate monitoring. Cover the front of the cage with a towel and use appropriate signage. Staff should wear gloves and changes smocks when handing the animals. When it is safe, appropriate antifungal therapy can be started.

**PREVENTION**

An excellent way to rapidly identify possible exposure risks is to have veterinary technicians routinely examine young cats presented for surgical neutering with a Wood's lamp or any cat under their care in which skin lesions are found. Obviously, proper training is needed.

**ELECTRIC CLIPPERS**

These are easily decontaminated by gross removal of debris and autoclaving. If the latter is not possible, meticulous removal of gross debris and thorough spraying of the blades and head with Clippercide (King Research, 45% isopropanol) is anti-fungal. Not less than 10 minutes of wetting time is needed. It is important to remember to clean the other surfaces of the electric clippers. Because contaminated clippers are one of the most common sources of fomite contamination, this should be repeated several times.

**FUNGUS IN THE AIR????**

Very commonly, the following occurs. A cat (usually a kitten) is diagnosed with dermatophytosis and in seven to 21 days later staff members develop lesions. Rumors of "*infection on the air currents"* start to circulate when a little investigation reveals that staff members, (very often non veterinary technicians, i.e. receptionists were cuddling, holding or playing with the kitten. Although spores have been found on contact plates placed in homes with infected animals, work with shelters had revealed that this is not a significant concern. Spores and hairs are trapped in furnace filters. Veterinary clinics adhering to routine and regular cleaning are unlikely to be contaminated by patient exposure.

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# NOTES

# DERMATOPHYTOSIS
# CLIENT EDUCATION: COMMON QUESTIONS AND ANSWERS

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The following are the most commonly asked questions that I answer in examination rooms or via email consultations. The goal of this session is to provide core information so that these questions can be easily and accurately answered. Some of the information needed to answer these questions is detailed in other notes in the session and will not be duplicated here.

*What is ringworm?*
*How did my cat contract it?*
*What do you mean this is a 'zoonotic disease'?*
*What is the risk to my family?*
*I have an immunosuppressed person in the house. What do I do?*
*Can my family be "carriers?"*
*What about my other cats?*
*What about my dog?*
*I have skin lesions what should I do?*
*How is this treated?*
*I can't give systemic antifungals. My neighbour said it is toxic and "chews up the liver". What should I do?*
*Topical therapy is too hard; do I have to do this?*
*I'm totally CONFUSED! You talk too fast? What is that treatment plan again?*
*I can't possibly 'confine' the kitten. It's too cute. What's the worst that can happen if I don't do this?*
*Environmental contamination! "You mean it's in MY HOUSE?" I'm getting rid of the kitten…*
*What do you mean by 'hard cleaning'?*
*What disinfectant should I use? I read that undiluted bleach is best.*
*How often do I need to clean?*
*The bottle says "One Step", why are you telling me I have to do more than spray this?*
*Do I have to throw out all the towels and blankets?*
*If vacuuming enough?*
*I heard this fungus lives for YEARS in the environment. What do I do????*
*When is the kitten cured?*

## PATHOGENS OF IMPORTANCE IN CATS

- Feline dermatophytosis is a superficial fungal skin disease of cats. The most commonly isolated pathogen is *Microsporum canis*; however infection with *M. persicolor*, *M. gypseum* and *Trichophyton spp* can occur.
- From a disease control perspective, the most important fungal pathogen of cats and in shelters is *M. canis*.
- *M. canis* is not part of the normal fungal flora of cats and isolation on a fungal culture indicates infection, fomite carriage from the environment, or cross contamination, i.e. via exposure to contaminated smocks or scrub tops.

## UPDATE ON TRANSMISSION AND PATHOGENESIS POINTS

- Transmission of the disease occurs most frequently from cat to cat contact.
- Culture positive status can occur from contact with infective arthrospores on blankets, bedding, toys, brushes, lab coats, leather gloves, or even external parasites.
- Spores can be airborne, but these are readily trapped in heating/cooling furnace filters. Spores readily adhere to dust and fall.

- The incubation period from exposure to *clinically* obvious lesions is approximately two to four weeks, but there is evidence that cats develop subclinical lesions and are infective much sooner.
- An experimental model of feline skin showed that *M. canis* arthroconidia started to adhere within two hours of contact and increased for up to six hours post inoculation. During experimental infection models, infected hairs (Wood's positive and direct examination positive) were found in less than a week post inoculation.
- Clearly the emerging information on how quickly spores can adhere and germinate emphasizes that prevention of contact with infective spores is important disease control point. In order for an infection to occur, infective spores must contact the skin surface and defeat host protective mechanisms (sebum, normal flora, grooming, and innate immunity.

**ZOONOTIC INFORMATION**

- Dermatophytosis is a zoonotic disease. The pathogen is considered a low level pathogen as disease is not life threatening and is easily cured.
- It is important to put the disease into perspective. Dermatophyte infections are common in people but they don't recognize this as such. Athlete's foot fungus is a dermatophyte infection. Most people have been in places (gym, showers, pools, etc.) where they already been exposed to large numbers of these spores. They may have even contracted it.
- Owners with skin lesions should be referred to their physician. However it is often comforting to client to tell them to expect their physician to tell them it is self-limiting disease and can easily be treated.
- Pet owners at risk for exposure to many zoonotic diseases some of which can cause serious illness or even be life changing.
- Children are at most risk for contracting dermatophytosis from an infected pet because they have not yet developed immunity to infection and because they interact with infected animals in ways that increases exposure, i.e. constant carrying of the kitten! (See notes on topical therapy and confinement).
- Immunocompromised people are at risk of contracting any disease, not just dermatophytosis. They should speak with their physician for more detailed information. They should not be involved in the treatment of infected or in contact animals until cured.

**IN CONTACT EXPOSED PETS**

- Animal to animal infection occurs through direct contact with another infected animal or via traumatic fomite inoculation.
- Household pets should be examined for skin lesions with a Wood's lamp. If cultures are pending, these pets can be washed with an antifungal shampoo several times a week.

**TREATMENT**

- The author uses the "CCATS" abbreviation to help explain treatment and help clients understand it.
- Treating consists of: **c**onfinement, **c**leaning, **a**ssessment, **t**opical therapy, **s**ystemic therapy.

**Confinement of the cat/kitten to an easily cleaned room**

- Confinement needs to be reasonable and appropriate for the pet. Kittens should not be left alone in a home unsupervised and older cats may not move around much and may have other diseases that require intense monitoring. Some older cats will not eat or be easily medicated if not in close contact with the owner.
- This shortens treatment time because it makes cleaning easier and minimized the spread of infective material into the home.
- Clients often protest, simply ask them to do what they would if the cat had diarrhea
- Treatment needs to be as short as possible because many infected animals are new family members and need to be socialized.

**Cleaning and Disinfection**

- Cleaning shortens treatment time because it minimizes the risk of false positive fungal cultures that would prolong treatment.
- Infective spores will be shed into the environment, it is CRITICAL to explain to understand that these spores do not grow or multiply in the environment, they are just like dust
- Unless it is clearly explained, many people equate "ringworm fungus" with mildew or black mold and become unnecessarily agitated about this risk
- The primary reason that cleaning of the home is necessary is to prevent infective material from collecting on the cat's hair coat and confounding culture results (false positive culture results).
- If case reports are any indication of occurrence, there is really little evidence of disease being contracted solely from contact with a contaminated environment (i.e. no direct contact with an infected host).
- See notes from other areas of this meeting for cleaning information.

**Assessment**

- Assessment refers to monitoring of treatment.
- **The recommendation is to treat cats until they have two negative fungal cultures**.
- The global cost of treatment needs to be considered when determining how the cat/kitten will be monitored. This global cost includes time and money spent on: systemic drugs, topical rinses and time to apply them/dry the cat/clean up after the treatment, money spent on buying cleaning supplies and time spent doing extra cleaning if what is requires is above what the client normally does, issues and time spent on confining the cat. The latter is an overlooked cost as this may be an emotional cost. It could also include money and time spent interacting with the kitten/cat and then washing clothes specifically identified for this purpose. If children are involved, this magnifies the problem as children are more likely to contract dermatophytosis and most likely to circumvent any 'confinement' measures.
- The author favors weekly fungal cultures to monitor treatment. The advantages of weekly fungal cultures are:
    - Provides more intense monitoring of the progress of treatment, clients have feedback
    - Documents cure (i.e. two negative fungal cultures) faster than culturing on other schedules
    - Allows for more rapid detection of treatment failures commonly due to lack of compliance with treatment
    - Decreases the time cats need to be reasonably confined during treatment
    - Decreases the number of topical applications used during therapy
    - Decreases the duration of antifungal therapy administered
    - Increases overall client compliance and minimizes the development of subclinical infections

**Topical Therapy**

- Treatment of dermatophytosis involves concurrent use of a topical antifungal whole body treatment and a systemic antifungal drug. Systemic antifungal drugs do not kill spores on the hairs, only spores in the hair follicle.
- Topical antifungal therapy is required to kill spores on the hair coat.
- Topical antifungal therapy will help prevent spread to other animals and possibly people, especially if lime sulphur is used.
- Topical antifungal rinses include lime sulphur, enilconazole, accelerated hydrogen peroxide (Pure Oxygen). In addition, antifungal shampoos that contain ketoconazole, miconazole or climbazole are antifungal.
- If children are present, the use of lime sulphur is strongly encouraged as this builds up on the hair coat and has a residual 'protective' effect for several days after repeated applications.
- Topical therapy should be applied no less than twice a week.

**Systemic Therapy**

- Systemic therapy is a necessary part of therapy of almost all cases of feline dermatophytosis.
- Systemic therapy kills spores in the hair follicle but not on the hairs that have emerged from the hair follicle; hence it is combined with topical therapy.
- The most commonly used systemic antifungal drugs are itraconazole, fluconazole, and terbinafine. All drugs are metabolized by some organ in the body and for these drugs it is the liver.
- The advantage of these drugs is that they accumulate and have residual antifungal activity after they have been discontinued.
- These drugs are commonly used in short term or pulse therapy protocols.

**Clipping of the hair coat**

- Clipping infected hairs grossly debulks the amount of infective material
- Clipping needs to be done carefully to avoid traumatizing the skin; electric clippers can cause thermal burns.
- Ideal in long haired cats, but sedation may be necessary.
- Scissor clipping is the recommended method to clip cats. Children's round tipped metal scissors are ideal. They are inexpensive so they can be discarded and are blunted so there is minimal risk of injury.
- May not be needed in most cases if through application/drenching of topical solution can be applied.

# NOTES

# SATURDAY

# CLINICAL SHORT COMMUNICATIONS SATURDAY

**NORTH AMERICAN VETERINARY DERMATOLOGY FORUM**
**Phoenix, AZ**

## SATURDAY, APRIL 12, 2014

## ACVD CLINICAL SHORT COMMUNICATIONS

| | | |
|---|---|---|
| 9:00 | Gotthelf | Clinical evaluation of a long lasting topical vehicle, TRI-726, containing ketoconazole 0.15% and hydrocortisone 1% in canine *Malassezia* otitis externa |
| 9:15 | Griffin | Prospective survey of reported reactions to allergen specific immunotherapy injections |
| 9:30 | Rossi | Mucocutaneous lupus erythematosus in 20 dogs: clinical signs and outcome |
| 9:45 | Cain | Clinical and histopathologic features of dorsally oriented furunculosis in dogs following water immersion or exposure to grooming products |
| 10:00 | Cain | Clinical response to medical management in 29 German shepherd dogs with perianal fistulae |
| 10:15 | Ferrer | Treatment of perianal fistulas in dogs with intralesional injections of autologous bone-marrow derived mesenchymal stem cells: a pilot study |
| 10:30 – 10:45 | | RESIDENT RESEARCH AWARDS |
| 10:45 - 11:00 | | BREAK |
| 11:00 | Frank | Serum cortisol concentrations in dogs with pituitary dependent hyperadrenocorticism and atypical Cushing's syndrome |
| 11:15 | Diesel | *Corynebacterium pseudotuberculosis* in a cat |
| 11:30 | Diesel | Chronic cutaneous protothecosis in a dog |
| 11:45 | Gonzales | Evaluation of Pregabalin (Lyrica®, Pfizer Inc.) in a canine model of flea-allergic dermatitis |
| 12:00 | Frank | Use of Oclacitinib (Apoquel®, Zoetis) for treatment of cutaneous mastocytosis in a cat |
| 12:15 | Navarro | Field effectiveness and compliance of cefalexine (Rilexine®) for Treatment of secondary superficial pyoderma in dogs |
| | | LUNCH |

**Clinical evaluation of a long lasting topical vehicle, TRI-726, containing ketoconazole 0.15% and hydrocortisone 1% in canine *Malassezia* otitis externa**

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**ABSTRACT:** TRI-726, a patented inert vehicle for drug delivery in both human and veterinary applications, rapidly changes from a solution to gel with increased temperature. When compounded with various pharmaceutical agents, the gel form of TRI-726 remains present in tissues for up to 15 days. The purpose of this clinical trial was to test the long acting properties of a preparation of TRI-726 containing ketoconazole 0.15% and hydrocortisone 1% in clinical canine *Malassezia* otitis externa cases. Twenty three affected ears were flushed with (dioctyl sodium succinate) and water, suction dried, then infused with the test material allowing 2 minutes for gelation. Study dogs were examined on each visit (infusion (day 0), days 7 and 14) with a video otoscope, evaluated cytologically, and pruritus was assessed. *Malassezia* counts (per HPF) were reported on a scale from 0 (none) to 4 (too numerous to count). Otic pruritus scores were reported by the owners based on a 0 (none) to 4 (severe) scale at days 0, 7, and 14. In the ears evaluated, 18/23 (78%) on day 7 and 14/23 (61%) on day 14 were successfully treated based on a reduction of *Malassezia* numbers to 0 or 1 on the *Malassezia* count. On day 0, 22/23 (96%) dogs were pruritic, while at day 7 and day 14, only 4/23 (17%) dogs were pruritic. In summary, a single infusion of this topical formulation is beneficial in treating canine *Malassezia* otitis externa and provides comfort from pruritus for up to 14 days.

This study was funded by TriLogic Pharma, LLC

Gotthelf, L. N. is a minority stockholder in TriLogic Pharma, LLC
Alur, H. H. is the Chief Scientific Officer at TriLogic Pharma, LLC

**Prospective survey of reported reactions to allergen specific immunotherapy injections**

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**Abstract:** Subcutaneous allergen specific immunotherapy (ASIT) has been associated with the induction of allergic reactions including pruritus, urticaria, and angioedema/anaphylaxis. This study was designed to determine the incidence of these reactions reported in dogs receiving ASIT. A prospective review of reported allergic reactions was done from August 1, 2011 to July 31, 2012. A log of all phone reports of probable allergic reactions reported from veterinarians who order ASIT solutions from Animal Allergy Specialists was kept. During that time 1,730 (1,679 canine and 51 feline) new allergen treatment sets were formulated. No cats and 27 dogs had allergic reactions. Significantly greater reactions occurred with the new set of allergens (25/27 dogs; $p<0.0001$) compared with only two reactions on refills. Those two dogs were restarting ASIT with the refill. Seven dogs reacted to the low concentration (1,000-2,000 PNU/ml) vial and 18 dogs reacted to the maintenance vial (10,000-20,000 PNU/ml). Anaphylaxis/angioedema was noted in 7 dogs [6 new sets (0.36%) (3 English bulldogs, 2 Pugs, 1 German Shepherd) and 1 refill in a Pitbull]. Two had concurrent urticaria. There were 5 cross bred dogs, including one Pug-cross and 10 purebred dog breeds reacting. Only three breeds had more than one case with a reaction reported. The incidence of allergic reactions in those breeds was 4/90 (4.4%) Boxers, 9/212 (4.25%) English Bulldogs, and 2/90 (2.22%) Pugs. Other breeds, affected with one case of an allergic reaction, included: Cocker spaniel, German Shepherd dog, Great Dane, Havanese, Pit bull, Rat terrier, and Welsh Corgi.

Self-funded

Conflicts of interest: All three authors work for Animal Allergy Specialists and Drs Griffin and Rosenkrantz own Animal Allergy Specialists and consult for Veterinary Allergy Reference Laboratory.

**Mucocutaneous lupus erythematosus in 20 dogs: clinical signs and outcome**

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**Abstract**: Mucocutaneous lupus erythematosus (MCLE) is a newly-recognized variant of chronic cutaneous lupus erythematosus in dogs; it clinically resembles mucocutaneous pyoderma except for the lack of response to antimicrobial therapy. Twenty dogs were diagnosed with MCLE based upon clinical and histological findings. Eleven (55%) were German shepherd dogs and their crosses. The age of onset varied widely (range: 3-13 years, median 6). The main historical signs were dyschezia and/or dysuria (13 dogs; 65%). At the time of diagnosis, lesions were seen on the vulvar/perivular (12 dogs; 60%) and/or anal/perianal regions (11 dogs; 55%). Lesions first appeared as well-demarcated erosions and ulcerations (19 dogs; 95%), erythema (13 dogs; 65%), and crusting (10 dogs; 50%). Hyperpigmentation was also commonly seen either perilesionally (14 dogs; 70%) or as a sequela to previous lesions (17 dogs, 85%). Immunomodulation was initiated after the lack of response to antimicrobial therapy. Where treatment information was available (17 dogs), topical/systemic glucocorticoid monotherapy (4 dogs; 24%), nicotinamide monotherapy (3 dogs; 18%), or polytherapy consisting of various combinations of these modalities in conjunction with modified cyclosporine, tacrolimus, and cycline antibiotics (10 dogs; 59%) were used. Spontaneous remission was not seen in any dog. Where outcome information was available, a majority of cases (9/16 dogs; 56%) underwent clinical remission within 30 days of beginning immunomodulation. Signs returned rapidly once therapy was discontinued. In conclusion, MCLE is a variant of chronic cutaneous lupus erythematosus that appears to have a fair prognosis for complete remission, but with frequent recurrences upon dose tapering.

# Clinical and histopathologic features of dorsally oriented furunculosis in dogs following water immersion or exposure to grooming products

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**Abstract:** A syndrome of acute-onset furunculosis has been described in dogs following bathing or grooming, though practitioners may be unfamiliar with typical features. The objective of this retrospective case series was to describe the clinical presentation, histopathologic features, and treatment of dogs with furunculosis following grooming product exposure or water immersion. Twenty-two dogs were included. The following information was collected from medical records: signalment; clinical signs; bathing or grooming procedure; diagnostic tests, including bacterial culture and histopathology; and treatment. German shepherd dogs (5/22; 23%) and Labrador retrievers (4/22; 18%) were most commonly affected. Skin lesions, particularly hemorrhagic pustules and crusts, were dorsally oriented in all dogs, and occurred a median of 2 days (range: 1 to 7 days) following water exposure. Twenty dogs (91%) were bathed at home or at a commercial grooming facility prior to lesion onset; one dog developed skin lesions following hydrotherapy in an underwater treadmill and one dog developed skin lesions after clipping and scrubbing for surgery. Lethargy, neck or back pain and fever were common clinical signs, mimicking discospondylitis or meningitis. *Pseudomonas aeruginosa* was most commonly isolated from the skin of dogs with bacterial culture performed (10/14; 71%). The main histologic feature was acute follicular perforation in the superficial dermis with suppurative inflammation and dermal hemorrhage. Systemic antimicrobial therapy, particularly oral fluoroquinolones (prescribed in 68% of cases), resulted in excellent clinical response in 16 of 22 (72%) cases. Recognition of this clinical syndrome is essential for accurate diagnosis and appropriate therapy of affected dogs.

This study was self-funded.

No conflicts of interest are declared.

# Clinical response to medical management in 29 German shepherd dogs with perianal fistulae

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**Abstract:** Immunosuppressive therapy is the mainstay of medical treatment for canine perianal fistulae. The objective of this study was to determine the effectiveness of medical management for perianal fistulae under field conditions. This retrospective study included 29 German shepherd dogs with perianal fistulae. Medical records were reviewed for: patient signalment; clinical signs; clinical lesion severity; starting and maintenance doses of immunomodulatory medications (ciclosporin with or without ketoconazole, tacrolimus, prednisone, or other) or antimicrobials; use of a diet trial; and complications arising during therapy. Median time to clinical response, defined as $\geq$ 50% improvement in lesion score, was determined using the Kaplan-Meier product limit method. Cox multivariable survival methods were employed to determine which factors were associated with time to clinical response. Median time to clinical response for all dogs was 500 days. Lesion scores were improved by 100% in 14/29 dogs (48%), by >50% in 8/29 dogs (28%), and by <50% in 7/29 dogs (24%). Of the 14 dogs achieving clinical remission, 10 (71%) received ciclosporin (Atopica: Novartis Animal Health, Greensboro, NC, or generic modified; mean dose: 3.4 mg/kg, range: 2-7.8 mg/kg) in combination with ketoconazole (mean dose: 6.8 mg/kg, range: 5-9.1 mg/kg) every 24 to 72 hours for maintenance. Five of these 14 dogs (36%) experienced relapse of perianal fistulae following discontinuation of maintenance medications, while no dogs relapsed with continuous medical management. None of the factors investigated were significantly associated with time to clinical response. This study confirms that long-term, uninterrupted medical management can successfully treat canine perianal fistulae.

This study was self-funded.

No conflicts of interest are declared.

# Treatment of perianal fistulas in dogs with intralesional injections of autologous bone-marrow derived mesenchymal stem cells: a pilot study

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**Abstract:** Intralesional injection of autologous mesenchymal stem cells (SC) has been demonstrated to be a safe and effective treatment for perianal fistulas associated with Crohn's disease in humans. The main objective of this pilot study was to determine the feasibility, safety and efficacy of intralesional injections of autologous bone marrow- derived mesenchymal SC in the treatment of canine perianal fistulas. Five dogs (4 German Shepherds, 1 Labrador retriever) diagnosed with perianal fistulas were included in the study. All were previously treated with ciclosporin and other drugs with minimal success. At the first visit, all dogs were examined, and the severity of the lesions graded (extension, depth). Cefalexin (30mg/kg/twice daily for 4 weeks) was prescribed. Twenty mL of bone marrow was collected from the proximal humerus. Mononuclear cells were isolated and SC expanded in cell culture. After 3-4 weeks of expansion, $20x10^6$ phenotyped SC were re-suspended in 10mL of PBS with 5% autologous serum and were injected in and around the fistulas. After the injection, the fistulas were sealed with fibrin glue. Two more SC injections were given 30 and 60 days after the initial treatment. No local or systemic side effects were observed in any of the dogs. One dog with very severe fistulas was withdrawn from the study; the remaining 4 dogs showed 30%-70% improvement after the third treatment. Although the treatment is apparently safe and feasible, increasing the potency or number of injected SC or combining it with other medications, will likely be required to obtain complete clinical cure.

Funding: Companion Animal Health Fund, Tufts University

Conflict of interest: none declared.

# Serum cortisol concentrations in dogs with pituitary dependent hyperadrenocorticism and atypical Cushing's syndrome

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**Abstract:** The purpose of this study was to compare cortisol concentrations and adrenal gland size in dogs with pituitary dependent hyperadrenocorticism (PDH) and atypical Cushing's syndrome (AHAC). Ten healthy dogs, 10 dogs with PDH, and eight dogs with AHAC were enrolled. Dogs were diagnosed with PDH or AHAC based on positive screening test results (PDH) or normal screening test results with abnormal extended adrenal hormone panel results (AHAC). All dogs had ultrasound performed to assess for adrenal-dependent disease and obtain transverse measurements for each adrenal gland. Blood was collected hourly from cephalic catheters for nine samplings. Serum cortisol concentrations and adrenal gland sizes were compared among the three groups. Mean cortisol concentrations (nmol/L) (control, 39.4; AHAC, 77.1; PDH, 136.4) and cumulative cortisol concentrations (nmol/L) (control, 410.5; AHAC, 755; PDH, 1195.8), excluding the first sampling time, differed significantly among the three groups ($P<0.05$). Adjusting for weight, the average adrenal gland diameter (mm) of control dogs (5.1 +/- 0.32) was statistically less than those of dogs with PDH (6.9 +/- 0.32; $P = 0.002$) and AHAC (7.5 +/- 0.36; $P < 0.001$). Adrenal gland diameter was not statistically different for dogs with AHAC or PDH ($P = 0.429$). In conclusion, sum and mean cortisol concentrations in dogs with AHAC were significantly increased compared to control dogs but less than those of dogs with PDH, while adrenal gland diameter was similar between dogs with AHAC and PDH. These findings suggest a role for cortisol in the pathomechanism of AHAC.

This study was funded by AKC Canine Health Foundation ACORN grant.

No conflicts declared.

# *Corynebacterium pseudotuberculosis* in a cat

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**Abstract:** A 2 year male castrated indoor/outdoor domestic shorthair cat was presented for cervicofacial pruritic dermatitis of one year duration. There were multifocal areas of alopecia, erosions, and excoriations along the face and neck, and a large oblong eroded plaque over the right lateral neck. No other systemic illness was reported, and peripheral lymph nodes were normal. Cytology of the lesions identified large numbers of cocci and rod shaped bacteria with numerous neutrophils. Culture was pursued at recheck evaluation when empiric antibiotics failed to resolve cytologically-reconfirmed infection (cocci and rods). Aerobic swab culture isolated *Corynebacterium pseudotuberculosis;* this was confirmed with biochemical tests and 16S rDNA sequence analysis. Histopathology showed perivascular eosinophilic and mastocytic dermatitis, scattered cocci bacteria, with granulation tissue formation, fibrosis, ulceration, and necrotic serocellular crusting suspected due to underlying allergic dermatitis/hypersensitivity. Bloodwork and thoracic radiographs did not identify any systemic involvement. Pradofloxacin (Veraflox: Bayer Animal Health, Shawnee Mission, KS) 10mg/kg once daily orally led to clinical resolution of infection in 8 weeks (scar remained). Flea allergy dermatitis was suspected as the underlying cause with secondary infection. *Corynebacterium pseudotuberculosis* primarily affects horses and goats; it has been reported only once in the veterinary literature in a cat (Australia) and is not identified as a small animal pathogen. This cat lived in a suburban area (Houston, TX, USA) with no access to large or farm animals and no travel history. Whether this is an under-recognized etiology or a novel presentation of clinical disease is uncertain. Further evaluation may be warranted.

Source of funding: NONE

**Chronic cutaneous protothecosis in a dog**

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**Abstract:** A 5 year female spayed Boston terrier dog was presented for multifocal nodular skin disease with ulceration, exudation, and crusting of several weeks duration. Haired and non-haired skin of the nasal planum, footpads, ear pinnae, head, sternum, and limbs was affected. Cytology identified neutrophilic inflammation, macrophages, intracellular and extracellular round to oval organisms measuring three to ten micrometers with many small round internal structures, and few cocci bacteria. Histopathology of lesions showed severe, diffuse, chronic granulomatous dermatitis and panniculitis with numerous previously described organisms. Aerobic tissue culture confirmed *Prototheca* species with secondary methicillin-resistant *Staphylococcus pseudintermedius* infection. Mild hyperglycemia and hyperglobulinemia were the only abnormalities on bloodwork. The dog had been diagnosed with cutaneous protothecosis two years prior to the recent presentation. At that time, medical therapy with oral doxycycline 14.5mg/kg twice daily and ketoconazole 14.5mg/kg daily was initiated and continued for 16 months; lesions resolved except for a persistent swelling over the right carpus. Discontinuation of therapy allowed the dog to remain otherwise lesion-free for 5 months. On representation, oral fluconazole (generic) 3.6mg/kg twice daily in combination with pentoxifylline (Trental: Sanofi-Aventis, Bridgewater, NJ) 14.5mg/kg led to clinical improvement within 4 weeks; she has remained lesion-free for 4 months with continued therapy. Protothecosis most commonly presents with severe gastrointestinal upset, weight loss, and/or neurologic abnormalities in dogs with access to contaminated water or environmental sources. Prognosis is considered severely guarded to grave. The patient described here had no obvious evidence of systemic disease and lived primarily indoors. Infection source was uncertain.

Conflict of interest: NONE
Source of funding: NONE

**Evaluation of Pregabalin (Lyrica®, Pfizer Inc.) in a canine model of flea-allergic dermatitis**

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**Abstract:** Pregabalin (Lyrica®, Pfizer Inc.) is an FDA-approved anticonvulsant used for the treatment of seizures, fibromyalgia, and a variety of pain indications in humans. It is believed to exert its therapeutic effects by binding with high affinity to the alpha2-delta site on voltage-gated calcium channels in neuronal tissue. Although not a label indication, pregabalin has been documented to reduce pruritus in dialysis patients where chronic pruritus is a common, debilitating side effect described by these patients. Consequently, there is an interest in determining the potential utility of pregabalin in pruritic conditions in veterinary species. The objective of this study was to evaluate the anti-pruritic activity of pregabalin in a canine model of pruritus associated with allergic skin disease. Using flea allergic mongrel dogs, a placebo controlled, masked, repeat dose study was performed. Dogs were treated orally, twice daily, for 7 1⁄2 days with either placebo (n = 8) or pregabalin (10 mg/kg/dose, *n* = 8), and pruritic behaviors were assessed continuously for 4 hours, beginning 1 hour after dosing via video recording. After 1⁄2 1 days of dosing, mean pruritic scores over the 4 hour observation window were 2708 seconds for the placebo group vs. 2863 seconds for the pregabalin treated group. After 7 1⁄2 days of dosing, mean pruritic scores were 1771 seconds for the placebo group vs. 2340 seconds for the pregabalin treated group. In summary, pregabalin administered at 10 mg/kg twice daily for 7 1⁄2 days did not significantly reduce pruritus in this population of dogs.

Funding: Zoetis Disclosure Conflict of Interest: All authors are current employees of Zoetis, formerly Pfizer Animal Health.

# Use of Oclacitinib (Apoquel®, Zoetis) for treatment of cutaneous mastocytosis in a cat

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**Abstract:** A 2 year old neutered male domestic shorthair Zoetis colony cat developed a focal lesion at the ear base that progressed from a focal reddened crusted area to a 2 cm in diameter raised oozing red area over 5 months. Cats in this room were observed for a minimum of about one hour per day. At the first sign of the lesion the cat was observed twice daily by a husbandry technician and once daily by the investigator; no pruritus was observed. There were no dermatological signs of pruritus (e.g. self-induced alopecia, excoriations). The lesion responded poorly to various topical treatments including corticosteroids and antimicrobials. One 4-mm punch biopsy was taken at the margin of the lesion. Histologically, the lesion was characterized by mild orthokeratotic hyperkeratosis and epidermal hyperplasia. There were moderate numbers of perivascular to diffuse mast cells in the superficial and deep dermis. Mast cells were well-differentiated with a central nucleus and granular cytoplasm, with granules staining positively with Toluidine blue stain. Mitotic figures were not observed. A few degranulated granulocytes were observed throughout the affected area. The lesion was most consistent with cutaneous mastocytosis. Treatment was initiated with 1 mg/kg twice daily orally Oclacitinib (Apoquel®, Zoetis, Kalamazoo, MI) for 31 days. The lesion healed progressively during treatment with Oclacitinib, and by the end of treatment was 99% resolved; by 2 weeks post treatment the lesion had completely resolved. The cat has been lesion-free for 2.5 months. No adverse effects were observed with Oclacitinib treatment.

Funding: Zoetis, Inc.

Conflict of Interest: All authors are current employees of Zoetis, formerly Pfizer Animal Health

# Field effectiveness and compliance of cefalexine (Rilexine®) for treatment of secondary superficial pyoderma in dogs

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**ABSTRACT:** Cefalexine is a first-line antibiotic for management of canine pyoderma. This multicentered, controlled, blinded and randomized field clinical trial sought to confirm the effectiveness of cefalexin (Rilexine®, Virbac, Fort Worth, USA) when administered orally at 22 mg/kg twice daily for 28 days. Dogs enrolled in the study had clinical lesions of secondary superficial pyoderma characterized as moderate or severe for at least one primary clinical sign (PCS): papules, pustules, and/or folliculitis. Following enrollment, all dogs were evaluated weekly for 5 weeks. Treatment was considered successful if all PCS were rated absent at the conclusion of the treatment and one week later. Seborrhea, erythema, pruritus and lesional spreading were assessed as secondary endpoints. Compliance was calculated according to the formula: compliance = (administered number of doses / recommended number of doses) x 100. One hundred dogs receiving cefalexin tablets and 49 dogs receiving placebo tablets were evaluated for efficacy. In the cefalexin group and the placebo group, compliance was superior to 95% with no statistical difference between groups ($p=0.1309$). At the end of the study, 70.0% of the dogs in the cefalexin group were considered a success (*versus* 14.3% of dogs in the placebo group, $p=0.0011$). There were statistically significant differences between the cefalexin and placebo groups on all individual clinical parameters (papules, pustules, folliculitis, seborrhea, erythema, pruritus ($p < 0.0001$)). Based on microbiological cultures, resistance to cephalexin was not noted before or after treatment. Rilexine® was effective for the treatment of secondary superficial bacterial pyoderma in dogs.

Source of funding: Virbac.

Conflict of interest: All of the authors are employees of Virbac.

# SCIENTIFIC SESSION PRESENTATIONS SATURDAY

# THE ROLE OF THE HOME IN STAPHYLOCOCCAL INFECTIONS

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Staphylococcal infections, particularly *S. pseudintermedius* and including *S. aureus*, are a leading cause of dermatitis in companion animals, especially dogs. Emerging pathogens, such as *S. schleiferi* and *S. lugdunensis*, also may cause disease. These staphylococcal bacteria share characteristics that enhance the opportunity for recurrent infections. These include carriage by human owners and survival on environmental surfaces in the home.

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA):

When companion animals develop MRSA infections, the strains isolated typically are the same ones that cause disease in people.[1] This has led some researchers to conclude that companion animals suffer from the "humanosis" of MRSA.[2] This is in contrast to strains found in horses (USA500) and livestock (ST398), which appear to be strains originally found in people that have become adapted to animal hosts over time.[3,4] Of note, livestock-associated ST398 has been shown to spill over into companion animal and human populations.[4,5]

A third of the human U.S. population may be colonized with *S. aureus* at any given time, with national prevalence rates for MRSA at 1.5% or higher.[6] In studies of pets in contact with MRSA-positive people, animals typically had lower rates of MRSA prevalence than their owners. For example, in one report, MRSA prevalence among 242 people, 132 dogs, and 161 cats was 3.3% for people, 1.5% for dogs and 0.0% for cats.[7] Typical risk factors for MRSA in companion animals include contact with veterinary healthcare settings, surgery, and antimicrobial use.[2,3] Contact with children and licking behaviors may enhance transmission from people to companion animals.[8] In studies evaluating concurrent presence of children and pets, both dogs and cats were found to carry MRSA.[9–11]

METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS (MRSP):

While the human epidemic of MRSA may spill over into companion animal populations, *S. pseudintermedius*, reclassified from *S. intermedius*,[12] is predominantly an animal pathogen.[13] That said, pet owners and veterinarians may carry *S. pseudintermedius*, and when this happens, they often carry the same strains as their companion animals.[14,15] Prevalence rates in people exposed to MRSP-positive pets may be 4% to 12%.[16–18] Hence, people may be important to consider in the dissemination and epidemiology of MRSP. One of the greatest challenges in treatment of *S. pseudintermedius* is the increasing frequency of methicillin- and multidrug-resistance profiles, particularly among isolates from dogs with veterinary healthcare contact.[19] Horizontal gene transfer conferring antimicrobial resistance among related staphylococci (MRSA and MRSP) has been demonstrated and may be important, but the rate of these events is unknown.

OTHER IMPORTANT STAPHYLOCOCCI:

*S. schleiferi*, which has a coagulase-positive subspecies (CPS: subsp. *coagulans*) and a coagulase-negative subspecies (CNS: subsp. *schleiferi*) is similar to *S. pseudintermedius* in that it typically causes disease in companion animals but may rarely cause disease in people.[20] *S. schleiferi* most commonly causes otitis and pyoderma in dogs, but also can affect cats or birds. Rates of methicillin- and multidrug-resistance in *S. schleiferi* appear to be increasing.[20]

*S. lugdunensis* is a coagulase-negative staphylococci that typically causes disease in people, primarily skin infections and endocarditis, but also may cause disease in companion animals.[20] Emerging CNS like *S. lugdunensis* and *S. schleiferi* demonstrate the potential for bacteria in the genus *Staphylococcus* to be important opportunistic pathogens of clinical relevance for both pets and people.

## THE ROLE OF THE HOME:

Pets may spend their whole lives within homes, and people spend 60-70% of their time there.[1] Unlike public areas, households are characterized by high-intensity contact among the same individuals (people and pets) over time.[1] Case reports, outbreaks, and larger studies have described MRSA-positive home environments of relevance to colonization and infections in people.[1,21] The literature on the role of the home environment in **transmission** among household members is sparse, but also has focused primarily on people in the context of MRSA. An emerging literature demonstrates that MRSP also may contaminate homes of positive companion animals.[16,18] Table 1 summarizes the literature on staphylococcal home contamination (MRSA and MRSP) of relevance to companion animals.

That homes may become contaminated, potentially serving as reservoirs for re-colonization of treated humans and companion animals, is perhaps expected based on what is known about these bacteria. The genus *Staphylococcus* is environmentally hardy, remaining viable even in dry environments from periods of weeks to months.[1] Colonized hosts, whether human or animal, shed bacteria into the environment through direct contact with surfaces, sloughing of skin cells with adherent bacteria, through aerosol discharge (sneezing), or through fecal shedding.[1] In households, both sites that are frequently touched and dusty surfaces have been shown to be positive, including toys, television remotes, door knobs, pillows, and bedding.[1] Because hosts shed into the environment and can also pick up bacteria from environmental surfaces, the entire cycle of home contamination is important to consider when choosing when and how to intervene. Household-wide interventions may be warranted with persistent infection, reoccurrence of infection, multiple infections within a household, or infection in the face of a history of owner infection or colonization.

## INTERVENTION STRATEGIES:

Few decontamination strategies have been tested in home environments. Previous case reports have explored use of gaseous ozone, carpet replacement or commercial steam cleaning, surface disinfection, and mattress replacement.[1] Laundering of bedding, towels, and clothing using low-temperature settings has been shown to be effective; however, textiles may become rapidly re-contaminated.[1] This indicates the need for harmonization of cleaning activities and repetition of laundering and cleaning activities during treatment.

The presence of organic material, such as food residues, may inhibit the effectiveness of detergents and cleaners. Combinations of chlorine and quaternary ammonium-based cleaners are effective disinfectants against *S. aureus* on kitchen sites, bathroom sites, and in pet-associated areas.[22] The prevalence of mutations conferring resistance to household disinfectants is unknown, but has been demonstrated previously.[23] This is important to consider for particularly difficult-to-eradicate strains.

For treatment that may involve in-contact pets or people, the literature is extremely sparse. In people, presumptive treatment of household members improves rates of MRSA reoccurrence in infected children, but does not fully eliminate *S. aureus* in the household.[24] Researchers have not yet shown whether targeted treatment based on testing or presumptive treatment of all household members (pets and people) will be effective. Regardless, decolonization treatment in people has been shown to have only short-term effectiveness, and similar treatments are not well studied in companion animals. This may mean that reservoirs within and outside the home are important for re-exposure of treated people and pets.

TABLE 1: Reports of household contamination with staphylococcal bacteria with relevance to companion animals. [1]

| Reference | Study design | Number of households | Household sites sampled | Results |
|---|---|---|---|---|
| Scott *et al.* 2008 & Scott *et al.* 2009 (Boston, USA)[10,11] | Cross-sectional study | 35 "healthy" households with a child in diapers and a pet in the home | *S. aureus* sampling: Moistened polyester swabs; kitchen, bathroom, TV remote, phone, computer keyboard & mouse, child's toy, infant changing mat & high chair, pet food dish | 97% of homes were positive for *S. aureus*; 26% of homes were MRSA-positive. Frequently contaminated sites included kitchen sponges and towels, a child's toy, and surfaces used for infants. Cat presence was associated with MRSA contamination. |
| Meandro-Felix *et al.* 2010 (Culiacan, Mexico)[22] | Longitudinal study | 60 households with a child under the age of 12 and a pet in the home | *S. aureus* sampling, weekly for five weeks: Moistened sampling sponge or peptone solution (for kitchen sponges); Kitchen: counter, sink, sponge, towel, cutting board; Bathroom: sink, shower, toilet; Pet area; child's toy | Mean log CFU of *S. aureus* ranged from 0 to 1.157, depending on the site sampled, and *S. aureus* was cultured from each site in at least one home; *S. aureus* was found most frequently in the toilet areas |
| van Duijkeren *et al.* 2011 (Netherlands)[18] | Cross-sectional study | 20 households with known MRSP-positive companion animal (index case) | *S. pseudintermedius* sampling: Human, companion animal (index case + in-contact companion animals), and environmental samples taken concurrently; Gauze samples were taken from 5-8 environmental surfaces: sleeping place of the index case (animal bedding), feeding site, floor underneath the sofa, door mat, window-sill, cupboard | 70% of households were contaminated with MRSP, particularly homes with concurrent index patient colonization or infection; feeding sites and bedding were frequently contaminated |
| Laarhoven *et al.* 2011 (Netherlands)[16] | Longitudinal study | 16 households with known MRSP-positive dogs (index case), with 12 households sampled at all visits | *S. pseudintermedius* sampling monthly for six months: Human (nasal swabs), companion animal (nasal & perineal samples from index case + in-contact companion animals), & environmental samples taken concurrently; moist environmental wipes from three sites (sleeping place of the index case, feeding place, and a site inaccessible to the animals) | 0-69% of households were contaminated at visits, and sometimes households were contaminated in absence of positivity in humans or animals; feeding sites were positive in 69% of households, sleeping places in 56% of households, and inaccessible sites in 38% of households |

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# NOTES

# ALTERNATIVE THERAPIES IN THE TREATMENT OF HUMAN AD

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## INTRODUCTION

"Alternative medicine" can serve as a blanket term from everything from Kirlian photography to chicken soup. It includes complex, fully-developed systems such as Traditional Chinese Medicine (TCM), to oversimplified "supplements." Perhaps the most functional definition of alternative medicine would be treatments that are *not* based on evidence, in stark contrast to the goal of evidence-based medicine. In controlled settings, many treatments have been tested and found not to work as claimed or hoped; we can safely and rightfully cross these off the list. However, a much larger number of therapies do not yet have sufficient evidence upon which to pass judgment. In some cases, data suggest that the "alternative" designation is merely temporary, and that some of these agents have a future of evidence-based healing.

The utility of alternative medicines in the treatment of various conditions depends on several factors. When Western medicines work (and do so safely), there is less drive for alternatives. For example, the belief that diet is the "root"of atopic dermatitis is an idea that some atopic dermatitis (AD) patients cling to very fiercely. A clever study by Dr. Jon Hanifin demonstrated that once the skin of patients with AD was under better control, patients significantly de-emphasized food alelrgies and diet as possible explanations, compared to when the skin was flaring initially.[1] However, when a disease is not curable, or when the treatments appear to be unsafe, many patients seek alternative approaches, often converging around 50% of AD patients.[2,3] Dissatisfaction with treatments and frustration with the chronic nature of the disease were frequently reported reasons.

## ESSENTIAL FATTY ACIDS

An older hypothesis holds that AD is caused by a deficiency of essential fatty acids (EFA), which has been known to mimic AD. Patients with AD have a possible deficiency of the delta-6 desaturase enzyme, which blocks conversion of linoleic acid to gamma-linolenic acid (GLA).[4] Thus, the idea of using EFAs to supplement GLA is suggested.

Essential fatty acid (EFA) deficiencies can be treated by topical application of sunflower oil, which is rich in EFAs and readily absorbed transcutaneously. Topical application of sunflower oleodistillate has been shown to increase synthesis of ceramides (thus improving barrier function) and to have anti-inflammatory effects—two highly-desirable properties in an AD therapy.[5] Linoleic acid is the major lipid that converts to arachidonic acid, which leads to prostaglandin E2, an inflammatory modulator, possibly via peroxisome proliferative-activated receptor-a (PPAR-a) activation.

As evidence for skin barrier repair, a study of 497 pre-term infants deemed high-risk for sepsis were given thrice-daily application of sunflower seed oil vs. Aquaphor vs. no treatment to see if skin barrier support would prevent systemic infection. Indeed, sunflower seed oil reduced sepsis by 41 percent, with a 26 percent reduction in mortality, significantly better than no treatment and similar to the effect of Aquaphor, but at a fraction of the cost.[6]

Another commonly discussed "natural" remedy is evening primrose oil (EPO), which has high levels of gamma-linolenic acid and omega-6 fatty acids. In one study, oral EPO was associated with an improvement by 96 percent in patients with AD, versus only 32 percent in the placebo group.[7] While these results are encouraging, the major issue in this study is that it used very high doses of EPO—up to 6,000mg per day— given as 12 capsules, which may make adherence to such a regimen difficult. In aggregate, studies have been mixed and conflicting, perhaps due to a lack of consistency in methodology and dosage. Similarly, borage oil is derived from the seeds of *borago officinalis*, rich with omega-6 essential fatty acids and with two to three times more gamma-linolenic acid than EPO. However, a recent

review in the venerable Cochrane Review concluded: "borage oil and evening primrose oil lack effect on eczema… further studies… would be hard to justify."[8]

**COCONUT OIL**

Coconut oil can decrease staphylococcus aureus colonization by 95 percent in patients with AD when applied topically twice daily for four weeks vs. 50 percent decrease in an olive oil control.[9] Taken in the context of current thinking about superantigens from staphylococcal colonization in AD, these findings make coconut oil a very interesting natural product for further study.

**PROBIOTICS**

Treatment with probiotics presents the compelling notion of "re-balancing" bacteria on and in the body. One study demonstrated that giving neonates Lactobacillus GG cut the development of AD in half vs. the control group.[10] Another study demonstrated an equally impressive finding; that twice-daily probiotics to children with moderate to severe AD demonstrated significant improvement over placebo.[11] However, additional studies have failed to reproduce benefit or prevention, calling these findings into question. Optimal dosing, timing, strain of probiotic, and patient selection all remain open questions as well.[12]

**VITAMIN D**

In one study examining the benefits of vitamin D, 11 children (mean age of seven years) with AD that worsened in the winter were randomly given 1,000 IU of D2 or placebo daily for one month. Results showed that 80 percent of patients who received the vitamin D saw significant improvement in their AD, as compared to just 17 percent in the placebo group.[13] A corroborating study evaluated 37 children with AD aged eight to 12 months. Serum 25(OH) D levels correlated significantly (and inversely) with disease severity, such that higher vitamin D levels correlated with less severity.[14]

**VITAMIN B12**

Cobalmin, or Vitamin B12, is an inhibitor of NO synthase and has been hypothesized to prevent flares in AD patients. In a Phase III RCT of topical B12 applied twice daily for eight weeks, patients treated with B12 saw significant improvement vs. placebo.[15] Another study in children aged six months to 18 years found significant improvement in as early as two weeks of use.[16] It is puzzling that such an impressive result has not been followed up with further study.

**TRADITIONAL CHINESE MEDICINE**

A complex system of medicine, TCM includes herbs which can have direct pharmacological effects on the body and skin, some of which may be synergistic and difficult to isolate as one specific compound. Acupuncture and acupressure have powerful placebo potential, but may work to modulate the nervous system in terms of pruritus perception, behavioral modification, and stress reduction. They may also have direct effects on inflammatory mediators that could also play a role. The Cochrane Review provocatively concluded that TCM herbal mixtures may be effective in the treatment of atopic eczema.[17]

**ACUPUNCTURE/ACUPRESSURE**

Acupuncture and acupressure have powerful placebo potential, but may work to modulate the nervous system in terms of pruritus perception, behavioral modification, and stress reduction. They may also have direct effects on inflammatory mediators which could also play a role. Several studies point to mechanistic possibilities for acupuncture helping inflammation or allergy,[18,19] while one small study revealed improvement over a control group with acupressure.[20]

## CONCLUSION

The studies reviewed offer some suggestive evidence that agents such as sunflower seed oil, coconut oil, oral vitamin D supplementation, TCM, acupuncture, and topical vitamin B12 may be useful adjuncts in treating AD. Evening primrose oil and borage oil may hold some promise in certain subgroups, but these, as well as probiotics, will require more research before being considered potentially viable options. Even in the case of some of the more promising agents, further investigation will be needed to better understand the mechanisms and to ensure robust effects and safety before their roles can be more clearly defined.

While conventional medicine still holds most of the answers, it may not have them all. Our patients are aware of this and are searching in the "alternative" realm. Even if incorporating alternative medicines is not the goal, knowing about them is worthwhile: tomorrow's evidence-based treatments must begin without evidence, and it is possible that they will begin as an "alternative" today.

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# NOTES

# THE BLEEDING, CRUSTING, AND OOZING EDGE: UPDATES IN HUMAN ATOPIC DERMATITIS

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## INTRODUCTION

Despite the tremendous amount of sophisticated research on atopic dermatitis (AD)—a disease that affects some 20 percent of children in industrialized countries—the etiopathogenesis remains obscure.[1,2] The current understanding involves several factors including impaired skin barrier function, abnormal nerve fibers, immune dysregulation, and microbiome abnormalities.[3,4] Yet, fundamental questions remain unanswered, such as whether AD is truly an allergic disease. The existence of at least two distinct subtypes, variably referred to as "extrinsic vs. intrinsic," or "atopic dermatitis vs. atopiform dermatitis," highlight the large subgroup that has no allergic features and thus may not be truly atopic at all.[5]

Four cardinal aspects of AD can be delineated, which help guide therapy choices: increased inflammation, barrier dysfunction, microbiome abnormalities, and itch. When a patient presents with more severe disease, maximizing each area may lead to powerful synergies, which, in turn, may minimize the need for more potent and potentially toxic treatments.

## INFLAMMATION

Immune dysregulation and inflammation in the skin are defining characteristics of AD.[6] Accordingly, topical corticosteroids, and to a similar extent, the topical calcineurin inhibitors tacrolimus and pimecrolimus, can be used successfully to calm the inflammation and improve the skin. Much of the literature is based on what might be called a "reactive" treatment approach: treating the skin when there is active inflammation and itch (a flare).[7] Newer studies suggest that a more "proactive" approach (treating the trouble areas twice per week, even in the absence of a flare) may reduce the the number of disease exacerbations as well as cost, while improving the quality of life.[8,9] Similar results were found with a topical calcineurin inhibitor or a low-mid potency topical corticosteroid used in this way. This proactive therapy, perhaps more than any other innovation, heralds a fundamentally new approach to managing AD and requires only wider adoption by clinicians. Analogous to the maintenance of asthma, moderate and severe cases of AD probably should be similarly maintained. Corticosteroid phobia (as well as concerns about topical calcineurin inhibitors) among patients and caregivers may limit this, however, and paradoxically result in more medication use over time due to poorly-controlled disease.[10]

## BARRIER DYSFUNCTION

The excitement surrounding the fairly recent discovery that mutations of the filaggrin gene (FLG) are associated with AD is justified, as it allows for an answer to the question: "why do I have AD?"—at least for some.[11] This protein is important for epidermal barrier function, but also degrades into Natural Moisturizing Factor (NMF) and helps maintain the acid mantle of the skin as well.[12] In the absence of sufficient filaggrin, the skin becomes "leaky," allowing increased water loss and enhanced penetration of allergens and irritants. It follows that restoring the skin barrier by moisturization is of paramount importance in AD. It has been shown convincingly that increased moisturizer use strongly correlates with decreased eczema severity.[13] Regular emollient use also appears to have a steroid-sparing effect and is associated with improved quality of life in AD as well.[14,15]

But what of those with normal filaggrin production: do these patients also have barrier dysfunction, and if so, why? A provocative paper in 2007 demonstrated that in the presence of inflammatory cytokines such as IL-4 and IL-13, there is significantly reduced filaggrin gene expression,

even in genotypic normal skin.[16] This functional filaggrin deficiency likely results in the same "leaky" skin seen in patients with an abnormal FLG gene, underscoring the importance of moisturization for all patients with AD.

A recent study suggested a potential ramification of maintaining the barrier through moisturization. The authors asked: "Can moisturizers prevent eczema in high-risk patients?" In the study, 22 neonates deemed high-risk for developing AD had moisturizer applied daily from birth. Excitingly, only 15 percent of these patients developed AD by two years of age, compared with a historic control prediction of 30 to 50 percent, suggesting a protective effect of moisturization.[17] Despite these positive findings, many unanswered questions remain regarding moisturizers. Selection of an "optimal" moisturizer (if there is such a thing), is still elusive and there is minimal comparative evidence in this domain. Additives and innovations in moisturizers have more recently resulted in suspiciously drug-like claims, while so-called "barrier repair creams" walk the line precariously as 510(k) devices rather than actual drugs in the eyes of the FDA.[18] On limited data—but bolstered by clinical experience—it seems that many mainstream over-the-counter moisturizers are comparable to the far more expensive barrier repair devices, and may even outperform them at a fraction of the cost.[19]

It is clear that skin barrier deficiency is at the root of AD and that moisturizers are currently our best way to address this aspect. In so doing, it seems plausible that beyond the improvement in symptoms, such treatment may actually alter the course of the disease as well.

**MICROBIOME ABNORMALITIES**

Frequent infections and abnormal colonization by bacteria such as *Staphylococcus aureus* are well-known features of AD.[6] Several converging lines of evidence help elucidate the role of antimicrobial treatments in AD, even without overt signs of infection. Multiple studies show a correlation between staphylococcus colonization and AD severity, and even without infection, the secretion of exotoxins by the bacteria seem to directly fuel inflammation in the skin via superantigens.[20] A recent report suggests that a specific protein called δ-toxin acts to directly activate mast cells, promote IgE and IL-4 production, and directly contribute to inflammation in the skin.[21] Work done by Kong, et al., models a flare of AD as beginning with increased colonization of staphylococcus on the skin, followed by inflammation, and ending with decreasing numbers of staphylococcus leading into healing.[5]

From a therapeutics perspective, the use of dilute bleach (sodium hypochlorite) baths has been shown to decrease the severity of AD, presumably by decreasing colonization of the toxin-secreting bacteria.[22] Clinically, the use of dilute bleach baths has dramatically decreased the frequency of infections, such that I find that I seldom need to prescribe oral antibiotics for my patients anymore. If dilute bleach baths were to become the standard of care, such a change could presumably result in massive cost savings, reduced adverse effects related to oral antibiotics, and decreased bacterial resistance from antibiotic overuse.

**PRURITUS**

AD is often known as "the itch that rashes," highlighting pruritus as the primary symptom. It seems to be driven by all of the other factors at once: the dryness of the skin and the infiltration of allergens and irritants through a dysfunctional barrier; direct and indirect inflammatory mediators by staphylococcus bacteria on the skin; the inflammation itself, including IL-2 and mast cells; and changes both qualitatively and quantitatively in periperhal nerve fibers.[23,24] It is disheartening that there are few specific treatments able to break the itch-scratch cycle. While oral antihistamines are generally thought to be ineffective for the pruritus of AD, there may be some patients who benefit, particularly from the sedative effects during the night.[25] Ultraviolet light treatments (phototherapy) can address several of these aspects simultaneously, and remains an important therapeutic consideration for AD. Anti-inflammatory, antimicrobial (via cathelicidin production, perhaps), and directly antipruritic, narrow-band ultraviolet B phototherapy is a powerful but safe option for moderate-to-severe AD.[26,27] Beyond this, we should consider systemic therapies, given the sufficient evidence available to justify use of cyclosporine, mycophenolate, azathioprine, and methotrexate in appropriate refractory cases.[25]

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# NOTES

# CONCURRENT SESSION PRESENTATIONS SATURDAY

**Itchy Pets: Case Based Dermatology**
Smokie. 5 year old, Male, Neutered, Domestic Medium Hair

**The Nonresponsive Allergic Cat**
Dana A. Liska, DVM, Diplomate, ACVD

**Smokie**
Neutered male domestic longhair cat, 10 pounds, 4 years of age on presentation in June.

**History**
Smokie's owner indicated the primary complaint as “face is scratchy, red, and bleeding." When asked to specify what helped relieve the cat's signs, the owner replied “nothing.” The referring veterinarian had given Vetalog (Fort Dodge Animal Health) injections in May 2 years earlier, in December of the previous year, and in January, 6 months before presentation. Oral prednisolone had been administered 3 months before the cat was presented. Neither treatment improved the condition.

**Clinical Signs**
The veterinary dermatologist who initially examined Smokie noted:
• Multiple excoriations.
• No other clinical abnormalities.
• Pruritus 10/10 [1,2]

**Initial Workup/Laboratory Results**
Wood's lamp: negative. Dermatophyte test medium (DTM): pending (ultimately negative).

**Differential Diagnosis**
• Food allergic dermatitis.
• Feline atopic dermatitis.
• Dermatophytosis.

**Next Steps**
The treatment history of response to intermittent steroids strongly suggested environmental allergies; however, head and neck pruritus is a predominant feature of food allergy in cats.[3,4] The attending dermatologist recommended the following approach, giving the client these directives:

• Start an elimination diet with Innovative Veterinary Diet Potato & Venison Cat Food (Royal Canin). No other food, treats, chews, flavored toys, or medications by mouth are to be given for the entire 8- to 12-week duration of the food trial. Do not give flavored heartworm preventive during the food trial. Compliance must be strict – 100% – to prove or disprove a food component as an allergen. Even an occasional transgression seriously limits the likelihood of reaching a diagnosis.
• If itch is resolved by the end of the strict food trial, please call and we will discuss the food challenge.
• If signs remain present despite a strict food trial, we need to recheck and discuss intradermal allergy testing for atopic dermatitis (environmental allergy).

• Dexamethasone will be administered to retest the steroid response. Give one 0.75 mg dexamethasone (0.17 mg/kg) tablet by mouth once daily for 10 days to retest steroid responsiveness.
• Use Soft Paws nail caps (Smart Practice) on the back claws.

**Return Checkups**
On his return visit 2 months later in the fall of that year, Smokie had improved dramatically. His facial pruritus was better (but did not resolve) with the food restrictions, and he was then responsive to oral dexamethasone. He underwent intradermal allergy testing and was found to have multiple positive reactions. In fact, he had multiple STRONG positive reactions to various allergens, which is an uncommon finding with feline intradermal allergy tests.[5] Allergen-specific immunotherapy (ASIT) was started, he continued on the elimination diet, and a dexamethasone taper was repeated (one 0.75 mg tablet [0.17 mg/kg] daily for 10 days, then every other day for 10 doses).

Smokie was maintained on ASIT, a limited-ingredient diet, and dexamethasone administered sparingly until fall of the following year when he flared again and was seen for a recheck examination. Skin cytology of his face was completed and revealed sheets of degenerative neutrophils with large numbers of intracellular and extracellular paired and single cocci. This superficial pyoderma was treated with an antibiotic dosed at 32 mg subcutaneously (Convenia – Pfizer Animal Health).

After the fall flare Smokie no longer received oral dexamethasone but was given methylprednisolone injections by his regular veterinarian 1 and 2 years later in the fall. The latest steroid injection did not seem as effective, and he was given a second steroid injection that fall. Again, the owner felt the anti-allergy effect had limited duration.

**Referral**
Smokie was presented to our clinic 2 months later with an ongoing flare. Skin cytology was collected from his face and revealed sheets of degenerative neutrophils with large numbers of

intracellular and extracellular cocci. This superficial pyoderma was treated with antibiotic therapy (Simplicef – Pfizer Animal Health; one half 100 mg tablet [10 mg/kg] by mouth once daily). The attending veterinary dermatologist recognized that Smokie had managed very well over the previous 4 years but that he seemed to be struggling more. Differentials included a new food allergy or new environmental allergies. In fact, when food was discussed the owner revealed that she had changed his food from the limited-ingredient venison diet that had been recommended to an over-the-counter diet. Before this examination, however, she had returned to feeding Smokie the limited-ingredient venison diet.

Smokie did well until early spring of the following year when he flared with facial dermatitis and pruritus. Both Simplicef and dexamethasone therapies were initiated but proved unrewarding. When he did not respond to the Simplicef regimen, he returned to the office and I assumed supervision of his case. His lesions were even more severe than at the time of his first visit to the clinic.

A swab sample was collected for bacterial culture and sensitivity (C&S) testing. Pending the results, the following changes were made:
• Switched the diet from venison based to rabbit based as the owner reported Smokie's facial pruritus seems worse after he eats.
• One 4 mg tablet methylprednisolone (Medrol – Pharmacia & Upjohn) daily (0.88 mg/kg) for 7 days, then every other day, to test for steroid tachyphylaxis (a condition described in the human literature [6,7] and observed in the animals we see).
• Dilute bleach bath solution (personal communication - Donald Leung, MD, PhD, during North American Dermatology Forum, Savannah, GA, April 2010): apply artificial tears ointment to both eyes. Soak cat's face with cool moist cloth to soften crusts, then follow with application of dilute bleach solution per recipe (1/4 cup bleach per 10 gallons of water).
• Elizabethan collar to prevent self-trauma.

C&S results were available one week later and showed *Staphylococcus pseudintermedius,* which was both methicillin resistant (MR) and multidrug resistant (MDR). On phoning to share the results of the C&S testing with the owner, Smokie was reported to be doing 70% better with topical therapy. Given that systemic therapy depended on the administration of amikacin or chloramphenicol, both of which can be associated with adverse effects,[8-12] the decision was made to continue the topical therapy. Smokie continued to do well in regards to his skin but developed feline urinary tract disease.

**Final Diagnosis**
• Food allergic dermatitis.
• Feline atopic dermatitis.
• Relapsing superficial pyoderma that ultimately developed both methicillin resistance and multidrug resistance.

**Key Points**
• Cats with severe dermatitis can have secondary infections with *Staphylococcus* sp and yeast. This occurs less commonly than in our canine patients but is common in the specific subset of patients referred to our clinic. Other veterinary dermatologists concur.[13]
• Tape cytology is a useful tool in ruling out secondary infections. One of the first studies to tout the importance of cytology was published in 1979.[14] To quote from two of the author's favorite sources: "An enormous amount of vital diagnostic data can be obtained by microscopic examination of stained material, such as smears of tissues or fluids, during a clinical exam ... often supplies sufficient data to narrow a differential diagnosis and develop a diagnostic plan."[15]

From a more recent publication on feline dermatology: "Cytology can give rapid results and may help to suggest or even confirm a diagnosis."[16]

• Patients can have multiple allergies, and it takes time to work through them.
• Cats can develop resistant *Staphylococcus pseudintermedius* infections.
• Topical therapy can be beneficial in resolving even resistant *Staphylococcus* sp infections.
• Changes of diet over time may be necessary for food-allergic patients.

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**Itchy Pets: Case Based Dermatology**
Chip. 3 year old, Male, Neutered, Labrador Retriever

**Thank you for giving me my dog back**
Dana A. Liska, DVM, Diplomate, ACVD

**Chip**
Neutered male, Labrador retriever, 67 pounds, 3 years of age on presentation in November 2011.

**History**
Chip's owner indicated the primary complaint as “itching of the face and armpits." When asked to specify what helped relieve the dog's signs, the owner replied “steroids is really the only thing we have found that touches this.” The referring veterinarian (rDVM) had given antibiotics (Simiplicef) 5 times in 2011 and prednisone tapers were prescribed 7 times.

**Clinical Signs**
• Patchy alopecia and mild papular eruption
• Pruritus 10/10 [1,2]

**Initial Workup/Laboratory Results**
• Deep skin scrapes: negative
• Skin cytology: Cocci amongst neutrophils
• IDAT

**Working Diagnosis**
Atopic dermatitis with secondary superficial staph pyoderma

**Next Steps**
• Complete Simplicef as prescribe by rDVM
• Start allergen specific immunotherapy (ASIT) injections
• Discontinue Atopica due to GI upset and cost was not justifying response
• Prednisone, 20 mg, 1/2 tablet by mouth once daily for 5-7 days then every other day

**Follow up**
The owner phoned in late December that Chip finished antibiotic one week ago and seemed to be flaring with "red bumps" again. She was hoping to avoid oral antibiotics again. The decision was made to start topica therapy with Triz Chlor 4 spray since the staph infection was confined to the ventral abdomen. Another phone call was received in early January with the report that the infection was spreading, cephalexin was prescribed as give one 500 mg + one 250 mg capsule by mouth every 12 hours for 30 days.

On his return visit 2 months later in March 2012, Chip had again relapse with a papular dermatitis and he had patchy alopecia on his head, trunk and limbs. At the time of the visit he was receiving fexofenadine daily and 10 mg of prednisone every other day at night "so everyone can sleep". The owner was giving Chip baths weekly with triz chlor 4 shampoo and was giving ASIT injections weekly. The rDVM had recently refilled a prescription for cephalexin. Skin cytology showed the cocci shaped bacteria persisted despite cephalexin therapy. A culture and sensitivity test was recommended but declined due to financial constraints. An empirical change of antibiotic was made to trimethoprim/sulfa: SMZ/TMP: 960 mg with the instructions to give 1 tablet by mouth every 12 hours initially. One refill was provided in case Chip was responding to therapy but the owner was advised that a culture and sensitivity would be necessary if the infection was not responding. Changes were made to the ASIT formula. Two weeks later the owner call to report that the "red bumps" were at least 60% improved and that his pruritus was waxing and waning.

Chip was maintained on ASIT and prednisone but the infection relapsed again in July. The owner inquired about a once daily medication, Primor ® (ormetiprim-sulfadimethoxine) was prescribed at 600 mg, give 1 + 1/2 tablet by mouth once daily for 30 days. The owner phoned in September to request a refill of predisone and reported that Chip's last antibiotic was approximately 6 weeks prior. This was promising news but proved to be short lived as Chip relapsed in early October and another prescription of Primor was administered. In late November the owner reported yet another relapse and requested the "less expensive" twice daily trimethoprim-sulfa medication. This antibiotic was necessary for another relapse in January 2013.

Chip returned to the dermatology clinic in March 2013. His current medications included Gives fexofenadine each morning and benadryl each evening, prednisone, 10 mg at night before bed so he would sleep. By this time most patients have transitioned to ASIT injections every 14 days but Chip needed them every 7 days due to itch trends noted before each injection was given. Pruritus level was rated 5-6/10. On examination a papular dermatitis was identified and skin cytology confirmed the prescence of cocci shaped bacteria amongst neutrophils. Deep skin scrapes were again negative. We had a prolonged discuss about therapy so far and explored important questions: namely have we used fewer antibiotics since starting ASIT (5 months on regular strength, 10 month on double strength for 10 months and the answer was no! We also asked whether he was less pruritus overall and needed less steroid, the answer was again no. The author may have encouraged another refill of ASIT; however, verbal and non-verbal cues clearly indicated frustration and financial constraints. The decision was made to start oral interferon as a

possible tool to supplement Chip's natural interferons which help fight secondary infections. [3] Prednisone was discontinued and changed to dexamethasone, 0.75 mg, 2 tablet by mouth daily for 7 days then every other day. [4,5]

Chip continued to maintain a reasonable level of comfort when on dexamethasone but attempts to discontinue therapy resulted in spikes in pruritus scores. A limited ingredient diet with Royal Canin rabbit and potato was instituted, including a change to non-flavored heartworm prevention. Over the next few months the owner was able to determine that the following steroid regimen kept Chip comfortable:
dexamethasone: 0.75 mg, 2 tablets every other day, alternating with 1/2 tablet every other day. The author felt this was a reasonable low dose steroid and the owner reported Chip was doing "very, very well" overall. The decision was made to continue the dexamethasone and interferon.

In November 2013 the owner phoned to report she was able to decrease the dexamethasone to 1.5 mg every other day and pruritus was approximately 3/10. When she tried every 3rd day dexamethasone therapy the pruritus increased to 6-8/10. The owner expressed concerned about weight gain and that Chip chewed a spot on the top of his tail to the point of hair loss. A recheck exam was recommended since this was a new lesion. An appointment was scheduled an the decision was made to continue interferon and try the new targeted itch therapy Apoquel®: 16 mg, give 1 tablet by mouth every 12 hours for 14 days then once daily for 16 days.

Three weeks later Chip visited ADRC. The following abnormalities were noted on examination: alopecia has progressed and Chip had a stripe of pelage along dorsum that was of course quality while the pelage on his lateral abdomen was softer and fluffier, much like a puppy coat. The dorsal tail had a patch of alopecia, erythema and hyperpigmentation with a prominent follicular pattern. The owner rated pruritus at 3-4/10 on twice daily apoquel but increased to 10/10 on once daily therapy. Testing this day consisted of deep skin scrapes which showed the tail patch to be positive for *Demodex canis* mites, moderate numbers in all life stages while scrapes from the shoulders back, head, and one paw to be negative. A discussion was held to discuss the demodex mites and how to proceed with therapy for Chip's allergies. The author believed the low dose steroid was ultimately responsible and should be avoided. The owner was in favor of avoiding steroids given Chip's weight gain from 65 to 77 pounds over time. Chip had previously received Atopica® for his allergies but gastrointestinal upset and the cost to low benefit ratio made this choice unfavorable for the owner. Apoquel® is contraindicated for dogs with demodicosis. The plan was made to continue interferon and continue apoquel®, 16 mg, but to try 1/2 tablet by mouth every 12 hours. Even though the lesion was localized the author recommended ivermectin at 300µg/kg, by mouth once daily.

One month later Chip returned for examination and he looked like a new dog. His coat quality was normal and full. His owner expressed her appreciation: "He is playing…like a different dog…like a puppy again". Repeat deep skin scrapes were negative for mites.
The plan was made to discontinue interferon and monitor for decline in coat quality or relapse of superficial pyoderma. He will continue Apoquel® and ivermectin until he is seen in 3-4 weeks for repeat skin scrapes. Information regarding future follow ups will not be included in these notes due to printing deadlines but will be shared at the time of case presentation.

**Final Diagnosis**
- Food allergic dermatitis – not challenge proven yet
- Canine atopic dermatitis
- Relapsing superficial pyoderma
- Demodicosis – focal, likely secondary to low dose steroid administration

**Key Points**

• Most patients respond to ASIT, approx 20% do not respond

•Apoquel is a new FDA approved allergy blocker, can't sustain high dose twice daily dosing due to concern for side effects

•Apoquel contraindicated for patients with demodicosis. It was used in this patient in an off label manner

•Skin can have changing dynamics over time, even if you've been working with a patient for years…don't forget new conditions may develop

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**Itchy Pets: Case Based Dermatology**
Barkley: 7 year old, Male, Neutered, Airedale Terrier

**Will the Owner Do the Rechecks When I Ask?**
**Allergic Dog with Highly Resistant Staph Infection**
Dana A. Liska, DVM, Diplomate, ACVD

**Barkley**
Neutered male Airedale terrier, 93 pounds, 7 years of age on presentation.
**History**
Barkley lived in the Dallas, Texas, area for the first 4 years of his life, moved to California for 3 years, and then returned to Texas. Medical records from his veterinarian in California show a history of intermittent dermatitis and pruritus responsive to cephalexin, tapering doses of prednisone, and Genesis Topical Spray (Virbac AH) consistent with atopic dermatitis.

Five months before his referral, Barkley experienced bilateral ear infections, which waxed and waned, along with waxing and waning skin infections, for 3 months. He was on and off topical and oral therapy for both conditions. The right ear infection responded to therapy, but infection in the left ear persisted. Three months before his referral a sample was collected from the left ear for bacterial culture and sensitivity, and growth showed coagulase-negative *Staphylococcus* bacteria . One month later a staph pyoderma was recognized as unresponsive to appropriate antibiotic therapy and was culture and determined to be *Staphylococcus pseudintermedius* sensitive only to amikacin. Because Barkley was moving to Dallas, no therapy was started.

Barkley's veterinarian in Dallas tested him for hypothyroidism. When the thyroid panel was within normal limits, she promptly referred Barkley to the author's clinic where he was examined

within 1 month of arriving in Dallas. At the time of referral he was eating a novel protein diet (Prescription Diet d/d venison formula – Hill's Pet Nutrition).

**Clinical Signs**
Barkley had active epidermal collarettes on his ventral abdomen. The interdigital skin (palmar, plantar, and dorsal) was moderately erythematous. An otoscopic examination of the left ear could not be performed due to severe stenosis and discomfort.

**Initial Workup/Laboratory Results**
Cytology of left ear: cocci bacteria too numerous to count (TNTC), with suppurative inflammation and a bipolar rod in each oil power field (1/OPF).
Skin cytology: Numerous cocci (extra and intracellular) among neutrophils from both the trunk and interdigital spaces.

**Diagnosis**
• Cocci bacterial otitis externa (left ear) – resistant *Staphylococcus schleiferi.*
• Superficial bacterial pyoderma – *Staphylococcus pseudintermedius* – methicillin (MR) and multidrug resistant (MDR).

**Differential Diagnosis for Recurrent Otitis and Dermatitis/Pruritus**
• Resistant organism – known.
• Reinfection from otitis media – unable to ascertain at time of referral.
• Secondary to food allergy – possible but less likely given the history is more consistent with waxing and waning dermatitis/otitis typical of atopic dermatitis
• Secondary to atopic dermatitis – historically more likely than food allergy
• Secondary to tumor in the ear canal.
• Perpetuated by chronic ear canal changes.

**Next Steps**
The history of waxing and waning pruritus, dermatitis, and otitis externa supported a working diagnosis of canine atopic dermatitis. Addressing the resistant infections was the short-term focus while working toward intradermal allergy testing. Barkley was eating a limited-ingredient diet, which allowed time to determine if he showed any degree of improvement with a diet trial.

Further short-term management included a simple ear flush (not a deep flush) to remove debris and infection, but the canal was so swollen that the author could not assess the tympanum. Topical therapy was started: antiseptic ear cleanser (Epi-Otic Advanced – Allerderm) as pretreatment against the cocci shaped microorganisms followed by ticarcillin/clavulanic acid (Timentin – SmithKline Beecham) therapy to address the infection specifically. Steroids are important for improving soft tissue swelling of severe otitis externa; therefore, prednisone at a dosage of 20 mg (0.5 mg/kg) was prescribed to be given as one tablet by mouth once daily until Barkley's recheck. His owner was counseled regarding side effects.

Regarding Barkley's skin, discussion included the high degree of antimicrobial resistance noted in the bacteria as well as starting amikacin injections and the possible side effects. Amikacin has long been known for its effects on the kidneys and middle ear/vestibular system.[1] A daily bath with antiseptic shampoo (Hexadene – Virbac) was prescribed, with instructions to lather affected areas and allow 10 minutes contact time before rinsing.[2,3] Additional topical therapy was prescribed for the opposite end of the day from the bathing; the owner was to apply chlorhexidine spray (TrizCHLOR 4 – DermaPet) to all lesions. A recheck examination in 2 weeks was recommended given the severity of the left ear infection. The owner was advised that multiple

rounds of ear flushing might be required before it could be determined whether medical management was going to be effective.

As commonly occurs, the owner returned with Barkley 4 weeks later rather than the recommended 2-week interval. Otoscopic examination still could not be performed due to severe stenosis and discomfort; however, the ear canal was opening, and Barkley was reportedly less painful at home. At this visit the author was able to pass a 10 French catheter for an ear flush. Barkley exhibited some coughing and swallowing during the flush, indicating that the eardrum was not intact. He had 3 active collarettes on his ventrolateral abdomen. The interdigital skin remained mildly to moderately erythematous.

Cytologic testing demonstrated the following results:

| **Test** | **Initial Visit** | **First Recheck** |
|---|---|---|
| **Ear cytology** | TNTC cocci bacteria with<br>1 bipolar rod in each OPF<br>Suppurative inflammation | 6-10 cocci per OPF<br>No rods seen<br>Suppurative inflammation |
| **Skin cytology**<br>From trunk and<br>interdigital skin | Numerous cocci<br>(extra- + intracellular)<br>amongst neutrophils | Low number cocci<br>bacteria |

The ear cleanser (Epi-Otic Advanced), ticarcillin/clavulanic acid (Timentin), and oral prednisone at the same dosage were continued. A recheck examination in 2 weeks was encouraged.

*Author's Note:* At the time that Barkley was due for his next recheck the Dallas area experienced a severe winter storm with dangerous ice resulting in a "veritable shutdown of the city." Instead of seeing Barkley as anticipated, the owner stopped by the referral clinic after about 6 weeks, rather than 2, to refill the ticarcillin/clavulanic acid for treating Barkley's ear infection. Barkley's owner reported that he was doing well until they ran out of prednisone and until they could not drive Barkley to the groomer for his daily bath during the ice storm. The owner mentioned that Barkley had not received prednisone for 10 days and that his affected ear had seemed worse over the previous few days.

The author saw the opportunity to take advantage of Barkley's period without steroids and formed a plan with the owner to wait 4 additional days and then perform intradermal allergy testing (IDT). The optimum time for corticosteroid withdrawal prior to IDT has not been established and probably varies in each clinical situation. In the absence of well-documented guidelines, current textbook recommendations for withdrawal of glucocorticoids prior to IDT are a minimum of 3 weeks for oral glucocorticoids and 8 weeks for injectable glucocorticoids.[4-6]

**Further Treatment**
**Physical Findings**
Management of Barkley's MDR staph infection had been possible with topical therapy alone and he was initially improving, but after bath therapy was discontinued he relapsed rapidly. Barkley's left ear continued to be inflamed and the author palpated more stenosis. While the canal was not completely ossified, the left canal seemed less pliable than on previous visits. The canal showed moderate erythema and a thick otic exudate. Papular dermatitis with epidermal collarettes, crusts, hyperpigmentation, and subtending erythroderma had relapsed.

About 2 months after Barkley's first recheck an intradermal allergy test was performed. Results indicated positive reactions to grasses, molds, weeds, trees, and others such as house dust, staphylococcal antigens and flea antigen. He continued to show cytologic improvement in his left ear despite continued soft tissue swelling. The staph pyoderma had relapsed off topical therapy. A culture and sensitivity test was repeated on the epidermal collarettes on his skin.[7] The author sees some patients in which avoiding systemic antimicrobial therapy for a prolonged period of time (several months) enables the staph bacteria to return to a less resistant state. In a recent publication, 31 dogs previously diagnosed with a clinical infection were sampled repeatedly for a minimum of 8 months or until two consecutive negative results were obtained. The overall median length of MRSP carriage was 11 months (range: 4.5-19).[8]

Cytology at this visit demonstrated these results:
**Test: Ear cytology –** two cocci/OPF
Skin cytology – moderate number of cocci shaped bacteria and degenerated neutrophils

This was the second ear cytology showing low numbers of bacteria yet the continued presence of severe otitis externa ± media. The author began encouraging surgical referral.
Oral prednisone dosed at 20 mg (0.5 mg/kg) was continued as was an appropriate dose of fatty acids and various antihistamines appropriate for Barkley's weight. The author and colleagues counsel clients to give 180 mg per 10 pounds of body weight of EPA (eicosapentanoic acid). Aggressive daily bath therapy Hexadene – (Virbac) was recommended pending culture results. Topical ticarcillin/clavulanic acid was continued to give the owner time to discuss surgical referral with the family.

**Working Diagnosis**
• Canine atopic dermatitis.
• Cocci bacterial otitis externa (left ear) – resistant *Staphylococcus schleiferi.* Numbers of bacteria continued to improve, but canal was swollen again.
• Superficial bacterial pyoderma – highly resistant *Staphylococcus pseudintermedius.* Sensitive only to amikacin; initially responded to topical chlorhexidine therapy but has relapsed.

**Differential Diagnosis for Recurrent Otitis**
• Resistant organism – known.
• Reinfection from otitis media.
• Secondary to food allergy – possible but less likely because Barkley is already eating a venison diet and history supports Atopic dermatitis.
• Secondary to tumor in the ear canal – no historical concern from family veterinarians.
• Perpetuated by chronic changes to the ear canal.

*1 week after IDT:* Bacterial culture and sensitivity test results were available, and the bacteria were sensitive to multiple systemic medications. Barkley's owner was updated and advised to administer 1,200 mg (28 mg/kg) of sulfadimethoxine/ormetoprim (Primor – Pfizer Animal Health) initially given as two tablets by mouth in a single oral dose. Then Barkley was to receive one tablet by mouth once daily for 30 days

*1 month later:* Barkley's owner phoned to report that the lesions had dramatically improved. The sulfadimethoxine/ormetoprim antibiotic was refilled and continued at the same dosage for an additional 15 days. Prednisone at the 20 mg dose was also refilled, and the recommendation was to give it once every other day until the next recheck. The owner was asked to ensure a recheck before medications were completed.

*2 through 3 months later:* The owner missed and rescheduled appointments multiple times, although the sulfadimethoxine/ormetoprim antibiotic was refilled to enable continued therapy until the actual recheck. Long-term therapy with poteniated sulfas raises concerns about side effects, such as fever, arthropathy, blood dyscrasias (neutropenia, thrombocytopenia, or hemolytic anemia), hepatopathy consisting of cholestasis or necrosis, skin eruptions, uveitis, or keratoconjunctivitis sicca.[9]

*4 to 7 months later:* Barkley basically continued on a low dose of prednisone administered every second to third day as well as topical ticarcillin and clavulanic acid, ASIT, and antihistamines. His owner reported that the ear was doing well and the skin looked great; however, at the end of the seventh month Barkley was presented again because his left ear was flaring with infection. Ear cytology showed TNTC cocci bacteria, and the author facilitated a referral to veterinary surgeons for a consultation regarding total ear canal ablation and bulla osteotomy.

The following month Barkley flared with *Staphylococcus* pyoderma. In most cases the author does not change the ASIT formula at the 6-month recheck because a majority of papers analyzing data about ASIT with aqueous allergens report that most dogs respond after 3 to 12 months of treatment.[10-12] In Barkley's case, however, the author increased the concentration of his formula given the severity of his allergies.

**Final Diagnosis/Case Discussion**

About a month after his last flare, instead of experiencing another truncal superficial pyoderma, he flared with severe pododermatitis on 3/4 paws. Tape strip cytology revealed TNTC *Malassezia*, which responded readily to ketoconazole at a dosage of 200 mg given as one tablet (4.6 mg/kg) by mouth once every 24 hours for 30 days[13] and prednisone at a dosage of 20 mg (0.5 mg/kg) given as one full tablet daily for 5 to 10 days, then every other day therapy and preferably every third day therapy. Given Barkley's history one might assume that this episode was just another flare of staph pododermatitis, yet a simple tape cytology test was beneficial in diagnosing *Malassezia* dermatitis and enabled an informed decision regarding appropriate antimicrobial therapy.

Barkley presently is seen by the author approximately every 5 to 6 months for allergic flares that result in staph pyoderma. He continues to respond to sulfadimethoxine/ormetoprim antibiotic therapy and tapering prednisone doses as previously prescribed.

**Key Points**

• Antimicrobial resistance patterns can improve if systemic antimicrobials can be avoided.
• Topical therapy for resistant staph pyoderma takes dedication on the part of the pet owner and can be rewarding.
• Ear infections do not recur because you have not found the right medication; they recur due to underlying allergy and chronic canal changes.
• Investigate and focus on the underlying allergy as a way to reduce the recurrent pruritus and relapsing infections.
• Refer recurrent otitis externa cases earlier rather than later to avoid an end-stage ear and the need for surgical intervention.
• Tape cytology is a useful tool in ruling out secondary infections. One of the first studies to tout the importance of cytology was published in 1979.[14-16] To quote from two of the author's favorite sources: "An enormous amount of vital diagnostic data can be obtained by microscopic examination of stained material, such as smears of tissues or fluids, during a clinical exam ... often supplies sufficient data to narrow a differential diagnosis and develop a diagnostic plan."[15] From a more recent publication on feline dermatology: "Cytology can give rapid results and may help to suggest or even confirm a diagnosis."[16]

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# NOTES

# PATHOPHYSIOLOGIC INTERSECTIONS OF PAIN AND ITCH

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## OBJECTIVE

This session will review the common neuroanatomic and neurochemical denominators of pain and itch, and illustrate new developments in the neurophysiology of itch. This knowledge is fundamental for the rational clinical management of patient with pruritus.

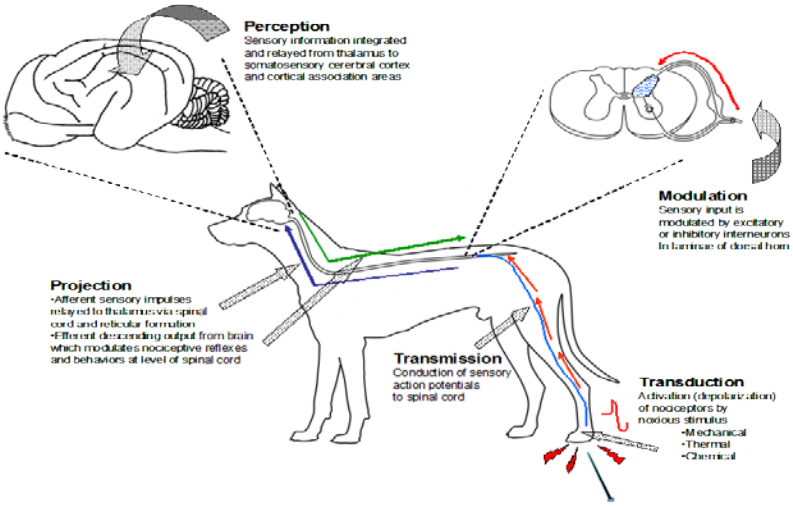
## INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.[1] Pruritus is regularly defined as an unpleasant sensation provoking the desire or reflex to scratch.[2] Pain and itch are extremely common presenting clinical complaints in veterinary medicine that can be caused by a vast number of cutaneous, systemic, and neurological disorders, and both have a significant and negative impact on quality of life.

## NEUROANATOMY AND PHYSIOLOGY OF PAIN AND ITCH

The neuroanatomic and neurochemical bases of pain and itch have a great deal in common. Both pruritoception and nociception have evolved as mechanisms to protect against or avoid noxious stimulation. Pain and itch are complex and dynamic sensations that involve five major processes: transduction, transmission, modulation, projection, and perception (Figure 1), each of which represents a potential therapeutic target. Research has just recently begun to elucidate the mechanisms responsible for the generation of and discrimination of itch.

**Figure 1- Physiology of Pain and Itch Sensations**




Current experimental evidence supports two major hypotheses regarding the manner by which itch signals are conducted from their origin in the periphery to the brain. The first, termed the *selectivity theory* states that there exist overlapping populations of polymodal itch and pain peripheral nerve fibers. Most fibers respond only to painful stimuli, but some respond to both pain and itch stimuli.[3] The higher density and numbers of pain-related C-fibers functions to exert an overall inhibitory influence on the smaller population of itch-sensitive C-fibers. Thus, itch is only perceived when the itch-transmitting C-

fibers are selectively activated.[3,4] A stimulus sufficient to activate both itch and pain, will result in masking of the itch input via activation of the population of C-fiber neurons transmitting the pain signal. Thus, the selectivity hypothesis posits that activation of dual-modal pain fibers serves to inhibit any itch sensation generated from these fibers. This hypothesis is supported by the clinical observation that strong and pain and itch sensations are not perceived simultaneously, and that scratching results in activation of pain fibers and thus often a relief from the itch sensation.[4] From an evolutionary perspective, the selectivity theory also confers the advantage of driving the organisms protective and avoidance efforts towards more threatening sensations (e.g. pain) when presented with multiple, noxious stimuli.

The second hypothesis, termed the *labeled-line theory*, postulates that there are itch-specific neurons in the peripheral and central nervous systems that are responsible for the transmission of itch sensations from the skin to the brain. It has long been recognized that there are itch-specific projection tracts in the spinal cord and brain, but only recently have itch-specific peripheral nerve fibers been definitively identified.[5] The labeled-line theory also holds that the generation of specific sensations involves crosstalk among distinct labeled lines. These features suggest that population coding is the mechanism underlying somatic sensation. Some authorities believe that ultimately elements of both hypotheses will be proven to be integral to itch physiology, and may demonstrate pathophysiological specificity depending on the type of itch and species being studied.

***Transduction- The Molecular Biology of Itch***

Pruritogens, defined as molecules that, after introduction into the skin, elicit both the sensation of itch and an urge to scratch following interaction with molecular receptors (Table 1), and several are also known mediators of pain. It is known that both keratinocytes and free nerve endings in the skin express molecules that interact with pruritogens and initiate the itch sensation. In many areas of the skin, free nerve endings are colocalized with keratinocytes, and the molecular signaling that occurs between keratinocytes and free nerve endings is an active area of research. Thus keratinocytes, and possibly other resident skin cells, and not just sensory nerves fibers, can serve as the molecular sentinels of itch.

**Table 1- Selected Mediators of Pruritoceptive Itch**

| Class | Specific Mediators | Receptor(s) |
|---|---|---|
| **Biogenic Amines** | Histamine | H1, H4 |
| **Cytokines** | Interleukin-31 | IL-31R |
| **Neuropeptides** | Substance P | NK1 |
| **Prostaglandins** | Leukotriene B4 | LTB4 |
| **Proteases** | Tryptase | PAR2 |
| | Cathepsin S/Cowhage | |
| | Kallikreins | |
| | Dust mite allergen | |

The molecular detectors relevant to itch can be receptors or ion channels present on sensory nerve fibers or keratinocytes. Many receptors identified to date are of the G-protein coupled receptor (GPCR) superfamily. GPCRs detect and respond to a diverse range of ligands or stimuli and are the target of many drugs. GPCRs that are relevant to itch respond to histamine, prostaglandins, neuropeptides, and proteases (Table 1).

The H4 receptor was discovered relatively recently and has been shown to produce acute itching separate from H1 activation. H4 receptor antagonists have been receiving attention as novel antipruritoceptive agents, as an H4 antagonist was shown to be superior to traditional antihistamines in the attenuation of experimental pruritus.[6] Numerous proteases can lead to the activation of protease-activated receptor-2 (PAR2). PAR2 is expressed on afferent neuron terminals and keratinocytes, can be activated by insect allergens, and is upregulated in patients with atopic dermatitis.[7] It has also been

demonstrated that PAR2 activation in spinal cord projection neurons leads to the release of substance P, which is also a tranducer of itch.[8] The release of substance P may exacerbate or intensify itch subsequent to mast cell activation. As PAR2 is a substrate for numerous protease pruritogens, PAR2 antagonists hold great clinical promise as anti-pruritic agents. Interleukin-31 (IL-31) has also emerged as an important mediator of pruritus. IL-31 has been detected in the skin of patients with atopic dermatitis.[9] Genetic mutations in the IL-31 receptor have been implicated in the pathogenesis of a form of localized pruritic disease known as familial primary localized cutaneous amyloidosis. It has been speculated that IL-31 exerts its pruritogenic effect by directly binding to IL-31 receptors on cutaneous nerves, but the precise mechanism remains unknown. Administration of anti-IL-31 antibodies has reduced scratching behavior in a mouse model of atopic dermatitis.[10]

The ion channels that appear to be principally involved in itch are members of the transient receptor potential (TRP) family. Although it is currently unknown if TRPs directly detect pruritogens, it is clear that TRPs are integral portions of the pathways involving transduction of itch and nociceptive stimuli in polymodal neurons. The transient receptor potential vanilloid receptor-1 (TRPV1) is a nonselective cation channel. TRPV 1 is expressed on sensory neurons, keratinocytes, mast cells, and endothelial cells. TRPV1 channels were originally presumed to be nociception-specific due to their activation by both the burning pain of capsaicin and the noxious temperatures. However, they have been implicated in pruritoceptive pathways because studies in TRPV1-deficient mice have shown diminished scratching in response to histamine or trypsin and TRPV1 has been found to be required in histamine and serotonin-induced itch.[5,11] Activation of TRPV1-expressing sensory neurons by pruritogens appears to utilize multiple different intracellular signal-transduction mechanisms.

***Transmission***

The primary sensory nerve fibers that innervate the skin are categorized into three groups based on their degree of myelination, diameter, and conduction velocity. Heavily myelinated Aβ fibers transmit tactile sensation, whereas the thinly myelinated Aδ and unmyelinated C-fibers are involved in the conduction of thermal and pain/itch sensations. Itch is transmitted predominantly by these unmyelinated, slow conducting C-fibers, which extend into the dermoepidermal junction with free endings penetrating into the epidermis where sensation is detected. The cell bodies for these C-fibers are in the dorsal root ganglia (DRG).[12]

The existence of itch-specific peripheral neurons has recently been confirmed. Han and colleagues genetically labeled and manipulated MrgprA3(+) neurons in the dorsal root DRG of mice and found that they exclusively innervated the epidermis and responded to multiple pruritogens.[5] Ablation of MrgprA3(+) neurons resulted in reductions in scratching behavior evoked by multiple pruritogens, while pain sensitivity remained intact.[5]

***Modulation***

The similarities between pain and itch processing are even more evident in patterns of central sensitization. It has been shown that touch- and pin prick-induced pain (allodynia and punctate hyperalgesia) correlate to touch- and pin prick-induced itch (alloknesis and punctate hyperknesis). Central sensitization, a from of which consists of hyperactivity of spinal cord dorsal horn neurons after tissue injury or persistent afferent input, such as C-fiber activation, is believed to cause hypersensitivity to itch.[13] Spinal cord long-term potentiation (LTP) is a form of synaptic plasticity and central sensitization. Recent evidence suggests that toll-like receptors, and specifically *Tlr3*, have a role in the central sensitization and spinal cord synaptic plasticity in chronic itch. LTP was unable to be induced in $Tlr3^{-/-}$ knockout mice, and subsequent behavioral expressions of central sensitization, such as capsaicin-induced secondary mechanical hyperalgesia was impaired in $Tlr3^{-/-}$ mice. Thus, *Tlr3* may control itch via novel neuronal mechanisms, including altering excitability of primary sensory neurons and modulating synaptic plasticity.[14]

***Projection***

From the DRG, both pain and itch sensations involve projection neurons that synapse in laminae of the dorsal horn and ascend via the contralateral spinothalamic tract to the thalamus. Approximately 5% of spinothalamic neurons of the cat are itch-specific.[15] Itch-selective spinothalamic neurons form a distinct pathway projecting from lamina I of the dorsal horn to the ventrocaudal part of the nucleus medialis dorsalis of the thalamus. This thalamic nucleus has projections to the anterior cingulate and dorsal insular cortex.

***Perception***

New developments in functional neuroimaging, such as positron-emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have revolutionized the mapping of the brain circuits that participate both in the perception of itch and scratching-induced euphoria (algedonic pleasure).[16] Itch and pain are complex sensations consisting of sensory discriminative, affective and motivational components.

The brain areas involved in itch and pain processing overlap significantly, and like pain, itch processing occurs in the dorsal posterior insula, anterior cingulate cortex (ACC), prefrontal cortex (PFC), thalamus, primary somatosensory cortex (S1), and cerebellum.[16] The temporal, spatial and intensity aspects of itch perception are processed in S1. Differences from pain mainly relate to the lack of secondary somatosensory (S2) cortical activation and the predominant inclusion of ipsilateral motor areas in pruritus. Ipsilateral motor area recruitment is correlated to the planning of the scratch response directed to the stimulated limb, as opposed to a classical pain response in which contralateral motor activation is required to withdraw the stimulated limb. The prefrontal cortex determines itch thresholds.

Studies using fMRI have also provided insight into the hedonistic aspects of itch. It has been shown that active scratching (patient delivered) results in more pronounced deactivation of the ACC and insula, when compared to passive scratching (investigator delivered). In addition, active scratching demonstrates involvement of the reward system including the ventral tegmentum of the midbrain, and deactivation of the periaqueductal gray matter (PAG), which suggests that itch modulation operates in reverse to endogenous analgesic mechanisms.[17]

**CONCLUSION**

Techniques such as microneurography, functional neuroimaging, and genetic knockout mice have lead to recent discoveries elucidating the transducers and mechanisms of itch, opening the door for therapies that specifically target the molecular biology of itch initiation and propagation. Targeted amelioration of pruritus offers the promise of significant improvements in the quality of life of patients, even in the face of a chronic and active underlying condition.

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# NOTES

# NEUROPATHIC PAIN AND ITCH: CLINICAL CONUNDRUMS?

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## OBJECTIVES

Using case examples, this session will illustrate the pathophysiologic mechanisms of itch and discuss the diagnostic and management approaches to the patient with various types of neuropathic itch.

## CLINICAL CLASSIFICATION OF ITCH

The sensation of itch can only be perceived in a few tissues, mainly the skin and superficial mucous membranes. Thus, while the clinical complaint of itch often indicates cutaneous inflammation or other pathology and remains the province of dermatologists, there exists a population of patients with significant itch in which there is no apparent dermatologic cause. Approximately 8% of all humans with chronic pruritus have neuropathic itch, and there is a growing body of evidence that neurological disorders can be a cause of focal or generalized pruritus in veterinary patients.[1,2]

The clinical subclassification of itch was first introduced by Twycross and colleagues, who distinguished etiologies according to the anatomical origin of itch-transmitting action potentials.[3] This original system has evolved to include anatomical, pathophysiological, and psychological factors, and has considerable utility when establishing a diagnostic and therapeutic plan for the patient, although there remains no universally accepted classification system for chronic pruritus.[4] Anatomical itch clinical classification schemes typically include four categories, which from the basis of this review:

1. Pruritoceptive: generated in the skin, usually by an inflammatory or other visible pathological process involving the skin.
2. Neurogenic: generated in the nervous system in response to circulating pruritogens, without evidence of neural pathology. Pruritus due to central excitation or abnormal processing of afferent sensory information from the periphery is also included within this category.
3. Neuropathic: due to anatomic lesions of the central or peripheral nervous system (e.g. nerve entrapment and tumors of the central or peripheral nervous system.
4. Psychogenic: considered psychiatric in origin, and may be associated with psychological comorbidities.

A given patient can have one or more types of itch. Following brief reviews of pruritoceptive and neurogenic itch, emphasis will be placed on the introduction and management of neuropathic itch syndromes.

More recently, a second classification system has been developed based on the clinical signs present in an attempt to limit the differential diagnostic considerations for each patient. This system divides pruritus into: 1) that associated with primarily inflamed skin, 2) that associated with non-inflamed skin, and 3) that associated with chronic secondary scratch-induced changes (prurigo).[5]

## PRURITOCEPTIVE ITCH

Pruritoceptive itch is the type most frequently encountered by veterinarians and veterinary dermatologists. It is generated in the skin either through inflammation or other skin damage. Most causes of pruritoceptive itch manifest with cutaneous changes visible upon

clinical examination, but itch can be a premonitory feature of an otherwise clinically silent dermatosis. Age-related changes in the barrier function of the skin or the structure and function of afferent sensory neurons can also lead to pruritoceptive itch. This type of itch accounts for the majority of clinical pruritus because everything from endogenous mediators and exogenous allergens that come into contact with the skin can induce pruritoceptive itch.

## NEUROGENIC ITCH

Neurogenic itch, which some sources refer to as "systemic itch" results from diseases affecting organs other than the skin. In humans chronic renal failure requiring hemodialysis, cholestatic liver disease, and hematopoietic malignancies are common etiologies of neurogenic itch. Although the itch sensation originates in the nervous system of these patients, there is no evidence of nervous system pathology. Neurogenic itch has been hypothesized to result, at least partially, as a response to intraspinal endogenous opioids released as a result of the underlying condition.[5] This would suggest that the administration of opioid antagonists might be expected to be effective in treating neurogenic itch, although clinical results have been mixed. Recent advances in itch research indicate that itch-selective neurons in the spinal cord are attractive and feasible targets for future neurogenic itch therapies. Systemic disease entities that fit current diagnostic criteria for neurogenic itch are poorly described in the veterinary literature.

## NEUROPATHIC ITCH

Neuropathic itch results from damage to central or peripheral sensory neurons, which leads to the activation of pruritogenic neurons without any cutaneous pruritogenic stimulation.[1] Neuropathic itch can be caused by lesions or dysfunction at any point along the afferent pathway of the nervous system.[1,6] Although relatively uncommon in veterinary medicine, neuropathic itch may be the only or predominant clinical sign of underlying neurological disease.

In general, therapy of neuropathic itch should be directed at the underlying etiology. However, symptomatic treatment is also important, because definitive diagnoses and disease-modifying treatments may not be found or readily available. Adjunctive therapies will be described with each of the neuropathic syndromes described below.

### *Trigeminal Trophic Syndrome (TTS) and Intracranial Lesions*

Numerous intracranial diseases have been associated with the development of neuropathic itch and TTS in humans including stroke, neoplasms, encephalitides, and multiple sclerosis. Approximately 20% of TTS cases in humans result from vertebrobasilar strokes.[7] TTS is the name of the facial neuropathic itch that progresses to scratching-induced erosions or ulcerations. Alternate names for TTS in the human in the literature include trigeminal neuropathy with nasal ulceration and trigeminal neurotrophic ulceration. For TTS to develop patients must have both intractable neuropathic itch but also concomitant cutaneous sensory denervation that makes the resulting scratching painless. Affected patients have essentially lost the physiological protective mechanism (e.g., pain) that inhibits scratching before it induces self-inflicted injury. In humans, TTS can occur in the absence dementia or other psychiatric disorders. In veterinary medicine, TTS has been observed as a rare manifestation of cavernous sinus syndrome, cranial nerve III and V tumors, lateral medullary stroke, and idiopathic trigeminal neuropathic syndromes.[8] Application of topical anesthetic patches, oral neuropathic analgesics, and use of physical barriers to scratching can be effective for TTS.

### *Seizure Disorders*

Compulsive facial itching, rubbing, or licking may accompany canine and feline seizure disorders as an aural, ictal, or post-ictal manifestation. This seizure semiology has been recognized most frequently in cats with complex partial seizures and hippocampal necrosis.[9] A proportion of these feline cases experience very brief periods of partial seizure activity, which

may be missed by the owner, followed by minutes to hours of orofacial automatisms, which prompt the owner to seek veterinary care. The author has seen affected cats with self-induced ulceration of the nasal planum or periorbital areas. Many of these cats will experience clinical improvement with phenobarbital, gabapentin, or zonisamide therapy, although other anti-convulsants may also be beneficial.

***"Spinal" Neuropathic Itch***

Various intramedullary spinal cord lesions have been shown to cause neuropathic itch in both humans and animals, attesting to the importance of the spinal cord as a site of itch modulation. Spinal itch has been associated with syringomyelia, spinal cord tumors, and traumatic spinal cord injury, and will typically affect the dermatome(s) corresponding to the level of the spinal cord lesion.[10,11] Intramedullary spinal cord diseases may induced pain and itch by altering sensory signal processing on the dorsal horn of the spinal cord, causing damage to intrinsic pruritogenic spinothalamic axons, or retrograde degeneration of peripheral sensory afferents.[10] Oral calcium channel and NMDA antagonists may be beneficial for spinal neuropathic itch, as they are for neuropathic pain. Patients with severe spinal neuropathic itch of the trunk and lower limbs may experience relief from intrathecal or epidural therapies with local anesthetics or alpha2 receptor-agonists.

***Radiculopathies***

Inflammation, entrapment, and compression of nerve roots can lead to neurogenic itch, which typically starts with a dermatomal distribution but may secondarily generalize. In humans, brachioradial pruritus, notalgia paresthetica, and shingles are common causes of radicular neuropathic itch.[1,6] Radicular compression or damage secondary to lateralized or foraminal intervertebral disk extrusions, peripheral nerve sheath tumors, and degenerative lumbosacral stenosis are common entities seen in veterinary neurology practice, and while clinical manifestations of neuropathic itch in these conditions are often present in the history and examination, they are frequently overlooked due to the presence of severe pain and/or motor deficits.[12] In this population of patients, surgical treatment of the underlying cause will usually result in satisfactory improvement or resolution of clinical signs. The adjunctive use of gabapentin, pregabalin, perineural nerve blocks, or epidural analgesics can also be beneficial.

Canine acute polyradiculoneuritis (Coonhound paralysis) and ganglioradiculitis are acquired inflammatory diseases of the nerve roots, spinal ganglia, and/or nerves of dogs that can result in scratching behaviors of sufficient intensity and severity to cause self-induced skin lesions, although other manifestations of motor and sensory dysfunction typically dominate the clinical presentation.[13]

***Sensory and Mixed Polyneuropathies***

Neuropathic itch can be a clinical feature of both inherited and acquired polyneuropathies. The clinical distribution of signs with small fiber sensory neuropathies usually assumes a "stocking and glove" distribution, Excessive licking of the feet or anogenital regions have been described as features that may precede more overt signs of self-mutilation in the inherited sensory neuropathies of the English pointer, Border collie, and long-haired Dachshund dogs.[14,15] These inherited neuropathies typically manifest with clinical signs beginning within the first 4-6 months of life.

Neuropathic itch may also be a part of numerous acquired sensory or mixed polyneuropathies, many of which have no definitive etiology even after extensive diagnostic testing is performed. The author has also observed neuropathic itch as a component of presumed paraneoplastic polyneuropathies associated with hepatocellular carcinoma, insulinoma, and myeloid leukemia.

## PSYCHOGENIC ITCH

Psychogenic itch is considered psychiatric in origin.[6] It typically presents with excessive impulses to scratch, pick, or lick at otherwise normal skin. Psychogenic pruritus involves brain or psychiatric abnormalities that are currently poorly defined, but multiple psychiatric diagnoses including depression, obsessive compulsive disorder, anxiety, somatoform disorders, mania, psychosis, and substance abuse have been associated with itch.[16] The incidence of patients in human dermatology clinics with psychogenic itch is estimated to be approximately 2%.[16] Psychogenic itch is generally a diagnosis of exclusion and requires ruling out other causes of pruritus. The most well documented potential examples of psychogenic itch that occur in veterinary medicine are feline psychogenic alopecia, tail chasing, flank-sucking, and "cryptogenic" anogenital pruritus.[2] It can be extraordinarily difficult to completely exclude underlying pathology of the nervous system in these patients.

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# NOTES

# RADIOTHERAPY FOR NEIOPLASTIC AND NON-NEOPLASTIC DISEASE

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## INTRODUCTION

Veterinary radiation oncology is the discipline which addresses the causes, prevention, and treatment of benign and malignant diseases to a variety of species with special emphasis on the role of ionizing radiation. With significant advancement in the field of radiation oncology and the increasing availability advanced technology in combination with the dedication and expectation of clients, expanded treatment options and significant improvements in optimal patient management are becoming a clinical reality. The purpose of this note set is to review the various aspects of radiation therapy, starting with the basic biologic understanding of ionizing radiation and its interactions with tissue, moving into a brief overview of the equipment and workflow, followed by normal tissue (skin) and tumor responses to radiation therapy, and ending on one potential novel application.

## RADIATION THERAPY

Radiation therapy is the therapeutic use of ionizing radiation in the treatment of patients. The aim of radiation therapy is to deliver a precise and well characterized dose to a well-defined target volume while minimizing damage to surrounding normal tissue. In general, there are three administration/delivery techniques available for clinical use in veterinary medicine which are all based on source location relative to the patient.

1) Teletherapy or external beam radiotherapy the radiation originates from a source located outside the patient and is administered through the use of isotope teletherapy units or linear accelerators.
2) Brachytherapy the radiation originates from radioactive sources encased in protective capsules called seeds implanted temporarily or permanently near or within the target.
3) Plesiotherapy or contact radiotherapy the radiation originates from a source located on the surface or in direct contact with the target.

## PHYSICS OF RADIATION THERAPY

From a classical perspective, the atom is the smallest unit of matter. The nucleus of the atom contains protons and neutrons and is surrounded by a cloud of electrons moving in distinct orbits. Electrons are held in their orbits by their binding energy. The absorption of energy by the atom from radiation resulting in the ejection of the electron is called ionization.

Ionizing radiations can be classified as either electromagnetic or particulate. Electromagnetic radiation is characterized by varying electric and magnetic fields which carry energy. X-rays and gamma rays are at the upper end of this spectrum and carry high energy. Particulate radiation is typically elementary particles, such as a proton or electron, with a positive or negative electric charge which also can carry high energy.

Ionizing radiation can be further subdivided as directly or indirectly ionizing. All charge particles are directly ionizing which means they have the ability to disrupt atomic structure as they pass through biological material. Electromagnetic radiation is indirectly ionizing which means they must interact with material first and liberate charge particles which have the ability to disrupt atomic structure and produce chemical and biological damage.

**EXTERNAL BEAM RADIATION THERAPY EQUIPMENT**

Up to about 15-20 years ago, most external beam radiotherapy (orthovoltage, cobalt-60, modern linac) was carried out with x-rays generated at relatively lower energies or with cobalt-60 teletherapy units. In the United States, subsequent availability of higher energy machines and the increasing demand for more modern linacs have resulted in a gradual demise of the inferior technology. However, these machines have not completed disappeared and are still in clinical use worldwide.

Orthovoltage – Orthovoltage units are similar in concept and appearance to the diagnostic x-ray machine. Lower energy x-rays are produced by bombarding a tungsten or tungsten alloy target with high energy electrons. The relative lower energy spectrum offers limited soft tissue penetration and a differential absorption of dose in bone (2 to 4 times more). In combination with the fact that orthovoltage units are operated at a relatively limited source-to-surface distance (around 50 cm) , the machine is more suitable for treating areas within 2 to 3 cm of the surface of the skin that do not involve bone.

Cobalt-60 – Cobalt-60 teletherapy irradiators contain a cylindrical source of 60CO within the head of the unit which produces radiation in the megavoltage range as it naturally decays. The beam produced has an average higher energy which results in greater penetrability to treat more deeply seated tumors, a larger source-to-surface distance (around 80cm), a dose build-up region called the skin sparing effect, and a more uniform dose deposition in bone. Some Cobalt-60 teletherapy irradiators are designed also for isocentric treatments which allow for the patient to be positioned only once for treatment while the source is rotated around the patient. Because of the source size and activity, the collimating system of the cobalt unit is more complex than the simple filters used in orthovoltage units. Unlike the orthovoltage unit or more modern linacs, the cobalt-60 source emits radiation constantly. This requires the source to be housed behind large amounts of lead shielding within the head of the unit even when the machine is off. In addition, the amount of dose per unit time is constantly decreasing as the source naturally decays necessitating the need for the source to be changed every 5 to 10 years.

Modern linear accelerator – Linear accelerators use electrical power converted by either a magnetron or klystron to produce high-frequency electromagnetic waves to accelerate electrons down a wave guide. The resulting electron beam can be directed by bending magnets in the head of the unit through a scattering foil and then to the patient for electron beam therapy (superficial) or alternatively can be caused to strike a heavy metal target producing x-rays (deep) that are collimated and then directed to the patient. As compared to the Cobalt-60 teletherapy irradiators, the collimators are typically more advanced and the beam produced has a higher average energy and dose rate which in combination results in greater penetrability to treat deeper tumors, an even greater skin-sparing effect, a larger source-to-surface distance (around 100 cm), larger treatment fields, shortened treatment times, and the ability to create complex geometrical shapes or beam intensities. In addition, the relatively small focal spot (source) limits the penumbra of the beam which results in a sharper treatment field edge and better port film discrimination.

## BIOLOGICAL BASIS OF RADIATION

DNA is the principle target for the biological effects of radiation. If any form of radiation, particulate or electromagnetic, is absorbed in biological material, there is a possibility that the DNA itself may be ionized leading to biological change. Alternatively, the radiation may interact with water molecules to produce free radicals that are able to diffuse far enough to reach and damage neighboring DNA. The damage produced by these free radicals in DNA can be more readily repaired under hypoxic conditions but may be made irreparable if molecular oxygen is present.

The clinical or cytotoxic effect of ionizing radiation is primarily due to the induction of DNA double and multiple single strand breaks (DSBs, SSBs). DSBs and SSBs are difficult to repair because the DNA helix is physically disrupted. DNA breaks may be repaired by numerous inducible repair mechanisms but defective recombination may result in terminal chromosomal aberrations.

In combination with repair, when DNA damage is detected, cells activate DNA-damage signal transduction pathways which arrest proliferating cells at specific cell cycle check points to give more time to repair. Following exposure to ionizing radiation, cells can repair the damage, activate programmed cell death (apoptosis), or continue through the cell cycle damaged ultimately resulting in some downstream mitotic catastrophe or necrosis.

## PRESCRIPTION (RADIATION IS A DRUG)

While radiation may be considered abstract clinically, as with any other drug utilized in veterinary medicine, radiation therapy must be prescribed. As with the stereotypical drug where the patient is given a number of administrations per day for a number of consecutive days to address a specific condition for optimal patient management, the same holds true for radiation. However, while some of the terminology is different, the underlying premise is the same. In general, the prescription should include the volume or site of administration, description of portals, radiation modality, energy (think of this as the specific drug), dose per fraction (how much drug per administration), number of fractions per day (how many administrations of drug per day), number of fractions per week (how many administrations of drug per week), total number of fractions (the total number of administrations), total target dose (total drug the patient receives), and prescription point (where the drug is treating).

## BIOLOGICAL BASIS FOR FRACTIONATION

The basis for fraction can be understood in simple terms. Dividing the total dose into several fractions or administrations creates a positive differential between normal tissue tolerance and tumor cell kill. Upon closer examination, four important biological processes are exploited:

1) Repair of sublethal damage by normal cells. Normal cells typically have better DNA repair and response pathways. Fractionation preferentially allows normal cells to repair sublethal damage as compared to tumor cells.
2) Repopulation of normal cells. The time interval between fractions allows the normal tissues to potentially repopulate preserving tissue function.
3) Reassortment of tumor cells into more radiosensitive phases of the cell cycle. Radiosensitivity varies throughout the cell cycle. Tumor cells are more likely to ignore DNA-damage signal induced arrest resulting in some proportion of cells cycling into more radiosensitive phases.

4) Reoxygenation of tumor cells. The majority of radiation induced DNA damage of cancer cells occur through the free radical production mechanism which is enhanced in the presence of molecular oxygen. The interfraction period allows additional perfusion of oxygen into hypoxic regions.

**NORMAL TISSUE TOXICITY (SKIN)**

Normal tissue morbidities associated with radiation therapy are commonly divided into two main categories, acute (early) or chronic (late), with each category demonstrating quite different patterns of response. The terms acute and chronic more accurately describe the time to development with most normal tissue toxicity dependent on the cell cycle time of the tissue in question. Classically, the terms are often used interchangeably to categorize the tissue type as either an acute responding tissue or a chronic responding tissue, but this is often misleading as all tissues are composed of discrete populations of cells with variable cell cycle times which have the ability to display both characteristics. In general though, acute toxicities typically present during or just shortly after the completion of therapy while late toxicities months to years after.

Its axiomatic in radiation therapy that fraction size and total dose are the dominant factors in determining the development of late tissue morbidities while weekly accumulation rate of dose, which is largely dependent on dose per fraction, number of fractions per week, and overall treatment time, determine the acute response of tissues. The rate at which radiation-induced toxicity develops and the time to clinical presentation is predominantly independent of dose and more determined by loss of cells from the terminally differentiated compartment. The skin possesses a hierarchical organization within the germinal components of the basal and suprabasal layers of the epithelia. The radiosensitivity of cells in these layers decreases during the maturation process. The preferential depletion of cells in the stem cell and early progenitor cell compartments results in a progressive decline in the number of cells available for terminal differentiation. Barrier function is maintained until post mitotic cells are lost through continuing normal cell turnover.

Clinically this leads to a predictable progression from erythema to dry desquamation and hyperpigmentation eventually to a moist desquamation, and if the dose is high enough, skin necrosis. Erythema is variable and more closely related to radiation effects (vasodialation) in the vessels. Epidermal changes are based on radiodepletion of the various cellular compartments. According to the average turn over time of canine epidermis of approximately 21 days, progression from dry to moist desquamation is typically seen about 2-3 weeks after the start of radiotherapy. Although skin tolerance experiences a significant volume and dose rate accumulation effect, erythema and dry desquamation typically present at cumulative doses around 20-30 Gy with progression to moist desquamation around 40-50 Gy in 2 Gy fractions. Epidermal appendages start to display a transient loss in function early in the protocol and may progress to permanent loss if doses are sufficient. After cumulative doses around 10-12 Gy in 2 Gy fractions, sebaceous gland and hair follicle dysfunction is observed.

There is no gold-standard approach in the prevention and management of progressive radiation dermatitis. Practice tends to be institution and site specific. Veterinary and human clinical trials have evaluated the efficacy of various treatment recommendations but results of clear benefit have been mixed. As preventatives, Aloe Vera and vitamin E topicals, zinc and pentoxifylline supplementation, corticosteroid and sucralfate creams are frequently prescribed. Use of supportive combination creams like Xclair or Miaderm in humans has shown some benefit during therapy but patient numbers were small.

From a general management perspective, the treatment site should be clean, dry, and protected from mechanochemical trauma and irritation. The site can be managed initially with warm water soaks or gentle washing with a mild soap and patting dry of the treated area. Hydrophilic moisturizing creams usually provide rapid relief for early dry desquamation and minor mechanical irritation. Low-dose topical corticosteroid ointments are usually beneficial for pruritus but should be discontinued if moist desquamation develops. Once moist desquamation develops, longer more frequent soaks should be used for natural debridement. Telfa or hydrogel wound dressings should be used to cover and protect the area. If moist desquamation develops with greater than 5-10 Gy remaining, debridement soaks should be continued and radiation held until new skin islets are visualized.

The severity of radiation-induced injury depends both on the extent of stem and progenitor cell depletion and the length of delay of maturation from stem cell to terminal differentiation (fully differentiated keratinocyte). Severity of injury increases with dose but dose fractionation can lessen the effects by allowing regeneration of the stem cell and progenitor cell compartments over the course of radiotherapy. The new skin formed after irradiation is often fragile, atrophic, and easily traumatized by mechanical or environmental influences. Within the treated areas, changes in hair color, texture, and quantity are present and glandular support to the skin is often compromised or absent giving the skin a dry, cracked appearance. In regions of high dose, melanocyte destruction can result in hypopigmentation while atrophy and contraction often result in subcutaneous fibrosis.

**PATIENT SELECTION**

When a clinician proposes the use of radiation therapy to a client for the optimal management of the patient's condition, additional fundamental questions must be addressed:

1) First, is there an indication to use radiation therapy? In other words, is there data which either shows or supports that radiation therapy would be efficacious for the patient. This is probably the most difficult question to answer initially as it relies on a global clinical awareness of potential benefit. In an ideal setting, gold standard data would exist for every possible permutation or condition clearly characterizing or demonstrating radiation therapy's value. This data would be widely disseminated and readily available. Unfortunately, the clinical reality is often that data is either unavailable, lacking, or absent blurring the obvious distinction of its clinical use. This is often complicated further by dogmatic declarations of evidence-based approach in veterinary medicine where the way we have always done it or lack of structured trials or initiatives is often confused with optimal management. In general though, radiation therapy can be justified in many cases either because it improves control and thus increases the probability of extending the life of the patient or improves quality of life of the patient by the amelioration of specific clinical signs.
2) Second, what is the owner's intent or ultimate goal? There are only two possible answers to this question. Definitive intent is used for the purpose of curing the patient. Implicitly this means the client and clinician are willing to risk the temporary loss of quality of life in the form of acute morbidities and the small but unknown risk of life long or chronic morbidities for the chance of a cure. Definitive radiation delivery strategies utilized in veterinary typically deliver small doses of radiation over the course of three to five weeks on a Monday through Friday schedule with weekends off. Irrespective of intent, patients have to be good anesthetic candidates to be considered. And

while anesthetic complications are potentially minimized as compared to surgical candidates, the risk is not fully removed.

Definitive radiation strategies are generally employed along with surgery either in a preoperatively or postoperatively setting based primarily on clinical presentation. This is an intuitive combination as surgery often fails to result in local tumor control primarily due to the presence of subclinical or microscopic disease beyond the margins of resection and radiation alone fails to control large masses. Postoperative strategies are based on three primary advantages: 1) surgery is not delayed and the detectable tumor is immediately removed, 2) the full extent of the tumor is often completely known after surgery, and post-surgical wound healing complications are not exacerbated by prior irradiation. In rebuttal, preoperative boast their own set of advantages: 1) the blood supply to subclinical disease is unperturbed increasing the likelihood of treating oxic populations, 2) there is no delay in radiotherapy secondary to post-surgical would healing complications, 3) the volume of irradiated tissue is based on naive treatment free with intact anatomical boundaries, 4) radiotherapy can reduce the growth of disseminated tumor cells prior to surgery, and 5) after irradiation, the tumor is often smaller allowing for a more conservative surgery or making a previously nonsurgical disease surgical. As with many areas of medicine, opinions will vary as to the optimal sequencing of these two modalities as each strategy has its own set of considerations.

In contrast to curative intent, palliative treatment is less intensive and used predominantly to manage specific clinical signs (pain, hemorrhage, space occupying aspects, obstruction, etc.). In general the goal of palliation is to improve the quality of life of the patient with little consideration necessarily to extending their life. To a much lesser extent as compared to definitive strategies, palliative treatments must balance patient, client, and clinical factors. It is considered poor palliative practice to induce treatment related morbidity while attempting to address the original clinical sign. Now, this definition is more purist and borrowed from our human counterparts who fail to have humane euthanasia as a commonly available treatment option. In veterinary medicine, our palliative approaches do extend life to some extent as they intervene prior to the time euthanasia would be considered or sought. Palliative radiation therapy delivery strategies utilized in veterinary medicine for the treatment of specific clinical signs typically deliver larger doses of radiation over the course of one to four weeks on a variable schedule.

3) Third, what is the true extent of the disease or the intended treatment volume? Imaging plays a large role in radiation treatment planning. In order to construct a radiation therapy treatment and minimize target and normal tissue uncertainties, the starting point is to acquire an image set of the area of interest to evaluate extent of disease and its impact on internal or underlying anatomy. Quantitative treatment planning is crucial in selection of the best plan parameters to deliver an optimal dose to the tumor while maximizing the sparing of normal tissues. The predicted consequences of treatment are based on the accuracy and precision with which the dose and irradiated volume are defined. In general, our ability to treat more aggressively is limited by the surrounding normal tissues. An imprecise treatment could result in a high incidence of normal tissue toxicity with paradoxically low tumor control or erroneously, could result in the lowering of target prescription (as a result of attempting to bring normal tissue morbidity back into clinical acceptability) resulting in even further loss of tumor control.

In facilities using manual techniques or older technologies, larger uncertainties are present but partially buffered by the lack of utilization of conformal techniques, complex beam orientations, intensity modulation, and as what the profession is clinically willing to let the patient tolerate. On the first day of treatment, patients are anesthetized or heavily sedated and the patient treatment position is determined. In combination with physical palpation and plain film radiography, determination of target volumes and organs at risk are identified, the treatment field geometry is verified, and correction-based manual dose calculations are made. Generation of port films for each treatment beam at the time of setup are acquired and used compare to future treatment time port films to help ensure correct patient setup. For a majority of clinical presentations, the disease and normal soft tissue anatomy is not readily visible, therefore beam blocks and field positions are typically determined only with respect to bony anatomical landmarks or rarely implanted fiducils (markers).

In more modern facilities utilizing 3D treatment planning techniques the simulation and treatment process are separated with the patient usually being simulated days prior to the start of radiation therapy. The simulation process typically requires the patient to be under general anesthesia. A CT image set (or multiple imaging modalities such as MRI, PET, etc) is acquired with the patient in the treatment orientation utilizing the appropriate position immobilizers for the specific area of the body. Once the CT image set is acquired, the clinician delineates or contours the target and the normal tissues or critical structures. Along with these contours, the clinician will also provide rough dose volume objectives and constraints to the treatment planning system. The treatment planning system uses algorithms to optimize and find a treatment plan that attempts to best satisfy the clinicians objectives.

**RADIATION RESPONSE**

The response to radiation can vary based on species, condition (malignant vs. benign disease), histology, histologic grade, stage of disease, age of patient, total dose, dose per fraction, etc. As previously mentioned, with such large variables to balance, there is a paucity of information detailing the use of radiation therapy for optimal management of benign and malignant diseases. Below is a list (not meant to be exhaustive) of outcomes based on condition, species, and response:

| Condition | Species | Treatment | Result |
|---|---|---|---|
| Aural ceruminous gland adenocarcinoma | Canine (n=5) and Feline (n=6) | Orthovoltage or Co-60 radiation +/- sx | 39.5-month progression-free survival [1], 56% 1 yr progression free survival |
| SCC in Situ (nasal planum) | Feline | Strontium-90 | 14 of 14 CRs with no recurrences. Overall survival > 3000 days[2] |
| SCC (nasal planum) | Feline | Strontium-90 | 9/11 achieved CRs with no recurrence during follow-up. 652 days median disease-free survival (range 134-2043 days)[3] |
| SCC (nasal planum) | Feline | Orthovoltage | 16.5-month median progression-free survival[4] |
| SCC (nasal planum) | Canine | Orthovoltage | 6-month median survival; |

| | | | |
|---|---|---|---|
| | | | median time to local failure 2.9 months[5] |
| SCC (nasal planum) | Canine | Megavoltage radiation +/- sx | RT only - 1 of 4 dogs cured, 3 of 4 recurred within 24 weeks (median 8 weeks)<br>RT + Sx – 0/7 cured, 7/7 recurred within 12 weeks (median 9 weeks)[6] |
| SCC (nasal planum) | Feline | Protons | 9/15 CRs, 5/15 PRs, 1/15 NR. 64% 1yr tumor control rate, median survival was 946 days +/- 516 days [7] |
| SCC (corneolimbal SCC) | Equine | Strontium-90 + keratectomy+graft | 24/38 1754 day +/- 1319 day mean tumor-free recurrence, 5/38 tumor recurrence at a mean of 449 +/- 339 days[8] |
| SCC (ocular) | Equine | Surgical debulking + 15/24 – Strontium-90<br>9/24 – Brachytherapy (iridium 192 implants) | 18/24 had no recurrence, 3/24 with local recurrence, 1/24 with local recurrence and extension, 2/24 with local recurrence and metastasis[9] |
| SCC (ocular adnexa) | Equine | Brachytherapy (Cesiums-137 and Cobolt-60 needles) | 14/19 controlled (did not return within 1 yr), 1/14 controlled returned after 2 years[10] |
| SCC (periorbital) | Equine | Stronitum-90 (8/17)<br>Brachytherapy (interstitial implants)(9/17)<br>16/17 +sx | 7/8 were controlled at 2 yrs<br>6/9 were controlled at 2 yrs[11] |
| SCC and Sarcoids (periorbital) | Equine | Brachytherapy (iridium-192)<br>N=115, 52 SCC, 63 Sarcoids | 81.8% and 86.6% 1 yr progression-free survival, 63.5% and 74% 5 yr progression-free survival[12] |
| Sarcoids (various locations) | Equine | Brachytherapy (iridium-192) N=22 horses with 23 lesions. 6/22 +sx, 3/22 +hyperthemia | 22/23 CRs. 94% 1 yr tumor-free incidence[13] |
| Sarcoids (periorbital) | Equine | Strontium-90 (N=3)<br>Brachytherapy (iridium-191)(n=53) | 3/3 CRs at > 1yr<br>52/53 CRs at >3 yr[14] |
| Cutaneous tumors (various) | Equine | Brachytherapy (iridium -191)(N=27) | 27/27 CRs, 100% 1 yr progression-free survival[15] |
| Mast cell tumor | Canine | Cobalt-60 +/- sx | 12-month median disease-free interval for measurable<br>54-month median disease-free interval for microscopic<br>32.7-month median disease- |

| | | | |
|---|---|---|---|
| | | | free interval overall[16] |
| Mast cell tumor (regional LN metastasis) | Canine | Cobalt-60 + sx | 36.5-month disease free interval[17] |
| Mast cell tumor (grade II) | Canine | Cobalt-60 + sx (N=45), 24 dogs had prophylactic regional lymph node irradiation | Median disease-free survival rate was not reached – 1 yr, 2 yr, and 3 yr disease-free survival rates were 80.6%, 67.1%, 67.1% resepctively[18] |
| Mast cell tumor (grade III) | Canine | Megavoltage + sx, (N=31), all dogs had prophylactic regional lymph node irradiation | 28-month median survival, 65% remission rate and 71% survival rate at 1 yr.[19] |
| Mast cell tumor (74% head, rest limbs and trunk) | Feline | Strontium-90 (N=54), 52/54 in the macroscopic disease setting | 53/54 CRs with local control with a median follow-time of 783 days. MST was 1075 days.[20] |
| Transmissible venereal tumor | Canine | Orthovoltage (N=18) | 25-month median disease-free progression[21] |
| Transmissible venereal tumor | Canine | Cobalt-60 (N=15), +chemo (N=6) | 21/21 CRs, 27-month median disease-free interval[22] |
| Cutaneous T-cell lymphoma (nonepitheliotropic) | Canine | Radiation + Pred (N=1) | CR, 36 month progression-free survival[23] |
| Cutaneous T-cell lymphoma (mycosis fungoides) | Canine | Orthovoltage | Clinical response after two fractions of 250 cGY total skin.[24] |
| Lick Granuloma | Canine | Megavoltage (N=13) | 11/13 favorable responses.<br>2/13 poor responses (lesions present over years)<br>5/13 had recurrence at 8 months to 3 years later (15-month mean time to recurrence)[27] |
| Infectious Dermatitis | Species unspecified | Superficial X-rays (N=6), refractory to all therapies | 3/6 CR<br>1/6 PD<br>2/6 unknown[28] |
| Lip Granuloma (Indolent ulcer) | Feline | Superficial X-rays (N=5) | 3/5 CR<br>2/5 PD[28] |

**NOVEL APPLICATIONS**

Over the past decade or so, several investigators have shown that administered radiation doses well below that of human conventional fractions (1-2 Gy) enhance the immune system. Of particular interest is low-dose irradiation's potential impact on immediate-type hypersensitivities or allergies. Immediate-type allergies are characterized by a population of allergen specific IgE antibodies and the over expression of Th2-mediated cytokines which causes persistent inflammatory cell recruitment. Cross-linking of IgE receptors on the surface of mast cells by complexes of IgE antibodies and captured allergen results the release of histamine and other mediators of the allergic response.

A subset of CD4+ T cells, $T_{regs}$, is known to protect the body from autoimmunity by controlling hyperimmune responses. In healthy individuals, Treg cells are usually present in high enough frequency to inhibit the development of Th2-mediated allergic responses. They accomplish this by directly suppressing the proliferation and cytokine expansion of Th2 and exhibit an inhibitory influence on dendritic cells and macrophages. Subtypes of Tregs, such as Tr1 and Th3 cells, are peripherally induced through antigenic stimulation in the presence of IL-10 and TGF-B. Increasing evidence has suggested impaired regulatory T cell activity can cause allergy.[25]

It has been reported in ovalbumin-induced allergic asthmatic mice that irradiation enhance Treg cells function and recruitment.[26] Data also suggests that irradiation inhibits airway hyperreponsiveness by reducing inflammatory cytokines as a result of suppressive activity of Tregs and reduces allergic inflammation and airway remodeling through inhibition of Th2-associated cytokines. Taken together, these observations suggest the possibility that low-dose radiotherapy could be used for the reduction of inflammation in patients with chronic hypersensitivies. Furthermore, by exploiting this inducible suppressive activity, strategies (such as allergen-specific immunotherapy) that expand antigen-specific natural T regs while inhibiting activation and expansion of effector T cells could improve tolerance.

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# NOTES

# DISORDERS OF PIGMENTATION

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Pigmentation of the skin and hairs of domestic animals is primarily determined by the amount of type of melanin present in the epidermis, dermis and hairs. Normal pigmentation is primarily determined by genetics, while disorders of pigmentation may be either genetic or acquired. Melanin is produced by melanocytes located in the basal layer of the epidermis and in the outer root sheath and hair matrix of hair follicles. Melanocytes originate in the neural crest and migrate to the skin during early embryogenesis. The production of melanin is affected by genetics, hormones, heat, injury, solar or ionizing radiation, heavy metals, and other factors. Changes in any of these factors can increase or decrease pigment production.

Hypopigmentation is the general term for a decrease in pigment. Decreased pigmentation may be due to melanocyte destruction, dysfunction, or an abnormal distribution of melanosomes. Hypopigmentation may be hereditary or acquired. Leukoderma is the term for a lack of pigment in the skin, leukotrichia is a lack of pigment in the hair. A decrease in pigment in the hair is called graying or poliosis.

Hyperpigmentation is the general term for an increase in pigment. Melanoderma is the term for increased pigment in the skin and melanotrichia is increased pigment in the hair. Hyperpigmentation may be hereditary, acquired, or associated with pigmented tumors. Aurotrichia is the term for hair that are gold-colored.

## GENETIC FORMS OF HYPOPIGMENTATION

Albinism is a partial or complete congenital absence of pigment in the skin, hair and eyes (oculocutaneous albinism) resulting from mutations in the tyrosinase gene. The eyes are usually blue. Albinism in cats and dogs is an autosomal recessive trait.

Chediak-Higashi Syndrome is an autosomal recessive disorder that affects Persian cats with yellow eyes and a blue smoke haircoat. This syndrome is also seen in white tigers, Hereford cattle, Aleutian minks and humans. It is characterized by partial oculocutaneous albinism and abnormal function of granulocytes and platelets. The hairs contain macromelanosomes and lysosomes in a variety of cells are larger than normal and exhibit impaired function resulting in bleeding disorders and an increased susceptibility to infections; most affected animals die at a young age.

Cyclic hematopoiesis in dogs results from homozygous recessive mutations in the gene coding dog adaptor protein complex 3. This is an autosomal recessive disease in which the dilute coat color is linked to a dysfunction of the pleuripotential stem cells in the bone marrow. Affected puppies have a silver-gray hair coat, lightly colored nose and cyclic decreases in hematopoiesis resulting in profound neutropenia every 10-12 days. This disease is most common in collies and affected puppies have recurrent episodes of sepsis. Affected dogs usually die before 6 months of age from secondary infections; those that survive longer often develop amyloidosis and die from renal failure.

Nasal hypopigmentation may occur as a season-associated lightening in color of the planum nasale during winter months. This is referred to as “snow nose” and is common in Siberian huskies, Golden retrievers, Labrador retrievers and Bermese Mountain dogs. The nose darkens in the spring and summer. Many other breeds of dogs (including Afghan hounds, Samoyeds, Siberian huskies, yellow Labrador retrievers, German shepherd dogs, Golden retrievers, poodles, Doberman pinchers, Irish setters, and pointers), have

a gradual fading of their nose from black at birth to a chocolate brown or paler color. The color change may be permanent or may wax and wane. A nose which undergo this form of depigmentation is commonly referred to as a "Dudley nose." Some dogs have more widespread congenital mucocutaneous hypopigmentation affecting their planum nasale, lips, eyelids, tongue and oral cavity.

Piebaldism is genetically determined white spotting. It is inherited as a dominant trait resulting in the absence of differentiated melanocytes in affected areas of the skin and hair coat.

Tyrosinase deficiency has been reported in Chow Chow puppies. Affected individuals have a pink tongue and portions of their hair shafts trun white. Melanin may spontaneously reappear in 2 to 4 months.

Vitiligo is a loss of pigmentation resulting from destruction of melanocytes in the epidermis. There are few, if any, melanophages in the superficial dermis—this helps to differentiate vitiligo from post-inflammatory hypopigmentation. Cases that develop white hairs also have destruction of melanocytes in the basal layer of the follicular bulb. Multiple pathologic mechanisms may be involved including mutations in the TYR gene and antimelanocyte antibodies have been identified in the serum of some affected dogs. Melanocytes of affected individuals may have an increased susceptibility to oxidative damage or antimelanocytic antibodies and cytotoxic lymphocytes. Breeds of dogs most commonly affected include Belgian Tervuren dogs, German shepherd dogs, collies, Rottweilers, Doberman pinschers and Giant Schnauzers. Vitiligo in dogs and cats usually appears as symmetric macular leukoderma and leukotrichia of the nose, lips, buccal mucosa and facial skin. Other areas of the haircoat and the footpads and claws may also be affected. Unilateral disease affecting the periocular skin in Siamese cats is called the Aguirre Syndrome and is associated with Horner syndrome, corneal ulcers, uveitis and upper respiratory tract infections. Biopsies typically reveal a complete absence of melanocytes in depigmented areas of skin.

Waarenburg-Klein Syndrome is due to a defect in the migration and differentiation of melanoblasts. Affected animals have blue or heterochromic eyes and white skin and hair. They are deaf and therefore should not be used for breeding. This syndrome is most commonly seen in cats, bull terriers, Sealyham terriers, collies and Dalmatians and is inherited as an autosomal dominant trait with incomplete penetrance.

Age-associated graying results from a reduction of melanocyte replication and is most commonly seen in German shepherd dogs, Irish setters, Labrador retrievers and golden retrievers.

**ACQUIRED FORMS OF HYPOPIGMENTATION**

Melanocytes and/or their function may be affected by inflammation, chemicals/drugs, hormonal/metabolic diseases, nutrition, neoplasia and many other factors.

Inflammation affecting the epidermis or basement membrane zone may result in destruction or "drop-out" of melanocytes and a loss of pigment. Discoid lupus erythematosus is a common cause of nasal depigmentation. Pemphigus erythematosus, systemic lupus erythematosus, pemphigus foliaceus, uveodermatologic syndrome, bullous pemphigoid, mucocutaneus pyoderma and drug eruptions may also result in depigmentation of the nose and other areas.

Infectious causes of nasal or skin depigmentation include leishmaniasis, blastomycosis, sporotrichosis, and bacterial folliculitis.

Contact dermatitis may result in depigmentation of the skin of the nose, lips and foot pads. Rubber may contain chemicals such as dihydroquinone, p-benzylhydroquinone and monobenzyl ethers which interfere with melanin production by blocking the activitiy of tyrosinase resulting in depigmentation of areas in contact with the rubber.

Drugs that have been associated with leukoderma and/or leukotrichia include glucocorticoids, progesterones, ketoconazole, procainamide and vitamin E. Cabergoline has been reported as causing a coat-color change from fawn to light yellow. Chronic chlorine and/or ultraviolet light exposure may also result in bleaching of hairs.

Nutrient deficiencies including zinc, pyridoxine, pantothenic acid and lysine may result in graying of the hair coat. Copper is another co-factor required for pigment production and a copper deficiency has been reported to cause black hairs to develop a reddish brown color. Recent studies in black cats have shown that diets low in tyrosine and/or phenylalanine develop reddish-brown hair coats that return to normal when the diet contains > 18 g/kg of tyrosine + phenylalanine.

Ovariohysterectomy may result in the growth of a lighter colored hair coat with a woolly texture. Endocrine disorders including hypothyroidism, hyperadrencorticism, hyperestrogenism, and hyperprogesteronism may also be associated with changes in coat color.

Cutaneous T-cell epitheliotrophic lymphomas, squamous cell carcinomas, basal cell tumors, mammary adenocarcinomas and gastric carcinomas have been associated with leukoderma and in some cases leukotrichia.

Leukotrichia and patchy hypopigmentation have been reported as idiopathic conditions in black Newfoundlands and also in black and chocolate Labrador retrievers. Siamese cats may be affected with unilateral or bilateral periocular leukotrichia that is sometimes associated with upper respiratory infections, pregnancy, dietary deficiencies or systemic illnesses while in other cases there is no apparent precipitating cause.

**CONGENITAL DISORDERS ASSOCIATED WITH HYPERPIGMENTATION**

Lentigenes are darkly pigmented macules that most often develop in the ventral abdomen. These may develop over a period of several months in healthy, adult dogs. These are sometimes referred to as “tar spots,” The skin in affected areas is heavily pigmented but otherwise normal. If the skin is thickened and scaly the lesion is probably due to a papillomavirus infection (discussed under acquired hyperpigmentation). Orange cats commonly develop hyperpigmented macules involving their lips, nose, gingiva and eyelids. This condition is termed lentigo simplex and has no adverse effects for the health of the cat.

Acanthosis nigricans is a cutaneous reaction pattern characterized by bilateral hyperpigmentation and lichenification of axillary skin. Dachshunds are thought to have a primary, hereditary, form of acanthosis nigricans which develops in dogs under 1 year old. Lesions start with hyperpigmentation of axillary skin and progress to alopecia, lichenification, and seborrheic changes that are often associated with bacterial and yeast infections. The lesions may spread to involve the forelimbs, ventral neck, chest, abdomen and others sites. Acanthosis nigricans developing in other breeds or in older Dachshunds is usually a post-inflammatory form associated with friction, intertrigo, allergies or endocrine disease with secondary bacterial and *Malassezia* dermatitis.

Acromelanism is the term used to describe the dark points seen on the points (feet, tail, ears) of Siamese, Himalayan-Persian, Balinese and Burmese cats are the result of a temperature-dependent enzyme controlling melanin production in hair bulbs. Temperatures above approximately 95 F (35 C) inhibit the enzyme resulting in production of lighter-colored hairs. Kittens of these breeds are born white with pigmented hairs replacing white ones on the cooler extremities during hair cycles following birth.

## ACQUIRED FORMS OF HYPERPIGMENTATION

One of the most common causes of hyperpigmentation is that occurring subsequent to inflammation (post-inflammatory hyperpigmentation). Leukotrienes, thromboxanes and other mediators of inflammation stimulate melanocytes to increase melanin production. Melanin down regulates inflammation by scavenging free radicals. Allergies, *Malassezia* dermatitis, bacterial pyoderma, dermatophytosis, demodecosis, scabies, actinic and intertrigo dermatitis are examples of inflammatory skin diseases commonly associated with cutaneous hyperpigmentation. Melanotrichia may develop as a response to inflammation affecting hair follicles, dermis or panniculus (e.g., sebaceous adenitis, panniculitis, vaccine reactions).

A variety of endocrinopathies including hyperadrenocorticism, hypoadrenocorticism, hypothyroidism, hyperestrogenism and other sex hormone imbalances may result in diffuse hyperpigmentation. ACTH and other pituitary hormones may stimulate melanogenesis. When hair loss precedes hyperpigmentation ultraviolet light may be the factor leading to increased melanin production. Dogs with hyperadrenocorticism treated with o,p'-DDD may develop a darker haircoat, perhaps due to increasing concentrations of ACTH or to direct affects of mitotane on the G (graying) locus.

Canine papillomaviruses can result in several different cutaneous lesions. Pugs appear to be at risk for the development of papillomavirus-associated slightly raised, scaly hyperpigmented macules and plaques involving their groin, abdomen, ventral thorax and neck. Similar lesions have also been described in miniature schnauzers, an American Staffordshire terrier and a Pomeranian. The lesions may transform into squamous cell carcinomas in some patients. Canine papillomavirus is also associated with exophytic papillomas which appear as cauliflower-like nodules or keratinous plaques which may be pink or flesh colored, or brown or black in color.

Many other tumors and tumor-like lesions are pigmented, with the color varying with the type of cells involved in the lesions. Apocrine cysts are generally bluish in color. Cutaneous hemagiomas and hemangiosarcomas may appear red, dark purple, or bluish-black in color. Histiocytic, lymphocytic and plasmacytic tumors may appear pink, red, or purple. Tumors which frequently appear black in color include melanomas, melanocytomas, and basal cell tumors. Other tumors which may appear dark brown or black include squamous cell carcinomas, trichoblastomas and fibromas.

## MISCELLANEOUS DISORDERS OF PIGMENTATION AND COLORATION

Acquired aurotrichia has been reported in miniature schnauzers and a bichon frise dog. The primary guard hairs changed color from silver or black to gold in patches on the dorsal thorax and abdomen. Pinnal and periocular regions were affected in some dogs. No underlying etiology was determined.

A variety of conditions can cause hair to become reddish in color. The most common is the reddish discoloration associated with salivary or tear staining of hairs. This is due to "rusting" of iron in the porphyrins found in saliva and tears. As previously noted, diets with inadequate content of aromatic amino acids and other nutrients may result in a coat-color change from black to red. A variety of endocrinopathies may also result in dark hair turning a reddish color. *Malassezia* infection of the claws has been associated with claws turning a reddish color, less frequently a similar color change is seen in association with bacterial paronychia.

Drugs may induce color changes in the skin, examples include a reddish-orange skin color in some individuals treated with rifampin, beta-carotene and clofazimine. Pinnal erythema has been reported in cats treated with enrofloxacin and ciprofloxacin.

Cutaneous flushing develops as a result of vasodilation of cutaneous blood vessels. This may occur due to emotional, autonomic or endocrine influences or by the direct action of vasodilators. In dogs, cutaneous flushing has occurred in association with pheochromocytomas and mast cell tumors.

## DIAGNOSTIC TESTS

Signalment, history and physical examination findings are helpful in formulating differential diagnosis for pigmentary disorders. Cutaneous cytology, skin scrapings and fungal cultures are helpful in the diagnosis of underlying causes for post-inflammatory hyperpigmentation. Skin biopsies for histopathological evaluation are fundamental for the diagnosis of hypopigmentation and for tumor-associated hyperpigmentation.

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# NOTES

# ISVD SESSION PRESENTATIONS SATURDAY

# MYSTERY SLIDE SESSION

**MYSTERY CASE #1:**

**Presenter: . Judith Nimmo.** ASAP Pathology, 53 Glenvale Cres, Victoria, Australia 3170.

A 4 year old male Maltese-cross dog had a > 2 year history of pruritic skin disease that had been treated with antibiotics, topical antifungal drugs, prednisolone and for the past 2 months with Atopica (cyclosporin A). The dog presented with a severe, progressive liquifactive cellulitis of the right foreleg. Despite aggressive antibiotic therapy the leg had to be amputated two weeks later**.**

**MYSTERY CASE #2.**

**Presenter: . Judith Nimmo.** ASAP Pathology, 53 Glenvale Cres, Victoria, Australia 3170.**Christina Mccowan**. University of Melbourne, Werribee, and Environment and Primary Industries, Bundoora , Australia.

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

**MYSTERY CASE #3**

**Judith Nimmo**. ASAP Pathology, 53 Glenvale Cres, Victoria, Australia 3170.

A 10 year old spayed female Labrador Retriever was presented with an ulcerative, lesion on nasal planum on the dorsal aspect of left naris that been present about a month. There had been no response to a week of antibiotics. There was no nasal discharge and the nasal mucous membranes look normal on scope. The nasal planum also appeared hyperplastic and dry.

**MYSTERY CASE # 4:**

**Presenter : . Emily Walder,** Independent Slide Consultation Service, Venice, CA, USA

Jacob is a 4-year-old, neutered male Chihuahua with a 4 month history of a nonpruritic, alopecic patch on the top of the head. More recently, a similar lesion with mild erythema and scale developed near the base of the right pinna. DTM and skin scrapes were negative. There was no response to Revolution or ivermectin.

**MYSTERY CASE #5,**

**Presenter : . Emily Walder,** Independent Slide Consultation Service, Venice, CA, USA

Sadie is a 7-year-old, neutered female Pomeranian with a history of moderate pruritus, partial to complete alopecia and scale on dorsal neck and trunk. She has polyuria/polydipsia and low T4. Low-dose dexamethasone suppression and ACTH stimulation tests were within normal limits

**MYSTERY CASE #6.**

**Presenter : . Emily Walder,** Independent Slide Consultation Service, Venice, CA, USA and Sonya Bettenay Tierdermatologie Deisenhofen, Germany.

A 12 year-old MC Yorkshire terrier was seen for a progressive pawpad lesion of 5 months‘ duration, non-responsive to antibiotics, starting as a small, superficial hole in the large RF pad and eventually involving the caudal haired skin junction. No other dermatologic or mucosal abnormalities were present.

# NOTES

# CORRELATION OF HISTOPATHOLOGY AND CLINICAL PRESENTATION

Verena K. Affolter DVM, PhD, Dipl. ECVP
Department of Pathology, Microbiology, Immunology, School of Veterinary Medicine,
University California Davis, Davis CA

## INTRODUCTION

The goal of this interactive session is to interpret pathology reports, formulate a morphologic diagnosis and correlate both with clinical presentations.
In an interactive session we will establish morphologic diagnoses, differential diagnoses of each case, determine which clinical lesions would be expected and what techniques and procedures will assist to further evaluate the cases.

**CASE 1: "Nantucket"**, 10 year old Westfalian gelding

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

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

**CASE 2:** "**Memphis Belle**", 8 year-old female spayed smooth coated collie

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

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

**CASE 3: "Romy",** 10 year-old Thoroughbred gelding

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

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

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

**CASE 4: "Aiden"** male castrated, 2 year-old Shepherd-mix

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

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

**CASE 5: "Molly"** female spayed, 10 year-old Labrador retriever

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

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

**CASE 6:** adult female Ring-tailed Lemur

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

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

**CASE 7:** "**Salsa**", 4 year-old, male castrated, DMH, orange tabby cat

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

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

**CASES 8:** "**Micky**", 5 year-old, male neutered DSH cat

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

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

**CASE 9: "Coco",** 5 year-old, mixed breed dog

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

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

**CASE 10: "Diego"**, 4 year-old, male Chihuahua-terrier mix

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

*Questions for discussion:*
*Morphologic diagnosis?*
*Major clinical skin lesions?*
*What are your differential diagnoses?*
*Do you have additional questions in regards to history?*

# NOTES

# Autosomal recessive congenital ichthyosis in American bulldogs is associated with decreased expression of ICHTHYIN (NIPAL4).

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

Funding:
The Commonwealth of Pennsylvania, International Society for Veterinary Dermatopathology, National Institutes of Health – OD010939

# NOTES

**Canine Epidermal Neural Crest Stem Cells – Characterization, Isolation and Expansion**

Barbara Gericota[1], Joseph S. Anderson[1], Gaela Mitchell[1], Dori L. Borjesson[2], Beverly K. Sturges[2], Jan A. Nolta[1], Maya Sieber-Blum[3]

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

**Funding**
This project was supported by Medical Research Council Grant 22358, UK (M.S.-B.), the Center for Equine Health, UC-Davis (B.G.), a gift from Dick and Carolyn Randall (J.N., D.B., B.G.), and by Fundacao para a Ciencia e a Tecnologia, Ministerio da Educacao e Ciencia, Portugal, under the Individual Doctoral Grant
SFRH/ BD/ 64871/2009 (B.G.).

**Conflict of Interest**
The authors indicate no potential conflicts of interest.

# NOTES

# THE MILLIONS OF MICROORGANISMS INHABITING THE SKIN: THE CANINE SKIN MICROBIOME

Rodrigues Hoffmann A[1], Patterson AP[2], Diesel A[2], Lawhon SD[3], Ly HJ[1], Stephenson C[4], Mansell J[1], Steiner JM[4], Dowd SE[5], Olivry T[6], Suchodolski JS[4]

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## INTRODUCTION

Molecular-based sequencing studies targeting the bacterial 16S rRNA gene have revealed that the skin surface of humans is inhabited by a diverse and variable microbial population composed of commensal, symbiotic, and pathogenic bacteria, defined as the microbiome. Recent studies have shown that an imbalance in these microbial populations may result in damage to the skin, and development of skin lesions, although it is still unknown if an altered microbiome is the cause of skin lesions, or the result of an altered skin barrier. [3-5,9]

The skin microbiome can be altered by host factors including presence of hair follicles, temperature, pH, moisture, environmental contact, and contact with mucous membranes. These different factors will influence the different skin microenvironments, which can be divided into dry, moist and sebaceous regions.[4] For instance, sebaceous areas are primarily colonized by *Propionibacterium* spp., whereas moist areas are more likely to be colonized by *Staphylococcus* spp. and *Corynebacterium* spp. Age is also considered to be one of the host factors influencing the composition of the skin microbiome, with the proportions of *Propionibacterium* spp. increasing in the forehead with age, whereas *Staphylococcus* spp. and *Streptococcus* spp. proportions often decrease with age.[1,10] These microbial populations can be also altered in disease states, which often result in lower diversity of these microbial populations.[2,6]

The environmental factors have been also considered to be an important factor responsible for changes in the skin microbiome. A recent study demonstrated that individuals cohabiting with dogs often had a more diverse microbiome, and that family members that cohabited with dogs were more likely to have share their skin microbiome.[8]

## THE SKIN MICROBIOME IN HEALTHY DOGS

In a recent study,[7] we evaluated the skin microbiome in different cutaneous and mucocutaneous regions in healthy dogs and in the axilla, groin, interdigital skin, and nostril of allergic dogs. A large scale DNA sequencing system (454 pyrosequencing) was used to sequence the bacterial 16S rRNA gene from skin swabs.

The sequence analysis revealed high individual variability between all samples collected from the different skin regions and between different dogs. Higher number of bacterial species (bacterial diversity) were observed on the haired skin (axilla, groin, periocular, pinna, dorsal nose, interdigital, lumbar) when

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

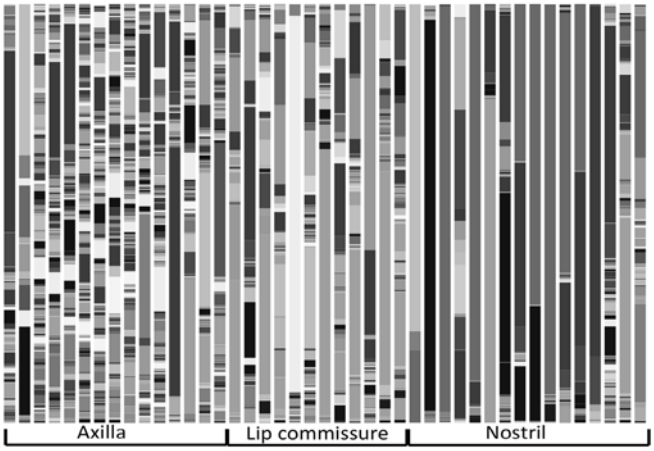


Figure 1. Representative taxa at the genus level showing the higher diversity in the samples from haired skin (axilla), followed by mucocutaneous junction (lip comissure), with the nasal mucosa having the lower diversity. Each color shade represents a different genus.[7]

Some of the most abundant phyla identified across all samples included Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes (Figure 2). The bacteria in the genus *Ralstonia* (phylum Proteobacteria, family *Oxalobacteraceae*) were significantly more abundant in the samples from the healthy dogs. The family *Moraxellaceae* was the most abundant in the nostril.

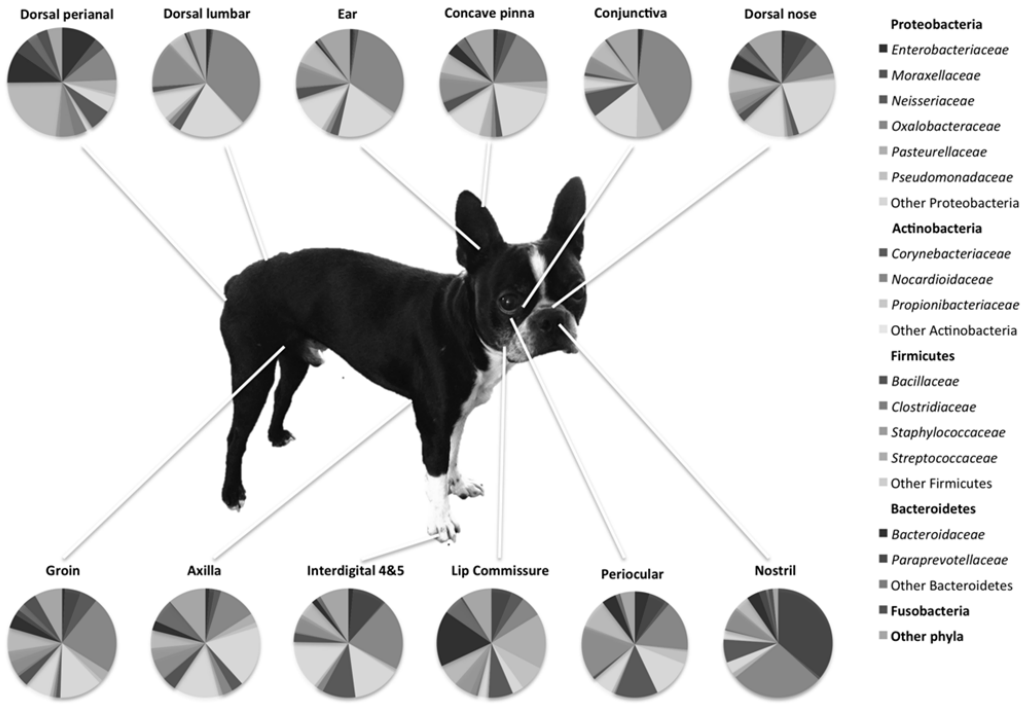


Figure 2. Average of most common bacterial phyla and families in the different skin regions in the healthy dogs. The group “Other phyla” includes 12 phyla that each were present in only low abundance. *PLOS one,* 2014: e83197.

## THE SKIN MICROBIOME IN ALLERGIC DOGS

Samples from the axilla, groin, interdigital skin and nose were collected from 6 dogs diagnosed with atopic dermatitis. None of the dogs had skin lesions at the time of collection or were being treated with antibiotics. The haired skin samples and nostril of dogs with allergic skin disease had lower number of observed bacterial species (median 125) and diversity when compared to the same skin sites of healthy dogs (median 239) (Figure 3).

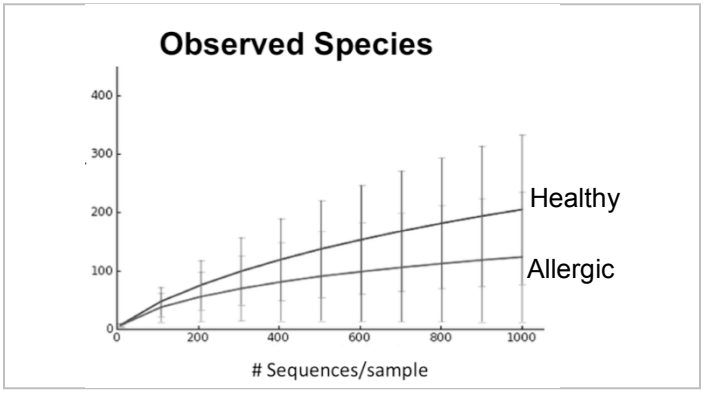


Figure 3. Number of observed bacterial species in pooled samples from the nostril, interdigital skin, axilla and groin from healthy versus allergic dogs. The top curved line represents the healthy dogs and the lower line represents the allergic dogs. The samples from the healthy dogs had higher number of observed species when compare to allergic dogs. *PLOS one*, 2014: e83197.

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

Similar to what was observed in this study, lower bacterial diversity has been previously described in the skin of children with atopic dermatitis (AD).[6] In AD children, reduction in microbial diversity, is followed by increased abundance of cutaneous *S. aureus* is described during skin flares. It is also described that increases in the abundance of *Staphylococcus* and reductions in microbial diversity precede an increase in the severity of AD. In AD children, antimicrobial or anti-inflammatory medications (hypochlorite baths) decreased, but did not eliminate, *S. aureus*. Interestingly, the changes in the microbial diversity during flares were reversed even before clinical improvement was seen. We have yet to evaluate the microbial diversity and composition of the skin following treatment with topical or systemic antimicrobial and anti-inflammatory drugs in dogs and other species.

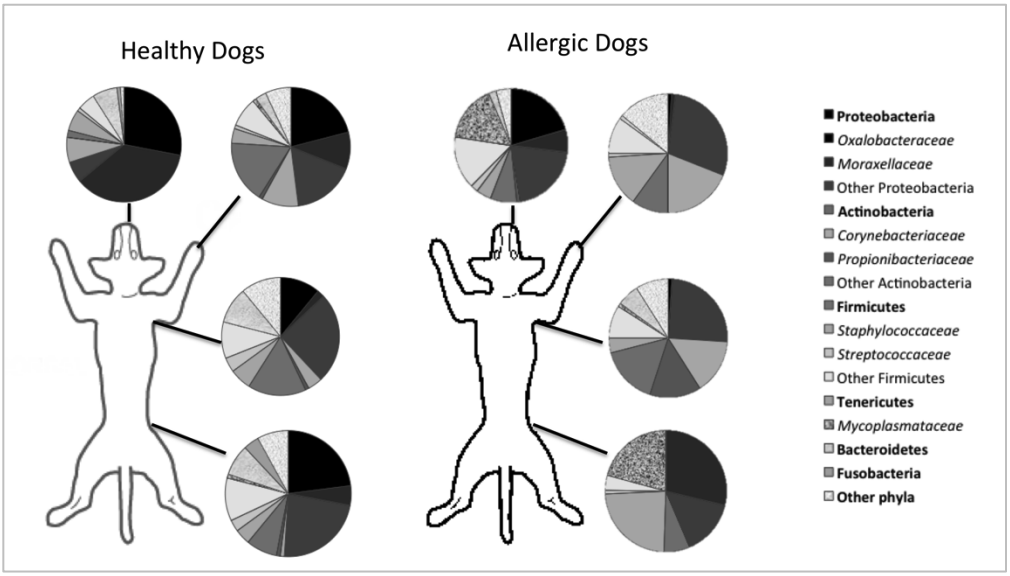


Figure 4. Average of most common bacterial phyla in the axilla, groin, and nostril of allergic versus healthy dogs. The group "Other phyla" includes 12 phyla that each were present in only low abundance. On average, the nostril and groin of allergic dogs were colonized with higher proportions of bacteria in the family *Staphylococcaceae*, and lower proportions of bacteria in the family *Oxalobacteriacea*e. Higher proportions of bacteria in the family *Corynebateriaceae* were also observed in the groin and interdigital region in allergic dogs.[7]

**FUTURE DIRECTIONS**

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

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# NOTES

# POSTER COMMUNICATIONS

**NORTH AMERICAN VETERINARY DERMATOLOGY FORUM**
**Phoenix, AZ**

## ACVD POSTER COMMUNICATIONS

| | |
|---|---|
| Collard | PK/PD of oclacitinib (Apoquel®) in an IL-31 induced model of pruritus in the dog |
| Craft | Feline Plasma Cell Pododermatitis in Littermates |
| Lam | Evaluation of agreement between intradermal and macELISA serum allergy test results in 47 cases |
| Layne | Impression smear compared to acetate tape preparation for cytologic sampling |
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# PK/PD of oclacitinib (Apoquel®) in an IL-31 induced model of pruritus in the dog

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**Abstract:** Interleukin-31 is a pruritogenic cytokine that involves JAK-STAT activation and has been implicated in the pathogenesis of atopic dermatitis in dogs. The objective of this study was to determine the pharmacokinetic-pharmacodynamic (PK/PD) relationship of oclacitinib (Apoquel®, Zoetis Inc., Florham Park, NJ), a selective JAK-1 inhibitor, in an IL-31 induced model of pruritus in healthy beagle dogs. Multiple groups were administered single oral 0.4 mg/kg oclacintib dose. At intervals over a 24 h period following the oclacitinib dose, dogs were administered an intravenous dose of canine rIL-31 to induce pruritus. The minutes of pruritic activity were determined over a 2 h period and expressed as a percentage of the maximum possible. Blood samples were collected and oclacitinib concentration determined by LC/MS/MS. PK/PD modeling was performed with WinNonlin. The observed PD response (% time with pruritus) closely mirrored oclacitinib pharmacokinetics from 0–24 h in this model. Maximum response was observed at 2–4 h for both PK and PD and there was no detectable lag in the PD response. A direct effect sigmoid Emax model best described the PK/PD relationship. This model predicted a maximum pruritic response (Emax) of 44.6% and a plasma concentration required to produce a 50% reduction in the maximum pruritic response (EC50) of 12.1 ng/mL. The EC50 observed in this model was similar to a previously determined IC50 in a cellular IL-31 functional assay. This study demonstrated the reduction in IL-31 induced pruritus by oclacitinib occurred rapidly after dosing and closely mirrored the PK of oclacitinib.

Sources of Funding: Self-funded

Conflict of Interest: Zoetis employees are Zoetis, Inc. shareholders

# Feline Plasma Cell Pododermatitis in Littermates

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**Abstract:** Two of four 4 year old indoor/outdoor domestic shorthair cat littermates living within the same household presented for a several week history of soft, swollen and discolored pawpads on all forefeet and hindfeet. The two affected neutered males occasionally exhibited mild lameness, but predominantly lacked clinical signs. The cats have lived in the same household since kittenhood. Physical exam abnormalities of the two affected cats included diffusely swollen and spongy metacarpal and metatarsal pads with fewer affected digital pads. All affected pads were dark purple-red and pad surfaces were coursed by many white, scaly, linear striae forming a cross-hatched pattern. Histopathologic findings in both cases included dense perivascular cuffs and diffuse infiltrates composed of myriad small to moderately-sized plasma cells mixed with low to moderate numbers of neutrophils and few mast cells, Mott cells and eosinophils. The cells formed sheets that extended from the superficial dermis through the deep dermis and often into the subcutis. The subcuticular infiltrate mildly to markedly expanded interlobular septa, effaced adipose tissue lobules, formed dense perivascular cuffs and was associated with mild to marked fibrosis. The histological interpretation was plasma cell pododermatitis. Laboratory testing for feline leukemia virus, feline immunodeficiency virus, and *Bartonella* were negative. Oral doxycycline was started at 5mg/Kg q12 hours with clinical improvement after 30 days. To the authors' knowledge, these are the first documented cases of plasma cell pododermatitis within related cats. At the time of submission, treatment was ongoing and a third littermate was developing similar pawpad lesions.

Source of Funding: self-funded

Conflict of Interest: none declared

**Evaluation of agreement between intradermal and macELISA serum allergy test results in 47 cases**

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**Abstract:** Intradermal allergy testing (IDAT) and monoclonal antibody cocktail-based ELISA (macELISA) serum allergy testing (SAT) are diagnostic methods used to identify allergens implicated in atopic skin disease for the design of patient-tailored immunotherapy. The study objective was to investigate the agreement between IDAT and SAT using Kappa (K), a measurement of relative concordance beyond the probability of chance. Forty-seven client-owned dogs, diagnosed with atopic dermatitis using Willemse's criteria by the Tufts Dermatology Service between November 2012 and November 2013, were included in the study. IDAT and SAT were performed on the same day. Thirty-four or 35 allergens were evaluated in each patient. General agreement between IDAT and SAT was considered poor. Six allergens in total showed fair to substantial agreement. Cedar/juniper, walnut, *Stemphylium* and *Mucor* had a K value falling between 0.2-0.4, consistent with fair agreement. Flea showed moderate agreement with a K value of 0.47. Substantial agreement was noted in dock/sorrel with a K value of 0.62. In 13/50 cases, IDAT results were negative whereas SAT results detected significant reactions to one or more allergens. Conversely, in 3/50 cases, SAT results were negative whereas significant reactions were noted on IDAT. Given that in 16/50 cases, performing a single test alone would have failed to identify any allergens, it may be justified to consider using both IDAT and SAT simultaneously to improve detection of potentially implicated allergens for formulating allergen-specific immunotherapy. Additional research is needed to understand the reasons and importance of the lack of agreement between IDAT and SAT.

This study was self-funded

Conflict of interest: none declared

# Impression smear compared to acetate tape preparation for cytologic sampling

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**Abstract:** Cytologic sampling techniques in veterinary dermatology are most commonly used to detect bacteria, yeast, and inflammatory cells for diagnosis and monitoring of treatment. Studies have examined slide evaluation techniques, ear swab cytology staining methods, and observer variations, however few studies compare common clinical sampling techniques.The objective of this study was to measure detection of micro-organisms and inflammatory cells by impression smear compared to two different acetate tape preparation staining methods. Twenty-eight lesions consistent with superficial pyoderma were sampled via impression smear and acetate tape preparation. Acetate tape preparations were either stained with modified Romanowksy stain solutions two and three or solution three alone. Impression smears were stained in the standard manner. One diplomate veterinary dermatologist and one general practitioner evaluated all slides. Bacteria, yeast, and neutrophils were evaluated using a quantitative scale [0-4]. The presence of melanin and overall ease of evaluation for each parameter was denoted with yes or no. When comparing quantitative scores impression smears demonstrated significantly more neutrophils, with a mean score of 2 [$p<0.05$]. Acetate tape preparations stained with solution three alone showed the most melanin [mean number of yes scores=2.4, $p<0.05$]. There was no significant difference between methods for detection of yeast or bacteria. Between each group there were significant differences in ease of evaluation with impression smears scoring more yes answers [mean=5, $p<0.05$] than acetate tape preparations for neutrophils. These results indicate detection of neutrophils may vary by sampling technique and evaluation ease of a slide is likely affected by sampling and staining method.

This study was self-funded.

Conflict of Interest: None declared.

**Apocrine cysts in cat ears (Cystomatosis) (12 cases) 2011-2013**

K. E. LOFT

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**Abstract:** Cystomatosis presents as characteristic bluish-black cystic lesions on the concave pinna, tragus region and the lower meatus. Literature reports cystomatosis to occur in middle aged to older cats with a tentative genetic predisposition in Persians and Himalayans. No known etiology is reported. Various nomenclatures have historically been used for cystomatosis, including apocrine cysts and ceruminous adenomas. This study aimed to report on 12 cats with cystomatosis that presented to the dermatology services for various degrees of secondary otitis (23 ears affected); ten cases were confirmed by histopathology (19 ears). Cystomatosis was noted in 0.06% of the general feline hospital population (n=21337). Affected breeds included Domestic Shorthair (DSH) (5 cats; 42%), Domestic Longhair (DLH) (3 cats; 25%), Domestic Medium hair (DMH) (2 cats; 17%), and Himalayan (1 cat; 8%). The average age at diagnosis was 9.6 years (range: 4.0 to 15.5 years). Cystomatosis was found in nine spayed females (75%), two intact males (17%) and one castrated male (8%). Cystomatosis was not noted on any other body areas and was not associated with other disorders, except for otitis externa. Three of the cases (4 ears) progressed from benign cysts to inflamed adenocarcinoma (n=3) or squamous carcinoma (n=1) identified on subsequent histopathology. Tissue from 16 ears was negative for PCR papillomavirus DNA. In this study, cystomatosis was found to be more prevalent in female spayed cats and in non-pure breed cats and does not appear to be associated with papilloma virus. Further studies are needed to investigate the etiology of cystomatosis.

Conflict of interest: None declared

Source of funding: None declared

# Field effectiveness and safety of an otic suspension (Easotic®) for treatment of otitis externa in dogs

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**Abstract:** Otitis externa (OE) is a common condition in dogs and is frequently caused by *Staphylococcus pseudintermedius* and/or *Malassezia pachydermatis*. This multi-centered, double-blinded, placebo-controlled and randomized clinical field trial determined the effectiveness and safety of an otic suspension containing hydrocortisone aceponate, miconazole nitrate and gentamicin sulfate (Easotic®, Virbac, Fort Worth, USA) when administered at 1ml per ear daily for 5 days. Dogs enrolled in the study had been clinically diagnosed, following cytological and microbiological examinations, with OE caused by bacteria and/or fungal pathogens normally susceptible to gentamicin and/or miconazole. The severity of their condition was assessed by rating each of the signs - malodour, discharge, pruritus, erythema, swelling, and pain, on a 0-3 scale. The dogs were evaluated with clinical and cytological examinations during 4 clinical visits over the course of 30 days. The criteria for treatment success were a clinical score of 2 or less at the fourth visit (V4), with no single parameter becoming worse at V4. Safety was determined for the 216 dogs having received at least one dose of a treatment. The incidence and severity of adverse events during the study were similar between the Easotic® treated group and the control treated group. The effectiveness analysis included 99 dogs in the Easotic® group and 51 dogs in the control group. Of the Easotic®-treated dogs, 72.3% were considered a treatment success at V4 (*versus* 23.7% in the control group, p=0.0198). Easotic® was safe and effective in treating otitis externa associated with *M. pachydermatis* and *S. pseudintermedius*.

Source of funding: Virbac.

Conflict of interest: All of the authors are employees of Virbac.

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