Dear Colleagues and Friends,

On behalf of the members of the Scientific and Local Organizing Committees, we would like to welcome you to this 31st European Veterinary Dermatology Congress 2019 in Liverpool, United Kingdom.

This year, as in recent ones, we have set up three concurrent sessions of increasing levels of complexity that, we hope, will satisfy attendees of all levels. In the “Practical Programme”, we invited our speakers to provide general practitioners with the latest information on clinical pharmacology, feline dermatology, exotics, dermatophytosis, skin biopsy approach, and ear surgery and management. In the “Advanced” and “Cutting Edge” Programmes, which were designed for specialists, we will offer “mini-symposium” sessions on the rapidly-evolving areas of cell biology, host-microbial interactions, and allergies and clinical immunology. In these, we have selected a comparative approach, having similar topics discussed by renowned academic medical and then veterinary dermatologists. An identical format was chosen for mast cell/eosinophilic skin manifestations and advances in the pathophysiology and treatment of itch. In addition, we will have a debate between clinical pharmacologists with a discussion on how to best utilize antimicrobials, understand the “myths” behind certain drugs and how to provide the best veterinary or medical standards of care.

We are delighted that we have been fortunate enough to attract world-renowned speakers in their respective areas to bring you the benefits of their experience and knowledge. It is needless to say that we will also have the traditional sessions: a Clinico-Pathological Conference, three Journal Clubs and the traditional short communications and posters. The ACC Liverpool on the former Kings Dock, opened in May 2008 within Liverpool’s event campus, will host us during our congress. The venue is a part of an interconnected arena, convention and exhibition centre, all positioned on the banks of Liverpool’s world heritage waterfront. Liverpool is a modern, vibrant city proud of its rich heritage, world trade roots, famous for its friendly people, and known worldwide for football and The Beatles! The venue is situated on a stunning waterside location in the heart of Liverpool’s city centre. There is an array of amenities within a 15 minute walk, including the Grade 1 listed Albert Dock (home to Tate Liverpool, the Beatles Story and a diverse range of cafes and restaurants), Liverpool ONE shopping centre and the terminal for the famous Mersey ferry. Furthermore, the venue is also very well served by circular bus routes, rail, underground and an extensive motorway network.

The Welcome Reception will take place on the 26th at the Liverpool Anglican Cathedral, designed by Sir Giles Gilbert Scott. It is the largest in the UK and the fifth largest in the world. This fascinating and unique building is a historic landmark famously described by Sir John Betjeman as “one of the greatest buildings in the world. The Dinner Dance evening is set to be at Revolución de Cuba: two stunning floors of rum bar and unrivalled cantina, right on the beautiful Liverpool Albert Dock. We are so thankful for the continuous support of our Long Term Partners (Bayer, DRN, ICF, Royal Canin and Zoetis) without whom it would be difficult to continue such a high quality meeting that promotes excellence in veterinary dermatology.

Welcome to Liverpool! We wish you a good congress.

Dr. Frane Banovic  
Chair of the Scientific Organizing Committee

Dr. Vanessa Schmidt  
Chair of the Local Organizing Committee
INDEX

COMMITTEES AND KEYNOTE SPEAKERS 06
SPONSORS AND EXHIBITORS 07
SCIENTIFIC PROGRAMME 08
PROGRAMME INDEX 14
SHORT COMMUNICATIONS INDEX 16
THURSDAY 26 SEPTEMBER 2019
PRACTICAL PROGRAMME 18
ADVANCED PROGRAMME 34
CUTTING EDGE PROGRAMME 44
FRIDAY 27 SEPTEMBER 2019
PRACTICAL PROGRAMME 46
ADVANCED PROGRAMME 60
SATURDAY 28 SEPTEMBER 2019
PRACTICAL PROGRAMME 70
ADVANCED PROGRAMME 88
CUTTING EDGE PROGRAMME 100
SHORT COMMUNICATIONS 112
AUTHORS INDEX 154
COMMITTEES

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KEYNOTE SPEAKERS
Nick Bacon Fitzpatrick Referrals Oncology and Soft Tissue, United Kingdom
Chiara Brachelente University of Perugia, Italy
Laura Buckley University of Liverpool, United Kingdom
Lluis Ferrer Universitat Autònoma de Barcelona, Spain
Joanna Hedley Royal Veterinary College, United Kingdom
Manfred Kietzmann University of Veterinary Medicine Hannover, Germany
Marcus Maurer Charité - Universitätsmedizin Berlin, Germany
Karen Moriello School of Veterinary Medicine, USA
Ralf Mueller Ludwig-Maximilians-University Munich, Germany
Thierry Olivry NC State University College of Veterinary Medicine, Raleigh, North Carolina, USA
Mark Papich NC State University College of Veterinary Medicine, Raleigh, North Carolina, USA
Aline Rodrigues Hoffmann Texas A&M University, USA
Vanessa Schmidt University of Liverpool, United Kingdom
Gil Yosipovitch University of Miami, USA
The Organizing Committee of the 31st European Veterinary Dermatology Congress, organized by ESVD/ECVD gratefully acknowledges contributions:

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PRACTICAL PROGRAMME | ROOM 11
WOUNDS & BURNS | Chair: Niksa Lemo
09:00 - 09:45 • Surgical planning for skin tumours - how to decide how much surgery we need?, N. Bacon (UK)
09:45 - 10:30 • Introduction to skin tumour surgery, N. Bacon (UK)

ADVANCED PROGRAMME | HALL 1A
SKIN BIOLOGY | Chair: Lucia Panakova
09:00 - 09:45 • Sterile pyogranulomatous dermatitis: update and future, A. Rodrigues Hoffmann (USA)
09:45 - 10:30 • Mast Cells: Can’t live with them - can’t live without them, M. Maurer (DE)

CUTTING EDGE PROGRAMME | HALL 1C
SHORT COMMUNICATIONS AND POSTERS | Chair: Kirsti Schildt
09:00 - 09:15 • The cutaneous and rectal microbiome of canine perianal fistulas and the effect of ciclosporin therapy, C.L. Cain
09:15 - 09:30 • Allergen-specific immunotherapy in dogs with atopic dermatitis: a comparison of subcutaneous, intralymphatic and sublingual administration, N. Fischer
09:30 - 09:45 • Two types of IgE in dog serum differ in glycosylation, A. Kumagai
09:45 - 10:00 • Characterization of the pro-inflammatory and pruritogenic transcriptome in experimental acute canine IgE-mediated skin lesions, A. Blubaugh
10:00 - 10:15 • Clinical Efficacy of Sublingual Allergen-Specific Immunotherapy in cats with nonflea nonfood-induced hypersensitivity dermatitis against mites, X. Hoj
10:15 - 10:30 • The potential of a recombinant anti-IgE mouse x dog chimeric antibody for treatment of canine IgE-mediated allergy, K. Masuda

10:30 - 11:15 BREAK

PRACTICAL PROGRAMME | ROOM 11
CLINICAL PHARMACOLOGY | Chair: Katarina Varjonen
11:15 - 12:00 • Principles of Pharmacokinetics for Specialists, M. Papich (USA)
12:00 - 12:45 • Clinically-Relevant Drug Interactions in Dermatology, M. Papich (USA)

ADVANCED PROGRAMME | HALL 1A
SKIN BIOLOGY | Chair: Jevgenija Kondratjeva
11:15 - 12:00 • Mast cell-driven diseases: Where do we stand? Where do we go? (), M. Maurer (DE)
12:00 - 12:45 • Mast cell-driven diseases: Where do we stand? Where do we go? (), M. Maurer (DE)

CUTTING EDGE PROGRAMME | HALL 1C
SHORT COMMUNICATIONS AND POSTERS | Chair: Monika Linek
11:15 - 11:30 • Natural history of atopic dermatitis (AD) in a cohort of West-Highland-White Terriers (WHWT), C. Favrot
11:30 - 11:45 • Early-life risk factors and heritability of canine atopic dermatitis: a birth cohort study from West Highland White Terriers, A. Rostaber
11:45 - 12:00 • An alternative to long-lasting elimination diet to diagnose food allergies in dogs with atopic dermatitis, C. Favrot
12:00 - 12:15 • Efficacy and safety of concurrent therapy of oclacitinib and 0.0584% hydrocortisone aceponate spray (Cortavance®) for control of atopic dermatitis in client-owned dogs: A randomized, double-blinded placebo-controlled study, J. Takahashi
12:15 - 12:30 • Comparison of the skin and ear canal microbiota of allergic and healthy German shepherd dogs, using next generation sequencing, N. Apostolopoulos
12:30 - 12:45 • Efficacy of allergen-specific immunotherapy in dogs with atopic dermatitis: a retrospective study of 145 cases, L. Ramio-Lluch
PRACTICAL PROGRAMME | ROOM 11
EXOTIC DERMATOLOGY | Chair: Ariane Neuber
15:00 - 15:45  Avian and reptile dermatology for general practitioners, J. Hedley (UK)
15:45 - 16:30  Small mammal dermatology for general practitioners, J. Hedley (UK)

ADVANCED PROGRAMME | HALL 1A
DERMATOPHYTOSIS | Chair: Claudia Nett
15:00 - 15:45  Evidence-based medicine for treatment and debunking ringworm folklore: Part I, K. Moriello (USA)
15:45 - 16:30  Evidence-based medicine for treatment and debunking ringworm folklore: Part II, K. Moriello (USA)

CUTTING EDGE PROGRAMME | HALL 1C
MINI SYMPOSIUM: HOST-MICROBIAL INTERACTION | Chair: Jacques Fontaine
15:00 - 15:45  Skin microbiome methods: from design to analysis, A. Rodrigues Hoffmann (USA)

PRACTICAL PROGRAMME | ROOM 11
CLINICAL PHARMACOLOGY | Chair: Anke Hendricks
17:15 - 18:00  Topical therapy in dermatologic diseases, M. Kietzmann (DE)

ADVANCED PROGRAMME | HALL 1A
JOURNAL CLUB | Chair: Sandrine Herbelet
17:15 - 18:00  Journal Club 1, R. Mueller (DE)

SHORT COMMUNICATIONS | HALL 1C
SHORT COMMUNICATIONS AND POSTERS | Chair: Kirsti Schildt
17:15 - 17:20  One Health approach to methicillin-resistance between Staphylococcus isolates from companion dogs affected with pyoderma and owners, J.H. Kang
17:20 - 17:25  Efficacy of fluralaner spot-on in cats affected by generalized demodicosis: 7 cases, M.B. Beccati
17:25 - 17:30  Chemotherapy induced palmar-plantar erythrodysesthesia (PPES) in a dog treated with liposome-encapsulated doxorubicin, C.G. Chibaudo
17:30 - 17:35  Cutaneous bullous mastocytosis in a Yorkshire terrier puppy, A. Petak
17:35 - 17:40  Wheal size after intradermal injection of histamine and saline in skin treated with local anesthetic in comparison with non treated skin, A.I. Cózar
17:40 - 17:45  Compassionate use of Allermune immunotherapy in a cat with DF associated skin and respiratory hypersensitivity, F. Martini
17:45 - 17:50  Analysis of correlation between allergen-specific antibodies and clinical symptoms by subcutaneous immunotherapy (SCIT) using recombinant Dermatophagoides farinae 2 conjugated pullulan (rDf2-p) on canine atopic dermatitis (CAD), K. Ueda
17:50 - 17:55  Determination of the synergistic, antagonistic or indifferent in vitro effect between an ear cleaner and four antibiotics against bacterial strains isolated from canine otitis, D. Fantini
17:55 - 18:00  Effect of afoxolener for the treatment of lice in zoo birds: Pavo cristatus, Ortalis vetula, and Phasianus colchicus, L. Komero
SCIENTIFIC PROGRAMME
FRIDAY 27 SEPTEMBER 2019

PRACTICAL PROGRAMME | HALL 1A
DERMATOPHYTOSIS | Anke Hendricks

08:30 - 09:15  • Phone call horrors: “Doctor I think I have an outbreak of ringworm” Steps anyone can do to help, K. Moriello (USA)
09:15 - 10:00  • Practical approach to fungal diagnostics, K. Moriello (USA)

ADVANCED PROGRAMME | ROOM 11
HOST-MICROBIAL INTERACTION | Chair: Jacques Fontaine

08:30 - 09:15  • Update on feline skin microbiome and disease association, A. Rodrigues Hoffmann (USA)
09:15 - 10:00  • Fungal microbiome in dogs and cats, A. Rodrigues Hoffmann (USA)

CUTTING EDGE PROGRAMME | HALL 1C
SHORT COMMUNICATIONS AND POSTERS | Chair: Pierre-Antoine Germain

08:30 - 08:45  • Dermis and subcutis of healthy dogs lack of a bacterial microbiota, R. Garcia-Fonticoba
08:45 - 09:00  • Transcriptome profiling of canine chronic cutaneous lupus erythematosus skin lesions using deep RNA sequencing, F. Banovic
09:00 - 09:05  • Is the canine dermatitis quality of life and treatment satisfaction questionnaire (CDQOL-TSQ) sensitive to differences in disease severity? A.K. Wright
09:05 - 09:10  • Medical honey for canine nasal intertrigo: how sweet is the placebo effect? G. Brosseau
09:15 - 09:30  • Understanding transcriptional connections of chronic cutaneous lupus erythematosus between humans and animal models, F. Banovic
09:30 - 09:45  • The pharmacokinetics of oclacitinib maleate in the cat, L. Ferrer
09:45 - 10:00  • Anticancer effects of betulinic acid derivative NVX-207 on equine melanoma cells and percutaneous permeation through isolated equine skin in vitro, L.A. Weber

10:00 - 10:45 B<i>REAK</i>

PRACTICAL PROGRAMME | ROOM 11
EAR SURGERY | Chair: Niksa Lemo

10:45 - 11:30  • Surgery for ear disease (I) in dogs and cats, N. Bacon (UK)
11:30 - 12:15  • Surgery for ear disease (II) in dogs and cats, N. Bacon (UK)

ADVANCED PROGRAMME | HALL 1A
CLINICAL PHARMACOLOGY | Chair: Monika Linek

10:45 - 11:30  • Usage of Antibiotics in Small Animal Dermatology. A Discussion: Part 1, M. Papich (USA) & M. Kietzmann (DE)
11:30 - 12:15  • Usage of Antibiotics in Small Animal Dermatology. A Discussion: Part 2, M. Papich (USA) & M. Kietzmann (DE)

CUTTING EDGE PROGRAMME | HALL 1C
SHORT COMMUNICATIONS AND POSTERS | Chair: Pierre-Antoine Germain

10:45 - 11:00  • Effect of phenol and formalin on mecA in methicillin-resistant Staphylococcus pseudintermedius (MRSP) as part of autogenous bacterin formulation, A. Loeffler
11:00 - 11:15  • Effects of Nano-sulfur against multi-drug resistant Staphylococcus pseudintermedius: an antimicrobial, anti-biofilm and cytotoxicity study, J. Santoro
11:15 - 11:30  • Ear canal microbiota and mycobiota - effect of preventive use of a topical steroid anti-inflammatory in atopic dog without any sign of active otitis, C.L. Leonard
11:30 - 11:45  • Longitudinal characterization of the anal sac microbiota in dogs with unilateral anal sacculitis treated with infusions of an antibiotic-steroid-antifungal suspension, C.C. Bergeron
11:45 - 12:00  • Prevalence of multidrug resistant Staphylococcus pseudintermedius and Staphylococcus aureus in dogs and people: a case control study, D. Santoro
12:00 - 12:15 • Testing the validity of the canine dermatitis quality of life and treatment satisfaction questionnaire (CDQOL-TSQ) through correlations with other measures, C. Noli

12:15 - 14:15 LUNCH
13:00 - 14:15 ESVD AGM | HALL 1C

PRACTICAL PROGRAMME | HALL 1A
GENERAL | Chair: Katarina Varjonen
14:15 - 15:00 • Cytology: the theory of everything, V. Schmidt (UK)

ADVANCED PROGRAMME | ROOM 11
SKIN BIOLOGY | Chair: Monika Linek
14:15 - 15:00 • Sterile neutrophilic dermatoses in dogs, C. Brachelente (IT)

CUTTING EDGE PROGRAMME | HALL 1C
SHORT COMMUNICATIONS AND POSTERS | Chair: Georg Lehner
14:15 - 14:30 • Major differences between canine atopic dermatitis and acute atopic models of house dust mite- and IgE-induced skin lesions determined by comparative global transcriptomic profiling, A. Blubaugh
14:30 - 14:45 • A prospective, randomized, double blind, placebo-controlled evaluation of the effects of an n-3 essential fatty acid supplementation (Agepi ω3®) on the clinical signs, and erythrocyte membrane, hair shafts and skin surface polyunsaturated fatty acid concentrations in dogs with poor coat quality, D. Combarros
14:45 - 15:00 • Evaluation of IL-17 and IL-22 positive cells in infected and noninfected chronic atopic skin, D. Santoro

15:00 - 15:45 BREAK

PRACTICAL PROGRAMME | HALL 1A
CLINICAL PHARMACOLOGY | Chair: Anke Hendricks
15:45 - 16:30 • Interpretation of culture and susceptibility testing, M. Papich (USA)

ADVANCED PROGRAMME | ROOM 11
CLINICOPATHOLOGICAL CONFERENCE | Chair: Monika Linek
15:45 - 16:30 • Clinicopathological conference, C. Brachelente (IT) & L. Buckley (UK)

CUTTING EDGE PROGRAMME | HALL 1C
SHORT COMMUNICATIONS AND POSTERS | Chair: Silvia Colombo
15:45 - 16:00 • Evaluation of an ear cleaner based on natural ingredients with antimicrobial, antibiofilm and anti-inflammatory properties in rabbits with otitis externa, C. Steinberg
16:00 - 16:15 • Evaluation of the cutaneous immunological milieu in dogs naturally affected by Leishmania infantum/chagasi: a preliminary study, D. Santoro
16:15 - 16:30 • Characterization of the pruritus responses and pruritic behaviors in an interleukin 31-induced canine model of pruritus, A. Blubaugh
SCIENTIFIC PROGRAMME
SATURDAY 28 SEPTEMBER 2019

PRACTICAL PROGRAMME | HALL 1C
GENERAL | Chair: Elisa Maina
09:00 - 09:45 • Feline allergy syndrom, L. Buckley (UK)
09:45 - 10:30 • Keep calm and carry on: dermatological emergencies, V. Schmidt (UK)

ADVANCED PROGRAMME | HALL 1A
CLINICAL PHARMACOLOGY | Chair: Ralf Mueller
09:00 - 09:45 • Immunosuppressive therapy in dogs and cats: selected topics from pharmacologist’s perspective (I), M. Kietzmann (DE)
09:45 - 10:30 • Immunosuppressive therapy in dogs and cats: selected topics from pharmacologist’s perspective (II), M. Kietzmann (DE)

CUTTING EDGE PROGRAMME | ROOM 11
MINI SYMPOSIUM ON ALLERGY AND CLINICAL IMMUNOLOGY | Chair: Thierry Olivry
09:00 - 09:45 • Neurobiology and mediators of itch, G. Yosipovitch (USA)
09:45 - 10:30 • Categories of itch and the new insights into the pathophysiology, G. Yosipovitch (USA)

10:30 - 11:15 BREAK

PRACTICAL PROGRAMME | HALL 1C
SKIN SURGERY | Chair: Niksa Lemo
11:15 - 12:00 • Reconstruction of large skin defects after tumour excision, N. Bacon (UK)
12:00 - 12:45 • Surgical to treat tumours of the digits and feet, N. Bacon (UK)

ADVANCED PROGRAMME | HALL 1A
GENERAL | Chair: Lucia Panakova
11:15 - 12:00 • Mast cells and eosinophilic skin diseases in dogs, cats and horses I, C. Brachelente (IT)
12:00 - 12:45 • Mast cells and eosinophilic skin diseases in dogs, cats and horses II, C. Brachelente (IT)

CUTTING EDGE PROGRAMME | ROOM 11
MINI SYMPOSIUM ON ALLERGY AND CLINICAL IMMUNOLOGY | Chair: Thierry Olivry
11:15 - 12:00 • Current topical and systemic therapies for itch, G. Yosipovitch (USA)
12:00 - 12:45 • Advances in understanding itching and scratching: a new era of targeted treatments, G. Yosipovitch (USA)

12:45 - 14:00 LUNCH
13:15 - 14:15 BVDSG AGM | HALL 1C

14:15 - 14:30 DECHA ABSTRACT AWARDS

PRACTICAL PROGRAMME | HALL 1C
GENERAL | Chair: Stefano Borio
14:30 - 15:15 • Help the pathologist to help you, C. Brachelente (IT)
15:15 - 16:00 • Management of otitis: what are good practice recommendations?, L. Buckley (UK)
ADVANCED PROGRAMME | HALL 1A
CLINICAL PHARMACOLOGY | Chair: Jevgenija Kondratjeva
14:30 - 15:15  • Strategies to Manage Antibiotic-Resistant Infections, M. Papich (USA)
15:15 - 16:00  • Pharmacokinetics of veterinary drugs with transdermal delivery, M. Kietzmann (DE)

CUTTING EDGE PROGRAMME | ROOM 11
MINI SYMPOSIUM ON ALLERGY AND CLINICAL IMMUNOLOGY | Chair: Ralf Mueller
14:30 - 15:15  • Comparative aspects of itch in companion animals and humans: pathogenesis, T. Olivry (USA)
15:15 - 16:00  • Comparative aspects of itch in companion animals and humans: treatment, T. Olivry (USA)

16:00 - 16:45 BREAK

PRACTICAL PROGRAMME | HALL 1A
ALOPECIA | Chair: Silvia Colombo
16:45 - 17:30  • 40 minute essential guide to hormonal hair loss, V. Schmidt (UK)
17:30 - 18:15  • Hair’s gone, normal bloods, now what?, V. Schmidt (UK)

ADVANCED PROGRAMME | ROOM 11
JOURNAL CLUB | Chair: Luc Beco
16:45 - 17:30  • Journal Club 2, L. Ferrer (ES)
17:30 - 18:15  • Journal Club 3, L. Ferrer (ES)
# PROGRAMME INDEX

## THURSDAY 26 SEPTEMBER 2019

### PRACTICAL PROGRAMME

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Surgical planning for skin tumours - how to decide how much surgery we need?</td>
<td>N. Bacon (UK)</td>
</tr>
<tr>
<td>21</td>
<td>Introduction to skin tumour surgery</td>
<td>N. Bacon</td>
</tr>
<tr>
<td>24</td>
<td>Principles of Pharmacokinetics for Specialists</td>
<td>M. Papich</td>
</tr>
<tr>
<td>26</td>
<td>Clinically-Relevant Drug Interactions in Dermatology</td>
<td>M. Papich</td>
</tr>
<tr>
<td>28</td>
<td>Avian and reptile dermatology for general practitioners</td>
<td>J. Hedley</td>
</tr>
<tr>
<td>30</td>
<td>Small mammal dermatology for general practitioners</td>
<td>J. Hedley</td>
</tr>
<tr>
<td>32</td>
<td>Topical therapy in dermatologic diseases</td>
<td>M. Kietzmann</td>
</tr>
</tbody>
</table>

### ADVANCED PROGRAMME

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Sterile pyogranulomatous dermatitis: update and future</td>
<td>A. Rodrigues Hoffmann</td>
</tr>
<tr>
<td>36</td>
<td>Mast Cells: Can’t live with them - can’t live without them</td>
<td>M. Maurer</td>
</tr>
<tr>
<td>38</td>
<td>Mast cell-driven diseases: Where do we stand? Where do we go? (I)</td>
<td>M. Maurer</td>
</tr>
<tr>
<td>38</td>
<td>Mast cell-driven diseases: Where do we stand? Where do we go? (II)</td>
<td>M. Maurer</td>
</tr>
<tr>
<td>40</td>
<td>Evidence-based medicine for treatment and debunking ringworm folklore: Part I</td>
<td>K. Moriello</td>
</tr>
<tr>
<td>40</td>
<td>Evidence-based medicine for treatment and debunking ringworm folklore: Part II</td>
<td>K. Moriello</td>
</tr>
<tr>
<td>42</td>
<td>Journal Club 1</td>
<td>R. Mueller</td>
</tr>
</tbody>
</table>

### CUTTING EDGE PROGRAMME

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>Skin microbiome methods: from design to analysis</td>
<td>A. Rodrigues Hoffmann</td>
</tr>
</tbody>
</table>

## FRIDAY 27 SEPTEMBER 2019

### PRACTICAL PROGRAMME

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>Phone call horrors: “Doctor I think I have an outbreak of ringworm” Steps anyone can do to help</td>
<td>K. Moriello</td>
</tr>
<tr>
<td>48</td>
<td>Practical approach to fungal diagnostics</td>
<td>K. Moriello</td>
</tr>
<tr>
<td>50</td>
<td>Surgery for ear disease (I) in dogs and cats</td>
<td>N. Bacon</td>
</tr>
<tr>
<td>53</td>
<td>Surgery for ear disease (II) in dogs and cats</td>
<td>N. Bacon</td>
</tr>
<tr>
<td>55</td>
<td>Cytology: the theory of everything</td>
<td>V. Schmidt</td>
</tr>
<tr>
<td>58</td>
<td>Interpretation of Culture and Susceptibility Testing</td>
<td>M. Papich</td>
</tr>
</tbody>
</table>

### ADVANCED PROGRAMME

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Update on feline skin microbiome and disease association</td>
<td>A. Rodrigues Hoffmann</td>
</tr>
<tr>
<td>62</td>
<td>Fungal microbiome in dogs and cats</td>
<td>A. Rodrigues Hoffmann</td>
</tr>
<tr>
<td>64</td>
<td>Usage of Antibiotics in Small Animal Dermatology. A Discussion: Part 1</td>
<td>M. Papich &amp; M. Kietzmann</td>
</tr>
<tr>
<td>64</td>
<td>Usage of Antibiotics in Small Animal Dermatology. A Discussion: Part 2</td>
<td>M. Papich &amp; M. Kietzmann</td>
</tr>
<tr>
<td>66</td>
<td>Sterile neutrophil dermatoses in dogs</td>
<td>C. Brachelente</td>
</tr>
<tr>
<td>68</td>
<td>Clinicopathological conference</td>
<td>C. Brachelente &amp; L. Buckley</td>
</tr>
</tbody>
</table>
SATURDAY 28 SEPTEMBER 2019

PRACTICAL PROGRAMME
70  • Feline allergy syndrom, L. Buckley
72  • Keep calm and carry on: dermatological emergencies, V. Schmidt
74  • Reconstruction of large skin defects after tumour excision, N. Bacon
77  • Surgical to treat tumours of the digits and feet, V. Bacon
79  • Help the pathologist to help you, C. Brachelente
81  • Management of otitis: what are good practice recommendations?, L. Buckley
83  • 40 minute essential guide to hormonal hair loss, V. Schmidt
86  • Hair’s gone, normal bloods, now what?, V. Schmidt

ADVANCED PROGRAMME
88  • Immunosuppressive therapy in dogs and cats: selected topics from pharmacologist’s perspective (I), M. Kietzmann
88  • Immunosuppressive therapy in dogs and cats: selected topics from pharmacologist’s perspective (II), M. Kietzmann
90  • Mast cells and eosinophilic skin diseases in dogs, cats and horses I, C. Brachelente
90  • Mast cells and eosinophilic skin diseases in dogs, cats and horses II, C. Brachelente
92  • Strategies to Manage Antibiotic-Resistant Infections, M. Papich
94  • Pharmacokinetics of veterinary drugs with transdermal delivery, M. Kietzmann
96  • Journal Club 2, L. Ferrer
98  • Journal Club 3, L. Ferrer

CUTTING EDGE PROGRAMME
100 • Neurobiology and mediators of itch, G. Yosipovitch
102 • Categories of itch and the new insights into the pathophysiology, G. Yosipovitch
104 • Current topical and systemic therapies for itch, G. Yosipovitch
106 • Advances in understanding itching and scratching: a new era of targeted treatments, G. Yosipovitch
108 • Comparative aspects of itch in companion animals and humans: pathogenesis, T. Olivry
110 • Comparative aspects of itch in companion animals and humans: treatment, T. Olivry
112 Comparison of the skin and ear canal microbiota of allergic and healthy German shepherd dogs, using next generation sequencing, N. Apostolopoulos

113 Transcriptome profiling of canine chronic cutaneous lupus erythematosus skin lesions using deep RNA sequencing, F. Banovic

114 The comparison of skin lesion transcriptomes between human and animal models of chronic cutaneous lupus erythematosus, F. Banovic

115 Efficacy of fluralaner spot-on in cats affected by generalized demodicosis: 7 cases, M.B. Beccati

116 Longitudinal characterization of the anal sac microbiota in dogs with unilateral anal sacculitis treated with infusions of an antibiotic-steroid-antifungal suspension: a pilot study, C.C. Bergeron

117 Characterization of the pruritus responses and pruritic behaviors in an interleukin 31-induced canine model of pruritus, A. Blubaugh

118 Characterization of the pro-inflammatory and pruritogenic transcriptome in experimental acute canine IgE-mediated skin lesions, A. Blubaugh

119 Major differences between canine atopic dermatitis and acute atopic models of house dust mite- and IgE-induced skin lesions determined by comparative global transcriptomic profiling, A. Blubaugh

120 Dermatophytes isolated from dogs and cats in France and their evolution in France from 2010 to 2018, P.J. Bourdeau

121 Medical honey for canine nasal intertrigo: how sweet is the placebo effect?, G. Brosseau

122 The cutaneous and rectal microbiome of canine perianal fistulas and the effect of ciclosporin therapy, C.L. Cain

123 A prospective, randomized, double blind, placebo-controlled evaluation of the effects of an n-3 essential fatty acid supplementation (Agepi ω3®) on the clinical signs, and erythrocyte membrane, hair shafts and skin surface polyunsaturated fatty acid concentrations in dogs with poor coat quality, D. Combarros

124 Wheal size after intradermal injection of histamine and saline in skin treated with local anesthetic in comparison with non treated skin, A.I. Cózar

125 Determination of the synergistic, antagonistic or indifferent in vitro effect between an ear cleaner and four antibiotics against bacterial strains isolated from canine otitis, O. Fantini

126 An alternative to long-lasting elimination diet to diagnose food allergies in dogs with atopic dermatitis, C. Favrot

127 Natural history of atopic dermatitis in a cohort of West Highland white terriers (WHWT), C. Favrot

128 The pharmacokinetics of oclacitinib maleate in the cat, L. Ferrer

129 Allergen-specific immunotherapy in dogs with atopic dermatitis: a comparison of subcutaneous, intralymphatic and sublingual administration, N.M. Fischer

130 Clinical efficacy of sublingual allergen-specific immunotherapy in cats with nonflea nonfood-induced hypersensitivity dermatitis against mites, R. Foj

131 Dermis and subcutis of healthy dogs lack of a bacterial microbiota, R. Garcia-Fonticoba

132 Chemotherapy-induced palmar-plantar erythrodysesthesia (PPES) in a dog treated with liposome-encapsulated doxorubicin, G.G. Chibaudo

133 First reported case of glomus tumor (glomus tympanicum) in an English setter dog treated with a diode laser in otoendoscopy, G.G. Chibaudo

134 Evaluation of IL-17 and IL-22 positive cells in lesional and non-lesional spontaneous atopic skin, J.B. Gillen
Evaluation of the cutaneous immunological milieu in dogs naturally affected by Leishmania infantum/chagasi: a preliminary study, A. Hernandez-Bures

One Health approach to methicillin-resistance between Staphylococcus isolates from companion dogs affected with pyoderma and owners, J.H. Kang

Two types of IgE in dog serum differ in glycosylation, A. Kumagai

Effect of the preventive use of a glucocorticoid anti-inflammatory topical formulation on the ear canal microbiota and mycobiota in atopic dogs without signs of otitis externa, C. Leonard

Effect of phenol and formalin on mecA in methicillin-resistant Staphylococcus pseudintermedius (MRSP) as part of autogenous bacterin formulation, A. Loeffler

Compassionate use of Allermune immunotherapy in a cat with mite associated skin and respiratory hypersensitivity, F. Martini

The potential of a recombinant anti-IgE mouse x dog chimeric antibody for treatment of canine IgE-mediated allergy, K. Masuda

Testing the validity of the Canine Dermatitis Quality of Life and Treatment Satisfaction Questionnaire (CDQOL-TSQ) through correlations with other measures, C. Noli

Cutaneous bullous mastocytosis in a Yorkshire terrier puppy, A. Petak

Efficacy of allergen-specific immunotherapy in dogs with atopic dermatitis: a retrospective study of 145 cases, L. Ramió-Lluch

Effect of afoxolaner for the treatment of lice in the zoo birds Pavo cristatus, Ortalis vetul, and Phasianus colchicus, C. Romero

Early-life risk factors and heritability of canine atopic dermatitis: a birth cohort study from West Highland White Terriers, A. Rostaher

Prevalence of multidrug-resistant Staphylococcus pseudintermedius and Staphylococcus aureus in dogs and people: a comparative dermatology case-control study, D. Santoro

Effects of nanosulfur against multi-drug resistant Staphylococcus pseudintermedius: an antimicrobial, anti-biofilm and cytotoxicity study, D. Santoro

Evaluation of an ear cleaner based on natural ingredients with antimicrobial, antibiofilm and anti-inflammatory properties in rabbits with otitis externa, G. Sheinberg

Efficacy and safety of a 0.0584% hydrocortisone aceponate spray to reduce flares of canine atopic dermatitis when tapering oclacitinib: a randomized, double-blinded, placebo-controlled trial, J. Takahashi

An analysis of the correlation between allergen-specific antibodies and clinical signs after subcutaneous immunotherapy with recombinant pullulan-conjugated Der f 2 in dogs with atopic dermatitis, K. Ueda

Anticancer effects of betulinic acid derivative NVX-207 on equine melanoma cells and percutaneous permeation through isolated equine skin in vitro, L.A. Weber

Is the Canine Dermatitis Quality of Life and Treatment Satisfaction Questionnaire (CDQOL-TSQ) sensitive to differences in disease severity?, A.K. Wright
Surgical planning for skin tumours - how to decide how much surgery we need?

N. J. BACON*†
* Fitzpatrick Referrals Oncology and Soft Tissue, Guildford, Surrey, UK
† University of Surrey School of Veterinary Medicine, Guildford, Surrey, UK

Surgical margins are defined by the tissue plane through which dissection and excision is done, and importantly the actual (or perceived) residual neoplastic disease in the wound bed. Classification of the margin achieved around the mass will help with judging the effectiveness of a surgical procedure in achieving local control of a tumour. Classification makes comparisons between different techniques possible, and rapidly communicates the intent of the surgery. Our understanding of margins is based largely on Enneking’s pioneering work in humans, whereby he classified them as intracapsular (intracapsular), marginal, wide or radical. An intracapsular margin is achieved by piecemeal removal (‘debulking’) of a lesion from within the capsule. This is also used if the capsule is accidentally entered during dissection as the surgical field is now contaminated. Gross and/or microscopic disease remains. Examples include incisional biopsy, curettage of bone lesions, infiltrative lipomas, and some hepatic masses. A marginal margin is achieved by an extracapsular dissection through the reactive zone around the mass. Both benign and malignant lesions may have extracapsular microextensions of disease, microsatellites (e.g. mast cell disease), and ‘skip’ metastases of high-grade lesions (e.g. soft tissue sarcomas). Classically these are termed ‘shell-outs’ and involve peeling the mass out from its tissue bed and off local attachments. A wide margin is achieved by en bloc removal of the lesion, its capsule and the surrounding reactive zone but always working in normal uncontaminated tissue within the compartment of the lesion. Non-neoplastic, non-reactive intracompartamental normal tissue is left at the margins and there is the possibility of ‘skip’ metastases arising in the remaining portion of the compartment (e.g. synovial cell sarcomas). A radical margin removes the lesion, reactive zone, and all the tissue of the associated compartment. There is no potential for residual neoplasm. The typical example is amputation, along with variants such as hemipelvectomy.

It is important to realize the surgery is defined by the margin it achieves, not the surgery itself, i.e. a hemipelvectomy can be both radical if extracompartamental, but also marginal if the reactive zone around the mass is entered. The margin is also defined by the least margin at any portion. A dissection that is 95% wide and 5% marginal is a marginal excision, i.e. excising 3cm of skin around a mast cell tumor will still be classified as marginal if the pseudocapsule is entered on the deep surface, the commonest site for incomplete excision.

This classification gives us an indicator of the completeness of surgical excision, but how generous this margin of normal tissue around the reactive zone of a solid tumour should be has not been defined for most veterinary tumour types or grades. There are however three fundamental approaches to delivering a surgical margin; the Metric Approach, whereby the mass is visualized as a homogenous tissue in three dimensions and is resected following direct measurement beyond the visible or palpable edge of the mass. The metric approach is most commonly employed for small to medium sized cutaneous or subcutaneous masses. Examples include margins of 2cm for grade 2 mast cell tumors, and 3-5cm for soft tissue sarcomas or feline vaccine sarcomas. The Barrier Approach relies on conceptualizing the mass being constrained within anatomical boundaries and dissecting upto an uninvolved barrier, or a barrier with great functional significance (e.g. sciatic nerve, vena cava, spinal cord). Many procedures are in fact a combination, i.e. the Metric/Barrier Hybrid, especially in areas where there is little non-functional tissue e.g. head, neck, thorax. Excision of cutaneous/subcutaneous masses often also rely on this hybrid approach, with a superficial metric skin margin, and a deep barrier margin of an uninvolved fascial plane. This approach often leads to a histopathological report of several cms clearance laterally, but only 1-2 mm clearance deep. These reports are often most easily interpreted by the surgeon who performed the surgery, and adequate communication with the pathologist who reviews the sample may be needed.
What to do with a ‘dirty’ margin

In both human and veterinary surgical oncology, universally accepted guidelines on treatment of solid tumours have been difficult to establish. One of the largest contributing factors to this is the indecisiveness over what to do with an incomplete surgical margin and its relevance on local recurrence and overall survival.

The impact of leaving residual tumour cells in the wound bed of an excised STS is a many fold increase in the rate of local recurrence (Kuntz). Several studies have reported the results of adjunctive radiotherapy in the management of STS with surgically incomplete margins and its success has generally been measured by its ability to provide consistently long overall survival times. The effect on local tumour control however, has been variable, with recurrence rates ranging from 17-60%. Retrospective investigation into the results of surgery alone (‘primary re-excision’) for the treatment of STS after incomplete resection studied 41 dogs that had undergone aggressive scar revision (attempted wide margins (1-3cm)/1 fascial plane deep) for incompletely excised STS. Complete margins were obtained after re-excision in 90% (37/41) of all the cases with mean margin widths of 2.7cm on the proximal portion of limbs and 1.4cm on the distal portion of limbs. Local tumor recurrence occurred in 15% (6/39) of the dogs at a median time to recurrence of 142 days. Correlations for recurrence were not identified and of particular note, in 4 out of the 6 recurrences, there was no evidence of tumour in the resected tissue and the scar was excised with clean margins. All the masses that recurred were grade 2, no recurrence was seen below the stifles or elbow, and only 2/6 dogs were ultimately euthanized for reasons related to the tumour.

Comparisons of the local control rates achieved with re-excision alone compared to radiation therapy suggest that the outcomes of surgery, when possible, may be equivalent to those achieved with adjunctive radiation therapy. In fact, when the costs and relative morbidity of radiation are factored in, an attempt at surgical excision alone may be a more desirable first line approach. The question then arises, what if the site of recurrence does not afford an extensive re-excision, or the owner declines further treatment after a tumour positive margin is confirmed? Veterinary opinion suggests that local recurrence rate will be high if ‘wait-and-see’ is employed but limited data has been published to support this assumption. A recent study evaluating the local recurrence rate of canine STS of the distal limbs treated by marginal excision alone followed 26 dogs with tumours of the distal antebrachium or pes who were determined to have tumour positive margins after marginal excision. All grades of STS were represented and follow-up intervals were long (median, 781 days, minimum 594 days). The rate of local recurrence was 37% (10/27 tumors) with only 12% (3/26) of the dogs being euthanized for problems relating to local disease. Interestingly, of the 10 recurrences, 4 were untreated and all 4 dogs died of tumour-related disease. The other 6 underwent further conservative surgery (intra-lesional, marginal) and only 2 died from tumour-related disease, suggesting that managing local recurrences by repeat marginal excisions may be an acceptable solution in some circumstances.

The reported recurrence rate following complete surgical excision of grade 2 MCTs is 11% with time to recurrence of 2-24 months, however published work shows that most incompletely excised MCTs do not recur (18-35% recurrence rate reported). This raises an interesting dilemma as clearly not all incompletely excised grade 2 MCTs recur, and so adjuvant therapies such as radiation or chemotherapy may not always be necessary. Developing local recurrence is typically prognostic for decreased overall survival however and so until data becomes available regarding the impact of omitting additional treatment following an incomplete excision, adjuvant therapy remains the standard of care.

Ultimately although substantial progress in our understanding of canine solid tumours has been made, and our staging and treatment plans have become ever more advanced, our appreciation for the ultimate biologic predictability of a particular individual’s tumor remains poor. Of particular significance and concern is the observed phenomenon of local recurrence after wide tumour negative margins have been achieved, or following wide resection of scars containing too few tumour cells to even
be detected histologically. Additionally, a causative relationship between local recurrence and systemic metastasis has yet to be elucidated. It is suggested that patient prognosis is dictated primarily by a multitude of independent biologic factors such as tumour grade, size and depth of invasion. Local recurrence is thought to serve more as a marker for, rather than a cause of diminished survival in patients with STS. As our individual experience increases, and our understanding of tumour biology improves, we hope we will soon be able to make decisions based more on the nature of recurrence rather than the fact that it has occurred at all.

**Conflict of interest:** none relevant to this lecture.

**Notes**
Animals with cancer often have very advanced (locally invasive or metastatic) disease. As a veterinary surgeon consulting owners it is imperative to have a strong knowledge base regarding the behaviour of the tumours that commonly occur in dogs and cats. Too little knowledge may result in performing inappropriate diagnostic tests or embarking on procedures that may be better performed by oncologic specialists. Many tumours demand an aggressive surgical and/or medical approach that can only be delivered by an experienced hand – in these cases, referral to a specialist should be considered. Remember, the best chance to remove a tumour is the first attempt. In addition to knowing when tumour resection is possible it is equally important to realize when the extent of disease has become too advanced to recommend surgery. Keeping the quality of our patients’ lives at the forefront can sometimes be challenging with owners who are struggling to accept the limitations of the therapies that are currently available.

**Curative intent resection**

A thorough knowledge of anatomy is necessary when palpating the extent of a primary tumour and planning the resection. Aside from tumour size, factors that influence resectability include proximity to non-expendable anatomical structures and degree of local invasiveness and fixation (which is dictated by tumour type). Advanced cross-sectional imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) provide a great deal of pre-operative information; however, there are still instances where it is not clear whether a given tumour is excisable until it is approached surgically. The final pre-operative physical evaluation is typically done just after induction of anaesthesia. In planning the resection it is essential that the surgeon knows the limits of various resection procedures and what surrounding normal anatomical structures can be sacrificed in order to achieve a complete excision and not cause unacceptable post-operative morbidity or complications. For most cutaneous malignancies, the general rule is to make a plan that will allow the surgeon to take all neoplastic tissue necessary for complete excision and consider wound closure a secondary concern. Of course this is not always possible due to proximity of important surrounding structures. Also, in some cases it may be prudent to plan a more conservative resection and accept that the surgical plan may result in microscopic residual disease. For example, a low grade subcutaneous soft tissue sarcoma of the extremity in a 14 year-old dog owned by a client who is opposed to the thought of wound management could be excised via a marginal excision. Although this approach alone would not likely affect a cure, it may be possible to “shell out” the mass and risk the possibility of local recurrence at a later date. If this same patient however is entirely asymptomatic and the tumour is slow-growing, a discussion as to whether to treat at all is warranted. If a wound following tumour excision cannot be closed routinely, closing the defect with a reconstructive surgical technique, such as a skin flap or graft, or allowing it to heal by second intention, are options to consider. Flaps should be used with caution; however, as they usually increase the size of the surgical field, and thereby complicate post-operative radiation therapy planning.

**Histopathology**

All excised tumours should be evaluated histologically. When submitting the specimen the surgeon should provide the pathologist with a concise but accurate history and help the pathologist maintain proper orientation of the tissue. This orientation may be communicated by making a drawing, placing a suture on a specified margin of the specimen. Knowing the orientation can be very helpful if tumour cells are observed extending to one of the tissue margins and further local therapy is indicated. Alternatively, small tumour bed samples can be harvested from areas of higher concern in the resulting wound bed.
just prior to closure. Following removal of the mass the surgical margins of the excised specimen should be painted with ink to document the original plane of dissection. Inking the margins can very valuable, particularly when it is necessary to re-evaluate the submitted specimen after the initial pathology read-out has been made. The ink is “painted” on with cotton tip applicators by the surgeon and should be allowed to dry for 5-10 minutes before the specimen is placed in formalin. There are multiple colours available but most pathologists seem to prefer black or yellow as these colours cannot be confused with the haematoxylin and eosin stains (blue and red) used by pathologists. Like suture tags, different ink colours can be used to direct the pathologist to areas of greater concern. The volume ratio of formalin to tumour mass should be 10:1 to insure proper fixing of tissue. With large tumours, near full-thickness fixative incisions should be made 1 cm apart throughout the tumour parenchyma (similar to slicing bread) to allow proper fixation. However, it is critical that the surgeon not allow fixative incisions to connect to the surgical margins as that will lead to confusion when the pathologist examines the tissue.

Incomplete resection and local recurrence
Local recurrence can often be predicted with a good histologic assessment of the surgical margins. When “dirty margins” are not treated, a mass might appear along or beneath the line of incision weeks, months or even years after the initial surgery (depending on the tumour identity). In this scenario, location, tumour identity and the results of re-staging dictate the course of action. For example, a recurrence from a previous aggressive local excision of a synovial cell sarcoma of the tarsal joint, with no evidence of lymph node metastasis is probably best treated by limb amputation. In other cases, additional surgery of the same operative site might be possible to remove the remaining neoplastic cells. In general, this approach is more likely to work soon after the initial surgery (i.e. when cells are still present at microscopic levels). Failing to include a fascial plane in the resection is one of the most common reasons for incomplete excision of subcutaneous masses. Although historically it was advised not to recommend that the client take the “wait and see” approach when incomplete margins have been documented histologically, an increasing body of evidence is showing that a reasonable percentage of these cases never develop local recurrence in the pet’s life time.

Adjunctive radiation therapy
Alternative therapies, such as radiation and chemotherapy, should also be considered in cases with incomplete excision, especially if an aggressive attempt at surgery was made during the initial procedure by an experienced surgeon. Although adjunctive chemotherapy is rarely effective in cleaning up dirty margins post-operatively, adjunctive radiation can be a very useful tool for this purpose. For example, post-operative radiation is often successful in treating incompletely resected mast cell tumours and soft tissue sarcomas of the extremity treated by marginal excision. In some instances radiation therapy is more advantageous prior to surgery.

Conflict of interest: none relevant to this lecture.
There are two major disciplines of pharmacology: pharmacodynamics and pharmacokinetics. Pharmacodynamics is the study of the effects of drugs, drug concentration-effect relationships, and mechanism of action. Pharmacokinetics is the study of the time course of change in concentration of drugs in blood and tissues of animals. It includes an understanding of the absorption, distribution, biotransformation (metabolism) and excretion of drugs. These processes together are sometimes referred to as drug disposition. Pharmacokinetic principles are important determinants of animal dosages for clinical applications. The affinity for receptors, pathogenic organism susceptibility, and cellular drug effect does not change appreciably among patients, between healthy animals and ill animals, or among animals with variation in physiologic parameters. Therefore, an understanding of pharmacokinetics is one of the most important pharmacologic principles for bench to bedside transfer of drug therapy and design of drug regimens in clinical patients. An understanding of clinical pharmacokinetics is important for formulating dosage regimens in animals with disease and translating dosages from one species to another. An understanding of clinical pharmacokinetic principles will benefit patients who may handle drugs differently than healthy adult animals that are used in preclinical experiments.

An important use of pharmacokinetic principles in veterinary medicine is to extrapolate drug use in one animal species to another, or from humans to animals, or to adjust for clinical conditions. There are four important determinants of drug dosage.

To derive dosages and adjust dosages in animals, one must understand these basic elements of pharmacokinetics:

1. Systemic availability (F) defines the dosage adjustment necessary for oral, SC, IM or other non-IV administration.
2. Clearance (Cl) defines the ability of the body to eliminate the drug. Two important organs of clearance are the kidney (Cl_K) and liver (Cl_L).
4. Elimination half-life (t½) is the time that it takes for half of the drug to be eliminated. Or, more properly stated, it is the time that it takes for the drug concentration to decline by one-half (drug concentrations in plasma can decline without elimination from the body). The t½ is most often used to define the dosing interval.

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 Clinically-Relevant Drug Interactions in Dermatology

M.G. PAPICH
College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Veterinarians often administer combinations of drugs without considering possible interactions that may occur. Many interactions and incompatibilities are possible considering the vast number of drugs available that may be used in combination. Interactions can result in a lack of therapeutic effect or produce adverse effects. This discussion will be limited to examples of interactions that are of clinical significance in veterinary medicine, particularly for dermatologists. A distinction should be made between drug interactions that occur in vitro (such as from mixing drugs in a syringe or vial) from those that occur in vivo (drug-drug interactions in the patient).

Pharmaceutical Interactions: Veterinarians frequently mix drugs together in syringes, vials, or fluids before administration to animals. These in vitro reactions also have been called pharmaceutical interactions. This type of drug interaction may result in a drug precipitate, a toxic product, or inactivation of one of the drugs to unknowingly administer an ineffective compound. Mixing drugs (compounding) that are incompatible is a source of an in vitro drug interaction that may be harmful to the patient.

Drug-Drug Interactions: in vivo interactions are reactions that occur in the patient when more than one drug is administered (drug-drug interactions, DDI). As the number of drugs administered to a patient increases, the number of drug interactions also increases. The consequences of drug interactions are most severe for drugs that have a narrow therapeutic index. (That is, when the ratio of toxic dose/effective dose is small.) in vivo drug interactions may cause changes in the drug absorption from the gastrointestinal (GI) tract, drug disposition, clearance, and excretion.

Absorption interactions occur when drugs chelate or bind to other constituents, such as when cations bind to fluoroquinolone and tetracycline antibiotics. Interactions can occur when acid-suppressing drugs (PPI) are administered with drugs that require acid for dissolution and absorption (oral azole antifungal agents). Clearance interactions occur when one drug can interfere with a transport process, or metabolism of another drug. The most common processes affected are Cytochrome P450 enzymes (CYP450) and p-glycoprotein membrane transport (p-gp). These process can be enhanced by inducers or decreased by inhibitors. Some of the most potent inducers are rifampin, and phenobarbital, which can increase clearance of other drugs. Some of the most important inhibitors are chloramphenicol, ketoconazole, and cyclosporine. These drugs can decrease the clearance of other co-administered drugs.

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Avian and reptile dermatology for general practitioners

J. HEDLEY
Exotics Service, Royal Veterinary College, UK

Avian and reptile dermatology can be challenging at times as skin disease is often secondary to underlying systemic health issues. Husbandry and diet deficits are also commonly involved, so on initial presentation, time should always be spent obtaining a full history in order to identify any underlying problems.

Most pet birds brought into the veterinary practice will be either psittacines (parrot-type birds) or passerines (perching birds). Owners may have noticed changes in feather colour or appearance, but in psittacines the most common presentation is feather-plucking which can progress quickly. Feather plucking may occur due to primary skin disease, secondary to systemic disease or due to behavioural causes. Primary skin problems such as ectoparasites can usually be easily ruled in or out on initial examination, but a full diagnostic work up is required to identify an underlying systemic problem. This may include haematology, biochemistry, imaging and coelioscopy. Viral diseases such as Psittacine Beak and Feather Disease (PBFD) and polyoma virus should also be considered and PCR tests are available to screen for these. Behavioural causes are the most challenging to treat and advice should be sought from an experienced avian behaviour specialist in severe or chronic cases.

Skin or shell problems in reptiles usually progress much more slowly and causes may include physical or thermal trauma, infections or neoplasia. The structure of reptile skin is vastly different to that of mammals or birds, with skin being regularly shed throughout an individual’s life, a process known as ecdysis. One of the most common reasons for seeking veterinary attention is a problem with shedding (dysecdysis). Treatment of dysecdysis is usually fairly simple and involves increasing environmental humidity, warm water baths and gentle manipulation of retained skin with a wet cotton bud to aid removal, but the underlying cause always needs to be identified to prevent problems with future sheds.

Finally, it is important to note that most treatments in reptiles are not licensed for use in these animals. Doses of unlicensed medications are usually extrapolated from those used in other companion animals. However, some drugs such as ivermectin are known to be toxic to species such as chelonians, so the most up to date exotic animal formulary should always be checked before using a drug in a species for the first time.

Conflict of interest: none relevant to this lecture.
Small mammal dermatology for general practitioners

J. HEDLEY
Exotics Service, Royal Veterinary College, UK

Most small mammals presented to a veterinary practice are prey species, so consequently likely to hide signs of underlying disease, pain or stress until problems are at an advanced stage. Skin diseases however, cannot be so easily hidden and may be either primary or more commonly secondary to an underlying problem. In addition to examination of the skin, a complete physical examination is therefore important to identify the underlying cause. Husbandry and diet deficiencies are also common predisposing factors, so time should be spent obtaining a full history of the animal’s lifestyle at home as well as information about any companions.

Ectoparasites are one of the most common reasons for presentation including mites, ticks, lice, fleas and flystrike. For most of these parasites, in low numbers they often appear to cause no obvious issues. In a confined captive situation however, animals may be under stress and immunosuppressed, especially if overcrowded, hygiene is poor or husbandry is inappropriate. In this situation, parasites can multiply rapidly and start to cause disease. Diagnosis and treatment of the individual is generally straightforward, but treatment of companion animals, the environment and any concurrent disease is important for a successful resolution.

Other infectious dermatoses include bacterial skin infections (usually secondary to other issues) and fungal infections such as dermatophytosis. Both *Trichophyton mentagrophytes* and *Microsporum canis* can affect small mammals, especially groups of young animals where husbandry is suboptimal. Although infection may be self-limiting, treatment is recommended due to the zoonotic risk and often close proximity of these pets to children in the household.

Less common causes of skin disease in small mammals include allergic skin disease, endocrine disease (e.g. cystic ovaries in guinea pigs) and neoplasia (e.g. epitheliotropic lymphoma in hamsters). These conditions can be extremely debilitating and will require a full diagnostic work up to identify the underlying aetiology.

Finally, it is important to note that most treatments in small mammals are not licensed for use in these animals. Doses of unlicensed medications are usually extrapolated from those used in other companion animals, but should always be checked in the most up to date formulary to ensure that they are suitable for the species.

**Conflict of interest:** none relevant to this lecture.
To guarantee the clinical efficacy of topically administered drugs, the active compound must reach the site of action (superficial layers, deeper layers of the skin, systemic circulation) in an appropriate concentration. Therefore, the physical and chemical properties of the compound and the pharmaceutical formulation are determining factors of the clinical efficacy. Based on the clinical diagnosis the therapy will be planned considering also benefits and disadvantages of a systemic or topical drug administration. A general advantage of the topical treatment of skin diseases should be the direct administration of the compound at the site of action, possibly with a reduction of undesired systemic effects. Disadvantages may be its low practicability or a local irritation caused by the active agents or other ingredients of the formulation used. The stratum corneum is not only the main barrier but also a reservoir for topically administered lipophilic compounds. This was demonstrated by the vasoconstriction (skin blanching) assay which is used to compare the potency of external glucocorticoids. The diffusion via the horny layer is significantly influenced by the pharmaceutical formulation. This was demonstrated by an experimental study performed in the isolated perfused bovine udder with pharmaceutical formulations containing betamethasone-17,21-dipropionate or non-steroidal anti-inflammatory drugs as active ingredients. The addition of penetration enhancers to a pharmaceutical formulation can enhance the transdermal flux significantly. Examples for penetration enhancers are dimethylsulfoxide, propylene glycol, various lipid formulations and also solvent combinations with isopropyl myristate and others. Although a large number of formulations is generally available, the use of solutions is mainly preferred in animals due to the hair coat. Formulations which are mainly used in human dermatology are various ointment formulations (hydrophobic, hydrophilic), creams, gels, pastes and others. Modern formulations are patches, microemulsions, formulations containing nanoparticles. An alternative route of drug administration is the use of shampoos. This is an interesting alternative for the drug administration in dogs. Active compounds can be used as ingredients of shampoo formulations which contain different detergents. The skin tolerability of the shampoo formulation has to be considered especially. Because the contact time of a shampoo (rinse-off formulation) is short, it has to be ensured that the active ingredient is entering the horny layer reaching there a sufficient concentration which guarantees that the compound will diffuse into the deeper layers. A further possibility is the use of spot-on or pour-on formulations or collars to reach systemic effects of the administered compound or to have a spreading effect with a horizontal diffusion in the stratum corneum.

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Sterile pyogranulomatous dermatitis: Update and future

A. RODRIGUES HOFFMANN*, C. OLDER* and F. BRUM ROSA†

* Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, USA
† Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Sterile pyogranulomatous dermatitis and panniculitis (SPDP) is a syndrome that is uncommon in dogs and rare in cats. Clinically, this syndrome is characterized by multifocal cutaneous nodules or plaques, which occasionally become ulcerated. Bacterial and fungal cultures and special stains are negative, and the lesions have therefore, been considered sterile. Histologically, these lesions are characterized by granulomatous to pyogranulomatous inflammation in a nodular to diffuse pattern often extending from the dermis and involving the panniculus. The histologic presentation is very similar to lesions caused by mycobacterial infections and certain fungal and protozoal infections, such as Leishmania, and reactive histiocytic disorders also need to be ruled out. A complete work up to rule out infectious agents, including bacteria, fungi, and Leishmania spp. (in endemic areas) must be performed before starting treatment for SPDP lesions. Many studies have investigated the presence of infectious organisms in these cases using culture, molecular techniques, and more recently, next-generation sequencing (NGS). Previous studies have identified the presence of Leishmania sp. by immunohistochemistry and PCR, and one study identified Serratia marcescens in skin samples of dogs diagnosed with sterile cutaneous nodular lesions. Mycobacterium spp. have also been investigated as a potential cause with PCR but with negative results. An NGS study targeting the bacterial 16S rRNA gene and fungal ITS region investigated if any bacteria or fungi were associated with skin lesions in fresh and formalin-fixed paraffin-embedded (FFPE) skin samples from SPDP-affected dogs and healthy controls. Significant differences between the samples of the different groups were only found for bacteria commonly identified on the canine skin surface, and no known invasive or novel pathogens were identified in SPDP samples. All these studies, together with the clinical response of lesions to immunosuppressive/immunomodulatory therapy, provide the support that SPDP lesions are likely sterile and associated with immune dysfunction. It is crucial for the practitioner to rule out infectious causes in these cases, as the recommended treatment in SPDP often requires immunosuppression, which could result in aggravation of occult infectious diseases. In the future, NGS will likely become available as one of the diagnostic tools to completely rule out infectious causes in these as well as other cases of sterile dermatitis.

Conflicts of interest: A.R.H. has consulted for the following company marketing products mentioned in this lecture: Zymo Research.
Mast Cells: Can’t live with them - can’t live without them

M. MAURER
Dermatological Allergology, Allergie-Centrum-Charité, Department of Dermatology and Allergology, Charité – Universitätsmedizin Berlin, Germany

Mast cells are key effector cells of skin inflammatory responses and chronic inflammatory skin conditions. When mast cell responses are directed to pathogens and other environmental threats, they have an important role in maintaining health and preventing disease. When mast cell inflammation is directed to allergens or autoantigens, these cells significantly contribute to the morbidity of chronic inflammatory skin disorders. Here, we will review recent findings that better define and characterize the role of mast cells in both, beneficial and detrimental skin inflammation. Examples will include the critical role of mast cells in raising innate immune responses to bacterial infections, their effects on wound healing and their protective role in the detoxification of venoms. Also, we will review the contribution of mast cells to allergic skin diseases and systemic allergic reactions, and we will discuss emerging evidence that mast cells play a critical role in type 1 autoimmune diseases of the skin, often also referred to autoallergy. Here, mast cells are activated by IgE directed to autoantigens (autoallergens), an important pathogenic mechanism in chronic spontaneous urticaria, bullous pemphigoid and other inflammatory skin diseases. For both, physiological and pathological functions of mast cells, the importance of cellular interactions, of the molecular mechanisms of their activation and the effects of their mediators, and key pathways of intracellular signal transduction will be reviewed. Finally, novel insights on known and new mast cell activators, receptors and mediators will be summarized and discussed with respect to their clinical relevance including their role as targets for new approaches in the treatment of mast cell-driven skin diseases.

Conflicts of interest: Grants and personal fees from Allakos, personal fees from Alnylam, personal fees from Aralez, grants from AstraZeneca, grants and personal fees from BioCryst, grants from Blueprint, personal fees from CSL Behring, grants and personal fees from FAES, grants and personal fees from Genentech, personal fees from Kalvista, grants and personal fees from Menarini, grants and personal fees from Novartis, grants from Leo Pharma, grants and personal fees from Moxie, grants and personal fees from MSD, grants from Pharming, personal fees from Pharvaris, grants and personal fees from Roche, grants and personal fees from Sanofi, grants and personal fees from Shire/Takeda, grants and personal fees from UCB, grants and personal fees from Uriach.
Mast cell-driven diseases: Where do we stand? Where do we go?

M. MAURER
Dermatological Allergology, Allergie-Centrum-Charité, Department of Dermatology and Allergology, Charité – Universitätsmedizin Berlin, Germany

Mast cells (MCs) contribute to the pathogenesis of a multitude of diseases that include purely MC-driven disorders such as urticaria, type I allergies, mastocytosis and mast cell activation syndrome (MCAS). They also contribute to chronic autoimmune disorders and other inflammatory conditions, and they may play a role in malignant tumors. Here, we will review and discuss the results of studies that identified and characterized how MCs contribute to disease and, importantly, what strategies are currently being explored to target MCs and MC-effects therapeutically. Specifically, we will discuss the most common approaches for investigating diseases for a relevant role of MCs. We will also review advances in our understanding of mastocytosis, MCAS, and chronic urticaria including the current therapeutic approaches used to modulate MC numbers, inhibit their activation, and protect patients from the effects of MC mediators. Chronic urticaria will serve as a model disease to discuss the evolution of pathogenic concepts in MC-driven diseases. Today, it is widely held that several different signals are responsible for the MC activation and degranulation that drive the development of urticaria signs and symptoms, and that chronic urticaria patients differ in which MC-activating signal is responsible for their urticaria. This process is modulated, i.e. inhibited and facilitated, by a wide array of different signals (cellular concept). We will review the evidence in support of different pathogenic concepts in chronic urticaria, particularly the cellular concept. Finally, we will address the unanswered questions and what research is needed to test and further develop our current view of what causes MC-driven disease including chronic urticaria. The overall aim of this contribution is to provide an overview of current views of the etiopathogenesis of MC-driven diseases and to review the diagnostic tools that have become available for their management as well as novel treatment options.

Conflicts of interest: Grants and personal fees from Allakos, personal fees from Alnylam, personal fees from Aralez, grants from AstraZeneca, grants and personal fees from BioCryst, grants from Blueprint, personal fees from CSL Behring, grants and personal fees from FAES, grants and personal fees from Genentech, personal fees from Kalvista, grants and personal fees from Menarini, grants and personal fees from Novartis, grants from Leo Pharma, grants and personal fees from Moxie, grants and personal fees from MSD, grants from Pharming, personal fees from Pharvaris, grants and personal fees from Roche, grants and personal fees from Sanofi, grants and personal fees from Shire/Takeda, grants and personal fees from UCB, grants and personal fees from Uriach.
Evidence based medicine for treatment and debunking ringworm folklore: Part I & II

K.A. MORIELLO
Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison Wisconsin USA

Dermatophytosis is a superficial fungal skin disease of dogs and cats. The primary pathogens are Microsporum canis, M. gypseum, and Trichophyton spp. There is a great deal of misinformation in lay literature about disease prevalence, transmission, clinical signs, diagnosis, treatment, and decontamination. Key take home points are summarized as follows. Dermatophytosis is not the most common skin disease of cats and overall its prevalence in small animal practice is 0-4%. The primary mode of transmission is via direct contact with spores on the hair coat. Disease transmission from the environment is rare in the absence of traumatic fomite inoculation. Clinical signs reflect the overall health of the host are best described as infections in otherwise healthy animals (simple), infection in animals with concurrent problems (complicated), or culture positive lesion free (fomite). There is no gold standard diagnostic test and diagnosis is made by using multiple tests. The only two diagnostic tests that confirm true infection in a hair follicle are skin biopsy and direct examination. The Wood’s lamp is a TOOL not a test and evidence based research had found M. canis fluorescence when exposed to a Wood’s lamp (365-400 nm) in >91% of untreated animals. Wood’s lamps and dermoscope tools are used to find hairs for direct examination. Direct examination is a cost effective simple diagnostic point of care diagnostic test. The best sampling method is a combination of scraping and plucking hairs that are examined using mineral oil; clearing agents in animals are not needed. Treatment is multi modal and includes limited confinement, cleaning, topical therapy, systemic therapy and monitoring. Confinement is to keep pets safe and minimize the area that needs to be cleaned. Cleaning is easier than we thought and “if it can be washed, it can be decontaminated”. Fungal spores can be removed with mechanical cleaning and washing with a detergent. Over the counter bathroom disinfectants are adequate for disinfection. Topical therapy (whole body and focal) is critical to cure as it disinfects the hair coat. Whole body treatments should be twice a week with daily or every other day application of focal topical therapy (1% miconazole, 1% terbinafine, miconazole/ chlorhexidine, or ketoconazole/miconazole formulations). Enilconazole, lime sulfur or miconazole+ chlorhexidine are effective as are residual mousse formulations for animals that cannot be wetted. Systemic therapy is needed to eradicate the infection within the hair follicle. Non-compounded systemic antifungals to use areitraconazole (cats and small dogs), ketoconazole (dogs), and terbinafine (dogs). Do not use fluconazole as it has poor efficacy against dermatophytes. Prolonged recovery is to be expected in animals with concurrent illnesses otherwise most animals cure within four to six week. Retrospective review of fungal culture data from cats cured of M. canis dermatophytosis revealed that in 90% of cats the first culture was predictive of mycological cure. These cats were lesional but otherwise healthy. Cats that were ill at the time of diagnosis required two negative fungal consecutive fungal cultures for mycological cure. Although this is a zoonotic disease, it causes skin lesions in people that are easily treated. The most common complication in animal acquired infection in immunocompromised people was slightly longer times to cure.

Conflicts of interest: none declared
Notes
Journal Club 1

R.S. MUELLER
Medizinische Kleintierklinik, Zentrum für klinische Tiermedizin, LMU München, Germany

In this Journal Club, we will explore an infectious based topic using a selection of papers published outside the field of veterinary dermatology.

In this session, I will continue on a theme discussed elsewhere in this congress: atopic dermatitis and infectious agents ("microbiome") as potential drivers of the disease. The specific question to be addressed will be: How Malassezia spp. manipulate the epidermal environment to promote atopic dermatitis skin inflammation?

Conflicts of interest: none relevant to this Journal Club.
When it comes to designing skin microbiome methods, there are a lot of details that need to be taken into consideration in order to make sure the microbiota is accurately represented. Selecting DNA free supplies is a crucial first step in these studies. Equally important are proper sample collection methods (e.g., superficial swabs, scrapings or biopsies), selection of DNA extraction kits for microbiome purposes, and choice of sequencing method. With PCR-based methods, primers also need to be carefully chosen in order to avoid amplification bias. Since cutaneous samples are considered to be of low biomass, controls need to be included to ensure any contaminating microbial DNA can be identified. All samples should be processed together or at least randomly assigned from DNA extraction to sequencing to avoid any bias between different experimental runs. The methods to process and statistically analyze the data should be chosen carefully due to the complex nature of microbiome data, and these should be well-documented within resulting manuscripts. While additional studies in companion animal skin microbiome are needed to optimize several aspects of experimental design, transparency in study design can enable future researchers to analyze data in the context of optimized methods and new technology. Understanding these technical aspects of skin microbiome studies, considering these while designing studies, and reporting this information will allow researchers and veterinarians alike to more critically interpret results and lead to a more well-rounded understanding of the companion animal skin microbiome.

Conflict of interest: none relevant to this lecture.
Phone call horrors: “Doctor I think I have an outbreak of ringworm”
Steps anyone can take to help

K.A. MORIELLO
Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison Wisconsin USA

Dermatophytosis is a superficial fungal skin disease of small animals. It is treatable, curable and will self-resolve in a healthy animal without treatment. It is not a common skin disease of small animals but is important because it is infectious and contagious and a low level zoonotic disease. Most veterinarians are well equipped to manage individual animal cases or cases involving several animals in the home. A situation where veterinarians are less familiar with is how to respond to a call for help from an organization where there is a suspicion of a suspected or confirmed outbreak. The most common outbreak encountered is from *Microsporum canis*. The following steps are helpful in responding.

**Step 1:** Collect as much information as possible and start by just asking open ended questions. Dermatophytosis is an emotional disease and an ‘unguided narrative’ from the caller will often provide information that won’t otherwise be shared.

**Step 2:** Treat the situation like any other outbreak situation. Instruct people to minimize animal movement, isolate suspect animals and CLEAN aggressively.

**Step 3:** Establish a “clean break” area which is one that will not house any animals that may have been exposed.

**Step 4:** Perform a site visit to examine and sort animals into groups by current infection risk.

**Step 5:** Use clinical data to divide animals into groups based on risk. The risk categories are truly infected/high risk (lesional, Wood’s lamp positive direct positive), infection suspected/moderate risk, or non-lesional/low risk.

**Step 6:** Move high risk animals (infected) away from the general population to stop disease transmission. Isolate suspect cats until test results confirm the presence or absence of disease. A topical antifungal shampoo or rinse is appropriate pending the results.

**Step 7:** Evaluate the results of pending fungal culture or PCR. Step 8: Move animals again based upon the laboratory diagnostics. In Step 5 cats were grouped into risk based upon initial screening. Any PCR or fungal culture positive cat groups as suspect should be examined. Suspect animals with no new or active lesions are unlikely to be truly infected and should be treated as dust mops.

**Step 9:** Evaluate the efficacy of environmental cleaning. Positive results on environmental cultures indicate some combination of poor cleaning or presence of cats with infection.

**Step 10:** Implement a long term plan to prevent reintroduction of infection.

**Conflicts of interest:** None declared
Dermatophytosis is a superficial fungal skin disease of animals. The most commonly isolated pathogens in small animals are Microsporum canis, M. gypseum and Trichophyton spp. The disease is treatable and curable and will resolve in infected hosts without treatment, however it contagious and a low level zoonotic disease. Early recognition and treatment will speed resolution of the disease and minimize spread to susceptible hosts. With regard to fungal diagnostics it is important to differentiate between diagnostic “tools” and “tests”. The Wood’s lamp (320 to 400 nm) is a diagnostic TOOL that is used to find fluorescing hairs of M. canis. Contrary to what is widely stated in the literature fluorescence is an inherent property of M. canis and is common in untreated animals (>91% in untreated animals). It is used to find hairs to examine for direct examination or for culture. A dermoscope is another diagnostic TOOL that is used to find hairs for direct examination or fungal culture. Fungal culture is a test that merely detects the presence or absence of fungal spores on the hair coat and needs to be carefully interpreted in light of clinical history and clinical findings. New studies have shown that >98% of DTM fungal cultures will be can be finalized by day 14 of incubation. PCR is a test is relatively new and is its advantage over fungal culture is that results are available in days vs weeks. It too merely detects the presence or absence of fungal DNA on the hair coat. Direct examination and histological examination of skin biopsy specimens are the only diagnostic tests that truly confirm the infection in the hair shaft. In rare cases of subcutaneous infection, cytological examination of exudate can confirm that skin lesions are due to a dermatophyte infection; fungal culture would be needed to confirm speciation. In a clinical practice setting, Wood’s lamp examination and/or dermoscope tools are highly useful for finding hairs for direct examination from cats. In general, skin scraping combined with hair plucking from suspect lesions are the best methods for collecting specimens for direct examination. Specimens should be mounted in mineral oil; clearing agents are not needed. Identification of infected hairs and scales is no more difficult than learning to identify mites, bacteria or yeast; it is skill that takes practice. Confirmation of infection using direct examination allows a veterinarian to start treatment on the day of first consultation. If fungal culture or PCR testing is preferred, it is important to collect specimens ONLY from the lesion. Fungal cultures can be performed in house with a high degree of accuracy provided both macro and microscopic examination of fungal cultures is performed and the user has had some training. The most commonly used fungal culture medium remains Dermatophyte Test Medium. Either PCR or fungal culture can be used to monitor response to treatment. Skin biopsy is not necessary in most cases and the diagnosis of dermatophytosis via histological findings occurs most commonly when the animal has an unusual infection.

Conflicts of interest: None declared
Surgery for ear disease (I) in dogs and cats

N.J. BACON *†
* Fitzpatrick Referrals Oncology and Soft Tissue, Guildford, Surrey, UK
† University of Surrey School of Veterinary Medicine, Guildford, Surrey, UK

Subtotal and Total Pinnectomy

Pinnectomy is a simple, uncomplicated procedure for management of pinna neoplasia, trauma, and tissue deficit in cats and dogs. The most common indication is feline SCC for regionally confined SCC of the pinna. Surgery is rapid, with the pinna being amputated with scissors at least 1 to 2 cm from the visible margin of crusting or ulceration. Few bleeding vessels are encountered, and these can be cauterized. The skin on the convex surface is pulled over the cut edge of the auricular cartilage and sutured to the skin on the concave surface with a simple continuous pattern of fine gauge, nonabsorbable monofilament material. Suturing the cartilage should be avoided. Maintaining an Elizabethan collar after pinnectomy is problematic; in the author’s experience, however, most irritation of the surgical site comes from inadequate analgesia, and after this is corrected, cats leave pinnectomy wounds alone. The cut edge of the excised pinna is inked and submitted for margin analysis.

Lateral Wall Resection

LWR is indicated when integumentary changes of otitis externa are considered reversible or when there is a small tumor of the tragus or lateral wall of the vertical ear canal not extending into the horizontal canal. Opening the lateral ear canal improves ventilation of the ear canal, reducing moisture, humidity, and temperature.

The patient is positioned in lateral recumbency as previously described. Parallel skin incisions are made along the rostral and caudal margins of the vertical ear canal, extending from either side of the tragus to a level just ventral to the junction of the vertical and horizontal ear canals, at which point the incisions converge. The skin is dissected free and removed, and a longitudinal incision is made through the subcutaneous tissues overlying the vertical canal. The tissue is retracted to expose the lateral cartilage of the vertical canal; the dorsal edge of the parotid salivary gland can be reflected ventrally to complete the exposure. Inadvertent sharp incision into the parotid salivary gland seemingly causes no problems. One blade of straight Mayo scissors is inserted down the ear canal, and the rostral and caudal edges of the vertical canal cartilage are transected to release the lateral 50% of the ear cartilage. Rotating the scissors 90 degrees so the handles are parallel with the skin helps create a cartilage incision parallel to the adjacent skin and helps in accurate mucocutaneous wound closure. Incisions should be made in stages to ensure that they remain in an appropriate orientation with the vertical canal as it spirals gently toward the horizontal canal and skull. The incisions are continued to the junction of the auricular and annular cartilages, at which point the lateral wall reflects ventrally with minimal tension.

The proximal (dorsal) two-thirds of the cartilage flap is removed and the ventral third reflected ventrally to form the drainage board. The drainage board decreases the amount of sutures near the stoma, lessening the risk of stricture, and shifts hair-bearing skin away from the stoma ventrally to minimize hair matting and reduce stoma maintenance long term.

Simple interrupted sutures of fine-gauge monofilament nylon are used to appose the aural epithelium to the surrounding skin, first passing the needle through the epithelium and exiting through the cut cartilage edge and then passing through the adjacent skin edge. It is advisable to place the deepest sutures first-those at the “hinge” at the junction of the auricular and annular cartilages. Sutures are then placed alternating rostral and caudal, moving from deep (closest to the horizontal canal) to superficial until the wound is closed. A one-layer closure is sufficient, and a drain is not necessary.
Self-trauma to the surgical site is the most common and most destructive postoperative concern but is often a result of underestimation of postoperative pain associated with the procedure and thus inadequate provision of analgesia. An Elizabethan collar is a sensible precaution. Bandaging the ears should be avoided, if possible, to allow the wound to be inspected and cleaned as necessary.

**Vertical Canal Ablation**

When the vertical ear canal is severely diseased but the horizontal canal is otherwise unaffected, a VCA is indicated. This allows for complete excision of vertical canal tissue with less postoperative exudate, postoperative pain, and incised cartilage, resulting in better healing and an improved cosmetic effect. Indications for VCA include irreversible hyperplastic otitis, severe trauma, and neoplasia limited to the vertical canal. It is unusual however, for neoplasia and otitis externa to be localized solely to the vertical canal, so VCAs are seldom performed. As noted, apposition of the lateral border of the horizontal ear canal to the skin with or without VCA has been used for the treatment of traumatic separation of the auricular–annular cartilage junction and congenital vertical ear canal stenosis.

The patient is positioned as described previously, and a skin incision is made over the vertical canal from the tragus to the level of the horizontal canal. Soft tissues are reflected off the lateral cartilage wall. The incision is extended circumferentially around the external auditory opening dorsal to all diseased tissue. Dissection is performed in the fascial plane as close to the cartilage as possible, especially when working around the medial pinna skin, to avoid inadvertent damage to the vessels supplying the pinna. Muscle and soft tissue attachments are dissected off the cartilage to the level of the horizontal canal, and the vertical canal is freed and reflected laterally through the skin incision. The canal is amputated at the level of the skin, and short dorsal and ventral flaps are made from the medial and lateral walls of the vertical canal to act as drainage boards. These also allow for a larger permanent stoma by moving the suture line away from the new opening and so minimizing any effect of stricturing. The dorsal skin wound is then closed in a T shape. Postoperative care is similar to that for LWR.

**Vertical Ear Canal Incision for Polyp Removal**

A lateral approach through the vertical canal has been described in cats to remove middle ear polyps extending into the external ear canal. A vertical skin incision is made beneath the tragus and extended to the level of the dorsal parotid tissue. The subcutaneous tissues and parotid are dissected free from the vertical ear canal, and the vertical and horizontal canals are exposed laterally just dorsal and ventral to the junction between the auricular and annular cartilages. The facial nerve is reflected ventrally, and a sharp vertical incision is made in the ear canal to access the polyp, which is grasped as close as possible to the osseous meatus and removed by traction. The canal wound is closed by suturing through the cartilage alone, and subcutaneous tissues and skin are closed routinely. Rarely, reexamination at 4 weeks reveals polyp regrowth, which can be removed at a second surgery as soon as it grows into the ear canal. No debriding of the middle ear is described, and long-term results for this approach are not available.

*Conflict of interest:* none relevant to this lecture.
Notes
Surgery for ear disease (II) in dogs and cats

N.J. BACON*†
* Fitzpatrick Referrals Oncology and Soft Tissue, Guildford, Surrey, UK
† University of Surrey School of Veterinary Medicine, Guildford, Surrey, UK

Total Ear Canal Ablation and Bulla Osteotomy (TECABO)

Studies often share similar demographics of dogs undergoing TECABO surgery, with cocker spaniels as the most common breed (43%-60%) undergoing the procedure. The mean age at time of surgery is 6.5 to 8.0 years, and the mean age of onset of ear disease is 5.2 years (range, 11 months–10.8 years). TECABO is the preferred technique for management of ceruminous gland adenocarcinomas, extensive benign disease, failed lateral ear resection, and extension of disease into the middle ear cavity. Dogs with chronic proliferative ear changes comprise up to 59% to 85% ear canal ablation patients. In one study of 52 ear canal ablations in cats, 50% had chronic inflammatory changes, and 41% had neoplastic disease. The patient is positioned in lateral recumbency as previously described. A T-shaped incision is made overlying the vertical canal, starting immediately ventral to the tragus. The subcutaneous tissues are separated to expose the lateral surface of the auricular cartilage. Incision of the dorsal parotid salivary tissue at the base of the vertical canal does not appear to be associated with any complications. The dorsal skin incision is extended around the external ear canal opening just outside the visible edge of proliferative change. To avoid ischemic necrosis of the pinna, the medial incision should not extend too far up the pinna. Soft tissues surrounding the ear canal are gently dissected free from the cartilage, best achieved by working deep to the perichondrium. The facial nerve is located caudoventral to the canal at the level of the terminal horizontal canal. Opinion differs as to whether the facial nerve should be identified and individually retracted with an encircling Penrose drain or deliberately avoided to prevent inadvertent iatrogenic trauma to the nerve. The author prefers the latter approach. The canal is then amputated as close as possible to the osseous external auditory meatus. The cut should be made with Mayo or cartilage scissors or a number15 blade; some surgeons recommend cutting from ventral to dorsal to protect the facial nerve trunk. The thickened, hyperplastic epithelium is then debrided from the meatus with a curette to accurately outline the opening into the tympanic bulla. The soft tissue overlying the lateral wall of the bulla, ventral to the osseus acoustic meatus, is reflected ventrally with a periosteal elevator. To prevent damage to vascular structures, stray dissection along the bulla wall should be avoided, especially rostrally (retroglenoid vein) and ventrally (carotid artery, maxillary vein). Rongeurs can then be used to remove the external osseus prominence ventrally and create a “keyhole” osteotomy to allow for curettage of the bulla floor, a common location for epithelium to be retained. Care is taken to avoid aggressive curettage dorsally where the round window is located. In minimally affected ears, the auditory ossicles are visible dorsally and are spared. With severely affected ears, the ossicles may not be evident among hair and debris and may be removed during curettage. Lavage with warm sterile saline throughout helps identify any epithelium attached to the bulla walls. The wound is infiltrated with bupivacaine. Dead space is closed with apposition of auricular muscles using monofilament absorbable material, and the skin closed in a T-shaped configuration. Immediately upon recovery, the function of the facial nerve is established because it is possible for temporary neuropraxia to develop 12 to 24 hours after surgery in the face of local postsurgical inflammation. The surgical wound is cold packed, and analgesia is provided as necessary. If the animal was not on preoperative glucocorticoids, a combination of systemic opioids and nonsteroidal anti-inflammatory drugs is recommended for initial postoperative pain relief. Alternatively, a constant rate infusion of local or systemic lidocaine or systemic fentanyl–lidocaine can be administered for the first 12 to 24 hours.

Conflict of interest: none relevant to this lecture.
Cytology, the theory of everything

V.M. SCHMIDT
Institute of Veterinary Science University of Liverpool, Leahurst Campus, Neston, UK

Introduction: Cytology is the ultimate in bedside tools and should be performed in all dermatological cases. Samples are easy to obtain (outside the body) and can give immediate diagnosis e.g. pyoderma, exclude disease e.g. demodicosis or aid therapeutic monitoring e.g. *Pseudomonas* otitis (saving time and money). Cytology can also help the interpretation of culture and susceptibility results e.g. intracellular coccoid bacteria with a heavy growth of *Staphylococcus* makes sense, whereas intracellular cocci with a light growth of *Pseudomonas* on culture does not.

What can I diagnose/suspect

- Ectoparasites
- Fungal/yeast infections
- Bacterial infections
- Protozoal infections
- Sterile skin disease
- Immune-mediated disease
- Hair shaft disorders
- Neoplasia

Which sample: parasites

- Acetate tape: cheyletiellosis, lice, other fur mites
- Hair plucks (trichograms): demodicosis (also dermatophytosis, hair shaft abnormalities)
- Skin scrapes: deep for demodicosis or superficial for cheyletiellosis, sarcoptes
- Indirect smear with paraffin: Otodectes cynotis, *Psoroptes cuniculi*

Which sample: micro-organisms

- Acetate tape: dry and scaly lesions
- Direct smear: moist lesions
- Indirect smear: greasy lesions, folds/ears
- Fine needle aspirate: large fluid filled lesions and nodules

Which stain?

Usually DiffQuik® (modified aqueous Romanowsky); time saving and sufficient for most preparations, but granules may not stain reliably. Staining time will depend on the preparation e.g. shorter if less cellular.

Knowledge of ‘normal’ and artefacts is needed to appreciate abnormal:

Keratinocytes

Common and largest cell. Usually from S. corneum (i.e. anucleate squames). Organisms may be adhered +/- melanin granules.

Acantholytic keratinocytes (AK cells) with pemphigus foliaceus (PF), *Trichophyton mentagrophytes* or superficial pyoderma; AK cells are round, dark-staining and nucleated; best viewed on lower magnification (e.g. x10).
Neutrophils
Common polymorphonuclear inflammatory cell (10-15 µm). Differentiate degenerate and non-degenerate. Non-degenerate (mature condensed chromatin and well-segmented purple lobes) seen in non-septic conditions e.g. foreign body, immune-mediated. Degenerate indicate sepsis so search for micro-organisms. Degenerate are more swollen with lighter nuclear staining (karyolysis) +/- pyknosis (karyorrhexis) with ‘nuclear (or DNA) streaming’. Main differential of streaming is damage due to rough handling or age; don’t confuse with fungal hyphae/fibrin.

Macrophages
Macrophages (histiocytes) are largest (2-4x neutrophils) inflammatory cell; round with an eccentric ‘bean-shaped’ nucleus. Suggest chronic inflammation: pyogranulomatous inflammation (mainly neutrophils); if >50% are macrophages, then granulomatous. May be sterile, infectious or neoplastic e.g. histocytoma. Activated cells are vacuolated (foamy); multinucleate giant cells prompt search for fungal organisms or foreign material.

Eosinophils
Binucleate with ‘red-orange’ staining granules on DiffQuik® (similar size to neutrophils); significant if >15% of inflammatory cells. Seen with allergy e.g. eosinophilic granuloma complex (EGC) and mosquito-bite hypersensitivity and herpes virus dermatitis in the cat. May be present with parasites, foreign bodies, immune-mediated or neoplasia e.g. MCT.

Other normal stuff/artefacts
- Melanin granules
- Crystals
- Environmental particles
- Inflammatory artefacts
- Stain artefact

Cellular inclusions
- Macrophages e.g. bacteria, atypical bacteria, fungi, protozoa, RBCs/pigment, neutrophils, cellular debris, lipid & foreign debris
- Neutrophils e.g. bacteria, RBCs
- Eosinophil (orange/red) and mast cell (blue to dark purple) granules (DiffQuik®)

Mites
Surface dwellers have long legs:
Otodectes cynotis (274-362 µm adult male; 345-451 µm adult female)
Cheyletiella spp. (385 µm)
Trombicula autumnalis (500 µm; larvae has 3 pair legs)
Leporacarus gibbus (240 x 440 µm adult males; 310 x 560 µm adult females)
Burrowing mites have short legs e.g.
Sarcoptes spp. (200-400 µm S. scabiei var canis; 200 x 240 µm N. cati)
Demodex spp. (250-300 µm D. canis; 330-370 µm D. injai)

Lice
Adults have six legs and are either chewing e.g. Trichodectes canis (dog) or Felicola subrostratus (cat) or sucking e.g. Linognathus setosus (dog).
Other pointers
- Flea dirt or immature stages e.g. six legged larvae
- Lice eggs adhere to hair shaft and have a lid (operculum)
- Cheyletiella (attached to hairs with fine threads), Sarcoptes (+/- faecal pellets) and Otodectes similar oval eggs
- Demodex canis eggs are lenticular shaped

Micro-organisms
- Cocci: round, dark blue-purple (DDX melanin)
- Malassezia: Russian dolls etc.

Superficial pyoderma has degenerative neutrophils with intracellular bacteria. Cocci in groups is usually *S. pseudintermedius*, occasionally other staphylococci and gram-negative bacteria (more common in deep infection). Cytology from deep pyoderma is similar with the addition of foamy macrophages, RBCs +/- eosinophils. Again usually *S. pseudintermedius*, occasionally other staphylococci or gram-negative and rarely anaerobic or atypical bacteria. Bacteria can be more difficult to find in deep infections so the search should be methodical.

Conflicts of interest: none to declare.

Notes
Interpretation of Culture and Susceptibility Testing

M.G. PAPICH
College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

On June 28, 2018 the Clinical Laboratory Standards Institute (CLSI) Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee published the newest in a series of standards for susceptibility testing (www.CLSI.org). These documents represent over 20 years of susceptibility testing standards for bacteria isolated from animals. CLSI-VAST is the only organization in the world that has published breakpoints for animal pathogens. The most recent CLSI standard document has approximately 180 breakpoints for drug-bacteria combinations in the major veterinary species. There are now breakpoints for practically every approved antimicrobial agent for the label indication in veterinary medicine. CLSI-VAST is working to fill the gaps for the few agents that do not have clinical veterinary breakpoints. Because there are many human-labeled antimicrobial agents used in veterinary medicine, CLSI also has developed clinical breakpoints for these agents in the non-food animal species.

CLSI-VAST develops the breakpoints through a process that includes pharmacokinetic data in the target species, MIC distributions for the pathogens targeted, and clinical data from the drug used under field conditions at the approved dose. An additional component is a pharmacokinetic-pharmacodynamic (PK-PD) analysis using Monte Carlo Simulations to show that at the approved dose, the drug attains PK-PD targets for the labeled pathogen.

The CLSI uses a consensus-driven process. The consensus process involves the development and open review of documents, revision of documents in response to discussion, and acceptance of a document as a consensus standard.

References:


Conflict of interest: Dr. Papich is the past Chairholder, current Vice-Chairholder, and member of the Clinical and Laboratory Standards Institute (CLSI) Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee.
Update on feline skin microbiome and disease association

A. RODRIGUES HOFFMANN, C. OLDER and A. MYERS
Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, USA

The skin of animals is colonized with a wide range of microorganisms, which influence skin homeostasis. An imbalance of these communities, referred to as dysbiosis, is associated with diseases, can lead to development or aggravation of skin lesions. A few studies have evaluated the bacterial and fungal communities inhabiting the skin of cats using both traditional microbiology and next-generation sequencing (NGS), which have demonstrated feline skin to be inhabited by diverse microbial communities. The bacterial communities are similar in composition to human and canine skin, consisting of primarily of Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Interestingly, cats seem to have more bacteria from the oral cavity/gut-associated phylum Bacteroidetes on their skin relative to what has been described in humans and dogs, indicating that the grooming habits may be important in shaping the cutaneous microbiome on cats. Fungal communities on feline skin appear diverse containing numerous taxa, which is distinct from those found on human skin which are dominated by Malassezia spp. Not surprisingly, breed and environment may contribute to the make-up of feline cutaneous communities, but not as much as the body site (for bacterial communities) and individual cat (for fungal communities) sampled. Similar to other species, the microbiome also plays a significant role in allergic skin disease. Even when lesions are not present, cats affected with allergic skin disease have an altered microbiota, with the bacterial communities in different body sites becoming more similar to each other and with a composition different from healthy feline skin communities. Overall, bacterial diversity is not different between healthy and allergic cats; although, higher relative abundances of Staphylococcus spp. are identified on allergic skin. Culture-based studies have shown that feline skin, both that of healthy cats and those with superficial pyoderma, is colonized by staphylococcal communities primarily composed of coagulase-positive S. aureus and S. pseudintermedius and coagulase-negative S. felis (initially characterized as S. simulans). Superficial pyoderma in cats is considered to be rare by most. However, some studies from referral practices have shown that superficial bacterial colonization can affect up to 20% of the feline population. While these bacteria can result in cutaneous disease in cats, the occurrence is much lower than dogs, likely due to the lower adherence of Staphylococcus spp. to feline corneocytes. Cats can also develop secondary skin infections when presented with cutaneous allergies, and in particular feline hypersensitivity syndrome. The most common secondary infections are caused by bacteria, especially Staphylococcus spp., and a few cases of fungal infections, primarily due to Malassezia spp., have also been reported. In NGS studies, healthy and allergic cats appear to harbor different communities in terms of the specific Staphylococcus species. The findings described above pave the foundation for future studies to better evaluate the role the skin microbiome in feline skin lesions.

Conflicts of interest: A.R.H. has consulted for the following company marketing products mentioned in this lecture: Zymo Research.
The skin is inhabited by a multitude of microorganisms, including bacteria, fungi, arthropods and viruses. Most studies to date have focused on evaluating the bacterial microbiota, with only a few studies targeting the fungal communities (mycobiota) inhabiting the skin. In whole-genome sequencing studies, which thus far have only been performed on human samples, fungi account for a very low percentage of all microbes inhabiting the skin. Despite its low relative abundance compared to other microbes, the mycobiome also plays a role in cutaneous homeostasis and diseases. The few studies investigating the cutaneous mycobiome of dogs and cats have demonstrated communities that are more diverse than those found on human skin and previously described in culture-based studies. The companion animal mycobiota tends to be similar across body sites, with higher fungal diversity identified in haired skin, and lower diversity in non-haired skin or mucocutaneous junctions. Their skin is mainly colonized with fungi from the phyla Ascomycota and Basidiomycota. Unlike human skin, which is primarily inhabited by Malassezia spp., the canine and feline skin mycobiota include many environmental fungi in the class Dothideomycetes (mainly the genera Cladosporium, Alternaria and Epicoccum). Although Malassezia spp. are one of the most common fungi isolated from the skin of dogs, next-generation sequencing studies found this microbe in low abundances in healthy canine skin. In one of these studies, dogs affected by allergic dermatitis had lower diversity and interesting alterations to their Malassezia communities. Analysis of these communities identified the lipophilic species M. restricted and M. globosa predominate, whereas M. pachydermatis has a higher relative abundance on the skin of affected dogs. The mycobiome is also different between healthy and allergic cats, even in the absence of lesions. From the body sites sampled, the axilla and interdigital space were particularly affected in terms of the structure and composition of the fungal communities. Together, these studies show that the fungal communities on the skin of companion animals are diverse, containing many organisms that are likely acquired from the environment, but with changes that may be important in disease. Given the importance of Malassezia spp. as a cause of secondary infections, further research into these communities specifically, as well as studies on the impact of topical and systemic antimicrobial treatment on the fungal microbiome, and its interactions with the bacterial microbiome, may provide valuable insight for better development of new treatment modalities for cutaneous infections in animals.

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There are many controversies regarding the use of antibiotics in dermatology patients. Although some drugs and treatment regimens have been tested, approved, and accepted, others are still in need of consensus. This discussion will be interactive and include the opinions of the two speakers. Among the areas (among others) that will be discussed are the following:

1. Can bactericidal and bacteriostatic combinations of antibiotics be used in the same patient?
2. Is it important to always use bactericidal antibiotics in patients with a compromised immune system (e.g., receiving corticosteroids or immunosuppressive therapy)?
3. Is it acceptable, and does it follow ethical standards in veterinary medicine, to use human-label antibiotics for treatment of methicillin-resistant *Staphylococcus* spp. in dogs and cats?
4. Should veterinarians be allowed to administer human-labeled antibiotics linezolid or vancomycin to dogs and cats for the treatment of methicillin-resistant *Staphylococcus* spp.?
5. Is pulse antibiotic therapy effective, and more likely to reduce recurrent infections? Or, more likely to promote resistance? (Pulse therapy regimens often include short courses of antibiotics on 1- or 2-day per week.)
6. Should tetracycline antibiotics be used for immune-mediated diseases (immunomodulation) or is this more likely to induce resistance?
7. Rifampicin use for treating *Staphylococcus* spp. infections: is single agent (monotherapy) effective, or is it necessary to use combination therapy?
8. Does the administration of 3rd-generation cephalosporins to pets produce drug-resistant bacteria that is an unacceptable public health risk, or risk to the animal? (Note: 3rd-generation cephalosporins include injections of cefovecin ‘Convenia’ in dogs and cats, oral cefpodoxime proxetil in dogs, and injections of cefquinome in other species.)
9. For infections caused by antibiotic-resistant *Pseudomonas aeruginosa, or ESBL-producing strains of Enterobacteriaceae, should veterinarians be allowed to use carbapenems (meropenem, or imipenem)?
10. Fluoroquinolones are often listed as a 2nd-tier antibiotics for skin infections in dogs and cats. Should their use continue for treatment in small animals, or is the risk of resistance too high?
11. Should veterinarians be allowed to use generic human-label fluoroquinolones in dogs such as ciprofloxacin and levofloxacin? (Note that these agents are often considerably less expensive than some veterinary-label fluoroquinolones, they have good in vitro activity, and ciprofloxacin is often more active against some strains than veterinary-labeled agents.)
12. Will the administration of highly protein-bound antibiotics such as cefovecin (Convenia) increase the risk of protein-binding interactions due to the displacement of other drugs from binding sites?
13. Is culture and susceptibility testing valid and reliable for animals, or no better than empirical selection? (That is, do susceptibility testing standards reflect clinical results?)
14. With the wide-spread use of topical antibacterial agents to treat skin infections, is there a chance that this will induce resistance against some agents (e.g., chlorhexidine)?

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Sterile neutrophilic dermatoses in dogs

C. BRACHELENTE
Department of Veterinary Medicine, University of Perugia, Italy

In human medicine, sterile neutrophilic dermatoses (SND) are a group of conditions with polymorphic clinical features but united by a similar histological aspect, characterized by the accumulation of mature neutrophils in the skin, in the absence of infection. The clinical manifestations of these dermatoses are heterogeneous and lesions can present as pustules, bullae, abscesses, papules, nodules, plaques and ulcers. They are often associated with extra-cutaneous signs (i.e. fever, malaise, anorexia) and involvement of other organs by the neutrophilic infiltration (lung, bones, joints, digestive tract, liver, spleen, pancreas, central nervous system, heart, and blood vessels). Sterile neutrophilic dermatoses can be subdivided into 3 main groups: (1) deep or hypodermal forms, whose prototype disease is pyoderma gangrenosum; (2) plaque-type or dermal forms, whose prototype disease is Sweet’s syndrome; and (3) superficial or epidermal forms, among which are the amicrobial pustulosis of the folds and the subcorneal pustular dermatosis. Histopathology shows variable degrees of infiltration by mature and non-degenerate neutrophils in the epidermis, dermis or subcutis. Neutrophils can destroy pre-existing follicular units and adnexal structures but are not primarily targeting them. Vascular lesions (necrosis and thrombosis) can be present but are considered secondary to the neutrophilic inflammation ("innocent bystander"). As this histopathologic pattern can also be observed in lesions caused by infectious agents, it is essential to perform bacterial and fungal cultures to rule out an infectious etiology. Neutrophilic dermatoses in humans are often idiopathic, but they have also been associated with inflammatory diseases (especially of the gastrointestinal and respiratory tract as well as of joints), malignancies, drug exposure, and trauma. Cutaneous lesions may precede, coexist or follow systemic signs. The pathogenesis of neutrophilic dermatoses has been thoroughly investigated in recent years and, although immune complexes and circulating auto-antibodies have been proposed to play a role, these diseases are now considered neutrophilic and cytokine-driven. In particular, the release of pro-inflammatory cytokines (IL-1β, IL-17, IL-8 and TNF-α) from an over-activated innate immune system is thought to play a role in the pathogenesis of these diseases, with a mechanism similar to autoimmune inflammatory diseases. The recognition of the pathogenic role of autoinflammation has opened the way to new therapeutic targeted options for these diseases, which are classically responding to immunosuppressive and immunomodulatory treatments. In veterinary medicine, sterile neutrophilic dermatoses are rarely reported. Single case reports or short case series have described dermatoses with clinical and histopathologic features similar to human pyoderma gangrenosum, Sweet’s syndrome and subcorneal pustular dermatosis, often in association with systemic clinical signs. In some of the canine Sweet’s-like descriptions, a drug association (NSAIDs) has been suspected whereas no malignancy-associated SND has been reported for dogs. Depending on the clinical signs, differential diagnoses include bacterial or fungal infections, spider bites, vasculitis and other immune-mediated diseases such as erythema multiforme, canine eosinophilic dermatitis, sterile pustular erythroderma of Miniature Schnauzers, pemphigus foliaceus, adverse drug reactions, and toxic shock syndrome.

Conflicts of interest: None to declare
Clinicopathological conference

C. BRACHELENTE* and L.M. BUCKLEY†
* Department of Veterinary Medicine, University of Perugia, Italy
† Small Animal Teaching Hospital, Institute of Veterinary Science, University of Liverpool, UK

During this session we will present a collection of interesting dermatological cases, where the diagnosis was obtained through a combination of clinical investigations and dermatohistopathology.

We will invite the audience to suggest differential diagnoses for both the clinical and histopathological findings. Any unusual or controversial clinical and histopathological features will be discussed and again, audience participation will be encouraged.

Conflict of interest: none declared
Feline allergy syndrome

L.M. BUCKLEY
Small Animal Teaching Hospital, Institute of Veterinary Science, University of Liverpool, UK

Feline hypersensitivity dermatitis usually presents with one or more of four cutaneous reaction patterns rather than the typical character and distribution of lesions seen in canine allergic skin disease. The term feline atopic dermatitis is generally avoided due to uncertainty over the significance of IgE in the development of skin lesions, and the term non-flea hypersensitivity dermatitis (NFHD) has been proposed. Cats present further challenges due to their solitary nature and secretive behaviour, meaning important clues may be missing from the clinical history. In terms of treatment of NFHD there may be challenges both in the administration of treatment and in the comparatively poor responses seen in some patients. The pathogenesis of NFHD is not fully understood. What is known is the histological pattern of cutaneous inflammation is similar to that seen in humans and dogs with atopic dermatitis. NFHD is thought to develop, as in human and canine disease, in association with internal (genetic) predisposing factors and external stimuli. The proposed major external ‘triggers’ of disease flares include food and environmental allergens. Exposure to these triggers drives clinical signs associated with food-induced hypersensitivity dermatitis (FIHD) and non-flea, non-food hypersensitivity dermatitis (NFNFHD), respectively. As for canine atopic dermatitis, the diagnosis of NFHD is a clinical one; compatible history and physical examination findings are used along with diagnostic criteria to achieve a diagnosis. Diagnostic tests are then used to eliminate differentials for the clinical presentation and diagnostic trials are performed to identify the triggering factor(s). The challenge in cats is in making the clinical diagnosis. A typical history includes a young age of onset (6 months - 3 years), presence of pruritus, absence of contagion and response to glucocorticoids. Unfortunately, for many cases, these important clues are not present as cats may present later in life, owners have not observed self-trauma and some cats remain pruritic on standard anti-inflammatory doses of glucocorticoids. Additionally, rather than the typical lesions of atopic dermatitis seen in humans and dogs, cats with NFHD present with one or more of four cutaneous reaction patterns. These include head and neck excoriations and pruritus, symmetrical self-induced alopecia, miliary dermatitis and lesions of the eosinophilic granuloma complex (eosinophilic ulcer, eosinophilic plaque and eosinophilic granuloma). Identification of one or more of the reaction patterns increases the suspicion of NFHD but does not confirm it. In 2012, Favrot and others published diagnostic criteria to assist in making a clinical diagnosis of NFHD. The criteria offer up to 90% sensitivity and 83% specificity and are therefore very useful for supporting what may be a challenging diagnosis. The criteria do not, however, confirm the diagnosis of NFHD and all suspected cases should undergo diagnostic testing to eliminate differential diagnoses (in particular ectoparasitism and microbial infection) and an elimination diet to differentiate between FIHD and NFNFHD. The management of NFHD involves anti-inflammatory/immunomodulatory treatment, avoiding flare factors such as ectoparasites and microbial infection, skin barrier care and where possible, allergen avoidance and allergen-specific immunotherapy.

Conflict of interest: none declared.
Keep calm and carry on: dermatological emergencies

V.M. SCHMIDT
Institute of Veterinary Science University of Liverpool, Leahurst Campus, Neston, UK

Usually dermatoses of sudden onset or sudden worsening or change; mostly immune-mediated disease, but infectious and allergic and severely pruritic conditions have also been seen on a Friday afternoon. Patients maybe systemically ill with fever, anorexia and malaise.

**Canine Eosinophilic Furunculosis (CEF) and Eosinophilic Dermatitis and Oedema (EDO)** are examples of acute skin disease with a predominance of eosinophils. CEF is characterised by acute onset of lesions over the bridge of the nose, muzzle and periorcular skin (rarely the limbs or glabrous skin of the trunk). Lesions consist of papules, nodules, crusts and exudative plaques. It may be pruritic or painful and the patient may be well or systemically ill. Pathogenesis is not proven, but suspected to be triggered by insect bites, possibly when ‘digging’. There is no predilection signalment, but young large-breed dogs are over-represented. History, clinical signs and cytology (eosinophils +/- secondary bacterial infection) is usually diagnostic. Cases respond well and promptly to steroids and recurrence is rare. EDO is characterised by acute onset of generalised skin disease. Lesions consist of erythematous macules that progress to wheels and coalesce into arciform or serpiginous plaques particularly over the trunk and pinnae +/- localised to generalised oedema; lesions may/not blanch on diascopy. Pathogenesis is unknown but some cases have gastroenteritis (GE) disease concurrent or prior to skin lesions and along with adverse drug eruption (treatment of GE) is a possible trigger. Confirmation is on histopathology. Usually respond within 1-2 weeks to drug withdrawal (if appropriate), steroids +/- anti-histamine +/- appropriate therapy for GE.

**Erythema Multiforme Complex** consists of Erythema Multiforme (EM), Steven Johnson’s Syndrome (SJS) and Toxic Epidermal Necrosis (TEN). There may be a trigger e.g. drug eruption, neoplasia or infection or idiopathic. Clinical signs are variable depending on the classification and true ‘target lesions’ are rare. In EM, lesions consist of bilateral symmetrical erythematous macules and papules forming annular or arciform patterns, urticarial plaques, bullae, vesicles, ulcers and crusts localised to the ventrum (groin and axillae), pinnae, MCJ, oral mucosae and footpads. Eruption of the mucosae (particularly oral) and footpads is reported in SJS/TEN. Pain can be marked. Idiopathic ‘old dog’ EM tends to affect the face and pinnae. The criteria for clinical classification is based on the presence of lesion (flat/raised, focal/multifocal, target/polycyclic), number of mucosal surfaces affected and the percentage of skin/mucosae involved +/- detached. Unless the diagnosis is EM minor, patients are usually systemically ill. The diagnosis is suspected on history and clinical examination, but confirmed by histopathology. Close examination of the patient’s drug history or further investigation for systemic disease e.g. bloods, urine, imaging etc. may reveal the trigger. Immune-suppressive therapy, drug withdrawal, supportive care and/or treatment of an underlying disease may be required.

**Vasculitis** is a cutaneous reaction pattern for a number of clinical syndromes. There are numerous reported triggers e.g. drug reaction, systemic disease (infectious, inflammatory, connective tissue), genetics or neoplasia or idiopathic. Usually only cutaneous, but in certain syndromes other organs may be affected e.g. Cutaneous and Renal Glomerular Vasculopathy. Cutaneous lesions occur over pressure points, extremities (pinnae, claws, pads, elbows, tail-tip), scrotum and oral mucosae. Lesions include non-blanching erythema (purpura), papules, plaques, bullae, ulcers, oedema, urticaria, nodular panniculitis, haemorrhage, cyanosis and alopecia. Confirmatory diagnosis is by histopathology, but this can be difficult. Thorough drug history and investigation for systemic disease as for EM may find a trigger.

**Necrotising fasciitis** (NF) and **Post-Grooming Deep Pyoderma** are examples of infectious dermatological emergencies. NF is an acute and serious life-threatening infectious disease requiring prompt treatment with the appropriate antibiotic. It consists of a
necrotising lesion with sepsis +/- toxic shock. Historically it was linked with bacterial toxins of *Streptococcus canis*, but other organisms e.g. *S. pseudintermedius*, *P. aeruginosa* or other gram-negatives may be responsible. The skin lesion is severely painful and necrotic and is rapidly progressive. It is usually localised but widespread e.g. a limb. Once the patient is stable and the disease process halted, surgical debridement and repair are required. PGDP is an acute onset of severe back pain. *Pseudomonas* spp. were originally reported, however other gram-negative bacteria may be involved. Infected shampoo combined with microtrauma e.g. grooming whilst wet, appear to be the cause. Diagnosis is by compatible history and clinical signs (histopathology supportive). Treatment should commence based on cytology and changed accordingly once culture and susceptibility results are received.

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Reconstruction of large skin defects after tumour excision

N.J. BACON*†
* Fitzpatrick Referrals Oncology and Soft Tissue, Guildford, Surrey, UK
† University of Surrey School of Veterinary Medicine, Guildford, Surrey, UK

Removal of skin and subcutaneous tumours makes up a large percentage of a practice surgical caseload. The cornerstone of oncologic management for almost any tumour remains ‘What is it? Where is it? What can we do about it?’ and this is especially true for large skin masses. Owners focus almost entirely on the third part whereas we can rarely answer that satisfactorily without paying attention to the first two.

What is it?

Few cutaneous masses are identifiable by sight alone. Cutaneous adenomas are often around the feet and face in older dogs and are the classic old dog ‘wart’. Histocytomas often suddenly appear as hairless raised red cutaneous ulcerated 1-1.5cm diameter masses in younger dogs. Even these two however may be misinterpreted as something else – a mast cell tumour could easily appear as both for example. When discussing ‘large’ skin masses however, their identity is key to treat appropriately. Large cutaneous masses should have a fine needle aspirate (FNA) first as the majority of cutaneous and subcutaneous masses will exfoliate cells easily (lipoma, adenoma, mast cell tumour, histiocytoma, plasmacytoma, lymphoma…..). Soft tissue sarcomas, which make up 15% of subcutaneous masses, often give a very low yield of cells in an aspirate, and a slide with little cellular material on it after staining should raise suspicion of this tumour type. A FNA is usually sufficient information to proceed to a surgical plan. Occasionally a tumour grade is desirable before surgery but in my experience this is the exception rather than the rule, and often links in with a need for more information regarding prognosis after surgery, ie differentiating between intermediate (grade 2) and high grade (grade 3) tumours. The majority of mast cell tumours and soft tissue sarcomas however are grades 1 and 2 and they can be assumed to be this if biological behaviour and natural history seems appropriate. If more tissue is needed then a Tru-cut is preferential in terms of ease, cost and morbidity (sedation only), but incisional biopsies are preferred by some. Incisional biopsies of masses suspected to be cutaneous mast cell tumours is generally a bad idea as mast cell granule release of proteases, heparin and histamine can all combine to make a non-healing wound.

Where is it?

For the large tumours in question this relates to both anatomic location and surgical constraint of key local structures, and staging of the tumour, ie whether there is regional nodal disease or distant metastasis. Staging tumours depends on the cell type; mesenchymal (sarcomas) have predominantly haematogenous spread and frequently go to lungs first; epithelial (carcinomas) have both lymphatic and haematogenous dissemination and so local lymph nodes as well as parenchymal abdominal organs, bone and lungs are possible sites of metastasis, and round cell tumours (melanoma, mast cell, plasma cell, lymphoma, histiocytic) have a pattern of spread more like carcinomas, with individual tumour idiosyncrasies (eg mast cell tumours rarely go to lungs). The location of the tumour becomes increasingly important on the extremities, including the elbow or stifl e or below, around the neck and face, and near the tail head or perineum. The lack of large amounts of skin, and sometimes lack of deep fascia, means both resection and reconstruction can be problematic. Difficulties in resection (eg proximity to eye, local nerves, digits, pinna, vulva etc.) mean conversations with the client need to occur ahead of surgery to determine where their priorities lie (cure versus cosmesis often being the greatest), plus also explain to the owner how you anticipate closing the defect, ie large clip for local flaps, skin graft, sacrificing the tail to use the skin etc....

What can we do about it?

When it finally comes to discussing surgery the broad principles of surgical ‘intent’ are curative versus palliative and these are
further grouped into intralesional (cutting into the tumour, gross tumour behind), marginal (shelling out), wide (margin of healthy tissue) and compartmental/radical (less commonly used in animals, often includes bone, limbs, multiple tissue types). Each of these can be explained to the owner in the context of the specific mass, and the pros/cons of each outlined in terms of the risk of local recurrence.

Consideration of the above will lead to a discussion including:
- identity of the mass
- likely local and systemic behavior without surgery
- stage now or stage later depending on clinical impression and owner resources
- role of pre-operative radiation (perhaps none)
- role of neo-adjuvant chemotherapy
- role of neo-adjuvant steroids (e.g. mast cell tumours)
- what is known about surgical margin for the tumour type
- outcomes of surgery depending on surgical ‘intent’
- plan A, B, C..., to close the resulting defect
- what to expect from the pathology report
- role of post-operative radiation
- role of adjuvant chemotherapy
- outlook for local control and the plan in the event of local recurrence
- outlook for systemic metastasis and overall survival

Specifics of surgery
Resection and reconstruction;
- wider clip than anticipated to factor in change of plan once tumour excised
- grab skin at proposed margins and determine degree of movement
- marker pen for the skin to mark out palpable tumour and then intended surgical margins
- sharp dissection of mass
- cautery to minimize blood loss and haematoma at surgical site
- consider exsanguinating tourniquet for tumours of digits/pads
- identify deep fascia or metric margin (distance) and dissect vertically down
- suture edges of resection to avoid tissue slip and keep resection en bloc
- examine specimen once removed to identify any areas where excision was too close to mass
- resect more from tumour bed if indicated
- close as simply as possible
- avoid large flaps unless confident high chance of curative excision performed
- avoid surgical drains
- mark excised tissue with India ink in areas of concern for pathologists to inspect

Conflict of interest: none relevant to this lecture.
Notes
Surgical to treat tumours of the digits and feet

N. J. BACON*†
* Fitzpatrick Referrals Oncology and Soft Tissue, Guildford, Surrey, UK
† University of Surrey School of Veterinary Medicine, Guildford, Surrey, UK

Tumours of the digits commonly encountered include melanoma, osteosarcoma, squamous cell carcinoma, mast cell tumour, haemangiopericytoma, and other malignancies. It is normally only affecting one toe, but very occasionally several can be involved. Surgery is the mainstay of therapy and positively impacts survival. Other factors such as patient age, tumour type, site and stage were not as important as a curative-intent surgical procedure and so that is where attention should be focused. Concern exists in surgery of the foot regarding appropriate margins to take, and how the resulting amputation is likely reconstructed. A number of options exist for surgery ranging from removal of P3 through to partial foot amputation. Staging is recommended prior to surgery and consists of foot radiographs, local lymph node palpation and aspiration, and chest radiographs. Techniques which will be covered in the lecture include;

BIER block
Named after August Bier, this is an intravenous regional anaesthesia technique in which the foot is anaesthetized for digit surgery. A distal vein is catheterized, the foot ex-sanguinated by use of an Esmarch tourniquet secured just proximal to the carpus or the hock, and then local anaesthetic solution instilled intravenously to infuse the tissues of the foot. The tourniquet ensures the local anaesthetic does not leave the foot, as well as ensuring a bloodless field to work in, making for faster more precise surgery. The beauty is also patients do not need full general anaesthesia, but heavy sedation is often sufficient. In an elderly patient, this can offer some real advantages and allows the surgery to performed on an out-patient basis. It is a useful, versatile, easily learnt technique.

Partial foot amputation
It is widely believed that only lateral toes can be removed, and amputation of even one central toe will result in lameness and decreased function. Partial foot amputation is used in reference to removal of typically two or more toes, including, at times, the two central toes. When this is performed, the remaining lateral and medial toes are partially filleted and the remaining skin dorsally and palmar/plantarly sutured together, including the pads.

Phalangeal fillet
A technique to preserve the skin over the digit, when it is not necessary to be excised in terms of achieving margins. It can be preserved with as wide a base as possible and used to rotate into defects or to cover exposed phalangeal bone.

Pad resection
Mast cell tumours, fibrosarcomas and melanomas and other malignancies are seen within pad tissue. The dense fibrous nature of pad tissue means that achieving margins can be simpler than expected even with limited pad ‘space’. Reconstruction techniques for pads include partial excision and fusion, undermining, and simple resection/repair.

Separation podoplasty
Typically when the central toes are removed, this is an alternative to the fusion podoplasty described above, and places less axial stress on the metacarpo/metatarso-phalangeal joints than the fusion. Concerns exist re function given the subsequent ‘splaying’ of the foot, but walking and running are still possible and largely normal.

Conflict of interest: none relevant to this lecture.
Help the pathologist to help you

C. BRACHELENTE
Department of Veterinary Medicine, University of Perugia, Italy

Skin biopsy represents a specific step of the correct diagnostic procedure. A biopsy is indicated when inflammatory diseases must be differentiated from neoplasia, when skin lesions have an unusual or atypical presentation, when they fail to respond to an apparently appropriate therapy or when new lesions appear during the course of therapy. Once the clinician has decided to take a skin biopsy, the choice of the area to be sampled can tremendously influence the outcome and the reliability of the histopathological diagnosis. The ideal situation would be to biopsy primary lesions (i.e. papules, pustules, nodules) in an active and florid stage, because these are the most diagnostic, whereas secondary lesions are often representative of complications and, sharing common features, are less diagnostic. For lesions that can be either primary or secondary, a good history can help the pathologist to interpret the findings. If there is variability in the clinical picture, more than one biopsy from lesions at different stages of development or from different body sites should be taken. Secondary lesions should be sampled when primary lesions are absent or when they represent a significant component of the disease. Crusts can be useful because they can reveal the presence of acantholytic cells or fungi. The sampling technique has also an impact on the outcome and the reliability of the histopathological diagnosis. Biopsy punches of 6 or 8 mm in diameter should be used rather than 4 mm ones. The latter should be restricted to areas such as mucocutaneous junctions, paw pads and margin of the pinnae. The rule of taking 50% lesional and 50% normal skin should not be followed in biopsy punches as this can lead to an insufficient lesional tissue to be analyzed. Even if a scalpel biopsy is taken, a good compromise would be to have 30% normal and 70% lesional skin. Biopsy punch technique should be used with caution for fragile lesions such as vesicles or pustules or deep lesions such as panniculitis, since sampling may cause rupture of the lesions or be insufficiently deep, respectively. For depigmented lesions, gray areas or the margin between pigmented and non-pigmented areas should be collected. For ulcerative lesions, one should collect the sample at the margin of intact skin extending into the ulcer (i.e. borders of a squamous cell carcinoma), but also the ulcer bed in its deepest region (i.e. vasculitis). For alopecic/hypotrichotic disorders, one should collect a range of samples from areas where the alopecia/hypotrichosis is most pronounced, to areas where it is less pronounced to normal haired skin. When multiple biopsies are taken, these should be fixed and labeled separately. On histopathological examination, samples are then classified according to a pattern analysis approach. Sometimes, the findings observed at the histopathological examination are not unique or pathognomonic of a single diagnostic picture and are therefore associated with a list of differential diagnoses. In other cases, histological findings cannot match or cannot explained the clinical signs described by the dermatologist. In all these cases, a clinical correlation is essential, as the correct diagnosis may require additional clinical information. Because clinical features usually are not available to the dermatopathologist when evaluating histologic specimens, it is important for clinicians to include as much information as possible in the pathology submission form, including clinical photographs or digital images.

Conflicts of interest: None to declare
Management of otitis: what are good practice recommendations?

L.M. BUCKLEY
Small Animal Teaching Hospital, Institute of Veterinary Science, University of Liverpool, UK

The management of otitis can be an onerous task due to complicating factors such as chronic inflammatory changes, multi-drug resistant bacterial infection and otitis media, and underlying diseases such as allergy, causing disease recurrence. Despite the difficulties, successful long-term management of otitis is possible, provided a treatment plan is established that targets the specific pathogenesis for individual cases. The pathogenesis of otitis may be split into primary and secondary causes and predisposing and perpetuating factors. Management of otitis starts therefore, with the identification of each of the contributing causes and factors. The management plan is then like following a recipe; a clear, step-wise process but each step being essential for a successful outcome.

This lecture covers an approach to the management of otitis including uncomplicated otitis externa and chronic otitis media. Options for treating multi-drug resistant bacterial otitis and cases where surgical management may be indicated will be discussed.

Secondary causes and perpetuating factors are the reason animals are presented for treatment. They cause considerable irritation and/or discomfort and therefore require prompt resolution. Microbial infection is the most common secondary cause and develops due to the altered ear canal environment resulting from primary causes and perpetuating factors. Microbial infection in many cases may be eliminated using otic cleaning or lavage to remove infectious debris and anti-inflammatory treatment to resolve ear canal pathology. The choice of antimicrobial agent is determined by cytological assessment of the otic discharge. If no microbes are identified then treatment is focused on resolution of inflammatory changes. Culture and susceptibility testing of the external ear canal is not useful for confirming/reassessing the presence of infection due to the possibility of identifying insignificant bacteria. Topical antimicrobials are likely to be more effective than systemic drugs due to 100-1000 fold higher concentration achieved with topical compared to systemic administration.

For most cases, perpetuating factors include chronic/recurrent epithelial and glandular inflammation leading to progressive pathological change. End-stage pathology such as epithelial fibrosis and mineralisation of cartilaginous tissue may require surgical management. Topical and/or systemic corticosteroids are the anti-inflammatory drugs of choice for management of most otitis cases. They decrease glandular secretions and epithelial exudation, reduce scar tissue and proliferative changes and are anti-pruritic.

Predisposing factors are unable to cause disease in their own right but should be addressed where possible to improve treatment outcomes. Systemic diseases causing debilitation or immune suppression, obstructive ear disease and inappropriate cleaning are potentially curable. For the remaining perpetuating factors steps should be made to reduce their impact e.g. additional topical management for regular swimmers and those with abnormal otic conformation.

The long-term control of primary factors is an essential part of management that is easy to overlook. Following the resolution of secondary causes and perpetuating factors, primary factors are those which lead to recurrent otitis episodes. The most common of these is allergic skin disease and, although different primary factors require different management strategies, like allergic disease, very few are curable, meaning otitis cases usually require life-long maintenance therapy to reduce disease recurrence.

Conflict of interest: none.
Notes
40 minute essential guide to hormonal hair loss

V.M. SCHMIDT
Institute of Veterinary Science University of Liverpool, Leahurst Campus, Neston, UK

Introduction: spontaneous alopecia can be classified as inflammatory or non-inflammatory. Inflammatory alopecia’s are caused by infections/infestations or immune-mediated. Non-inflammatory alopecia’s are due to defective hair synthesis or hair cycle arrest. Causes can be excluded, suspected or deprioritised based on history, clinical examination and basic tests. More advanced testing may follow, however achieving a definitive diagnosis can be extremely challenging.

Infections/ infestations:
- superficial pyoderma
- demodicosis
- dermatophytosis
- leishmaniosis

Immune-mediated:
- Alopecia areata
- sebaceous adenitis
- dermatomyositis

Defective hair synthesis:
- congenital alopecia
- pattern baldness
- colour dilution
- follicular dysplasia
- anagen defluxion

Hair cycle arrest:
- telogen defluxion
- canine flank alopecia
- post-clipping alopecia
- injection alopecia
- paraneoplastic alopecia
- pituitary dwarfism
- endocrinopathies
- Alopecia X

Endocrinopathies: The hair cycle
- anagen (growth phase) to telogen (resting phase). Hormones e.g. thyroxine promote anagen, and excessive hormones e.g. steroids/oestrogens suppress it. Hairs cycle into telogen and are gradually lost; first lost from frictional e.g. collar, dorsal muzzle, ventral tailhead and flanks, but it may generalise. The presence of typical clinical signs and abnormalities on routine bloods and urine and exclusion of the differentials are paramount to diagnosis. Function tests should only be performed in such cases.

Hyperadrenocorticism:
Pathogenesis: functional pituitary or adrenal tumour or iatrogenic steroid excess.
History: common signs include PU/PD, polyphagia, panting, hepatomegaly and pendulous abdomen, muscle weakness and systemic hypertension. Usually in a middle-old-aged dog (range 2-16; median 7-9 years); higher frequency in females. All breeds and crosses can be affected, but predispositions in Bostons, Boxers, Dachshunds and Poodles.
Clinical examination: non-pruritic truncal alopecia, hyperpigmentation, comedones, poor regrowth, hypotonic skin atrophy and striae, poor coat quality/lightening, seborrhoea, prominent vessels and bruising, phlebectasia, milia, poor wound healing and calcinosis cutis +/- signs due to pyoderma, Malassezia dermatitis or demodicosis.
Cytology: secondary pyoderma, yeasts, demodicosis or calcinosis cutis.
Urinalysis: hyposthenuria (<1.008; <= 1.018-1.020), proteinuria, calcium oxalate crystals +/- urinary tract infection +/- diabetes mellitus; UCCR can exclude as 99% sensitivity, but poor specificity (collect morning samples at home to improve this).
Haematology: stress leucogram, thrombocytosis and mild erythrocytosis.
Biochemistry: increased ALP; raised ALT, cholesterol, triglycerides and glucose.
Function tests: non-adrenal illness increases false-positives. ACTH stimulation 85-87% sensitivity for PDH and 50-61% for ADH; good specificity (64-86%) and choice if co-morbidities e.g. DM; LDDST greater sensitivity up to c. 97% (95% PDH and >95% for ADH), but poor specificity (44-73%) particularly with non-adrenal illness (can differentiate between PDH and ADH c.30% dogs); test of choice for spontaneous HAC.
Imaging: abdominal ultrasound may be supportive of HAC; differentiates PDH from ADH. MRI if neurological signs for pituitary mass.
Atypical (or occult) Cushing’s is a term to account for patients with compatible clinical signs/routine bloods, but function tests are within normal limits. It is suspected to be associated with an overproduction of adrenal sex hormones, but never proven.
**Hypothyroidism:**
Pathogenesis: 90% acquired lymphocytic thyroiditis/necrosis and atrophy.
History: Weight gain, lethargy, mental depression, heat seeking, weakness/myopathy, reproductive abnormalities or neurological signs; may be completely normal with only dermatological signs. Any breed or cross, but increased frequency in e.g. Golden retrievers, Labradors and Dobermans. No sex predilection, but neutered animals may be at higher risk. Age onset 6-10 years, but earlier e.g. 2-3 years in large/giant/predisposed breeds.
Clinical examination: dull, dry and brittle coat and lightening, post-clipping alopecia, alopecia of frictional areas (rat tail) to full truncal alopecia, poor wound healing/easy bruising, scaling, seborrhoea and hyperpigmentation, tragic facial expression (myxoedema), clinical signs of secondary infections or demodicosis; bradycardia, ocular abnormalities e.g. corneal lipidosis. Cytology: NAD or telogenisation of hairs, secondary skin/ear infection or demodicosis. Urinalysis: NAD; proteinuria particularly if chronic pyoderma.
Haematology: mild chronic non-regenerative anaemia (normocytic, normochromic) in c.30% cases; decreased platelet/clotting factor function and bleeding.
Biochemistry: raised cholesterol (50-75% cases), marked CK (<50% cases) and mild-marked raised ALT/ALP.
ECG: NAD, bradycardia or arrhythmia.
Function tests: T4/TSH (specificity 90-100% compared to 75% if T4 alone) and/or fT4 (especially if non-thyroidal illness suspected); euthyroid sick syndrome and many drugs can influence.
Imaging: ultrasound +/- other modalities may help differentiate from nonthyroidal-sick-syndrome.

**Hyperoestrogenism:**
Causes: intact male dogs with functional testicular tumour, usually Sertoli cell; intact female dogs with cystic ovaries or rare functional ovarian disease; any dog with oestrogen administration or inadvertent contact e.g. oestrogen cream.
*Sertoli cell tumour*
History: 10X more likely if cryptorchid; older intact dog, gradual onset of alopecia (if rare bone marrow suppression then weakness, bleeding etc.).
Clinical examination: alopecia of perineum, inguinal and flanks, comedones, seborrhoea, macular melanosis/hyperpigmentation, testicular enlargement/palpable lesion (but may be palpably normal), linear preputial erythema, pendulous prepuce, feminisation syndrome, concurrent prostatic hypertrophy/prostatitis.
Cytology: NAD or secondary skin infection.
Urinalysis: may be NAD or consistent with prostatitis etc.
Haematology: rare myelosuppression (thrombocytopenia, neutropenia, anaemia)
Biochemistry: NAD.
Imaging: testicular lesion that can be sampled (+/-local lymph nodes) and thoracic radiographs (metastasis rare).
Histopathology: confirms diagnosis.

**Conflicts of interest:** none to declare.
Hair’s gone, normal bloods, now what?

V.M. SCHMIDT
Institute of Veterinary Science
University of Liverpool, Leahurst Campus, Neston, UK

A good history can make some diseases more or less likely than others, for example:

Telogen defluxion: stressful event causes hairs to synchronise into telogen and shed 1-3 months later; triggers include pregnancy, lactation, severe illness/fever, drugs/surgery.

Anagen defluxion: immediately following a stressful event e.g. chemotherapy, infectious, endocrine or metabolic disease damages the growing follicle; alopecia is patchy to generalised. Rare in dogs.

Post-clipping alopecia: regrowth takes several months (normal <=3 months), usually plush coated; exclude endocrine/follicular dysplasia if suggestive.

Pattern baldness: unknown pathogenesis, slowly progressive miniaturisation of hairs in young dogs (6 months-1 year); several short-coated breeds at bilateral symmetrical localisations e.g. Greyhound caudal thigh alopecia, Dachshund and Yorkshire Terriers convex pinnal alopecia and hyperpigmentation.

Follicular dysplasia: familial disorders that may be colour-coat-linked e.g. Black Hair Follicular Dysplasia (BHFD) with alopecia of black patches in puppies (defect in melanisation) OR non-colour-linked in young adult dogs of certain breeds e.g. Irish water spaniels, Portuguese water dog, Curly coat retriever or Airedale, dysplastic follicles; hairs fracture with progressive alopecia (trunk, neck and proximal limbs).

Canine flank alopecia:
Pathogenesis: unknown; localised, cyclic follicular dysplasia; day length trigger.
History: usually 3-6 years, but range 1-11 years; many breeds, but Boxers, Airedales, British bulldog and Schnauzers common.
Clinical examination: non-inflamed (unless secondary pyoderma) alopecic patches over the flanks and dorsum with well-defined linear or scalloped/serpiginous borders; skin is hyperpigmented; alopecia may be sporadic, recurrent or continuous; regrowth is variable but usually within 3-8 months; new hairs are usually darker or a different shade and/or texture. Surrounding haircoat and skin usually normal (contrast to endocrine alopecia).
Cytology: NAD unless secondary pyoderma.
Histopathology: confirmatory ‘witches feet’ (follicular atrophy and infundibular hyperkeratosis); diagnosis often on history and clinical.

Alopecia X:
Pathogenesis: follicular arrest of unknown cause; local hair follicle factors e.g. hormone/receptor/growth factor problem?
History: Pomeranians, Chow, Keeshonds, Alaskan malamutes, Samoyeds, miniature and toy poodles and others. Age range 1-10 years; no sex or neuter status predilection. Neutering may resolve clinical signs in intact animals, but relapse within 1-2 years.
Clinical examination: dull and dry coat, primary hairs lost first (‘puppy coat’) with progression to total truncal alopecia and hyperpigmentation; frictional areas worse affected.
Urine/bloods: NAD.
Histopathology: excludes other inflammatory causes; consistent with endocrine +/- follicular dysplasia; supportive not diagnostic. Hair growth at the biopsy sites.

Colour dilution alopecia:
Pathogenesis: unknown, genetic defect in melanisation (transport and storage) in dilute colour coated dogs (blue or fawn). Dilute hairs have enlarged pigment granules (macromelanosomes) causing fragility, fracture and loss; progressive and with time follicular activity stops.
History: colour dilute breed with alopecia +/- pruritus. Age of onset 6 months-3 years. Clinical examination: patchy hypotrichosis/alopecia, scaling +/- secondary bacterial pyoderma; usually starting dorsal trunk +/- recurrent pyoderma. Cytology: trichograms show macromelanosomes and distorted/fractured hairs. Histopathology: is supportive.

Sebaceous adenitis:

Conflicts of interest: none to declare.

Notes
Decades ago, therapeutic options were limited in cases of immune-mediated diseases, like atopic dermatitis or autoimmune disorders. A new era started with the introduction of systemically and topically used glucocorticoids because of their anti-inflammatory, antiproliferative and immunosuppressive effects. If glucocorticoids are used only because of their anti-inflammatory potency, the immunosuppression should be an undesired (side) effect. However, if treating immunological disorders, the immunosuppressive and antiproliferative effect (especially on inflammatory cells) is desired. Based on the rapidly increasing knowledge on immune functions and the pathophysiological involvement of the immune system in skin disorders, various new immunomodulatory compounds (i.e., ciclosporin, oclacitinib and lokivetmab) were introduced in the veterinary drug market broadening the spectrum of options for the treatment of immune-mediated diseases. Initially, the peptide ciclosporin was introduced as an immunosuppressive drug for the use in organ transplantation in humans. Ciclosporin, a calcineurin inhibitor, has a selective inhibitory effect on lymphocytes, suppressing their cellular response to antigenic and regulatory stimuli and inhibiting the production of cytokines. Since many years, ciclosporin is registered as a veterinary drug for the treatment of dogs with atopic dermatitis. In contrast to human beings, ciclosporin is well-tolerated by dogs. Oclacitinib is the first Janus kinase (JAK) inhibitor approved for veterinary use against itch caused by atopic dermatitis. Oclacitinib is a selective inhibitor of the Janus kinase 1 (JAK 1). The JAKs (JAK1, 2, 3 and TYK2) play a pivotal role in the intracellular signaling of hematopoietic and immune cells. Upon cytokine binding, the JAKs autophosphorylate thus stimulating their kinase activity. The activated JAKs phosphorylate associated receptors generating binding sites for specific downstream proteins (STATs). As a JAK 1 inhibitor, oclacitinib interacts therefore with the signaling pathway of numerous cytokines (e.g. IL-4, IL13, IL-31). The drug is well tolerated by dogs. Nevertheless, it should be considered that a large number of cytokines including the IL-6 family signal through receptors that rely on JAKs signal transduction which might be blocked by JAK inhibitors. In 2016 (USA) and 2017 (Europe), the monoclonal antibody lokivetmab against canine IL-31 was registered for the treatment of atopic dermatitis in dogs. The IL-31 cytokine is involved in the “pruritic circle”. The antibody is administered subcutaneously with a duration of 30-60 days. The duration of therapeutic action is about two months. Beginning with the prefix (lo in lokivetmab), the name of the therapeutically used antibodies describes their origin and therapeutic use. The word lo-ki–vet–mab contains the information that it is a monoclonal antibody (mab) for veterinary purpose (vet) and interacts with a cytokine (ki). The increasing knowledge on immune functions explains the rapid development in the field of immunopharmacology. We can expect further innovations for the development of new principles (e.g. monoclonal antibodies) for the treatment of immune-mediated diseases in animals.

Conflicts of interest: The author declares that there are no conflicts of interest.
Mast cells and eosinophilic skin diseases in dogs, cats and horses I – II

C. BRACHELENTE
Department of Veterinary Medicine, University of Perugia, Italy

Mast cells (MCs) derive from CD34+ precursor cells from the bone marrow that circulate in the blood and migrate to peripheral tissue where they differentiate into mature mast cells. Mast cells are the major cell population involved in different mast cell-driven diseases such as urticaria, acute and chronic type I allergic reactions and mastocytosis. Many “mastocytic skin diseases” are recognized in veterinary medicine: indeed, mast cells, often in association with eosinophils, are the predominant inflammatory cells in a variety of skin diseases of dogs, cats, and horses such as urticaria and angioedema, atopic dermatitis, food hypersensitivity, flea/bite/insect bite hypersensitivity and cutaneous mastocytosis (urticaria pigmentosa). All these diseases are considered mast cell-driven as mast cells can be identified in increased numbers in lesional skin of affected animals. However, despite recognizing their presence and their increase in number in these diseases, understanding what is their exact role and contribution to the development of these diseases is more challenging. Eosinophils are granulocytes that have a major role in allergic reactions and parasitic infections. Their migration and chemotaxis to tissue and sites of inflammation is influenced by interactions between endothelial cells and eosinophils, as well as eosinophils and peripheral tissues. Eosinophils destroy parasites and remove foreign material/inciting antigens by phagocytosis and degranulation of enzymes and toxins contained in their granules and by releasing extracellular traps. A number of diseases characterized by a prominent eosinophilic inflammation and referred to as “eosinophilic skin diseases” are recognized in dogs, cats, and horses. Examples of these are: the eosinophilic granuloma complex, the eosinophilic folliculitis and furunculosis, the eosinophilic dermatitis and oedema (CAEDE, Wells-like syndrome) in dogs; the miliary dermatitis, the eosinophilic granuloma complex, the mosquito bite hypersensitivity, and the ulcerative dermatitis caused by feline herpesvirus 1 in cats; the insect-bite hypersensitivity, the angioedema and urticaria, the eosinophilic granuloma and the multisystemic eosinophilic epitheliotropic disease in horses. However, similarly to what has been discussed for the mast cells, the exact role of eosinophils in the different diseases they are associated with is not clear. The current perspective is therefore that, according to an eosinophil-related pathophysiology, numerous disorders may benefit from therapies targeted to reduce or eliminate eosinophils. More targeted therapeutic approaches have indeed lead to the use, in association or in replacement with traditional steroidal and non-steroidal anti-inflammatory drugs, of selective immunomodulating biological substances (i.e. antibodies targeting Th2 cell signalling pathways, pruritic pathways, and inflammatory pathways). The application of similar strategies is beginning to be the subject of research and reporting of therapeutic protocols for the treatment of mast cells and eosinophilic skin diseases in veterinary medicine as well.

Conflicts of interest: None to declare
Strategies to Manage Antibiotic-Resistant Infections

M.G. PAPICH
College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Antibiotic-resistant bacterial infections present significant challenges to veterinarians, particularly dermatologists. Many guidelines have appeared in published proceedings, review papers, consensus documents, and textbooks to treat these infections; but, when empirical treatment fails, or when resistance is suspected, a culture and susceptibility test is needed to guide therapy.

The bacteria that cause the most problems for dermatologists because of clinical resistance are: Methicillin-resistant Staphylococcus spp., most often S. pseudintermedius (MRSP), Pseudomonas aeruginosa, and occasionally bacteria of the Enterobacteriaceae (E. coli, Klebsiella pneumoniae, Enterobacter spp. and Proteus spp.). Enterococci (Enterococcus faecium, Enterococcus faecalis) may be cultured in some infections, but are often disregarded unless there is evidence of clinical infection.

The problem with the resistant bacteria listed above is that when tested using the approved CLSI breakpoint, they often show clinical resistance to commonly available veterinary-approved antibiotics. When an isolate is resistant to an approved veterinary antibiotic, the clinician may have to consider a human antibiotic extra-label (off-label). If the amenable to topical treatment (antibacterial shampoo, rinses, sprays, etc.) and owner can comply, this option should be explored.

For methicillin-resistant Staphylococcus spp. the antimicrobial agents often demonstrating activity against veterinary isolates are chloramphenicol, aminoglycosides (e.g., gentamicin, amikacin), rifampin, and occasionally a tetracycline (minocycline). These agents have issues with adverse effects and easy-of-use, and staphylococcal isolates are not consistently susceptible to tetracyclines. Other less-often used agents for these infections include vancomycin (prohibited in some regions) and linezolid.

Resistant strains of Pseudomonas aeruginosa, may require injectable antibiotics, as the only oral agents active against susceptible strains of this organism are fluoroquinolones. The injectable agents with activity may include amikacin (not gentamicin), ceftazidime, piperacillin-tazobactam, or a carbapenem (e.g., meropenem). Because many drug-resistant infections occur in the ear, topical therapy is preferred if possible.

Resistant bacteria of the Enterobacteriaceae may be resistant to the commonly used fluoroquinolones, amoxicillin-clavulanate, and cephalosporins. Some of these isolates may be producers of extended-spectrum beta-lactamase (ESBL). Often the only systemic antibiotic active against these strains is amikacin or a carbapenem.

**Conflict of Interest:** The author has no conflicts of interest relating to the medications mentioned in this presentation.
Pharmacokinetics of veterinary drugs with transdermal delivery

M. KIETZMANN
Institute of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Foundation, Germany

To reach deeper skin layers and the systemic circulation, drugs have to pass various skin layers. The main barrier is the stratum corneum, like a brick and mortar wall containing corneocytes (brick) and lipids (mortar). While lipophilic and non-ionised agents may easily penetrate the stratum corneum, this barrier is only poorly (or not) permeable for hydrophilic and ionised compounds. The diffusion via the horny layer is significantly influenced by the pharmaceutical formulation. The formulation is releasing the active compound (liberation) which penetrates thereafter the stratum corneum. It has to be considered that the stratum corneum is not only a barrier but also a reservoir for topically administered lipophilic compounds. The viable part of the epidermis and the dermis are permeated mainly by diffusion. Taken together, the physical and chemical properties of topically administered compounds as well as the pharmaceutical formulation are determining factors of the clinical efficacy. Transdermal therapeutic systems were developed to achieve the influx of a compound throughout a defined treatment period. This influx can be described as a zero-order input. The elimination can be described by means of as a first-order process. Modern transdermal delivering systems formulations are patches containing the active ingredient in different pharmaceutical formulations, for example formulated as a microemulsion. The direct contact between patch and skin must be guaranteed over the time of administration. Therefore, the adhesive compounds of patches are important. Species differences have to be considered also. Patches developed for human use are not always suitable for animals. The absorption rate of a topically administered compound depends on various parameters. Examples are the properties of compound and formulation, inclusion of penetration enhancers in the formulation as well as patch size and others. Additional examples for veterinary drugs with transdermal delivery are antiparasiticides formulated as spot-on or pour-on formulations as well as collars.

In summary, essential pharmacokinetic factors of topically administered drugs are

- the liberation from the pharmaceutical formulation
- the penetration into and/or through the horny layer
- the permeation through the skin (epidermis, dermis)
- a possible biotransformation in the skin
- the absorption, systemic distribution and elimination

Conflicts of interest: The author declares that there are no conflicts of interest.
Journal Club 2

L. FERRER
Department of Animal Medicine and Surgery, Universitat Autònoma de Barcelona, Barcelona, Spain

Therapy with mesenchymal stem cells: cutting-edge therapy or snake oil?
Recent publications (see, for instance, Villatoro et al, Vet record 2018) have shown good results in the treatment of immune-mediated skin diseases with mesenchymal stem cells and have triggered a certain euphoria. Stem cells seem to be the ideal therapy: safe, effective and accessible. However, there are numerous critical voices pointing to many dark areas in relation to this therapy. Some even consider stem cell therapy (SCT) an alternative medicine, closer to homeopathy than to evidence-based personalized medicine.

In this JC we will discuss key publications on SCT and critically assess this new therapy.

Conflict of interest: none relevant to this Journal Club.
Notes
Times They Are A-Changing: clinical metagenomics and the culture-independent diagnosis of infectious diseases.

The advent of next-generation sequencing (NGS) technologies in 2005 jump-started the metagenomics field. For the first time, millions to billions of reads could be generated in a single run, permitting analysis of the entire genetic content of a clinical or environmental sample. This approach allowed the characterization of the microbial communities of the intestinal tract or of the skin, for instance. However, it is important to underline that these studies were carried out by amplifying and sequencing relatively short fragments of the genome of the microorganisms. This fact limited the precise identification of the microorganisms (beyond the genus) and the detailed knowledge of its biology (resistance, pathogenic factors). The focus of the studies was in the microbial community (abundance, diversity). The recent development of techniques that allow a whole genome sequencing (WGS) in a fast and affordable way (e.g. Nanopore technologies) are revolutionizing the field of infectious diseases (https://www.cdc.gov/amd/what-we-do/new-tech.html). Clinical metagenomic next-generation sequencing (or simply clinical metagenomics) is the comprehensive analysis of microbial and host genetic material (DNA and RNA) in samples from patients. This analysis can help not only to identify an infectious agent, but also to characterize it (resistance, pathogenicity factors, toxin production) and also to know the host immune response. Thus, clinical metagenomics can be a key driver for precision diagnosis of infectious diseases, advancing precision medicine efforts to personalize patient care in this field (for review see: Chiu CY & Miller Sa. Clinical metagenomics. Nature Reviews Genetics. 2019).

In this journal club we will analyze the situation of clinical metagenomics in veterinary dermatology through two examples: the diagnosis of leishmaniosis and of staphylococcal skin infections.

Conflicts of interest: none relevant to this Journal Club.
Neurobiology and mediators of itch

G. YOSIPOVITCH
Dr Phillip Frost Department of Dermatology Miller School of Medicine FL

Itch is encoded by two major neuronal pathways: histaminergic (in acute itch), and non-histaminergic (in chronic itch). This lectures provides an overview of the current understanding of the molecular, neural, and immune mechanisms of itch: beginning in the skin, proceeding to the spinal cord, and eventually ascending to the brain, where itch is processed. Furthermore a review of our growing understanding of peripheral and central neuro-sensitization as an important factor of the chronicity of itch will be discussed in detail.

Conflict of interest: none relevant to this lecture.
Categories of itch and the new insights into the pathophysiology

G. YOSIPOVITCH
Dr Phillip Frost Department of Dermatology Miller School of Medicine FL

Itch could be divided to two major categories acute and chronic itch. Acute itch physiology is induced by environmental changes such as an insect bite, or skin irritation by a plant or allergen resulting in a signal sent to the brain that is interpreted as itch followed by a motor response to scratch away and remove the culprit inducing itch. In this type of itch histamine and TRPV1 channels have a significant role. However chronic itch defined by the International Forum for the Study of Itch (IFSI) as itch that lasts more than 6 weeks is filled with complexities and dynamic processes. There is no single chronic itch, there are many categories that include chronic skin inflammatory diseases such atopic eczema, psoriasis and bullous disorders and chronic parasitic infections, there are systemic underlying diseases causing itch, neurological diseases and also psychogenic conditions such as depression anxiety and chronic stress. A detailed understanding of the pathophysiology that underlies chronic itches of different types such as itch in scabies, atopic eczema and bullous disorders will be discussed. These advances enable us to develop targeted therapeutic approaches.

Conflict of interest: none relevant to this lecture.
Current topical and systemic therapies for itch

G. YOSIPOVITCH
Dr Phillip Frost Department of Dermatology Miller School of Medicine FL

Itch has many pathogenic mechanisms with numerous mediators involved. These include histamines, proteases, neuropeptides such as opioids and Substance P, cytokines such as IL31 and IL17’s. Therefore, there is no single universally effective anti-itch treatment available to treat all itches. First line treatments include topicals moisturizers, low pH emollients, cooling agents such as menthol, topical anesthetics such as pramoxine and lidocaine. Ion channel blockers like topical capsaicin, strontium and topical amitriptyline and ketamine. Anti-inflammatory medications like topical steroids and calcineurin inhibitors and topical PDE4 inhibitors. Treatments with systemic therapies vary according to the underlying etiology. For histaminergic itch such as urticarial itch antihistamines both non-sedating and sedating are still the mainstay therapy. For non-histaminergic itch which encompass the majority of the types of chronic itch oral anticonvulsants such as gabapentoinoids, anti depressants such as mirtazapine as well as mu antagonists and kappa opioid agonists like butorphanol and NK-1 inhibitor aprepitant, and biologic therapies targeting cytokines such as dupilumab are used. The lecture will provide therapeutic ladders for different types of itch.

Conflict of interest: none relevant to this lecture.
Advances in understanding itching and scratching: a new era of targeted treatments

G. YOSIPOVITCH
Dr. Phillip Frost Department of Dermatology Miller School of Medicine FL

There are significant advances in our understanding of itch including inflammatory, immune-mediated itch, which has manifested into the development of targeted treatments such as interleukin-4 (IL-4) and IL-13 inhibitors (e.g., dupilumab), IL-31 inhibitors, and Janus kinase (JAK) inhibitors. In addition, our understanding of neural mechanisms of itch lead to development of emerging systemic therapies including neurokinin 1 inhibitors, drugs acting on the kappa and the mu opioid receptors. Topical therapies targeting Tropomyosin receptor kinase A inhibitors, Transient receptor channels, Nav channels, cannabinoids and botulinum toxin Type A.

Conflicts of interest: Dr. Yosipovitch serves as member of SAB of Trevi, Menlo, Sienna, Galderma, Sanofi Regeneron, Pfizer, Eli Lilly, Kiniksa, Abbvie, Novartis, Bayer, Ortho.
Notes
Comparative aspects of itch in companion animals and humans: pathogenesis

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

In humans, dogs and cats, itch (pruritus) is a common symptom seen in a variety of conditions of dermatologic and neural origin. While the pathogenesis of the various forms of itch often is similar across species, it occasionally is not entirely translatable from animals to humans and vice versa. This lecture will examine areas where the itch pathogenesis is similar across species, but we will focus more on where it is different.

We will begin with the review of the latest classification of human itch, that of the International Forum of the Study of Itch (IFSI), and will discuss where it is applicable to dogs and cats.

We will continue with the latest information on the neuroanatomy of dermatologic (inflammatory) itch highlighting, where known, the receptors expressed by dorsal root ganglia and spinal cord neurons in dogs and cats; similarities and differences between canine pruritogen receptors, especially MRGPRs, and those of humans will be pointed out.

We will then review the two most-commonly studied inflammatory pruritogens (histamine and IL-31) and how they induce itch in mice, humans and dogs.

Finally, we will explore the latest hypotheses why dogs (and cats) do not appear to be pruritic when affected with the cholestasis that is associated with a debilitating chronic itch in humans.

Conflict of interest: none relevant.
Comparative aspects of itch in companion animals and humans: treatment

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

There has been much progress in the understanding of the pathophysiology of itch in people, and research on this topic is only starting in dogs and cats. In humans, a better understanding of the mechanism of itch has led to the generation of a clinical classification that has helped in the selection of the drugs most likely to help relieve this often-debilitating symptom.

We will again start with the IFSI classification and will look at the drugs used in humans and dogs to relieve pruritus in the three most common itch categories: dermatologic, neuropathic and psychogenic itch.

Whenever possible, a parallel will be drawn between the effect of a particular drug (or class of drugs) for a specific pruritic disease/itch category in humans, dogs and cats; similarities and differences in the magnitude of the antipruritic response will be discussed where relevant.

When a specific drug has been reported to be effective in humans, but does not seem to have been much explored in dogs and cats, we will present a brief summary of its mechanism of action with some possible indications in dogs and cats.

We will finish with the “new frontier”, the interference with the transmission of itch at the level of the spinal cord interneurons and will discuss the possible targets adding the companion animal data, wherever available.

With this lecture, we hope to expand the panoply of antipruritic drugs available to treat our animal patients, hoping to attract some attention to the research needs of our community.

Conflict of interest: none relevant.
Comparison of the skin and ear canal microbiota of allergic and healthy German shepherd dogs, using next generation sequencing

N. APOSTOLOPOULOS*, S.P. GLAESER†, B. BAGWE†, U. MAYER*, R. NEIGER§, C. EWERS¶, P. KÄMPFER† and N. THOM**

* AniCura Kleintierspezialisten Augsburg GmbH, Augsburg, Germany
† Institute for Applied Microbiology, Justus Liebig University Giessen, Giessen, Germany
§ Tierklinik Hofheim, Hofheim, Germany
¶ Institute for Hygiene and Infectious Diseases of Animals, Giessen, Germany
** Small Animal Clinic - Internal Medicine, Justus Liebig University, Giessen, Germany

The diversity of canine ear canal and skin microbiota has been documented by few next generation sequencing studies so far. Bacterial dysbiosis is associated with canine atopic dermatitis. Our objective was to evaluate if the skin and ear canal of allergic German shepherd dogs (GSDs) without pyoderma or infectious otitis have an altered microbiota composition and a reduced skin microbiota diversity than healthy GSD. Swabs of axilla, interdigital skin, groin and ear canals were collected from 12 allergic and 12 healthy GSDs. Total DNA was extracted and 16S rRNA gene sequence-based Illumina amplicon sequencing was performed. The bacterial community composition was compared by non-metric multidimensional scaling based on a Bray-Curtis distances. Similarity Percentage (SIMPER) analysis was used to determine which taxa mostly contributed to the dissimilarity among samples. The Shannon, CHAO 1, Dominance and Evenness indices evaluated the alpha diversity. Between allergic and healthy GSDs, axilla (p=0.048), groin (p=0.036) and ear canal (p<0.0001) microbiota showed significant differences, while the interdigital skin microbiota (p=0.1548) did not (One way ANOSIM). The skin microbiota of allergic dogs showed a low relative abundance of *Macrococcus* and *Brevibacterium* (both 0.2%) but a high relative abundance of *Staphylococcus* (4.9 and 4.6%, respectively). These genera had the major contribution to microbiota differences. The axilla microbiota of allergic GSDs was characterized by a significantly lower species richness (CHAO 1) than healthy GSDs (p=0.032). These observations suggest that allergic GSD without skin or ear canal infection have a site-specific skin microbiota composition with decreased diversity.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Transcriptome profiling of canine chronic cutaneous lupus erythematosus skin lesions using deep RNA sequencing

F. BANOVIC, A. BLUBAUGH, T. DENLEY and R.M. GOGAL
University of Georgia College of Veterinary Medicine, Athens, Georgia, USA

Dogs spontaneously exhibit chronic cutaneous lupus erythematosus (CCLE) lesions that clinically and histologically mirror the human disease homologue. The global patterns of gene expression and inflammatory pathways leading to local skin damage in canine CCLE has not been reported. Our objectives were to characterize the skin lesional transcriptome in 10 dogs with CCLE (12 specimens; 8 DLE, 1 ECLE, 1 MCLE), while eight samples from six healthy dogs served as controls. We extracted the total RNA from skin biopsies and analyzed the transcriptome using RNA sequencing. The comparison of the mRNA expression of canine CCLE skin lesions with that of healthy skin identified 1,887 differentially expressed genes (DEGs; 1.5-fold change, \( P \)-adjusted value \( \leq 0.05 \)). The top upregulated DEGs were Th1 and interferon-related markers (\( \text{STAT1} \), \( \text{OASL} \), \( \text{MX1} \), \( \text{GZM2B} \), \( \text{SG15} \), \( \text{IFNG} \), \( \text{CXCR3} \), \( \text{IL12A} \)) and the T-cell trafficking chemokines \( \text{CXCL10} \) and \( \text{CXCL11} \). We found no significant differences in the expression of most Th2 and Th17 cytokine genes, except for those encoding \( \text{IL-33} \) and \( \text{IL-17F} \). Pathway analyses showed a strong activation of both innate and adaptive immune responses while the activated innate pathways included nucleic acid recognition mechanisms (TLR signaling, cytosolic DNA sensing, RIG-I-like receptor signaling). The most upregulated network processes showed a strong upregulation of interferon signaling, lymphocyte proliferation, the JAK-STAT pathway and NK-cell cytotoxicity. In conclusion, canine CCLE skin lesions exhibit robust immune and inflammatory responses with a predominance of Th1 and interferon-associated inflammation. The activation of pathogen recognition receptor pathways through nucleic acids is a potential key mechanism in such lesions.

Source of funding: Self-funded.

Conflicts of interest: None declared.
The comparison of skin lesion transcriptomes between human and animal models of chronic cutaneous lupus erythematosus

F. BANOVIC, A. BLUBAUGH, T. DENLEY and R.M. GOGAL
University of Georgia College of Veterinary Medicine, Athens, Georgia, USA

Chronic cutaneous lupus erythematosus (CCLE) lesions consist of severe inflammatory processes leading to decreased quality of life and potential skin scarring with disfigurement. The treatment of CCLE needs novel effective therapeutics first testable on animals. To determine the best model for the biological pathways of human CCLE, we compared a meta-analysis of the human CCLE skin transcriptomes with those of their spontaneous canine and murine (MRL/lpr) homologues. Utilizing microarray data of three human CCLE studies, we determined a consensus shared gene list of 245 differentially expressed genes (DEGs) (>2-fold enhanced, \( P < 0.05 \)). The Th1 and interferon (IFN)-related genes (\( \text{STAT1}, \text{OASL}, \text{MX1}, \text{IFNG}, \text{GZMB}, \text{ISG15} \)) and those encoding T-cell trafficking chemokines (\( \text{CXCL9}, \text{CXCL10} \) and \( \text{CCL11} \)) were among the strongest upregulated genes. Using the Metacore overlap analysis, the top enriched process networks of human CCLE were upregulations of type I interferon and IFNG signaling, innate immune response to RNA viral infection, NK-cell cytotoxicity and the JAK-STAT pathway. The comparative analysis between species revealed that canine and murine CCLE lesions contained 56% (139/245) and 24% (59/245) of the DEGs of human CCLE skin lesions, respectively. There was a moderate-to-strong positive significant correlation between canine and human CCLE DEGs (Pearson \( r = 0.52-0.67 \)). The shared canine and human CCLE lesional transcriptome signature reflected a strongly activated pathway of type I IFN signaling involving JAK-STAT, the antiviral actions of type I interferon and IFNG signaling. In conclusion, the skin lesions of canine CCLE appear to reproduce the main immunologic signature of human CCLE.

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**Conflicts of interest:** None declared.
Efficacy of fluralaner spot-on in cats affected by generalized demodicosis: 7 cases

M.B. BECCATI*, P.P. PANDOLFI† and A.D. DI PALMA§
* Centro Medico Veterinario ADDA, Capriate san gervasio, Italy
† Ambulatorio Veterinario Dott.ssa Pandolfi, Filottrano, Italy
§ Department of Agricultural Sciences, Food and Environment, Foggia, Italy

Feline demodicosis due to Demodex cati is an unusual, rare skin disease frequently associated with a dysfunction of the immune system. There are no licensed options for feline demodicosis treatment and protocols generally consist of prolonged and empiric therapies based on rinses, subcutaneous injections, oral drugs or repeated spot-on formulations. We aimed to evaluate the efficacy of fluralaner (Bravecto® topical solution for cats, MSD) as a single spot-on therapy for cats affected with demodicosis. Seven adult spayed cats (European DSH), weighing between 2 and 6 kg, presented with pruritus with multifocal alopecia, erythema, crusts and ulcerations. The skin scraping revealed Demodex cati, confirming the suspected diagnosis of feline demodicosis. Three cats were feline immunodeficiency virus-positive whereas all the cats were feline leukemia virus-negative. Methylprednisolone acetate, cyclosporine or cephalexin were given to 5/7 cats for several weeks. A single dose of topical fluralaner spot-on was initiated in all cats and negative skin scrapings were achieved within 14 days of treatment along with pruritus resolution. At the 30 day recheck, erythema, crusts and ulcerations were completely resolved. There were no relapses of feline demodicosis clinical signs at the 6-month follow up after the single fluralaner administration. In conclusion, successful treatment of feline demodicosis can be achieved using a one-time application of topical fluralaner. If possible, it is crucial to control the triggering factors in feline demodicosis, which may require long and complex therapies for each cat.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Longitudinal characterization of the anal sac microbiota in dogs with unilateral anal sacculitis treated with infusions of an antibiotic-steroid-antifungal suspension: a pilot study

C.C. BERGERON, F. SAUVÉ, L. BERNARDI DE SOUZA and M.C. COSTA
Université de Montréal, Saint-hyacinthe, Canada

Anal sacculitis (AS) represents a frequent disorder in dogs, usually resulting from impaction or bacterial infection. We aimed to characterize the bacterial microbiota of 4 castrated male dogs with unilateral AS by comparing the infected anal sacs with the healthy unaffected counterlateral sacs. The diagnosis of AS was based on clinical signs and cytologies compatible with infection (degenerative neutrophils and intracellular bacteria). Furthermore, we investigated the treatment impact of a once weekly local therapy with gentamicin, mometasone furoate and clotrimazole (Mometamax, Merck Animal Health, Canada) for 4 weeks in the infected anal sacs. Swabs from both anal sacs and rectum were collected at day (D) 0, D7, D14, and D28 for 16S rRNA next-generation sequencing. Fusobacteria, Bacteroidetes and Firmicutes were the main phylums found in the rectum, healthy and infected anal sacs. There was a great variability between dogs whereas the microbiota of healthy anal sacs remained stable overtime. There was a significant difference in species composition between anal sacs and rectum (AMOVA, p<0.001) and in community structure of infected anal sacs compared to rectum (AMOVA, p=0.018) and to healthy anal sacs (AMOVA, p=0.035). Three out of 4 dogs responded clinically and cytologically to the local treatments. Topical AS treatment was associated with changes in bacterial taxa composition, but did not reach the composition of healthy anal sacs. The canine anal sac microbiota is a complex environment, richer than previously reported with culture-based studies. Anal sacs with AS had different microbiota compared to healthy anal sacs.

Source of funding: AKC Canine Health Foundation.

Conflicts of interest: None declared.
Characterization of the pruritus responses and pruritic behaviors in an interleukin 31-induced canine model of pruritus

A. BLUBAUGH, T. DENLEY and F. BANOVIC
University of Georgia College of Veterinary Medicine, Athens, Georgia, USA

Experimental induction of acute itch in dogs has proven difficult. Previous study revealed that intravenous, subcutaneous and intradermal interleukin-31 (IL-31) administration induced pruritus in 9/10 healthy beagles over four hours. Unfortunately, the observation period started 30 min after IL-31 administration and there was no in-depth characterization of pruritic behaviors or the acute itch responses. In the present study, we evaluated the immediate/delayed pruritus responses and the pruritic behaviors observed in an IL-31-induced pruritus model in 15 healthy beagles. Dogs were randomized to two groups to receive an intravenous injection of either recombinant canine IL-31 (1.75 µg/kg) or phosphate buffered saline (PBS). After a 2-week recovery period, a crossover design was employed and the same dogs received the alternative IL-31 or PBS injection. After injections, the dogs were video-recorded for 4.5 hours to evaluate pruritic behavior. A blinded evaluation revealed that both intravenous IL-31 injections (mean pruritic seconds 3056 and 4293 for IL31_01 and IL31_02, respectively) induced strong and a long-lasting, significant increase in pruritic seconds in all dogs over 4.5 hours (Friedman’s test, p<0.05 for all comparisons). Intravenous IL-31 induced significant immediate itch responses in dogs in the first 30 min and a long-lasting pruritic effect over the following 4 hours (p<0.05 for all comparisons, respectively). No significant difference was observed between IL31_01 versus IL31_02 in pruritic seconds over 270 min (p=0.99). Chewing, scratching and head shaking were behaviors significantly increased after IL-31 challenge (p<0.05 for all comparisons). In conclusion, intravenous IL-31 reproducibly induces acute itch responses in healthy dogs.

Source of funding: Boehringer Ingelheim Animal Health.

Conflicts of interest: None declared.
Characterization of the pro-inflammatory and pruritogenic transcriptome in experimental acute canine IgE-mediated skin lesions

A. BLUBAUGH, T. DENLEY, D. RISSI, K. HOOVER and F. BANOVIC
University of Georgia College of Veterinary Medicine, Athens, Georgia, USA

Intradermal injection of anti-immunoglobulin E (IgE) antibodies in dogs grossly and histologically resemble naturally occurring atopic dermatitis (AD). However, the activated inflammatory and pruritic pathways have not been characterized. The objectives of this study were to characterize the inflammatory transcriptome of experimental acute canine IgE-induced lesions. Biopsies were collected at 6 and 24 h after intradermal injections of anticanine-IgE antibodies to eight healthy male castrated Beagles; healthy and saline-injected skin served as controls. We extracted total RNA from skin biopsies and analyzed transcriptome using RNA-sequencing. Gene expressions of IgE-induced biopsies was compared to that of controls from the same subject (1.5-fold change, \( P \)-adjusted value \( P \leq 0.05 \)). Acute IgE-mediated lesions had a significant upregulation of pro-inflammatory (e.g., LTB, IL-1B, PTX3, CCL2, IL6, IL8, IL18) and T helper-(Th)2 (e.g., IL4R, IL5, IL13, IL33 and POSTN) genes, as well as Th2 chemokines (CCL17, CCL24). The expression of thymic stromal lymphopoietin (TSLP) and IL22 (Th22) cytokines was downregulated. The Th1/IFN\( \gamma \) signal (e.g., STAT-1, OASL, MX-1, CXCL10, IL-12A) as well as the T-cell trafficking chemokine (CCR7) and its ligand (CCL19), were also among the upregulated genes. There was also significant upregulation of genes encoding other known pruritogenic proteins and pathways, such as cathepsin S (CTSS) and CTSC, nerve growth factor (NGF), and histamine-synthesis enzyme and receptors (HDC, HRH4). Pathway analysis revealed strong significant upregulation of JAK-STAT, histamine, IL-4 and IL13 signaling. In conclusion, acute canine IgE-mediated skin lesions exhibit a multipolar immunological axis upregulation (Th1, Th2 and Th17) in healthy dogs, resembling spontaneous canine AD lesions.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Atopic dermatitis (AD) is caused by a complex interplay between immune and barrier abnormalities. Canine models (Immunoglobulin-E induced (IgE) and House Dust Mite (HDM)) have been used to simulate AD for preclinical assessments treatments in their comparative transcriptomic profiles with canine spontaneous AD, but with a lack of stringent criterion. We sought to evaluate the transcriptomic profiles of all canine AD models and determine how they relate to canine spontaneous AD lesional skin. Gene expression data of dogs with spontaneous AD and house dust mite (HDM) patch and tape stripping canine models were obtained and re-analyzed from microarray published cohorts; transcriptomic profiling of IgE-induced model was performed using RNA sequencing. Criteria of False Discovery Rate (FDR)<0.05 and Fold Change (FC)>2 were used for analysis across all samples. Gene Set Variation Analysis (GSVA) was performed for phenotypically unbiased analysis of gene sets (Th1, Th2, Th17, Th22). Spontaneous AD, patch and tape stripping HDM, and IgE modeling comparisons to healthy canine skin revealed 41 common DEGs: upregulation of pro-inflammatory (IL8, CCL2, MMP9); IFNγ (CXCL10, ISG15), and T helper-(Th)2 genes (IL13RA2).

Transcriptomic comparison of DEGs between canine models revealed IgE contained 63.5%, whereas tape stripping and patch HDM induced model comprised 49%, of spontaneous AD genes. Multipolar pathways were observed among spontaneous and AD models in GSVA, with robust Th1/Th2 activation. Th17 was significantly enriched in IgE and spontaneous AD, but not HDM. Further studies of spontaneous AD with larger sample sizes utilizing next-generation RNA sequencing and atopic models should be conducted.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Dermatophytes isolated from dogs and cats in France and their evolution in France from 2010 to 2018

Veterinary school of Nantes, Nantes, France

There is little current information on the frequency and dynamics of dermatophytes isolated from dogs and cats. We analyzed the results obtained from samples sent by veterinary practices from all over France to our laboratory. We compared two periods of the years 2010, 2014 and 2018: January/February (JF) and September/October (SO) using a generalized linear model. Nine dermatophyte species were identified in 2008 isolates; most frequently Microsporum canis (n=1303), Microsporum gypseum (= Nannizia gypsea) (n=169). In cats M. gypseum is the most frequent dermatophyte (p-value <0.0001) with decreasing frequency from 75% to 49% in dogs and 87% to 77% in cats in JF and 46% to 25% in dogs and 83% to 69% in cats in SO. In contrast, Trichophyton mentagrophytes was increasingly isolated from 15% to 31% in dogs and 19% to 25% in cats in JF and from 28 to 39% in dogs and 18% to 27% in cats in SO. M. gypseum also increased from 5 to 10% in dogs and 1% to 4% in cats in JF, and from 17% to 30% in dogs in SO. Globally all dermatophytes are increasingly isolated (p-value=0.0007). The increase of M. gypseum in dogs and Trichophyton mentagrophytes in dogs and cats was significant (p<0.0001 in dogs and p=0.001 in cats). In 2018, 68% of dermatophytes isolated in dogs and 25% in cats were not M. canis.

Source of funding: Laboratory DPM (Laboniris).

Conflicts of interest: None declared.
Medical honey for canine nasal intertrigo: how sweet is the placebo effect?

G. BROSSEAU*, N. PAGÉ*, C. DE JAHAM* and J.R.E. DEL CASTILLO†

* Centre Vétérinaire DMV, Montréal, Canada
† Quebec’s Animal Pharmacology Research Group, Université de Montréal, Saint-hyacinthe, Canada

Antimicrobial resistance is a global health emergency and alternatives to prolonged anti-infective therapies are needed. A pilot study reported that medical-grade honey was effective against canine surface pyoderma but lacked placebo control. Our objectives were: (1) compare the therapeutic efficacies against intertrigo of Manuka honey (Medihoney®: Derma Sciences, Toronto, Canada) and of a 1% ethyl phenylacetate placebo hydrogel; (2) characterize the pre- and post-treatment polymicrobial burdens. We performed a bioethically approved randomized, placebo-controlled, double-blinded trial. New canine intertrigo cases fulfilling prespecified inclusion criteria were randomized across the Test and Placebo treatment arms. Once daily for 21 days, owners gently washed the affected sites with water, sponged and applied a thin film of the products. Cytological and lesional scores, owner-assessed pruritus, and microbial cultures were performed prior to treatment and on Day-22. The fixed effects of time, treatment, pruritus and microbial burden scores on the composite cytological and lesional scores, accounting for random dog effect, were estimated with generalized linear mixed models for repeated count outcomes ($\alpha=0.05$). Placebo was given to 19 dogs and honey to 16 dogs. Five dogs were lost to follow-up. Despite having higher baseline cytological scores (Trt: 0.60±0.16; $P=0.0008$), placebo dogs tended to improve faster than with medical honey ($\text{Time}\times\text{Trt}: -0.016\pm0.009; P=0.08$). Microbial burden increased the severity of cytological scores ($\text{Mi}\_\text{score}: 0.06\pm0.02; P=0.0011$). Clinical scores improved at similar rates in both groups ($\text{Time}: -0.010\pm0.003; P=0.0025$) and only cytological score increased their severity ($\text{Cy}\_\text{score}: 0.04\pm0.02; P=0.0249$). Medical honey did not significantly outperform placebo in treating canine nasal intertrigo.

Source of funding: Canadian Academy of Veterinary Dermatology Research Grant Centre Vétérinaire DMV.

Conflicts of interest: None declared.
The cutaneous and rectal microbiome of canine perianal fistulas and the effect of ciclosporin therapy

C.L. CAIN*, M.D. GROGAN†, L. CITRON*, D.O. MORRIS*, E.A. GRICE† and C.W. BRADLEY*
* School of Veterinary Medicine, University of Pennsylvania, Philadelphia, United States of America
† Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

Perianal fistulae are painful ulcers or sinus tracts that spontaneously occur around the anus; microbial populations have been proposed to play a role in pathogenesis. This study included 26 German shepherd dogs: 11 dogs with perianal fistulae and 15 healthy dogs. Swabs were collected from the axilla, rectum, and perianal skin (unaffected) or fistula site for each dog at days 0 and 30 and for affected dogs only at day 60. Affected dogs were treated with ciclosporin (Atopica: Novartis Animal Health, Greensboro, NC, USA or generic equivalent; mean dose: 3.0 mg/kg/day, range: 2.1-4.4 mg/kg/day) and ketoconazole (mean dose: 6.9 mg/kg/day, range: 5.1-8.5 mg/kg/day). Fistula number, severity, and extent were evaluated at each visit. Microbiome analysis of each site was performed via sequencing of the V4 region of the 16S rRNA gene. Affected dogs had a mean of two fistulas (range: 1-4) at day 0 and a mean of one fistula (range: 0-2) at day 60. Greater than 50% resolution was achieved at day 30 in 5/11 dogs (45%) and at day 60 in 9/11 dogs (82%), respectively. Bacterial community composition for affected dogs differed significantly between sites at day 0 (p < 0.05) and day 60 (p < 0.05), but did not differ significantly between visits. Alpha diversity (Shannon index) for affected dogs did not differ significantly between visits for any site or across all sites (p = 0.9). Overall, the microbiome of perianal fistulas approximated the rectal microbiome; consistent microbial shifts were not observed with treatment or fistula resolution.

Source of funding: Penn Vet Center for Host Microbial Interaction Pilot Award.

Conflicts of interest: None declared.
A prospective, randomized, double blind, placebo-controlled evaluation of the effects of an n-3 essential fatty acid supplementation (Agepi ω3®) on the clinical signs, and erythrocyte membrane, hair shafts and skin surface polyunsaturated fatty acid concentrations in dogs with poor coat quality

D. COMBARROS*, E. CASTILLA-CASTANO*, L.A. LECRU*, N. AMALRIC†, C. PRESSANTI* and M.C. CADIERGUES§

* Department of clinical sciences, Université de Toulouse, ENVT, Toulouse, France
† Synelvia, Labège, France
§ UDEAR, Université de Toulouse, INSERM, ENVT, Toulouse, France

Dietary supplementation with ω-3 essential fatty acids (EFA) has a beneficial effect on dog skin and coat quality. However, little is known about necessary dose and treatment duration. The objective was to determine the kinetics of the hair and skin quality improvement after starting n-3 EFA supplementation with Agepi®ω3 (IVP, MP Labo, France), the duration of the effects after its withdrawal and its association with erythrocyte membrane (EM) and hair shaft lipid content. Twenty-four dogs (same owner, environment and diet at baseline) were enrolled in this prospective, randomized, double-blinded, placebo-controlled study. Dogs received 1 capsule/10kg of placebo or IVP daily for 90 days (1 capsule IVP = 110 mg eicosapentaenoic acid (EPA)/68 mg docosahexaenoic acid (DHA)). Clinical assessment (Skin Seborrhic Index, SSI) and blood and hair lipids were evaluated at days 30, 60, 90, 120, 150 and 180; SSI was significantly reduced in the treatment group from day 30 reaching a plateau after 2 months. SSI remained stable for 1 month after withdrawal and progressively returned to baseline levels at d180. EPA and DHA content on EM increased significantly from day 30 and decreased rapidly after withdrawal in the treatment group. Total lipid content in hair shafts increased until day 60 and remained stable 90 days after withdrawal. No side effects were observed. The hair and skin improvement after IVP supplementation is maximal after two months. One month after withdrawal the maximal beneficial effect is still present, then the SSI goes up until baseline levels at day 90 after withdrawal (d180) and is associated with marked increase of EPA and DHA content in EM and total lipid content on hairs shafts.

Source of funding: MP Labo, Grasse, France.

Conflicts of interest: DC, ECC, LAL, and CP declare that they have no competing interests. MCC has previously been consultant for MP Labo.
Wheal size after intradermal injection of histamine and saline in skin treated with local anesthetic in comparison with non treated skin

A.I. CÓZAR* and A. DALMAU†
* CV Musicos, Tres Cantos, Spain
† Hospital Veterinario Mediterrani, Reus, Spain

Intradermal testing is usually performed under sedation to avoid discomfort and stress and to shorten the procedure. This need for sedation or anesthesia could lead an owner to reject the procedure because of pre-existing conditions such as heart, liver or renal disease. Herein, we wished to assess the wheal size when saline and histamine controls are injected intradermally in the skin pretreated with an anesthetic cream compared to that without such application. Twelve privately-owned dogs were sedated with medetomidine and a 10x10 cm area was clipped on the medial aspect of both thighs. Lidocaine 40 mg/g cream (Lambdalina, Isdin, Barcelona, Spain) was applied to one of the areas while the other one was left untreated. After 20 min, paired injections of histamine and saline controls (Diavet-Diater, Madrid, Spain) were intradermally-injected and reactions were assessed after another 20 min using a digital caliper. Wheal sizes in treated and non-treated zones were highly correlated. The ratio of treated-to-untreated wheal diameters was narrowly-distributed with an average value very close to 1. In summary, the lidocaine cream application did not significantly affect the size of the histamine and saline wheals after intradermal injections, thus suggesting the cream’s potential for performing intradermal tests in nonsedated animals.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Determination of the synergistic, antagonistic or indifferent in vitro effect between an ear cleaner and four antibiotics against bacterial strains isolated from canine otitis

O. FANTINI, D. GALLAND, D. MYOTTE-DUQUET and F. EL GARCH
Vétouquinol, Paris, France

The aim of this study was to determine potential interactions between the ear cleaner, Sonotix (Vétoquinol; Lure, France) and four antibiotics (marbofloxacine, gentamicin, polymyxin B or florfenicol) against five strains of S. pseudintermedius and P. aeruginosa. The synergistic (Fractional Inhibitory Concentration Index (FICI) ≤ 0.5), antagonistic (FICI ≥ 4) or indifferent (0.5 < FICI < 4) effects were determined by a microdilution checkerboard assay. The bacterial concentration in each well was about 5.10^5 CFU/mL. The tested ranges for the checkerboard assays varied between 1/8 and 1/512 dilution for Sonotix: 0.015 and 8 µg/mL when antibiotic MICs were between 0.12 and 2 µg/mL and between 0.25 and 128 µg/mL with MICs between 4 and 64 µg/mL. A sterility and a growth control were used in each test. The interaction between Sonotix and the four antibiotics was not antagonistic for any of the tested strains. A partially synergistic (0.5 < FICI < 1) effect was observed in 20% and 40% of P. aeruginosa strains with Sonotix combined with gentamicin or marbofloxacine and polymyxin B, respectively. A partial synergy between Sonotix and florfenicol was found for all P. aeruginosa isolates. For 40% and 80% of S. pseudintermedius strains the combinations of Sonotix and gentamicin or polymyxin B were partially synergistic. The results of this study show that Sonotix does not antagonize the antibacterial effect of the four tested antibiotics that are most commonly used in topical otic formulations. Moreover, a partial synergistic effect was observed between Sonotix and the antibiotics for some strains.

Source of funding: Vétoquinol.

Conflicts of interest: All authors are Vétoquinol employees.
An alternative to long-lasting elimination diet to diagnose food allergies in dogs with atopic dermatitis

C. FAVROT*, P. BIZIKOVA†, N. FISCHER*, A. ROSTAHER* and T. OLIVRY†
* Department of Small Animal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
† Department of Clinical Sciences, College of Veterinary Medicine, Raleigh, NC, United States of America

Food allergy is a possible trigger of atopic dermatitis (AD) in dogs, and it is typically diagnosed following an eight-week elimination diet (EDT) and a provocation with the original food items. This lengthy procedure might be difficult for owners and its interpretation unclear. Herein, we explored the feasibility of a shorter-duration EDT with prednisolone administration during its first weeks. The goal was to allow for food challenges earlier than traditionally-recommended. Fifty-three dogs with AD were included in the study. They were fed an extensively-hydrolysed protein-based commercial pet food (Anallergenic, Royal Canin, Aimargues, France) and treated with prednisolone at least two weeks to reduce pruritus and inflammation. Dogs were challenged two weeks after prednisolone discontinuation, provided that no flare had occurred; those with relapsing signs were fed the hydrolysate for a total duration of 8 weeks with or without prednisolone. Ten out of 53 dogs (19%) did not have a flare of AD after being two-week off the prednisolone; they were subsequently challenged with their regular food, had a relapse of signs and were diagnosed with a food-induced AD. In the other dogs, signs were never controlled without prednisolone or they relapsed rapidly after its discontinuation; they were considered non-food allergic after the 8-week EDT. These results suggest that a shorter EDT is possible if the allergic itch and inflammation are initially controlled with a short course of glucocorticoids. This shorter trial is likely to improve owner adherence and facilitate the diagnosis of food allergy.

Source of funding: The study was funded by Royal Canin, Aimargues, France.

Conflicts of interest: None declared.
Atopic dermatitis (AD) is a frequent disease and West Highland white terriers (WHWT) are predisposed for this condition. As dermatologists are usually consulted when the disease is already developed, very little is known on the natural AD progression. The goal of the study was to evaluate AD development and progression in WHWT for three years. Puppies were initially examined at the breeder’s and the owners were contacted at least two times a year; if signs of allergy were observed, the owners and encouraged to visit the veterinarian. The diagnosis of AD was made based on established criteria and when pruritus and skin changes were chronic or relapsing (at least two episodes/year after onset). We assessed duration, extension and treatments needed for AD severity. From 108 puppies that were included, 92 were followed during three years and AD was diagnosed in 47 dogs. The AD severity was mild in 26/47 (36%) AD dogs and 37/47 (79%) developed the first signs during the first year, that included gastro-intestinal signs in four dogs. Generalized and localized (less than three body areas) AD was observed in 25 (53%) and 22/47 (47%) of AD dogs; most frequently affected areas were the paws, ears and abdomen. Extracutaneous signs were frequently observed, especially gastro-intestinal (15 dogs) but also conjunctivitis and rhinitis. Elimination diet was carried out in 31 AD dogs and led to an improvement in 12 dogs. This first longitudinal study sheds new light on the early development of the AD disease in WHWT dogs.

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**Conflicts of interest:** None declared.
The pharmacokinetics of oclacitinib maleate in the cat

L. FERRER, C. CRISTÒFOL, I. CARRASCO and A. PUIGDEMONT
Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain

Oclacitinib is a Janus kinase inhibitor that is effective and safe for the treatment of allergic dermatitis in dogs. Its use in cats has been limited by the absence of data on the pharmacokinetics. The objective of this study was to determine the pharmacokinetic parameters of oclacitinib in cats after oral and intravenous administration. Six healthy adult domestic shorthair cats were enrolled in a two-period, two-treatments study design. Cats received two treatments, oclacitinib maleate intravenously (IV) and orally (PO), at a dose of about 0.5 mg/kg and 1 mg/kg, respectively. The one-week interval of washout was allowed between the two treatments, and the cats received each treatment only once. The plasma concentration of oclacitinib was determined by HPLC at times 0, 5 min, 15 min, 30 min, 1h, 4h, 6h, 10h, and 24h after intravenous administration, and at 0, 15 min, 30 min, 1h, 2h, 4h, 6h, 10h, and 24h after oral administration. After oral administration, oclacitinib was absorbed rapidly and almost completely, as shown by an absolute bioavailability of 87%, and a Tmax of 35 min. The elimination of the drug was also very rapid, as shown by a half-life of 2.41 hours and a clearance calculated as 6.28 mL/min/kg. The pharmacokinetic parameters of oclacitinib in the cat are similar to those described for the dog, although absorption and elimination are somewhat faster and variability between individuals is somewhat greater. Larger doses or shorter dosing intervals would be recommended in cats to achieve similar blood concentrations to those in dogs.

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Conflicts of interest: L.F. has received unrelated funding for lecturing from Zoetis. No conflicts of interest have been declared by the other authors.
Allergen-specific immunotherapy in dogs with atopic dermatitis: a comparison of subcutaneous, intralymphatic and sublingual administration

N.M. FISCHER, A. ROSTAHER and C. FAVROT
Department of Small Animal Medicine, University of Zurich, Zurich, Switzerland

Allergen-specific immunotherapy (ASIT) is the only causative treatment of atopic dermatitis in dogs. Distinct routes of ASIT administration are available, but comparative studies between routes are lacking. Therefore, this study aimed to compare the efficacy and safety of subcutaneous (SCIT), intralymphatic (ILIT) and sublingual (SLIT) immunotherapy in 30 dogs. Depending on the owner’s decision, dogs were included in either ILIT (n:12), SCIT (n:8) or SLIT (n:10) groups and ASIT was administered following current protocols. Pruritus visual analogue scale (PVAS), canine atopic dermatitis extent and severity index (CADESI), concurrent medications and adverse events were recorded initially and after one, three, six and 12 months. The main outcome measure was the return to a normal state, namely CADESI <12, PVAS <2.5 and a medication score <10. Drop-out cases were evenly distributed in all groups and 23 dogs finished the study (ILIT n:10, SCIT n:6 and SLIT n:7); adverse events were rare. Groups were not statistically different at inclusion. After 12 months of ASIT treatment, a reduction of CADESI and pruritus score was obvious in the ILIT and the SCIT (P<0.05 for both for all scores). All three scores deteriorated in the SLIT group. Return to the normal state was achieved in 6/10 (60%) of dogs receiving ILIT, compared to 1/6 (17%) and 1/7 (14%) in the SCIT and SLIT groups, respectively. Our study is the first one comparing several protocols of immunotherapy in dogs; SCIT and ILIT were both effective, but ILIT was associated with a much higher “return to normal rate”.

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Conflicts of interest: None declared
Clinical efficacy of sublingual allergen-specific immunotherapy in cats with nonflea nonfood-induced hypersensitivity dermatitis against mites

R. FOJ*, I. CARRASCO†, F. CLEMENTE§, F. SCARAMPELLA¶, A. CALVET**, A. PRATS**, P. BRAZIS* and A. PUIGDEMONT††

* Laboratorios LETI, Barcelona, Spain
† Vetland Sant Boi, Barcelona, Spain
§ Ambulatorio Veterinario Associato San Luca, Bologna, Italy
¶ Studio Dermatologico Veterinario, Milano, Italy
** Clinica Felina Barcelona, Barcelona, Spain
†† Universitat Autònoma de Barcelona, Bellaterra, Spain

Sublingual immunotherapy (SLIT) has shown beneficial effects in humans and dogs with atopic dermatitis. So far, no studies have been reported SLIT efficacy in cats with hypersensitivity reactions. This study aimed to evaluate the clinical efficacy of SLIT in cats with nonflea nonfood-induced hypersensitivity dermatitis associated to storage or house dust mites. Twenty-two cats with clinical signs and dermatological lesions compatible with a mites hypersensitivity were treated with SLIT for 6 months. After 3 and 6 months of SLIT administration, dermatological lesions evaluated through Scoring Feline Allergic Dermatitis (SCORFAD) were significantly reduced from 22 to 7.8 and 5.7, respectively \( (P < 0.001 \text{ for both}) \). In addition, the owner pruritus score was reduced from an initial 7.9 to 4.9 and 3.6 \( (P < 0.001 \text{ for both}) \) at 3 and 6 months. Improvement in clinical signs was followed by a significant decrease in specific IgE levels against mites from 115 to 87.5 and 53.3 at 3 and 6 months, respectively \( (P < 0.05 \text{ for both}) \). There were no changes observed in mite-specific IgG levels over the follow-up. Three cats withdrew from the study for reasons non-related to the treatment. None of the animals presented adverse effects associated with the administration of the SLIT. In conclusion, mite-specific SLIT induced a significant improvement of clinical signs and pruritus in hypersensitive cats after three months of treatment. Therefore, SLIT should be considered a rapid, effective and safe treatment in cats with nonflea nonfood-induced hypersensitivity dermatitis.

Source of funding: Animal Health, B.U. Laboratorios LETI.

Conflicts of interest: Rubén Foj and Pilar Brazis are employees of Laboratorios LETI. They participated in the design of the study and helped to the draft of the manuscript. No conflicts of interest have been declared by the other authors.
Dermis and subcutis of healthy dogs lack of a bacterial microbiota

R. GARCÍA-FONTICOBA, L. FERRER, O. FRANCINO and A. CUSCÓ
Universitat Autònoma de Barcelona, Barcelona, Spain

Studies using highly sensitive molecular techniques have recently detected bacterial communities below the human epidermis. Depending on their abundance and composition, this finding could be clinically relevant. This possibility, however, has not been investigated in the dog so far. The aim of this study was to determine if bacteria can be detected in the dermis and subcutaneous tissue of dogs using two different approaches: traditional cultures and next-generation sequencing (NGS) of the V4 region of bacterial 16S rRNA gene. Seven healthy dogs were included, and two sets of samples were collected from each subject. Samples sets were composed by one environmental blank sample, one skin surface swab and one 6-mm sterile abdominal skin biopsy, split in epidermis, dermis and subcutis. From each dog, one set of samples was submitted for bacterial culture and the other one for bacterial DNA amplification and sequencing. Five different bacterial genus (Staphylococcus, Bacillus, Corynebacterium, Streptococcus and Enterococcus) were isolated in 5/7 epidermal surface samples with traditional culture methods. Cultures from all the other samples were negative in all seven subjects. Interestingly, the bacterial isolates were not the most abundant bacteria constituting the skin microbiota according to the NGS results. Although some DNA could be amplified from epidermal, dermal and subcutaneous tissue samples, the results of the NGS were similar to those of the blanks, revealing the absence of a microbiome.

The results of this study do not support the presence of a dermal or subcutaneous microbiota in healthy dogs.

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Conflicts of interest: None declared.
Chemotherapy-induced palmar-plantar erythrodysesthesia (PPES) in a dog treated with liposome-encapsulated doxorubicin

G.G. GHIBAUDO, C.C. CATALUCCI and V.P. VALENTI
Clinica Veterinaria Malpensa, Samarate, Italy

Palmar-plantar erythrodysesthesia (PPES) is a cutaneous reaction caused by some drugs, including liposome-encapsulated doxorubicin (Caelyx, Janssen Cilag, Cologno Monzese, Italy), which is a chemotherapeutic used for different malignancies. Skin changes of PPES include erythema, hyperemia, edema, alopecia, severe crusting, ulceration and epidermal necrosis; they occur in the paws, axillary and inguinal region, are usually self-limiting and resolve after discontinuation of the drug. The pathophysiology is poorly understood and it seems associated with excretion of the drug into the sweet glands. We describe a 10 years old, male intact, Labrador Retriever, that was referred for a stage 2 splenic hemangiosarcoma and doxorubicin-based chemotherapy was initiated. After first dose administration, the patient developed severe gastrointestinal toxicity. Therefore Caelyx was introduced in place of doxorubicin. After one dose of Caelyx, the patient developed alopecia and hyperpigmentation on the muzzle, ventral abdomen and right leg; the skin appeared shiny and exudative. A biopsy of the lesions showed atrophic dermatopathy and perivascular deep lymphohistiocytic dermatitis with perifollicular fibrosis. Based on the clinical onset and the histological aspect, the lesions were considered suggestive of PPES. The treatment with Caelyx was discontinued and after 1 month of oral pyridoxine and topical supportive care, improvement of the lesions was observed. Chemotherapy-induced dermatological toxicity should be considered in the differential diagnosis of patients receiving chemotherapeutic, for which this side effect has been described.

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Conflicts of interest: None declared.
First reported case of glomus tumor (glomus tympanicum) in an English setter dog treated with a diode laser in otoendoscopy

G.G. GHIBAUDO*, C.C. CATALUCCI†, V.A. VERCELLI† and S.T. TARAGLIO§
* Clinica Veterinaria Malpensa, Samarate, Italy
† Clinica Veterinaria Città di Torino, Torino, Italy
§ Serv. Anat. Patol. Osp. S.Giovanni Bosco, Torino, Italy

Glomus tumors are typically benign but highly vascular neoplasia, described in people which arise in a small and limited space behind the eardrum. Here, we report a case of glomus tumor of the eardrum in an 11 years-old, non-castrated male English setter dog presented for unilateral deafness and acute bleeding from the left ear canal. The otoendoscopic examination showed the presence of a vascularized mass that was incorporated in the tympanum. A computer tomography (CT) confirmed the presence of tissue attenuation material at the level of the last tract of the ear canal and eardrum. Brainstem auditory evoked response (BAER) audiometric tests showed a unilateral left hearing loss. Histopathological examination of the mass revealed hyperchromatic cells with rich vascular stroma and no signs of atypia or mitosis, leading to a diagnosis of benign glomus tympanicum tumor. Laser diode therapy, at 980 nm wavelength in continuous wave mode, induced marked reduction of the tumor mass; there was no pain or bleeding after the surgical procedure. The affected ear was treated with topical ear solution containing silver sulfadiazine, clotrimazole and dexamethasone once daily for four weeks. Recheck examinations at one and six months revealed a partial pseudo-tympanic fibrotic formation, without tumor relapse, and a discrete improvement in hearing in the affected ear using BAER test. No relapse of the tumor was observed in the following 18 months. To the best of our knowledge, this is the first report of a glomus tympanic tumor in a dog.

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Conflicts of interest: None declared.
Evaluation of IL-17 and IL-22 positive cells in lesional and non-lesional spontaneous atopic skin

J. B. GILLEN, A. HERNANDEZ-BURES and D. SANTORO
University of Florida, College of Veterinary Medicine, Gainesville, USA

Interleukin (IL)-17 and IL-22 have been associated with the pathogenesis of human atopic dermatitis (AD). There have been limited investigations of IL-17 and IL-22 pathway in spontaneous canine AD. Thus, this study aimed to evaluate the amount and distribution of IL-17- and IL-22-positive cells in the non-lesional (n=6 dogs) and lesional (n=7 dogs; erythema and papules n=2, erythema alone n=3, pustules n=4) atopic skin; healthy skin (n=10 dogs) served as control. Skin biopsies were collected and eight sequential sections were stained using commercially available antibodies against canine IL-17 and IL-22. The positive cells were counted in the dermis of each section and the average of positive cells per section was compared between the groups. There was no statistical difference in the number of positive IL-17 and IL-22 dermal cells between healthy (0.2 average cells for IL-17, respectively) and atopic lesional (P=0.7 for IL-17, 8.6 cells; P=0.7 for IL-22, 8.6 cells; P=0.3 for IL-17, 4.1 cells; P=0.9 for IL-22, 0.4 cells) and atopic non-lesional skin samples. Keratinocytes in healthy and atopic sections were also positive for IL-22, but this was not considered for the statistical analysis. This is the first study evaluating IL-17- and IL-22-positive cells in the skin of atopic and healthy dogs. A significant difference in positive dermal immune-cells between atopic and healthy dogs was not observed in this subset of AD dogs. However, larger studies are needed to confirm the role of cutaneous or systemic IL-17 and IL-22 pathways in spontaneous canine AD.

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Evaluation of the cutaneous immunological milieu in dogs naturally affected by Leishmania infantum/chagasi: a preliminary study

A. HERNANDEZ-BURES*, J.B. GILLEN*, K. APOSTOLIDIS†, M. SARIDOMICHELAKIS†, A. DI LORIA§ and D. SANTORO*

* University of Florida, College of Veterinary Medicine, Gainesville, USA
† University of Thessaly, Karditsa, Greece
§ University of Napoli, Napoli, Italy

Canine leishmaniosis is associated with an aberrant cutaneous immune response. Few studies have assessed the presence of immune cells in the skin of infected/diseased dogs versus clinically healthy dogs. The aim of this study was to evaluate the number and distribution of several immune-cells, macrophages, activated neutrophils, T helper (Th)1, Th2, T regulatory (Treg) cells, and interleukin (IL)-17-producing cells, in the skin from the same anatomic region of 12 non-infected-clinically healthy, 12 infected-clinically healthy, and nine infected-diseased dogs. Diagnosis of canine leishmaniosis was based on compatible clinical signs and the detection of Leishmania amastigotes in cytological aspirates from lymph nodes and bone marrow. Paraffin-embedded skin biopsies were processed for routine immunofluorescence and positive cells were identified using commercially available anti-canine specific antibodies. The number of positive cells between healthy and infected dogs was statistically analyzed. Compared with non-infected-clinically healthy skin, a significantly higher number of neutrophils (p<0.05), macrophages (p<0.05), and Treg (p<0.01) was observed in both non-lesional and lesional skin. Compared with healthy skin, more Th2 cells (p<0.05) were present in non-lesional skin. Similarly, more IL-17-positive cells (p<0.01) and a higher Th1/Th2 ratio (p<0.05) was seen in lesional compared with healthy skin. There was no difference in amount of any of the immune-cells tested between non-infected and infected but clinically healthy dogs. This is the first study showing higher presence of IL-17-producing cells in the skin of dogs with leishmaniosis. Larger studies are needed to assess the correlation between immune-cells, macroscopic skin lesions, systemic signs, and clinical stage of canine leishmaniosis.

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Conflicts of interest: None declared.
One Health approach to methicillin-resistance between Staphylococcus isolates from companion dogs affected with pyoderma and owners

J.H. KANG and C.Y. HWANG
* Seoul National University, Seoul, South-Korea

Staphylococcal cassette chromosome mec (SCCmec) are mobile genetic element contained mec gene inducing methicillin-resistance. The SCCmec is known to transfer between staphylococcal species, but One Health approach to the issue of SCCmec transfer in the clinical environment is still limited. We evaluated genetic relatedness of SCCmec between staphylococci from companion dogs affected with pyoderma and their owners. Thirty-one pairs of companion dogs and owner participated in this study with consent. Clinical isolates were obtained from canine pyoderma lesions, nasal and finger swab of the owners. Species identification, oxacillin resistance screening agar, and antimicrobial susceptibility testing were performed to select methicillin-resistance staphylococci (MRS). The SCCmec of MRS was characterized genetically by PCR-based SCCmec typing, direct-repeat unit (dru, variable-number tandem repeat sequences in SCCmec) typing, and whole genome sequencing (WGS). Among total 31 pairs of companion dogs and owners, 12 pairs (S. pseudintermedius from dog and S. epidermidis from the owner) had the same type of SCCmec (type V) but different dru type. One pair carrying same SCCmec type V and dru type 11a was detected and submitted for WGS. The WGS revealed SCCmec region of J3, mec gene complex, ccr gene complex, and J2 had 98.8% homology within S. pseudintermedius from dog and S. epidermidis from the owner. SCCmec region of J1 was heterogeneous. Previous studies mainly focused transfer of pathogen itself in One Health approach to antimicrobial resistance. However, this study suggests S. pseudintermedius from dog and S. epidermidis from the owner could have a remarkable genetic relationship in methicillin-resistance.

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Two types of IgE in dog serum differ in glycosylation

A. KUMAGAI*, M. SAITAMA*, T. TSUKUI† and K. MASUDA*
* Animal Allergy Clinical Laboratories, Sagamihara, Kanagawa, Japan
† Nippon Zenyaku Kogyo Corporation (Zenoaq), Koriyama, Fukushima, Japan

The main function of serum ("pathogenic") IgE is to bind to FcεRI on mast cells, and this property is lost after a heat-induced conformational change. In canine sera, we discovered a new type of IgE that does not bind to the FcεRI without first heating the serum ("non-pathogenic IgE"). These are detectable by the novel anti-dog IgE monoclonal antibody CRE-DR that recognizes a heat-induced conformational change of pathogenic IgE. Since glycosylation is important in IgE conformation, we hypothesized that pathogenic IgEs in the dog serum have a different glycosylation pattern. An ELISA using CRE-DR to detect IgE against the major Dermatophagoides farinae allergen Der f 2 was performed with or without serum glycosidase digestion. Sera were collected from three atopic dogs spontaneously sensitized to and three healthy beagles experimentally sensitized to Der f 2, which were known to contain high levels of pathogenic or non-pathogenic IgE, respectively. Before glycosidase digestion, Der f 2-IgE in the atopic dogs was detected at low levels ranging from 0 to 39 ng/mL, and these increased from 194 to 226 ng/mL after digestion with peptide-N-glycosidase F (PNGase F). Neuraminidase digestion did not show such an increase after digestion in the atopic dogs. In the laboratory dogs, both glycosidase digestions did not affect the IgE serum levels. These data suggest that there is a difference in glycosylation between pathogenic and non-pathogenic IgEs. Pathogenic IgE appears to have high mannose type glycans that are digested by PNGase F but not by neuraminidase.

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Conflicts of interest: KM is a stockholder of the Animal Allergy Clinical Laboratories and TT is an employee of the Zenoaq corporation.
Effect of the preventive use of a glucocorticoid anti-inflammatory topical formulation on the ear canal microbiota and mycobiota in atopic dogs without signs of otitis externa

C. LEONARD, J. NGO, P.A. FALL, G. DAUBE, B. TAMINIAU and J. FONTAINE
University of Liege, Liege, Belgium

As described for the proactive topical therapy skin lesions of dogs with atopic dermatitis, the use of a topical glucocorticoid to control the inflammation of the external ear canal (EEC) could represent an alternative to antimicrobials for maintaining the balance of the bacterial and fungal flora. Ten dogs with atopic dermatitis are selected. Mometasone 0.1% lotion (Elocon, MSD Brussels, Belgium) was applied at 0.3 ml twice weekly during 4 weeks in the right EEC, the left ear canal left untreated. Clinical and cytological examinations of the EEC were performed on Day 0, 14 and 28 and scored with a global index (0-12 grades). Samples were collected at each visit for microbiota (evaluation of hypervariable segments V1-V3 of the 16S DNA) and mycobiota (identification of the variables in ITS areas) evaluations. The global indices were not significantly different between the right and left EECs on either Day 0 and 28. In contrast, indices were significantly different during treatment in the right ear canal (paired t-test, $P = 0.024$), but not in the untreated left EEC. There were no significant differences in the richness, evenness or diversity of the bacterial or fungal microbiota between the treated or untreated EECs (Kruskal-Wallis or Friedman tests). In conclusion, proactive therapy with topical glucocorticoids could be useful to prevent otitis in atopic dogs. This treatment does not appear to change the microbiota and mycobiota during a 4-week period. These preliminary results should be confirmed in a longer duration and a larger number of dogs.

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Conflicts of interest: None declared.
Effect of phenol and formalin on mecA in methicillin-resistant Staphylococcus pseudintermedius (MRSP) as part of autogenous bacterin formulation

A. LOEFFLER, C. SCOTT and S.M. FROSINI
Royal Veterinary College, London, United Kingdom

Autogenous Staphylococcus pseudintermedius bacterins are formulated by rendering bacteria non-viable using phenol and formalin, while aiming to preserve bacterial cell wall antigens. The emergence of MRSP has raised concern over dispersal of mecA through bacterin therapy, particularly since a variable effect of formalin on DNA has been reported. We investigated the presence and integrity of mecA after bacterin formulation in 19 staphylococcal isolates (7 MRSA, 12 MRSP) from canine pyoderma. Pellets from overnight broth incubation were washed three times with 0.5% sterile phenol saline, followed by the addition of 0.1ml of 10% formol saline to 10ml of the phenol saline suspension. Bacterin sterility was confirmed and DNA extracted using a standard genomic extraction kit and another recommended for formalin-fixed tissue samples (FFT). The presence of mecA was determined after PCR and its integrity examined in five randomly selected samples after sequencing. Five isolates were also examined after phenol or formalin processing only. In all 19 bacterins from methicillin-resistant isolates, mecA was detected following FFT extraction and products aligned fully to a reported mecA sequence. After standard DNA extraction, mecA was seen in 16/19 samples. Preparations of separate phenol or formalin were not sterile (80%) and mecA remained unaffected. The persistence of mecA following bacterin processing suggests a risk of dispersal of an important resistance mediator through MRSP bacterin therapy. While the transmissibility of naked mecA remains unclear, it seems prudent to initially resolve MRSP infection and subsequently formulate autogenous bacterins from mecA-negative S. pseudintermedius for long-term management of pyoderma.

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Conflicts of interest: None declared.
Compassionate use of Allermune immunotherapy in a cat with mite associated skin and respiratory hypersensitivity

F. MARTINI*, C. FAVROT*, F. BAUMANN†, A. ROSTAHER* and N. FISCHER*
* Department of Small Animal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
† Department of Clinical Services, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Allermune (Zenoaq, Japan) is a recombinant Dermatophagoides farinae 2 (Derf2)-pullulan-based immunotherapy vaccine whose efficacy on house dust mite allergic dogs has been demonstrated in several studies. There are no data available on the efficacy of Allermune in allergic cats. A thirteen-year-old neutered female European shorthair cat was presented with a long-lasting history of non-seasonal self-induced alopecia, pruritus and asthma. The patient’s symptoms have been managed using fluticasone-propionate (0.125 mg once daily), oclacitinib (0.6 mg/kg once to twice daily) and hydrocortisone aceponate spray once daily. Despite these treatments neither medication dosage reduction nor long-lasting improvement of the clinical signs was possible. Initially, oral prednisolone induced a transient diabetes mellitus and treatment with oral glucocorticoids was abandoned. The patient showed elevated dust mite Derf-specific IgE in serum and Allermune treatment was initiated once weekly for 6 weeks then every month for 3 months. The other medications were discontinued, with the exception of the fluticasone-propionate inhaler. After 6 weeks, the client reported significant strong pruritus reduction. After 22 weeks, hair regrowth was almost complete and fluticasone-propionate for asthma treatment was successfully reduced to every third day. No side-effects were seen at any time of the treatment. These results should be interpreted cautiously and further studies involving more animals should be carried out. The results can be regarded as promising and this treatment could potentially help many cats presenting skin or respiratory signs of mite-hypersensitivity.

Source of funding: Allermune was gifted by Zenoaq.

Conflicts of interest: C.F. has lectured for Zenoaq.
The potential of a recombinant anti-IgE mouse x dog chimeric antibody for treatment of canine IgE-mediated allergy


* Animal Allergy Clinical Laboratories, Inc., Sagamihara, kanagawa, Japan
† Vaccine Innovation Laboratory, RIKEN Baton Zone Program, RIKEN, Yokohama, kanagawa, Japan
§ Yamaguchi University, Yamaguchi, Japan

The ideal therapeutic antibody for immunoglobulin E (IgE)-mediated allergy would recognize and kill IgE-producing B cells without mast cell degranulation. A mouse monoclonal antibody against dog IgE, CRE-DR, was examined for its potential as a therapeutic anti-IgE antibody. In flow cytometry, CRE-DR recognized IgE-positive B cells among dog peripheral blood mononuclear cells (PBMCs) and an IgE-producing dog myeloma cell line; in contrast, it did not recognize canine IgE-coated RBL-2H3 rat mast cells. A pharmacokinetic (PK)-test performed in a laboratory dog, in which a high-IgE serum and CRE-DR were injected, was negative, thereby indicating that CRE-DR did not cross-bridge IgEs to degranulate mast cells in vivo. A chimeric antibody was made by CHO cells transfected with genes of complementarity determining the region of CRE-DR grafted with the dog IgG-B gene. The reactivity of this chimeric antibody for canine IgE was nearly similar to that of CRE-DR by ELISA. The percentages of chimeric antibody-induced cytotoxicity on IgE-producing dog myeloma cells as target cells and dog PBMCs as effector cells increased, up to 89%, in an antibody concentration-dependent manner, while those of a recombinant control dog IgG-B remained as 4%. The direct killing effect of the chimeric antibody on the myeloma cells without PBMCs was 25 % at the highest chimeric antibody concentration. In summary, this chimeric antibody would be useful as a therapeutic antibody to eliminate IgE-producing B cells without anaphylaxis by mast cell degranulation in dogs with IgE-mediated allergy.

Source of funding: Self-funded.

Conflicts of interest: KM and TM hold stocks in Animal Allergy Clinical Laboratories, Inc.
Testing the validity of the Canine Dermatitis Quality of Life and Treatment Satisfaction Questionnaire (CDQOL-TSQ) through correlations with other measures

C. NOLI*, A. WRIGHT†, J.R. WELLS§, P. GRIFFITHS§ and E. BROHAN§
* Servizi Dermatologici Veterinari, Peveragno, Italy
† Zoetis, Parsippany, NJ, USA
§ Patient-Centered Outcomes, Adelphi Values, Bollington, UK

The measurement of quality of life (QoL) is valuable to assess disease burden and treatment benefit. The Canine Dermatitis Quality of Life and Treatment Satisfaction Questionnaire (CDQOL-TSQ) was developed to assess the QoL of dogs with dermatitis and their owners. We wished to assess the convergent validity of the CDQOL-TSQ through examining how the instrument correlates with previously published QoL and clinical assessment instruments. The owners of 71 dogs with allergic dermatitis completed the CDQOL-TSQ, the QoL questionnaire for dogs with skin disease developed by Noli (QoLQDSD), and a pruritus visual analogue scale (PVAS). Meanwhile, veterinarians assessed the disease severity using the CADLI and CADESI4. Correlations were assessed between the CDQOL-TSQ and all other measures. Moderate-to-high correlations (Spearman’s $r = 0.30-0.50$) were expected between the QoL evaluation tools, as domains measure similar concepts, while lower correlations ($r < 0.30$) were expected between the CDQOL-TSQ and the disease severity measures, as lesions and pruritus are different from the QoL. The CDQOL-TSQ demonstrated moderate-to-strong correlations with the QoLQDSD, thus suggesting a convergent validity (Dog’s QoL; $r=0.42-0.49$, Owner’s QoL; $r=0.47-0.63$). Lower correlations were found between the disease severity measures and the CDQOL-TSQ ($r=0.19-0.29$). The moderate correlations between the CDQOL-TSQ and the QoLQDSD confirms that these questionnaires measure similar concepts, while the lower correlations between the QoL and the disease severity measures suggests that they are conceptually different. The CDQOL-TSQ is a valuable tool for QoL assessment that could be used, alongside clinical symptoms measures, to fully understand disease burden in dogs with dermatitis.

Source of funding: Zoetis.

Conflicts of interest: CN has received lecture and consultation fees from Zoetis; AW and JR are employee of Zoetis.
Cutaneous bullous mastocytosis in a Yorkshire terrier puppy

A. PETAK*, I. C. ŠOŠTARIC-ZUCKERMANN†, A. GUDAN KURILJ† and N. LEMO*

§ Faculty of Veterinary Medicine of University of Zagreb, Clinic for internal diseases, Zagreb, Croatia
† Faculty of Veterinary Medicine of University of Zagreb, Department of veterinary pathology, Zagreb, Croatia

Diffuse cutaneous mastocytosis (DCM) is rare variant of cutaneous mastocytosis, representing 1-5% of all cases in human paediatric medicine. Blistering and bullae may be the presenting symptoms and the blisters can be hemorrhagic. A 7-month-old Yorkshire terrier puppy had cutaneous bullous lesions that started at 6 weeks of age with occasional vomiting, painful defaecation and hypotensive episodes. Skin changes were generalised but the ventral abdomen was severely affected. The skin was alopecic, severely thin, and hypotonic with prominent blood vessels. Bullae were seen mostly in the inguinal and axillary regions, with ulceration and hemorrhage. Histopathological examination showed severe diffuse infiltration of mast cells. Immunohistochemistry for C3, C9, and mixed IG depositions ruled out other autoimmune bullous diseases. The C-kit receptor expression was characterized by none or faint membrane staining yet intensive and partially granulated cytoplasmic reaction (most consistent with KIT pattern 2, as described by Kiupel, 2017). The dog was treated with ranitidine, cetirizine, methylprednisolone and a restrictive diet. A second biopsy revealed no significant improvement. With Masitinib mesylate (Masivet: AB Science, Paris, France) at 9.6 mg/kg once daily for 2 months, and every other day for one more month, hemorrhagic bullae reduced in size and severity. A third biopsy after 10 months of treatment revealed a decreased number of mast cells but severe dermal atrophy. To the best of the authors’ knowledge, this is the first described case of cutaneous bullous mastocytosis in a puppy and, therefore, should be included in differential lists where bullae are the dominant feature in young animals.

Source of funding: Self-funded.

Conflicts of interest: None declared.
Efficacy of allergen-specific immunotherapy in dogs with atopic dermatitis: a retrospective study of 145 cases

L. RAMIÓ-LLUCH*, P. BRAZÍS*, L. FERRER† and A. PUIGDEMONT§

* Animal Health, B.U. Laboratorios LETI, Barcelona, Spain
† Dept of Animal Medicine and Surgery, Univ. Autonòma de Barcelona, Barcelona, Spain
§ Dept of Pharmacology, Therapeutics and Toxicology, Univ. Autònoma Barcelona, Barcelona, Spain

Allergen-specific immunotherapy (ASIT) has been used for years in dogs with atopic dermatitis (AD), although evidences of efficacy are limited. The aim of this study was to review retrospectively a large number of dogs with AD treated with ASIT to better understand the factors that may influence its efficacy. One hundred and forty-five privately-owned dogs diagnosed with AD between 2016 and 2018 and treated with ASIT were included. Thirty-three of the dogs (23%) discontinued the ASIT before 10 months, due to lack of efficacy (11%) or for other reasons (owner compliance, cost, development of other diseases) (12%). Approximately half of the dogs that discontinued the treatment did not refill it and therefore they were treated only for < 8 months. Of the 112 dogs treated with ASIT for >10 months, 81 (72%) showed a significant improvement in clinical signs, measured in a disease severity score from 0 to 10 (p<0.001, Student’s paired t-test). The clinical signs began to improve at a mean time of 4.7±2.7 months after beginning of the treatment. ASIT also was associated with a sparing effect of concomitant medication (specifically in local and systemic corticoids, oclacitinib, systemic and local antibiotics, p<0.001). After this period, 58% of the dogs were treated exclusively with ASIT. Approximately 50% of the withdrawn animals did not refill the prescription and this may be one cause for the limited success of ASIT reported in the literature.

Source of funding: This study was supported in part by Laboratorios LETI.

Conflicts of interest: LR and PB are employees of Laboratorios LETI; they participated in the design of the study and helped to the draft of the manuscript. LF and AP have received unrelated funding from LETI. No conflicts of interest have been declared by the other authors.
Effect of afoxolaner for the treatment of lice in the zoo birds *Pavo cristatus*, *Ortalis vetul* and *Phasianus colchicus*

C. ROMERO*, E. YARTO†, C. SHEINBERG†, R. HEREDIA* and A.M. CORDERO‡

* Dermatología Veterinaria, México City, México
† Centro Veterinario México, Mexico City, México
‡ VETDERM, Guadalajara, México

Several louse species affecting birds, such as *Pavo cristatus*, *Ortalis vetula* and *Phasianus colchicus* have been reported worldwide. These lice can cause stress, anorexia, weight loss and anemia, especially during severe infestations. The isoxazolines could be a valuable alternative to the prevailing treatments for such parasites, as this class of drugs has been used successfully in poultry without adverse effects on health or production. Our goal was to evaluate the effect of afoxolaner for the treatment of lice in zoo birds of these three genera. Fourteen peacocks, 36 pheasants and 11 plain chachalacas naturally-infested by the *Goniodes pavonis* lice were included. They were divided into two groups: Group 1 (nine peacocks, 21 pheasants and three chachalacas) was treated with 2.5 mg/kg of afoxolaner, orally, once daily with a dose calculated using an allometric scale; the group 2 (5 peacocks, 15 pheasants and 8 chachalacas) was left untreated. Lice were collected using the acetate-tape technique for their microscopic identification on Days 1 and 28. The proportion of peacocks, pheasants and chachalacas positive for lice on Day 28 (3%) was significantly lower than that of untreated birds (100%; Fisher’s test, \( P = 0.02 \)). We observed no adverse effects attributed to afoxolaner. The safety, efficacy and ease of administration of oral afoxolaner make it a promising treatment for insect ectoparasites in exotic birds.

**Source of funding:** Boehringer Ingelheim Animal Health.

**Conflicts of interest:** None declared.
Early-life risk factors and heritability of canine atopic dermatitis: a birth cohort study from West Highland White Terriers

A. ROSTAHER*, N.M. FISCHER*, G. DOLF†, S. AUDERGON*, L. ZWICKL§ and C. FAVROT*

* Vetsuisse faculty Tierspital Zurich, Zürich, Switzerland
† Vetsuisse faculty Bern - Institute of genetics, Bern, Switzerland
§ Salina Vetteam GmbH, Rheinfelden, Switzerland

In human allergology accumulating evidence suggests that environmental experiences during the first months of life can influence the development of allergic disease. To the authors' knowledge so far no study evaluated this in veterinary medicine. Therefore the aim of this study was to assess early-life risk factors for canine atopic dermatitis (AD) and secondly to estimate its heritability. A West Highland White Terrier birth cohort (n: 107) was followed up to age 3 years to record the development of AD. The effect of environmental factors (house dust mites (HDM), hygiene, feeding, life style) and early life determinants (breeder, mode of delivery, birth season, sex, litter size, early life IgE levels) was assessed. After data editing 92 dogs of which 48 (52%) were deemed atopic entered the statistical analyses using Stata SE 15.1. Heritabilities were estimated using the R program packages MCMCglmm and Qgglmm. Male gender (P=0.06), delivery by cesarian (P=0.12), breeder (P=0.06), presence of HDM (P=0.11) and hygiene standard (P=0.15) were identified as possible influence factors by univariate analyses. In the multivariate analysis male gender was significantly associated with the development of AD in the offspring (P=0.03, OR 0.39), as was maternal atopic dermatitis (P= 0.013, OR 3.3). The heritabilities were 0.31 (direct) and 0.04 (maternal genetic). This study adds to the understanding of canine AD epidemiology. Our study suggests several environmental factors that could influence the occurrence of canine AD but also clearly shows the genetic influence.

Source of funding: This study was funded by the Swiss National Science Foundation.

Conflicts of interest: None declared.
Prevalence of multidrug-resistant Staphylococcus pseudintermedius and Staphylococcus aureus in dogs and people: a comparative dermatology case-control study

D. SANTORO, M. BOHANNON, A. FOXWORTH, L. VELEZ and A. DE BENEDETTO
University of Florida, College of Veterinary Medicine, Gainesville, USA

In the past decade, the incidence of methicillin-resistance (MR) and multidrug-resistance (MDR) has dramatically increased in humans and dogs. In dermatological patients, recurrent staphylococcal infections, S. aureus (SA) in people and S. pseudintermedius (SP) in dogs, often require multiple cycles of antibiotics selecting for MDR. This retrospective study aimed to evaluate the prevalence of MRD (resistance to ≥3 antibiotic classes) in patients (canine and human) presenting to the dermatological service of the same institution over six years (2011-2017). One-hundred bacterial cultures from 72 dogs and 228 cultures from 190 people were reviewed. Age, sex, atopic status, bacterial sensitivity, previous history of antibiotics, MR and MDR status were recorded. Frequency of MDR was evaluated by logistic regression. Forty-six MDR-SP (46%, canine cohort) and 54 MDR-SA (21.9%, human cohort) isolates were identified in this study. Logistic regression highlighted MR status as a major risk factor for MDR. In dogs, 35/46 (54%) MDR-SP were MRSP, whereas in people, 48/54 (88.9%) MDR-SA were MRSA. The odds to having MDR were three times (OR: 3.08; 95% CI: 1.19-8; p=0.02) higher in dogs with MRSP and 39 times (OR: 39.9; 95% CI: 10.3-153.7; p<0.0001) higher in people with MRSA. Malassezia dermatitis was negatively associated with MDR (OR: 0.2; 95% CI: 0.05-0.7; p=0.01) in dogs. Finally, a significantly higher prevalence of MR (p<0.0001) and MDR (p<0.0001) was seen in dogs compared to people. This is the first comparative study showing the higher prevalence of MDR among MR staphylococci independently from atopic status and previous exposure to antibiotics.

Source of funding: Self-funded

Conflicts of interest: None declared
Effects of nanosulfur against multi-drug resistant *Staphylococcus pseudintermedius*: an antimicrobial, anti-biofilm and cytotoxicity study

D. SANTORO, L.D. KHER, D.J. GIBSON and G. SCHULTZ
University of Florida, College of Veterinary Medicine, Gainesville, USA

Because of the increase rate of antimicrobial resistance, there is a constant need to develop newer and safer antimicrobials. In dermatology, elemental sulfur has been used for centuries for its antimicrobial effects. Few antimicrobials have been developed as nanoparticles to increase their efficacy and reduce their side effects. The objective of this study was to assess the antimicrobial/anti-biofilm activity of nanosulfur against multi-drug resistant *Staphylococcus pseudintermedius* (MDR-SP). Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of nanosulfur (47nm, orthorhombic) and nanosilver (50nm, spherical) were tested against ten clinical isolates of MDR-SP in planktonic (microbroth dilution) and biofilm (canine skin explants) states. Twelve (233.3 to 0.11 mg/mL) and five (1,866.6 to 233.3 mg/mL) two-fold serial dilutions of both nanoparticles were tested for planktonic and biofilm formation, respectively. All the concentrations were tested for cytotoxicity using LDH and ATP assays. Seven of ten (70%) MDR-SP isolates were susceptible to nanosulfur (MIC/MBC: 233.3µg/mL). A biofilm bactericidal concentration for nanosulfur was achievable in 6/10 isolates (933.3µg/mL). Nanosilver did not show any antimicrobial or anti-biofilm activity at any concentrations tested. Both nanoparticles showed no cytotoxicity with LDH assay. At high concentrations (933.3 and 1866.6 mg/mL), nanosulfur tended to precipitate resulting in a significant reduction in ATP levels. This is the first study showing the antimicrobial and anti-biofilm activity of nanosulfur for MDR-SP with an absence of cytotoxicity. Nanosulfur has the potential to be used in veterinary and human medicine as an effective, safe, and cheap alternative to the antimicrobials/anti-biofilm agents currently available.

**Source of funding:** American Kennel Club.

**Conflicts of interest:** None declared.
Evaluation of an ear cleaner based on natural ingredients with antimicrobial, antibiofilm and anti-inflammatory properties in rabbits with otitis externa

G. SHEINBERG*, A.M. CORDERO†, C. ROMERO‡, A. FLORES# and R. HEREDIA#

* Centro Veterinario Mexico, Mexico City, Mexico
† VETDERM, Guadalajara, Mexico
‡ Universidad Autonoma del Estado de Mexico, Toluca, Mexico

Rabbits have become popular pets and otitis externa is challenging to treat in this species due to the type of exudate they produce. Our objective was to evaluate the efficacy of a commercial otic solution (PYOclean Oto, LDCA, Castres, France) composed of natural ingredients such as green apple lipoaminoacids, honey and propolis, combined with red myrtle essential oil and N-acetylcysteine. Thirty New Zealand rabbits with otitis externa due to *Psoroptes cuniculi* were divided randomly into two groups: 15 were treated with the above solution for 4 weeks while the other 15 were untreated controls. The ear canal was evaluated visually and cytologically on Days 0, 7, 14 and 28 to determine the presence of bacteria, *Psoroptes* and *Malassezia*. The treated group showed a gradual decrease in the presence of ectoparasites, and the cytological detection of bacteria was significantly reduced at D7 (53%, *p* < 0.01); bacteria were no longer found on Day 21. *Malassezia* yeast were not seen on any of the cytological examinations. All rabbits from the control group remained infected with ectoparasites and bacteria. A progressive decrease in the severity of otitis was observed in the treated group: 73% of ear canals presented severe lesions on Day 7 while all were lesion free on Day 28. Adverse effects were not observed in any treated rabbit. In summary, the PYOclean Oto effectively eliminated the presence of *Psoroptes cuniculi* and cocci bacteria over three weeks, and signs of otitis decreased without the use of additional topical or systemic medications.

Source of funding: Laboratoire de Dermo-Cosmétique Animale, Castres, France.

Conflicts of interest: None declared.
Efficacy and safety of a 0.0584% hydrocortisone aceponate spray to reduce flares of canine atopic dermatitis when tapering oclacitinib: a randomized, double-blinded, placebo-controlled trial

J. TAKAHASHI*, S. KANDA* and K. IYORI†
* Noah Animal Hospital, Kofu, Japan
† Vet Derm Tokyo, Tokyo, Japan

Both oclacitinib (Apoquel; Zoetis JP, Tokyo, Japan) and an 0.0584% hydrocortisone aceponate (HCA) spray (Cortavance; Virbac, Osaka, Japan) have been approved for treatment of canine atopic dermatitis (AD). Atopic dogs treated with oclacitinib sometimes exhibit a flare when decreasing its administration frequency. This randomized, double-blinded, placebo-controlled trial aimed to evaluate the efficacy of the HCA spray to prevent exacerbation of signs when tapering oclacitinib. Two groups of nine atopic dogs were randomized to receive oclacitinib (0.4-0.6mg/kg twice daily for 14 days then once daily) with either the HCA spray (once daily for seven days then every other day) or placebo. Owners assessed pruritus with a visual analog scale while veterinarians scored the CADESI4 every seven days until Day 28. Blood samples were collected every two weeks for safety evaluation. On Days 7, 14, 21 and 28, HCA-treated dogs had a 35, 52, 55 and 59% reduction from baseline in CADESI4, respectively, compared with a 29, 45, 34 and 30% reduction in the placebo group. From Days 14 to 21, the mean CADESI4 score of HCA-treated dogs decreased (P = 0.4334) while that of the placebo group increased significantly (P = 0.0038). The mean reduction in PVAS of HCA-treated dogs was significantly higher than that of the placebo group on Day 21 (P = 0.0243). One HCA-treated dog was withdrawn due to diarrhea. Alkaline phosphatases activity increased in three placebo-receiving dogs. In conclusion, the combination treatment of oclacitinib and the HCA spray in atopic dogs helps prevent flares when tapering oclacitinib.

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Conflicts of interest: None declared.
An analysis of the correlation between allergen-specific antibodies and clinical signs after subcutaneous immunotherapy with recombinant pullulan-conjugated Der f 2 in dogs with atopic dermatitis

K. UEDA*, T. KAWAI*, Y. SUGIYAMA*, A. CHIDOI†, D. MINEGISHI† and T. TSUKUI†

* Yokohama Yamate Dog and Cat Medical Center, Yokohama, Kanagawa, Japan
† Nippon Zenyaku Kogyo Corporation (Zenoaq), Koriyama, Fukushima, Japan

The specific mechanisms of clinical sign improvement during subcutaneous immunotherapy (SCIT) are not known. In this study, we performed SCIT in 12 dogs with AD using recombinant pullulan-conjugated Der f 2 (rDF2-p), and we examined the evolution of allergen-specific antibody levels and clinical signs. Before, 2 and 4 months after starting SCIT, serum rDF2-specific IgE and IgG and rDF2-p-specific IgG were measured by ELISA while clinical signs were evaluated using the CADESI4. Recombinant Der f 2-specific IgE (in OD values average ± SD) were 0.9 ± 1.0 (before), 0.9 ± 1.0 (2 months) and 0.7 ± 0.7 after 4 months. Recombinant Der f 2-specific IgG were 1.0 ± 0.5 (before), 1.2 ± 0.7 (2 months) and 1.3 ± 0.8 after 4 months. Similarly, rDF2-p-specific IgG were 0.8 ± 0.5 (before), 1.6 ± 0.9 (2 months) and 1.7 ± 0.9 after 4 months. Finally, CADESI4 score decreased from 23.8 ± 10.2 (before) to 10.2 ± 8.4 (2 months) and 6.7 ± 7.2 after 4 months. Recombinant DF2-p-specific IgG significantly increased (Friedman’s test, p < 0.01) while CADESI4 scores significantly decreased during rDF2-p SCIT (Friedman’s test, p < 0.001). A mild negative correlation was confirmed between rDF2-p-specific IgG and CADESI4 scores (Spearman’s r = -0.3, p = 0.1). These results suggest that the improvement of clinical signs of AD during SCIT are linked to the production of allergen-specific blocking IgG.

Source of funding: This study was Self-funded.

Conflicts of interest: None declared.
Anticancer effects of betulinic acid derivative NVX-207 on equine melanoma cells and percutaneous permeation through isolated equine skin in vitro


* Clinic for Horses, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany
† Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary M, Hannover, Germany
§ Biosolutions Halle GmbH, Halle (saale), Germany
¶ Biozentrum, Martin Luther University Halle-Wittenberg, Halle (saale), Germany
** University Equine Clinic, University of Veterinary Medicine Vienna, Vienna, Austria

Aging grey horses frequently suffer from equine malignant melanoma (EMM). Current therapies such as surgical excision can be challenging. Thus, more feasible topical treatment options should be considered. Therefore, betulinic acid-trisester (NVX-207), a derivative of the naturally occurring betulinic acid, was assessed for its anticancer activity on primary equine melanoma cells and primary equine dermal fibroblasts and evaluated for its percutaneous permeation in vitro. Cells were treated with different NVX-207 concentrations for 5, 24, 48 and 96 h. Cell proliferation and viability were assessed by means of crystal violet staining and CellTiter 96® AQueous One Solution (MTS) assays, respectively. Half-maximal inhibitory concentrations (IC\textsubscript{50}) were determined in 6-8 biological replicates. “Basiscreme DAC” with 1% NVX-207 was evaluated for its permeation through isolated equine skin (800 µm thickness; n=6) using Franz-type diffusion cells. NVX-207 was extracted from skin and a depth profile of the compound in the permeated skin was determined by HPLC analysis. NVX-207 showed antiproliferative and cytotoxic effects on both equine melanoma cells and fibroblasts in a time- and dose-dependent manner (IC\textsubscript{50} values in cytotoxicity assay after 96 h 0.1 µmol/L and 7.7 µmol/L for melanoma cells eRGO1 and MelDuWi, respectively). Detected amounts of NVX-207 in the different skin layers by far exceeded the calculated IC\textsubscript{50} values. In conclusion, NVX-207 is a promising compound for topical EMM treatment. Safety and in vivo antitumoral effects of the pharmaceutical formulation need to be assessed in prospective clinical studies in horses.

Source of funding: This study was funded by the German Federal Ministry for Economic Affairs and Energy.

Conflicts of interest: None declared.
Is the Canine Dermatitis Quality of Life and Treatment Satisfaction Questionnaire (CDQOL-TSQ) sensitive to differences in disease severity?

A.K. WRIGHT*, J.R. WELLS†, P. GRIFFITHS† and E. BROHAN†
* Zoetis, Parsippany, NJ, USA
† Patient-Centred Outcomes, Adelphi Values, Bollington, UK

The measurement of quality of life (QoL) is valuable to assess disease burden and treatment benefit. The Canine Dermatitis Quality of Life and Treatment Satisfaction Questionnaire (CDQOL-TSQ) is an owner-completed measure to assess the QoL of dogs with dermatitis and their owners. Our objective was to examine whether the CDQOL-TSQ could distinguish between dogs with lower and higher disease severity using the known-groups method of establishing questionnaire validity. The CDQOL-TSQ and disease severity measures (pruritus visual analogue scale; PVAS, CADLI and CADESI4) were completed. Seventy-one dogs with allergic dermatitis were separated into those with lower and higher disease severities, informed by the thresholds for normal-to-mild atopic dermatitis suggested by the CADLI and CADESI4 included in the Core Outcome Set for Canine Atopic Dermatitis (COSCAD). The size of differences in QoL between severity groups was assessed against established psychometric thresholds (small: $d=0.20$; medium: $d=0.50$; large: $d=0.80$) with the expectation that dogs with higher disease severity would have worse QoL scores. The QoL was consistently worse in dogs with more severe disease. The size of the difference between severity groups was medium for the Dog’s ($d=0.37-0.64$) and the Owner’s QoL ($d=0.43-0.65$) scales. These results support the validity of the CDQOL-TSQ, showing that it can distinguish between dogs with varying disease severities. The magnitude of effect-size was such that this difference in QoL would be clinically recognisable. The CDQOL-TSQ, when used alongside clinical measures, can enhance the assessment of treatment benefit and aid discussions with dog owners regarding disease burden.

Source of funding: Zoetis.

Conflicts of interest: AW is an employee of Zoetis while JRW, PG and EB are employed by Adelphi Values.
AUTHORS INDEX

A
Alexandre, F., 120
Amalric, N., 123
Apostolidis, K., 135
Apostolopoulos, N., 112
Audergon, S., 127, 146
B
Bacon, N.J., 18, 21, 50, 53, 74, 77
Bagwe, B., 112
Banovic, F., 113, 114, 117, 118, 119
Baumann, F., 140
Beccati, M.B., 115
Bergeron, C.C., 116
Bernardi De Souza, L., 116
Bizikova, P., 126
Blubaugh, A., 113, 114, 117, 118, 119
Bohannon, M., 147
Bourdeau, P.J., 120
Brachelente, C., 66, 68, 79, 90
Bradley, C., 122
Brazis, P., 130, 144
Brohan, E., 142, 153
Brosseau, C., 121
Brun Rosa, F., 34
Buckley, L.M., 68, 70, 81
C
Cadiergues, M.C., 123
Cain, C.L., 122
Calvet, A., 130
Carrasco, I., 128, 130
Castilla-Castano, E., 123
Catalucci, C.C., 132, 133
Cavalleri, J.M.V., 152
Chidoi, A., 151
Citron, L., 122
Clemente, F., 130
Combarros, D., 123
Cordero, A.M., 145, 149
Costa, M.C., 116
Cózar, A.I., 124
Cristófol, C., 128
Cusco, A., 131
D
Dalmau, A., 124
Daube, C., 138
De Benedetto, A., 147
De Jahn, C., 121
Del Castillo, J.I.E., 121
Delarocque, J., 152
Denley, T., 113, 114, 117, 118, 119
Di Loria, A., 135
Di Palma, A.D., 115
Dolf, C., 146
Dumont, C., 120
E
El Garch, F., 125
Ewers, C., 112
F
Fall, P.A., 138
Fantini, O., 125
Favrot, C., 126, 127, 129, 140, 146
Feige, K., 152
Ferrer, L., 96, 98, 128, 131, 144
Fischer, N., 126, 127, 129, 140, 146
Flores, A., 149
Fujikake, K., 141
G
Galland, D., 125
Garcia-Fonticoba, R., 131
Ghibaudo, G.G., 132, 133
Gibson, D.J., 148
Gillen, J.B., 134, 135
Glaser, S.P., 112
Gogal, R.M., 113, 114
Grice, E.A., 122
Griffiths, P., 142, 153
Grogan, M.D., 122
Gudan Kurilj, A., 143
H
Hedley, J., 28, 30
Heredia, R., 145, 149
Hernandez-Bures, A., 134, 135
Hoover, K., 118, 119
Hubert, F., 120
Hwang, C.Y., 136
I
Iyori, K., 150
J
Jeanneau-Imparato, L., 120
K
Kalbitz, J., 152
Kämpfer, P., 112
Kanda, S., 150
Kang, J.H., 136
Kawai, T., 151
Kher, L.D., 148
Kietzmann, M., 32, 64, 88, 94, 152
Kumagai, A., 137, 141
L
Lecru, L.A., 123
Lemo, N., 143
Leonard, C., 138
Loeffler, A., 139
M
Martini, F., 140
Masuda, K., 137, 141
Maurer, M., 36, 38
Mayer, U., 112
Meason-Smith, C., 62
Meißner, J., 152
Michaelis, A., 152
Minegishi, D., 151
Mizuno, T., 141
Morinello, K.A., 40, 46, 48
Morris, D.O., 122
Mueller, R.S., 42
Myers, A., 60
Myotte-Duquet, D., 125
AUTHORS INDEX

N
Nara, T., 141
Neiger, R., 112
Ngo, J., 138
Nol, C., 142

O
Older, C., 34, 44, 60
Olivry, T., 108, 110, 126

P
Pagé, N., 121
Pandolfi, P.P., 115
Papich, M.G., 24, 26, 58, 64, 92
Paschke, R., 152
Petak, A., 143
Prats, A., 130
Pressanti, C., 123
Puigdemont, A., 128, 130, 144

R
Ramió-Lluch, L., 144
Rissi, D., 118
Rodrigues-Hoffmann, A., 34, 44, 60, 62
Romero, C., 145, 149
Rostaher, A., 126, 127, 129, 140, 146
Roux, C., 120

S
Saitama, M., 137
Saito, T., 141
Santoro, D., 134, 135, 147, 148
Saridomichelakis, M., 135
Sauvé, F., 116
Scarampella, F., 130
Schmidt, V.M., 55, 72, 83, 86
Schultz, G., 148
Scott, C., 139
Sheinberg, G., 145, 149
Šoštaric-Zuckermann, I.C., 143
Sugiyama, Y., 151
Suzuki, C., 141

T
Takahashi, J., 150
Taminiau, B., 138
Tanaka, T., 141
Taraglio, S.T., 133
Thom, N., 112
Thorin, C., 120
Torihama, K., 141
Tsukui, T., 137, 151
Uchida, K., 141
Ueda, K., 151
Valenti, V.P., 132
Velez, L., 147
Vercelli, V.A., 133
Watanabe, S., 141
Weber, L.A., 152
Wells, J.R., 142, 153
Wright, A.K., 142, 153
Yarto, E., 145
Yosipovitch, G., 100, 102, 104, 106
Zwickl, L., 127, 146
The influence of shampooing or water immersion regarding the transmission of canine leishmaniosis has not been examined. It is shown that monthly shampooing or water immersion does not significantly shorten the 8 months efficacy duration for ticks. Reduction of the risk of infection with *Babesia canis vogeli* and *Ehrlichia canis* from the tick vector *Rhipicephalus sanguineus*, thereby reducing the risk of canine babesiosis and canine ehrlichiosis for 7 months. Reduction of the risk of infection with *Laxinum argenteum* as transmission by sand fleas for up to 8 months. For treatment of biting/chewing lice (*Trichodectes canis*) infestation. Ideally, the collar should be applied before the beginning of the flea or tick season. Dogs: for the treatment and prevention of *Rhipicephalus sanguineus* and *Ixodes ricinus* infestation for 8 months. Precautions: The collagen should be applied before the beginning of the flea or tick season. Ideally, the collar should be applied before the beginning of the flea or tick season..

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