WELCOME TO VALENCIA!

Dear Friends and colleagues,

We are very happy to welcome you to Valencia, “The City of Light and Flowers” for this 26th Annual Congress of the European Society and College of Veterinary Dermatology.

As for previous European dermatology congresses, the Scientific Organizing Committee has organized two separate lecture tracks: the Continuing Education Program (which is to be simultaneously interpreted from English to Spanish) has lectures on the diagnosis and treatment of the main alopecic and pruritic dermatoses while the Advanced Program was set with high level lectures about the skin barrier, topical therapy, itch and autoimmune skin diseases. As for the 2011 congress, we have reserved a third room for expanded oral and poster shortcommunication theme-based sessions.

The Local Organizing Committee chose the city of Valencia because of its luminosity and strategic situation next to the Mediterranean Sea. This location provides all of us, clinicians and scientists alike, with the possibility to expand beyond veterinary dermatology to meet an ancient culture, to be exposed to the sun (hopefully) and to taste the wonderfully famous local gastronomy.

Valencia is located in the central area of the Spanish Mediterranean coast, and it was funded as a Roman colony in 138 BC. Valencia was an important “Muslim Taifas Kingdom” since the 8th century; it became a Christian city after the 13th century. In the 15th century, Valencia became a commercial Empire and wonderful civil buildings, such as “La Lonja of the Silk” were built then. The Old Town can be visited using our tramway, or by walking around the old Wall. In that area, you will find the main historic buildings, such as the Cathedral and the Los Serranos bridge and tower. Not only do we have historic monuments, but the modern architecture of the “City of Arts and Sciences”, designed by Santiago Calatrava, might surprise you as well.

As you already know, Valencia is internationally known because of “The Paella”, the most representative dish of the Spanish gastronomy. What you may not know, however, is that the paella made with rice, fish and seafood (the so-called “Paella Marinera”) is not the authentic “Paella Valenciana”. We invite you to try our authentic typical paella, which is made with rice from “la Albufera”, chicken meat, snails, fresh vegetables and legumes; it is found in the old Valencia center neighborhood “Barrio del Carmen”. You might also wish to taste the other local dishes of “Fideuá” and “Arroz Negre” (black rice cooked in squid ink). If you would like to try a local refreshing beverage, ask for a very cold “Horchata”. If you would rather have something stronger, try the “Mistela-Moscatel” wine or the “Agua de Valencia” cocktail made with Cava, triple sec liqueur and orange juice.

It will be a pleasure to meet all of you at the Valencia Conference Centre, which seems to have been specifically designed to suit the needs of the Annual ESVD-ECVD Congress by Sir Norman Foster.

We would like to thank all our sponsors for their support and their involvement and collaboration to help organize this meeting that is being awaited with high expectation by many veterinarians in Europe. Finally, we would like to thank our congress organizer, Pauwels Congress Organisers (PCO) from Maastricht, the Netherlands, for the quality of their management skills and the professionalism of their staff.

We hope that this congress will fulfill your expectations, both scientifically but also socially.

Best wishes to all,

Dr. Maite Verde
Chair of the Local
Organizing Committee

Dr. Thierry Olivry
Chair of the Scientific
Organizing Committee
COMMITTEES

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### KEYNOTE SPEAKERS

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<td>Tierdermatologie Deisenhofen Consultations, Germany</td>
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<td>Thomas Bieber</td>
<td>University of Bonn, Germany</td>
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<td>Petra Bizikova</td>
<td>North Carolina State University College of Veterinary Medicine, USA</td>
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<td>Mona Boord</td>
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<td>Lluis Ferrer</td>
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<td>Thierry Olivry</td>
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<td>Susan Paterson</td>
<td>Rutland House Veterinary Hospital, United Kingdom</td>
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<td>Ian Ramsey</td>
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<td>University of Liverpool, United Kingdom</td>
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<tr>
<td>Jessica Stahl</td>
<td>University of Veterinary Medicine Hannover, Germany</td>
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<td>Maja Suter</td>
<td>University of Bern, Switzerland</td>
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SCIENTIFIC PROGRAMME
THURSDAY 19 SEPTEMBER 2013

CONTINUING EDUCATION SESSION

THE PRURITIC DOG
Chair: Pilar Brazis
9:00 - 9:45 • Approach to the pruritic dog - A. Fondati
9:45 - 10:30 • Management of the pruritic dog - A. Fondati

THE PRURITIC DOG
Chair: Pilar Brazis
11:15 - 12:00 • What to do when treatment fails - A. Fondati
12:00 - 12:45 • Improving client compliance - S. Bettenay

ENDOCRINE ALOPECIA
Chair: Sonya Bettenay
14:30 - 15:15 • Non inflammatory alopecia - S. Paterson
15:15 - 16:00 • Cushing’s Syndrome and Hypothyroidism: Case Discussions 1 - I. Ramsey

ENDOCRINE ALOPECIA
Chair: Sonya Bettenay
16:45 - 17:30 • Cushing’s Syndrome and Hypothyroidism: Case Discussions 2 - I. Ramsey
17:30 - 18:15 • Update on treating Cushing’s disease - I. Ramsey

TOPICAL THERAPY: PRINCIPLES AND USE IN ALLERGIC PATHOLOGY
Chair: Dominique Heripret
16:45 - 17:30 • Topical anti-inflammatory in veterinary dermatology: current status and future concepts - J. Stahl / M. Kietzmann
17:30 - 18:15 • Journal Club (1): a review of landmark papers published in the medical or veterinary literature - T. Olivry

SCIENTIFIC SESSION

SKIN BARRIER: FROM BIOLOGY TO ALLERGIC PATHOLOGY
Chair: Luc Beco
9:00 - 9:45 • Structure of the skin barrier and its relevance for skin disease - M. Suter
9:45 - 10:30 • Interspecies differences in skin barrier and transdermal penetration - J. Stahl / M. Kietzmann

SKIN BARRIER: FROM BIOLOGY TO ALLERGIC PATHOLOGY
Chair: Luc Beco
11:15 - 12:00 • Skin barrier in human atopic dermatitis - T. Bieber
12:00 - 12:45 • The skin barrier in canine atopic dermatitis - T. Olivry

TOPICAL THERAPY: PRINCIPLES AND USE IN ALLERGIC PATHOLOGY
Chair: Dominique Heripret
14:30 - 15:15 • Principles of topical therapy (formulations, penetration, permeation) - J. Stahl / M. Kietzmann
15:15 - 16:00 • Topical management of human atopic dermatitis - T. Bieber

10:30 - 11:15 BREAK

12:45 - 14:30 LUNCH

16:00 - 16:45 BREAK
FRIDAY 20 SEPTEMBER 2013

CONTINUING EDUCATION SESSION

DEMODICOSIS
Chair: Carmen Lorente

8:45 - 9:30  • The many faces of demodicosis - M. Boord
9:30 - 10:15  • Therapy of demodicosis - S. Paterson

IMMUNOMODULATORS IN VETERINARY DERMATOLOGY
Chair: Thierry Olivry

8:45 - 9:30  • Mechanisms of autoimmunity: From hypotheses to clinical cases - P. Bizikova
9:30 - 10:15  • Autoimmune skin diseases: From structure to skin lesions - M. Suter

10:15 - 11:15 BREAK

MALASEZIA AND ANTIFUNGAL THERAPY
Chair: Carmen Lorente

11:15 - 12:00  • The many faces of malassezia dermatitis - M. Boord
12:00 - 12:45  • Antifungal therapy - S. Paterson

AUTOIMMUNE DISEASES
Chair: Thierry Olivry

11:15 - 12:00  • Immunosuppression: mechanism of action of selected drugs - P. Bizikova
12:00 - 12:45  • Immunosuppression of autoimmune skin diseases: How to more specifically target disease pathogenesis - P. Bizikova

12:45 - 15:00 LUNCH
13:00 - 15:00 ESVD AGM, Auditorio 1

ANTIBIOTIC THERAPY
Chair: Susan Paterson

15:00 - 15:45  • The impact of antibiotic therapy on antimicrobial resistance mechanism - V. Schmidt
15:45 - 16:30  • Practice guidelines for antibiotic therapy - V. Schmidt

CLINICOPATHOLOGICAL CONFERENCE
Chair: Richard Harvey

15:00 - 16:30  • Clinicopathological conference: Is this disease autoimmune?
  Session 1: oral and perioral erosions
  Session 2: footpad sloughing - M. Suter / T. Olivry

16:30 - 17:15 BREAK

SURGERY
Chair: Susan Paterson

17:15 - 18:00  • Surgical treatment of chronic pododermatitis - M. Boord

JOURNAL CLUB
Chair: Richard Harvey

17:15 - 18:00  • Journal Club (2): a review of landmark papers published in the medical or veterinary literature - L. Ferrer
SHORT COMMUNICATION SESSION

MICROBIOLOGY
Chair: Pilar Brazis
Oral communications
11:15 - 11:30 • Genetic insights into the emergence of multidrug-resistance in meticillin-resistant Staphylococcus pseudintermedius - A. Loeffler
11:30 - 11:45 • Retrospective assessment of previous antibiotic therapy in dogs diagnosed with meticillin-resistant Staphylococcus pseudintermedius pyoderma - N. Okunaka
11:45 - 12:00 • Case-control risk factor study for meticillin-resistant Staphylococcus pseudintermedius (MRSP) infection in dogs and cats in Germany - G. Lehner
12:00 - 12:15 • Toll-like receptor 2 is overexpressed in dogs with demodicosis, Malassezia dermatitis and cutaneous bacterial infection - A. Rivas
12:15 - 12:20 • Antimicrobial susceptibility monitoring of dermatological pathogens isolated from diseased dogs and cats across Europe (ComPath I, 2008-2010) - C. Ludwig

12:20 - 12:45 Interaction with the following poster presenters
• Antimicrobial susceptibility monitoring of dermatological pathogens isolated from diseased dogs and cats across Europe (ComPath I, 2008-2010) - C. Ludwig
• In vitro activity of pradofloxacin against canine and feline pathogens recovered from skin infections in four European Union countries - C. Ludwig
• Development of an enzyme-linked immunosorbent assay for the serodiagnosis of ringworm infections in cattle - B. Mignon

PARASITOLOGY
Chair: Sonya Bettenay
Oral communications
15:00 - 15:15 • Canine nasal dermatitis: histopathological and immunopathological features of discoid lupus erythematosus and leishmaniosis - M. De Lucia
15:15 - 15:30 • Histopathological characteristics and expression of Toll-like receptor 2 in lesional skin of dogs with papular dermatitis due to Leishmania - L. Ordeix
15:30 - 15:35 • A cross-sectional survey of leishmaniosis in clinically normal and sick cats in Greece with indirect immunofluorescence antibody test and enzyme-linked immunosorbent assay - M. Chatzis
15:35 - 15:40 • Development of a PCR technique specific for Demodex injai in biologic specimens - I. Ravera
15:40 - 15:45 • Identification of three different Demodex species in cats using a novel PCR assay - D. Ferreira

15:45 - 16:30 Interaction with the following poster presenters
• A cross-sectional survey of leishmaniosis in clinically normal and sick cats in Greece with indirect immunofluorescence antibody test and enzyme-linked immunosorbent assay - M. Chatzis
• Development of a PCR technique specific for Demodex injai in biologic specimens - I. Ravera
• Identification of three different Demodex species in cats using a novel PCR assay - D. Ferreira
• Thyroid function in dogs with leishmaniosis due to Leishmania infantum before and during treatment - M. Saridomichelakis
• First report of straelensiosis in cats and unique features of the canine disease in Israel - R. Kaufmann
• Coproscopic detection and treatment of Demodex gatoi infestation in a Cornish rex cat in Austria - K. Silbermayr

16:30 - 17:15 BREAK
SATURDAY 21 SEPTEMBER 2013

CONTINUING EDUCATION SESSION

CLINICOPATHOLOGY CONFERENCE
Chair: Laura Ordeix
8:45 - 10:15 • Clinicopathological conference - S. Bettenay / S. Paterson

ADVANCED LEVEL: Auditorio 2

FELINE HYPERSENSITIVITY DERMATOSES
Chair: Jacques Fontaine
11:15 - 12:00 • Update on feline hypersensitivity dermatoses - C. Favrot
12:00 - 12:45 • Cyclosporine therapy for feline hypersensitivity dermatitis - C. Favrot

WHAT'S NEW IN CLINICAL DERMATOLOGY
Chair: Maite Verde
17:15 - 18:00 • What's new in clinical dermatology? - V. Schmidt

10:15 - 11:15 BREAK

ITCH
Chair: Claudia Nett
8:45 - 9:30 • Mechanism of atopic itch in humans and dogs: Selected (a)topics - T. Olivry
9:30 - 10:15 • An update on target-based pharmacotherapy of itch - T. Olivry

LASERS IN VETERINARY DERMATOLOGY
Chair: Claudia Nett
11:15 - 12:45 • Lasers in veterinary dermatology from theory to practice - M. Boord

12:45 - 15:00 LUNCH
13:30 - 15:00 ECVD AGM, Auditorio 1

LASERS IN VETERINARY DERMATOLOGY
Chair: Emmanuel Bensignor
15:00 - 16:30 • Lasers in veterinary dermatology: Case discussion - M. Boord

16:30 - 17:15 BREAK

LEISHMANIOSIS
Chair: Maite Verde
15:00 - 15:45 • Update on leishmaniosis diagnosis in dogs and cats - L. Ferrer
15:45 - 16:30 • Update on leishmaniosis treatment in dogs and cats - L. Ferrer

ULTRA-CHALLENGING CASES
Chair: Emmanuel Bensignor
17:15 - 18:00 • "The most bizarre case that we have ever seen" - L. Beco, P. Bizikova and T. Olivry
SATURDAY 21 SEPTEMBER 2013

SHORT COMMUNICATION SESSION

MISCELLANEOUS
Chair: Ursula Mayer

Oral communications
8:45 - 9:00  • The use of deslorelin to promote hair regrowth in dogs with Alopecia X - R. Cerundolo
9:00 - 9:05  • Prevalence of papillomavirus EcPV2 in clinically healthy horses in Switzerland - N. Fischer
9:05 - 9:10  • Sero- and genoprevalence of FdPV2 in healthy cats in Switzerland - M. Geisseler
9:10 - 9:15  • Dermoscopic features of dermatophytosis in 11 cats with M. canis infection - F. Scarampella
9:15 - 9:20  • Progressive tail necrosis in a rabbit colony - N. Thom
9:20 - 9:25  • Cutaneous larva migrans in an immunocompromised dog with a multiple nematode infection - E. Bensignor

9:25 - 10:15 Interaction with the following poster presenters
• Prevalence of papillomavirus EcPV2 in clinically healthy horses in Switzerland - N. Fischer
• Sero- and genoprevalence of FdPV2 in healthy cats in Switzerland - M. Geisseler
• Dermoscopic features of dermatophytosis in 11 cats with M. canis infection - F. Scarampella
• Progressive tail necrosis in a rabbit colony - N. Thom
• Cutaneous larva migrans in an immunocompromised dog with a multiple nematode infection - E. Bensignor
• A pilot uncontrolled open study on the use of Oxalic (Medeor International) for treatment of sebaceous gland adenoma/ hyperplasia in dogs - J. Ngo
• A case of generalized verrucosis associated with papillomavirus 9 infection in a dog - P. Cavana
• Efficacy of the low-level laser therapy on hair regrowth: a preliminary study on 8 cases of non-inflammatory alopecia in dog - L. Olivieri
• Spontaneous alopecia in Lagotto Romagnolo dogs: a prospective questionnaire and a retrospective case study of Swedish dogs - C. Thomassen
• Oro-dental diseases and dermatological disorders are highly associated in pet rabbits: a case-control study - D. D’Ovidio
• Leporacarus gibbus infestation in client-owned rabbits and their owner - D. D’Ovidio

SKIN BIOLOGY/PATHOLOGY
Chair: Laura Ordeix

Oral communications
11:15 - 11:30  • Expression patterns of selected desmosomal, tight and adherens junction proteins in an experimental model of canine atopic dermatitis skin lesions - T. Olivry
11:30 - 11:45  • Canine epidermal tight junction proteins: comparison of their immunoreactivity in normal and experimental atopic canine skin - A. Roussel
11:45 - 12:00  • Intradermal injection of recombinant human type VII collagen restores collagen function in a canine model of dystrophic epidermolysis bullosa - D. Pin
12:00 - 12:15  • Certifect-triggered pemphigus foliaceus in dogs: clinical, histological and immunological characteristics - P. Bizikova
12:15 - 12:20  • Keratinocyte differentiation and cornification abnormalities in hereditary nasal parakeratosis in Labrador retrievers - J. Bannoehr
12:20 - 12:45 Interaction with the following poster presenters
• Keratinocyte differentiation and cornification abnormalities in hereditary nasal parakeratosis in Labrador retrievers - J. Bannoehr
• Chitin and lipopolysaccharide modulate innate immune responses of the canine keratinocyte cell line CPEK - M. Bardagi
• Fatty acid composition of lipids derived from isolated canine sebaceous glands and epidermis - A. Watson
• Proof-of-concept for the use of spectrophotometry to describe coat colour in dogs - A. Watson

10:15 - 11:15 BREAK

12:45 - 15:00 LUNCH
13:30 - 15:00 ECVD AGM, Auditorio 1
**ATOPIC DERMATITIS**

**Chair:** Thierry Olivry

**Oral communications**

- Specific increased level of peripheral blood CD34+ cells in dogs with canine atopic dermatitis - V. Bruet
- Beneficial effects of immunotherapy with Gordonia bronchialis on canine flea allergic dermatitis - G. Tártara
- Evaluation of patch testing with proteins, carbohydrates and commercial foods for diagnosis of canine adverse food reactions - C. Johansen
- Reproducibility of allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories - J. Plant
- Sublingual immunotherapy (SLIT) in the dog: where to apply the allergen? Analysis of the distribution of immune cells in the canine oral mucosa - S. Villanueva-Saz
- Selection of an efficacious dosing regimen of oclacitinib (Apoquel®, Zoetis) for the control of atopic dermatitis in client-owned dogs using visual analog scale and CADESI scores - S. Cosgrove
- Oclacitinib (Apoquel®, Zoetis) is a novel Janus kinase inhibitor that has activity against canine pro-allergic and pro-inflammatory cytokines - A. Gonzales
- The effect of flea treatment on the efficacy of oclacitinib (Apoquel®, Zoetis) for the treatment of pruritus associated with canine allergic dermatitis - S. Cosgrove
- Breed differences in transepidermal water loss and pH among dogs with atopic dermatitis - N. Sanchez
- Use of activity monitors to assess pruritus in an acute model of canine atopic dermatitis - R. Schwab-Richards
- Evaluation of the usefulness of Doppler blood flow in the diagnosis of canine cutaneous adverse food reactions - M. Ordas
- Evaluation of pruritic reflexes used for the diagnosis of flea-related dermatoses in dogs - V. Bruet
- A retrospective study on the prevalence and causative allergens of food-induced atopic dermatitis in France - P. Fiora

16:05 - 16:30 Interaction with the following poster presenters

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31 • The pruritic dog: What to do when treatment fails - A. Fondati
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38 • Non inflammatory alopecia - S. Paterson
43 • Cushing’s Syndrome and Hypothyroidism: Case Discussions 1 - I. Ramsey
46 • Cushing’s Syndrome and Hypothyroidism: Case Discussions 2 - I. Ramsey
51 • Update on treating Cushing’s disease - I. Ramsey

SCIENTIFIC SESSION
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62 • Interspecies differences in skin barrier and transdermal penetration - J. Stahl / M. Kietzmann
65 • Skin barrier in human atopic dermatitis - T. Bieber
68 • The skin barrier in canine atopic dermatitis - T. Olivry
74 • Principles of topical therapy (formulations, penetration, permeation) - J. Stahl / M. Kietzmann
77 • Topical management of human atopic dermatitis - T. Bieber
81 • Topical anti-inflammatory in veterinary dermatology: current status and future concepts - J. Stahl / M. Kietzmann
84 • Journal Club (1): a review of landmark papers published in the medical or veterinary literature - T. Olivry
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91 • Therapy of demodicosis - S. Paterson
95 • The many faces of Malassezia dermatitis - M. Boord
97 • Antifungal therapy - S. Paterson
102 • The impact of antibiotic therapy on antimicrobial resistance mechanism - V. Schmidt
107 • Practice guidelines for antibiotic therapy - V. Schmidt
112 • Surgical treatment of chronic pododermatitis - M. Boord

SCIENTIFIC SESSION
115 • Mechanisms of autoimmunity: From hypotheses to clinical cases - P. Bizikova
121 • Autoimmune skin diseases: From structure to skin lesions - M. Suter
126 • Immunosuppression: mechanism of action of selected drugs - P. Bizikova
128 • Immunosuppression of autoimmune skin diseases: How to more specifically target disease pathogenesis - P. Bizikova
130 • Clinicopathological conference: Is this disease autoimmune? - M. Suter / T. Olivry
  Session 1: oral and perioral erosions
132 • Session 2: footpad sloughing
134 • Journal Club (2): a review of landmark papers published in the medical or veterinary literature - L. Ferrer
CONTINUING EDUCATION SESSION

138 • Clinicopathological conference - S. Bettenay / S. Paterson
140 • Update on feline hypersensitivity dermatoses - C. Favrot
144 • Cyclosporine therapy for feline hypersensitivity dermatitis - C. Favrot
147 • Update on leishmaniosis diagnosis in dogs and cats - L. Ferrer
150 • Update on leishmaniosis treatment in dogs and cats - L. Ferrer
154 • What’s new in clinical dermatology? - V. Schmidt

SCIENTIFIC SESSION

158 • Mechanism of atopic itch in humans and dogs: Selected (a)topics - T. Olivry
164 • An update on target-based pharmacotherapy of itch - T. Olivry
169 • Lasers in veterinary dermatology from theory to practice - M. Boord
172 • Lasers in veterinary dermatology: Case discussion - M. Boord
174 • “The most bizarre case that we have ever seen” - L. Beco, P. Bizikova and T. Olivry

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179 • Bardagi - Chitin and lipopolysaccharide modulate innate immune responses of the canine keratinocyte cell line CPEK
180 • Bensignor - Cutaneous larva migrans in an immunocompromised dog with a multiple nematode infection
181 • Bizikova - Certifect-triggered pemphigus foliaceus in dogs: clinical, histological and immunological characteristics
182 • Bruet - Evaluation of pruritic reflexes used for the diagnosis of flea-related dermatoses in dogs
183 • Bruet - Specific increased level of peripheral blood CD34+ cells in dogs with canine atopic dermatitis
184 • Cavana - A case of generalized verruosis associated with papillomavirus 9 infection in a dog
185 • Cerundolo - The use of deslorelin to promote hair regrowth in dogs with Alopecia X
186 • Chatzis - A cross-sectional survey of leishmaniosis in clinically normal and sick cats in Greece with indirect immunofluorescence antibody test and enzyme-linked immunosorbent assay
187 • Cosgrove - Selection of an efficacious dosing regimen of oclacitinib (Apoquel®, Zoetis) for the control of atopic dermatitis in client-owned dogs using visual analog scale and CADESI scores
188 • Cosgrove - The effect of flea treatment on the efficacy of oclacitinib (Apoquel®, Zoetis) for the treatment of pruritus associated with canine allergic dermatitis
189 • De Lucia - Canine nasal dermatitis: histopathological and immunopathological features of discoid lupus erythematosus and leishmaniosis
190 • D’Ovidio - Leporacarus gibbus infestation in client-owned rabbits and their owner
191 • D’Ovidio - Oro-dental diseases and dermatological disorders are highly associated in pet rabbits: a case-control study
192 • Ferreira - Identification of three different Demodex species in cats using a novel PCR assay
193 • Fiora - A retrospective study on the prevalence and causative allergens of food-induced atopic dermatitis in France
194 • Fischer - Prevalence of papillomavirus EcPV2 in clinically healthy horses in Switzerland
195 • Geissler - Sero- and genoprevalence of FdPV2 in healthy cats in Switzerland
196 • Gonzales - Oclacitinib (Apoquel®, Zoetis) is a novel Janus kinase inhibitor that has activity against canine pro-allergic and pro-inflammatory cytokines
197 • Johansen - Evaluation of patch testing with proteins, carbohydrates and commercial foods for diagnosis of canine adverse food reactions
198 • Kaufmann - First report of straelensiosis in cats and unique features of the canine disease in Israel
Lehner - Case-control risk factor study for meticillin-resistant Staphylococcus pseudintermedius (MRSP) infection in dogs and cats in Germany

Loeffl er - Genetic insights into the emergence of multidrug-resistance in meticillin-resistant Staphylococcus pseudintermedius

Ludwig - Antimicrobial susceptibility monitoring of dermatological pathogens isolated from diseased dogs and cats across Europe (ComPath I, 2008-2010)

Ludwig - In vitro activity of pradofl oxacin against canine and feline pathogens recovered from skin infections in four European Union countries

Mignon - Development of an enzyme-linked immunosorbent assay for the serodiagnosis of ringworm infections in cattle

Ngo - A pilot uncontrolled open study on the use of Oxalic (Mederor International) for treatment of sebaceous gland adenoma/hyperplasia in dogs

Okunaka - Retrospective assessment of previous antibiotic therapy in dogs diagnosed with meticillin-resistant Staphylococcus pseudintermedius pyoderma

Olivieri - Efficacy of the low-level laser therapy on hair regrowth: a preliminary study on 8 cases of non-inflammatory alopecia in dog

Olivry - Expression patterns of selected desmosomal, tight and adherens junction proteins in an experimental model of canine atopic dermatitis skin lesions

Ordas - Evaluation of the usefulness of Doppler blood flow in the diagnosis of canine cutaneous adverse food reactions

Ordeix - Histopathological characteristics and expression of Toll-like receptor 2 in lesional skin of dogs with papular dermatitis due to Leishmania

Pin - Intradermal injection of recombinant human type VII collagen restores collagen function in a canine model of dystrophic epidermolysis bullosa

Plant - Reproducibility of allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories

Ravera - Development of a PCR technique specific for Demodex injai in biologic specimens

Rivas - Toll-like receptor 2 is overexpressed in dogs with demodicosis, Malassezia dermatitis and cutaneous bacterial infection

Roussel - Canine epidermal tight junction proteins: comparison of their immunoreactivity in normal and experimental atopic canine skin

Sanchez - Breed differences in transepidermal water loss and pH among dogs with atopic dermatitis

Sandomichelakis - Thyroid function in dogs with leishmaniosis due to Leishmania infantum before and during treatment

Scarampella - Dermoscopic features of dermatophytosis in 11 cats with M. canis infection

Schwab-Richards - Use of activity monitors to assess pruritus in an acute model of canine atopic dermatitis

Silbermayr - Coproscopic detection and treatment of Demodex gatoi infestation in a Cornish rex cat in Austria

Tártara - Beneficial effects of immunotherapy with Gordonia bronchialis on canine flea allergic dermatitis

Thom - Progressive tail necrosis in a rabbit colony

Thomassen - Spontaneous alopecia in Lagotto Romagnolo dogs: a prospective questionnaire and a retrospective case study of Swedish dogs

Villanueva-Saz - Sublingual immunotherapy (SLIT) in the dog: where to apply the allergen? Analysis of the distribution of immune cells in the canine oral mucosa

Watson - Fatty acid composition of lipids derived from isolated canine sebaceous glands and epidermis

Watson - Proof-of-concept for the use of spectrophotometry to describe coat colour in dogs
CONTINUING EDUCATION SESSION

Approach to the pruritic dog
Alessandra Fondati
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The definition of pruritus as an unpleasant sensation provoking the desire to scratch, given by S. Haffnerfer more than three centuries ago, is still the most apt to describe this sensory phenomenon.

Acute pruritus acts an alarm system with the aim to remove potentially noxious agents from the skin, thus it serves as an innate defence mechanism. Nevertheless, chronic pruritus has lost its protective function and is mostly bothersome.

In humans, induction, perception and exacerbation of pruritus involve a complex network of sensory nerves, epidermal and dermal cells (keratinocytes, T lymphocytes, mast cells, eosinophils) and numerous mediators, including histamine, leukotrienes, prostaglandins, proteases, substance P, nerve growth factor, IL-2 and IL-31. In addition, it is worth remarking that pruritus is experienced as a sensation arising in the skin but, analogous to other skin sensations, is “created” by the brain. Responses to pruritogenic stimuli differ with the animal species, breed and individual, and in dogs comprise scratching (sensu stricto), licking, nibbling, biting, rubbing and rolling. The site of pruritus also influences the way of scratching (sensu lato). Scratching is considered a “pleasurable” response that alleviates itch but at the same time in dogs provokes alopecia, erythema, excoriations, ulcers, lichenification and hyperpigmentation.

While pruritus is a subjective perception difficult to measure objectively, scratching activity (sensu lato) can be quantified. The severity of clinical signs due to pruritus is commonly used in practice to estimate scratching intensity. However, in those pruritic dogs with absent-mild cutaneous lesions, as it might be observed e.g. in atopic Jack Russels, according to author’s experience, severity of pruritus as well owner and dog’s discomfort may be underestimated.

In humans, the causes of chronic pruritus are subdivided into four major categories: i) dermatologic causes (e.g. atopic eczema and scabies), ii) systemic causes (e.g. myeloproliferative disorders, liver and kidney disease), iii) neuropathic and iv) psychogenic causes. The first step in the evaluation of chronic pruritus in humans is to determine whether it can be attributed to a dermatologic disease or to a non-cutaneous cause.

In the vast majority of cases canine pruritus has dermatological causes and is related to inflammatory skin diseases. It is in fact frequently associated with allergic dermatitis towards environmental (atopic dermatitis (AD*)) and/or flea and/or food allergens, and with parasitic diseases, particularly sarcoptic mange. Furthermore, Malassezia and bacterial overgrowth-infection are commonly superimposed and widely contribute to provoke pruritus. Itch is “multifactorial” and the approach to the itchy dog is finalized to discover, and possibly remove, all the identifiable pruritogenic factors.

Canine pruritus is uncommonly due to other dermatological problems, as adverse drug reactions, irritant/allergic contact dermatitis, arthropod bite reactions and epitheliotropic cutaneous T cell lymphoma. It is also rarely associated to neuropathies (e.g. Chiari-like malformation and syringomyelia* and acral mutilation syndrome*) or behavioural disorders (e.g. acral lick dermatitis). Sporadic cases of canine pruritus attributable to systemic causes (i.e. portosystemic shunt* and pseudorabies*) have also been reported.

In the author’s opinion, signs suggestive of pruritus of non-cutaneous origin are the lack of cutaneous inflammatory changes (e.g. lichenification and hyperpigmentation) and the presence of well-demarcated excoriations, ulcers and scars with mild perilesional inflammation. In some cases skin inflammation is completely absent. Other useful clues to diagnose pruritic diseases of non-cutaneous origin include breed predilection (e.g. Chiari-like malformation in Cavalier King Charles Spaniels), typical distribution of lesions (e.g. distal extremities in acral mutilation syndrome) and presence of systemic signs (e.g. pseudorabies).

Whenever a non-cutaneous cause of pruritus is suspected, skin biopsies, complete blood count, basic chemistries, urinalysis, and any other test deemed necessary to make the diagnosis should be performed. Furthermore, the diagnosis should be ideally discussed with a neurologist, a behaviourist or an internist. If behavioural problems are suspected, a complete dermatologic workup is recommended anyhow.

In “standard” dermatologic cases the diagnostic approach to a pruritic dog proceeds in a stepwise fashion through progressive elimination of pruritogenic components associated with parasites, bacteria and yeast, and is commonly followed by exclusion of allergic components. The workup has to be sequentially ordered and methodically performed, otherwise unreliable and inconsistent conclusions are achieved. It includes 2 main steps:
1. Rule out and treat sarcoptic mange and other parasitic dermatoses, besides *Malassezia* and bacterial overgrowth-infection. Skin scrapings and other diagnostic procedures useful to rule out parasitic mites have to be performed. If negative results are obtained but the presence of mites (except *Demodex*) is still suspected, a therapeutic trial is recommended. Strict flea control has also to be instituted.

Cytological examinations to rule out *Malassezia* and bacterial overgrowth-infection have to be carried out and oral and/or topical antimicrobial treatments should be prescribed, if needed.

Once problems related to parasites, bacteria and yeasts have been ruled out and/or effectively treated, if pruritus persists, as long as clinical signs are compatible, an underlying allergy has to be searched.

2. Rule out allergic problems through:
- Maintenance of flea control
- Diet trial (4-8 weeks) to determine whether food allergens play a role
- If a reduction of pruritus is obtained, a provocative test with previous diet has to be performed
- If no reduction of pruritus is obtained, with consistent signalment, history and clinical signs, AD can be diagnosed

The need to carry out a complete diagnostic workup varies with the duration of pruritus: acute (≤ 1 week, in the author's opinion) versus chronic (≥ 6 weeks, according to human criteria)

Acute pruritus: the workup can be limited to step 1, and be followed by specific therapy or short-term anti-inflammatory treatment (see "Management of the pruritic dog")

Chronic pruritus: a complete workup (steps 1&2) has to be carried out

Pruritus of ≥ 2 weeks to ≤ 5 weeks duration: the decision can be individualized

Typically, dogs with chronic itch are presented with a variety of provisional diagnoses, commonly including a rarely demonstrated food allergy and/or contact dermatitis. Their skin shows evidence of long-term scratching, and *Malassezia* and/or bacterial overgrowth-infection is common. Normally these dogs have changed numerous diets and have already undergone extensive investigations, sometimes including blood work and skin biopsies. However, data from history are often not convincing (e.g. incorrect diet trials, ineffective flea control...), and the diagnostic workup must be frequently restarted. Thus owners must be actively motivated to go again through those diagnostic procedures they have already experienced.

*In the entire text AD has to be intended as AD *sensu stricto* (i.e. non food induced)

**Conflicts of interest**
The author declares no conflicts of interests

**Selected references**
Management of the pruritic dog

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Treatment of pruritus should be directed at the underlying cause, when possible. If pruritus has removable dermatologic causes, e.g. parasites, bacteria, yeasts, food, drug and contact allergens, normally the offending element is eliminated or a specific therapy is instituted. However, if itch is due to common but not removable causes, i.e. environmental allergens, it tends to become chronic. If allergen-specific desensitization is not performed or is not effective, chronic pruritus due to atopic dermatitis (AD) or atopic-like dermatitis must be treated. Severe and unrelenting chronic pruritus results in miserable dogs and frustrated and frustrating owners, inasmuch as they both need to be “managed”. In the vast majority of cases, management of pruritus in dogs corresponds to the management of AD and is therefore based on anti-inflammatory and immunomodulatory agents (treatment of pruritus due to non-cutaneous causes is outlined in “The pruritic dog: What to do when treatment fails”).

The chronicity of pruritus is a problem in atopic dogs because requires prolonged treatment, thus i) the risk of adverse effects increases and ii) owners’ adherence tends to decrease. In order to obtain a better compliance, the author suggests to clearly explain to owners that AD cannot be cured. The aim of treatment in atopic dogs is to maintain pruritus at a level acceptable both for dogs and owners. The episodes of worsening should be reduced to no more than 2/year and this objective should be ideally achieved using the least aggressive therapy. Adverse effects of treatments in fact must not be worse than the pruritus, as suggested in the IV century BC by Hippocrates “First, do not harm” (Primum non nocere)!

The anti-inflammatory and immunomodulatory agents most commonly used to treat pruritic atopic dogs are oral steroids and calcineurin inhibitors.

Oral steroids. Their anti-pruritic effect is related to anti-inflammatory properties. In fact, they decrease the production of numerous inflammatory mediators. Short-acting oral steroids (prednisone, prednisolone, methylprednisolone) are preferred. Prednisone and prednisolone are commonly used by the author at the initial dose of 0.5-1 mg/Kg daily and methylprednisolone at 0.4-0.8 mg/Kg daily. Once inflammation is alleviated, steroids are decreased to the lowest effective dose. Oral steroids have limited utility in the long-term management of persistently pruritic dogs because of adverse effects, including polyuria, polydipsia, polyphagia, elevated liver enzymes and urinary tract infections. Nevertheless, short courses (≤3 weeks, according to the author’s opinion) are useful to obtain a rapid and temporary relief of pruritus and may also be cyclically repeated. Dogs treated with steroids should undergo periodic pertinent screening including complete blood cell count, biochemistry profile and urinalysis.

Oral ciclosporin is a calcineurin inhibitor that at low dose exerts an anti-inflammatory and immunomodulatory effect, through inhibition of T-cell activation. Ciclosporin is administered orally at the dose of 5 mg/Kg daily for at least 4 weeks because clinical benefit has slow onset. In case of good response, the dose is then adjusted as needed for therapeutic effect. Vomiting and diarrhoea are common but usually self-limiting. Risk of drug interactions, e.g. with azole antifungals, has to be considered. Prior to and during therapy it is advisable to perform periodic laboratory investigations, including blood count, biochemistry profile and urinalysis and in Mediterranean countries anti-Leishmania antibodies should also be titrated.

Management of pruritus in atopic dogs is based on an integrated treatment strategy aimed to reduce the use of oral anti-inflammatory and immunomodulatory therapies. Topical steroids and calcineurin inhibitors might be considered as “systemic steroid-ciclosporin sparing” agents. In addition, topical and oral adjuvant therapeutic measures (see below) are commonly used in pruritic dogs and they might also possess this sparing effect. Topical adjuvant treatments are particularly suitable for atopic dogs because mostly finalized to normalize skin barrier.

Topical steroids have an anti-inflammatory effect and represent the mainstay of therapy for bringing human AD under control. They are divided in VII classes of potency, from low (class VII) to high (class I). Hydrocortisone 1% (class VII) is approximately equivalent to 0.1%-0.5% triamcinolone and to 0.05% betamethasone. The clinical efficacy and the risk of local (e.g. skin thinning and milia) and systemic (i.e. hypothalamus-pituitary axis suppression due to systemic absorption) side effects correlate with potency class and duration of use and/or frequency of application. As suggested in human AD, lesions should be cleared with daily application of steroids for 1-2 weeks, then the same product should be intermittently used (e.g. 2-3 times/week) even if visible lesions have disappeared. This proactive treatment approach reduces the risk of flares and extends the time of remission. The proactive application of hydrocortisone aceponate twice-weekly is beneficial in atopic dogs.

Topical tacrolimus. The mechanism underlying its ability to reduce pruritus is unclear. As calcineurin inhibitor, it has an anti-inflammatory and immunomodulatory effect but it seems also able to activate and then desensitize receptors (TRPV1).
located on peripheral nerve fibres. Tacrolimus ointment (0.1%) applied twice daily has been useful for treating localized skin lesions of canine AD. Owners should be instructed to wear gloves. Clinical benefit has slow onset and initial cutaneous irritation may occur. Proactive application 2-3 times-weekly might be considered for maintenance of lesions once stabilized with topical steroids or with daily tacrolimus, as reported in humans. The advantage of tacrolimus over steroids is that prolonged use does not cause adverse effects (e.g. skin thinning). In Italy it is not licensed for use in dogs.

**Adjuvant therapy**

Moisturizers and skin barrier protectors Moisturizers are widely used, nevertheless, their anti-pruritic efficacy should be further studied. Commercial products contain a combination of humectants, emollients and occlusive ingredients. It is unpredictable which product will be the most beneficial for each subject, therefore different moisturizers can be tried. Moisturizing shampoos should be used initially 2-3 times/week, or even daily in more severe cases. The frequency of bathing is then reduced according to skin conditions and owners’ adherence. Shampooing is beneficial for pruritic dogs probably due to improved skin hydration and hygiene. Moisturizing sprays should be applied once-twice daily and after bathing and they should be let air-dry. They are expected to have residual effects longer than shampoos, because they are not rinsed off. Lipid-based emulsions and essential fatty acids spray on-sprays should contribute to repair abnormalities of stratum corneum described in atopic dogs and, subsequently, to restore barrier function. Nevertheless, further studies are needed to explore the potential beneficial role of these products in the clinical field.

**Topical antimicrobials.** Anti-bacterial/yeast solutions should decrease microbial burden on the skin surface and thus reduce barrier dysfunction. Inflammation and pruritus should improve especially in those atopic dogs prone to infections. However, their prophylactic and therapeutic efficacy in atopic dogs has not been assessed yet. Antiseptics are preferred over antibiotics because their use is not associated with increased risk of antibiotic resistance selection. However, short courses of topical antibiotics, with/without combined steroids, are commonly used to treat localized lesions. Shampoos containing anti-bacterial/yeast active ingredients, including chlorhexidine, benzoyl peroxide, ethyl lactate and azoles should be used twice a week. Moisturizing and antimicrobial shampoos may be alternated too. Antiseptic solutions are also useful but can cause contact reactions, especially if applied at high concentration on erythematous skin, and do not possess the same “cleaning” activity of shampoos. On the other hand they are easier to apply because they do not need to be rinsed off. They should be preferably applied on those areas in which microbial overgrowth is more frequent and hair does not interfere with the application (e.g. muco-cutaneous junctions, intertriginous areas, abdominal and inguinal skin). Normally they are applied once daily and towel dried in moist areas (e.g. ventral interdigital skin). Then the frequency of application can be decreased. Chlorhexidine solution (up to 4%) represents one of the most commonly used antiseptic in dogs. However, sodium hypochlorite solution might be considered when chlorhexidine causes contact reactions or its cost is prohibitive for the owner. There is evidence of the efficacy of a 0.005% bleach solution to control clinical signs in atopic children. In addition, in vitro studies have demonstrated that 1:32 diluted bleach possesses bactericidal activity on canine skin-isolated MRSP strains.

**Oral H1 antihistamines** block H1 receptors and are widely used but demonstration of their anti-pruritic efficacy is inconclusive. From a recent systematic review appears that there is currently no sufficient evidence to support or refute the efficacy or safety of oral H1 antihistamines as monotherapy for human eczema. Due to their sedating properties, in humans they are considered more useful to promote sleep rather than to control pruritus. Oral hydroxyzine (1 mg/Kg twice daily) and cetirizine (1 mg/Kg daily) are the most commonly used antihistamines in atopic dogs and, anecdotally, they are reported to be beneficial in approximately 20% of cases.

**Essential fatty acids (EFAs)** have anti-inflammatory and immunomodulating properties and seem to improve barrier function. There are numerous products containing different amounts and ratios of omega-3 and omega-6 fatty acids. Before evaluating their effect they should be administered for at least 2 months. However, their efficacy to control pruritus still remains controversial. Selected commercial diets with high EFA-content, given for 2 months, have been reported to contribute to decrease pruritus in atopic dogs.

**Endocannabinoids.** Palmitoylethanolamide (PEA) and adelmidrol belong to the aliamide family, a group of fatty acid derivatives with cannabimimetic properties, able to downregulate mast cell degranulation. Both products have caused a reduction of antigen-induced wheals in Ascaris hypersensitive experimental dogs. In Italy PEA is available in oral formulation combined with EFAs and biotin, whereas adelmidrol is available in topical formulations combined with antimicrobials (spray) or moisturizers (fluid). The oral product should be administered for at least 2 months and the topical spray and fluid should be applied 2-3 times daily for at least 10 days. Furthermore, a plethora of products has been reported to be more or less useful to treat atopic dogs, including probiotics, tricyclic antidepressants, leukotriene and phosphodiesterase inhibitors, misoprostol, dexamethasone, tyrosine-kinase inhibitors (masitinib), interferons (recombinant feline interferon omega and recombinant canine interferon gamma), plant-derived extracts, Chinese herbal
products and a variety of topical medication (ciclosporin, budesonide, triamcinolone acetonide, diphenhydramine and capsaicin). Due to low-quality evidence of clinical efficacy, risks of adverse effects, prohibitive cost and/or unavailability in Italy, they are rarely used by the author. Therapeutic decisions in atopic pruritic dogs have to be tailored to the owner (time and financial resources) and patient’s (age, general health status, co-medications) profiles, as well as to duration of itch and severity and extent of skin lesions.¹

Acute pruritus. In this case a relatively “simplified” diagnostic workup can be carried out (see “Approach to the pruritic dog”) before instituting anti-pruritic therapy. Short courses of topical steroids are recommended to treat localized lesions. If pruritus is generalized, or localized but causes discomfort, a short course of oral steroids can be administered. Topical adjunctive therapy can be considered too.

Chronic pruritus. It is worth remarking that pharmacological treatment of pruritus can be initiated only when clinical diagnosis of AD has been confirmed (see “Approach to the pruritic dog”) and in dogs who still scratch after clearing microbial overgrowth-infections. Furthermore, it must be accompanied by strict flea control. In chronically pruritic dogs, development of an effective treatment plan should ideally include different therapeutic options and be individualized and modified over time, as dog’s needs require and owner’s adherence allows. Normally it takes 2-4 months to optimize it. If the cause of pruritus has not been previously searched-identified, long-term anti-inflammatory and immunomodulatory therapy must be avoided because i) it prevents the achievement of etiological diagnosis and ii) represents a risk for the dog’s health.

Chronic pruritus with no observable-mild skin lesions (e.g. focal self-induced alopecia, erythema, salivary staining). Oral antihistamines can be used, plus adjunctive therapy and/or topical steroids, or tacrolimus for localized lesions. Especially if there are no observable lesions, the use of oral steroids or ciclosporin should be avoided. Remember Hippocrates’ suggestion!

Chronic pruritus with severe skin lesions (e.g. lichenification, hyperpigmentation and ulcers). The therapeutic approach in chronically pruritic dogs is normally conservative and proceeds in a step-wise fashion.
1. Remission of pruritus can be achieved with a short course of oral steroids, accompanied and followed by continuous maintenance with adjunctive therapy plus topical steroids, or tacrolimus for localized lesions.
2. In case of prompt relapse with tapering or discontinuation of oral steroids ciclosporin might be administered, accompanied and followed by continuous maintenance with adjunctive therapy plus topical steroids. The concomitant use of oral steroids should be avoided, except in case of flares, which should be promptly suppressed with short cycles of oral steroids.

*In the entire text AD has to be intended as AD sensu stricto (i.e. non food induced)

Conflicts of interest
The author declares no conflicts of interests

Selected references
Readers are referred to the articles cited below for detailed information on the drugs mentioned in these notes.
7. Valdman-Grinshpoun Y, Ben-Amitai D, Zvulunov A. Barrier-Restoring Therapies in Atopic Dermatitis: Current Approaches and Future Perspectives. Dermatology Research and Practice 2012; Article ID 923134, 6 pages
The pruritic dog: What to do when treatment fails
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Treatment failure normally refers to the lack of adequate response of presumed atopic dogs to both oral steroids and ciclosporin. In these cases, all the diagnostic and therapeutic steps must be thoroughly revised and the following alternatives should be assessed.

If an exacerbation of signs occurs in dogs in which remission had been previously obtained with conventional steroids or ciclosporin treatment, flare factors have to be searched for, including parasitic infestations, microbial overgrowth-infections, systemic infections (e.g. leishmaniosis in Mediterranean countries) or metabolic disturbances able to obscure the initial response to therapy. In addition, if pruritus and lesions are confined to areas where topical products are applied, a contact dermatitis might be hypothesised.

If the diagnosis of atopic dermatitis (AD) is considered to be correct and a lack of response to therapy is reported to have occurred, the dose and duration of treatment should be rechecked, as well as owner’s adherence to therapeutic recommendations.

Furthermore, considering that owners tend to interpret a temporary response as a lack of response, a true lack of response to steroids should be verified by prescribing 1-1.5 mg/Kg daily of short-acting steroids for at least one week. In the author’s experience, when prednisone seems ineffective, switching to prednisolone or methylprednisolone can be useful. If the lack of response to a correct dose of ciclosporin is reported, before increasing the dose, ciclosporin levels might be checked, although in atopic dogs blood concentrations and clinical response do not seem to be correlated.

If the lack of response to steroids and ciclosporin is definitely proved and all the alternative explanations for treatment failure have been correctly revised, the two diagnostic alternatives are: i) a case of refractory pruritus associated to AD (in fact not 100% atopic dogs respond to oral steroids or ciclosporin), ii) an incorrect diagnosis.

Histopathological examination of skin biopsies can help to orientate the diagnosis. The presence of an inflammatory cutaneous disease can be confirmed and dermatological problems unresponsive to conventional anti-pruritic therapy can be ruled out (e.g. epitheliotropic cutaneous T cell lymphoma). If clinical and histopathological features of AD or atopic-like dermatitis are present, more aggressive immunosuppressive therapy might be considered to control inflammation and pruritus in those refractory cases.

Before initiating systemic immunosuppressive therapy, patients and owners must be carefully selected, always keeping in mind that treatment must not be more harmful than the disease (think of Hippocrates’ recommendation *Primum non nocere*). In addition, prior to start immunosuppressive therapy, oral steroids or ciclosporin should be withdrawn for approximately 3 to 4 weeks and complete blood and urine analyses should be obtained.

**Immunosuppressive therapy**

Azathioprine has been used to treat refractory canine AD at the oral dose of 2-2.5 mg/Kg daily but with scarce efficacy. In addition, myelosuppression and hepatic toxicity (reversible increase of ALT and ALP activities) have been commonly reported, thus, frequent blood monitoring is mandatory. In human severe atopic dermatitis, due to the fact that the onset of azathioprine efficacy is delayed, it is suggested to initiate therapy once the flare has subsided and, at least initially, to associate oral and/or topical steroids. In humans there is also concern on the incompletely understood long-term risk of malignancy of azathioprine. Owners should be instructed to wear gloves before handling the tablets.

Also methotrexate has been used in atopic dogs. Oral methotrexate given once weekly at an oral dose ranging from 5 to 10 mg/Kg (depending on dog’s clinical conditions) has been reported as beneficial in 5/10 atopic dogs. Vomiting post-administration was observed in 5/10 dogs but on the whole the drug was considered safe. In Italy azathioprine and methotrexate are not licensed for use in dogs.

Pruritus non originating from the skin is frequently unresponsive or mildly responsive to anti-inflammatory and immunosuppressive therapy. If a non-cutaneous problem is suspected (see “Approach to the pruritic dog”), consultation with the specific branch specialist is recommended. In case of neuropathic or pain-associated pruritus, therapy must be directed to a non-cutaneous target and neuroactive medication can be considered. It must be stressed that, to the author’s knowledge, there are no published reports on the use of the following drugs to control pruritus in dogs.
Neuroactive therapy

Gabapentin is an anticonvulsive agent and pain modulator used in humans also to treat uremic and neuropathic itch. Its action should be related to the reduced release of excitatory neurotransmitters. Suggested starting dose is 5-10 mg/kg given orally every 12 hours. Sedation and ataxia are the most common side effects. In Italy it is not licensed for use in dogs. Maropitant is a neurokinin-1 (NK1) receptor antagonist that inhibits the action of substance P with resulting anti-pruritic effect. A similar drug, aprepitant, has been reported to reduce pruritus due to systemic and dermatological causes in humans. Maropitant is licensed for use in dogs to prevent and treat vomiting at daily oral dose of 2 mg/kg. The same dose, given long-term, is suggested to control pruritus.

Neuroactive therapy could be taken into consideration also in severely affected atopic or atopic-like dogs who cannot be treated with oral steroids or ciclosporin (e.g. dogs with diabetes mellitus or liver-kidney disease, patients who do not tolerate steroids and ciclosporin, or whose owners are steroid- or ciclosporin-phobic) and/or cannot be immunosuppressed (e.g. dogs with generalized demodicosis or leishmaniosis). In the author’s experience these circumstances are relatively more common than “true” refractory pruritus.

*In the entire text AD has to be intended as AD sensu stricto (i.e. non food induced)

Conflicts of interest

The author declares no conflicts of interests

Selected references

CONTINUING EDUCATION SESSION

The pruritic dog: Improving client compliance
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Pruritus is one of the major reasons for a dermatologic presentation. The degree of pruritus can vary, it can be acute, chronic or recurrent. Atopic dermatitis is typically chronic and relapsing. Pruritus has many causes and although the clinical picture can give diagnostic clues, the work-up of the case may involve multiple tests, often taken in multiple steps. The management of a case of (chronic) canine atopic dermatitis can be particularly challenging.

There are many causes of pruritus which are curable, mostly ectoparasitic, however for most animals an allergy will be the cause of the clinical signs of pruritus and will not be „cured“. A successful outcome will depend on the client learning how to manage the clinical signs. A great deal of the clinical success with allergic patients depends ultimately upon the owner and their level of compliance. Improving client compliance is a key to improving the success rate of not only diagnosis but also management of pruritic patients.

Canine pruritus impacts on owners: From the owner viewpoint, pruritus can be frustrating, they see their beloved pet suffer, feel helpless to do anything. More than 70% of owners in a study by Linek in 2010 perceived that the atopic dermatitis of their dog had a major impact on the health related quality of life. In severe cases, owners may be additionally personally sleep deprived - which is a recognised cause of depression. These factors can ultimately lead to a general feeling of dissatisfaction with ones’ veterinarian. These negative feelings are especially commonly observed in owners experiencing a recurrence of symptoms. Indeed chronic / recurrent pruritus is one of the major reasons for seeking a second opinion.

Before we discuss how to improve client compliance, we need to ask some questions:

What are the aims in the diagnosis of pruritus?
Owner goals: typically the owner seeks a permanent cure. They believe - in most cases justifiably - that once we know the cause, we can „fix it“.
„Vet“ goals: to identify the root of the pruritus and if possible effect a cure.

What are the aims in the management of pruritus?
Owner goals typically are for a treatment that is not harmful, not expensive and also one which impacts minimally on their own busy life. Linek’s study found that while most owners of atopic dogs do not complain about major disturbances to their family or social lives, the burden and cost of treatment itself cause major concerns. The fact that some 50% of the owners believed that previous treatments did not provide long term improvement, suggests that at least some owner expectations were not for management but rather cure?
„Vet“ goals, where a cure is not possible, are to find a treatment regime which is safe, cost-effective and which will minimise the number of revisits and recurrent superficial infections - if these are an issue - and which their client can undertake.

What does our owner seek, when they come for an appointment?
Modern society has never been more varied and not all owners are the same. My husband discusses diagnostic and treatment choices by broadly categorising owner groups into socio-economic and financial categories. He uses the „bicycle group“ the „volkswagen group“ and the „mercedes group“. Although this is acknowledged as an enormous oversimplification, it is really very useful. Another classification of owner groups can be based on emotional needs: in this case the pets can be considered as: „child substitutes“, „family members“ and „tools and equipment“.

Where a diagnostic and / or treatment plan involves financial and personal time input, the owner-animal relationship will influence the ultimate choices made. Before we can obtain client compliance we need to be able to determine where our clients’ needs and views fall. In order to establish long term compliance we need to work hard at establishing realistic expectations and a supportive role on behalf of he animal health practitioner.

The aims of owners and vets may be a little different, but overall I believe the aims are similar enough - to achieve the best outcome in as short a time as possible with minimal expense... and then, in the case of chronic pruritic disease, to be able to maintain this. Interestingly, there is a large degree of denial within the group of owners of dogs with atopic dermatitis. In Linek’s study, some 30% of owners had not accepted that their dogs would require life-long treatment or care.
What tools / techniques may help?

> Communicate effectively.

1. Written information will help the owners to recall what was discussed and to share that information with the rest of the family members. This should provide:
   • background information - in the form of a client information sheet on the disease in question, where possible.
   • a summary of the treatment and / or diagnostic plan when this involves more than one test / form of medication.

2. Handouts should:
   • be in a language that is understandable to the lay person
   • not be too long
   • include different styles of presenting the information, to accommodate the different recognised learning styles (visual versus hearing versus word-based for example)
   • include a point form summary at the end.

3. Receptionists / nursing staff are a great resource to help build / maintain the momentum and to check that a therapy is actually suitable / working:
   • via telephone follow-ups, which can be especially helpful during long term diagnostic therapy such as an elimination diet - is the dog eating the food?
   • via rechecks which do not involve a professional fee, such as is used in weight reduction programmes
   • actually asking what the result of a shampootherapy was? Did it help? Did it create more pruritus? To determine whether the owners have actually used the recommended flea therapy The answers to these questions may be of more value in the first week after the visit than at the recheck 3 weeks later.

4. Ensure the entire plan is transparent for all staff members, so that there is no switching or changing which will lead to owner confusion and possibly damage the initial

> Provide measures of success

We all need these - we are trained from school age to „tick off lists or boxes”.
Almost all successful teaching programs encompass / contain objectives which the student / participant aims to achieve. We also need to give these to our owners. These can include observation of the skin lesions, measuring the levels and style of pruritus, observing a benefit - or at least not a worsening - following medication administration.

> Motivation tools:

In business, effective and motivational leadership is a required role for a successful team / project manager. Many studies have been undertaken and indeed university courses now exist to help groom effective leaders. Although veterinarians spend an enormous amount of their time working with their owners, they are not typically trained in leadership skills. In previous generations the social status of the veterinarian often played a role in obtaining owner compliance, but the internet and the wide availability of large numbers of textbooks has diluted this „authority effect”. and individuals question more and require different forms of motivation.

Effective leaders differ from effective achievers, or „best performers”. Best performers have a tendency to strive for dominance, outperform, or put one another down which can lead to disengagement and apathy. The most effective leaders, exhibit communication style flexibility whilst respecting each individual with an aim to establish rapport. An effective leader in the veterinary practice sense will be able to convey the message that there is a solution, life is great and that they will work together with the owner as a team to arrive at this solution. They need to be able to break the mold of helplessness and feelings of alienation and „life sucks” which frustrated and depressed owners may have.

> Owner support groups:

Little is documented of the value of patient support groups. In 2002 a prospective randomised study on human asthmatics revealed that an educational program improved the quality of life in asthmatic subjects, mainly in patients with moderate-to-severe asthma. A cochrane review revealed that there was not only a short term (within 6 months) but also a long term benefit from patient education programs. An owner education group for atopic dog owners has been pioneered by Linek in Germany.
References
1. Influence Beyond “Me” - Igniting Inspiration for Accelerated Performance
CONTINUING EDUCATION SESSION

Non inflammatory alopecia
Susan Paterson
Rutland House Referral Hospital, Abbotsfield Road, St Helens, Merseyside, UK

Hair loss can be either inflammatory or non inflammatory. Inflammatory causes of hair loss include diseases that cause direct damage to the hair follicle such as parasitic, infectious, immune mediated diseases and neoplasia and diseases where the hair is damaged as a bystander such as vasculitis or trauma. Non inflammatory alopecia is caused either through hair cycle arrest or through the formation of abnormal hair such as the different dysplastic diseases. However in many cases the aetio-pathogenesis of the different alopecic conditions is poorly understood. (see table 1)

Investigation of non inflammatory alopecia
It is important to rule out endocrine disease and inflammatory causes of alopecia when investigating alopecia skin disease. To do this skin scrapes, hair plucks and fungal culture should be undertaken as part of a minimum data base together with routine haematology, biochemistry, thyroid function tests and a urine cortisol: creatinine test. Where blood and urine samples are unremarkable and skin scrapes and fungal culture fail to reveal signs of parasites and dermatophytes respectively then hair plucks remain the next most useful diagnostic test before biopsies are undertaken (table 2).

Hair cycle arrest alopecia
Hair cycle abnormalities are the most common cause of alopecia in the dog. Various hormones play important roles in either stimulating anagen or prolonging telogen. Hypothyroidism and Hyperadrenocorticism will be dealt with elsewhere in these proceedings and will not be discussed further here. Other causes of hair cycle arrest alopecia include Alopecia X, anagen defluxion, telogen defluxion, canine flank alopecia and post clipping alopecia

Alopecia X (growth hormone responsive dermatosis/adrenal sex hormone responsive dermatosis/congenital adrenal hyperplasia/castration responsive dermatosis) This disease represents a category of hair cycle arrest alopecia that is poorly understood, initial thought were that this was a hormonal disease it is now known not to be. Cycle arrest occurs in all cases so that hairs stay in telogen in suspended animation. Several theories exist for the pathogenesis including abnormal adrenal sex hormone imbalance and a growth hormone deficiency. Most recent work suggests that hair loss may be due to a local follicular receptor dysregulation. It is an uncommon disease only recognised in dogs. Clinical signs are most commonly reported in young dogs usually aged 2 - 5 years. Predisposed breeds include the Chow chow, Samoyed, Keeshond, Pomeranian, Alaskan malamute, Siberian husky and miniature poodle. The dog’s hair coat becomes faded and truncal alopecia often starts around the perineum and inguinal areas. Hair loss is progressive leading to complete alopecia of neck, tail, caudodorsal areas, perineum and caudal thighs. The head and neck tend to be spared but the coat is made of secondary hairs which give the appearance of “puppy coat” Areas of alopecic skin may become hyperpigmented and thinned. Secondary seborrhoea and pyoderma can be present.

Telogen defluxion is when an abrupt stressful medical or surgical intervention e.g. pyrexia, systemic illness, pregnancy, or major surgery leads to cessation of the anagen phase of cycle. Hairs become synchronised in catagen then move through to telogen to be shed 1-3 months after the initial insult. Dogs usually present with sudden and widespread hair loss that progresses rapidly over a period of a few days. Hairs can be easily epilated. Hair loss is generalised but tends to spare the head. The skin is non inflamed and there is no evidence of pruritus.

Anagen defluxion occurs when the anagen growth phase is temporarily halted leading to abnormalities of the hairshaft. The hair is usually lost within days of insult as the resulting dysplastic change is incompatible with normal hair growth. An uncommon disease in dogs and cats, it can be caused by a range of factors including antimitotic drugs e.g. cancer, chemotherapy, infectious disease and endocrine disease or metabolic disease. Hair loss is usually diffuse and widespread hair loss over most of the body, often sparing the head. Shed hair appears to be relatively normal. No primary lesions are present.

Canine flank alopecia. In this condition hair cycle arrest occurs at particular times of the year leading to alopecia on the flanks. The hair regrowth can be triggered in some breeds by changing photo period leading to the assumption that the pineal gland may be important, in combination with prolactin production, in stimulating hair growth. No specific hormonal abnormalities have been identified in animals. It is an uncommon condition in dogs and not recorded in cats. Predisposed breeds include the boxer, Airedale and bull dog although signs can occur in any breed. In the Northern hemisphere hair loss occurs between November and March. It will normally regrow 3 - 8 months later. Dogs can lose and
then regrow hair annually, biannually or not at all. Hair is lost from the thoracolumbar region and typically is bilaterally symmetrical although asymmetrical hair loss can occur. Unlike endocrine hair loss the borders of alopecia tend to be well demarcated borders and are often geometric in shape. The skin is usually hyperpigmented but not inflamed and there is no pruritus. Dogs show no signs of systemic ill health.

**Post clipping alopecia** is recognised in dogs where hair fails to grow in areas that have been clipped either due to a pre-surgical clip or part of the normal grooming process. It is an uncommon disease only found in dogs. Any breed can be affected but the plush coated breeds such as the Chow chow and Siberian Husky appear to be predisposed. Cycle arrest occurs at the time of clipping, hair will normally start to regrowth after 3 - 4 months. Unclipped areas of hair appear to be normal whereas clipped areas appear alopetic due to presence of short stubble.

**Dysplastic alopecia**

**Congenital Alopecia /hypotrichosis.** Dogs and cats with congenital hypotrichosis are born without hair. Strictly speaking they do not have a follicular dysplasia but an ectodermal defect. Most dogs and cats with congenital hypotrichosis are born with a complete hair coat or with only partial loss of hair which progresses through dystrophic change to almost whole body alopecia within four months. In some cases whiskers, claws and papillae on the tongue can be affected. Predisposed dog breeds: poodles (toy and miniature), whippets, beagle, Rottweiler, Yorkshire terrier, American cocker spaniel, Belgian shepherd dog, Labrador retriever. The Chinese Crested Dog is a hairless breed where alopecia is caused through follicular dystrophy

**Colour dilution alopecia (colour mutant alopecia)** This condition is associated with blue (dilute black) or fawn (dilute brown) coat colours. Genetic factors appear to be important. Dysplastic change is associated with dilute coloured hair which has defective hair pigmentation in the form of large pigment granules which leads to the formation of abnormal hair. In some cases lethal pigment changes cause hair loss due to shaft fractures. Predisposed breeds include Doberman, dachshund, Great Dane, Yorkshire terrier, whippet, greyhound, miniature pinscher, Saluki, Chow Chow, Boston terrier, Shetland sheepdog, Chihuahua, poodles and Irish setter. The condition is tardive as the dog’s hair coat appears normal at birth but hair loss becomes noticeable from 6 months of age. Lesions start dorsally as hypotrichosis usually with bacterial folliculitis and can become generalised. The none colour dilute areas remain unaffected.

**Black/dark hair follicular dysplasia** This is a familial disease which is only seen in bicoloured or tricoloured puppies, only the black/dark hair is affected. A defect in hair growth is thought to be associated with disorder of pigment transfer. Predisposed breeds include the bearded collie, basset hounds, Saluki, beagle, dachshund, pointer, also bi and tri-coloured cross breeds. Dogs appear to have a normal hair coat at birth however progressive loss of weaken black hairs occurs due to shaft fracture from four weeks of age. Alopecic areas can give the appearance of complete alopecia or short stubble

**Follicular dysplasia** In follicular dysplasia the hair loss is caused by abnormal hair follicle development or else structural abnormalities. Hair loss is not colour linked and shows no seasonal pattern. It is usually tardive in onset and has been recognised in a variety of different breeds. It usually progresses slowly from puppyhood.

**Doberman Pinscher,** Miniature pinschers and Manchester terriers - black or red dogs affected; hair loss is often seen first at 1- 4 years of age. Alopecia starts on the flanks and will spread caudo-dorsally, secondary bacterial infection is common. Siberian Husky, Malamute - hair loss is seen on trunks at 3- 4 months of age, typically the coat turned reddish. Areas clipped for biopsies do not regrow. **Irish Water Spaniel,** Portuguese Water Dog usually loose hair on the ventral neck and tail is normal for water dogs. In the Irish Water Spaniel dysplastic alopecia affects neck, flanks, trunk and thigh. In males hair loss starts in middle age and is non seasonal and progressive, in females hair loss starts 6-8 weeks after first second season and will regrow 3 -4 weeks later. In the Portuguese Water Dog dysplastic alopecia affects the flanks, caudodorsal trunk and periocularly. Hair loss waxes and wanes but will grow back in many dogs. New hair is of poor quality and at each cycle less hair grows back leading to permanent alopecia. **Airedale,** Boxer, English Bulldog, Staffordshire Bull terrier, wire haired griffons and Affenpinschers loose hair between 2 and 4 years of age. Alopecia is usually restricted to the flanks and or saddle regions. Hair loss can be cyclical in other it is persistent.

**Pattern alopecia** This represents an idiopathic hair cycle arrest which leads to alopecia. It is thought to be associated with miniaturisation of hairs. It is an uncommon disease only recognised in dogs. In all cases thinning of the hair progresses to complete alopecia of the affected area with time. Hair loss is usually symmetrical and hair in non alopecic areas does not tend to epilate easily. Three syndromes are commonly recognised. The first is in male dachshunds who get alopecia of the pinnae which usually starts at 6 - 9 months of age. The second is of American water spaniels and Portuguese water dogs who develop alopecia of ventral neck, caudo-medial thighs and tail from 6 months of age. The final one affects female
Dachshunds, Chihuahua, whippet, greyhound and presents with alopecia of the post auricular area, ventrum and caudo-medial thighs. This disease starts in early adulthood.

**Other causes of alopecia**

*Traction and compression alopecia* Hair loss is seen on the top of the head of dogs that have elastic/rubber bands or bows to tie up their hair. If these are applied tightly for prolonged periods hair loss can occur. It is an uncommon condition only recognised in the dog. The initial lesion is an erythematous plaque which progresses to hair loss. Typically lesions present as well circumscribed areas of hair loss associated with area where hair was tied up, usually on the top of the head.

### Table 1 Non inflammatory alopecia

<table>
<thead>
<tr>
<th>Hair cycle arrest alopecia</th>
<th>Dysplastic alopecia</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperadrenocorticism</td>
<td>Congenital alopecia</td>
<td>Traction and compression alopecia</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>Black hair follicular dysplasia</td>
<td></td>
</tr>
<tr>
<td>Alopecia X</td>
<td>Colour dilution alopecia</td>
<td></td>
</tr>
<tr>
<td>Anagen defluxion</td>
<td>Follicular dysplasia (various breeds)</td>
<td></td>
</tr>
<tr>
<td>Telogen defluxion</td>
<td>Pattern alopecia</td>
<td></td>
</tr>
<tr>
<td>Canine flank alopecia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post clipping alopecia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2 Interpretation of hair plucks

<table>
<thead>
<tr>
<th>Part of the hair affected</th>
<th>Finding</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip</td>
<td>Finely tapered hair shaft</td>
<td>No sign of trauma to the tip hair loss is unlikely to be traumatic</td>
</tr>
<tr>
<td></td>
<td>Hair shaft ragged at tip</td>
<td>Hair tip has been chewed suggesting hair loss may be traumatic</td>
</tr>
<tr>
<td></td>
<td>Hair shaft sharply truncated</td>
<td>Hair has been cut, unhelpful in establishing cause of hair loss</td>
</tr>
<tr>
<td>Shaft</td>
<td>Parasites seen along hair shaft. Spores seen along the shaft</td>
<td>Follicular parasites most commonly Demodex canis (dog) or Demodex cati (cat). Spores on shaft consistent with dermatophytes</td>
</tr>
<tr>
<td></td>
<td>Shaft is damaged by fragmentation or nodules</td>
<td>Shaft damage usually associated with trauma suggesting hair loss is traumatic. Nodules on the hair shaft often called Trichorhhexis nodosa may be congenital or traumatically induced</td>
</tr>
<tr>
<td></td>
<td>Pigment within the hair shaft is abnormal</td>
<td>Pigment abnormalities most common seen with colour dilute alopecia or black hair follicular dysplasia</td>
</tr>
<tr>
<td></td>
<td>Prominent follicular casting on individual hairs</td>
<td>Keratinisation disorders (Vitamin A responsive disease, sebaceous adenitis), demodicosis, dermatophytosis, hyperadrenocorticism, hypothyroidism</td>
</tr>
<tr>
<td></td>
<td>Prominent follicular casting with hairs grouped together</td>
<td>Most commonly canine flank alopecia also Alopecia X</td>
</tr>
<tr>
<td>Bulb</td>
<td>Anagen hair bulb</td>
<td>Hair in the growing phase can be lost due to anagen defluxion</td>
</tr>
<tr>
<td></td>
<td>Telogen hair bulb</td>
<td>Resting hair can be seen in telogen defluxion or can be a normal finding</td>
</tr>
</tbody>
</table>
References and selected reading
5. Frank LA Hnilica KA Oliver JW. Adrenal steroid hormone concentrations in dogs with hair cycle arrest alopecia (Alopecia X) before and after treatment with melatonin and mitotane. Vet Dermatol. 2004; 15, 278-284
CONTINUING EDUCATION SESSION

Cushing’s Syndrome and Hypothyroidism: Case Discussions 1
Ian K. Ramsey
School of Veterinary Medicine, University of Glasgow, Bearsden Road Bearsden, Glasgow, UK

Case 1. Tyson: 9 year old M Akita

History and clinical examination: Presented with a history of symmetrical non-pruritic alopecia of his tail and ventrum with excessive thirst / urination. Routine blood tests and urinalysis were performed by a colleague and are shown below. The dog is not insured but the owners can afford treatment and some further diagnostics.

<table>
<thead>
<tr>
<th>Biochemistry</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>(60-80)</td>
</tr>
<tr>
<td>Albumin</td>
<td>(30-40)</td>
</tr>
<tr>
<td>Globulin</td>
<td>(30-45)</td>
</tr>
<tr>
<td>Urea</td>
<td>(3.5-8.5)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>(45-155)</td>
</tr>
<tr>
<td>ALT</td>
<td>(21-59)</td>
</tr>
<tr>
<td>AP</td>
<td>(3.142)</td>
</tr>
<tr>
<td>GGT</td>
<td>(0-10)</td>
</tr>
<tr>
<td>Glucose</td>
<td>(3.3-5.5)</td>
</tr>
<tr>
<td>Sodium</td>
<td>(136-159)</td>
</tr>
<tr>
<td>Potassium</td>
<td>(3.4-5.8)</td>
</tr>
<tr>
<td>Chloride</td>
<td>(105-120)</td>
</tr>
<tr>
<td>Calcium</td>
<td>(2.2-3.0)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>(0.6-1.3)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>(0-0.10)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>(2.7)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>(0-0.6)</td>
</tr>
</tbody>
</table>

Question 1. How important are routine blood tests and urinalysis in the assessment of this case?

- a) Very important, I would not give specific endocrine therapy without them
- b) Important, I would not perform a specific endocrine test without them
- c) Useful, but I would perform a specific endocrine test without them
- d) Interesting, but not that important: more for academic completeness
- e) Unnecessary, a waste of time and money: I would not bother in my own dog

Question 2. What other tests would you consider to be important in this case?

- a) Blood pressure measurement
- b) Radiography and ultrasonography
- c) T4/cTSH or freeT4ed/TSH
- d) Water deprivation test
- e) ACTH stimulation test or a low dose dexamethasone suppression test or UCCR
- f) Anti-thyroglobulin antibodies
- g) Endogenous ACTH
Case 2. Mia 13 year old FN Collie crossbred

History and clinical examination: 7 months history of gradual alopecia and coat colour change. In last 3 months polyphagia, polydipsia (130ml/kg/day) increased panting and increased liver enzymes. The dog is not insured but the owners are prepared to spend money for the best treatment for their much loved pet.

<table>
<thead>
<tr>
<th>Biochemistry</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tot. prot.</td>
<td>61 g/l (60-80)</td>
</tr>
<tr>
<td>Albumin</td>
<td>26 g/l (29-36)</td>
</tr>
<tr>
<td>Globulin</td>
<td>35 g/l (28-42)</td>
</tr>
<tr>
<td>Urea</td>
<td>5 mmol (2.5-8.0)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>67 μmol/l (45-150)</td>
</tr>
<tr>
<td>ALT</td>
<td>326 iu/l (21-59)</td>
</tr>
<tr>
<td>Alk. Phos</td>
<td>6401 iu/l (3-142)</td>
</tr>
<tr>
<td>GGT</td>
<td>21 iu/l (0-10)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.4 mmol/l (3.4-5.3)</td>
</tr>
<tr>
<td>Sodium</td>
<td>146 mmol/l (135-155)</td>
</tr>
<tr>
<td>Potassium</td>
<td>5.3 mmol/l (3.5-5.8)</td>
</tr>
<tr>
<td>Chloride</td>
<td>102 mmol/l (105-120)</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.5 mmol/l (2.5-2.7)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.44 mmol/l (0.6-1.2)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>2 μmol/l (0-5)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>25.5 mmol/l (2.0-7.0)</td>
</tr>
<tr>
<td>Bil acid</td>
<td>20 mmol/l (0-5)</td>
</tr>
</tbody>
</table>

Endocrine results:
- Sodium: 146 mmol/l (135-155)
- Potassium: 5.3 mmol/l (3.5-5.8)
- Chloride: 102 mmol/l (105-120)
- Calcium: 2.5 mmol/l (2.5-2.7)
- Phosphate: 1.44 mmol/l (0.6-1.2)
- Bilirubin: 2 μmol/l (0-5)
- Cholesterol: 25.5 mmol/l (2.0-7.0)
- Bil acid: 20 mmol/l (0-5)

Question 3. What form of hyperadrenocorticism does this dog have?
- a) Adrenal dependent
- b) Pituitary dependent (like 85% of all dogs with HAC)
- c) Ectopic ACTH
- d) Iatrogenic
- e) Non-ACTH dependent, food associated
- f) It is not possible to tell
- g) It is not possible to tell (and also not important)

Question 4. What further tests would you perform on this dog?
- a) Endogenous ACTH
- b) Abdominal and thoracic radiography
- c) Computed tomography (CT)
- d) Magnetic resonance imaging (MRI)
- e) High dose dexamethasone suppression test
- f) Hickey-Hare test
- g) Urine culture
- h) Blood pressure measurement
Case 3. Kara 5 year old FN Labrador

**History and clinical examination:** 2 months of rapidly progressive obesity following OHX. Now weighs 45 kg (had been 37 kg 6 months previously). Has also developed massive PU/PD (12 litres per day). Also has lethargy, a ravenous appetite and a mild alopecia. There are also mammary masses (showed to be lipomas). The dog is insured and the owners want the best treatment for their much loved pet.

### Biochemistry

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<tbody>
<tr>
<td>Tot. prot. 67 g/l</td>
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<tr>
<td>Albumin 40 g/l</td>
<td>(29-36)</td>
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<tr>
<td>Globulin 27 g/l</td>
<td>(28-42)</td>
</tr>
<tr>
<td>Urea 5.5 mmol/l</td>
<td>(2.5-8.0)</td>
</tr>
<tr>
<td>Creatinine 106 μmol/l</td>
<td>(45-150)</td>
</tr>
<tr>
<td>ALT 67 iu/l</td>
<td>(21-59)</td>
</tr>
<tr>
<td>Alk. phos 835 iu/l</td>
<td>(3-142)</td>
</tr>
<tr>
<td>GGT 6 iu/l</td>
<td>(0-10)</td>
</tr>
<tr>
<td>Glucose 6.1 mmol/l</td>
<td>(3.4-5.3)</td>
</tr>
<tr>
<td>Sodium 150 mmol/l</td>
<td>(135-155)</td>
</tr>
<tr>
<td>Potassium 4.2 mmol/l</td>
<td>(3.5-5.8)</td>
</tr>
<tr>
<td>Chloride 115 mmol/l</td>
<td>(105-120)</td>
</tr>
<tr>
<td>Calcium 3.0 mmol/l</td>
<td>(2.5-2.7)</td>
</tr>
<tr>
<td>Phosphate 1.12 mmol/l</td>
<td>(0.6-1.2)</td>
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<tr>
<td>Bilirubin 0 μmol/l</td>
<td>(0-5)</td>
</tr>
<tr>
<td>Cholesterol 12.8 mmol/l</td>
<td>(2.0-7.0)</td>
</tr>
</tbody>
</table>

### Question 5. What should we do when ACTH stimulation and low dose dexamethasone do not give us the answer we are expecting?

- a) Total thyroxine (T4) and canine thyroid stimulating hormone (cTSH)
- b) Repeat the ACTH stimulation test
- c) Measure 17 hydroxyprogesterone
- d) Perform a urine cortisol: creatinine ratio
- e) Give trilostane anyway
- f) Do some form of diagnostic imaging of the adrenal glands (CT, radiography or ultrasonography)
- g) Do some form of diagnostic imaging of the pituitary (MRI or CT)

### References


### Recent Reviews

Case 4: Milli, 4 years old, FN, Chinese Crested Dog (?)

**History and clinical examination:** 6 month history of lethargy and intermittent inappetence. Clinically overweight, subdued and affected by various dermatopathies including otitis externa, scaling, seborrhoea, hyperpigmentation and interdigital dermatitis. The dog is not insured but the owners are prepared to spend some money for the treatment of their pet.

<table>
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<tr>
<td>ALT</td>
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</tr>
<tr>
<td>Alk. Phos</td>
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<tr>
<td>GGT</td>
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</tr>
<tr>
<td>Potassium</td>
<td>(3.4-5.8)</td>
</tr>
<tr>
<td>Chloride</td>
<td>(95-115)</td>
</tr>
<tr>
<td>Calcium</td>
<td>(2.3-3.0)</td>
</tr>
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<td>Phosphate</td>
<td>(1.3-2.9)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>(0-5)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>(2.0-7.0)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>(0 – 0.6)</td>
</tr>
</tbody>
</table>

**Question 6. What tests should be performed in this case?**

a) Total thyroxine (T4)  
b) Free T4 measured by equilibrium dialysis (fT4ed)  
c) Canine thyroid stimulating hormone (cTSH)  
d) Recombinant human thyrotropin (rhTSH) stimulation test  
e) ACTH stimulation test  
f) Low dose dexamethasone suppression test  
g) Thyroxine treatment trial
Case 5: Aimi 6 year old FN Toy Poodle

**History and clinical examination:** Given steroids and developed diabetes mellitus. Steroids stopped but increasing insulin resistance noted. In addition the owner has noted weight gain and that the dog is ‘quieter than normal’. There is alopecia but no skin thinning. The dog is obese, (BCS = 7/9). It is currently on cephalexin for pyoderma. It is not significantly polyuric or polyphagic. It does not have cataracts. The dog is insured but the owners are disheartened with the treatment so far.

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<td>USG = 1.025</td>
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<td>6.7 mmol</td>
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<td>72 μmol/l</td>
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<td>ALT</td>
<td>111 iu/l</td>
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<td>(0-40)</td>
<td>RBC = 5 x 10^{12}/l, PCV = 0.35 l/l</td>
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<tr>
<td>Sodium</td>
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<td>(135-155)</td>
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<td>17.1 mmol/l</td>
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<td>Fructosamine</td>
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Case 6: Gyp, 3 year old M Collie

History and clinical examination: Long term history of inflammatory bowel disease currently controlled with prednisolone 2.5mg once daily and 200mg metronidazole twice daily. Presented with a one month history of lethargy. Variable appetite but drinking less than usual but urinating more frequently with reportedly concentrated urine. Poor coat quality with areas of alopecia seemingly bilaterally symmetrical over his thorax and flank as well as a patch on his dorsum. Mildly overweight. The dog is insured and the owners want the best treatment for their much loved pet.

Biochemistry

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<td>Triglyceride</td>
<td>0.58 mmol/l (0 – 0.6)</td>
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</table>

Question 8. Which of the following drugs are likely to have a clinically significant effect on thyroid hormone (and what effect)?

a) Prednisolone
b) Cephalexin
c) Meloxicam
d) Phenobarbitone
e) Insulin
f) Bromide
g) Ciclosporin
h) Metronidazole
i) Trimethoprim-sulphonamides
Case 7: TJ, 7 year old MN Irish Setter

**History and clinical examination:** Presented with a history of dramatic hair growth in last month (with increased shedding) and weight gain (38 to 46kg). There is a slightly increased thirst and the appetite is described as good. The dog is not lethargic or on treatment. The dog is insured and the owners want the best treatment for their much loved pet.

**Question 9. How should we proceed with this case?**

- a) Dietary management (weight reduction programme)
- b) Monitor but no further diagnostics
- c) ACTH stimulation test (cortisol +/- 17 OH progesterone)
- d) Low dose dexamethasone suppression tests
- e) T4/cTSH rTSH stimulation tests
- f) Thyroxine treatment trial
- g) Start trilostane treatment

**Recent reviews**


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<td>Cholesterol 7.9 mmol/l</td>
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**Biochemistry**
- **Urine:** SG = 1.012 No blood, ketones or glucose
- **Haematology:** Lympho/c = 0.6 x 10⁹/l
- **Rest NSAD:**

**Biochemistry**

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<td>Bilirubin</td>
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<td>Cholesterol</td>
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</table>
Update on treating Cushing’s disease
Ian K. Ramsey
School of Veterinary Medicine, University of Glasgow, Bearsden Road Bearsden, Glasgow, UK

Introduction
In general, all dogs with hyperadrenocorticism should be treated. However, cases identified fortuitously during routine health checks may not require immediate treatment. The risks of not treating hyperadrenocorticism, especially when more advanced, include the development of pancreatitis, diabetes mellitus, and calcium oxalate urolithiasis. Treatment of hyperadrenocorticism may be associated with the unmasking of steroid-responsive diseases, including arthritis and atopic dermatitis. The sudden reduction in cortisol concentrations may result in rapid growth of a pituitary tumor, leading to neurologic signs such as ataxia, depression, apparent blindness, inappetence, aimless walking, seizures, and alteration in normal behavior patterns. Treatment may also be associated with a unilateral facial nerve paralysis; it is often unclear if this is a result of the disease or the treatment, but it is seen with both trilostane and mitotane therapy. There are at least three effective treatments for hyperadrenocorticism. No one regimen is perfect for all cases. Local laws and personal experience are important factors in determining the advice that is offered. These notes provide an update on the current treatment of Cushing’s disease focusing on the use of trilostane. For a detailed description of the more well-known aspects of this disease and other treatment options, readers should consult one of the standard textbooks.

Trilostane
Trilostane is a synthetic non-hormonal steroid that is used in the treatment of canine hyperadrenocorticism (HAC), and in particular the pituitary dependent form of the disease (PDH). It has been evaluated in several major studies from across the world.

1. Mode of action
Trilostane primarily acts as a competitive, and therefore reversible, inhibitor of the 3ß-hydroxysteroid dehydrogenase enzyme system which blocks adrenal synthesis of glucocorticoids, mineralocorticoids and sex hormones. However, 17-hydroxyprogesterone concentrations do not change or increase in dogs treated with trilostane. In addition cortisone concentrations in dogs with HAC (which are normally increased, in contrast to the situation in humans) are reduced by trilostane therapy. This has been suggested as evidence for an effect on 11ß-hydroxylase and possibly on the interconversion of cortisol, and cortisone by 11ß-hydroxysteroid dehydrogenase (11ß-HSD)\(^2,3\). The effects of trilostane on dogs may be different to the effects on humans because steroid synthesis is subtly different between the species.

2. Starting dose and frequency of administration
The starting dose recommended by the manufacturer is in the range 3 to 6 mg/kg. A range of capsule sizes (10, 30, 60 and 120 mg) is available in the UK, but in other countries reformulation may be necessary for small dogs. The tablets are more effective if administered with food. There are no studies that directly compare different frequencies of trilostane administration. However it has been demonstrated that the effect of trilostane on basal and ACTH stimulated cortisol is considerably less than 24 hours in most cases\(^4\). When some poorly controlled dogs are switched to twice daily dosing, their clinical condition improves. However, the overall results obtained by twice daily dosing are not superior to those obtained by once daily\(^5,6\) and therefore it is unnecessary to divide the starting dose for all dogs. In one study in which trilostane was used twice daily in all dogs there was a higher rate of adverse incidents when compared to other studies\(^6\). Later studies using lower starting doses demonstrated a lower rate of adverse events (equal to that achieved with single daily dosing) and lower total daily doses when compared with other studies using once daily dosing\(^7\). Whether the convenience of single daily dosing is outweighed by the benefit of a lower total daily dose will be a client dependent decision. The long term effects of twice daily dosing are unknown. Nevertheless, individual dogs may respond better to a twice daily dosing regimen, and dogs treated once daily may require higher total drug quantities\(^8\). Anecdotal evidence suggests that about 20% of dogs with hyperadrenocorticism in first opinion practice are more stable if trilostane is given twice daily.

Care should be exercised when using trilostane with aldosterone antagonists (such as spironolactone), and the effects of angiotensin-converting enzyme inhibitors may be potentiated. The drug should be used with greater caution in animals with preexisting renal or cardiac disease. No specific data on drug interactions exist, and these comments are purely precautionary. No unwanted drug interactions have been seen in dogs receiving trilostane and various nonsteroidal antiinflammatory drugs, antibiotics, insulin preparations, and levothyroxine.

3. Monitoring
Dogs on trilostane should be monitored using the clinical signs and ACTH stimulation test results every 3 to 6 months. Dose adjustments should be small and, if increasing the dose, should not be more than 50% higher than the current dose. Most animals will stabilise within the range of 2 to 7 mg/kg however a small number of animals may require doses...
significantly in excess of 10 mg/kg. Various cortisol target concentrations for the ACTH stimulation test have been used to monitor trilostane therapy. The lower the target range the greater the possibility of the animal developing signs of hypoadrenocorticism. The author currently recommends a target range for the post ACTH cortisol concentration of 40 to 120 nmol/l for ACTH stimulation tests started 2 to 4 hours after dosing; however if dogs have a post-ACTH cortisol concentration of 120-200 and are responding well to treatment then an increase in monitoring rather than dose may be more acceptable to the owners. The short duration of action of trilostane has a protective effect against the development of hypoadrenocorticism. Many dogs with no serum cortisol response to ACTH stimulation 2 - 4 hours post trilostane dosing do not develop signs of hypoadrenocorticism.

If the post-ACTH cortisol concentration is lower and there are clinical signs of hypoadrenocorticism, trilostane is stopped for 5 to 7 days and reintroduced at a lower dose. If clinical signs are not apparent (and cortisol concentrations are below 40 nmol/l) then trilostane may be continued but the frequency of monitoring should be increased. If the post-ACTH cortisol concentration is higher, the dose of trilostane may need to be increased, depending on the resolution of clinical signs. However, if the post-ACTH cortisol concentration is between these two values and the patient appears not to be clinically well controlled, the trilostane may need to be given twice daily (and increasing the total daily dose by approximately 50%). This can be investigated by performing an ACTH stimulation test 24 hours after the administration of trilostane. Post ACTH cortisol concentrations that are greater than 250nmol/l 24 hours after the administration of trilostane in dogs with clinical signs of hyperadrenocorticism but apparently adequate control 3 to 5 hours after administration are an indication for twice daily dosing.

4. Efficacy
Trilostane has been found to be between 67 per cent and 90 per cent effective in resolving the various signs of hyperadrenocorticism over 3 to 6 months. The reported median survival times of PDH dogs treated with trilostane range from 662 to 900 days and is comparable or better than the median survival times of dogs treated with mitotane (which ranged from 708 to 720 days in the same studies)\(^9,10\). Trilostane has also been shown to be effective in adrenal dependent hyperadrenocorticism. In the largest series, the survival of 22 dogs treated with trilostane was compared to 13 dogs treated with mitotane\(^11\). There was no significant difference between the median survival time for animals treated with trilostane (353 days (range 4-1341)) when compared to the median survival time of mitotane treatment (102 days (range 33-982)). The one-year survival fraction for dogs on mitotane was 23% (95% CI 5-47%) whereas for trilostane it was 45% (95% CI 23%-65%). Although trilostane is not cytotoxic and thus has no effect on the growth of the tumor or of metastases, experience has shown that the same recommendations in dogs with adrenal-dependent hyperadrenocorticism are valid as for dogs with pituitary-dependent hyperadrenocorticism. Interestingly, the doses required to achieve clinical stabilization do not seem to increase significantly with time in dogs that do respond to the initial therapy.

One small series of eight dogs with concurrent diabetes mellitus and hyperadrenocorticism has been published\(^12\). This study showed that, following trilostane treatment of diabetic dogs with hyperadrenocorticism, the median insulin dose actually increased (from 1.1 IU/kg/dose to 1.5 IU/kg/dose). Despite this increase in insulin requirement in the surviving dogs, the median fructosamine concentration also increased from 401 micromol/l (range 244 - 600 micromol/l) to 438 micromol/l (range 325-600 micromol/l). Although limited by the small number of dogs, this study showed that insulin requirements and fructosamine concentrations do not consistently reduce during trilostane treatment for HAC. Furthermore prospective reductions in insulin doses at the start of trilostane treatment are probably not warranted. It seems logical to give the trilostane and insulin at the same frequency (i.e. either both once a day or both twice a day) but no study has examined this issue.

5. Effect on adrenals
In general trilostane appears to be well tolerated by most dogs. If the numbers of dogs from 6 major clinical trials are combined then only 39 dogs out of 244 dogs (16%) treated with trilostane developed adverse effects which the authors considered may have been attributable to trilostane\(^1\).

Short term adverse effects associated with overdosage are probably the most common. If this happens, trilostane should be stopped, and prednisolone given for 1 or 2 days. Some cases will require glucocorticoid and mineralocorticoid supplementation for the rest of their lives. In some cases, the dog may require very much lower doses of trilostane for the remainder of their life\(^13,14\).

Long-term adverse effects have not been documented, but adrenal glands increase in size in response to therapy, probably as a result of chronic overstimulation with endogenous ACTH. There is histopathological evidence that, as well as adrenal hyperplasia, subclinical mild adrenal necrosis is common in treated dogs. This may be due to the hypersecrecion of ACTH that, as well as increasing the size of the adrenals, may also, paradoxically, result in necrosis and haemorrhage of the adrenal glands. There have been no documented instances of adrenal tumors developing in trilostane treated dogs. Rarely adrenal necrosis may lead to more serious signs of vomiting, diarrhea, or lethargy which develops due to
hypoadrenocorticism. This has been documented in 2 case reports, one fatal and the other requiring permanent glucocorticoid therapy\textsuperscript{15,16}. Necrosis of the adrenal cortex can not be directly explained by the competitive inhibition of steroidogenesis. The development of adrenal necrosis could be due to the hypersecretion of ACTH as it has been demonstrated that trilostane causes an increase in ACTH concentrations\textsuperscript{17}. Furthermore it has been shown that even short periods of administration of ACTH can also, paradoxically, result in degeneration, focal necrosis and haemorrhage of human adrenal glands\textsuperscript{18}.

References
Figures

Figure 1 A flow chart illustrating the use of trilostane
A. Introduction

Since the epidermis is crucial for the barrier functions of the skin, this structure will be the focus of this presentation. The epidermis, a multilayered stratified squamous epithelium, is a tightly sealing membrane providing a barrier between the inner milieu of the body and the environment. It guarantees protection from loss of inner homeostasis such as transepidermal loss of water, electrolytes, and proteins, and from harmful influences from the outside. However, it is not just sheltering from hostile external influences such as mechanical, chemical and physical insults that require a sturdy structure, or from pathogens which require a close interaction between the epidermis and the skin immune system (SIS). It is also a highly versatile sensory organ allowing the perception of external influences such as temperature changes or tactile and electrical stimuli. These various barrier functions require a highly regulated and adaptive composition of various structural and functional components of which some of them only recently were revealed. Furthermore, since the epidermis is constantly renewing itself without losing its barrier functions, its cells need to interact via a lively cross-talk to allow the orchestration of the structural and functional adaptations essential for epidermal homeostasis. Disruption of epidermal structure and functions not only leads to altered epidermal differentiation as in ichthyoses but also to aberrant allergen sensitization as seen in atopic dermatitis. Understanding these structures and functional processes is therefore essential to allow the approaching of many skin diseases with new therapeutic strategies.

Barrier functions that will be discussed, are:

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<tr>
<td>Mechanical protection</td>
<td>Sturdy multilayered epidermal membrane with corneocytes “glued” together by lipid lamellae</td>
</tr>
<tr>
<td>Chemical/toxic protection</td>
<td>Lipid layer of stratum corneum (SC), tight junctions</td>
</tr>
<tr>
<td>Antimicrobial defense</td>
<td>Mechanical membrane/lipid layer; low pH, antimicrobial peptides, skin immune system</td>
</tr>
<tr>
<td>Protecting inner homeostasis</td>
<td>Tight junctions/lipid layer</td>
</tr>
<tr>
<td>Sensory functions</td>
<td>Touch corpuscles, Merkel cells, free unmyelinated nerve endings - neuroimmunologic interlace; cholinergic anti-inflammatory pathway.</td>
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To fulfill these functions, the epidermis has developed three different barriers:
- a liquid-liquid barrier by tight junctions in the stratum granulosum
- an air-liquid barrier provided by the corneocyte and the intercellular lipids
- an immune barrier by an interplay of different structural and functional components of the epidermis and the skin immune system (SIS)

A fourth barrier can be regarded as the separation of the body’s vascular structure from the outside by the epidermis.

B. Structure of the Epidermis and the EpidermalBarrier

The Transition of the Keratocyte from a Living Cell to a Corneocyte

In the stratum granulosum (SG), the keratinocyte prepares for terminal differentiation to become a corneocyte embedded in a water-impermeable intercellular mass of lipid lamellae, i.e. to form a strong barrier between the inner and the outer milieu. This is a highly orchestrated process of programmed cell death and consists of a specific set of changes which include:
1. The adaptation of intercellular adhesion
2. The formation of the cornified envelope
3. The development of keratin macrofilaments
4. The secretion of intercellular lipids
5. Stratum corneum maturation and desquamation

Many Ca++ dependent mechanisms of differentiation and cornification are facilitated by the rising calcium levels during the upward migration of keratinocytes.
1. The Adaptation of Intercellular Adhesion

In the epidermis, strong intercellular adhesion is conferred by desmosomes and to a lesser extent also by adherens junctions. They both have a similar architecture with single-span transmembrane cadherins connected to a set of intracellular plaque proteins. In the basal layer, the desmosomal cadherins desmoglein (Dsg) 3 and desmocollin (Dsc) 3 are mainly expressed, while in the suprabasal layers, including the SG, Dsg 1 and Dsc 1 predominate, in addition to Dsg 4 which is restricted to the SG and SC. In contrast, E-cadherin represents the major adherens junction cadherin that is expressed in all epidermal layers. The plaque proteins are the armadillo proteins plakoglobin (PG) and plakophilins (PPhs), and plakin family members which include desmoplakin (DP) in desmosomes, and the armadillo proteins β-catenin and PG as well as α120 and –catenin in adherens junctions. The cadherins interact in “cis” (lateral) as well as extracellularly in “trans” to confer adhesion. While desmosomes anchor intracellularly to keratin filaments, adherens junctions bind to actin filaments. At the border to the SC, desmosomes are fortified by addition of corneodesmin, an extracellular component secreted by lamellar bodies, and are then called corneodesmo-somes. The central importance of these desmosomal proteins in epidermal integrity is shown by the ectodermal dysplasia-like phenotype of Chesapeake Bay Retriever dogs with a plakophilin-1 mutation, the autoimmune disease pemphigus foliaceus with antibodies to either desmoglein 1 or desmocollin 1 or “Peeling Disease” in man with a dyscohesion in the SC due to a corneodesmin mutation. Furthermore SG keratinocytes (in mice in the second SG layer called SG2) express a “strand” of tight junctions separating the “internal” and “external” water-soluble interstitial fluid by forming a paracellular fluid barrier. Like desmosomes, they have several transmembrane and plaque proteins, i.e. contain the 4-spanning transmembrane occludin, several claudin family members and JAM-1 that link to the intracellular plaque proteins ZO-1 & 2, and cingulin. These plaque proteins anchor this multimeric protein complex to the actin filaments. The barrier function of the tight junctions is exemplified by the ichthyosis phenotype and rapid dehydration of Claudin-1 knock-out mice or infants with the NISCH syndrome, a Claudin-1 gene defect. Like desmosomal and classical cadherins, claudins are Ca²⁺ binding proteins, which require this ion to adopt the tertiary structure necessary for transadhesion.

2. The Formation of the Cornified Envelope (CE)

The CE initially forms just underneath the plasma membrane and then develops into a 10 nm thick layer of highly cross-linked insoluble proteins including the plasma membrane. CE proteins such as involucrin and two members of the plakin family, envoplakin and periplakin, associate with the plasma membrane in a Ca²⁺-dependent manner. Transglutaminase is expressed in these epidermal layers to crosslink the CE proteins to form a monomolecular layer along the inner surface of the plasma membrane spanning the desmosomes and leading to the formation of a scaffold. Subsequently, further CE proteins, mainly loricrin and small proline-rich proteins (SPRPs), are crosslinked to the CE to reinforce the scaffold. The transglutaminase-1 gene mutation in a recessive lamellar ichthyosis-like disease in Jack Russell Terrier demonstrates the importance of this protein for epidermal integrity.
3. The Development of Keratin (K) Macrofilaments

The intermediate filaments, K1 and 10 heterodimers, and K2 as a newly synthesized keratin in the SC, are responsible for structural integrity of keratinocytes in the upper epidermal layers. At the border of the SG and the SC, these intermediate filaments are cross-linked to form the mechanically highly resistant macrofilaments of the corneocyte and are linked to the CE. This occurs by the action of filaggrin, a monomeric protein produced by dephosphorylation and proteolysis of profilaggrin that is released from keratohyalin granules upon increasing levels of extracellular Ca++. The importance of these keratins and filaggrin in the process is exemplified by the skin phenotype of genetically modified mice and skin diseases in man and dog such as epidermolytic hyperkeratosis of the Norfolk Terrier, a keratin 10 mutation, or atopic dermatitis in which filaggrin mutations present the most significant risk factor and varying filaggrin immunofluorescence patterns suggest altered filaggrin expression and processing in the dog.

4. The Secretion of the Intercellular Lipids

Concurrently with the formation of the CE and the macrofilament formation in the SG, lamellar bodies bring barrier lipids (mainly ceramides) to the plasma membrane. These lipids are delivered into the extracellular milieu. In particular the w-hydroxy-ceramides have long fatty acid chains that span the lipid bilayer of the plasma membrane and project into the cell where they are crosslinked to the CE proteins by the action of transglutaminase connecting them to the scaffold. In this stage most of the other cell organelles and structures are degraded except for the keratin macromolecules that are crosslinked to the CE. The importance of the ceramide content in the SC is highlighted by gene defects of lipid metabolism proteins in a congenital ichthyosis of Golden Retriever dogs and the correlation of decreased ceramides in dogs with atopic dermatitis or seborrhoea.

5. Stratum Corneum Maturation and Desquamation

In the upper part of the SC preserving water is essential to maintain skin plasticity, a process thought to be mediated by free amino acids and smaller proteins resulting from proteolysis of filaggrin into naturally moisturizing factors. As the corneocyte migrates towards the surface, exfoliation of corneocytes is facilitated by several hydrolytic enzymes that degrade corneodesmosomes, a prerequisite for desquamation. Furthermore, serine proteases such as Kallikrein 5 and 7 and others regulate the desquamation process by cleaving corneodesmosomes, and by degrading lipid processing enzymes in the SC.

C. Epidermal Barrier and the Skin Immune System (SIS)

The skin as the largest organs of the body has an immense surface bordering the environment. The maintenance of the inner homeostasis requires therefore a constant surveillance of possibly harmful influences such as pathogens. Langerhans cells (LC), the antigen-presenting dendritic cells of the epidermis, are positioned in the basal layer with dendrites extending into the upper layers while tightly adhering to the neighboring basal keratinocytes by expression of E-cadherin. Besides LCs, the keratinocyte itself can also act as a primary sensor of cutaneous injury and intrusion of pathogens, in particular via its Toll-like receptors (TLR) and Protease-Activated Receptor-2 (PAR-2). After perturbation of the SC air-liquid interface barrier, e.g. by exogenous proteases, keratinocytes may get activated via PAR-2 signalling, and secrete inflammatory mediators such as IL-1, TNF- or TSLP. These in turn activate LC. Interestingly, in a quiescent state, LC dendrites reach up to the most inner SG layer but do not cross the tight junction liquid-liquid barrier. In contrast when activated, they pass the tight junction barrier by forming tricellular tight junctions and reaching into the “external” liquid layer of the outer most SG where they are able to get into contact with a new set of allergens/pathogens. This mechanism is believed to be involved in atopic dermatitis. With these two “warriors”, the keratinocyte and the LC at the front, the SIS is always alert and ready to react.

D. Epidermis, a Sensory Organ in the Neuro-Immunologic-Cutaneous System

The epidermis is not just a protective measure from harmful external influences and for the maintenance of inner homeostasis but a true sensory organ of great importance for perception of environmental stimuli including touch, pain, itch and direct electromagnetic stimulation, i.e. it represents a sensory tissue. Touch corpuscles, e.g. contain up to 50 specialized tactile cells and adhere via desmosomes to basal keratinocytes, and each one links to its sensory nerve fiber ending reacting to pressure changes. Also single nociceptive A fibers end in the epidermis and are activated by electrical current transmitting a pricking sensation or pain. Interestingly, new ingrowth of nerve endings into the epidermis...
were seen in hyperplastic skin. Merkel cells are multisensory cells receiving most of the environmental stimuli including electromagnetic, UV-radiation, temperature, and humidity and are proposed to transfer this information to the brain. However, sensor proteins and neuropeptides are expressed by all epidermal cells including keratinocytes, melanocytes, LC and Merkel cells suggesting that all of these cells perceive external stimuli forming a neuro-immuno-cutaneous system. Particularly important sensory proteins are of the transient receptor potential (TRP) family which each transmits specific sensations, e.g. TRPV-1 pain and neurogenic inflammation. Furthermore, a subpopulation of dorsal root ganglia neurons that lead to epidermal nociceptors, have been specifically linked to itch.

In conclusion, it has become evident in recent years that the epidermis is a complex multicellular organ which is capable of a highly orchestrated crosstalk between its cells to fulfill the overwhelming variation of its essential functions.

References
The topical administration of drugs has become very popular in the last decades, not only in human medicine but also in veterinary practice. However, the mammalian skin represents an effective barrier against endogenous water loss and the intrusion of environmental substances. Interestingly, the topical application of a compound to different species results in marked interspecies differences\(^1\)\(^-\)\(^3\). What are the reasons for these interspecies varieties? Studies in the past have demonstrated that the main skin barrier is maintained by the outermost skin layer, the stratum corneum, which comprises corneocytes embedded in a lipid-rich matrix in a so called “brick and mortar model”. Thus, a topically applied drug has to pass the stratum corneum, which is assumed to take place in three possible ways, intercellular through the lipid rich domains, through hair follicles or through skin glands (Figure 1).

Consequently, the transdermal drug diffusion can be influenced by the intercellular lipid content and composition as well as morphological skin characteristics.

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Consequently, the transdermal drug diffusion can be influenced by the intercellular lipid content and composition as well as morphological skin characteristics.

The diffusion behavior and may vary between different species. According to the Fick’s first law of diffusion a thicker stratum corneum of a species is proportional to a longer diffusion pathway of a topically applied compound. This could be demonstrated by a comparison study, in which the permeation of several test compounds through the skin of four different species was compared. The comparison of skin permeability of several substances with different physicochemical drug characteristics through skin of four species, exhibited a general rank order of permeability: pig < dog < cattle < rat. Although the different species exhibit a special lipid profile, no single lipid class could be characterized in this study to mainly determine transdermal drug diffusion.

Moreover, the length of the intercellular lipid pathway of a topically applied drug has to be taken into account for the diffusion behavior and may vary between different species. According to the Fick’s first law of diffusion a thicker stratum corneum of a species is proportional to a longer diffusion pathway of a topically applied compound. This could be demonstrated by a comparison study, in which the permeation of several test compounds through the skin of four different species was compared. The comparison of skin permeability of several substances with different physicochemical drug characteristics through skin of four species, exhibited a general rank order of permeability: pig < dog < cattle < rat. Although the different species exhibit a special lipid profile, no single lipid class could be characterized in this study to mainly determine transdermal drug diffusion.

Moreover, skin lipid classes consist of various molecules differing in their structure. For example, the lipid class of the ceramides consists of several ceramide types, all of which can be distinguished by their molecular structure. Cer1 or Cer[EOS] contains ester-linked fatty acids, omega-OH fatty acids and sphingosines while Cer3 or Cer[NP] contains non-OH fatty acids and phytosphingosines. So, each ceramide may play a special role in transdermal drug diffusion. Due to the different skin lipid compositions, species exhibit different permeation profiles. The comparison of skin permeability of several substances with different physicochemical drug characteristics through skin of four species, exhibited a general rank order of permeability: pig < dog < cattle < rat. Although the different species exhibit a special lipid profile, no single lipid class could be characterized in this study to mainly determine transdermal drug diffusion.

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References


Figure 1
Figure 1: Diffusion pathways through the stratum corneum according to Hadgraft (2001).  

Conflict of interests
All authors declare to have no conflicts of interests.
Skin barrier in human atopic dermatitis

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Introduction
Atopic dermatitis is characterized by two important phenomena: (i) On one hand a marked xerosis (dry skin) with a disturbed epidermal barrier function and (ii) an underlying chronic inflammatory reaction. Since the 80., the dermatological community was discussing the question as to what was first? The chicken or the egg? Has the chronic inflammation its origin in a genetically determined barrier dysfunction or is the latter a result of a chronic inflammation? Both dogma have been heavily discussed over the last 30 years, and those who were defending the epidermal dogma (“inside-outside” concept) often argued that any kind of IgE sensitization is clinically not relevant but must be seen as an epiphenomenon. On the other hand, there were enough clinical and biological arguments in favor of an immunological/allergological concept (“outside-inside” concept) such the positive atopy patch-tests which clearly shown by their specificity the role of allergens in triggering the inflammatory reaction in patients with atopic dermatitis.

Allergy or epidermal barrier function: what do genetics say?
While due to modern technologies, research in the field of monogenetic diseases has done tremendous progress in the last years, the so called genetic complex diseases such as psoriasis or atopic dermatitis have benefited of these new developments such as the genome wide association studies (GWAS). The discovery of the Filaggrin gene mutations as an important origin for the epidermal barrier dysfunction in patients with atopic dermatitis has opened new perspectives in our understanding the biochemical mechanisms underlying the dry and sensitive skin in these patients. Interestingly the Filaggrin itself is not so important but instead its metabolites such as the natural moisturizing factors (NMF) are critical and remain under the further control of several other factors besides genetics. The intra epidermal pH as well as the protease/anti-protease balance and the underlying inflammatory reactions with mainly Th2 cytokines are able to dramatically up or down-regulate the expression of Filaggrin and of its metabolites. Meanwhile, about 50 different Filaggrin mutations and gene variants have been described and the hot spots in the genes are different in Caucasian and Asian populations. The functional consequences of these gene mutations can be quite different such as an increase in the adherence of Staphylococci, the induction of an unspecific reaction in the skin and a decrease in the hydration of the stratum corneum combined with the important defect in the barrier function and increased permeability to environmental allergens. This disturbance of the barrier function bears a number of clinically relevant consequences. Besides a high risk to develop an early and severe form of atopic dermatitis, these patients also have the highest risk to develop a peanut allergy, viral complications such as eczema herpeticum and last but not least they also have a higher risk to make an atopic career, i.e. to have allergic asthma for the rest of their life.

Although the importance of the Filaggrin mutations for the epidermal barrier function have been widely recognized and reproduced in many papers, it must be acknowledged that they may only explain 30 to 50 % of the patients with atopic dermatitis while the genes responsible for the remaining patients have not yet been clearly identified. More recently it has been shown that other genes such as Claudin-1 or genes encoding for the protease/anti-protease system of the epidermis such as SPINK5 may play an important role. Moreover, there must be a number of other genes which have yet to be discovered which may be instrumental in the disturbance of the epidermal barrier function in these individuals.

Besides the genes relevant for the epidermal barrier function, we also have to consider the more immunologically relevant genes related to the inflammatory reactions and the IgE sensitization. These candidate genes for the atopic condition are classically divided in two main families: (i) the genes of the innate immunity and (ii) the genes of the acquired immunity. Besides the genes directly relevant for the IgE such as IL-4 and IL-13 and their receptors, a number of other genes have been suspected in the last years such as the gene encoding for Thymic Stromal Lymphopoietin (TSLP). In animal models, it has been shown that variants of the TSLP gene have an important role in the IgE sensitization and development of asthma which has been sensitized epicutanously. TSLP is highly suspected because it has a key role in directing the dendritic cells to initiate a Th2 response in T cells. Therefore, beside IL-4, TSLP is also currently one of the key targets for new biologicals in the treatment of atopic dermatitis and atopic diseases.

Genetics and inflammation are key in the control of the barrier function
It appears that the epidermal barrier function in atopic dermatitis is controlled on one hand by the genetic background and on the other hand by the underlying inflammatory reaction. This is of importance since we know that in the very initial face of atopic dermatitis in infants, there is no evidence for an IgE sensitization and we assume that in this very early phase, there must be a kind of unspecific inflammatory reaction which is not linked to an allergic reaction but rather the results of some intrinsic and genetically determined inflammations starting from the epidermal compartment. In this very early phase, genes like those involved in the epidermal protease/anti-protease cascade may play an important role. Here, genetic variants of the SPINK 5 gene as an initial trigger for this allergen-independent inflammatory reaction would be of interest for new therapies.
could be an interesting candidate. There is some evidence from animal models that variants of this gene may indeed play a role that could even induce locally increased production of TSLP and thereby represent the *primum movens* of the disease in the very early phase. This knowledge will have a tremendous impact in our understanding the mechanisms underlying atopic dermatitis and represent the basics for a more personalized approach in prevention and management of this disease.

**References**


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Notes
Introduction

The new “outside-inside-outside” theory for the pathogenesis of human AD postulates that genetic (i.e. primary) skin barrier defects enhance allergen penetration leading to atopic cutaneous inflammation that results in decreased stratum corneum (SC) lipid and protein secretion, as well as increased desquamation, all of these contributing to further (i.e. secondary) barrier dysfunction.1

Because of the latest change in paradigm and focus regarding the pathogenesis of human atopic dermatitis (AD), the question whether or not a cutaneous barrier dysfunction exists in dogs with the homologous disease is timely and relevant.

This lecture will briefly review the evidence existing for skin barrier anomalies in dogs with AD. We will successively discuss changes in skin permeability, SC lipids, superficial epidermal proteins, the implications of skin barrier dysfunction on the process of sensitization and the results of trials employing topical formulations aimed at skin barrier restoration.

Do dogs with AD have dry skin and lose water through their skin?

In humans, the assessment of skin barrier function has traditionally relied on the measurement of transepidermal water loss (TEWL) with either open or closed chamber evaporimeters. There is indisputable evidence that human patients with AD have increased TEWL in normal-appearing skin, and that skin lesions and higher disease severity increase these values.2, 3 At this time, the usefulness of TEWL measurements in dogs remains debatable, either with open or closed chamber devices, as there is very high day-to-day, site-to-site and dog-to-dog variability that makes comparisons between patients unreliable in clinical studies.4, 5

In spite of the limitations stated above, Shimada and colleagues reported that, in ten dogs with spontaneously arising AD, closed-chamber TEWL measurements of both clinically nonlesional and lesional skin were markedly higher than those of normal dogs at similar sites.6 Similar findings were reported in house dust mite (HDM) sensitized beagle dogs that had significantly higher open-chamber TEWL values at six of ten clinically normal body sites compared to normal beagles (n = 21)7 Furthermore, challenges with HDM resulted in significantly higher TEWL values in young dogs, and these measurements were highest at AD predilection sites that normally develop mite-induced skin lesions. 7

A recent study established that closed-chamber TEWL values were significantly higher in the concave pinnae of dogs with active AD, compared to those of dogs whose AD was in remission after immunotherapy or cyclosporine. Values of the latter group overlapped with those of normal dogs.8 A second study documented the reduction of lesional TEWL values in dogs with AD treated successfully with an hydrocortisone aceponate spray (Cortavance, Virbac).9

In summary, measuring TEWL in dogs does not appear to be the most optimal method to assess skin barrier function in dogs due to the very high variability and lack of reproducibility of measurements. In spite of these limitations, values from dogs with spontaneous AD seem markedly higher than those of normal dogs, with measurements in lesional skin exceeding those of visibly normal skin. In an experimental dog model of AD skin lesion development, allergic challenges appear to increase TEWL values compared to pre-provocation ones, and this change is most prominent at AD predilection sites. Importantly, treatment of AD lesions, which results in their remission, leads to normalization of TEWL values.

Do dogs with AD have abnormal SC lipids?

Anomalies of SC lipid lamellae

A decade ago, we reported that the deposition of lipid lamellae in the SC of dogs with AD was very heterogeneous compared with that of normal canine skin. Many areas of atopic SC were found to be devoid of lipid lamellae. When present, the lamellae often exhibited an abnormal and/or an incomplete structure. Lamellar bodies extruding lipids appeared fairly normal in number and morphology. When compared between normal and atopic canine SC, scores for continuity and thickness of intercellular lipid lamellae were found to be significantly lower in nonlesional atopic than normal canine SC.10 Similar changes were reported recently in the nonlesional SC of five dogs with naturally occurring AD.11
Finally, in an experimental model of canine AD, wide intercellular spaces, disorganized lipid lamellae and intra-corneocyte retention of lamellar bodies were found in visibly nonlesional SC of HDM-sensitized but not normal beagles. In these hypersensitive dogs, HDM challenges led to further disorganization of corneocytes and lipid lamellae arrangement as well as increased widening of intracellular spaces in the SC.

In summary, anomalies in the amount and arrangement of intercellular lipid lamellae appear to exist in the clinically nonlesional SC of dogs with AD. Furthermore, in experimentally sensitized dogs, allergen challenge seems to further worsen SC lipid anomalies.

Anomalies of SC lipid composition

In 2009, two studies showed that SC lipids isolated from dogs with spontaneous AD had a lower proportion of ceramides than those of normal dogs. In these atopic dogs, the relative amount of SC ceramides was negatively correlated with TEWL values, thereby indicating the possible contribution of these ceramides to epidermal water permeability.

Further evidence of a ceramide deficiency in atopic dogs came from the recent demonstration of a significant reduction in the quantity of SC total free ceramides and their subclasses CER[EOS] (ceramide 1), CER[NS]/[NDS] (ceramide-2/10), CER[NP] (ceramide-3), CER[AS]/[NH] (ceramides 5/8) and CER[EOP] (ceramide 9) in both lesional and nonlesional skin of ten dogs with AD compared to that of ten healthy age- and breed-matched dogs. Similar results were found in another study that also revealed lower quantities, not only of free, but also protein-bound ceramides with CER[EOP] and CER[AH] (ceramide-7) being barely detectable. Interestingly, there was a concurrent increase in the amounts of both free and protein-bound glycosylceramides in the SC of atopic dogs compared to normal dogs. This increase suggests the possible existence of an abnormal ceramide metabolic pathway in the skin of atopic dogs, for example a deficiency in beta-glucocerebrosidase, the enzyme converting glycosylceramides into ceramides. Of importance is the observation of a noticeable variation in the lipid composition between SC layers in dogs with AD, suggesting that successive waves of inflammation might transiently have altered lipid biosynthesis.

Finally, we recently demonstrated, in an experimental model of acute AD skin lesions, that HDM challenges led to a selective decrease in SC total ceramide and some of their fractions, not only at the site of allergen challenge, but also at areas distant from it. Stratum corneum ceramide levels returned to normal within 2 months after challenge. In contrast, cholesterol and fatty acid SC levels did not change significantly. These results suggest that changes in SC ceramides might occur secondarily to inflammation.

In summary, there is a reduction in total ceramides and some free and protein-bound ceramide subclasses in the nonlesional SC of dogs with AD. This ceramide reduction is associated with increased TEWL. There is evidence suggesting that inflammation leads to reduction in SC ceramide levels, which can return to normal once inflammation subsides. These results cast some doubts on the existence of a primary SC lipid defect in dogs with AD.

Do dogs with AD have abnormal SC proteins?

In 2010, Chervet and colleagues reported results of indirect immunofluorescence performed in normal skin of 16 healthy dogs and nonlesional skin of 18 dogs with AD using antibodies specific for either the carboxy or aminoterminus of filaggrin. Four distinctive filaggrin expression patterns were identified in non-lesional skin. Ten dogs exhibited an identical staining pattern for both antibodies with comparable (category I; 3/18, 17%) or reduced (category II; 7/18, 39%) fluorescence intensity compared to that of controls. In contrast, 4/18 dogs (22%) displayed aberrant large vesicles revealed by the C-terminal but not the N-terminal antibody (category III). Finally, 4/18 dogs (22%) had normal N-terminal but no detectable C-terminal filaggrin (category IV). The observation of reduced filaggrin expression in dogs from category II is compatible with a possible decrease secondary to atopic inflammation, while the anomalies seen in dogs from category IV, and possibly in those from category III, are suggestive of mutations in the FLG gene that result in the abnormal or loss of function of the filaggrin protein. In another study, the expression of filaggrin, but also that of involucrin, was found to be reduced in canine lesional AD skin compared to nonlesional and normal canine skin. In an experimental model of acute AD skin lesions, the expression of filaggrin by immunohistochemistry did not appear to be different before or after challenge, or after resolution of skin lesions.

The transcription of LCN was found to be significantly lower in nonlesional skin of five West Highland white terriers (WHWT) with AD compared to that of three normal WHWTs. In contrast to these results, the transcription of genes encoding filaggrin and involucrin was found to be significantly higher in lesional canine AD skin compared to nonlesional or normal canine skin. The latter results are contrary to those of immunostaining studies by the same authors.
At this time, linkage analyses have excluded the FLG gene as being associated with AD in WHWT in Australia, the UK, and the USA. In contrast, the filaggrin gene region appears to be linked to AD in Labradors in the UK.

Finally, in a pilot study, we confirmed that the expression of the tight junction protein claudin-1 and that of the corneocyte desmosome protein corneodesmosin became discontinuous after HDM patch testing in mite-sensitized dogs. In contrast, the expression of E-cadherin, desmoglein-1 and desmocollin-1 appeared normal after patch testing. (Olivry, unpublished 2013).

In summary, there is evidence that a fraction of dogs with spontaneous AD have anomalies in their filaggrin carboxy-terminus. At this time, however, mutations in FLG have not been confirmed to exist in dogs with this disease. Atopic inflammation appears to lead to abnormal expression of some superficial epidermal proteins.

What are the consequences for dogs of having a defective SC?

Two studies have reported the changes that occur after SC tape removal in normal and atopic canine skin. In 2009, using a closed-chamber evaporimeter, Shimada and colleagues established that TEWL values increased linearly with increasing numbers of corneocyte-removing tape strips.

Furthermore, sensitization to epicutaneously-applied HDM occurred earlier and was stronger, as shown by allergen-specific peripheral blood T-lymphocyte activation, allergen-specific IgE serum levels and intradermal testing, in atopic laboratory dogs in which the SC was removed by tape-stripping compared to dogs in which the SC was not altered.

In summary, in dogs as in other species, an intact SC appears to be a barrier indispensable for the limitation of water evaporation and cutaneous allergen sensitization.

Are "barrier repairing interventions" of clinical benefit for dogs with AD?

At the time of this writing, there are four small randomized controlled trials (RCTs) reporting the effect of topical lipid-based interventions aimed at restoring the skin barrier in dogs with AD.

The first RCT enrolled 47 dogs with AD, which were allocated to receive either a phytosphingosine-containing shampoo (Douxocalm, Sogeval), the phytosphingosine-containing shampoo and then a spray with similar ingredients (Douxocalm microemulsion spray, Sogeval) or a control shampoo containing antiseptics, fatty acids and complex sugars (Allermyl, Virbac). After 20 days, the CADESI score had decreased in the three treatment groups by 40 to 46% while the pruritus grade had been reduced by 23 to 30%. There were no apparent differences in efficacy between these three topical lipid-containing interventions.

In a second RCT, 14 dogs with AD were treated either with a weekly spot-on (Dermoscent Essential 6, LDCA) or a daily spray containing a similar type of ingredients (Dermoscent Atop 7, LDCA). After eight weeks, the CADESI scores and the pruritus visual analog values had decreased in 13/14 and 9/14 dogs with AD, respectively. Nevertheless, there were no differences in perceived effect between the two groups. Additionally, the tested topical medications did not appear to have any effect in improving TEWL values. In this trial, some atopic dogs had, at the time of study onset, clinical scores or TEWL values in the range of those of normal dogs or atopic dogs with mild disease. Such low disease severity at study onset is bound to render the interpretation of treatment effect difficult.

Furthermore, in the two RCTs above, the lack of a control group treated with a non lipid-based topical formulation is a likely source of bias, as a placebo effect is commonly seen in clinical trials enrolling dogs with AD.

In a third RCT, 48 dogs were treated topically once weekly for 8 weeks with a fatty acid and essential oil-containing spot-on (Dermoscent Essential 6, LDCA) or a placebo. Lesion and pruritus scores significantly improved in dogs treated with the active but not with the placebo spot-on. Comparisons of scores between groups were not reported, however, so a valid assessment of treatment effect could not be made.

The fourth RCT tested the efficacy of a topical ceramide and fatty acid (Allerderm Spot On, Virbac) or a placebo solution applied three times weekly for 4 weeks onto the pinnae, antebrachium, axillae and groin in dogs with AD. After 4 weeks, the CADESI lesional scores were significantly lower in dogs treated with the active compared to the control solution. The magnitude of improvement was not reported in the abstract. Changes in TEWL values during treatment were found to be variable between dogs.
Finally, it is important to remember that, in none of these trials, was the diet controlled between dogs. As diets can have a wide range of fatty acids, which could, by themselves, normalize SC lipid lamellae and ceramide levels, the lack of diet standardization between groups could likely affect study results.

In summary, four RCTs provide low quality evidence of still inconclusive clinical benefit of lipid-based topical therapy in dogs with AD. Additional trials of higher quality (e.g. with diet controls) are clearly needed. Whether or not the addition of topical lipids is of greater benefit than oral fatty acid administration, in diets or in supplements, remains to be determined. Similarly, one must determine if the addition of such topical lipid formulations are of benefit in dogs receiving standard of care anti-allergic therapy.

Conclusions and implication for practice
At the time of this writing, there is increasing evidence that a skin barrier defect likely exists in dogs with AD. This barrier dysfunction is characterized by abnormal intercellular SC lipid lamellae, abnormal SC morphology, reduced and abnormal ceramide content and, in some dogs, abnormal expression of filaggrin and other superficial epidermal proteins. As a logical consequence of these barrier changes, TEWL is normally increased. Allergen-induced atopic inflammation appears to further worsen these anomalies. When lesions disappear spontaneously or during treatment, many of these changes appear to return to normal. It is currently not known if only some or all dogs with AD have similar skin barrier dysfunction. Furthermore, whether this defect, if present, is primary (i.e. of genetic origin) and/or secondary to atopic skin inflammation itself remains to be determined. The recent observation of normalization of SC lipids and TEWL values after remission of skin lesions currently argue for a secondary barrier defect in canine AD. At the time of this writing, there is no evidence of the existence of a primary barrier defect in this disease. That changes can be seen in nonlesional (i.e. visibly normal) canine AD skin cannot be as proof of existence of a prelesional barrier defect, as, nonlesional skin already exhibits microscopic inflammation and contains proinflammatory cytokines similar in nature to those of lesional atopic skin. Clinically, the optimal intervention to correct a possible barrier dysfunction (e.g. nutritional lipid supplementation and/or topical lipid application) and the therapeutic benefit - if any - of correcting such barrier defect remain largely unknown and unproven.

Conflict of Interest
The author does not declare any conflict of interest relevant to this review except for research funding and consulting honorarium received from Virbac France and lecturing and consulting honorarium given by Sogeval; both companies currently market topical lipid formulations aimed at restoring skin barrier function in dogs.

Note: these notes were derived, summarized and actualized from the following article: Olivry T. Is the skin barrier abnormal in dogs with atopic dermatitis? Veterinary Immunology and Immunopathology 2011; 144: 11-16.

References


Notes
To ensure the clinical efficacy of a topically administered active compound, this must reach the site of action (superficial layers, deeper layers of the skin, systemic circulation) in an appropriate concentration. Therefore, the physico-chemical properties of the compound and the pharmaceutical formulation are determining factors of the clinical efficacy.

A general advantage of the topical treatment of skin diseases is the direct administration of the compound at the site of action, possibly without undesired systemic effects. Disadvantages may be its low practicability and a local irritation caused by the active agents or other ingredients of the formulation used.

The basic mechanism in transdermal absorption is the passive diffusion via the horny layer and via the other epidermal layers and the dermis. Lipophilic and non-ionised agents may easily penetrate the stratum corneum as the most important barrier. In contrast, the horny layer is only poorly permeable for hydrophilic and ionised compounds. It has to be considered that the stratum corneum is not only a barrier but also a reservoir for lipophilic compounds. This was demonstrated by the vasoconstriction (skin blanching) assay which is used to compare the potency of external glucocorticoids. Glucocorticoids induce a blanching reaction of the skin after topical administration (occlusive conditions). Without further treatment, occlusive conditions after the decay of the blanching reaction may re-induce a vasoconstriction also after days. This was explained by an enhanced flux of the glucocorticoids stored in the horny layer into the dermis.

The diffusion via the horny layer is influenced by the pharmaceutical formulation. This was demonstrated by an experimental study performed in the isolated perfused bovine udder where betamethasone-17,21-dipropionate was administered onto the skin as an ingredient of an ointment, cream and solution. The lowest transdermal permeation was found after administration of the solution. After deterioration of the barrier function of the horny layer, the penetration and permeation were enhanced. Further studies demonstrating the influence of pharmaceutical formulation were performed with ibuprofen. These studies confirm that there exist significant differences of transdermal penetration, permeation and absorption between external formulations. The use of microneedles was also tested as a possibility to enhance the transdermal penetration.

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. As in each other formulation, the skin tolerability of the shampoo formulation has to be considered. 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.

The addition of penetration enhancers to a pharmaceutical formulation can enhance the transdermal flux significantly. Examples for penetration enhances are dimethyl sulfoxide, propylene glycol, various lipid formulations and also solvent combinations with isopropyl myristate and others.

A further interesting topic is the use of spot-on or pour-on formulations to reach a systemic effect of the administered compound or to have a spreading effect with a horizontal diffusion in the stratum corneum. Locally administered drugs are used actually to protect the whole body area against fleas, ticks and other ectoparasites. There exist different formulations which ensure the diffusion over the whole body. This was demonstrated in own studies for the pyrethroids permethrin and flumethrin. Within hours (permethrin) after local administration in the neck the compound was measurable in different body regions. Flumethrin which is used in dogs and cats as an ingredient of a collar needs a little bit longer because of the liberation process.
In summary, essential pharmacokinetic factors of topically administered drugs are:

- the liberation from the pharmaceutic 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

Drug formulations which ensure a sufficient passage of the active ingredient via the horny layer as the most important barrier of the skin will support the dermatological therapy.

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Conflicts of interest
The authors declare that there are no conflicts of interest.
Topical management of human atopic dermatitis

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Introduction
Atopic dermatitis (AD) is the most common chronic inflammatory skin disease whose increasing prevalence presents a major public-health problem in industrialized world areas and also an increasing issue in developing countries. Characteristic features of AD include pruritus and chronic or chronically relapsing dermatitis, usually beginning mostly at infant age (early onset) but can also start in adulthood (late onset). AD is a paradigmatic genetic complex disease and is often accompanied or followed by other atopic disorders such as allergic rhino-conjunctivitis or allergic bronchial asthma. These diseases may appear simultaneously or develop in succession during the course of disease. AD is characteristic for early childhood, while pollen allergy and allergic asthma predominate in adolescence. This characteristic, age-dependent sequence has been postulated as the "atopic march". Thus AD often represent the beginning of this atopic carrier while asthmatic diseases usually mark its full expression. Besides the allergological workup, management of AD should no more concentrated solely on the treatment of acute flares but be directed towards the improvement of the genetically determined dysfunction of the epidermal barrier function and early intervention against skin inflammation which may prevent subsequent sensitization.

Management of atopic dermatitis
As AD is a chronic relapsing disease, in the past, the classical targets of the therapy were mainly the short time treatment of acute flares or superinfections, i.e. a rather reactive management. More recently, based on the new insights into the genetics and pathophysiology, a merely long-term proactive management became more popular. Therefore, the current management of AD is based on distinct important aspects:

- Avoidance of trigger factors, including allergic and microbial factors,
- A basis therapy (formerly called skin care) which aims to compensate the genetically determined xerosis and epidermal barrier function,
- An anti-inflammatory therapy aimed to better control the underlying and subclinical inflammation assumed to provide the background for the flares.
- In some selected cases, an adjunctive or complementary therapy may be useful.

As a rule, the management should be adapted to the severity of the disease. While mild cases may be well controlled by basic therapy, moderate form will require basic therapy associated to a topical proactive management with anti-inflammatory compounds and phototherapy. In more severe and refractory cases, the use of systemic drugs may be required to control the disease. An appropriate educational programme is now considered as an integral part of the modern management of AD patients.

Avoidance of trigger factors
There are multiple environmental and psychological trigger factors like stress, allergens, sweating, soap, wool or cigarette smoke which are individual in each patient and may be identified by a careful anamnesis and allergological testing. These should be performed when clinical symptoms are present but are only meaningful in the IgE-associated form. RAST is performed to quantify specific IgE antibodies against allergens. Results are determined as RAST classes (from 1-6) and in kU/l. IgE-sensitization has to be positive (>150kU/l) to meet the general criteria of IgE-associated AD. Provocation tests are additionally performed to determine the clinical significance of positive laboratory tests since skin tests and in vitro testing should complement one another yet do not always have to be concordant. The atopy patch test (APT)7, as a provocation test, is used to provoke an eczematous reaction through the application of aeroallergens and food allergens epidemaneously to a patient’s back. Its major usefulness resides in its specificity rather than the sensitivity. The avoidance of these triggering factors is essential for the prevention of new flares in the IgE-associated form of AD.

As S. aureus, which densely colonises the skin in majority of AD patients, is known to amplify the skin inflammation in AD by various pathophysiological mechanisms (see above), the reduction of the bacterial load plays an important role in the treatment of AD. Antiseptic soap-free detergents containing octenidin may be used or triclosan (1-5%), clioquinol or octrinidin may be added to the basic emollient. Topical antibiotics such as fusidic acid, retapamulin or gentamycin can be used in superinfected eczema, but their use should be restricted in order to prevent bacterial resistances. The administration of systemic antibiotics should be limited to the short-time treatment of severe impetiginisation. According to the susceptibility situation, first generation cephalosporins such as cefalexin are the antibiotics of first choice, cefuroxim and amoxicillin/clavulanate can be used if a broader spectrum is required8. More recently diluted bleach-bathes associated to intranasal application of mupirocin have been reported to improve the clinical severity in AD. 

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Basis therapy
Recent findings about the high impact of the impaired skin barrier on the pathophysiology of AD are underlining the importance of a consequent and continuous basic therapy with emollients even in periods in which the skin is not affected.

The galenic formula of the emollient should be adapted to the dryness of the skin, the localisation, the acceptance by the patient and the season. It should not contain potentially allergy provoking ingredients like perfume, lanolin or herbal extracts. The addition of moisturizing factors, which are able to bind water such as glycerol or urea leads to an increased hydration of the epidermis. While adult patient tolerated rather high concentrations (5-10%) of urea in emollients, in children and on excoriated skin, urea should not be used at concentrations above 4% because it may burn on the sensitive skin. Recently novel promising emollients containing lipids, cholesterol fatty acids and ceramides have been developed which aim to substitute the missing components of the epidermis. The basic therapy should be applied twice daily on the entire skin. Patients should use mild soap-free detergents or oil-bath and afterwards apply an emollient on the humid skin.

Topical anti-inflammatory therapy
Topical steroids and topical calcineurin inhibitors are the most important anti-inflammatory agents in the treatment of AD.

For the treatment of acute flares, class I-III topical steroids (depending on the localization and severity) are still the therapy of first choice because of their potent anti-inflammatory and anti-pruritic effects. They provoke a suppression of several transcription factors which leads to a reduced expression of pro-inflammatory cytokines, but also to an inhibition of cell growth and a reduced synthesis of proteins and collagen, which explains their side effects such as skin atrophy.

Topical steroids from the di-ester class, which have a favourable therapeutical index such as prednicarbate, hydrocortisone butyrate, methylprednisoloneacetonate, fluticasone or mometasonfuorate should be preferred. Tapering of the dose is important to avoid a rebound. The dose and strength of the topical steroid has to be adapted to the severity of the eczema, the treated localization and the age of the patient in order to avoid side effects such as skin atrophy, hypertrichosis and telangiectasiae.

Recent studies have shown that a proactive management is able to highly efficiently control the disease, even in moderate to severe forms. This regimen (twice weekly application) applied for up to 4 months with topical steroids enables to avoid further flares in more than 80% of the patients. The topical calcineurin inhibitors tacrolimus ointment and pimecrolimus cream have been shown to suppress the T-cell activation and proliferation, the release of mediators by basophils and mast cells, to have an impact on IDEC and to modulate the cytokine secretion and apoptosis. Their efficacy in treatment of AD has been proven in several clinical trials. There has been concern because of reported cases of lymphoma and viral super-infections under the therapy with topical calcineurin inhibitors, which could not be confirmed by further investigation. Recent studies comparing the proactive application of tacrolimus ointment twice weekly to a reactive therapy in adults as well as in children over a period of one year showed that the proactive therapy is effective to prevent new flares without a higher consumption of medication.

References


Inflammatory skin diseases - especially allergic diseases of dogs - are frequently diagnosed. Reviewing the scientific literature the International Task Force for Canine Atopic Dermatitis (AD) recommended a “multi-faceted approach to treat dogs with AD”. The general purposes of treatment are decrease of pruritus, decrease of the inflammatory reaction and an immunosuppression. Additionally, an improved healing of skin lesions and the treatment of secondary infections are relevant.

A combination of nonirritating baths and topical glucocorticoids was recommended for acute flares in addition to oral glucocorticoids and antimicrobial therapy when necessary. In dogs with chronic AD, the use of nonirritating shampoos is recommended. A good evidence of high efficacy is reported for topical and oral glucocorticoids, and calcineurin inhibitors such as oral ciclosporin and topical tacrolimus. The dose and frequency of administration of these drugs varies individually considering the efficacy, adverse effects and also the costs.

Since many decades, systemically or topically administered glucocorticoids are known as important drugs for the treatment of inflammatory skin diseases (especially for the treatment of allergic skin diseases) because of their antiinflammatory, immunosuppressive and antiproliferative action. Nevertheless, it has to be considered that their pharmacological effects only cause symptomatic improvement. Additionally, these agents have a long list of remarkable adverse effects. The onset of these adverse effects can be reduced by an optimised treatment schedule.

Because the spectrum of systemic adverse effects is reduced thereby, the topical treatment may be preferred. Using glucocorticoids topically their anti-inflammatory potency has to be considered. The pharmacokinetic properties (penetration via the stratum corneum and permeation via the epidermal and dermal layers) and the pharmacodynamic potency are determining factors for the drugs potency. Therefore, there exist differences between locally and systemically administered glucocorticoids.

In contrast to the ranking of potency for systemically administered “glucocorticoids”, topically administered glucocorticoids are divided into groups of drugs with very low, low, high and very high potency. It must be considered that the increasing potency is accompanied by an enhanced onset of adverse effects (especially skin thinning). Therefore, the use of agents with a lower potency is recommended. Glucocorticoids with a very high potency are only used over short periods in small treatment regions. The ranking of topically administered glucocorticoids is based on the vasoconstriction assay. The obvious difference in the ranking of systemically and topically administered glucocorticoids is explained by their pharmacokinetic properties. For example, the systemically very potent glucocorticoid dexamethasone exhibits a lower potency after local administration onto the skin because of its poor penetration and absorption through the skin. To minimise the adverse effects after topical administration, the treatment can be started with a more potent steroid. After clinical improvement, the treatment is continued with a glucocorticoid of lower potency. Additionally, the periods between administrations have to be prolonged in order to minimise the adverse effects after topical administration. The onset of these adverse effects can be reduced by an optimised treatment schedule.

Not all available pharmaceutical formulations (mainly for human use) can be used in animals because of the hair coat. Veterinary formulations of glucocorticoids are mainly solutions. An example is a hydrocortisone aceponate spray (0.0584 %) which is successfully used in atopic dogs. Reviewing all published clinical studies for the hydrocortisone aceponate spray, a “moderate-quality of evidence of the efficacy” was stated by Olivry and Bizikova. While hydrocortisone has a low potency, a moderate potency is reported for hydrocortisone aceponate. Hydrocortisone aceponate is member of the group of so-called “soft steroids” with good clinical efficacy and a limited occurrence of side effects. “Soft steroids” (budesonide, ciclesonide, loteprednol, hydrocortisone-21-aceponate) are used locally (dermal administration, inhalation, ophthalmic use). In Australia, a leave-on conditioner containing budesonide (0.025 %) was described as efficacious in atopic dermatitis.

As a modern development SEGRAs (selective glucocorticoid receptor agonists) are developed which may have selective therapeutic benefits and less side effects. Actually, no SEGRAs are registered for topical use in human beings or animals.

An improvement of the therapeutic options for atopic dermatitis was reached by the introduction of the systemically administered calcineurin inhibitor cyclosporine A for the treatment of atopic skin diseases in dogs and cats. Other calcineurin inhibitors are tacrolimus and pimecrolimus which are available for topical use. Various authors reported about a successful use of these compounds in canine atopic dermatitis. They describe tacrolimus as efficacious and safe, when it is used topically at an equivalent dose of up to 0.3 mg/kg. Lacking side effects like skin thinning or increased risk of cutaneous infections were reported as advantages of the topically administered calcineurin inhibitors.
Additionally, compounds which inhibit the phosphodiesterase 4 (PDE4 inhibitors), sphingosine-1-phosphate or its analogs as well as drugs with effects mediated via the histamine H4 receptor are possible new tools for the topical treatment of inflammatory skin diseases. But no specific compounds are registered as veterinary drugs yet.

References

Conflicts of interest
The authors declare that there are no conflicts of interest.
Journal Club (1): a review of landmark papers published in the medical or veterinary literature
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Notes
The many faces of demodicosis
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The objective is to present a variety of clinical cases to assist in the understanding of Demodicosis.

Demodex canis is present in normal dogs making it part of the normal fauna, but in small numbers. Also described in dogs are the Demodex sp. injai and cornei. Demodex spp. as part of the normal fauna should live in numbers small enough not to harm their host. Their entire life cycle is spent on their host. There have been reports of “kissing lesions” occurring in dogs living and sleeping together but this is not common. Perhaps it is a virulence factor of the mite or an immune deficiency of the host but generalized demodex in the juvenile host can be a life threatening disease due to secondary infections and deep cellulitis. When diagnosed with generalized demodicosis at 2-5 years of age it is suspected these patients could have had disease since they were young or that an immune suppressive therapy or systemic illness is contributing to the overgrowth of the mite numbers. True adult onset generalized demodicosis is less common than juvenile onset and is often associated with underlying disease such as hypothyroidism, spontaneous or iatrogenic hyperadrenocorticism, leishmaniasis, or chemotherapy for cancer or immune mediated disease. It is not known why some dogs develop demodicosis while others do not, when all dogs harbor the mites. Nonspecific immunity and humoral immunity does not appear to be the cause. Complement, neutrophil function and anti-body levels are normal. There does appear to be T cell hypo-reactivity. This decrease in cellular immunity appears to be due to an abnormal function of the T cells not the numbers of T cells.

It is important to recognize and diagnose demodex when a case presents. Not all cases have a typical history or clinical signs. Left undiagnosed, the patient suffers progressive cutaneous changes especially in the case of chronic pododermodicosis. There are many breeds reported to be predisposed such as the Pug, English Bulldog, Boston Terrier and West Highland white terrier, but any dog may be affected. The lesions in the initial stages of localized demodicosis may be little more than a thinning of the hair coat and erythema. Some cases have a silvery scale of desquamation. Depending on the host response and presence of a secondary infection the lesion may be non pruritic or severely pruritic. Some dogs may traumatize the skin and the presenting clinical signs may include a deep folliculitis and furunculosis or acute pyotraumatic lesion. Lesions may come and go for several months and then resolve on their own. The most common sites affected are the face and legs. Occasionally the ear is affected. Generalized demodex will begin similarly to the localized form of the disease but with more sites affected and typically a more rapid progression of disease with lymphadenopathy. Patients may present with varying clinical signs of folliculitis. Some will present with comedones, others with pustules, and still others with follicular casts. Other cases may present with seborrheic changes to the skin with greasy exudate, with hyperpigmentation, lichenification and alopecia. Bacterial infections are very common with generalized demodex and will affect the clinical presentation. Besides infection with Staphylococcus sp., Proteus mirabilis and Pseudomonas aeruginosa can cause severe complications. Dogs will present with severe pododermatitis with deep scarring proliferative tissue changes causing permanent damage to the anatomy to the paw. The pain, swelling and ruptured follicles with foreign body reactions to the free hair shafts and demodex mites cause many cases to be euthanized. It is not only important to identify the mites and initiate treatment, it is also important to rule out or treat underlying diseases such as leishmaniasis, hypothyroidism or hyperadrenocorticism. A second type of demodex mite describe in the dog is Demodex injai. Based on reports the terrier breeds appear predisposed to this mite, particularly the West Highland white terrier and the Wirehaired Fox terrier. The clinical signs with this parasite are different from Demodex canis with a distribution focussed on the hair follicles of the dorsal midline. The most striking and consistent clinical sign is a very greasy/oily dorsal midline and pruritus. On skin scraping these mites are very long in comparison to Demodex canis. Previous therapy with glucocorticoids and hypothyroidism have been reported as underlying primary causes of disease. Demodex cornei is the third type of demodex mite described in the dog. This is a short bodied mite also found in the superficial stratum corneum. It is also associated with sebaceous hyperplasia and greasy seborrheic changes. It often resolves with treatment of the primary skin disease and is seen frequently with a malassezia overgrowth. The three types of demodex mentioned above have been compared phylogenetically and molecular studies of amplification and sequencing yielded the conclusion, D. canis, D. injai, and Demodex sp. cornei are polymorphisms of the same species.

Feline demodicosis is caused by Demodex cati. Localized demodicosis is rarely a clinical problem and when presented is usually associated with alopecia and patchy erythema on the periocular skin or the head and neck. Generalized demodicosis is less common and usually associated with underlying systemic disease such as FeLV, diabetes mellitus,
or spontaneous or iatrogenic hyperadrenocorticism. The second type of demodex mite reported in the cat is *Demodex gatoi*. *D. gatoi* is markedly different from the other forms of demodicosis. It is a contagious, pruritic skin disease with the additionally asymptomatic carrier cats. It inhabits the stratum corneum. The clinical signs of marked pruritus, alopecia, excoriations on the head, neck and elbows can mimic notoedres, feline allergy or adverse food reactions. A report from Finland showed that unlike most demodex sp. this mite is contagious between cats. Unfortunately it also appears there are cats that are asymptomatic carriers. It is suspected there is a hypersensitivity reaction to the mites in some cats. This form of demodex is also more challenging to treat. There are multiple reports of failures to treat with lime sulphur dips, imidacloprid-moxidectin, selamectin, and ivermectin. Amitraz did appear effective at 0.0125-0.025% concentration but is not approved for use in cats. There is a third demodex mite described in the literature but it remains unnamed. It has been reported from Germany and Japan. In these cases older cats were presented with alopecia and variable pruritus on the face and neck areas. Both cats responded to ivermectin therapy. The cat from Germany had a mixed population of *Demodex cati* and the unnamed mite after receiving several depo-corticosteroid injections for flea bite hypersensitivity.

References
CONTINUING EDUCATION SESSION

Therapy of demodicosis
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Demodex is a normal haired skin commensal. In dogs, demodex is recognised currently as *Demodex canis* - inhabitant of canine pilosebaceous unit (hair follicle, sebaceous duct and gland) *Demodex injai* - inhabitant of canine pilosebaceous unit (hair follicle, sebaceous duct and gland), *Demodex cornei* - inhabitant of the stratum corneum, but these three have been reported to be the same species.

All cases should be assessed before therapy can be started. Important factors that must be considered and will affect therapy are:

- **Age of onset** - adult or juvenile onset disease (< or > 3 years of age)
- **Degree of infection** that is present - pustular or squamous demodicosis
- **Extent of the disease** - whether it is localised (< 6 patches, otitis or one single foot) or Generalised disease (>6 patches; 2 or more feet affected);

Juvenile onset demodicosis is recognised in animals less than 3 years of age. Approximately 90% of these cases will self resolve with minimal amounts of therapy providing any secondary bacterial infection is controlled, the animal is maintained on a good plane of nutrition and glucocorticoid therapy is avoided. In those cases of juvenile demodicosis that do not resolve more intensive therapy is required.

Adult onset demodicosis is caused through immunosuppressive disease and it is important to identify and treat any underlying factors to maximise the success for therapy. As in cases of juvenile onset disease.

**Localised demodicosis**
Most cases of localised disease resolve spontaneously after 6-8 weeks providing that glucocorticoids are not prescribed. Where pyoderma is identified this must be treated. No difference is seen in the healing between treated and untreated cases. If the clinician feels that therapy is needed topical therapy may be used in the form of gentle anti-parasitic treatments e.g. lime sulphur, selenium sulphide or follicular flushing agents e.g. benzoyl peroxide. Anti-parasitic therapy is rarely required but any of the miticidal therapies used for generalised demodicosis may be used if necessary. The animal’s general health status should be monitored to ensure a good plane of nutrition and an adequate worming and vaccination protocol is in place. The progression of cases may be monitored with repeat skin scrapes to ensure resolution. Deterioration of the dog’s condition indicates progression to generalised demodicosis. Where the demodex affects the ear and the ear drum can be visualised, then amitraz in mineral oil 1:9 dilution daily as an off/extra-label use may be administered if necessary with care. Localised pododemodicosis can be very difficult to treat. Any of the therapies for generalised disease can be used. In addition Amitraz foot soaks may be useful using 0.125% solution every 1 - 3 days (keep feet dry between treatments).

**Generalised demodicosis**
Generalised forms of the disease can be difficult to treat and good owner compliance is essential. The dogs’ general health and management should be checked and intact bitches should be neutered as oestrous or pregnancy will result in relapse of the disease. In adult onset disease it is essential to identify and treat the underlying condition where possible. Any form of glucocorticoid is contraindicated. When secondary pyoderma is present antibiotic therapy is typically used for a minimum of 4 weeks for superficial infection and up to 12 weeks for deep infection and for at least 10 days after clinical resolution. Not all dogs need antiparasitic therapy. 30 - 50% of cases of generalised disease in dogs less than 1 year of age will resolve spontaneously.

Antiparasitic therapy may be given orally or applied topically. Treatment should be continued until deep skin scrapes are negative and then ideally for an additional 30 to 60 days. Parasitological cure is assessed to be when skin scrapes from dogs contain no live or dead mites at any stage of development. Ideally 4 - 6 sites should be sampled and the same areas should be chosen at each visit for comparative purposes. No dog should be considered “cured” until 12 months after treatment has stopped.
Amitraz is licensed in the USA and in many areas of Europe as a treatment for demodicosis. It is used in the UK as a 5% solution. In order to maximise the success rate the dog should be clipped down to short stubble all over, and the haircoat should be kept short throughout treatment (sedation may be necessary for clipping). The dog should be bathed in an antiseborrhoeic shampoo to remove crust and scale if present e.g. benzoyl peroxide or sulphur/salicylic acid containing products. The amitraz should be sponged onto the skin at a dilution of 1:100 (0.05%) in water to the whole body. Applicators should be warned to wear gloves, protective clothing and work in a well ventilated area. The solution should be allowed to dry on the dog. Treatment should be repeated every seven days until parasitological cure can be achieved. Adverse effects in dogs include sedation 12-24 hours duration, pruritus, allergic reactions (rare), weakness, ataxia (very rare). Cure rates are reported to vary between zero to 90% (Miller 2012). More recently it has been produced in a spot on formulation combined with metaflumizone (Promeris, Fort Dodge), registered for both demodex and tick treatment. Clinical work has shown it to be effective when applied topical to treat demodex. Work by Fournie et al showed when applied topically every two weeks it achieved a 99.6% reduction in mite numbers in 84 days and 62.5% of dogs were mite free at the end of the period. When applied monthly the data is equally impressive showing mite numbers were reduced by 98.6% and 42.9% of dogs were mite free in the same time period. There can be no doubt that topical application works well although long term studies are needed to assess the continued progress of animals. Unfortunately though, this product has also been identified as causing drug eruptions. Work by Oberkirchner et al reported in 2011 suggested this combination produces a contact induced pemphigus foliaceus like disease, which in some of the dogs required long term immunosuppressive therapy. It is no longer available as a topical product in the USA.

Avermectins and milbemycins have also been employed to treat demodex in dogs. Use of many of these drugs constitutes an off-label use to treat demodociosis and therefore in these circumstances their use should be justified using the veterinary cascade relevant for the prescribing veterinarian. Certain dogs have been identified with a multi drug resistance (MDR1) / ABCB1-1Delta mutation which leads to an inability to pump a number of drugs away from the brain after they penetrate the blood brain barrier. Avermectins are among these drugs. Particularly susceptible breeds include the Australian Shepherd, Border Collie, GSD, Long haired Whippet, OES, Rough Collie, Sheltie and Smooth Collie. A test is available to check for the presence of this genetic defect. Where this test is not performed as a screening test then drugs within this group can only safely be used under their licence application i.e. selamectin (Stronghold, Pfizer), milbemycin (Milbemax, Novartis) and moxidectin (Advocate, Bayer). Drugs to be avoided systemically in susceptible dogs include ivermectin, doramectin and moxidectin. This is unfortunately not the only mechanism for toxicity, a “clear test result” does not guarantee safety. (Bissonette 2009)

A range of studies have described the use of ivermectin, selamectin, milbemycin and moxidectin given orally off licence to treat demodex in non-susceptible breeds. With the exception of selamectin (Schnabl, 2010) these drugs have been shown to be beneficial in treating demodex.

Ivermectin can be administered orally at a dose of 0.2 - 0.6 mg/kg po sid until 30 -60 days beyond microscopic and clinical cure (Ristic 1995, Medleau 1996, Fondati 1996, Guaguere 1998, Muller 1999, Muller 2004). Treatment courses range from 35 - 210 days. Dose should be started at 0.1 mg/kg daily and increased daily by 0.1mg/kg up to 0.6 mg/kg to minimise the risk of side effects. It is contraindicated in collie’s and related breeds. Clinical cure rates varies from 80 - 100%. Adverse effects include incoordination, weakness, diluted pupils, blindness, ataxia and in rare cases collapse and coma and death.

Milbemycin has also been given orally at a dose of between 0.5 to 2.0mg/kg daily with good success rates. It is not readily available in the UK and many other parts of Europe as a single product. Treatment courses range from 60 - 300 days and should be given until 30 - 60 days beyond parasitological cure. Clinical cure rates range from 15- 92% depending on the dose used and the type of demodicosis treated. Dogs with juvenile onset disease responded more favourably than those with adult onset disease (Miller 1995, Holm 2003, Guaguere 2002, Muller 2004). It is available as an endoparasitic treatment, where milbemycin is combined with praziquantel as Milbemax® (Novartis) or else it may imported into the UK and other non-licensed countries from licensed European countries (e.g.Italy) under special licence as milbemycin as Interceptor® (Novartis). Adverse effects are uncommon but if they occur are similar to those for described for ivermectin.

Moxidectin has been used off label as the large animal product (Cydectin®, Pfizer) given orally at a dose of 0.2 - 0.4 mg/kg daily with similar clinically beneficial effects to ivermectin and milbemycin (Burrows 1997, Bensignor 1998, Wagner 2000). Adverse effects similar to those for ivermectin are reported to be more common than for ivermectin. Recent changes to the label for the moxidectin/imidocloprid spot on Advocate (Bayer) has improved the effectiveness for topical therapy. A recent study (which has led to a change in the label claim for Advocate) using the spot on showed that it was more effective when applied weekly compared to monthly with cure rates approaching oral ivermectin (Paterson 2009). A second study confirmed it to be very safe when given at this frequency, making it a useful licenced alternative as an initial
therapy. This topical form of miticidal therapy has been demonstrated to be less effective with more severe clinical signs and a high number of mites on skin scrapings. (Mueller, R 2009?)

Doramectin has been used both orally (Murayama 2002) or by subcutaneous injection (Johnstone 2002). In both cases a dose of 0.6 mg/kg was used once weekly until 30 days beyond clinical cure (course may be prolonged). When used by subcutaneous administration all 23 dogs treated achieved negative scrapes at between 5 - 20 weeks of therapy. When used orally only 72% of 29 dogs achieved negative scrapes. Adverse effects are uncommon but similar to ivermectin.

Immunostimulants have been reported to have a variable benefit. Drugs that have been investigated include vitamin E, levamisole, thiabendazole, Propionibacterium acnes and muramyldipeptide-parapoxvirus combinations (Mojzisova 1997, Miller 2012)

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The many faces of Malassezia dermatitis
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The objective is to present a variety of clinical cases to assist in the understanding of malassezia dermatitis (MD) and how underlying primary epidermal structure changes or disease may trigger malassezia dermatitis.

Malassezia pachydermatis is a commensal microflora. When performing cytologic evaluations it is important to understand normal levels of Malassezia spp. and the distribution of malassezia on the host. Malassezia spp. may play a pathogenic role as a secondary factor in many primary causes of dermatitis. Certain skin diseases and the therapy associated with treating these diseases may yield M. pachydermatis numbers 10,000 times higher than populations on healthy dogs. The author finds it amazing that only certain types of skin disease tend to yield malassezia overgrowth. For example, why is primary idiopathic seborrhea often associated with malassezia dermatitis yet the seborrheic changes that occur with canine tail gland hyperplasia are not? Another example linked with malassezia overgrowth is thymoma associated feline generalized exfoliative dermatitis. Histologically this disease shares similarities to discoid lupus erythematosus (DLE), yet rarely is malassezia found associated with DLE despite the disease being associated with mucocutaneous junctions which malassezia tend to favor. Colonization versus yeast infection may have to do with the host’s skin structure, nutrition, hormonal balance, environmental temperatures/humidity, hygiene, and/or immune response to the yeast or to other factors. The changes involved between the host and yeast organisms appear complex and are not fully understood. Primary diseases of the skin may lead to hyperhydrosis, increased sebum production and with time, may lead to lichenification and intertrigo. The secretion of hydrolyses, lipases, phospholipases, proteases, and sphingomyelinases are associated with malassezia overgrowth. These changes provide an environment which promotes malassezia overgrowth and adherence to the stratum corneum. M. pachydermatis has been shown to be allergenic in canine atopic dermatitis (CAD).

It is important to recognize the differences in clinical presentations. MD may occur in many different breeds yet certain breeds are reported to be predisposed: the Basset hound, American Cocker spaniel, and West Highland white terrier. In the initial stages of MD there is little more than erythema. The erythema tends to be more diffuse and less macular. Papules are not present without other concurrent factors. Pruritus is a consistent symptom. Once present the skin tends to respond in one of two ways; with a generalized exfoliative scale/dander or with greasy, waxy exudate. The exudate may form heavy waxy casts on the proximal hair shafts. The exudate may be a dark brown to black color, often seen with the Labrador Retriever, or yellow in color, seen more with the American Cocker Spaniel. With chronicity, the skin becomes more diffusely hyperpigmented as compared to the reticulated hyperpigmentation seen with CAD. The color is also gray vs. the black hyperpigmentation of Alopacia X or cyclic flank alopecia. Lichenification with an irregular cobblestone texture develops and may progress to deep skin folds. Partial to diffuse alopecia is often present. The patients often have a strong odor described as rancid. Concurrent staphylococcal pyoderma is reported in approximately 40% of dogs diagnosed with MD and this will affect the clinical signs presented to the clinician. The distribution of lesions typically is similar to the normal colonization areas of malassezia; muzzle, ears, perineal, and clawfolds (paronychia). The ventral neck area is rarely associated with primary skin disease except when secondary MD is present. When the MD is secondary to other primary diseases the distribution may follow the distribution of that disease. Terriers and American Cocker Spaniels can be very difficult to manage as they may have several predisposing factors to MD such as CAD, keratinization disorders, and hormonal imbalances.

Malassezia dermatitis in cats appears to be much less common. The characteristic clinical sign in cats is a very dark brown to black debris. Cats may also develop malassezia otitis. This may create proliferative swelling at the oseal of the canal making topical therapy by the owner more difficult. There tends to be a marked production of black waxy debris which requires frequent cleaning until well controlled. This type of debris can also be seen with feline acne, paronychia and with Idiopathic Facial Dermatitis of the Persian cat. In these cases the malassezia is secondary as treatment with topical and or systemic therapy alone does not yield success. There is also a nice report out of Italy describing MD in allergic cats. It was a retrospective report looking at 16 cats diagnosed with atopic dermatitis, one with adverse food reaction and one cat whose underlying disease was unknown. The report states the cat’s clinical signs improved with anti-fungal therapy. These 18 cats were not compared to the number of cats diagnosed with feline atopic dermatitis with or without MD over the same time period so the incidence cannot be appreciated. There was also a control group in which 7 of the 18 cats sampled showed the presence of Malassezia spp. Malassezia dermatitis has also been seen in 2 forms of feline paraneoplastic skin disease seen in older cats, in which acquired alopecia is the initial presenting sign. The first presents with an acute and rapidly progressive alopecia of the ventrum and legs, usually associated with weight loss and decreased appetite. The face and ears tend to be spared until later in the disease. The hair is easily epilated and the epithelium has a smooth and shiny appearance. The paw pads may be involved and appear shiny and smooth, or they may be crusted and fissured. The cats may be pruritic. If cytology is performed malassezia is frequently found. Treatment of the malassezia will help with the pruritus but the disease will progress. Most cats with this presentation have had pancreatic carcinoma or bile duct adenocarcinoma. The prognosis
is grave as the tumor may metastasize, but if caught early surgical removal of the tumor results in regrowth of hair. The second paraneoplastic dermatitis associated with MD in cats is an exfoliative dermatitis of the head and pinnae that is often non-pruritic. As the disease progresses it involves most of the body with alopecia, erythema and pruritus. Waxy deposits may accumulate on the claws, lips, ear canals and periocular skin and malassezia may be identified. Thoracic radiographs may reveal a mediastinal mass. Biopsy or mass removal and histopathology reveal thymoma. The disease resolves with removal of the mass. This has also been reported in a rabbit but no malassezia were found.

In horses *Malassezia spp.* has been reported to cause tail head pruritus responsive to topical anti-yeast therapy. One group evaluated *Malassezia spp.* carriage in the intermammary region of healthy mares and the preputial fossa of healthy geldings. *Malassezia spp.* were identified by cytologic examination in 40 of 44 samples. The conclusion was malassezia found on cytologic examination in the horse may not determine its role as a pathogen.

**Notes**
**Antifungal therapy**

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Fungal infections in companion animals can be superficial, subcutaneous or systemic. Subcutaneous and systemic fungal infections are rare causes of skin disease whereas superficial diseases are recognised commonly. Superficial mycoses are fungal infections that involve superficial layers of the skin and claws. The organisms involved include dermatophytes (Microsporum, Trichophyton), Malassezia, Candida and Trichosporon (Piedra). The first two represent the superficial mycoses of greatest significance in companion animal health. There are only occasional reports of other superficial mycoses. This review of antifungal therapy will focus on the therapy of dermatophyte infection and Malassezia.

**Dermatophytosis**

The most commonly isolated pathogens to cause dermatophytosis in dogs and cats are Microsporum canis, M. gypseum and Trichophyton mentagrophytes. Any age, sex or breed of animal is susceptible to dermatophytosis but young, sick debilitated and elderly animals appear to be more prone to developing infection. Where possible therefore, when therapy is to be undertaken, the animal’s immune status should be assessed and where immunosuppressive factors are present they should be treated. Therapy can be considered as topical or systemic as well as through vaccination.

**Topical therapy**

Topical therapy should be used if possible in all cases of dermatophytosis. Topical therapy not only speeds the resolution of the infection but reduces the risk of spread to human contacts. Penetration of the active ingredient is best achieved by clipping the animal. Although clipping may spread the lesion it has the advantage of removing infected hairs (DeBoer 1995(1), Moriello 1996). Clipped hair should be removed and disposed of. Topical therapy for dermatophytosis has been evaluated both in vitro using isolated infected hairs and in a range of different in vivo studies (Moriello 1995, White-Weithers 1995, DeJaham 1998, Moriello 1998, Paterson 1999, Mason 2000, Guillot 2002, Hnilica 2002, Perin 2003). Current recommendations for treatment are to use a combination of topical and systemic therapy (Moriello 2004, Miller 2013). In vitro studies from different investigators (Rycroft 1991, White-Weithers 1995, Moriello 1995, Moriello 1998) have considered a range of topical products including Lime sulphur, enilconazole, miconazole, miconazole/chlorhexidine, chlorhexidine alone, captan and povidone iodine. Although in vitro techniques have disadvantages; such as their inability to quantify and standardise the amount of infective material that is being tested and the effect of soaking hairs which can lead to maceration and the release of spores to re-infect a sample; they can give useful information about the efficacy of different products. Using such techniques lime sulphur (1:16), enilconazole, miconazole with and without chlorhexidine have been shown to have good activity against M. canis. Captan, chlorhexidine and povidone iodine have been shown to be ineffective when tested in this way (Rycroft 1991, White-Weithers 1995, Moriello 1995, Moriello 1998). In vivo studies have also considered a range of topical drugs. 2% Chlorhexidine (DeBoer 1995(2)) was found to be ineffective when used as a sole form of therapy when cats were dipped twice weekly for 150 days after their coats had been clipped. Enilconazole has been used at a concentration of 0.2% in two studies (DeJaham 1998, Hnilica 2002) to treat Persian cats as a sole form of therapy and has been shown to be effective in both. Possible adverse effects associated with enilconazole therapy include hypersalivation, weight loss and inappetance. The most effective topical drugs appear to be lime sulphur, enilconazole and miconazole. These appear to be best employed when used twice weekly as a whole body application. Lime sulphur and enilconazole are used as leave on dips, a combined miconazole and chlorhexidine shampoo is generally recommended to have a 10 minute contact time. Creams and lotions may be used for focal lesions. They should be applied every 12 hours and include a 6cm margin around the outside of the lesion. Cream containing miconazole or terbinafine may be useful. Multifocal or generalised skin disease is best treated with rinses and leave on dips used twice weekly. 2% lime sulphur (1:16), a combined 2% miconazole with 2% chlorhexidine shampoo and 0.2% enilconazole are the most effective products. Shampoos are less effective because they macerate hairs which can lead to a release of spores and increase the risk of contagion.

**Systemic therapy**

Griseofulvin, ketoconazole, itraconazole, terbinafine and lufenuron have been used to treat dermatophytosis in domestic animals. Griseofulvin which for many years was the mainstay of dermatophyte therapy has largely been superseded by newer drugs such as itraconazole and terbinafine. Ketoconazole is an imidazole whose antifungal activity is due to its ability to impair ergosterol synthesis in fungal cell walls. It is an effective therapy for dermatophytosis in both dogs and cats. It is normally given at a dose of 10mg/kg every 24 hours. It is poorly tolerated in cats as it frequently causes inappetance, weight loss and hepatotoxicity. Itraconazole is a synthetic triazole whose mode of action is to alter fungal cell membrane permeability through inhibition of ergosterol synthesis (Debruyne 2001). At a low dose it is fungistatic
and at high doses it is fungicidal. When used as a sole form of therapy it has been shown to be partially effective at the dose range of 1.5 - 3.0mg/kg (Mancianti 1998) but very effective at a dose of 10mg/kg (Moriello 1995, Colombo 2001). The most commonly effective dose for itraconazole is 5-10mg/kg once daily for 28 days then for alternate weeks (Moriello 2004). Adverse effects are less common than with ketoconazole although vomiting, diarrhoea and inappetance can be seen. Terbinafine is an allylamine antifungal drug that suppresses the biosynthesis of ergosterol by inhibition of the fungal enzyme squalene epoxidase. It is a fungicidal drug for dermatophytes. Several studies have described the use of terbinafine to treat dermatophytosis in dogs and cats. Dose ranges varying from 5 - 40mg/kg daily have been reported in a range of clinical trials (Mancianti 1999, Chen 2000, Castanon-Olivares 2001, Kotnik 2001, Kotnik 2002). Although all of the dose rates appear to have some benefit the higher end of the dose range appears to be most effective. Current recommendations suggest it should be used at a dose rate of 20-30mg/kg every 12-24 hours for dogs and 20-40mg/kg every 24-48 hours for cats (Miller 2013). All of the studies report the terbinafine to be well tolerated with vomiting and anorexia being the most commonly recorded adverse effect. Lufenuron is a benzoylphenylurea drug whose principal use is as a flea treatment. Its mode of action is to disrupt chitin synthesis. As fungal cell walls contain chitin it was hoped that it may have activity against fungi. However although initial studies suggested it may be a useful therapy (Ben-Ziony 2000) subsequent work including placebo controlled studies showed that, although lufenuron may slow down the rate at which infection becomes established (DeBoer 2003) it does not prevent or treat dermatophytosis (Moriello 2002(2)). It can not therefore be recommended as a therapy for dermatophytosis in either dogs or cats.

Recommendations for systemic therapy
Systemic therapy should be considered for animals with multifocal lesions, long haired animals and those in multiple animal environments. Animals who have failed to show a response to topical therapy within 2 - 4 weeks should also have systemic therapy. Drug to be considered should be: Dogs - ketoconazole, itraconazole or terbinafine. Cats - itraconazole or terbinafine. Different recommendations have been made for the end point of therapy. Currently it is suggested therapy should continue until 3 successive fungal cultures performed at weekly intervals are negative.

Vaccination
Several studies have investigated the use of fungal vaccines in cats to try and prevent dermatophytosis (DeBoer 1994, DeBoer 1995, DeBoer 2002). In the first two of these studies a killed cell wall vaccine was evaluated but not shown to be protective against challenge exposure. Similar results were seen in the third study when a combined live-inactivated dermatophyte vaccine and a killed commercially available dermatophyte vaccine were also found to be ineffective. The only benefit the investigators suggested could be gained from these vaccines was a slight reduction in the severity of the infection.

Environmental control
*M. canis* can remain viable in the environment for up to 18 months, therefore environmental therapy is important. The following procedures should be performed. Minimise cross infection by people who should wear protective clothing when entering a contaminated area. Thoroughly clean all non porous surfaces by vaccum cleaning and disinfection. Effective disinfectants include undiluted bleach (5.25% sodium hypochlorite) and 1% formalin (Moriello 1998). However due to their corrosive nature neither product is completely suitable as an environmental product. Enilconazole works more slowly but is effective in 8 hours and is a better choice. It is licensed in some European countries for poultry and can be used as a spray or a fogger. Destroy all bedding, scratch poles, rugs etc if they can not be effectively sterilised. Cages may be cleaned with a 1:10 solution of bleach.

References
6. DeBoer DJ, Moriello KA. Clinical update on feline dermatophytosis Part II. Compend Cont Educ, 1995 (1) 17:1471
7. DeBoer, DJ, Moriello, K.A. Inability of topical treatment to influence the course of experimental feline dermatophytosis. Journal of the American Veterinary Medical Association 1993; 207: 52-7(2)


Malassezia

A similar range of topical and systemic medication that have been described for therapy in dermatophytosis can also be used to treat Malassezia infection in dogs and cats.

Topical therapy

Topical drugs that can be used to treat Malassezia include shampoo formulations of miconazole with and without chlorhexidine, chlorhexidine alone, selenium sulphide, ketoconazole, tar and boric acid. Leave on solutions that have been shown to be useful contained enilconazole or lime sulphur. An evidence based review of therapy for Malassezia (Negre 2009) suggested that at the time of the review there was only one topical treatment with good evidence of control which was a 2% miconazole and 2% chlorhexidine shampoo (Malaseb®). A study comparing a 2% miconazole and 2% chlorhexidine shampoo (MCS) with a selenium sulphide shampoo (Bond 1999) showed the MCS to be more effective than selenium sulphide. A more recent randomised controlled trial comparing MCS with a 3% chlorhexidine shampoo (Maynard 2013) has shown no significant difference between the two groups. A double blind placebo controlled study to investigate a 1 and 2% miconazole conditioner (Marsella 2000) showed a reduction in yeast numbers compared to the water control but no difference in clinical scores between the three groups.
Current recommendations for topical therapy
2% miconazole /2% chlorhexidine shampoo twice weekly or 3% chlorhexidine shampoo twice weekly

Systemic therapy
Systemic drugs that can be used are ketoconazole, itraconazole and terbinafine. In the same evidence based review in 2009 Negre et al suggested there was fair evidence for the use of two systemic therapies with azole derivatives. These were ketoconazole 10mg/kg daily and itraconazole 5mg/kg daily. In a study in 2002 Pinchbeck compared pulse administration versus once daily administration of itraconazole for the therapy of Malassezia for both dermatitis and otitis. Both regimes were successful for Malassezia dermatitis but not for otitis. Once daily therapy with itraconazole at a dose of 5mg/kg once daily for 3 weeks was as efficacious as twice weekly therapy at the same dose. Different studies have compared oral ketoconazole with terbinafine (Guillot 2003) and the same two drugs combined with cephalaxin (Rosales 2005). In the first study ketoconazole at a dose of 10mg/kg sid was found to be as effective as terbinafine at 30mg/kg sid given daily for 3 weeks. Similarly in the second study ketoconazole at a dose of 5-10mg/kg twice daily was found to be as effective as terbinafine at 30mg/kg sid. A more recent study (Berger 2012) compared once daily versus twice weekly terbinafine at a dose of 30mg/kg sid for Malassezia dermatitis. No significant difference was seen between the two groups.

Current recommendations for therapy with systemic drugs
Ketoconazole 5-10mg/kg once daily po 3 weeks, Itraconazole 5-10mg/kg once daily po 3 weeks or 5mg/kg once daily twice weekly for 3 weeks, Terbinafine 30mg/kg once daily po 3 weeks or 30mg/kg once daily twice weekly

References
The impact of antibiotic therapy on antimicrobial resistance mechanism
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“There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring ‘fastness’ [resistance].”-Alexander Fleming, 1946.

Introduction
Even before antibiotics were used therapeutically, microorganisms possessed antimicrobial resistance (AMR) mechanisms to combat natural antimicrobials. The use of antibiotics will select for pre-existing populations of resistant bacteria (pathogenic, commensal and environmental) and increased use has led to the emergence and spread of multidrug resistance (MDR). In people, antibiotic therapy has been linked to increased infections with fungi, C. difficile, staphylococci and coliforms and a number of veterinary studies report antimicrobial therapy as a risk factor for carriage or infection with AMR bacteria (See Table 1: Antibiotics as risk factors for AMR).

Competition - fitness costs
In bacterial populations, antibiotic pressure may kill susceptible cells and allow cells with AMR to that particular antibiotic to survive and proliferate and transfer AMR to daughter cells. The new AMR population may colonise and transfer their resistant determinants to other bacteria of the same or different genera or species. AMR may be shed into the environment or food chain or transferred to in-contact individuals (See Figure 1: Environmental flow of AMR- E. coli). AMR may not be naturally eliminated or reversed so different traits may accumulate over time resulting in MDR strains. AMR genes may exert a fitness cost to the host so AMR isolates can be out-competed by antimicrobial susceptible isolates in the absence of a selection pressure. However, genetic adaption by natural selection over time may overcome fitness costs so that the AMR strains may thrive.

Molecular basis of AMR
Intrinsic AMR
Is an inherent tolerance of an entire bacterial species to ≥1 antimicrobial e.g. resistance of \( \textit{Pseudomonas aeruginosa} \) to tetracycline or sulphonamides.

Acquired AMR
Acquired resistance arises from gene mutation (altered physiological processes and/or cellular structures), or via horizontal gene transfer of mobile genetic elements (MGEs) by transformation, transduction or conjugation (See Table 2: Mechanisms of horizontal gene transfer between bacteria). MGEs are discrete pieces of DNA that encode factors allowing them to mobilise within or between genomes. They frequently transfer and/or exchange, carrying AMR and/or virulence genes, and are responsible for AMR dissemination between bacteria (See Table 3: Examples of MGEs).

AMR mechanisms include:
1. Prevent entry: Antimicrobials enter the cell via porin channels. Some gram-negative bacteria can modify the channel frequency, size and selectivity to reduce the uptake of certain antibiotics e.g. \( \textit{Pseudomonas} \) - carbapenems
2. Efflux pumps: To prevent the intracellular accumulation of antimicrobials, bacteria have efflux pumps (membrane proteins) to export the drug. Some pumps are specific for certain antibiotics e.g. \( \textit{E. coli} \) - tetracycline, while others are multiple drug resistance pumps and can remove a variety of structurally diverse antimicrobials including antiseptics e.g. \( \textit{S. aureus} \) - fluoroquinolone, quaternary ammonium compounds (QACs) and dyes.
3. Enzymatic drug inactivation by degradation or chemical modification
Staphylococcus spp.
\( \beta \)-lactam antibiotics bind to penicillin binding protein (PBP) to prevent synthesis of the bacterial peptidoglycan cell wall. To prevent this some staphylococci produce \( \beta \)-lactamase enzyme, encoded by the \( \textit{blaZ} \) gene, which cleaves the \( \beta \)-lactam ring of the antibiotic.

Gram-negative bacteria
Extended spectrum \( \beta \)-lactamase genes have emerged among gram-negative bacteria and include SHV, TEM, CTX-M, OXA, carbapenem-hydrolysing and AmpC-type. These genes encode for broad \( \beta \)-lactam resistance, including third and fourth generation cephalosporins, and in the case of AmpC and OXA-type, clavulanic acid. The genes may be chromosomal or carried on plasmids, transposons or integrons with multiple AMR genes inferring MDR
4. Modification of the target site:
Meticillin resistance
Target modification used by \( \textit{Staphylococcus spp} \) for resistance against \( \beta \)-lactamase resistant \( \beta \)-lactam antibiotics i.e. meticillin, flucoxacinil, carbepenams and cephalosporins. The staphylococcal antibiotic target site PBP is altered to PBP2a by a mutation encoded by the resistance gene \( \textit{meic} \)A resulting in reduced affinity.
5. Chromosomal mutation

Fluoroquinolones

Mutations in DNA gyrase and topoisomerase IV genes result in resistance to fluoroquinolones. High-level resistance usually has multiple mutations as well as other mechanisms such as efflux pumps.

The impact of antibiotic therapy on antimicrobial resistance mechanism encompasses the selection, proliferation, colonisation and dissemination of AMR and MDR isolates and the potential for them to become stable and prosper even in the absence of antibiotic pressure. Treatment of bacterial infections is compromised by the emergence of MDR bacteria.

Meticillin-resistant Staphylococcus pseudointermedius (MRSP) as an example:

AMR mechanism

β-lactam resistance in staphylococci is conferred by carriage of the mecA gene resulting in a modification of the antibiotic target site (see above). The mecA gene is carried on a large MGE the SCCmec cassette. Differences in SCCmec are defined by the combination of resistance (mecA) and recombinase (ccr) genes. Eleven types (I-XI) and several subtypes are currently recognised in MRSA. Transfer of this element may occur between staphylococci and the integration of SCCmec into the host genome is usually stable. The smaller classes, e.g. IV, may be more easily transferred between strains and have less fitness cost. Since 2006 antimicrobial MRSP isolates have emerged with the spread of two major clones: MLST ST71-spa t02-SCCmec II-III in Europe and MLST ST68-spa t06-SCCmec V in North America. Multiple different SCCmec have been acquired by different MRSP lineages (SCCmec II-III, III, IV, V, VII). SCCmec II-III is a combination of SCCmec II from MR-Staphylococcus epidermidis and SCCmec III from MRSA, highlighting potential spread of SCCmec elements between co-inhabiting staphylococcal species.

Unlike MRSA, MRSP is usually MDR including β-lactams, aminoglycosides, macrolides, lincosamides, tetracyclines, trimethoprim, chloramphenicol and fluoroquinolones. MDR has been linked to acquisition of numerous AMR genes on SCCmec and in some MRSP isolates, mutation of DNA gyrase and topoisomerase IV genes, that confer fluoroquinolone resistance, can be carried on SCCmec II-III and VII cassettes in conjunction with mecA gene. Consequently the use of either antibiotic may enhance selection of AMR to both. Some staphylococcal strains that appear susceptible to clindamycin on routine antimicrobial susceptibility testing develop resistance during treatment. This inducible resistance trait is present in a small number of MRSP isolates and is due to the carriage of erm gene that encodes for an altered target site for macrolides and lincosamides (methylation of 23s rRNA). Antibiotic susceptibility testing by disc diffusion with erythromycin and clindamycin (D-test) or PCR is required for detection.

Virulence factors and antimicrobial resistance

Staphylococcus pseudointermedius (SP), like S. aureus (SA), carry virulence factors that are involved in colonisation, invasion and dissemination. Strains of SP have also been reported to form biofilms, which protect the bacteria from the host’s immune system and antibiotic treatment. SA isolates in biofilms have increased mutability, which potentially accelerates the emergence of heritable AMR, and have increased ability to acquire/disseminate AMR determinants by horizontal gene transfer.

MRSP Carriage - a reservoir of resistance?

The increasing number of dogs carrying MRSP may represent a risk for in-contact animals and people. The majority of studies report a low prevalence of MRSP carriage in healthy dogs (0.4-5%) and healthy cats (1.2-4%)10. However, carriage appears to be higher among veterinary staff, owner’s of infected pets and dogs with concurrent skin disease20.11. Longitudinal studies have reported long-term carriage of MRSP in dogs following infection12,13, which was extended by three or more weeks of β-lactam treatment14.

Sharing - dissemination

The transfer of indistinguishable staphylococcal strains between humans and pets and vice versa has been reported in numerous studies. While zoonotic transmission and infection has been reported, isolation of MRSP from people tends to be transient carriage or contamination13 apart from one study where two veterinarians were MRSP-positive on two occasions, one month apart14. On the other hand, transfer between veterinary patients and in-contact household animals is much more common12,14.

Environment as a source of dissemination:

MRSA have been shown to survive 6 months+ in hospital dust (in-vitro). Similar studies have not been performed for MRSP isolates but positive environmental samples have been reported even after cleaning12,14. It is not clear if this represents re-contamination or survival, never the less, infected environments may act as sources of AMR.
Summary

The mucosa and faeces of animals and people and the environment provide a reservoir of AMR genes that could rapidly spread within bacterial populations under the selection pressure exerted by antimicrobial therapy. Clinical guidelines on responsible antibiotic use are therefore required.

References


Conflicts of Interest

PhD funding from Zoetis Animal Health.
Table 1: Risk factors for carriage/infection of AMR staphylococci or E. coli

<table>
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<th>Bacteria</th>
<th>Population base</th>
<th>Risk factors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolonged carriage of MRSP (Sweden)</td>
<td>Dogs with wounds or skin infections</td>
<td>β-lactam therapy for ≥ 3 weeks</td>
<td>13</td>
</tr>
<tr>
<td>Infections with MRSA (UK)</td>
<td>Dog and cat clinical isolates submitted to nationwide laboratory</td>
<td>&gt;3 antimicrobial courses, &gt;1 day admitted to veterinary clinics or surgical implants</td>
<td>17</td>
</tr>
<tr>
<td>MRSA infections (USA and Canada)</td>
<td>Clinical isolates from dogs presenting to referral hospital</td>
<td>Receipt of antimicrobial drugs (β-lactams or fluoroquinolones) or IV catheterisation</td>
<td>18</td>
</tr>
<tr>
<td>MRS carriage and infection (Spain)</td>
<td>Presentation of healthy, first time pyoderma and chronic pyoderma dogs to University clinic</td>
<td>AMR to β-lactam or fluoroquinolone antibiotics or MRS if chronic pyoderma</td>
<td>19</td>
</tr>
<tr>
<td>MRSP carriage (Germany)</td>
<td>Presentation of dogs to University clinic</td>
<td>Hospitalisation and antibiotic treatment within the last six months</td>
<td>20</td>
</tr>
<tr>
<td>Faecal carriage of MDR and ESBL-E. coli (Chile)</td>
<td>In-patients treated with fluoroquinolones compared to healthy controls</td>
<td>Fluoroquinolone treatment</td>
<td>21</td>
</tr>
<tr>
<td>Rectal carriage MDR E. coli during hospitalisation (Australia)</td>
<td>Retrospective case-control study of dogs admitted to hospital; primary care or referral</td>
<td>Hospitalisation &gt; 6 days, treatment with cephalosporins prior to admission, and/or treatment for &gt;1 day during hospitalisation</td>
<td>22</td>
</tr>
</tbody>
</table>

Diagram 1: Potential flow of AMR E. coli in the environment

Table 2: Mechanisms of horizontal transfer between bacteria

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformation</td>
<td>Uptake of free DNA or plasmids into the chromosome</td>
<td>Not staphylococci</td>
</tr>
<tr>
<td>Conjugation</td>
<td>Synthesis of pillae/pores to allow passage of plasmids or transposons between cells</td>
<td>E. coli &gt;&gt; staphs</td>
</tr>
<tr>
<td>Transduction</td>
<td>DNA (chromosomal or MGE) is packaged into a bacteriophage head and injected into the recipient cell</td>
<td>Usually staphs</td>
</tr>
</tbody>
</table>

Table 3: Examples of MGEs

<table>
<thead>
<tr>
<th>MGE</th>
<th>Description</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmids</td>
<td>Circular pieces of dsDNA that can multiply independently of chromosomal DNA</td>
<td>Encode for ≥1 AMR genes &amp; MDR; can be multiple</td>
</tr>
<tr>
<td>Transposons</td>
<td>Small pieces of DNA that can change their position in the genome “jumping genes”</td>
<td>Integrate into other DNA (chromosomal or other MGE) and encode AMR</td>
</tr>
<tr>
<td>SCCs (staphylococcal cassette chromosome)</td>
<td>Large pieces of DNA; relatively stable and move infrequently compared to other MGE</td>
<td>Usually carry mecA gene (SCCmec); can also carry other AMR genes</td>
</tr>
<tr>
<td>Integrons</td>
<td>Integrate into other DNA like transposons</td>
<td>Carry genes encoding recombination enzymes +/-AMR</td>
</tr>
<tr>
<td>Bacteriophages</td>
<td>Viruses that infect and can replicate in bacteria; common in S. aureus</td>
<td>Encode for toxins, virulence factors; highly species-specific and lineage-specific</td>
</tr>
</tbody>
</table>
Practice guidelines for antibiotic therapy
Vanessa Schmidt
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Introduction
We are in no doubt that antimicrobial resistance (AMR) to commonly used antibiotics is increasing at an alarming rate making it difficult or even impossible in some cases to adequately treat or prevent bacterial infections in humans and other animals. We cannot rely on the release of new antibiotics to combat this crisis, as development is challenging, costly and slow. The perpetuation of AMR is inevitable and infections will always occur. Therefore we need good antimicrobial stewardship, as well as hygiene and infection control strategies to help increase the life of our existing antimicrobials. Many human hospitals have successfully implemented antimicrobial stewardship guidelines. Some of these recommendations are applicable to veterinary health care. In addition, specific guidelines have been proposed for the veterinary profession by a number of groups. This lecture will present a list of recommendations for the use of systemic antimicrobials (SAM) in the treatment of canine pyoderma based on current knowledge.

Should I use SAM in this patient?
1. Is there evidence of a bacterial infection?
Evidence of pyoderma includes compatible history, clinical signs, cytology +/- culture and antibiotic susceptibility testing. The causative bacterium in dogs is usually Staphylococcus pseudintermedius. Rarely other staphylococci, gram-negative bacteria or anaerobes may be involved, particularly in deep pyoderma. The clinical signs of pyoderma are described and are to be found in veterinary dermatology text books. Confirmation of pyoderma is based on finding groups of degenerative neutrophils with intracellular bacteria, which may be coccioid and/or rod-shaped. Small coccoid bacteria present in clusters of 2-4 are typical of Staphylococcus spp. With deep infections the inflammation is commonly pyogranulomatous (degenerate neutrophils and macrophages). Why is there bacterial pyoderma?
In the majority of cases there will be underlying disease that compromises the skin barrier or immune function. In order to treat successfully, it is necessary to identify and treat/manage the primary disease. In my experience, atopic dermatitis is the most common primary disease.

2. Can the infection be treated without using systemic antimicrobials?
Topical antiseptics have been used successfully as sole therapy in cases of canine pyoderma
and as adjunct therapy to SAM to reduce the time to cure. Therefore, topical therapy alone may be efficacious for surface infections and some superficial infections and a good adjunct to SAM for severe or widespread superficial or deep pyoderma. Wherever possible topical treatment in place of SAM is recommended by the guidelines, in particular for surface and superficial pyoderma and otitis. SAM as prophylaxis is strongly discouraged; this would include the use of pulse antibiotics for recurrent and/or idiopathic cases of pyoderma. Once again, in addition to addressing the underlying disease, regular topical therapy would be beneficial in these cases. While topical therapy may be efficacious and useful in many cases, its selection and success will be totally dependent on owner compliance.

3. How do I choose the correct antibiotic?
Clinicians should be familiar with commonly used antimicrobials and their spectrum of activity.

First choice antimicrobials should be prescribed based on culture and susceptibility, provided that no first-choice agents are appropriate: e.g. cefovecin, cefpodoxime, moxifloxacin**, enrofloxacin**, marbofloxac in**, orbifloxacin** and pradofloxacin**. Fluoroquinolone resistance in staphylococci appeared soon after their introduction and appears to be increasing.

Second choice antimicrobials should only be prescribed for serious and life-threatening infections, based on culture and susceptibility, where no first- or second-choice agents are appropriate: e.g. amoxicillin, amoxicillin-clavulanic acid, trimethoprim-sulfadiazine (TMS) and lincosamides. Where compliance is an issue, cefpodoxime and cefovecin should be considered. Resistance to TMS, macrolides and lincosamides may occur in 25% of cases, however tetracyclines and TMS may be useful for MRS based on susceptibility testing.

Third choice antimicrobials should only be prescribed for serious and life-threatening infections, based on culture and susceptibility, where no first- or second-choice agents are appropriate: e.g. aminoglycosides, azithromycin, ceftazidime, chloramphenicol, clarithromycin, florphenicol, fosfomycin, piperacillin, rifampin, tiamphenicol and ticarcillin. Many are not licensed for animal-use and data on use, safety and efficacy is lacking. Clinicians should follow the guidelines of use for their country of work. Drugs of critical importance to human medicine should not be used in veterinary patients. These include glycopeptides, oxazolidinones, streptogramins, ketolides, carbapenems and tigecycline.
countries it is prohibited to use human antibiotics that are not licensed for animals including azithromycin, ceftazidime, clarithromycin, imipenem, piperacillin, rifampicin and ticarcillin.

**The BSAVA and BVA guidelines place fluoroquinolones in the 3rd tier with third- and fourth-generation cephalosporins. There are concerns that we are at risk of restrictions preventing veterinary use of these drugs.**

**Situations where empirical antibiotic therapy may be appropriate**
- Superficial pyoderma with coccoid bacteria on cytology
- First-time pyoderma
- Absence of risk factors for AMR (see below)
- Non-life-threatening infection* (see below)

Empirical choice encompasses cytology, experience, knowledge of current recommendations and local AMR patterns when available\(^1\)\(^3\)\(^4\).

**When to perform culture and susceptibility tests**
Emphasis should be placed on obtaining samples for culture and susceptibility whenever possible so that treatment change can be implemented if necessary\(^1\)\(^3\) and local patterns can be monitored.
- Recurrent or chronic infection
- History of previous, particularly multiple, courses of broad-spectrum antibiotics
- Poor response to empirical therapy
- Rod-shaped or unusual organisms on cytology
- Degenerate neutrophils but absence of bacteria on cytology
- Deep infections
- Non-healing wounds
- Post-operative or other nosocomial infections
- Owner or animal or in-contacts have had recent health care contact
- Life threatening infection*

*Life-threatening conditions require immediate treatment. Therefore FECAVA advises empirical prescription, whilst awaiting culture and susceptibility results. De-escalation, if possible to a narrow-spectrum antimicrobial, following receipt of the results is recommended. Overall narrow-spectrum is recommended over broad spectrum if possible\(^1\)\(^3\)\(^4\) and de-escalation or escalation following susceptibility result has also been recommended by Beco et al. This is in line with human guidelines\(^1\) that recommend streamlining and de-escalation following susceptibility results to eliminate redundant or combination therapy, improve pathogen targeting, decrease antimicrobial exposure and save costs.

**The Minimum inhibitory concentration (MIC)**
MIC is the lowest concentration of an antimicrobial required to inhibit growth of a particular bacteria. The results are quantitative. MIC is compared to published cutoffs (breakpoints)\(^17\) to determine if the isolate is susceptible, intermediate or resistant. To be susceptible, the antibiotic level at the site of infection should be greater than the MIC for the specific organism. The lower the MIC, the more susceptible the organism. Breakpoints are based on the attainable level of antibiotic in each body compartment based on a standard fixed dose e.g. MIC breakpoints for oxacillin against *S. pseudintermedius* clinically susceptible ≤0.25 mg/L, clinically resistant ≥0.5 mg/L.

**4. What dose and frequency?**
Optimal dosing regimes that maximize bacterial killing and minimize the window for resistance development* need to consider patient characteristics, pathogen, site of infection and pharmacokinetic (PK) and pharmacodynamic (PD) data\(^1\). PK pertains to drug concentration and time in the patient, while PD describes the concentration- and time-dependent interactions of antibiotics against pathogens in the patient. As the skin is the largest organ of the body, and its blood supply is comparatively poor, antibiotics are used at the upper end of their dose range\(^8\). However dose adjustments may need to be made based on the MIC, PK and PD e.g. dose increase of concentration-dependant- or frequency increase of time-dependant- antibiotics. Bacteriostatic drugs may have both concentration-dependant and time-dependant properties (see table 1 for examples).

*How can you improve compliance?*
Client education is important for successful treatment. Detailed verbal and written instructions are encouraged - the frequency is best given in hours rather than number of times per day\(^1\)\(^3\)\(^8\). As compliance decreases with increased treatment frequency and number of treatments it is recommended to keep everything to a minimum and to give regular follow-up and support.

**Are there contra-indications in this patient?**
Consider important host factors such as age, previous allergy, renal or immune-dysfunction. Adverse effects may arise from effects on non-target commensal bacteria, expected dose-related pharmacological activity or idiosyncratic. Owners
should be warned about common and mild adverse effects, such as transient gastrointestinal tract upset, to improve compliance.

5. What duration of therapy?
The length of treatment will depend on the depth of the infection. Treatment of superficial pyoderma usually requires 2-4 weeks of treatment. Deep pyoderma, particularly with fibrosis, is usually more protracted often with an initial rapid response followed by slower improvement (4-12 weeks). In the absence of further improvement, culture and susceptibility should be repeated. Treatment is usually prolonged and should extend until clinical, cytological and palpable resolution. Treatment is best assessed by regular re-examinations every 7-14 days.

Do you have practice guidelines?
Practices are encouraged to create their own guidelines along with treatment and outcome records to encourage good stewardship and allow evaluation of therapeutic regimes. Guidelines should be flexible to allow regular re-evaluation and updates. Local antimicrobial resistance information can be incorporated if it is available. It is also advised that any treatment failure be reported to the appropriate body; in the UK it is the Veterinary Medicine Directorate (VMD). This will assist in monitoring of emerging resistance.

Summary
To slow the spread of AMR it is important to stop inappropriate and overuse of SAM. Other therapies, including topical antiseptics, can provide good alternatives for the treatment of canine bacterial pyoderma. When SAM are required, guidelines may assist the clinical decision-making process. Recommendations aim to maximise therapeutic success and minimise development of AMR and while appropriate for the majority of cases, some cases may require alternative treatment regimes. Owner and patient compliance is paramount to success.

References:
12. Loefller A, Cobb MA, Bond R. Comparison of a chlorhexidine and a benzoyl peroxide shampoo as sole treatment in canine superficial pyoderma. Veterinary Record 2011; 169: 249-U91.


Conflicts of Interest
PhD funding from Zoetis Animal Health.

Table 1: Examples of time-dependant & concentration-dependant antibiotics

<table>
<thead>
<tr>
<th>Time-dependant antibiotics with no postantibiotic effect (PAE)</th>
<th>Concentration-dependant antibiotics with PAE</th>
<th>Time-dependant &amp; concentration enhanced antibiotics with PAE</th>
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</thead>
<tbody>
<tr>
<td>Beta-lactams</td>
<td>Aminoglycosides</td>
<td>Clindamycin</td>
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<td></td>
<td>Fluroquinolones</td>
<td>Erythromycin</td>
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<td></td>
<td>Azithromycin</td>
<td>Tetracycline</td>
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<td>Optimal bactericidal effect when drug concentrations are maintained 2-4 times above the MIC throughout dosing interval</td>
<td>The peak concentration and area under the concentration curve (AUC) determine efficacy of these antibiotics</td>
<td>Efficacy of this group is determined by the 24-hour AUC to MIC ratio (AUC/MIC)</td>
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Surgical treatment of chronic pododermatitis
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Pododermatitis is a common problem in the dog and is less common in the cat. The term pododermatitis refers to an inflammatory skin disease that affects the paws. It is a pattern of distribution not an etiologic diagnosis. The paw has many different structures (skin, skin folds, claw folds, claws, haired skin, pad junction, and pads) and distribution of the disease on the paw helps to determine which differential diagnoses are more or less likely. In order to have the best clinical response to therapy it is important to have not only an accurate diagnosis, but also to understand that with chronic disease, chronic pathologic change to the tissue may occur. This change can and often does hinder therapeutic success.

Pathogenesis
A complicating factor in determining the underlying etiology of pododermatitis in the dog is the large range of diseases that may cause pedal disease. The pathogenesis of pododermatitis can be evaluated in the following way which can help improve therapeutic success. Look for predisposing factors, primary factors, secondary factors, and perpetuating factors. Failure to address any of these factors will lead to recurrence of disease or failure of therapy.

Perpetuating Factors
Perpetuating factors occur because of alterations in the normal structure and physiology of the paw as a result of predisposing and primary causes as well as the response to secondary infections. Perpetuating factors often prevent the resolution of pododermatitis even when the primary factors are controlled. Pathologic responses are very common with chronic pododermatitis and can lead to permanent changes in the anatomy leading to persistence of the disease. Inflammation in the skin stimulates epidermal hyperkeratosis and acanthosis, dermal fibrosis, edema, glandular hyperplasia/dilation and in some cases folliculitis and furunculosis. The tissue swelling that occurs leads to deep folds and fibrotic nodules in the interdigital tissues. These folds also act as sites for the perpetuation and protection of secondary microorganisms. Regardless of the initial disease process the clinician is now faced with treating deep fold dermatitis. These folds and swelling then cause abnormal weight bearing. The pet no longer walks on the pads but starts to walk on the haired interdigital skin causing further folliculitis and furunculosis, swelling and fibrosis.

Clinical evaluation of the paw
Physical evaluation of the patient with pododermatitis should determine multiple clues to the underlying etiology. First assess the conformation of the paw. Is normal weight distribution present? The anatomy of the paw is complex. Determine the distribution of the disease on the paws including how many paws are diseased. The claws, claw folds, pads, and dorsal and ventral interdigital areas should be evaluated. Evaluate the paws for erythema, swelling, comedones, follicular casts, scaling, ulceration, crusting, hemorrhagic furuncles, proliferative growths and/or discharge. If discharge is present, quantify and qualify the type present. Palpate the tissue to evaluate for painfulness, and fibrosis or deep nodules. Scarred, nodular lesions warrant a more guarded prognosis. Chronic interdigital nodular tissue may not return to normal and may be difficult to successfully manage with medical therapy alone.

Therapy
Prior to recommending therapy, it is important to discuss with the client the multiple potential causes of their pet’s symptoms. This should include a discussion about the perpetuating problems that the clinician perceives and the more guarded prognosis when chronic proliferative changes are present. Then discuss which diseases are most likely and what the most helpful diagnostics will be to determine the main cause of disease. This is most important when the pet has chronic or recurrent disease. If there is concern about disease that will require longer term maintenance therapy, it is important at this point to explain to the owner your suspicions about why the pet is having recurrent problems and if you are correct, what long term maintenance may be required to control the pet’s problems. Discuss with the client that most infections are secondary and will recur unless the primary disease is addressed. Doing this may help prevent some client from changing veterinarians multiple times looking for a “cure”.

The next step is to treat any secondary problems like bacterial and yeast infections. The length of therapy depends on the response to therapy, the occurrence of super infections, and the presence of deep microabscesses and fibrosis. At this point in time starting steroids is usually contraindicated. Using both antibiotics and steroids together initially makes evaluating the response to antibiotics much more difficult.

If the history and clinical signs are compatible with atopic dermatitis or food allergy consider having allergy testing done or starting an elimination diet for 8 weeks. It is also important if an elimination diet is recommended that the owner understands nothing else except the specialty diet is to pass the pet’s lips other than water for a period of 8 weeks.
When chronic perpetuating factors cause abnormal weight bearing and deep follicular cysts prevent continued improvement or cause relapses then surgical removal of interdigital nodules or deep interdigital folds may become necessary. This procedure is called fusion podoplasty. The first article presented in 1991 by Swaim and a newer publication in 2011 by Papazoglou, have reviewed cases and describe this surgical procedure in detail. This is a salvage procedure similar to a total ear canal ablation being a salvage procedure in chronic proliferative otitis i.e. medical management will not resolve the physical changes. A complete podoplasty involves removing all of the interdigital skin medial and lateral to the digits and all the haired skin on the ventral paw. This is a tedious surgery, but can be achieved. For most patients there is a marked improvement in the quality of life and marked decrease in recurrent disease. Potential complications can include fissures at the junction of the central pad and digital pads ventrally.

A partial fusion podoplasty is an option when only part of the paw is involved in the disease process. The therapeutic aim is to remove the scar tissue and improve the weight bearing of the patient.

**Surgical procedure**

The paw is surgically clipped and prepared with surgical scrub. A tourniquet is applied over the metacarpal or metatarsal area and must be released strategically during the surgery. The incision is started on the dorsal paw. The incision is made to remove the lateral and medial skin from the digits leaving the skin dorsally to cover the dorsal digit. On the lateral fourth digits and medial third digit enough skin must remain on the paronychia to cover the digit to allow for the second and fifth digits being shorter. Blunt dissection is used to separate the scarred tissue from the digital nerves and blood vessels. Depending on the amount of time this takes the tourniquet is released for several minutes to allow blood flow and prevent thrombi. Then the ventral interdigital haired skin is excised. The only tissue that should remain is the central and digital pad tissue. One should attempt to removal all hair follicles. The paw is then soaked in diluted chlorhexidine and flushed with saline. A 6mm penrose drain is placed between the central pad and digital pads. The digital pads are apposed to each other and to the central pad. The dorsal skin is then closed. The paw is bandaged in a splint to prevent extension of the paw. The bandage should apply enough pressure to prevent excessive bleeding but not be so tight as to create ischemic damage. The bandage is changed in 6-12 hours. The bandage is initially changed every other day. The drain is removed in 2 days. In 10-14 days the bandage can be applied without the splint. The main complication with this procedure is a separation of the digital and central pads. This will however, typically then heal by secondary intention. In one study evaluating 7 dogs one developed necrotized faciitis.

A variation on the partial fusion podoplasty has been published for dogs with abnormal weight bearing which has created thickened scar tissue on the medial aspects of the fifth digits and deep interdigital cysts. In this surgery the CO2 laser is used to ablate the abnormal tissue and the skin is allowed to heal by second intention.

**References:**

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

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

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

1. Overexpression of self-antigen
Lymphocyte clonal deletion, a part of central tolerance mechanisms, is more likely to affect lymphocyte clones with high receptor affinity to self, while clones with low affinity to self are more likely to escape and reach the periphery. These clones, that escape deletion, remain inactive in the periphery due to their receptor low affinity, which limits their activation, and because the self-antigens are only present in miniscule amounts. This immunological ignorance can be disrupted when larger amounts of self-antigen become released (trauma, impaired clearance, etc) in a supporting cytokine milieu (e.g. inflammation). For example, one of the core hypothesis for lupus pathogenesis involves an impaired clearance of apoptotic cells, leading thus to accumulation of nuclear antigens, which can then be recognized by the immune system and actively targeted. 5 Additionally, these accumulated self-antigens might become modified during the process of apoptosis and necrosis (methylation, acetylation, etc) forming so called danger signals that promote inflammation. In genetically susceptible individuals and with appropriate environmental triggers (sex hormones, drugs, UV, infection, etc), these accumulated self-antigens and danger signals trigger an autoimmune response resulting in autoantibody production and tissue pathology. Moreover, accumulated dead cells trigger chronic tissue inflammation, which makes the cytokine milieu in the tissue even more supportive for the development of autoimmunity. Interestingly, people suffering with cutaneous lupus exhibit delayed clearance of UV-induced apoptotic cells (up to 72hr) in contrast to healthy individuals (up to 24hr), a phenomenon that could explain the role of UV-light in induction of the disease. 6

2. Exposure of cryptic or sequestered self-antigen
Proteins in the body are built from amino acids, which are the key components of epitopes recognized by immune cells. Epitopes that are preferentially expressed and recognized are called dominant, while those that are physically hidden by conformation of the molecule, stereochemical alteration or sequestration in immune privileged site are called cryptic. In the thymic selection process, it is clear that immune cells recognizing dominant self-epitopes are deleted and those recognizing cryptic epitopes have a higher chance to escape to the periphery. Similarly, in the periphery, hidden or cryptic epitopes are only minimally presented to immune cells and thus have only limited ability to establish peripheral tolerance. Events leading to the exposure of cryptic epitopes (e.g. trauma, infection) are thought to trigger autoimmunity in susceptible individuals. Similarly, immune responses to cryptic epitopes play a role in the epitope spreading phenomenon. In this phenomenon, an immune response that had developed against one tissue epitope (for example in an autoimmune reaction started by a...
different mechanism, etc.) leads to tissue destruction over time, which exposes a second tissue epitope (not cross-reactive with the original triggering epitope) that was originally hidden from an immune surveillance in a healthy tissue. Sympathetic ophthalmia represents a classical example of disease caused by exposure of a sequestered self-antigen in one eye due to an injury, followed by a breakdown of tolerance and evasion of the privileged site of the other, originally uninjured eye.8 Three decades of research have confirmed that the hair follicle represents another immune privilege site with features like no MHC class I and II expression by hair follicle cells, reduced presence of antigen presenting cells and presence of special Langerhans cells with limited MHC class II expression and increased expression of pro-tolerogenic molecules including indolamine 2,3-dioxygenase (IDO), IL-10, TGFB and α-MSH.9 Disruption of this immune privilege and exposure of originally sequestered antigen(s) of the hair follicle is the leading theory of alopecia areata pathogenesis.9 Loss of immunological tolerance in the follicle is associated with initiation of expression of MHC I molecules, which present originally hidden hair follicle antigen(s) to the immune system.

3. Aberrant expression of MHC molecules

Expression of MHC class II molecules in a tissue, which usually does not express such molecules, can lead to presentation of self-antigens to autoreactive CD4+ T cells and thus initiate or potentiate an autoimmune response to those self-antigens. Pemphigus gestationis (PG) is an example of disease in which aberrant expression of MHC class II has been proposed as the initial step in development of autoimmunity. This disease is characterized by urticaria or blister-formation starting usually around the umbilicus of women during the second or third trimester of their pregnancy. The disease shows spontaneous remission with average of 4 and 60 weeks for the blisters and urticarial lesions, respectively.10 NC16A epitope of collagen XVII has been identified as the major target antigen in this disease, the source of which in PG is the placenta. The cause for production of the antibodies is not fully clear, but several lines of evidence suggest an important role for aberrant MHC class II expression by stromal and trophoblast cells of the placenta.21

4. Epitope spreading

Epitope spreading theory, first described by Lehmann et al. in 1992, is considered to be an important mechanism contributing to the dynamic and evolving character of autoimmune diseases. The main principle of this theory is that continual tissue damage (e.g. due to infection or already existing autoimmune disease) exposes new epitopes and will cause autoreactive T cells to become activated de novo to these newly uncovered epitopes within the original molecule (intramolecular epitope spreading) or on a new molecule (intermolecular epitope spreading). There are multiple examples of epitope spreading in human as well as veterinary dermatology. In people, for example, intermolecular epitope spreading has been proposed to a play role in the pathogenesis of paraneoplastic pemphigus13, in the transition of pemphigus foliaceus to bullous pemphigoid14, in development of bullous systemic lupus erythematosus (SLE) from SLE15, etc. Although intermolecular epitope spreading has not been well documented in veterinary dermatology, possible examples of this process can be found in the published literature, including mixed subepidermal autoimmune blistering dermatosis in dogs with autoimmunity against lamin 332 (laminin-5) and collagen VII16, paraneoplastic pemphigus or bullous SLE17, etc. The phenomenon of intramolecular epitope spreading is often discussed in dermatology in conjunction with an entity called endemic pemphigus foliaceus (PF), also called Fogo Selvagem (FS).18,19 This disease presents as a superficial blistering skin disease and shares clinical, histological and immunological similarities with the classical PF. The major target antigen is desmoglein-1 and, like in the classical human PF, and possibly in classical canine PF, the pathogenic antibodies are of IgG4 subclass.19 Intramolecular epitope spreading is one of several key events in the pathogenesis of FS. Studies showed that while healthy inhabitants and FS patients in remission express anti-DSG1 antibodies recognizing the EC5 domain of the protein, patients with active disease produce antibodies against additional epitopes localized in the EC1 or EC2 domains.18 The pathogenicity of the anti-DSG1 EC1/EC2 antibodies was demonstrated by a blister-formation after a passive transfer to mice, while the anti-DSG1 EC5 antibodies failed to induce blisters.19

5. Molecular mimicry

Although the small number of amino acids used to make proteins allows for numerous combinations, identical sequences (less than 10 amino acids) can be found among self- and nonself-proteins. As a consequence, an immune response mounted against an epitope present in a pathogen (viral, bacterial or parasitic), which is similar or identical to a self-antigen, can trigger immune response not only against a pathogen, but also against self. This phenomenon is known as molecular mimicry and there are numerous examples throughout human and veterinary medicine. Probably the most well known disease with an underlying molecular mimicry mechanism is rheumatic fever, in which the antibody response mounted against a group A Streptococcus cross-reacts with self-antigen expressed in the heart, skin, joints and brain. The consequence is clinical disease characterized by polyarthritis, endocarditis, erythema marginatum or subcutaneous nodules, etc. It has been long hypothesized that molecular mimicry is one of the critical pathomechanisms of FS. Indeed, several lines of evidence support this hypothesis: a strong geographical link, an association with rural life-style, the resolution of clinical signs after leaving the geographical location, a reduction of antibody titer with increasing distance from the reservation, an increased prevalence at the end of the rainy season, an association with other vector-borne infections and exposure to
insects. Interestingly, it has been demonstrated that anti-DSG1 IgG response precedes the development of clinical signs and that anti-DSG1 IgG antibodies can be detected in more than half of healthy people living in the Limao Verde reservation. In contrast, the same study showed that only approximately 2% of healthy people from USA and Japan tested positively for anti-DSG1 IgG antibodies. This observation suggested that an environmental trigger present in Limao Verde plays a critical role in the initiation of the disease. In more direct support of this hypothesis, in 2012, Qian and colleagues were able to demonstrate that the salivary gland antigens from Lutzomyia longipalpis, specifically the LJM11 salivary protein is recognized by FS antibodies. Moreover, mice immunized with LJM11 generated anti-DSG1 antibodies, thus confirming that insect salivary antigen delivered into the skin can lead to development of cross-reacting antibodies, which likely occurs in genetically susceptible individuals.

6. Failure of central tolerance

A syndrome called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) represents an example of a failure of central tolerance caused by a defect in the AIRE gene, which is responsible for self-antigen presentation in the thymus and deletion of autoreactive T cells ([OMIM] number 240300). Thus autoreactive T cells, instead of being deleted, enter the periphery, where they cause a multi-organ autoimmunity leading to hypoparathyroidism, hypoadrenocorticism, hypothyroidism, hypogonadism, vitiligo, alopecia areata, hepatitis, etc. Disruption of central tolerance has been proposed as one of the mechanisms leading to autoimmunity associated with thymoma. Indeed, more than 50% of people with thymic neoplasia were diagnosed with some form of an autoimmune disease, and relatively frequent reports of thymic neoplasia-associated autoimmunity can be found also in the veterinary medicine (e.g. paraneoplastic pemphigus and thymic lymphoma, myasthenia gravis and thymoma, erythema multiforme and thymoma, etc). Hypotheses explaining this increased prevalence of autoimmune diseases include: 1) Theory of immaturity (immature T cells lacking sufficient self-tolerance escape to the periphery); 2) Genetic theory (increased T cell proliferation leads to increased production of autoreactive clones, and/or impaired expression of HLA in neoplastic epithelial cells causes education and possible selection of autoreactive clones).

Conclusions

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


Autoantibodies of bullous dermatoses target adhesion structures of the epithelium and the basement membrane, provoking dyshesion of cell-matrix or cell-cell junctions leading to severe skin blistering. For several of these dermatoses a direct causal link between autoimmunity and disease has been shown by animal models. While steric hindrance of trans-adhesion between junctional molecules has long been postulated as the only pathogenetic mechanism, it has become clear, for at least in some of the diseases, that adhesion molecules have also signaling functions and that the cooperation of steric hindrance with downstream signaling events is necessary for the full blown lesions recognized in the clinic. The identification of the autoantigen has allowed a correlation of the clinical presentation of the lesion with the location of autoantigen expression within the epidermis or basement membrane, respectively, and with specific body locations. Bullous skin diseases can therefore be divided into superficial and deep subepidermal, and intraepidermal dermatoses (Fig. 1). For a better understanding of these differences, the epidermal turnover is briefly reviewed.

B. Epidermal Turn Over

1. From progenitor cells to corneocytes: an overview

The epidermis is renewed throughout adulthood by proliferation and terminal differentiation of its main constituent, the keratinocyte. Under normal homeostatic conditions, progenitor cells that reside in the basal layer divide asymmetrically to give rise to cells determined for terminal differentiation. At that stage they have a firm anchorage to the underlying basement membrane. When exiting the cell cycle and starting the differentiation process, they leave the basal layer and migrate upwards through the epidermis until they reach the stratum corneum as terminally differentiated corneocytes or squames. There, they form an impermeable tightly sealing membrane together with intercellular lipids. Under homeostatic conditions the progenitor derived cells are thought to be sufficient to renew the overlying epidermis while in case of severe and deep epidermal damage as in wounding, epidermal or hair follicle bulge stem cells can be recruited to reconstitute the tissue. During the journey through the multilayered epidermis, the keratinocytes maintain tight cell-matrix and cell-cell adhesion in spite of their constant migration. Disturbance of this adhesion by autoantibody binding leads to bullous dermatoses, that is blistering skin diseases.

2. Cell-Matrix Adhesion

Basal layer keratinocytes firmly anchor to the underlying basement membrane via hemidesmosomes and focal adhesions, both being composed of transmembrane adhesion molecules and plaque proteins, anchoring intracellularly to keratin and actin filaments, respectively. In regard to autoimmune diseases, we will concentrate on hemidesmosomes and focal adhesions, both being composed of transmembrane adhesion structures of the epithelium and the basement membrane provoking dyshesion of cell-matrix or cell-cell junctions leading to severe skin blistering. For several of these dermatoses a direct causal link between autoimmunity and disease has been shown by animal models. While steric hindrance of trans-adhesion between junctional molecules has long been postulated as the only pathogenetic mechanism, it has become clear, for at least in some of the diseases, that adhesion molecules have also signaling functions and that the cooperation of steric hindrance with downstream signaling events is necessary for the full blown lesions recognized in the clinic. The identification of the autoantigen has allowed a correlation of the clinical presentation of the lesion with the location of autoantigen expression within the epidermis or basement membrane, respectively, and with specific body locations. Bullous skin diseases can therefore be divided into superficial and deep subepidermal, and intraepidermal dermatoses (Fig. 1). For a better understanding of these differences, the epidermal turnover is briefly reviewed.

B. Epidermal Turn Over

1. From progenitor cells to corneocytes: an overview

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3. Cell-Cell Adhesion
In the epidermis strong intercellular adhesion is conferred by desmosomes and to a lesser extent by adherens junctions. Regarding the structure of these two junctions refer to the handout on the structure of the epidermal barrier in these proceedings. Here we concentrate on desmosomes, due to their importance in intraepidermal autoimmune bullous dermatoses. For the understanding of these diseases, the assembly, a multistep process, and the turnover of desmosomal proteins to form keratin anchored and transadhesive desmosomes is essential. Desmosomal cadherins in association with the plaque proteins plakoglobin and plakophilin are continuously targeted to the plasma membrane and again recycled until they establish stable transadhesion with cadherins of a neighboring cell. Once stabilized, they become linked to desmoplakin and the intermediate keratin filament network which represents the initial step of desmosome assembly forming a cell-cell spanning network. Desmosomes then mature from calcium dependent to calcium independent (hyperadhesive) desmosomes. Two forms of functionally distinct desmosomal cadherin complexes exist therefore at the plasma membrane which can be biochemically distinguished; they are referred to as “non-junctional” (without keratin anchorage) and “junctional” (in desmosomes with keratin anchorage) desmosomal cadherins. While the junctional complexes mainly confer adhesion, adhesive proteins of non-junctional desmosomal cadherins function as signaling receptors at the plasma membrane with the ability to sense the cellular microenvironment.

Furthermore, the level of expression of each individual cadherin depends on body location. For example, Dsg3 is more strongly expressed in the oral mucosa while Dsg1 is highly expressed in the skin. These distributions are important for lesion localisation since the patients preferentially present lesions at sites where one of these antigens is predominantly expressed and cannot be functionally compensated by the second one. This is exemplified by the distribution patterns of lesions in pemphigus foliaceus (PF) and vulgaris (PV): PF with anti-Dsg1/anti-desmocollin 1 (in the dog) antibodies affects the superficial epidermis of haired skin, while PV with anti-Dsg3 antibodies mainly manifest in the suprabasal mucous membranes and mucocutaneous junctions (see Table 1). Accordingly, PV patients with antibodies against Dsg3 and Dsg1 exhibit both oral and skin blisters. The antibody titer has been shown to correlate with disease activity in canine PF (Nichifuji 2005). The importance of the integrity of these cadherins in intraepidermal adhesion is further exemplified by the PF-like subcorneal blisters seen in canine epidermis exposed to Staphylococcal exfoliative toxin EXI that digests Dsg1. The antibody titer in PV patients with antibodies against Dsg3 and Dsg1 exhibit both oral and skin blisters. The antibody titer has been shown to correlate with disease activity in canine PF (Nichifuji 2005). The importance of the integrity of these cadherins in intraepidermal adhesion is further exemplified by the PF-like subcorneal blisters seen in canine epidermis exposed to Staphylococcal exfoliative toxin EXI that digests Dsg1.

4. Autoimmune Bullous Dermatoses and Cell Signaling
Over recent years, evidence has accumulated that transmembrane adhesion molecules not only have structural functions, that is to assemble into multimeric adhesion structures but also swiftly modulate intercellular signaling pathways depending on their adhesive status to change cell fate. Studies on cultured epithelial cells have revealed that engagement of adhesion molecules with their substrate (cell-matrix adhesion) or adhesion molecules of neighboring cells (cell-cell adhesion) trigger a variety of signals even before cytoskeleton-linked multiprotein adhesion complexes are assembled. This indicates that adhesion receptors are non-junctional proteins. Consistent with this notion, non-junctional integrin and cadherin receptors accumulate in lipid-containing signaling platforms at the plasma membrane in association with a variety of signaling molecules as well as growth factor receptors.

Due to their signaling capabilities which depend on their adhesive status, adhesion molecules instantaneously communicate to the cell about attachment to the basement membrane, contact with a neighboring cell or no contact promoting cell migration and division by outside-in signaling. For example, non-junctional β4 integrin receptors associate with Src family kinases and growth factor receptors to trigger cell migration. When the basal cell becomes sessile, β4 integrins are integrated into hemidesmosomes attenuating the signaling functions. If uncontrolled, integrin-mediated signaling as well as loss of cadherin signaling have been implicated in many epithelial tumors. It is therefore plausible that autoantibodies of bullous diseases, which functionally interfere with adhesion molecules, alter the signaling make up of their target cells. This is best documented so far for the autoimmune disease pemphigus vulgaris (PV).

In PV, Dsg3 is the major antigenic target and can directly alter the signaling pathways upon antibody binding, which itself is sufficient to induce blisters. This disease represents an unique example of the importance of adhesion molecules for cell fate. Furthermore, the understanding and identification of pathogenic signals represents an excellent approach to develop therapies without systemic immunosuppressive effects.

In dog, mouse and humans, PV antibody binding triggers cell proliferation and retards differentiation even in absence of a visible loss of functional desmosomes. Furthermore, as shown in the mouse model, the epithelial growth factor receptor (EGFR), which is under control of Dsg3, becomes activated and contributes to PV pathogenesis. Pathogenicity was revealed by passive transfer of PV antibodies into neonatal mice together with clinically used EGFR inhibitors, which abrogate epidermal blistering. This drug therefore represents a promising candidate for clinical treatment approaches.

In conclusion, integrating adhesion-mediated signaling into our current understanding of cell fate decisions provides the basis to unravel the underlying mechanisms and develop novel treatment approaches in autoimmune bullous diseases.
### Table 1: Autoimmune and Inherited Diseases of Adhesion Molecules in Domestic Animals

(Olivry 2006, Olivry 2009 Vet Derm)

<table>
<thead>
<tr>
<th>Autoantigen</th>
<th>Disease</th>
<th>Location of split</th>
<th>Distribution</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autoantibodies of the hemidesmosomal adhesion complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Superficial (split above L. densa):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen type XVII (BPAG II)</td>
<td>BP, MMP</td>
<td>Subepidermal</td>
<td></td>
<td>Olivry &amp; Dunston, 2010</td>
</tr>
<tr>
<td>LAD-1 (soluble coll. XVII fragm.)</td>
<td>LAD, MMP, JEB, mixed AISBD</td>
<td>Subepidermal</td>
<td></td>
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<tr>
<td>Laminin 332</td>
<td></td>
<td>Subepidermal</td>
<td></td>
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<tr>
<td><strong>Deep (below lamina densa):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>collagen type VII</td>
<td>EBA, BSLE, mixed AISBD</td>
<td>Subepidermal</td>
<td></td>
<td></td>
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<tr>
<td><strong>Antigens of desmosomes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pemphigus complex</td>
<td>Intraepidermal</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Desmogleins (Dsg)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Dsg 1 (minor Ag in dog)</td>
<td>PF &amp; PF-variants (PE, PPP)?</td>
<td>Subcorneal</td>
<td>Skin</td>
<td>Olivry et al, 2006</td>
</tr>
<tr>
<td>Dsg 3 (+/- Dsg 1)</td>
<td>PV</td>
<td>Suprabasal</td>
<td>Skin only mucosa only</td>
<td>Nishifuji et al 2003, Winfield et al 2013</td>
</tr>
<tr>
<td>- Dsg 3 only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Dsg 3 and Dsg 1</td>
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<tr>
<td><strong>Desmocollins (Dsc)</strong></td>
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<tr>
<td>Dsc 1 (major Ag in dog)</td>
<td>PF</td>
<td>Subcorneal</td>
<td>Skin</td>
<td>De Bruin et al 1999, Nishifuji et al 2007</td>
</tr>
<tr>
<td><strong>Envoy- and Periplakin, Dsg 3</strong></td>
<td>Paraneoplastic pemphigus (PNP)</td>
<td>Suprabasal (with apoptotic cells)</td>
<td>Skin &amp; oral mucosa</td>
<td>Olivry et al, 2006</td>
</tr>
</tbody>
</table>

**Abbreviations:** BP = bullous pemphigoid; MMP = mucous membrane pemphigoid; EB = epidermolysis bullosa (J= junctional, A= acquisita, D= dystrophic); BPAG = bullous pemphigoid-antigen; LAD = linear IgA dermatosis; BSLE = bullous systemic lupus erythematosus; PPP = panepidermal pustular pemphigus; subcorn. = subcorneal; intraepid. = intraepidermal; mucocutan = mucocutaneous; DP = desmoplakin

**References**


Immunosuppression: mechanism of action of selected drugs
Petra Bizikova
Department of Clinical Sciences, College of Veterinary Medicine and Center for Comparative Medicine and Translational Research, NC State University, Raleigh, North Carolina, USA

Note from the Scientific Organizing Committee (SOC):
This committee is indebted and very grateful to Dr. Petra Bizikova for having accepted to replace a previously-scheduled speaker and for having spent her summer developing new lectures with a short notice. Because of this last minute change in speakers, detailed notes could not be included in these congress proceedings. The notes for this lecture will be available for download from the following website: www.esvd-ecvdcongress.com until October 20, 2013

Notes
Immunosuppression of autoimmune skin diseases: How to more specifically target disease pathogenesis
Petra Bizikova
Department of Clinical Sciences, College of Veterinary Medicine and Center for Comparative Medicine and Translational Research, NC State University, Raleigh, North Carolina, USA

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Notes
**Clinicopathological conference: Is this disease autoimmune?**

**Session 1: oral and perioral erosions**

Maja M. Suter*, Thierry Olivry†

* DermFocus, Inst. Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland
† Department of Clinical Sciences, College of Veterinary Medicine and Center for Comparative Medicine and Translational Research, NC State University, Raleigh, North Carolina, USA

**Notes**
Clinicopathological conference: Is this disease autoimmune?
Session 2: footpad sloughing
Maja M. Suter*, Thierry Olivry†
*DermFocus, Inst. Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland
†Department of Clinical Sciences, College of Veterinary Medicine, and Center for Comparative Medicine and Translational Research, NC State University, Raleigh, North Carolina, USA

Notes
Journal Club (2): a review of landmark papers published in the medical or veterinary literature
Lluís Ferrer
Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, Massachusetts, USA

Notes
**Clinicopathological conference**

Sonya V. Bettenay*, Susan Paterson†
* Tierdermatologie Deisenhofen, Munich Germany
† Rutland House Referral Hospital, Abbotsfield Road, St Helens, Merseyside, UK

**Case 1**

7 year old neutered terrier cross
Acute onset generalized pruritic skin disease of approximately 4 months duration. Owner reports a partial response to antibiotic therapy.

**Histopathology**

**Diagnosis**

**Case 2**

18 month old flat coat retriever
Dog presents with rapidly progressing, acute onset, pustular disease of 2 months duration. Disease has been unresponsive to topical therapy with shampoo, hydrocortisone aceponate spray, systemic antibiotics and prednisolone given at a dose of 1mg/kg sid po.

**Histopathology**

**Diagnosis**
Case 3
A 7 month old Doberman.
A severely debilitated semi collapsed dog with generalized deep pyoderma of 4 months duration. Demodex mites have been identified on skin scrapings but the dog has shown no response to therapy with topical moxidectin.

Histopathology

Diagnosis

Case 4
10 year old Yorkshire terrier
Long standing non inflammatory alopecia of several years duration. Dog has shown no response to a food trial and in vitro allergy blood test results have been unremarkable.

Histopathology

Diagnosis

Case 5
4 year old Domestic short haired cat
Cat was presented with a generalized, mildly pruritic, scaling skin disease of unknown duration.

Histopathology

Diagnosis
CONTINUING EDUCATION SESSION

Update on feline hypersensitivity dermatoses
Claude Favrot
Dermatology Service, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Hypersensitivity dermatitides (HD) are often suspected in companion animals and these include flea (and other insect) bite hypersensitivity dermatitis, cutaneous adverse food reactions, urticaria, angioedema, contact dermatitis and atopic dermatitis (AD). Some of these conditions are rare in cats (angioedema, contact dermatitis) and the term “atopic dermatitis” itself may be regarded as inadequate because the role of IgE in the development of this condition is not definitively proven.

Clinical signs
Most cats with HD present with one or more of the following reaction patterns: head and neck excoriations and pruritus, miliary dermatitis, self-induced (symmetrical) alopecia, and eosinophilic dermatitis. In one recently published study, 46% of the Non Flea HD cats presented with at least 2 of the reaction patterns mentioned above. It is however worth noting that atypical forms such as pododermatitis (including plasma cell pododermatitis), seborrhoeic reactions, exfoliative dermatitis (mural folliculitis), facial erythema, pruritus sine material (without any obvious skin changes) and ceruminous otitis have been described by some authors and represented 6% of Non Flea HD cats included in the previously cited study.

Miliary dermatitis refers to a papulocrustous dermatitis developing usually on the face and dorsal aspects of the body. These lesions are usually very small and may be difficult to see. This pattern is often associated with other facial lesions and/or alopecia.

Head and neck excoriations and pruritus refers to papular and erythematous changes occurring on the face and neck of cats often in association with self-induced lesions, alopecia, crusts, miliary dermatitis and/or seborrhoeic changes. Associated pruritus may be extremely severe and self-induced lesions may be impressive.

Self-induced alopecia is characterized by usually symmetrical changes occurring mostly on the flanks, abdomen and dorsum and caused by excessive licking (overgrooming). This behaviour is easily recognized because hair tips in and around the lesions are broken. Some owners do not associate these changes with excessive licking and present the cat for a suspicion of spontaneous alopecia.

Eosinophilic dermatitides consist of eosinophilic plaques or granulomas and/or indolent ulcerations.

Indolent ulcer is a unilateral or bilateral erosive to ulcerative lesion of the upper lips. The lesions may be very severe but do not cause major discomfort.

Eosinophilic plaques are raised, erythematous, exudative and intensely pruritic lesions developing on the abdomen, inguinal, medial and caudal aspects of the thigh area and less frequently on the neck and face.

Eosinophilic granulomas may present as linear or diffuse to plaque-like swollen and usually firm lesions occurring mostly in the oral cavity, interdigital areas, chin (fat chin) and limbs (linear granulomas).

HD cats usually present with intense pruritus. In one study, owners evaluated this itching as 5 or above 5 in a scale ranging from 0 to 10 in 88% of the patients. Pruritus is however not always recognized by owners (especially in cats with self-induced alopecia), and trichoscopy (to identify broken hair tips) is useful to demonstrate overgrooming.

HD cats may also present with some non-dermatological signs. In one study, 6% presented with sneezing and/or coughing, 14% with digestive signs (Soft tools, diarrhea, vomiting), 7% with conjunctivitis and 16% with otitis externa and/or media.

Flea HD, Food HD and non flea, non food HD cats
It has been reported that cats with Food HD present more frequently with head and neck excoriations than cats with other type of hypersensitivity. This report was not confirmed by the more recently published study, in which no statistically significant differences in terms of distribution patterns, were found. One of the main conclusions of this study was that Food and not Food HD are virtually indistinguishable on clinical criteria alone.

Flea HD may present with the very same signs as other forms of feline HD but the relative frequency of each pattern and localization varies. In Flea HD cats, dorsal and lateral aspects of the body are clearly more often affected while face, ventral parts and limbs lesions are more often associated with Food or non Flea non Food HD.
As far as reaction patterns are concerned, miliary dermatitis is more often observed in cats with flea HD when compared to other HD cats. On the other hand, the three other reaction patterns appear more typical for Food HD and non Flea, non Food HD. It is also worth noting that these three conditions may present with two or more than two of these patterns. These associations are more frequently observed in Food or non Flea, non Food HD cats.

**Diagnosis**

None of the clinical signs or reaction patterns mentioned above is pathognomonic and ruling out diseases which may present with similar clinical signs is consequently a compulsory step in the HD work-up. Ectoparasites such as otodectes, notoedres, demodex, lice, neotrombiculids, and bacterial and fungal diseases should be ruled out in virtually all cats. Additionally, depending on the clinical presentation, some other differential diagnoses should be considered and corresponding tests should be carried out (see Table below).

<table>
<thead>
<tr>
<th>Reaction pattern</th>
<th>Main differential diagnoses</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miliary dermatitis</td>
<td>Fleas</td>
<td>Comb, therapeutic trial</td>
</tr>
<tr>
<td></td>
<td>Ectoparasites</td>
<td>Scrapings, therapeutic trial</td>
</tr>
<tr>
<td></td>
<td>Dermatophytes</td>
<td>Cultures, trichoscopy, wood’s lamp</td>
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<td></td>
<td>Folliculitis</td>
<td>Cytological examination</td>
</tr>
<tr>
<td>Self-induced alopecia</td>
<td>Internal diseases</td>
<td>Trichoscopy (hair tips not broken)</td>
</tr>
<tr>
<td></td>
<td>Folliculitis</td>
<td>Cytological examination</td>
</tr>
<tr>
<td></td>
<td>Psychogenic alopecia</td>
<td>Diagnosis of exclusion, Therapeutic trial</td>
</tr>
<tr>
<td></td>
<td>Ectoparasites (demodex)</td>
<td>Scrapings</td>
</tr>
<tr>
<td>Eosinophilic dermatitis</td>
<td>Gingivitis</td>
<td>Histopathology</td>
</tr>
<tr>
<td></td>
<td>Ectoparasites</td>
<td>Scrapings</td>
</tr>
<tr>
<td></td>
<td>Skin tumors (mast cell tumor, cutaneous lymphoma, metatases)</td>
<td>Histopathology</td>
</tr>
<tr>
<td>Head and neck pruritus</td>
<td>Ectoparasites</td>
<td>Comb, scrapings, therapeutic trial</td>
</tr>
<tr>
<td></td>
<td>Fungal diseases (dermatophytes, maalssaezia)</td>
<td>Cytological examination, wood’s lamp, trichoscopy, culture</td>
</tr>
<tr>
<td></td>
<td>Bacterial diseases (Staph., mycobacteriosis, nocardiosis)</td>
<td>Cytological examination, histological examination, culture, PCR</td>
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<tr>
<td></td>
<td>Viral diseases (herpesvirus, papillomavirus, calicivirus, poxvirus, FelV virus)</td>
<td>Histological examination, PCR</td>
</tr>
<tr>
<td></td>
<td>Skin tumors (cutaneous lymphoma, squamous cell carcinomas, mast cell tumors)</td>
<td>Histopathological examination</td>
</tr>
</tbody>
</table>

Criteria for the diagnosis of feline HD dermatitis have been recently proposed and will be presented during the lectures. When Non Flea, non Food HD cats are compared to all other cats with chronic pruritus, the sensitivity (including those with Flea HD) and specificity of these criteria is about 75%: When flea HD cats are excluded, other criteria may be used and are associated with a sensitivity of 90% and a specificity of 82%; one must consequently keep in mind that using these criteria alone would be associated with a substantial amount of incorrect diagnoses and that a thorough work-up is needed in all cats with a suspicion of HD dermatitis. The work-up should include tests for fleas and flea control, scrapings for other ectoparasites, cytological examination of the skin when inflamed, a properly undertaken elimination diet and specific allergy tests.

**References**


CONTINUING EDUCATION SESSION

Cyclosporin therapy for feline hypersensitivity dermatitis
Claude Favrot
Dermatology Service, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Cyclosporine A (CsA) is an immunomodulatory drug belonging to the group of calcineurin inhibitors. This lipophilic drug easily penetrates the cell membranes and binds in the cytoplasm to cyclophilin. CsA-Cyclophilin complex inhibits calcineurin, leading to an impaired activation of Nuclear Factor of Activated T cells (NF-AT). As NF-AT is one of the major activator of cytokine gene transcription, CsA administration results in an impaired expression of cytokines.

At the cellular level, CsA inhibits T lymphocyte activation, eosinophil recruitment, functions and growth of antigen-presenting cells, especially Langerhans cells, pro inflammatory cytokines secretion of keratinocytes and IgE mediated mast cell degranulation.

After oral or intravenous administration, peak and trough levels are highly variable in cats. It has, however, been shown that serum concentration and clinical efficacy are not correlated which implies that routine evaluation of CsA blood concentration is not necessary, in most cases. The peak concentration is obtained one hour after oral administration of 5mg/kg CsA and ranges between 100 and 1600 ng/ml when measured by high-pressure liquid chromatography. It is worth noting that CsA values vary with the technique used and immunoassays provide typically much higher values, when compared with high pressure chromatography.

The bioavailability of CsA in cats is rather low (29%) and bioaccumulation is usually not observed. As mentioned above, CsA is a very lipophilic drug, which explains the high volume of distribution (6L/kg) as well as the high concentration in the skin (4 times higher than in serum). Feeding before CsA administration is associated with a lower bioavailability but this decrease does not seem to impair clinical outcomes.

CsA interacts with cytochrome P 450 3A4 and drug interactions are consequently numerous. Very few have however been demonstrated in clinical studies and these interactions may be influenced by several factors such as age, concurrent diseases, dosage etc. In cats, an interaction between CsA and ketoconazole, itraconazole and clarithromycin have been demonstrated. Concomitant treatment should be associated with a reduction of CsA administration. Other possible interactions with ranitidine, omeprazole, cimetidine, metoclopramine, allopurinol, erythromycin, digoxin, furosemide, ciprofloxacin, verapamil and trimethoprim- sulfa are likely.

Adverse-effects of CsA include gastrointestinal signs, anorexia, weight loss and gingivitis. Otitis and cystitis are also sometimes observed during CsA treatment, even though causality is not firmly established. Outdoor cats should be tested for toxoplasma IgG before treatment and negative cats should either not be treated or should be kept indoors during the whole treatment. They should also not be fed uncooked meat. On the contrary, IgG positive cats are protected. Cats with chronic infectious diseases such as FIV, FeLV, dermatophytosis should also not be treated with CsA. Cats with chronic renal insufficiency and diabetes mellitus could treated with CsA but should be monitored carefully.

In a Novartis internal study (freedom of information), cats were first vaccinated and, then, treated 4 months afterwards with CsA 24mg/kg during 8 weeks. These cats were subsequently re-vaccinated after this period of treatment: Titers were lower than in the control group but still within the normal range which suggests that booster vaccination could be made during CsA treatment.

The first evidence for the efficacy of CsA for the treatment of hypersensitivity cats was provided by an open study published by Noli and coworkers. The first controlled study was published by Wisselink and coworkers who compared two groups of allergic cats, the first group was treated with CSA 5 mg/kg and the second with Prednisolone 0.5mg/kg. Improvement of the clinical signs were seen in both groups. Larger studies were published afterwards and evidence was provided that a more effective dosage for allergic cats was 7 mg/kg. Some other studies have been carried out afterwards but are still not published. It was however demonstrated that after an initial phase of treatment of 6 to 8 weeks, about 70% of allergic cats only need every other day treatment. After 4 additional weeks, 55 % were treated only twice a week while 20 % were still on every other day treatment and 15% on daily administration. Only 10% did not respond to the treatment.

All in all, studies show that CsA is a good treatment option for allergic cats and that most of them tolerate the treatment well.
References
**Update in leishmaniosis diagnosis in dogs and cats**
Lluis Ferrer
Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, Massachusetts, USA

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

The most useful diagnostic approaches for investigation of infection in clinically healthy infected dogs include detection of serum anti-*Leishmania* antibodies by a quantitative serological assay (ELISA, IFAT) and demonstration of the parasite DNA in tissues by applying molecular techniques.

In general, good sensitivities and specificities are gained with quantitative serological methods. Measurable antibody titres, even when the titres are low, indicates infection. Dogs from non-endemic areas are consistently negative for anti-*Leishmania* antibodies. High antibody titres are usually associated with disease and a high parasite load and for this reason; they are considered conclusive of a diagnosis of clinical leishmaniosis. Low antibody titres have a more difficult interpretation. However, they are always indicative of contact with the parasite. The complete elimination of the infection in dogs is a controversial issue. Most authors consider that this is an uncommon event if it ever happens. Therefore, a seropositive dog (with low or even borderline titres) has to be considered an infected dog, probably with a very low parasitic load (infection controlled by the immune response). We have to keep in mind, however, that this situation can change (infections, immunosuppressant treatments, aging,..). In non-endemic areas, however, a positive serology has high diagnostic value. It is important to remember also that cross reactivity has been reported with other species of *Leishmania*, and *Trypanosoma cruzi*. Vaccinated animals are also seropositive, usually with a low titre and for a few months. However, we do not know if these positive titres increase or persist after several years of vaccination because the vaccine was launched only last year.

The management of the seropositive dog without clinical signs is one of the most challenging areas of canine leishmaniosis. However, this is not an uncommon situation because many practitioners, in endemic areas, perform annual serologic surveys as a strategy to prevent clinical leishmaniosis. The rationale behind this strategy would be the detection of the infection and of the clinical disease at early stages, when the prognosis is best. The main problem with this approach, in endemic areas, lays in the difficulty to distinguish those with an infection that will progress to clinical leishmaniosis from those dogs with a controlled, subclinical infection. In endemic areas, treating all seropositive dogs, even those with borderline or low titres, is probably not indicated, and can have risks for the individual dogs (side effects of the drugs) and for the public health (resistances). On the other hand, simply recording the titre and suggesting a recheck in X months or scheduling a visit when some clinical signs appear doesn’t seem to be a good medical practice.

The introduction of the vaccine in Europe will make this situation even more complex, because most vaccinated dogs are seropositive. At the present time, one intervention that seems to be effective and safe for clinically healthy but seropositive dogs is the administration of the immune-potentiating drug domperidone.

Several PCR assays with various target sequences using genomic or kinetoplast DNA (kDNA) have been developed for CanL. Assays based on kDNA appear to be the most sensitive for direct detection in infected tissues. PCR can be performed on DNA extracted from tissues, blood, biological fluids or from histopathologic specimens. PCR on bone marrow, lymph node, spleen or skin is most sensitive and specific for the diagnosis of CanL. PCR on whole blood, buffy coat, and urine is less sensitive than the aforementioned tissues. PCR on aspirates of lymph node and bone marrow has been shown to be more sensitive than microscopic detection of amastigotes in stained smears or parasite culture. Quantitative real-time PCR can detect extremely low parasitic loads and allows the quantification of *Leishmania* loads in tissues of infected dogs, that is important for diagnosis as well as for follow-up during the treatment of CanL. PCR is not the first confirmatory assay recommended for dogs with clinical signs suspected of CanL because in endemic areas, a large portion of the dog population is likely to harbour *Leishmania* without associated clinical disease, or while suffering from a different medical condition.
**Diagnosis of clinical leishmaniosis**

The diagnosis of canine leishmaniosis (CanL) requires an integrated approach (clinicopathological diagnosis and specific laboratory tests), which includes careful documentation of the clinical history, a thorough physical examination and several diagnostic tests such as CBC, biochemical profile, urinalysis, urine protein/creatinine ratio, serum electrophoresis, and a coagulation profile. Imaging of the abdomen by radiographs and ultrasound can assist in raising the suspicion index for this disease.

Since high serological titres are closely associated with clinical disease and less frequent among clinically healthy carriers of *Leishmania*, quantitative serology would be the first recommended specific assay for the disease. However, the decision to treat clinically healthy dogs with anti-Leishmanial medication based on positive PCR alone is not recommended. The diagnostic approach for sick dogs suspected of suffering from leishmaniosis and living in an endemic area as recommended by the Leishvet group is shown in Figure 1.

**Common problems in the diagnosis of canine leishmaniosis**

Despite the wealth of information, and the recent publication of several guidelines, the diagnosis of the disease remains challenging. In the last part of the lecture three specific situations with a difficult management will be discussed:

1. A dog with a diagnosis of leishmaniosis and currently on treatment develops new skin lesions. Is this a consequence of the disease, of a concomitant disease or of an adverse drug reaction?
2. A dog diagnosed with leishmaniosis years ago (has remained since then seropositive), that develops new clinical signs, compatible with a relapse of the disease. Is this a relapse of leishmaniosis or a different disease?
3. A vaccinated dog develops clinical signs compatible with the disease (scaling, lymphadenopathy); the dog is tested and is seropositive. Is this a case of vaccine failure or a different disease in a vaccinated and seropositive dog?

**References**


**Figure 1. Diagnostic approach for sick dogs suspected of suffering from leishmaniosis in endemic areas (Solano-Gallego et al, 2011).**
Notes
Update in leishmaniosis treatment in dogs and cats
Lluis Ferrer
Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, Massachusetts, USA

Management of clinically healthy infected dogs in endemic areas
The management of clinically healthy but infected dogs in areas where canine leishmaniosis (CanL) is endemic is of major importance for practitioners. Healthy dogs should be screened for *Leishmania* antibodies as an initial indication for the presence of infection if: (1) They are scheduled to travel or be exported to non-endemic areas (2) They serve as blood donors. In addition to this, many practitioners recommend to perform a serological analysis at least every 12 months for early detection of infection. When the result of the tests is positive, the decision of starting a treatment depends on (1) the titre of antibodies, (2) the presence of other clinic-pathologic abnormalities (plasma protein abnormalities) and (3) the results of qPCR (quantification of parasites in bone marrow).

According to the results, basically three different interventions are possible:

<table>
<thead>
<tr>
<th>Intervention/treatment</th>
<th>Serology titer</th>
<th>Clinical Pathology</th>
<th>qPCR - Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment. Repeat serology in a 3-6 months.</td>
<td>Borderline or low titer.</td>
<td>No abnormalities.</td>
<td>Negative or low parasitic load (0-10 parasites ml).</td>
</tr>
<tr>
<td>Domperidone.</td>
<td>Low or medium</td>
<td>No abnormalities or mild changes.</td>
<td>Low - medium parasitic load (10-100 parasites ml).</td>
</tr>
<tr>
<td>Domperidone+allopurinol. Recheck in one month. Consider conventional treatment.</td>
<td>High antibody titer and/or changes in serum proteins and/or high parasitic load (&gt;100 parasites/ml).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clinical staging and treatment of sick dogs
Dogs are considered sick when they present clinical signs or clinic-pathologic changes as a consequence of the *Leishmania* infection or of the immune response against the infection. Usually these dogs, together with characteristic clinical signs, present high anti-*Leishmania* antibody titres, high parasite loads (qPCR on bone marrow or blood) and characteristic clinic-pathologic changes. A clinical staging system to decide on the therapy most suitable for each patient and also to consider a prognosis has been proposed by the LeishVet group. The sick dog is staged at a certain moment in time but later on, the stage can change as it deteriorates or improves. The proposed system includes four clinical stages, based on clinical signs, clinic-pathological abnormalities and serological status (see table 1).

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

- **In stages II and III** the treatment is based on the use of anti-parasitic (leishmaniostatic or leishmanicidal) drugs. To date, an active principle that is 100% effective has not been found since, generally speaking, most dogs improve from a clinical or pathological point of view but a “parasitological cure” is not achieved. In many cases, relapses occur after a period of time, which depends on the drugs used and the individual immune response of each patient.

Pentavalent antimonials and miltefosine still remain the drugs of choice for the treatment of CanL, as they usually induce clinical remission, and partial reduction on the parasitic burden although they do not prevent relapses; for this reason they are normally combined with allopurinol. A controlled clinical trial demonstrated a similar efficacy of meglumine antimoniate (100 mg/kg/q24h; 4 weeks) and miltefosine (2 mg/kg/q24h; 4 weeks), together with allopurinol (10 mg/kh/q 12h).

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

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

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

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

**Immune-potentiating and immune-modulating therapy**
Clinical CanL is both an infection and an immunodeficiency. Dogs with clinical leishmaniosis develop a type of immune response that is unable to control the progression of the infection and the development of lesions and clinical signs. Once the disease has developed the animals show signs of immune-deficiency and immunopathologic abnormalities. Numerous attempts have been made to help the immune system to control the infection (immunotherapy), including:

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

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

3. **Vaccines**. There are several studies and trials demonstrating that vaccines can be used as therapeutic drugs in dogs with clinical leishmaniosis. In a clinical trial in Brasil the Leishmune® vaccine reduced the clinical signs and the parasite load, modulating the outcome of the infection and the dog’s potential infectivity to phlebotomines. In another trial, the subunit vaccine Leish-111f + MPL-SE was effective in the treatment of dogs with mild disease but not of dogs with severe clinical leishmaniosis. In Spain, an autologous (auto) - vaccine prepared with parasites isolated/cultivated from the sick dog is marketed. Immunotherapy in canine leishmaniosis is certainly challenging, but has clearly some advantages that make it very attractive. Less and milder adverse effects than traditional chemotherapy, absence of resistance and the possibility of using it in combined protocols together with parasiticidal drugs, are some of them.

**Forbidden drugs and drug interactions**
Canine leishmaniosis is a prevalent (in endemic areas) and chronic disease and it is common to be confronted with patients on leishmania treatment (allopurinol, mife sosine, meglumine antimoniate, domperidone) that need treatment for a concomitant disease (allergy, immune-mediated disease, neoplasia, seizures) or that have to be treated with preventative medications or vaccinated. Which drugs should never be used on these patients? Which are the most common adverse interactions observed in these patients? Which precautions should be adopted? This will be discussed in the last part of the lecture.

**References**
Table 1. Clinical staging of CanL according to Leishvet Group

<table>
<thead>
<tr>
<th>Clinical stages</th>
<th>Serology *</th>
<th>Clinical signs</th>
<th>Laboratory findings</th>
<th>Therapy</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I Mild disease</td>
<td>Negative to low positive antibody levels</td>
<td>Dogs with mild clinical signs such as peripheral lymphadenomegaly, or papular dermatitis</td>
<td>Usually no clinicopathological abnormalities observed</td>
<td>Scientific neglect/allopurinol or meglumine antimoniate or miltefosine/allopurinol + meglumine antimoniate or miltefosine**</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal renal profile: creatinine &lt; 1.4 mg/dl, non-proteinuric; UPC &lt; 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II Moderate disease</td>
<td>Low to high positive antibody levels</td>
<td>Dogs, which apart from the signs listed in stage I, may present diffuse or symmetrical cutaneous lesions such as exfoliative dermatitis/hypergammaglobulinemia, ulcerations, planum naeale, footpads, bony prominences, mucocutaneous junctions, anemia, weight loss, fever, and eosinophilia</td>
<td>Clinicopathological abnormalities such as mild non-necrotizing anemia, hyperglobulinemia, hypalbuminemia, serum hyperurocaryoid syndrome</td>
<td>Allopurinol + meglumine antimoniate or allopurinol + miltefosine</td>
<td>Good to guarded</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Substages</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>a) Normal renal profile: creatinine &lt; 1.4 mg/dl, non-proteinuric; UPC &lt; 0.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>b) Creatinine &gt; 1.4 mg/dl, UPC = 0.5-1</td>
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<tr>
<td>Stage III Severe disease</td>
<td>Medium to high positive antibody levels</td>
<td>Dogs, which apart from the signs listed in stages I and II, may present signs originating from immune-complex lesions: vasculitis, arthritis, uveitis and glomerulonephritis</td>
<td>Clinicopathological abnormalities listed in stage I</td>
<td>Allopurinol + meglumine antimoniate or allopurinol + miltefosine</td>
<td>Guarded to poor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chronic kidney disease (CKD)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stage I with UPC &gt; 1 or Stage II (creatinine 1.4-2 mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IV Very severe disease</td>
<td>Medium to high positive antibody levels</td>
<td>Dogs with clinical signs listed in stage III: Pulmonary thromboembolism, or nephrotic syndrome and end stage renal disease</td>
<td>Clinicopathological abnormalities listed in stage I</td>
<td>Allopurinol ( alone)</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD IRS stage III (creatinine 2-5 mg/dl) and stage IV (creatinine &gt; 5 mg/dl)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Nephrotic syndrome; marked proteinuria; UPC &gt; 5</td>
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</tbody>
</table>

* Dogs with negative to medium positive antibody levels should be confirmed as infected by other diagnostic techniques such as cytology, histology, immunohistochemistry or PCR. High levels of antibodies, defined as a 3-4 fold elevation above the cut off level of a well established reference laboratory, are conclusive of a diagnosis of CanL. ** Dogs in stage I (mild disease) are likely to require less prolonged treatment with one or two combined drugs or alternatively monitoring with no treatment. However, there is limited information on dogs in this stage and, therefore, treatment options remain to be defined.
CONTINUING EDUCATION SESSION

What’s new in clinical dermatology?
Vanessa Schmidt
Department of Infection and Global Health, University of Liverpool, UK

If it is dry - add moisture; if it is moist - add dryness. Congratulations, now you are a dermatologist! (www.make4fun.com)

Introduction
As the world of veterinary clinical dermatology is ever evolving there are many new things to discuss however one current and major issue that we are facing across all health-care disciplines is antimicrobial resistance; most importantly for us is the emergence and spread of MRSP. Due to the multidrug resistance (MDR) shown by these strains there is often a need to use topical therapy. Topical antimicrobials, antiseptics and biocides have become the new “buzz” words for dermatologists - therefore this lecture will look at what is new and/or newsworthy in clinical dermatology for topical therapy of skin and ears.

SKIN
Topical antiseptics
A recent review examined the literature (in vitro and in vivo) on topical antimicrobial therapy for domestic species. Good evidence was found to recommend shampoo with either 2-3% chlorhexidine or 2% chlorhexidine + 2% miconazole (2C2M), and to a lesser degree benzoyl peroxide (BP), for the treatment of canine pyoderma. However there was only fair evidence for the efficacy of fusidic acid, silver sulphadiazine and medical honey.1

Chlorhexidine
The available products include shampoos (0.8%, 2%, 2C2M, 3%, 4%), conditioners (3% gluconate), wipes (Triz-EDTA+zinc gluconate) and gel (0.45%, 0.3% with Triz-EDTA). The susceptibilities of various common dermatological bacterial isolates, including susceptible and resistant strains of S. pseudintermedius (SP) and P. aeruginosa (PA), to different chlorhexidine formulations have been investigated in different studies. In one study 2C2M, 3% and 4% formulations had similar efficacy and significantly better efficacy than the other shampoos tested in the study including 10% ethyl lactate (EL) and BP.4 There were similar findings reported for a 2% chlorhexidine acetate product when examined over different time points and compared to EL and BP and a third study, that compared chlorhexidine digluconate to other biocides and oils also reported it to be effective.5 In addition, both 2C2M and 3% chlorhexidine +/- 3% conditioner formulations were found to have the best residual antibacterial activity on canine hair samples against SP isolates when compared to 0.8% and 4% concentrations and EL and BP.6,7 In vivo studies have corroborated the in vivo results. Chlorhexidine 2% and 3% shampoos have been found to be effective as a sole treatment of canine superficial pyoderma.8 In addition Murayama et al 2011 did not find any difference in efficacy between three different doses (57 mL/m2, 29 mL/m2, 19 mL/m2 body surface area) or between 1 minute and 10 minutes contact time using the 2% product. There was no difference in the susceptibility of the antimicrobial resistant strains compared to the susceptible strains in any of the studies.

2.5% Benzoyl Peroxide
While BP did not perform well in the in vitro studies, its use in vivo as sole therapy for canine pyoderma proved useful with half of the enrolled dogs reported as improved or much improved after three weeks of therapy.8 As skin contact may be necessary for its antimicrobial action, it is possible that the in vitro studies may have underestimated its efficacy.9

Anything new?
Other products that have been looked at include benzalkonium chloride, triclosan, accelerated hydrogen peroxide (AHP), geranium oil, tea tree oil and grapefruit seed extract (GSE). Triclosan had the highest efficacy against SP and MRSP isolates however all biocides and oils other than GSE had minimum inhibitory concentrations (MIC) that would be exceeded with topical application. While these products may have antibacterial potential, further investigations of efficacy and safety are needed.10

Bleach baths
In people the use of bleach baths (0.005%) for the reduction of S. aureus skin colonisation associated with atopic dermatitis or skin and soft tissue infections (SSTI) has recently gained popularity11-13. Reported adverse effects are uncommon and mainly consist of irritation and pruritus. However if the skin barrier is poor due to concurrent atopic disease and/or the concentration of the bath is incorrect there is a risk of bleach-related xerosis or burning. Unfortunately, there is currently a lack of evidence regarding this treatment. A recent systematic review of interventions to reduce S. aureus in the management of atopic eczema reported that although anti-staphylococcal interventions reduced S. aureus skin colonisation there did not appear to be any obvious clinical benefit.14
Topical antibiotic and antiseptic resistance in people
Of note are the increasing reports of resistance among human S. aureus strains and CoNS isolates to topical antimicrobials (mupirocin, fusidic acid) and antiseptics (chlorhexidine) that are commonly employed for decolonisation and prevention of nosocomial infections or recurrent SSTI in outpatients. While it is expected that topical therapy concentrations will overcome resistance, treatment failures have been reported and outbreaks of resistant strains have occurred in hospitals. High-level mupirocin resistance (smrA gene) and chlorhexidine resistance genes (qacA/B and smr) are carried on plasmids and can therefore readily disseminate between bacterial isolates. In addition, both genes may be potentially linked to other genes conferring resistance to systemic antimicrobials and a potential for co-selection.

Topical antibiotics and antiseptic resistance in dogs
Mupirocin is not authorised for animal use in Europe and should be restricted due to its critical importance in human medicine and ensuing resistance. Fusidic acid is authorised in some countries for dogs and cats as monotherapy eye ointment or as combination therapy with corticosteroids +/- anti-fungal and is widely used for focal pyoderma and otitis. In some cases the eye preparation has been used for decolonisation of dogs carrying MRSA or MRSP, however further studies are required to determine the benefits/indications of this procedure in animals.

Loeffler et al. 2008, reported resistance to fusidic acid in canine MRSA isolates, however all of the SP or MRSP isolates were susceptible to fusidic acid and mupirocin. Two recent studies have also reported an absence of fusidic acid or mupirocin resistance or chlorhexidine resistance for SP and MRSP isolates. In addition, this last study did not find any isolates carrying qacA/B or smr genes. Although these resistance mechanisms have not yet emerged (to our knowledge) among SP or MRSP isolates, due to the potential of transfer of resistant determinants among staphylococci of different species continued monitoring and stewardship for topical therapies would seem sensible.

EARS
Allergic
Similar to use of topical steroids in human atopic dermatitis, where it is recommended to reduce the frequency to intermittent maintenance therapy as soon as stable, two veterinary studies have investigated twice weekly maintenance therapy with hydrocortisone aceponate in dogs with CAD. The first study reported a good response of affected skin and the second a reduction in the episodes of recurrent allergic otitis.

- infections
As a sole agent Triz-EDTA is not antibacterial, however as it may damage bacterial cell walls, it can potentiate the effects of other antimicrobials. One recent in vivo study has examined Triz-EDTA with 0.15% chlorhexidine for the treatment of bacterial and/or Malassezia overgrowths or purulent canine otitis with a positive outcome. A second in vitro study demonstrated that Triz-EDTA significantly potentiates the bactericidal activity of silver sulfadiazine against multi-drug resistant Pseudomonas aeruginosa.

Stability of compounded products
Quite often for cases of Pseudomonas otitis we prescribe compounded topical treatment. Up until now, evidence-based-information regarding the stability, storage and subsequent efficacy of these products has been desperately missing. The first study investigated the stability of 0.9% enrofloxacin in four cleaners including Triz-EDTA (TrizEDTA®;TE), Triz-EDTA + 0.15% chlorhexidine (TrizChlor®; TC) and 2.5% lactic acid, 0.1% salicylic acid and 0.1% parachlorometaxylenol (Epi-Otic; EO), kept at room temperature, and their in vitro efficacy against SP and PA over 28 days. 0.9% enrofloxacin compounded with sterile water, TE, EO and EO- advanced maintained chemical stability and bactericidal efficacy for 28 days in contrast when compounded in TC stability was only for 14 days. A similar study investigated Timentin® diluted in Methopt for stability and bactericidal activity against PA. The results showed that a stock solution can be kept at –20°C for at least 12 months and in the fridge or at room temperature for 28 days.

Contamination of cleaners
Reassuringly bacterial contamination of ear cleaners appears to be uncommon however risk factors appear to be the tips, expired products, or the presence of Triz-EDTA.
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Conflicts of Interest
PhD funding from Zoetis Animal Health.
SCIENTIFIC SESSION

Mechanism of atopic itch in humans and dogs: selected (a)topics
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Introduction
The last five years have seen an explosion of information on the mechanism of itch in humans with spontaneous atopic dermatitis (AD), as well as in mice and rodent models of allergic skin inflammation or pruritus. New receptors and molecules have been shown to be relevant for pruritus induction or transmission on a quarterly basis. In contrast, there is scarcity of investigations on the pathophysiology of itch in companion animals.

Relevance for human AD itch
There is controversy whether or not histamine levels are elevated in human AD skin lesions. Intradermal injections of histamine in normal human (and mouse) skin cause wheal-and-flare immediate reactions associated with pruritus. In contrast, histamine challenges lead to reduced itch in humans with AD compared to normal subjects (reviewed in Ref. 1). A recent systematic review established that there is currently no high-level evidence to support or refute the efficacy or safety of oral H1R antihistamines as monotherapy for treatment of human AD. Furthermore, latest treatment guidelines concluded that “there is not enough evidence to support the general use of both first and second generation H1R antihistamines for treatment of pruritus in atopic eczema”.

Relevance for canine AD itch
There are several arguments that indicate that histamine could be important in the genesis of lesions and/or pruritus in dogs with spontaneous AD. Firstly, skin histamine levels are higher in dogs with AD compared to those of normal dogs; skin histamine levels do not correlate with their respective plasma concentrations, however. Furthermore, the epicutaneous application of house dust mites (HDM) to the skin of sensitized dogs results in a transient rise of dermal histamine. The total histamine content per isolated skin mast cell is higher in dogs with AD than in control dogs, and mast cells isolated from canine AD skin release more histamine than those of normal skin when stimulated with mast cell degranulating agents. Interestingly, immediate reactions after intradermal injections of histamine are consistently smaller compared to those seen in normal dogs; this lower histamine responsiveness suggests a possible downregulation of H1R after their activation.

In contrast to the observations above, there are several arguments pointing against a major role of histamine and H1R in the genesis of atopic itch and skin lesions in dogs. Firstly, the intradermal injection of histamine does not appear to lead to either noticeable pruritus induction or persisting skin lesions in dogs. Secondly, the preadministration of either H1R or

Histamine and histamine receptors
Structure and Biology
The biogenic amine histamine has been one of the most extensively studied molecules in medicine. Large amounts of histamine are set free by mast cell degranulation with peak local tissue concentrations in the millimolar range. The effects of histamine are mediated by four G protein coupled histamine receptors which are named in the order in which they were discovered: histamine 1 (H1R), H2 (H2R), H3 (H3R), and H4 receptor (H4R), the latter having been first described in 2000. These receptors are widely distributed in the body; to simplify, the H1R is found on smooth muscle, endothelial as well as immune cells, and it plays a role in the genesis of immediate type hypersensitivity reactions; the H2R plays a role in gastric acid production, whereas the H3R is mainly found in the central nervous system and on peripheral neurons. Interestingly, the H4R is mainly expressed on hematopoetic cells (neutrophils, eosinophils, monocytes, dendritic cells, Langerhans cells, T-lymphocytes, basophils, mast cells), fibroblasts, endocrine cells and neurons, thereby leading to a suspected role in allergy and inflammation. Importantly, histamine-induced pruritis in mice seems to be mediated via the histamine H1 and H4 receptors, while the H3R appears to have a negative regulatory role.

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H4R antagonists do not prevent the development of experimental atopic skin lesions in dogs (Note: this experimental canine model of acute AD skin lesions did not allow for an assessment of pruritus inhibition). Finally, while clients reported in a survey that H1R antihistamines were perceived to be effective to treat dogs with AD, when used as part of a multi-intervention regimen, two systematic reviews of randomized controlled trials (RCTs) have reported that there is no consistent and conclusive evidence of the antilesional and/or antipruritic efficacy of topical or oral H1R antihistamine monotherapy in dogs with AD.

Nerves, nerve elongation and nerve repulsion

Structure and biology

The growth of nerve endings (neurites) in the skin is believed to be dependent upon the balance between "nerve elongation factors" (e.g. nerve growth factor [NGF], amphiregulin, TNF-alpha…) and "nerve repulsion factors" such as semaphorin-3A (Sem3A) and anosmin-1.

Relevance for canine AD itch

The microscopic examination of human AD skin has revealed a higher neurite density in the superficial lesional dermis and epidermis of affected patients, thereby suggesting the hypothesis that this higher nerve ending density might be at least partly responsible for the intense itch seen in this disease. The expression of the nerve elongation factors NGF, amphiregulin and TNF-alpha is higher in lesional AD than in normal human skin. The reverse has been shown for the expression of Sem3A and anosmin-1, which is lower in atopic skin. Overall, this imbalance between elongation and repulsion is suspected to result in the neurite growth seen in human AD skin. The injection of anti-NGF antibodies reduces itch levels and skin lesions in a mouse model of AD.

Relevance for human AD itch

At this time, there is no firm documentation of increased nerve density and epidermal neurite sprouting in the dogs with AD. However, we recently demonstrated increased NGF and reduced Sem3A immunoreactivity in the lesional epidermis of dogs with AD as well as after HDM patch testing in sensitized dogs (Olivry, unpublished data). The increased amount of TNF-alpha mRNA and protein found in canine AD skin lesions could theoretically promote neurite elongation in the skin of dogs with this disease.

Proteases and proteinase-activated receptors (PARs)

Structure and biology

In the skin, there are several endogenous proteases (mostly from kallikreins and cathepsin families as well as mast cell proteases) that are tightly regulated by a variety of local inhibitors, and this balance between activation and inhibition is involved in the processes of desquamation, cutaneous inflammation, host defense, chemotaxis, cytokine expression, vascular function, tissue repair, and apoptosis. Exogenous proteases, such as those of mite, insect, mold and some pollen allergens, as well as those of microbes, can also be involved in the initiation of allergic inflammation.

Proteases bind to one of four recognized proteinase-activated receptors (PARs), all members of the G-protein-coupled receptor family. These proteases trigger the activation of PARs by clipping a small segment near the aminoterminal end of the receptors. The newly unmasked aminotermini self-activate their own receptors, thereby triggering cell activation. In the skin, the second PAR (PAR-2) is expressed by keratinocytes, fibroblasts, endothelial cells and afferent nerve endings. Its activation on dorsal root ganglia (DRG) neurons triggers both itch and pain because of the co-activation of TRPV1 (the "capsaicin receptor") triggering the release of neuromediators such as substance P (SP) by the same neurons.

Relevance for human AD itch

Proteases and PARs might be relevant for human AD and its itch via the association of AD with genes encoding either endogenous proteases (e.g. KLK7) or protease inhibitors (e.g. SPINK5), or by an increase in superficial epidermal pH resulting in protease hyperactivation and secondary higher desquamation and barrier defects. Furthermore, exogenous allergen proteases, such as those of mite allergens, can also activate keratinocytes to secrete proinflammatory cytokines in a PAR-2 dependent fashion. Finally, PAR-2 is likely relevant for the pathogenesis of human AD, as suggested by its detection at high levels in lesional AD skin, and by the consistent induction of itch with PAR-2 agonists such as the tropical legume cowhage-derived mucunain and the epidermal cysteine protease cathepsin S.

Relevance for canine AD itch

There is increasing evidence showing the likely relevance of proteases and PAR-2 for inflammation and itch in dogs. This receptor has been found to be expressed on the canine progenitor epidermal keratinocyte (CPEK) cell line, and keratinocyte stimulation with PAR-2 agonists results in increases in the transcription of genes encoding TNF-alpha, CCL17 (TARC) and CXCL8 (IL-8); stimulation of these keratinocytes with the mite protease allergen Der f 1 similarly increases the transcription of the same genes and also that coding for GM-CSF. Stimulation with a HDM extract also results in increased transcription of the TSLP proallergic cytokine gene, but not if HDM were heat...
treated, which inactivates HDM proteases. Sensitization of Maltese-beagle atopic dogs with proteolytically inactive Der f 1 results in lower HDM-specific IgE and IgG, decreased mast cell degranulation and less intense dermal infiltration at application sites than when using proteolytically active Der f 1 (Pucheu-Haston et al, unpublished).

Recently, our laboratory confirmed that PAR-2 is expressed in the stratum granulosum of canine normal and atopic skin as well as on differentiated CPEK and C2 mast cell lines. Stimulation of C2 mast cells with canine PAR-2 agonist peptides results in a strong histamine release (Olivry and Bizikova, unpublished). The transcription of PAR-2 mRNA does not appear significantly different between either lesional or nonlesional canine AD and normal canine skin. Finally, the relevance of PAR-2 activation for canine itch is shown by the induction of pruritus with native cowhage spicules, but not when proteases have been heat inactivated. Similar pruritus induction can be provoked with cowhage extract and microneedle epidermal piercing (Olivry and Bizikova, submitted).

Interleukin-31 and IL-31 receptor
Structure and biology
First identified in 2004, interleukin-31 (IL-31) is an helical cytokine that belongs to the IL-6 superfamily. It is secreted preferentially, but not exclusively, by Th2 (T-helper2) lymphocytes after their activation and by skin homing memory T-lymphocytes. It is also secreted by mast cells, monocytes, macrophages and dendritic cells. Interleukin-31 binds to a heterodimeric receptor complex consisting of the IL-31 receptor alpha (IL-31RA) and the oncostatin M receptor (OMSR). This receptor is transcribed, among other tissues, in the skin (keratinocytes), brain, peripheral neurons and blood leukocytes such as activated monocytes. Binding of IL-31 to its receptor activates several signaling pathways, one of them involving Janus kinases (JAK1, JAK2 and TYK2) and downstream STAT (-1-3,-5) signaling molecules. Interleukin-31 and its receptors are involved in the regulation of progenitor cell hematopoiesis and the induction of secretion of numerous chemokines (e.g. CCL17/TARC) and proinflammatory cytokines.

Relevance for human AD itch
There is evidence suggesting the relevance of IL-31 in the pathogenesis of AD and its itch. Transgenic mice overexpressing IL-31, or mouse skin injected with IL-31, both developed skin lesions and pruritus resembling those of AD. Furthermore, injections of IL-31 antibody during the onset of skin lesions reduces scratching in a mouse model of AD. There is a higher number of IL-31-expressing cells in the skin of humans with AD, and serum levels of IL-31 are elevated in humans with AD, especially those with severe signs. In one study, however, there was no correlation between pruritus and serum IL-31 levels. Polymorphisms in the IL-31 encoding genes appear associated with the development of both human (extrinsic) AD and (intrinsic) nonatopic eczema. Drugs like ciclosporine can lead to reduced IL-31 serum levels, as the patient’s atopic lesions and itch improve.

Relevance for canine AD:
Canine IL-31 was cloned from activated peripheral blood mononuclear cells, with activation in itself increasing gene transcription. Interleukin-31 mRNAs was not detected in normal canine skin. The transcription of both chains of the IL-31 receptor (IL31-RA and OSMR) have been found in canine dorsal root ganglia, as in other species. The injection (intradermal, subcutaneous or intravenous) of recombinant canine IL-31 into dogs induces transient and variable manifestations of pruritus. Interleukin-31 injection-induced pruritus manifestations are inhibited by both prednisolone and the JAK inhibitor oclacitinib, but only if these are given at specific times before IL-31 challenges. In contrast to that of CCL17, which is elevated in canine AD skin, the transcription of the canine IL-31 gene was not detected in either lesional or lesional canine AD skin. Whereas IL-31 was not detectable in the serum of nonatopic or laboratory dogs, even after HDM or flea sensitization, it was found to be elevated in the serum of approximately half of dogs with spontaneous AD.

In summary, while IL-31 injection induces pruritus in dogs, the relevance of this cytokine in the pathogenesis of canine AD and its associated pruritus remains unclear at the time of this writing.

Conflict of Interest
The authors do not declare any conflict of interest directly relevant to the subject of itch mechanism except for research funding provided to NC State University (TO) by Novartis Animal Health.
References


An update on target-based pharmacotherapy of itch
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Introduction
The preceding lecture highlighted several novel pathways that appear involved in the pathogenesis of itch, in particular that associated with AD of both humans and dogs. The discovery of these and other pathways has not only provided an explanation for the antipruritic mechanism of action of old molecules, but it has also given a rationale for the use of novel or nontraditional drugs to alleviate pruritus. Some of these drugs have already been shown to benefit human patients with itch associated with AD and other diseases.

The objective of this lecture is to familiarize the audience with selected topical and systemic medications that have been shown to have an effect for the control of itch manifestations in humans and/or small animals. Of note is that these molecules were chosen because of their unique mechanism of action as well as an antipruritic potential that deserves further investigations in small animals.

Due to the time limitation of this lecture, currently used and well-known drugs shown to have antipruritic effect in dogs will not be reviewed herein (e.g. glucocorticoids, calcineurin inhibitors [cyclosporine, tacrolimus], antidepressants, H1R antihistamines). For more exhaustive information on the current guidelines and therapy of itch in humans, the audience should refer to recently published reviews on this topic.¹⁻⁵

Topical treatments of pruritus
Menthol
Mechanism of action
Menthol is a monoterpen isolated from the essential oils of plants from the Mentha genus. Among other effects, menthol activates TRPM8 (transient receptor potential M8), an ion channel. In sensory neurons TRPM8 functions as a cold thermosensor. As a result, the topical application of menthol, but also that of other monoterpenes present in eucalyptus, lemongrass, and citronella oils, to TRPM8 leads to a cooling skin or mucosal sensation (8-28°C) and a resulting analgesic and antipruritic effect.

Antipruritic efficacy in humans
Menthol, at concentrations of 1 to 3% has been shown to relieve pruritus in humans; higher concentrations can cause irritation and can also worsen transepidermal water loss. The application of menthol has been reported to benefit human patients with lichen amyloidosis, and hydroxyethyl, histamine and mustard gas-induced itch.

Antipruritic efficacy in small animals
Evidence of the use of menthol as an antipruritic agent could not be identified in the veterinary literature.

Capsaicin
Mechanism of action
Capsaicin is a vanillylamide and the main capsaicinoid derived from hot chili peppers of the Capsicum genus. The main action of capsaicin results from its binding to TRPV1 (transient receptor potential vanilloid 1), an ion channel that also responds to other stimuli such as heat greater than 43°C, acid and other plant derived compounds (e.g. piperine, ginerol, eugenol...). Capsaicin specifically activates TRPV1 receptors on polymodal mechano- and heat-sensitive primary sensory C nerves as well as mechano- and heat-sensitive A-delta fibers, both of these normally transmitting pain and itch. The binding of capsaicin to TRPV1 results in calcium and sodium influx, nerve depolarization and substance P (SP) release, which causes an initial intense burning and stinging perceived as pain or itch. Repeated applications of capsaicin leads to long-lasting nerve desensitization, by exhaustion of SP nerve reserves, with ensuing decreases in pain and itch. As a result, repeated applications of capsaicin can block peripheral nerve transmission of itch, thereby “numbing” peripheral nerve pathways.

At high concentrations (e.g. in 8% patches), capsaicin also causes reversible peripheral nerve ending degeneration.

Antipruritic efficacy in humans
Topically, capsaicin has been reported to be helpful for treatment of humans with localized itch, such as that associated with the chronic sensory neuropathy notalgia paresthetica, but also that seen with prurigo nodularis, aquagenic or idiopathic pruritus. The higher the initial concentration of capsaicin and the more frequent the applications, the earlier nerve desensitization appears and the antipruritic effect occurs. Once pruritus control is obtained, the frequency of application of capsaicin can be reduced. To enhance patient compliance, topical anesthetics can be applied 20 minutes before capsaicin application but a concentration of 0.025% is generally tolerated by most patients. In case of lack of antipruritic effect, the concentration of capsaicin can be increased. For sensitive mucosae, a lower starting concentration (e.g. 0.006%) is suggested.
Antipruritic efficacy in small animals
We could only find one controlled trial reporting the effect of topical capsaicin for treatment of itch associated with an animal skin disease. Twelve dogs with AD were randomized to be treated with either a 0.025% capsaicin lotion or placebo onto lesional areas, twice daily for 6 weeks. After a wash-out of 4 weeks, treatments were reversed. After 6 weeks of capsaicin, the owner pruritus scores, but not those assessed by investigators, were significantly lower than those after placebo application. Owner scores did worsen after 1 week of capsaicin application. There are anecdotal reports of the use of capsaicin for treatment of acral lick dermatitis in dogs (Vetderm list, April 2013).

Cannabinoids
Mechanism of action
Cannabinoid receptors CB1 and CB2 are expressed on sensory nerve fibers, keratinocytes and mast cells. Treatment with topical cannabinoid receptor agonists reduces histamine-induced itch and vasodilation in healthy humans. In mice, CB antagonists induce a dose-dependent itch. Palmitoylethanolamide (PEA, palmidrol) is an endogenous fatty acid amide that targets mainly the peroxisome proliferator-activated receptor (PPAR)-alpha; it has a cannabinoid-like effect but does not seem to bind directly to CB1 or CB2.

Antipruritic efficacy in small animals
Palmidrol, compounded as a cream, has been shown to reduce itch in human patients with AD, prurigo nodularis, lichen simplex chronicus, pruritus of unknown origin and urticarial pruritus.

Antipruritic efficacy in small animals
Palmidrol cultured with canine mast cells reduces the anti-IgE induced release of histamine, PGD2 and TNF-alpha. An experiment confirmed that the oral administration of PEA at 10 mg/kg to six Ascaris hypersensitive beagle dogs significantly reduced mast cell degranulation-induced wheals by a maximum average of approximately 30%. The topical application of 2% aldemidrol, an azelaic acid derived PEA analog, reduced antigen-induced wheal diameter and mast cell numbers in the skin of Ascaris-hypersensitive beagles. In another study, beagles sensitized to house dust mites (HDM) were treated for 4 days with either PEA or placebo at 15 mg/kg once daily. After a wash-out of 4 weeks, treatments were reversed. While PEA appeared to delay the occurrence of skin lesions after challenge, there were no significant differences in treatment effect between PEA and placebo treatments. Finally, 10/15 cats (67%) with eosinophilic plaques or granulomas had an improvement in skin lesion scores after 30 days of PEA at 10 mg/kg twice daily.

In summary, PEA and related compounds might be of value as anti-allergic agents in dogs and cats due to their effect on mast cell activation. To our knowledge, these agents have not been tested for any antipruritic effect in animals with spontaneous allergic skin diseases.

Systemic treatments of pruritus
Opioid receptor antagonists/agonists
Mechanism of action
Endogenous opioids (e.g. dynorphins, enkephalins, endorphins, endomorphins, nociceptin) and opiates (e.g. morphine) bind to one of four opioid G-protein-coupled receptors (OP1 to 4) also known as delta (DOR), kappa (KOR), mu (MOR) or nociceptin receptors, respectively. Mu opioid receptor agonists induce mast cell histamine release. Epidermal keratinocytes and peripheral sensory neurons express both MORs and KORs. Recent data suggest that stimulation of MORs induces itch while that of KORs antagonizes it. These results are concordant with the observation that KOR stimulation inhibits MOR effects in both peripheral and central nervous systems.

Antipruritic efficacy in humans
Several randomized controlled trials have reported a significant antipruritic effect of the MOR antagonists naloxone, naltrexone, nalmefene in humans with cholestatic pruritus, chronic urticaria, and AD. Additional case reports suggested that MOR antagonists might be effective to reduce itch in human prurigo nodularis, epitheliotropic lymphoma, postburn pruritus, aquagenic pruritus, hydroxyethyl starch-induced pruritus and that of unknown origin. Interestingly, the topical application of MOR antagonists reduces itch in humans with AD suggesting a peripheral rather than, or in addition to, a central effect. Nalfurafine, a KOR agonists, has been shown to be effective to reduce uremic pruritus. Tachyphylaxis occasionally occurs in human patients treated in the long term with MOR antagonists due to the upregulation of MORs.

Antipruritic efficacy in small animals
A double-blinded crossover trial suggested the efficacy of a single injection of the MOR antagonist naloxone (1 mg/kg subcutaneously) in cats with excessive grooming. Naltrexone, another MOR antagonist, was reported to induce the remission of skin lesions in 7/11 dogs (64%) with acral lick dermatitis at the dosage of 2.2 mg/kg once daily per os. A beneficial effect was similarly reported in dogs with acral lick dermatitis treated with naltrexone (1 mg/kg subcutaneously) or nalmefene (1-4 mg/kg subcutaneously). The anecdotal observations above suggest that MOR antagonists, and possibly KOR agonists, deserve further investigations as antipruritic agents in small animals.
**NK1 receptor antagonists**

**Mechanism of action**

Substance P (SP) is a tachykinin oligopeptide that is released in the central nervous system and in primary afferent sensory neurons where it functions as an excitatory neurotransmitter; it is also secreted by endothelial cells, fibroblasts, keratinocytes and immune cells, such as mast cells. Of the three neurokinin (NK) receptors, SP binds preferentially to NK1R. Upon binding to its receptor, SP induces neurogenic inflammation. If injected in the skin, SP causes itch via both mast cell-mediated histamine release and mast cell-independent mechanisms. Finally, the NK1R appears to be involved in itch transmission at the spinal cord level. Aprepitant (Emend, Merck) and maropitant (Cerenia, Zoëtis) are both NK1R blockers, and they therefore serve as SP antagonists; they are approved for their effect as antiemetics.

**Antipruritic efficacy in humans**

Aprepitant has been shown to significantly reduce itch scores in ten human patients with atopic diathesis and refractory pruritus. It has been shown to be effective also in some individuals with prurigo nodularis, nephrogenic pruritus, Sézary syndrome, paraneoplastic and drug-induced pruritus.

**Antipruritic efficacy in small animals**

Evidence of the use of maropitant as an antipruritic agent could not be identified in the veterinary literature. However, in an informal internet survey, maropitant was perceived as providing only partial antipruritic benefit, and mostly as an adjuvant therapy, in 12/62 dogs with AD (Olivry, VetDerm list, March 2013). The effect might be stronger in cats with hypersensitivity dermatitis (Olivry, VetDerm list, March 2013). In summary, whereas its effect as an antipruritic drug in dogs with AD appears to be limited, maropitant might be deserving of further investigation in dogs with pruritus of unknown origin. The antipruritic effect of maropitant in cats needs to be documented formally.

**Gamma-aminobutyric acid (GABA) analogs**

**Mechanism of action**

Gabapentin and pregabalin are both GABA structural analogs that are approved for the treatment of epilepsy and for neuropathic diseases causing pain in humans. These drugs elicit their pharmacological effect by binding to the alpha2delta-subunit of voltage-dependent calcium channels, especially at the spinal cord level. This binding inhibits presynaptic calcium influx and decreases glutamate release and associated synaptic transmission. Furthermore, these molecules also inhibit the inflammation-induced release of SP and calcitonin-gene-related peptide (CGRP).

**Antipruritic efficacy in humans**

There is anecdotal evidence of the antipruritic efficacy of gabapentin and/or pregabalin in humans with neuropathic (e.g. brachoradial, postherpetic) pruritus and that associated with prurigo nodularis, cutaneous T-cell lymphosarcoma, burns or wound healing. Some patients with chronic pruritus of unknown origin have had rapid reductions in itch levels with GABA analogs. Randomized trials suggest the efficacy of gabapentin and pregabalin to reduce pruritus of chronic kidney disease and that following burns.

**Antipruritic efficacy in small animals**

Oral gabapentin (10 mg/kg every 8 to 12 hours), pregabalin (2-4 mg/kg every 8 hours) and/or carprofen (2 mg/kg every 24 hours) were found to induce complete cessation of scratching in 9/48 Cavalier King Charles spaniels (19%) with Chiari-like malformation and associated syringomyelia. Evidence for antipruritic efficacy of gabapentin and pregabalin for other diseases was not found.

**JAK inhibitors**

**Mechanism of action**

The Janus kinase (JAK) family encompasses four intracellular tyrosine kinases (JAK1, JAK2, JAK3 and TYK2) that transduce signals of numerous cytokine and chemokine receptors via the STAT signaling pathway. Oclacitinib (Apoquel, Zoëtis) is a novel JAK inhibitor that has been shown to inhibit the function of JAK1-dependent cytokines involved in allergic inflammation (IL-2, IL-4, IL-6, IL-13); it appears to have minimal activity against JAK-2 dependent cytokines involved in hematopoiesis or those associated with the innate immune response (ESVD-ECVD congress 2013). Oclacitinib reduces IL-31-induced pruritus in dogs, likely because of its interference with the IL-31 receptor signal transduction.

**Antipruritic efficacy in humans**

There is preliminary evidence of the efficacy of JAK inhibitors for treatment of human psoriasis, but details on antipruritic effect were not found.

**Antipruritic efficacy in small animals**

Recent randomized controlled trials have documented the rapid efficacy of oclacitinib (0.4 mg/kg twice daily) to decrease itch, as well as skin lesions, in dogs with flea allergy dermatitis and atopic dermatitis.
Conflict of Interest

The authors do not declare any conflict of interest directly relevant to the subject of this lecture.

References

Lasers in veterinary dermatology from theory to practice
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Can a laser help my practice?
The laser is a tool which will augment your diagnostic and therapeutic options. There are many procedures that can be performed more easily with the laser than with traditional surgery. There are also procedures that cannot be done or wouldn’t normally be attempted with traditional surgery. The laser will also enhance the public image of a practice. The public is well educated and desires less painful alternatives to traditional surgery. Improved healing and increased comfort are non-economic factors that may lead to increased numbers of procedures and referrals.

How do lasers interact with tissue?
The energy from the laser light is transferred to the tissue. Depending on the wavelength of the laser light, the optical properties of the irradiated tissue and the technique of the surgeon, the laser energy interacts with the target tissue. There are different types of lasers available, which produce different wavelengths of light, different beam intensity, and different temporal patterns of the laser beam. The wavelength of the beam depends on the lasing media. The lasing media is usually the product the laser is named after. For example, in a CO\(_2\) laser the lasing media is CO\(_2\) and it produces a wavelength of 10,600nm. This wavelength is in the infrared range and is invisible to the human eye. Helium neon was the lasing media of the older red laser pointers. Argon, Krypton and KTP lasers produce light in the blue green range. Eximer lasers produce light in the ultraviolet range. There are dye and diode lasers with adjustable wavelengths.

The beam intensity is the amount of energy per area of tissue. A 10 watt laser applied into a 2mm tip will deliver 320 Watts/cm\(^2\). This is only enough to warm tissue. A CO\(_2\) laser needs to deliver 4500-5000 Watts/cm\(^2\) to ablate tissue efficiently. A 10 Watt laser through a 0.8mm spot will only deliver 2000 Watts/cm\(^2\). Changing to a 0.4mm tip will increase the beam intensity to 8000 Watts/cm\(^2\). The energy delivered to tissue creates heat. The longer it takes to cut tissue the more time the peripheral tissue is heated thus creating collateral thermal damage. Thermal relaxation is the time it takes for tissue to cool. This knowledge has been utilized to produce lasers with different temporal pattern to deliver pulsed beams versus continuous beams of energy. This allows improved surgical precision and decreases unwanted collateral tissue damage. By applying a high energy in rapid pulses there is specific selection of the energy being delivered to the specific tissue selected and not the surrounding tissue.

The interactions of laser light with tissue include:
1. reflection of light without penetration of the target tissue
2. transmission of light through the tissue
3. scattering of light within the tissue
4. absorption into the tissue and transforming the energy into a photo-thermal reaction
5. photo disruption where the high energy pulse generates a shock wave
6. photochemistry is which the light energy reacts with a chemical and stimulates a chemical process in the tissue

What determines which of the above interactions that will occur is based on the composition of the target tissue and the wavelength of the light beam. The various wavelengths are preferentially absorbed by tissue based on the tissues composition. Thus a laser is chosen for a procedure based upon the optical properties of the target. In our case, this is skin. Tissues have different energy absorption coefficients based on their water, hemoglobin, melanin, and protein contents. Melanin and hemoglobin are present in the skin. Tissues of this color absorb blue and green wavelengths but not red wavelengths. Therefore a krypton or KTP laser is a better choice to remove a port wine stain or an angiomatosis lesion. Water is clear but does absorb light of longer wavelengths in the infrared range. Also the shorter the wavelength the deeper the light beam may penetrate tissue. This may cause damage to tissues deeper than the target tissue and must be taken into account. Therefore the best laser for a procedure depends upon what type of tissue is being lased.

Which laser is best for which type of tissue?
CO\(_2\) has a very long wavelength of 10,600nm. It is highly absorbed by water creating a photothermal interaction. This results in vaporization of tissue with a high water content with minimal scatter, shallow penetration, and minimal peripheral tissue injury. These features make the CO\(_2\) laser very useful for most cutaneous procedures. Due to the long wavelength of the CO\(_2\) laser, it can only be delivered by hollow waveguides and articulated arms; therefore, it cannot be utilized for endoscopic procedures. Argon and potassium titanyl phosphate (KTP) lasers have short wavelengths of 524 and 532 nm respectively. For tissues high in hemoglobin, argon lasers and KTP lasers are preferentially absorbed. The problem with these lasers is their short wavelength allows them to transmit through tissue that does not have high hemoglobin content. This can result in peripheral thermal tissue injury. To minimize damage the amount of energy delivered to the target tissue should be closely monitored. Between these two lasers are diode lasers and neodymium yttrium aluminum garnet (Nd:YAG) lasers with wavelengths of 635 - 740nm, and 1,064nm respectively. Water and
hemoglobin will absorb these wavelengths but not as well as CO\textsubscript{2} laser is absorbed by water and argon is absorbed by hemoglobin. Therefore the depth of thermal injury to surrounding tissue may be greater if the amount of energy being delivered to the tissue is not closely monitored. Dye lasers have variable wavelengths of 400 - 1000 nm depending on the dye. They have been used together with photosensitizers for photodynamic therapy. Overall, for the most daily procedures in the veterinary dermatology practice, the CO\textsubscript{2} laser is the laser of choice.

What are the risks or safety issues when using laser in the veterinary practice?
With proper training and conscientious adherence to safety protocols the risks are very manageable. Most of the lasers being used in veterinary clinics produce heat and vaporization of the tissue. This creates a plume of smoke. The plume may contain viable organisms (bacterial or viral) as well as cells and can be irritating when inhaled. There are laser safe surgical masks available and the plume must be removed with a smoke evacuator. The evacuator has a filter that should be changed based on hours of use. The surgical technician is responsible for logging the amount of time the evacuator is in use.

As mentioned previously, there is heat generated by the laser, therefore surgical preparation should not include flammable products such as alcohol. Accidental fire can occur with flammable liquids, oxygen, paper drapes, or methane gases. It is imperative to prevent endotracheal tube fires. There are laser-safe endotracheal tubes available or the practitioner can protect standard tubes with saline or sterile water soaked gauze when using lasers well absorbed by water. If surgery is being performed around the anal area, water soaked gauze should be placed in the anus.

If proper technique is not utilized, the surgeon, staff, or patient may receive accidental skin burns. Remember the laser is a light energy much like a laser pointer used in lecturing. If the laser pointer is aimed at a hole in the projection screen the light will continue on to whatever is behind it. When cutting through tissue it is common to cut through one area more quickly than another. If the laser is passed over the area already incised it continues on and burns the tissue beyond. This may be the surgeon’s finger or another tissue on the patient. Burns can be minimized by directing the beam at the surgical site, accurately using the foot pedal to activate the laser beam and using sterile water soaked gauze or tongue depressors as a backstop. The surgical technician should put the laser in standby mode when not in use to prevent accidental discharge and burns.

The light from the laser can also be reflected and if reflected into the eye can create damage. Care should be taken not to aim the light at surgical instruments. There are laser instruments available with ebonized or burnished finishes which decrease reflected light. Personnel in the operating room must all wear protective eyewear. The type of eyewear needed varies with the type of laser. Regular glasses or safety lenses are sufficient for the CO\textsubscript{2} laser. The patient’s eyes should also be protected. Again sterile water soaked gauze may be placed over the eyes or there are special eye-cups and masks available. For more information, the American National Standards Institute publishes a book of safety standards and regulations called Safe Use of Lasers in Health Care facilities.

What are the advantages of using laser over traditional surgery?
One of the big advantages with most photothermal lasers is the sealing of small blood vessels. Using the laser provides a very dry surgical field even in highly vascular areas. A feline rhinectomy to remove squamous cell carcinoma may have taken 30 - 45 minutes with traditional surgery to control the hemorrhage, and with the CO\textsubscript{2} laser the procedure takes about 15 minutes.

Another very important benefit is decreased post-operative pain. The laser energy is painful at the time of surgery and general or local anesthesia must be used. However, post-operatively patients seem less painful and return to normal behavior more quickly. This can best be seen following a feline onychectomy or rhinectomy. The cats that have had a rhinectomy often are grooming and eating normally within 4 hours of their procedure. Another benefit is decreased swelling if used properly. The laser energy does not crush or tear tissue; it vaporizes it. It also seals lymphatics thereby decreasing swelling. However if there is significant peripheral tissue damage due to thermal damage additional swelling will occur.
Lasers in veterinary dermatology: Case discussion
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What are the applications for the CO₂ laser in dermatology?
The CO₂ laser has been very helpful in patients that have multiple epidermal lesions to be removed. Depending on size, these lesions once removed can heal by secondary intention as quickly as lesions closed with primary closure. Examples of more common lesions would include multiple sebaceous adenomas, hemangiomas, keratoacanthomas, and feline ceruminous cystomatosis. There are multiple breeds in which multiple sebaceous adenomas can affect the quality of life due to pruritus and secondary infections. The removal of these lesions can significantly decrease the requirement for medical therapy.

Highly vascular areas are more easily handled using the laser. One of the more common procedures laser has been recommended for is feline rhinectomy and/or pinnectomy. In the hands of an experienced laser surgeon, this procedure takes about 15 minutes, with more hemorrhage occurring from the sutures then the tissue removal. Masses may be removed or biopsied from the oral cavity. Remember care must be taken when oxygen is being administered. It can be used to remove melanomas on the penile sheath with less pain and hemorrhage. The laser is also very helpful in removing epidermal lesions difficult to close with primary closure, and in some cases laser is the only therapy with good success. Feline ceruminous cystomatosis is an example. This is an entity in which multiple fluid filled ceruminous cysts form initially on the tragal folds of the pinna. If left untreated, the cysts extend down into the canal, occlude the canal, and secondary otitis externa and/or media develops. The cystic tissue is very thin and often adjacent to the pinnal cartilage. This makes removal of the entire cyst difficult with traditional surgery and without the removal of the entire cyst recurrence is likely. Another example is cutaneous angiomatosis. In veterinary patients, due to the progressive proliferative nature of this condition, previous recommendations included wide surgical excision or amputation. If the lesions occurred in a location where this was not possible (such as the face) the pets were euthanised. Laser therapy has been reported to be a successful alternative in these cases.

Infected tissue can be vaporized or removed using the laser. Surgical technique is very important so that the healthy tissue to remain is not contaminated with infected cells. The types of infections where this is important include papilloma virus, herpes dermatitis, mycobacterial granuloma and sarcoids. The utilization of the laser with proper surgical technique has resulted in decreased recurrence rates post therapy. Another type of infected tissue includes chronic proliferative infected tissue. This is most commonly seen in cases of chronic otitis in certain breeds such as the American Cocker Spaniel. If this problem is identified prior to calcification of the canal occurring, the duration of medical therapy may be shortened or total ear canal ablation may be avoided. Once the deep folded cauliflower like tissue is removed medical therapy is much more effective.

Summary
In short the laser is a tool now available in veterinary medicine that allows us to provide state-of-the-art patient care. It allows procedures to be performed that could not otherwise be accomplished. It also provides a sterile, dry surgical field and improved healing, while also decreasing post surgical pain. If not used properly the patient and staff may be injured with the laser so training and experience are very important.

References

SCIENTIFIC SESSION
SCIENTIFIC SESSION

“The most bizarre case that we have ever seen”
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Notes
Keratinocyte differentiation and cornification abnormalities in hereditary nasal parakeratosis in Labrador retrievers

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Hereditary nasal parakeratosis (HNPK) is an inherited disorder affecting Labrador retrievers. The main clinical feature is a non-pruritic parakeratotic hyperkeratosis of the nasal planum, but the exact mechanism leading to the clinical signs is currently unknown. Very recently, HNPK has been associated with a specific mutation affecting the \textit{SUV39H2} gene, which encodes for histone H3-K9 methyltransferase-2. Histones and their modifications influence chromatin structure, thereby resulting in transcriptional alterations of affected genes. The biological effect and relevance of the mutated \textit{SUV39H2} gene in Labrador retrievers with HNPK remains unknown. The aim of this study was the phenotypic characterization of the altered keratinocyte differentiation and cornification process in affected Labrador retrievers having a mutation of the \textit{SUV39H2} gene. Formalin-fixed biopsies of the nasal planum of dogs with HNPK (\(n=6\)) and non-affected control dogs (\(n=6\)) were collected and screened by immunofluorescence for the presence and distribution of selected epidermal proliferation and differentiation markers including Ki-67, involucrin, loricrin, desmogleins 1 and 2 (DSG 1/2) and keratins (K)1, 10 and 14. Ki-67 staining results suggested that epidermal proliferation was not enhanced. Immunostaining for K14, K1, K10 and DSG1/2 was similar between affected and control biopsies. In contrast, the expression of loricrin and involucrin was altered in biopsies from affected dogs. Our study results suggest that mutations in the \textit{SUV39H2} gene cause abnormal terminal differentiation of nasal planum keratinocytes.

Source of funding
Self-funded

Conflict of interest
None declared
SHORT COMMUNICATIONS

Chitin and lipopolysaccharide modulate innate immune responses of the canine keratinocyte cell line CPEK

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Toll-like receptors (TLRs) play a central role in the cutaneous innate immune system. Chitin is a component of several pathogens, including ectoparasites and fungi, and it can be recognized by the cutaneous innate immune system. Lipopolysaccharide (LPS) is found in the outer membrane of Gram-negative bacteria and it is known to elicit strong immune responses. We hypothesized that chitin and LPS exposure would activate innate immune responses of keratinocytes by modulating TLR expression and chemokine secretion. The CPEK canine cell line was cultivated and then stimulated with sonicated chitin (0.2 mg/mL and 2 mg/mL) and with LPS (10 μg/mL and 100 μg/mL). The expression of TLRs 1-9, interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) were measured after 3, 24 and 48 h. Non-stimulated CPEK cells (control cells) expressed TLRs 1 to 6 and TLR-9, and did not express TLRs 7, 8 and 10. Chitin induced a marked increase in the expression of TLR4 and TNF-α and slightly downregulated the expression of TLRs 3, 5, 6, 9 and IL-8. At high concentrations chitin strongly induced expression of TNF-α and was toxic to keratinocytes at 48h. Lipopolysaccharides upregulated the expression of TLR-4 and TNF-α, and downregulated the expression of IL-8. These results suggest that canine keratinocytes can recognize chitin and LPS, which appear to modulate the innate immune response. TLR-4 upregulation, as observed in other species, seems to play a central role in this process.

Source of funding
Affinity Petcare

Conflict of interest
None declared
Cutaneous larva migrans in an immunocompromised dog with a multiple nematode infection

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Angiostrongylus vasorum is a nematode classically responsible for respiratory, neurological, gastro-intestinal signs and/or bleeding in dogs. Skin lesions associated with this parasite are very unusual. We report here one case of cutaneous larva migrans compatible with this diagnosis. A 3-year-old female Weimaraner was referred for acute lesions on the nose, ear pinnae and one foot. The dog was otherwise healthy but had been under long-term treatment with glucocorticoids and azathioprine for aseptic meningitis for two years. Clinical signs included erythema, alopecia, papules and raised plaques with hyperkeratosis on the bridge of the nose and the ear pinnae and alopecia, swelling and penonyxis on one foot. No pruritus was reported. Biochemistry was unremarkable except for increased ALP. A complete blood count revealed a mild leucocytosis without eosinophilia. Histopathological examination demonstrated numerous dermal pyogranulomas with eosinophils centred around parasitic elements (200-300 µm long) compatible with larvae of Angiostrongylus vasorum. A Baermann’s test demonstrated the presence of numerous larvae of A. vasorum. Coproscopic examination also demonstrated eggs of Uncinaria stenocephala and Eucoleus boehmi. A few days later the dog developed respiratory distress and lethargy. Chest radiographs showed an alveolar and interstitial opacity compatible with angiostrongylosis. The dog was treated with fenbendazole 20 mg/kg/day for 3 weeks. A marked improvement of the skin lesions was reported 5 days after the first dose. To the best of our knowledge this is the first case of a dog infested with larvae of Angiostrongylus vasorum that presented initially with only skin lesions.

Source of funding
Self-funded

Conflict of interest
None declared
SHORT COMMUNICATIONS

Certifect-triggered pemphigus foliaceus in dogs: clinical, histological and immunological characteristics

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A recently launched topical ectoparasiticide containing fipronil, amitraz and S-methoprene (Certifect, Merial, Duluth, GA, USA) has been associated with the development of an acantholytic pustular dermatitis similar to that of Promeris-triggered pemphigus foliaceus (PF). Our objectives were to describe the clinical and immunological features of this PF-like cutaneous adverse drug reaction (CADR). Twenty dogs with a probable or definitive (Naranjo scale) diagnosis of PF-like CADR were identified between May 2012 and February 2013. Most dogs were middle-aged or older (median: 9 yrs) and of large size (median: 24 kg). In six dogs (30%), the PF-like lesions remained confined to the site of application, while 14 dogs (70%) exhibited lesions at distant sites. One or two applications of Certifect were sufficient to trigger PF-like lesions in seven (35%) and six (30%) dogs, respectively. Systemic signs were reported in eight dogs (40%), all with lesions extending to sites distant from application areas. Tissue-bound antikeratinocyte IgG were detected in the lesional epidermis of 8/18 (44%) cases by direct immunofluorescence, while serum antikeratinocyte IgG were detected in 9/14 (64%) cases by indirect immunofluorescence. Autoantibodies were found to target canine desmocollin-1 in 11/14 dogs (79%), but not canine desmoglein-1, by indirect immunofluorescence on transfected cells. These immunological findings were similar in cases with localized and distant disease. In conclusion, Certifect is capable of triggering the development of an acantholytic pustular dermatosis that clinically, histologically and immunologically closely matches Promeris-triggered PF and naturally occurring autoimmune PF in dogs.

Source of funding
Self-funded

Conflict of interest
None declared
Evaluation of pruritic reflexes used for the diagnosis of flea-related dermatoses in dogs

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The purpose of this study was to evaluate the usefulness of induced pruritus reflexes on previously identified pruritic areas to differentiate dogs with flea infestation (FI, abdomen) or flea-bite hypersensitivity (FBH, dorsolumbar area) from those with skin diseases not related to fleas. Two body areas were scratched for a maximum of 30 sec by the clinician: the umbilicus and the dorsolumbar area. A reflex was considered positive if the scratching triggered a pedalling motion, a labial motion, licking or chewing. This study included 191 dogs: 90 with flea-related dermatoses (FRD) (31 FBH, 59 FI) and 101 dogs with skin diseases not due to fleas (non flea-related dermatoses; NFRD). Umbilical and dorsolumbar reflexes were positive in 52 (58%) and 47 dogs (52%) with FRD, respectively; both were negative in 27 of these dogs (30%). Umbilical and dorsolumbar reflexes were positive in five dogs each (5%) with NFRD; both were negative in 92 of these dogs (91%). The positivity of either umbilical or dorsolumbar reflexes, or of both, was not correlated specifically to FI or FBH, but to FRD as a group. For the diagnosis of FRD, a positive reflex at either umbilicus or dorsolumbar areas had a sensitivity of 77%, a specificity of 87%, a positive predictive value of 87% and a negative one of 77%. In conclusion, umbilical and dorsolumbar reflexes appear useful in helping to diagnose FRD in the absence of visible fleas or of classic lesions of FBH.

Source of funding
Self-funded

Conflict of interest
None declared
**SHORT COMMUNICATIONS**

**Specific increased level of peripheral blood CD34+ cells in dogs with canine atopic dermatitis**

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The bone marrow might be involved in human atopic diseases, as shown by the specific release of CD34+ cells into the peripheral blood. The purpose of this study was to determine if a specific increase of CD34+ cells was also seen in atopic dogs. Three groups of dogs were included: those with non-food induced atopic dermatitis (NFIAD; 27), healthy dogs (13) and dogs with non-allergic inflammatory diseases (16). Dogs with NFIAD were selected after fulfilment of Favrot’s criteria with the exclusion of other pruritic dermatoses, rigorous flea control, and after no improvement following a hypoallergenic diet trial. Healthy dogs did not have any history or clinical signs of cutaneous or systemic diseases. Blood samples were obtained from all dogs and CD34+ cells were stained with phycoerythrin-conjugated anti-canine CD34 and enumerated using a flow cytometer. Kruskal-Wallis, Mann-Whitney and non-parametric Spearman’s rank correlation tests were used to analyse the data. Numbers of peripheral CD34+ cells in dogs with NFIAD (median: 1.7) were statistically higher than in dogs with other nonallergic inflammatory diseases (median: 1.0; P = 0.01) or healthy dogs (median: 0.9; p = 0.009). In dogs with NFIAD, a correlation was not noted between the numbers of CD34+ cells and the lesional (CADESI-03) or the pruritus (visual analog scale) scores. Results of this study suggest a possible involvement of CD34+ cells in dogs with NFIAD. These observations are consistent with those seen in human AD, although the role of these cells in the disease itself remains unclear.

**Source of funding**
Self-funded

**Conflict of interest**
None declared
A 7-year-old female American Staffordshire bull terrier was presented for generalized alopecia and recurrent demodicosis. Physical examination revealed pyrexia, depression and peripheral lymphadenopathy. Skin lesions consisted of generalized alopecia, hyperpigmentation, multiple dark exophytic warts and slightly prominent raised plaques affecting mainly the abdomen and coalescing on the distal limbs. Deep scrapes on alopecic areas demonstrated numerous *Demodex canis* mites. Histopathological examination of the exophytic lesions revealed a prominent granular layer with large keratohyalin granules. Keratinocytes had a pale cytoplasm and oval hypochromic nuclei with margined chromatin. Eosinophilic intracytoplasmic aggregates and intranuclear viral inclusions were visible; mitoses were frequently observed. An infection with canine papillomavirus 9 (CPV9) was confirmed by PCR. The dog was treated orally with ivermectin (0.5 mg/kg once daily; Ivomec, Merial, Villeurbane, France) and interferon-α (60 IU/day, Roferon-A, Roche, Boulogne, France). The demodicosis underwent remission within 4 months, but no improvement was noted for the papillomatosis. To our knowledge, this is the second isolation of CPV9 infection in a dog but the first report of this unusual clinical presentation. We propose to define this condition generalized verrucosis. This term was employed previously to describe a dog with a diffuse oral CPV infection that spread to the skin. In humans, however, generalized verrucosis is used for widespread cutaneous PV infections with up to thousands of lesions across the body; it is generally observed in patients with acquired immunodeficiency status or congenital immunodeficiencies. In our case, an immunodeficiency was suspected because of coexisting demodicosis, but it remained unproven.

**Source of funding**
Self-funded

**Conflict of interest**
None declared
The use of deslorelin to promote hair regrowth in dogs with Alopecia X

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Alopecia X affects dogs such as Nordic breeds, Pomeranians and miniature poodles. Its pathogenesis is not completely understood; it may be different from breed to breed. Treatment with hormones, mitotane, neutering and trilostane has given inconsistent results. Deslorelin (Suprelorin, Virbac, Bury St Edmunds, UK) is a nonsteroidal, peptide-based contraceptive implant containing a GnRH-agonist licensed for the induction of temporary infertility in healthy, non-castrated adult male dogs. Whether it exerts any role on the hormonal receptors at the skin/hair follicle levels is unknown. Our aim was to study whether the deslorelin implant promoted hair regrowth in dogs with alopecia X. Three chow chows, two keeshonds, one Chihuahua, one Pomeranian and one toy poodle were diagnosed with alopecia X, after ruling out other causes of alopecia by performing routine dermatological tests, an adrenal-gonadal and thyroid hormonal evaluation and skin biopsy. All dogs received a subcutaneous implant of deslorelin (4.7 mg/dog), and all treated dogs showed a progressive and profuse regrowth of hair within 2 to 4 months. Adverse effects were not noted, other than a decreased testicular size in intact males. Our findings suggest that deslorelin could be used to promote hair regrowth in dogs with alopecia X. This therapeutic approach may provide a more cost-effective treatment, as it appeared to be well-tolerated in this group of dogs. Further studies are required to determine the long-term efficacy and safety of deslorelin for treatment of alopecia X in dogs.

Source of funding
Self-funded

Conflict of interest
None declared
A cross-sectional survey of leishmaniosis in clinically normal and sick cats in Greece with indirect immunofluorescence antibody test and enzyme-linked immunosorbent assay


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Cats living in areas where canine leishmaniosis is endemic may become exposed to the parasite and develop anti-Leishmania antibodies. The aims of the present survey were to evaluate a population of clinically normal cats and cats with various diseases that lived in an endemic area for the presence of anti-Leishmania IgG and IgM, to compare the results of indirect immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) for anti-Leishmania IgG, and to investigate for possible associations between seropositivity to Leishmania spp. and several possible risk factors. Fifty clinically normal and 50 cats with various diseases were screened for anti-Leishmania IgG by IFAT and ELISA and for anti-Leishmania IgM by IFAT. Cut-off values for either test were established using serum samples from 25 clinically normal and 50 sick cats from a non-endemic area (Texas, USA). Low levels of anti-Leishmania IgG were detected by IFAT in 10/100 (five clinically normal and five sick cats) and by ELISA in 1/100 (one IFAT-negative clinically normal cat), whereas IgM antibodies were present in a single clinically normal cat. Seropositivity for Leishmania was not associated with either signalment, living conditions, health status or with seropositivity to feline leukemia virus, feline immunodeficiency virus, feline coronavirus, Toxoplasma gondii and Bartonella henselae. The low serum levels of anti-Leishmania IgG and the discordant results between IFAT and ELISA may challenge the validity of using serology in epidemiological studies in cats. The reasons for this discordance in serological results must be explored further.

Sources of funding
European Union (European Union (European Social Fund; ESF) and Greek national funds through the Operational Program Education and Lifelong Learning of the National Strategic Reference Framework (NSRF)-Research Funding Program: Heracleitus II-Investing in knowledge society through the European Social Fund

Conflict of interest
None declared
SHORT COMMUNICATIONS

Selection of an efficacious dosing regimen of oclacitinib (Apoquel®, Zoetis) for the control of atopic dermatitis in client-owned dogs using visual analog scale and CADESI scores


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Oclacitinib inhibits the function of multiple pro-inflammatory and pruritogenic cytokines that are dependent on Janus kinase (JAK) enzyme activation. Oclacitinib preferentially inhibits JAK1 dependent cytokines. The efficacy of three doses and regimens of oclacitinib for the control of canine atopic dermatitis compared to placebo was evaluated. Fourteen dermatologists enrolled 220 dogs with a history of chronic nonseasonal AD. Dogs were randomly allocated to one of four treatment groups: (T01) placebo, (T02) oclacitinib 0.4-0.6 mg/kg twice daily from Day 0-14 followed by once daily until Day 112, (T03) oclacitinib 0.4-0.6 mg/kg once daily from Day 0-112, and (T04) oclacitinib 0.2-0.3 mg/kg once daily from Day 0-112. Treatment success (TS) was defined as a ≥2 cm reduction from baseline for owner VAS score for pruritus or by a ≥50% reduction from baseline CADESI-02 scores. For pruritus assessments on Days 28, 56, 84 and 112, the three oclacitinib-treated groups showed a larger percentage of TS than the placebo-treated controls. Within the oclacitinib-treated groups, T02 had a larger percentage of TS (85%, 76%, 66%, 69%) than T03 (70%, 68%, 54%, 56%) and T04 (42%, 36%, 35%, 28%). Treatment success for the placebo-treated group was ≤5% at all timepoints. CADESI-02 assessments mirrored these findings: T02 had a larger percentage of TS (58%, 59%, 61%, 59%) than T03 (47%, 47%, 42%, 50%) and T04 (24%, 26%, 31%, 28%). Treatment success for the placebo-treated group was ≤7% at all time points. The twice-daily/once-daily dosing regimen (T02) was selected for later clinical trials.

Source of funding
Pfizer Animal Health, now Zoetis

Conflict of interest
All authors are current or former employees of Zoetis, formerly Pfizer Animal Health
The effect of flea treatment on the efficacy of oclacitinib (Apoquel®️, Zoetis) for the treatment of pruritus associated with canine allergic dermatitis


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Oclacitinib inhibits the function of multiple pro-allergic, pro-inflammatory and pruritogenic cytokines that are dependent on Janus kinase (JAK) enzyme activity. Four hundred and seven client-owned dogs with moderate-to-severe owner-assessed pruritus and a presumptive diagnosis of allergic dermatitis were enrolled. Dogs were randomized to receive either oclacitinib at 0.4 to 0.6 mg/kg orally twice daily or an excipient-matched placebo. A 10.0 cm long visual analog scale (VAS) was used by owners to assess pruritus severity from Days 0 to 7. Treatment success (TS) was defined as achieving a ≥2 cm reduction from baseline VAS score on at least 70% of the study treatment days assessed (i.e. Day 1 to Day 7). Treatment success on Day 7 was compared between dogs receiving flea control or not on Day 0, but regardless of whether fleas were present at that time. Sixty-five of 407 dogs (16%), (27/204 [13%] placebo-treated and 38/203 [18%] oclacitinib-treated) received flea products on Day 0. On Day 7, the percentage of TS for oclacitinib-treated dogs was approximately the same whether or not they were treated for fleas (63% versus 67%). By contrast, initiating flea treatment in placebo-treated dogs on Day 0 doubled the percentage of TS (52%) compared to dogs not treated for fleas (26%). In summary, adding flea treatment on Day 0 did not appear to impact the efficacy of oclacitinib for pruritus control in dogs with allergic dermatitis, but it could explain up to half of the pruritus reduction observed in placebo-treated dogs.

Source of funding
Pfizer Animal Health, now Zoetis

Conflict of interest
All authors are current or former employees of Zoetis, formerly Pfizer Animal Health
Canine nasal dermatitis: histopathological and immunopathological features of discoid lupus erythematosus and leishmaniosis

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In areas where canine leishmaniosis (CanL) is endemic, the most important clinical differential diagnoses for nasal planum erosive-ulcerative dermatitis in dogs are discoid lupus erythematosus (DLE) and CanL. The objective of this study is to compare histopathological and immunopathological features of nasal biopsies from dogs with DLE and CanL, both diagnosed on the basis of compatible clinical signs, histopathology results and response to treatment. Furthermore, CanL was confirmed through the demonstration of intralesional *Leishmania* by immunohistochemistry (IHC) using a standard protocol and a polyclonal anti-*Leishmania* spp. antibody. Sections of paraffin-embedded samples from 14 cases of DLE and seven of CanL were stained with haematoxylin-eosin. Additionally, serial sections were immunostained for T-lymphocytes (CD3), B-lymphocytes (CD20) and macrophages (Mac387) with positive stained cells counted in the dermis using an image analysis software. Superficial band-like and perivascular mononuclear cell-rich inflammation with basal cell damage was observed in both DLE (13/14) and CanL (6/7). A nodular-to-diffuse superficial and/or deep mononuclear cell-rich infiltrate was only seen in CanL (4/7). CD20-positive cells predominated over both CD3- and Mac387-positive cells in both DLE and CanL. The number of dermal Mac387 positive cells was higher in CanL compared to DLE. In conclusion, a band-like lymphoplasmacytic dermatitis with basal cell damage, a pattern suggestive of chronic DLE, was also found commonly in nasal biopsies from dogs with leishmaniosis. As a result, where CanL is endemic, the presence of *Leishmania* should be investigated by IHC in samples showing a histopathological pattern suggestive of DLE.

**Source of funding**
Hill’s Pet Nutrition (Italy), after agreement of the Canine Leishmaniasis Working Group

**Conflict of interest**
None declared
Leporacarus gibbus infestation in client-owned rabbits and their owner

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Leporacarus gibbus is an uncommonly reported rabbit fur mite infesting laboratory and pet rabbits. It usually causes subclinical infection and only rarely pruritic dermatitis. Two pet rabbits, living in the same household, presented with moderate scaling, erythema, pruritus and alopecia. The lesions were located mainly on the neck in both rabbits. A pruritic papular dermatitis was present on the owner’s arms and legs. Parasitological examination of the rabbits’ skin and fur revealed L. gibbus. Skin cytology and fungal culture were negative for bacterial and dermatophyte infections, respectively. Both rabbits were treated with a single application of a spot-on formulation of 1% moxidectin and 10% imidacloprid (Advocate, Bayer Animal Health, Leverkusen, Germany). The environment was also disinfected with a miticide. After treatment, the clinical signs of these two rabbits improved markedly, and the lesions on the owner’s arms disappeared. Leporacarus gibbus dermatitis in humans has only been reported once in the UK. The main lesions described here included small cutaneous papules on the owner’s arms and legs. Due to its zoonotic potential, even though it is uncommon, L. gibbus should always be considered as a possible differential diagnosis in pet rabbits when their owners are exhibiting a papular dermatitis.

Source of funding
Self-funded

Conflict of interest
None declared
SHORT COMMUNICATIONS

Oro-dental diseases and dermatological disorders are highly associated in pet rabbits: a case-control study

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Dermatological disorders (DD) and oro-dental diseases (ODD) are a major source of morbidity in pet rabbits. Additionally, ODD has anecdotally been associated with cutaneous disorders. The purpose of this study was to analyse the possible association between DD and ODD, in particular whether ODD increased the risk of DD development in pet rabbits. Medical records of 222 pet rabbits seen in 2010 in twenty private veterinary clinics in and around Naples (Italy) were retrospectively evaluated. Records of rabbits diagnosed with DD were selected. The frequencies of ODD and other variables were evaluated in rabbits with and without DD using logistic regression. Rabbits seen during the same time period without a diagnosis of DD were included as controls. The prevalence of DD was 28% (63/222) and that of ODD was 23% (51/222). There was a significant association between DD and ODD: rabbits diagnosed with ODD were 63 times more likely to be diagnosed with DD compared to rabbits without ODD (OR: 63; 95% CI: 23.9–170.2; P < 0.0001). Rabbits with ODD appear to be at greater risk of developing cutaneous disorders. Although coat condition and hair quality can be influenced by many biological and environmental factors, ODD should be carefully considered as a possible underlying condition in rabbits with DD. Further prospective studies are needed to evaluate a causal association and pathological factors.

Source of funding
Self-funded

Conflict of interest
None declared
Identification of three different Demodex species in cats using a novel PCR assay

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Demodex cati and Demodex gatoi are considered the two Demodex species of cats. However, several reports have also identified Demodex mites morphologically different from these two species in cats. DNA amplification/sequencing has been used effectively to identify Demodex mites in humans and dogs. The goals of this investigation were to develop a PCR technique to identify feline Demodex mites and to use this technique to investigate the prevalence of Demodex in cats. Demodex cati mites were obtained from a 16 year-old DSH cat with bilateral ceruminous otitis. Demodex gatoi mites were obtained from a 2-year-old Cornish red cat with pruritic dermatitis. Demodex mites, classified morphologically as a third species, were obtained from a 3-year-old DLH cat with partial alopecia. DNA was extracted and a 301 or 331bp fragment of the 16S rDNA was amplified and sequenced. Sequences of D. cati and D. gatoi mites shared 100% identity with those published in GenBank. The sequence of the third unnamed species was different, as it exhibited only 79% and 77% identity with the D. gatoi and D. cati sequences, respectively; this confirmed that it was a distinct Demodex species. Further hair samples from eleven cats were taken from ten skin locations. Three cats were positive for Demodex DNA. In one case the sequence corresponded to that of D. canis. A larger epidemiology study is underway, but preliminary results indicate that at least three Demodex species can be found in cats, and that D. canis mites can be found occasionally on feline skin.

Funding
Self-funded

Conflict of interest
None declared
SHORT COMMUNICATIONS

A retrospective study on the prevalence and causative allergens of food-induced atopic dermatitis in France

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Cutaneous adverse food reactions (CAFR) are known to trigger flares of atopic dermatitis (AD) in some dogs. The aim of this retrospective study was to determine the prevalence of CAFR in dogs with nonseasonal AD (i.e. food-induced AD; FIAD) in a French specialty clinic. From July 2009 to January 2012, 578 dog files were randomly selected. Canine AD was diagnosed in 336/578 dogs (58%) based on Favrot’s criteria. After exclusively feeding a commercial hydrolyzed diet (CHD; z/d ULTRA, Hill’s Pet Nutrition, Sophia-Antipolis, France) for 8-to-10 weeks, 123/336 dogs (37%) were determined by the dermatologist to be in full remission. Of these, 36/123 dogs (29%) were exclusively fed the CHD and remained in remission for at least 1 year (presumed FIAD). Fifty of 123 dogs (41%) underwent selected food challenges that triggered pruritus (confirmed FIAD). Positive challenges were found for zero, one, two, three or four food items in 12/50 (24%), 22 (44%), 11 (22%), 3 (6%) and 2 (4%) cases, respectively. Beef, chicken, lamb or pork meats, dairy products, rice and wheat were the offending allergens in 16/50 (32%), 13 (26%), 10 (20%), 8 (16%), 7 (14%), 3 (6%) and 1 (2%) dogs, respectively.

In this group of French dogs with nonseasonal AD, the prevalence of confirmed and presumed FIAD was 11% (38/336 dogs) and 14% (48/336 dogs), respectively. Regrettably, approximately one third of owners did not agree to follow the restrictive part of the diet with complete provocative food challenges to confirm the diagnosis of FIAD.

Source of funding
Self-funded

Conflict of interest
None declared
Prevalence of papillomavirus EcPV2 in clinically healthy horses in Switzerland

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The development of several genital disorders in horses, including penile papillomas and squamous cell carcinomas, has been proposed to be dependent on an infection with equine papillomavirus type 2 (EcPV2). However, little is known about the prevalence of this virus. Therefore, the aim of this study was to determine the geno- and seroprevalence of EcPV2 in clinically healthy horses in Switzerland. Cytobrush samples from the penis or vulva and serum samples were collected from 50 horses displaying neither skin or mucous membrane pathology nor severe signs of other diseases.

To determine the genoprevalence of EcPV2, DNA was extracted from the cytobrush samples and tested for viral DNA with a PCR assay that amplifies a 338 basepair fragment of the E7/E1 region of the viral genome. To determine the seroprevalence of this virus, an ELISA was designed to specifically detect antibodies against the major capsid protein (L1) of EcPV2. EcPV2 DNA was amplified by PCR, and further sequencing confirmed viral identity in 9/50 horses (18%). Antibodies against EcPV2 were detected alone in 31/50 horses (62%), while viral DNA and EcPV2-specific antibodies were found together in 4/50 horses (8%). This high seroprevalence suggests that EcPV2 is circulating intensely in the Swiss equine population, whereas active infections seem to be less common. The discrepancy between geno- and seroprevalence indicates different stages of infection in the tested population.

Source of funding
Self-funded

Conflict of interest
None declared
SHORT COMMUNICATIONS

Sero- and genoprevalence of FdPV2 in healthy cats in Switzerland

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Felis domesticus papillomavirus type 2 (FdPV2) is considered to play a major role in the development of viral plaques, Bowenoid in situ carcinomas and some invasive skin carcinomas. As there are only scarce data regarding the prevalence of FdPV2, our objectives were to determine its sero- and genoprevalence in clinically healthy cats in Switzerland. Serum and cytobrush samples were collected from two different body regions of 100 cats without clinical dermatological or other severe diseases. To determine viral seroprevalence, a GST-capture ELISA for the detection of specific antibodies against the major capsid protein (L1) was designed. To evaluate viral genoprevalence, we established a highly sensitive quantitative real-time PCR (qPCR) to concurrently determine copy numbers of FdPV2 DNA. Detectable levels of antibodies against FdPV2 were found in the serum of 19/100 cats. In 98 cats, viral DNA was detected in at least one of the two cytobrush samples. FdPV2 DNA amplification products were compared to those of the housekeeping gene GAPDH in each sample, and ratios ranged from 0.0004 to 202,664 indicating a very high variability in virus load among cats. A correlation between number of virus copies and OD values was not found. The variation in virus load, and the low percentage of cats having FdPV2-specific antibodies, might indicate that FdPV2 is often just carried by the animal, or that it induces subclinical infections without a strong antibody response. In conclusion, our results suggest a very high prevalence of FdPV2 among cats in Switzerland with widely varying virus loads.

Source of funding
Self-funded

Conflict of interest
None declared
Oclacitinib (Apoquel®, Zoetis) is a novel Janus kinase inhibitor that has activity against canine pro-allergic and pro-inflammatory cytokines

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Cytokine dysregulation can orchestrate a variety of cellular and molecular changes that lead to chronic conditions including allergy in dogs. Many cytokines thought to trigger such changes bind receptors that activate Janus kinase (JAK) enzymes. The objective of this study was to determine if the novel JAK inhibitor oclacitinib could reduce the activity of a variety of cytokines thought to induce many of the clinical signs associated with allergic conditions in dogs. Using isolated enzyme systems and in vitro human or canine cell models, the potency and selectivity of oclacitinib was evaluated against individual JAK family members as well as cytokines dependent on JAK activation. Oclacitinib inhibited JAK family members by 50% at concentrations (IC_{50}s) ranging from 10-99nM and did not inhibit a panel of 38 other non-JAK kinases (IC_{50}s > 1000nM). Oclacitinib was most effective at inhibiting JAK1 (IC_{50} = 10nM). Oclacitinib also inhibited the function of JAK1-dependent cytokines involved in allergy and inflammation (IL-2, IL-4, IL-6, and IL-13), as well as those that cause pruritus (IL-31) at IC_{50} ranging from 36-240nM. Oclacitinib had minimal activity against JAK2-dependent cytokines involved in haematopoiesis (erythropoietin and GM-CSF; IC_{50} > 1000nM), and it did not inhibit other JAK2-dependent cytokines involved in innate immune responses (IL-12, IL-23; IC_{50} >3000nM). These results demonstrate that oclacitinib selectively inhibits JAK1-dependent cytokines involved in allergy, inflammation and pruritus. As a result of this widespread yet role-specific anti-cytokine activity, oclacitinib is likely to be effective to treat clinical signs of allergic diseases in dogs.

Source of funding
Pfizer Animal Health, now Zoetis

Conflict of interest
all authors are current or former employees of Zoetis, formerly Pfizer Animal Health, or employees of Pfizer
Evaluation of patch testing with proteins, carbohydrates and commercial foods for diagnosis of canine adverse food reactions

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The gold standard for diagnosing canine adverse food reactions (AFR) remains a dietary restriction-provocation trial. Recently, patch testing with food allergens was reported to be helpful for choosing ingredients for an elimination diet. The aim of this study was to further evaluate patch testing with proteins, carbohydrates and commercial foods in dogs. In 25 dogs suspected of AFR, patch testing was performed with raw and cooked meat (n=16), salmon and carbohydrates (n=11) and with their own commercial foods (n=4); the median number of patches per dog was 21 (range 17-30). After 48 hours, skin reactions were evaluated. In each dog, patch test results were compared with the outcome of sequential oral food challenges. Overall, the sensitivity of patch testing was 78% and its specificity 82% (138 comparisons patch versus challenges). For proteins (meats and salmon), the sensitivity was 100% and specificity 69% (62 comparisons), for carbohydrates 70% and 83% respectively (49 comparisons). For commercial foods, the sensitivity was low (22%) but the specificity highest (100%); 27 comparisons). A positive patch reaction was observed to both raw and cooked proteins in 93% of cases, and to raw proteins in the others; there were no reactions solely to cooked meats. In conclusion, patch testing seems a useful tool to predict food antigen reactivity--especially against meat proteins--with the majority of positive reactions being seen against raw proteins.

Source of funding
Royal Canin

Conflict of interest
C. Johansen’s residency is supported by Royal Canin; C. Mariani is an employee of this company; in the last five years, R. Mueller has obtained funding, lectured or consulted for Royal Canin
First report of straelensiosis in cats and unique features of the canine disease in Israel

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Straelensia cynotis is a parasitic larva that causes nodular dermatitis in outdoor dogs. To date, the disease has been documented in dogs in France, Spain and Portugal. Herein, we report the first cats infested with this parasite, as well as some unique aspects of canine straelensiosis in Israel. Two cats and ten dogs were diagnosed with straelensiosis between February 2003 and January 2012 in Israel. Both cats exhibited erythematous macules and nodules on the abdomen; one was extremely pruritic, while lesions were incidentally detected during neutering in the other cat. The histopathology of feline straelensiosis appeared similar to that of the canine disease. Clinical signs in dogs included multiple small erythematous or crusted papules scattered over the head, dorsum and limbs. Additionally, this disease was associated with severe pruritus in 6/10 dogs (60%). Treatment consisted of different combinations of drugs, including systemic avermectins and topical insecticides or acaricides. All animals experienced a complete resolution of clinical signs with any therapeutic regimen used; amitraz was the most effective treatment as it led to fast (median: 5 weeks) and complete resolution of signs in the five dogs that received it. In 4/5 dogs, treatment consisted of 1:200 amitraz dips every 4 to 7 days, while the last dog wore an amitraz collar. In summary, we report herein that cats are also susceptible to straelensiosis, which causes erythematous ventral lesions in this species. Furthermore, in Israel, canine straelensiosis is often very pruritic and appears to respond best to amitraz.

Source of funding
Self-funded

Conflict of interest
None declared
Meticillin-resistant Staphylococcus pseudintermedius (MRSP) has emerged as a highly drug-resistant pathogen. Although often isolated from outpatient in veterinary clinics, there is concern that MRSP could follow a similar epidemiology as meticillin-resistant Staphylococcus aureus (MRSA), that is an important nosocomial pathogen for humans. Our objective was to identify risk factors for MRSP infections in dogs and cats in Germany. Clinical data from cases of MRSP (n=150) and meticillin-susceptible S. pseudintermedius (MSSP) controls (n=133) six months prior to staphylococcal isolation were analysed by multivariable logistic regression. The identity of all MRSP isolates was confirmed genotypically through demonstration of S. intermedius-group specific nuc and mecA. In the final model, cats (OR 22, 95% CI 2-239; P = 0.01), animals that had been hospitalized (OR 105; 95% CI 21-518; P < 0.001), had visited veterinary clinics more than once (OR 2, 95% CI 1-3; P = 0.001) as well as those that had received topical ear medication (OR 5, 95% CI 2-15; P=0.003) or glucocorticoids (OR 20, 95% CI 6-64, P < 0.001) were associated with MRSP infection, whereas S. pseudintermedius isolates from ears were more likely to belong to the MSSP-group (OR 0.09, 95% CI 0.03-0.34, P < 0.001). These results indicate a likely association of MRSP with veterinary clinic or hospital settings, and perhaps also an association with chronic skin inflammation. The unexpected lack of association between MRSP isolation and antimicrobial therapy requires further investigation; it may indicate that this bacterium adapted to canine skin with little need for selective pressure.

Source of funding
ESVD research grant

Conflict of interest
None declared
Short communications

Genetic insights into the emergence of multidrug-resistance in meticillin-resistant Staphylococcus pseudintermedius

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Staphylococcus pseudintermedius (SP) is a common canine pathogen and most infections are treated successfully with antimicrobials. However, meticillin-resistant strains (MRSP) with a multidrug-resistant phenotype have recently emerged. In MRSP infections, all classes of clinically relevant antimicrobial agents may be ineffective and zoonotic infections can occur. Our objectives were to identify mobile genetic elements (MGE) responsible for the multidrug-resistant phenotype of MRSP. Antimicrobial resistance genes, putative MGEs and their genomic location were identified by sequencing and analysing five MRSP genomes using the Illumina HiSeq platform. A collection of 60 meticillin-susceptible (MS) SP and 64 MRSP from the UK and Germany were screened by PCR for the presence of MGEs including \textit{mecA} and the transposase of \textit{Tn}\textsubscript{5405}. Related phenotypic resistance was assessed by agar dilution screening including erythromycin, kanamycin, streptothricin and trimethoprim. Amongst \textit{mecA}-positive SP, two genetically distinct types were found: strain ST71 possessed a \textit{Tn}\textsubscript{5405}-like transposon, carrying five resistance genes (\textit{aphA}, \textit{sat}, \textit{aadE}, \textit{ermB}, \textit{dfrG}) and \textit{IS}\textsubscript{tetM}\textsubscript{916} (\textit{aac-aph}); \textit{Tn916} (\textit{tetM}) and \textit{IS}\textsubscript{1272} (\textit{aac-aph}) were characteristic for other types. Resistance to \(\beta\)-lactam antibiotics correlated with the presence of \textit{mecA}, while the multidrug-resistant phenotype (13\% of MSSP, 88\% of MRSP) was associated with the presence of the \textit{Tn}\textsubscript{5405}-like transposon \((p<0.001)\). Despite the seemingly sudden emergence of MRSP, at least two different MRSP genetic backgrounds (ST71 and ST118) have evolved independently in Europe. Each has unique transposon and resistance profiles, involving four distinct MGEs. These MGEs in MRSP are a risk for horizontal gene transfer into the human pathogen \textit{S. aureus} and are a public health concern.

Source of funding
Royal Veterinary College

Conflict of interest
None declared
SHORT COMMUNICATIONS

Antimicrobial susceptibility monitoring of dermatological pathogens isolated from diseased dogs and cats across Europe (ComPath I, 2008-2010)

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ComPath is the first pan-European antimicrobial susceptibility monitoring program for pathogens isolated from diseased dogs and cats not recently treated with antimicrobials. Samples were collected from animals with skin, ear and soft tissue infections in 10 European countries. Aerobic bacteria were isolated and identified by standard biochemical methods in national laboratories (one strain per bacterial species per animal per owner). Minimal inhibitory concentrations (MICs) were determined for 14 commonly used antibiotics in a central laboratory by agar dilution according to CLSI M31-A3 standards. Results were interpreted using CLSI breakpoints where available. In total, 1,182 canine and 226 feline strains were recovered. In dogs, *Staphylococcus pseudintermedius* (n = 556) susceptibility varied from 70-80% for penicillin, clindamycin and chloramphenicol to ≥91% for amoxicillin/clavulanic acid, ampicillin, oxacillin, gentamicin, enrofloxacin, marbofloxacin and orbifloxacin, and for *Staphylococcus aureus* (n = 45) from 49-58% for penicillin and ampicillin to ≥91% for amoxicillin/clavulanic acid, oxacillin, gentamicin, clindamycin, chloramphenicol, enrofloxacin, marbofloxacin and orbifloxacin. For canine streptococci (n = 167), resistance against penicillin, amoxicillin/clavulanic acid, ampicillin, chloramphenicol, enrofloxacin, marbofloxacin and orbifloxacin was very low (<2%). Canine *Escherichia coli* (n = 108) showed good susceptibility (≥86%), except to ampicillin, whereas resistance was frequently seen in *Pseudomonas* spp. (n = 173). Generally, susceptibility ranges for the feline isolates were comparable to those for dogs. In total, 44 staphylococci harboured a meca gene, 36 from dogs and 8 from cats. This is the first international antimicrobial susceptibility monitoring program to use standardized methods and centralized MIC determination.

**Sources of funding**

Bayer, MSD, Novartis, Vetoquinol, Virbac and Zoetis

**Conflict of interest**

All authors are full-time employees or consultants of the above listed veterinary pharmaceutical companies.
In vitro activity of pradofloxacin against canine and feline pathogens recovered from skin infections in four European Union countries

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Bayer Animal Health, Leverkusen, Germany

Pradofloxacin (Veraflox, Bayer Animal Health, Leverkusen, Germany) is a novel fluoroquinolone approved for the treatment of bacterial infections in dogs and cats. It has enhanced antibacterial activity compared with conventional fluoroquinolones, covering major Gram-positive and Gram-negative aerobic and anaerobic pathogens. The aim of this survey was to study the pradofloxacin susceptibility of common aerobic bacterial pathogens isolated from acute skin and soft tissue infections in dogs and cats across Europe. Pre-treatment bacterial isolates from dogs and cats with skin infections were obtained from local diagnostic laboratories in Germany, Hungary, Sweden and the United Kingdom between 2007 and 2012. Minimum inhibitory concentration (MIC) values were determined by agar dilution method (CLSI; M31-A3, 2008) and MIC_{50} and MIC_{90} were calculated. In total, 1049 isolates were tested, 77 % from dogs and 23 % from cats. The most frequently isolated species was Staphylococcus pseudintermedius (n=469), followed by Escherichia coli (n=90), Streptococcus canis (n=84), Pasteurella multocida (n=68), coagulase-negative staphylococci (n=46), Pseudomonas aeruginosa (n=38), Staphylococcus aureus (n=33) and various minor species (n=221). For the major species, MIC_{50} values ranged from 0.008 to 0.06 µg/mL, except for S. canis (0.125 µg/mL) and P. aeruginosa (0.25 µg/mL). The MIC_{90} values varied between 0.015 and 0.125 µg/mL, except for P. aeruginosa (0.5 µg/mL). There were no notable differences in MIC patterns among the four countries. In conclusion, the survey demonstrates high in vitro activity of pradofloxacin against major canine and feline pathogens, particularly Gram-positive bacteria, isolated from skin infections.

Source of funding
Bayer Animal Health

Conflict of interest
The authors are employees of Bayer Animal Health
Development of an enzyme-linked immunosorbent assay for the serodiagnosis of ringworm infections in cattle

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Several enzyme-linked immunosorbent assays (ELISAs) have been developed to evaluate antibody responses in dermatophytosis of animals, but only few focused on detecting specific antibodies in cattle dermatophytosis. The goal of this study was to develop an in-house ELISA based on recombinant antigens for the serological diagnosis of cattle dermatophytosis. Antigens consisted of available recombinant forms of either *Trichophyton rubrum* dipeptidyl peptidase V (TruDppV) or leucin aminopeptidase 2 (TruLap2), which are 98% identical to *Trichophyton verrucosum* orthologues. Sensitivity (Se), specificity (Sp), positive (PPV) and negative (NPV) predictive values of both ELISAs were determined using field serum samples from 135 cattle with dermatophytosis, diagnosed by microscopy and PCR analyses, and from 55 healthy cattle without history of dermatophytosis (negative controls). Differences between the optical density (OD) mean values obtained in both animal groups were highly significant, showing that our ELISAs can discriminate between infected and healthy animals (Mann-Whitney U test; \(P < 0.0001\)). Using a cut-off point equal to the mean OD + 2 standard deviations of control sera, the ELISA detecting specific antibodies against DppV had the following performance: 90% Se, 93% Sp, 97% PPV and 78% NPV. The recombinant TruLap2-based ELISA exhibited 88% Se, 91% Sp, 96% PPV and 76% NPV. This is the first ELISA based on recombinant antigens to assess the immune response in bovine dermatophytosis. It could be useful in epidemiological studies and for the evaluation of vaccines and/or vaccination procedures.

Sources of funding
Grant 3.4558.10 from Fonds de la Recherche Scientifique Médicale (FRSM, Belgium) and 594/2012 from Executive Unit for Higher Education, Research, Development and Innovation Funding (UEFISCDI, Romania). This research was made possible thanks to the agreement that binds the Wallonia-Brussels (WBI) and Romania

Conflict of interest
None declared
A pilot uncontrolled open study on the use of Oxalic (Medeor International) for treatment of sebaceous gland adenoma/hyperplasia in dogs

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Oxalic is a solution containing nitric acid, organic acids and metallic salts; it is proposed as a topical alternative to surgery for management of sebaceous gland adenoma/hyperplasia (SGAH). We report herein the results of an open uncontrolled trial to evaluate the efficacy of Oxalic (Medeor International, Braine-l’Alleud, Belgium) for treatment of SGAH in dogs. Healthy dogs of all ages with at least one verrucoid lesion typical of SGAH were included in the study. The presence of preexisting local inflammation and the use of topicals other than antiseptics or antibiotics were exclusion criteria. Oxalic was applied, following the manufacturer’s instructions, once on Day 0; it was reapplied 14 days later if the clinical response was considered unsatisfactory. Treated lesions size (diameter and height) and subjective evaluation of treatment response were recorded at two-week intervals. Twenty-nine dogs were included and 35 SGAH lesions (mean size on Day 0: 7.7 X 4.3 mm) were treated. Nine of 35 masses (26%) needed retreatment on Day 14. On Days 14 and 28, significant mean reductions of 34% and 71% (ANOVA, P < 0.001) respectively for lesion diameter, and 61% and 86% (P <0.001) respectively for lesion height were observed. The owners and veterinarians subjectively evaluated the treatment response as excellent in 89% and 83% of dogs, respectively. The tolerance of Oxalic was reported as excellent by both owners and veterinarians in 97% of dogs. In summary, Oxalic appears to be an effective and well-tolerated topical alternative to surgical treatment for lesions of SGAH in dogs.

Source of funding
Oxalic was provided free of charge by Medeor International

Conflict of interest
J. Fontaine consults for Medeor International
Retrospective assessment of previous antibiotic therapy in dogs diagnosed with meticillin-resistant Staphylococcus pseudintermedius pyoderma

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The emergence of meticillin-resistant Staphylococcus pseudintermedius (MRSP) has become a significant animal health problem. A recent study has indicated that previous antibiotic exposure is a factor in the development of canine MRSP pyoderma. The purpose of this study was to identify any association between prior antibiotic use and MRSP pyoderma in dogs presented to a veterinary teaching hospital. The medical records of canine MRSP and meticillin-susceptible Staphylococcus pseudintermedius (MSSP) pyoderma diagnosed between January 2006 and November 2012 were reviewed. These included cases of deep or superficial, chronic or recurrent MRSP or MSSP pyoderma with at least a twelve-month drug history prior to the diagnosis. Fifty-three MRSP and 45 MSSP cases met the inclusion criteria: 52/53 (98%) MRSP and 42/45 (93%) MSSP cases received at least one course of antibiotics prior to their diagnosis. The number of antibiotic prescriptions in MRSP cases (mean: 4.5) was higher than for MSSP (mean: 2.5) (P < 0.0001). The number of different antibiotic classes prescribed in MRSP cases (mean: 2.5) was higher than in MSSP (mean: 1.9; P = 0.0086). The percentage of MRSP cases given beta-lactam antibiotics (98%) was higher than that of MSSP (82%; P = 0.0066). Finally, the percentage of MRSP cases receiving concurrent anti-inflammatory therapy (e.g. glucocorticoid and ciclosporin) was higher (62%) than that of MSSP (42%; P = 0.048). These results suggest that the number of antibiotic prescriptions, exposure to multiple antibiotic agents or a certain class of antibiotics, and concurrent anti-inflammatory therapy may be associated with MRSP pyoderma.

Source of funding
Self-funded

Conflict of interest
None declared
Efficacy of the low-level laser therapy on hair regrowth: a preliminary study on 8 cases of non-inflammatory alopecia in dog

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The term ‘non-inflammatory alopecia’ embraces a diverse group of dermatological diseases; these can be challenging conditions to manage. We aimed at testing the clinical efficacy of low level laser therapy (LLLT) on hair regrowth in dogs with non-inflammatory alopecia. Eight dogs of different age, breeds and genders were included in the study after a clinical and histopathological diagnosis of non-inflammatory alopecia. Each dog was treated (5 min/administration) twice weekly for a maximum of 2 months with a type BTL 4000 (BTL Italia, Salerno, Italy) therapeutic laser with a cluster probe, equipped with three different wavelengths emerging simultaneously from 21 foci: 13x16 mW (470nm), 4x50 mW (685 nm) and 4x200 mW (830 nm). A predetermined alopecic area was left as an untreated control. Seven dogs ended the study; 4/7 received 16 laser treatments and 3/7 only 10. In 5/7 animals the coat regrowth was complete, and in 2/7 an improvement in the hair density and length was noticed. Biopsies were collected from treated and non-treated sites at the start and end of the study from one dog: on transverse sections the majority of hair follicles in the non-treated sites remained in the kenogen phase as at diagnosis. In contrast, the majority of follicles were in anagen in the treated areas. In this small number of dogs, low-level laser therapy appeared to be beneficial for treatment of non-inflammatory alopecia. Further studies with larger numbers are required. Histologically, transverse sections appear to facilitate the assessment of the percentage of kenogen follicles in alopecic conditions.

Source of funding
Società Italiana di Dermatologia Veterinaria (SIDEV)

Conflict of interest
None declared
Expression patterns of selected desmosomal, tight and adherens junction proteins in an experimental model of canine atopic dermatitis skin lesions

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In recent years, the stratum corneum was found to be important for providing a functional barrier against environmental allergens in humans and animals with atopic dermatitis (AD). Recently, the expression of intercellular epidermal tight junction proteins was shown to be reduced in human AD, thereby providing further evidence for defective epithelial permeability in this disease. We studied the expression of selected upper epidermal desmosomal, tight and adherens junction proteins in an experimental model of canine AD. Two types of control and house dust mite (HDM) extract-containing patches were applied to the skin of six Maltese-beagle atopic dogs hypersensitive to HDM. Patches were left on for 48 h and biopsies were collected 24 h after removal. Normal canine skin served as another control. Frozen skin sections were stained by indirect immunofluorescence for corneodesmosin (CDSN), desmoglein-1 (DSG1), desmocollin-1 (DSC1), claudin-1 (CLDN1) and E-cadherin (CDH1). Each expression pattern was assessed for its continuity on the entire epidermis of each section. The immunostaining of DSG1, DSC1 and CDH1 was intercellular and continuous in all control and HDM patches. In contrast, the immunoreactivity of CDSN and CLDN1 was discontinuous in 12/12 and 8/12 HDM patches, respectively, but in none of the control patches and normal skin (Fisher’s exact test, P < 0.001). These observations suggest that HDM allergens, either via proteolytic digestion and/or because of induced allergic inflammation, might affect the integrity of corneodesmosomal and tight junction proteins. Ensuing intercellular junction alterations could promote further penetration of allergens through the epidermis.

Source of funding
Self-funded

Conflict of interest
None declared
Evaluation of the usefulness of Doppler blood flow in the diagnosis of canine cutaneous adverse food reactions

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A simple diagnostic tool that could indicate whether a diet is likely to be a flare factor in canine cutaneous adverse food reactions (CAFRs) would be very helpful. In this study, the range of the resistive (RI) and pulsatility indices (PI) in the cranial mesenteric and celiac arteries were determined in eight healthy dogs fed various diets, and in 12 dogs with previously diagnosed CAFRs when fed appropriate hydrolysed or novel protein diets, as well as during dietary provocations. Resistive index and PI were calculated using Doppler ultrasonography with a micro-convex 7.5 MHz probe. Each examination consisted of five ultrasound assessments: pre-prandial, and 20, 40, 60 and 90 minutes post-prandial. Dogs with CAFRs showed a RI < 0.82, whereas healthy dogs had a RI > 0.82 in both mesenteric and celiac arteries 60 minutes after the administration of the provocation diet (P < 0.005). There were no significant differences between groups at the other time points, or when the hypoallergenic diet was fed. A significant difference in PI values was not found between groups. Using an RI < 0.82 cutoff in the mesenteric and the celiac arteries after 60 minutes of dietary provocation in a group of 21 dogs diagnosed with non-food-induced atopic dermatitis or CAFR yielded a test with 100% sensitivity, 76% specificity, 50% positive predictive value and 100% negative predictive value for the diagnosis of CAFR. These results suggest that Doppler ultrasonography might have some value in helping diagnose CAFRs in dogs.

Source of funding
Self-funded

Conflict of interest
None declared
Histopathological characteristics and expression of Toll-like receptor 2 in lesional skin of dogs with papular dermatitis due to Leishmania

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Papular dermatitis caused by Leishmania infantum (PDL) is considered a mild form (Stage I) of canine leishmaniosis. It is associated with negative to low positive antibody levels and specific cell-mediated immune responses, and with spontaneous resolution and a good prognosis. Dogs affected with PDL could mount a protective immune response useful to future studies. However, this condition is not fully characterized. The aim of this retrospective study was to define the histopathological pattern, the parasite load and the expression of Toll-like receptor 2 (TLR-2) in skin biopsies of dogs with PDL. Routine histology, and Leishmania and TLR-2 immunohistochemistry (IHC) were performed in skin biopsies from 11 patients and six healthy dogs from a non-endemic area. Moderate to severe granulomatous to pyogranulomatous dermatitis with several histopathological patterns was noted in all the PDL patients. The amastigote numbers ranged from 0 to 200 / high power field (HPF) (mean ± SD = 49 ±76 amastigotes / HPF). TLR-2 IHC was strongly positive in the hyperplastic epidermis overlaying the dermal infiltrate. The mean number ± SD of TLR-2 positive mononuclear dermal cells was 26 ± 13 cells/HPF. TLR-2 IHC of normal skin showed only scattered positive basal and suprabasal epidermal and epithelial follicular cells, positive grouped cells in the follicular ostium and scattered positive perivascular mononuclear cells in superficial and mid dermis. In conclusion, PDL exhibited a granulomatous to pyogranulomatous dermatitis with a variable number of intralesional amastigotes. The TLR-2 expression was increased compared to normal skin, as seen in human cutaneous leishmaniosis.

Source of funding
ESVD research grant

Conflict of interest
None declared
Intradermal injection of recombinant human type VII collagen restores collagen function in a canine model of dystrophic epidermolysis bullosa

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Dystrophic epidermolysis bullosa (DEB) is an incurable skin fragility disease due to mutations in the collagen VII (C7)-encoding gene. Collagen VII is the major component of anchoring fibrils (AFs) in the basement membrane zone (BMZ). In our canine spontaneous recessive DEB (RDEB) model that reproduces the main features of human RDEB, we made one injection of purified recombinant human (rh)C7 intradermally into sites situated around lesions on the lips and ears on one side of the body. Saline injections were used as control on the other side of the body. Skin biopsies taken 1, 2, 4, and 5 weeks after the injection were subjected to immunostaining and immunoelectron microscopy labelling with an antihuman C7 monoclonal antibody that does not cross react with dog C7. Clinically, the injected sites were less inflamed and had no erosions after C7 injection compared with the same sites before injection or nontreated control sites. One week after intradermal injection, the injected rhC7 was incorporated into the affected dog’s BMZ, and it corrected dermoepidermal separation. The localization of the injected rhC7 was confirmed within the BMZ by co-labelling the same sections of dog skin with a polyclonal antibody that recognizes both canine and human C7; immunoelectron microscopy further established that the injected rhC7 formed AFs. This injected rhC7 remained incorporated into the dog’s BMZ for at least 5 weeks. This first study on protein-based therapy in a spontaneous large animal model establishes that intradermally injected human C7 can incorporate into the BMZ and restore C7 function.

Sources of funding
VetAgro Sup and Shire Human Genetic Therapies

Conflict of interest
M. de Souza is an employee of Shire Human Genetic Therapies
SHORT COMMUNICATIONS

Reproducibility of allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories

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Canine allergen-specific IgE assays in the USA are not subjected to an independent laboratory reliability monitoring program. The objective of this study was to evaluate the agreement of diagnostic results and treatment recommendations of four serum IgE assays available commercially from four laboratories in the USA. Replicate serum samples from ten atopic dogs were submitted to each of four laboratories in the USA (ACTT, Bio-medical Services, Austin, TX; VARL Liquid Gold, Veterinary Allergy Reference Laboratory, Pasadena, CA; Allercept, Heska, Loveland, CO and Greer Aller-g-complete, IDEXX Laboratories, Westbrook, ME). The inter-laboratory agreement of standard regional panels and ensuing treatment recommendations were analyzed with the kappa statistic ($\kappa$) to account for agreement that might occur merely by chance. Six comparisons of pairs of laboratories and overall agreement among laboratories were analyzed for ungrouped allergens (as tested) and also with allergens grouped according to reported cross-reactivity and taxonomy. The overall diagnostic agreement between laboratories was only slightly better than expected by random guessing ($\kappa = 0.14$). No two laboratories displayed even moderate chance-corrected agreement ($\kappa > 0.40$) with each other. The overall agreement of the treatment recommendations was also poor ($\kappa = 0.11$). Altogether, 85% of ungrouped allergen treatment recommendations were unique to one laboratory or another. Our study results indicate that the choice of a specific commercial allergen-specific IgE assay is likely to have a major influence on the obtained results and ensuing treatment recommendations.

Source of funding
Self-funded

Conflict of interest
J. Plant is the owner of RESPIT, LLC
Development of a PCR technique specific for *Demodex injai* in biologic specimens

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The identification of *Demodex injai* as a second *Demodex* species of dogs has opened new questions and challenges in the study of *Demodex*-host relationships. To advance our understanding of canine demodicosis we developed a PCR technique with primers based on published genome sequences of *D. injai* from the Genbank. This technique amplified a 238 bp fragment corresponding to a region of the mitochondrial 16S rDNA of *D. injai*. The PCR was positive in DNA samples obtained from mites morphologically identified as *D. injai*, which served as positive controls, and also in samples from *D. injai* three cases of demodicosis in terrier dogs associated with proliferation of mites identified as *D. injai*. Samples of *D. canis* and *D. folliculorum* were consistently negative with this assay. Hairs with roots plucked from five sites in 19 healthy dogs were investigated for the presence of *D. injai* DNA. Two were positive, confirming that *D. injai* is also a normal inhabitant of canine skin. This sampling technique, however, probably underestimates the prevalence of *D. injai* because these mites are suspected to live in the sebaceous glands and ducts. Skin samples from seven dogs with generalized demodicosis caused by *D. canis* were all negative with the *D. injai* specific PCR, demonstrating that the mite proliferation in these dogs was species-specific. This technique may be a useful tool in diagnosis as well as in epidemiologic and pathogenesis studies.

Source of funding
ESVD-ECVD PhD scholarship grant

Conflict of interest
None declared
Toll-like receptor 2 is overexpressed in dogs with demodicosis, *Malassezia* dermatitis and cutaneous bacterial infection

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Toll-like receptors (TLRs) are transmembrane proteins that function as pattern-recognition receptors. TLR-2 is activated by peptidoglycan of Gram-positive bacteria, yeast zymosan, and bacterial lipoproteins among other compounds. The aims of the present study were to investigate the expression of TLR-2 in normal canine skin (n=9), canine demodicosis (n=6), *Malassezia* dermatitis (n=6), and bacterial infection (n=4) using immunohistochemistry (IHC). TLR-2 IHC was considered positive in case of brown membranous and/or granular cytoplasmic specific staining of cells. Epithelial positivity was graded from absent (only scattered cells positive), mild (weak staining of groups of cells) and strong (strong staining of numerous cells). Normal skin showed scattered positive basal and suprabasal epidermal and epithelial follicular cells, with positive grouped cells in the follicular ostium and mononuclear cells mainly in perivascular superficial and mid dermis. TLR-2 positivity was observed in epidermal and follicular epithelium and in mononuclear inflammatory cells in all lesional samples with the exception of one bacterial infection. Endothelial cells and fibroblasts were positive in 100%, 75% and 50% of cases with demodicosis, bacterial infection and *Malassezia* dermatitis, respectively. TLR-2 staining in the epidermis and hair follicles was graded as severe in 83%, 75% and 50% of cases with demodicosis, bacterial infection and *Malassezia* dermatitis, respectively. TLR-2 staining was associated with epidermal hyperplasia and/or spongiosis. In conclusion, these commensal microorganisms seem to stimulate skin innate immunity, and TLR-2 appears to play a role in the inflammatory response in these cutaneous infections in dogs.

Source of funding
Self-funded

Conflict of interest
None declared
SHORT COMMUNICATIONS

Canine epidermal tight junction proteins: comparison of their immunoreactivity in normal and experimental atopic canine skin

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Epidermal tight junctions (TJ) have been well characterized in humans, and they appear involved in many skin diseases such as atopic dermatitis (AD). In dogs, there is no information regarding the implication of TJ in skin diseases such as AD. The aim of this study was to compare the expression and the distribution of ZO-1, occludin and claudin-1 tight junction proteins in normal and experimental atopic canine formalin-fixed, paraffin-embedded skin. Biopsies from six experimentally sensitized atopic beagles were used; these dogs had been sensitized to house dust mites at a young age, and they were known to develop an AD-like pruritic dermatitis following allergen exposure. Samples were obtained prior to allergen challenge from clinically non-lesional skin. Skin specimens from nine healthy dogs without skin lesions were also obtained. Manual immunoperoxidase staining was used to study the immunoreactive pattern of ZO-1, occludin and claudin-1 in the nonlesional epidermis of both groups of dogs. Positive controls were healthy human skin samples. Immunoreactive patterns were blindly assessed by two investigators. Comparisons between groups were performed using Wilcoxon-Mann-Whitney test. The mean expression score of claudin-1 was significantly lower in atopic skin compared to that of healthy subjects. Atopic dogs had a significantly lower expression of claudin-1 and ZO-1 along the membranes of the basal cells and a higher cytoplasmic staining for ZO-1 in the stratum granulosum than controls. Occludin expression remained similar between groups. These results suggest a possible defect in claudin-1 and ZO-1 expression in experimental canine atopic epidermis.

Source of funding
Self-funded

Conflict of interest
None declared
Breed differences in transepidermal water loss and pH among dogs with atopic dermatitis

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Canine Atopic Dermatitis (AD) is associated with changes in surface epidermal barrier in affected animals. This study evaluated the transepidermal water loss (TEWL) and the skin pH in atopic dogs from three different breeds: French bulldogs, cocker spaniels and Labrador retrievers, as well as that of normal dog controls. Transepidermal water loss was measured with a closed-chamber evaporimeter (Vapometer wireless, Delfin Technologies, Kuopio, Finland) and pH was measured with a pH meter (Mettler Toledo, Barcelona, Spain) on the nonlesional inguinal and axillary skin. Sixty dogs were evaluated: 29 with AD (12 bulldogs, 9 cockers and 9 Labradors) and 31 controls (11 bulldogs, 9 cockers and 11 Labradors). French bulldogs with AD exhibited significantly higher inguinal and axillary pH values than control bulldogs (mean ± SD: 7.9 ± 0.2 vs. 6.9 ± 0.2; P = 0.007 and, 7.9 ± 0.2 vs. 7.1 ± 0.3; P = 0.016, respectively). In contrast, atopic cockers tended to have an axillary pH lower than that of control Cockers (6.9 ± 0.4 vs. 7.8 ± 0.3; P = 0.063). For TWEL, bulldogs with AD had significantly higher axillary TEWL values than control bulldogs (19.3 g/m²/h ± 7.2 versus 11.4 g/m²/h ± 1.2; P = 0.004); there were no further differences in TEWL values between other sites or groups. In conclusion, atopic French bulldogs exhibit significant differences in TEWL and pH values than atopic Labrador retrievers, cocker spaniels and normal dogs. These observations suggest that further investigations on skin barrier differences in different breeds of atopic dogs are warranted.

Source of funding
Affinity Petcare

Conflict of interest
N. Sanchez and C. Torre are employees of Affinity Petcare
Thyroid function in dogs with leishmaniosis due to *Leishmania infantum* before and during treatment

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Hypothyroidism may predispose to the development of canine leishmaniosis or it may appear during the course of the latter due to infiltration and destruction of the thyroid gland by infected macrophages. The main purpose of this study was to evaluate thyroid function through measurement of serum total thyroxin (TT4), free thyroxin (FT4), and canine thyroid stimulating hormone (cTSH) concentrations in 36 dogs with leishmaniosis, before, and after 2 and 4 weeks of treatment with allopurinol with or without meglumine antimonate. Before treatment 27/36 (75%) dogs had serum TT4 concentrations below the lower limit of the reference interval, but only two dogs had concurrent serum FT4 concentrations below the lower limit of the reference interval and none had increased serum cTSH concentrations. During treatment, there were no significant changes in serum TT4 or FT4 concentrations, whereas a significant increase in serum cTSH was observed. Two dogs had decreased serum TT4 and FT4 but normal cTSH concentrations before treatment; two other dogs had decreased serum TT4 and increased cTSH, but normal FT4 concentrations during the treatment period. Although hypothyroidism could not be definitively excluded in these dogs, it was considered unlikely based on their overall hormonal profile, clinical presentation, and response to treatment. In summary, hypothyroidism does not appear to be an important predisposing disease or a frequent complication of canine leishmaniosis. However, clinicians should be reminded that dogs with leishmaniosis may exhibit sick euthyroid syndrome, characterised by low TT4 levels, before treatment.

**Source of funding**
Self-funded

**Conflict of interest**
None declared
Dermoscopic features of dermatophytosis in 11 cats with *M. canis* infection

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Scalp dermoscopy has been shown to be a useful tool for the diagnosis of congenital and acquired hair shaft abnormalities in people, and the dermoscopic characteristics of human tinea capitis have also been reported. The aim of this report was to describe the use of dermoscopy in eleven cats with multiple patchy lesions due to *Microsporum canis* infection by using a conventional non-polarized dermoscope (Heine Delta 20, Heine Optotechnik, Herrsching, Germany). All cats were presented with multifocal alopecia and scales; 8/11 were European shorthaired, and the others were one Abyssinian, one British shorthair and one Persian; ages ranged from 2 to 194 months. *Microsporum canis* infection was confirmed by fungal culture in all cats. At a 10-fold magnification, the most common findings observed in circumscribed lesions of 9/11 cats (82%) were broken hair with a sharp slanted end, a homogeneous thickness and a variable amount of white-to-yellow greasy scales. Six of these nine cats (67%) were also positive by Wood’s lamp and microscopic examination. The remaining three were negative by Wood’s lamp, but microscopic examination of the broken, thickened hair seen with the dermoscope confirmed the presence of hyphae and spores along hair shafts. These first observations suggest that dermoscopy could be a useful screening test for *Microsporum canis*-induced dermatophytosis in cats, and, in cases where Wood’s lamp examination is negative, it might help selecting infected hairs for microscopic examination. This method is non invasive, fast and relatively inexpensive, and it could be easily used in everyday clinical practice.

Source of funding
Self-funded

Conflict of interest
None declared
Use of activity monitors to assess pruritus in an acute model of canine atopic dermatitis

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We developed a canine model of acute atopic dermatitis to evaluate the potential of compounds to treat pruritus and skin lesions induced in Dermatophagoides farinae (Df)-sensitised dogs. The aim of this study was to investigate the effectiveness of long-term recording activity monitors (AMs; Actical, Mini Mitter, Bend, OR, USA) to assess pruritus induced by allergen provocation. Twenty-eight Df-sensitised dogs were challenged on three consecutive days with a Df slurry applied to clipped skin on the abdomen. In two blinded crossover trials, dogs fitted with AMs were either treated with prednisolone (1 mg/kg once daily for 5 days, starting one day before challenge) or left untreated. The activity of dogs treated with prednisolone was significantly lower between midnight and 3am and between 3am and 6am compared to untreated dogs (repeated measures ANCOVA; P < 0.0001). To determine if the recorded night time activity corresponded with observable pruritic behaviours (i.e. scratching, chewing, licking or rubbing), we compared AM and video recordings in four dogs for two periods (4:30 pm to 8:30 pm, midnight to 3am) from two nights before and every night during a Df challenge. The correlation between night time AM activity and time seen engaged in pruritic behaviours was highly significant (test of correlation coefficient versus zero; R = 0.57; P < 0.0001). In conclusion, determining night time activity with AMs after allergen challenge appears to be an objective and practical way to assess pruritus in this experimental model of canine atopic dermatitis.

Source of funding
Novartis Animal Health

Conflict of interest
The first five authors are currently employed by Novartis Animal Health. P. Roosje is a consultant for this company
Coproscopic detection and treatment of *Demodex gatoi* infestation in a Cornish rex cat in Austria

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We report herein the first observation of *Demodex gatoi* infestation in Austria and the use of coproscopy for mite detection. A two-year-old Cornish rex cat - acquired as a kitten in the Czech Republic - was presented with pruritic dermatitis and alopecia. Skin scrapes revealed *D. gatoi* mites. Scrapes were negative on an asymptomatic housemate, a three-year-old Thai cat that had never travelled abroad. Faecal floatation with sugar and zinc solutions permitted the detection of *D. gatoi* in both cats. Fewer mites were detected by coproscopy in the asymptomatic cat compared to the affected one. Both cats were treated with 250 µg/kg ivermectin (Ivomec, Merial, Lyon, France) orally every other day. After three months, treatment was stopped in the asymptomatic cat, as faecal examination was negative. The affected cat was treated for more than 4 months, as coproscopy remained positive for *D. gatoi* in spite of the cat not having visible skin lesions. At that time, ivermectin had to be stopped because the cat developed inappetence and hind leg ataxia. To confirm the identity of the mites as *D. gatoi*, PCR of mite mitochondrial 16S rDNA gene was performed on the scrapes of the affected cat. This assay yielded a 325 base pair DNA fragment, whose sequence was 100% identical to that of an American *D. gatoi* mite. This is the first report of demodicosis due to *D. gatoi* in Austria, and our observations suggest the validity of using coproscopy to detect mites in affected cats and nonaffected in-contacts.

**Source of funding**
Self-funded

**Conflict of interest**
None declared
Beneficial effects of immunotherapy with *Gordonia bronchialis* on canine flea allergic dermatitis

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Flea allergy in dogs occurs in sensitised animals, and the skin lesions are thought to result from a Th2-polarised response. As *Gordonia bronchialis* (Gb) has been identified as a promising candidate for immunomodulation of Th2 responses, our objective was to study whether killed suspensions of Gb could be used to successfully treat signs of canine flea allergic dermatitis (FAD). Following standard flea control, 31 dogs with FAD were randomly allocated to receive two intradermal injections of either 0.1 ml of a saline placebo (n=15) or a 10 mg/ml suspension of Gb (n=16) on Day 0 and Day 20. Skin lesions were scored using the CADESI-03 while pruritus was assessed using a visual analog scale (PVAS) on days 0, 20, 40 and 60. Twenty days after the second injection of Gb or placebo, the median relative decreases in CADESI-03 and PVAS from baseline were significantly greater in dogs injected with Gb (90% and 95% improvement, respectively) than with placebo (54% and 44%, respectively; Mann-Whitney U test: CADESI-03 P = 0.0051; PVAS P = 0.0011). Dogs injected with Gb had a five time higher chance of improvement compared to placebo recipients. Our observations suggest that injections of Gb could be helpful to reduce clinical lesions and pruritus associated with canine FAD.

*Source of funding*
*The study received partial support from BioEos*

*Conflict of interest*
*The last five co-authors are unpaid advisory directors of BioEos*
Progressive tail necrosis in a rabbit colony

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Acral necrosis due to mycotoxin-contaminated feed has been described in cattle and swine, but not in rabbits. This report describes an outbreak of progressive tail necrosis in a rabbit colony where 15/103 rabbits (15%) were affected. Animals were kept in outdoor cages, on shavings and straw, and they were fed hay, pellets and water ad libitum. General clinical examination revealed no abnormalities apart from variable alopecia, scales, multifocal crusted erosions and ulcerations on the distal tails; other acral sites were not affected. Ischemia was suspected and two tails were amputated for histopathology. This revealed serocellular crusts, epidermal hyperplasia, superficial perivascular neutrophilic dermatitis, granulation tissue formation and muscle oedema with hyalinised fibres, without signs of vascular damage. Vasoconstriction was likely, but vasculitis could not be excluded. There was no evidence of infection on clinical examination, or haematology or biochemistry profiles. The only environmental factor that could have triggered acral ischemia was the cold temperature (down to 0°C). Toxin analysis of roughage did not reveal any abnormality, but the pellets contained markedly elevated levels of ergot alkaloids compared to 44 reference specimens. After withdrawal of the contaminated feed, the disease did not progress further, and all lesions eventually healed spontaneously. Acral necrosis due to ergotism has not been described in rabbits before, but a high susceptibility of this species to ergot alkaloids is already known. In conclusion, ergotism should be included in the differential diagnoses for progressive tail necrosis in rabbits.

Source of funding
Self-funded

Conflict of interest
None declared
Spontaneous alopecia in Lagotto Romagnolo dogs: a prospective questionnaire and a retrospective case study of Swedish dogs

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Alopecia affecting the trunk has been recognized in Lagotto Romagnolo dogs in Sweden. Our aim was to evaluate the prevalence of spontaneous alopecia in Swedish Lagotto Romagnolo, and to further characterize this condition. Information was collected by questionnaires sent out to all members of the Swedish Lagotto Romagnolo Association. Furthermore, medical records from dogs reported to have alopecia were retrospectively analysed. Information from 277 dogs (a response rate of 20%) belonging to Swedish Lagotto Romagnolo Association members was reviewed. Alopecia was reported in 68/277 dogs (25%). Owner information and the medical records reported a non-pruritic and non-inflammatory alopecia. This most commonly presented as bilateral trunk alopecia that spared the head and extremities. The age of onset was less than 4.5 y in 77% of cases. The hair loss usually started in the autumn/winter, and seasonal cycling was common (54%); a gender predisposition was not noticed. Alopecia onset or worsening of the condition was associated with oestrus. Affected dogs were otherwise healthy with normal hormonal profiles. Histopathology revealed dilated, keratin filled infundibula with fragmented hair shafts in infundibula. Telogen, sometimes pleomorphic, hair follicles often dominated and sebaceous glands were mostly intact. Based on the collected information in Swedish dogs, this spontaneous alopecic dermatosis of Lagotto Romagnolo dogs appears to share similarities with both canine recurrent seasonal flank alopecia and follicular dysplasia of Irish water spaniels and Portuguese water dogs.

Source of funding
Self-funded

Conflict of interest
None declared
Sublingual immunotherapy (SLIT) in the dog: where to apply the allergen? Analysis of the distribution of immune cells in the canine oral mucosa

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Sublingual immunotherapy (SLIT) has been recently developed as an alternative to subcutaneous immunotherapy in canine atopic dermatitis. In humans, the allergen is applied on the sublingual mucosa to exploit the tolerogenic milieu of this region, which is characterized by low numbers of mast cells (MCs) and Langerhans cells. Such information, however, is not yet available in dogs. We performed a histologic and immunohistochemical study of six regions of the canine oral mucosa of six dogs: dorsal tongue, sublingual, hard palate, vestibulum, bucca and gingiva. Microscopic sections were stained by haematoxylin-eosin and immunohistochemically for CD3 (T-lymphocytes), CD79 (B-lymphocytes) and MHCII (antigen presenting cells). The highest density of MCs was observed in the mucosa of dorsal tongue (average: 14 MCs/high power field [hpf]). In all other areas, the density ranged from 1-5 MCs/hpf. T-lymphocytes (CD3+) were present in the epithelium and lamina propria of all locations, with densities ranging from 6-13 cells/hpf, except in the sublingual mucosa, where the density was significantly lower (5 cells/hpf). Only scarce CD79+ B-lymphocytes were observed in some of the samples. Class II MHC+ cells were found in the epithelium and lamina propria of all locations in small numbers (1-5 cells/hpf), without statistically significant differences between locations. In summary, tested sites did not seem to be markedly different from each other, but the oral vestibulum and the gingiva, which both combine low numbers of MCs, moderate numbers of T-lymphocytes and antigen-presenting cells and easy access, may be considered an adequate site to apply the allergen.

Source of funding
Self-funded

Conflict of interest
None declared
Fatty acid composition of lipids derived from isolated canine sebaceous glands and epidermis

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To date, studies of canine skin lipids have been performed using extracts lifted from the skin surface, thereby disguising relative contributions made by the two primary lipid sources, the epidermis and the sebaceous glands. By micro-dissection of small excised skin samples from adult Labrador retrievers (two males and two females), we separated the sebaceous and epidermal lipid components to identify the lipid classes and fatty acids (FAs) present. Following dissection, the purity and integrity of the skin components was checked via microscopy. Bligh and Dyer lipid extracts were then analysed using a combination of high performance liquid, liquid, gas and thin layer chromatography techniques. The major components of canine sebum were identified as triglycerides, wax esters and wax diesters. The predominant FAs present in sebum triglycerides were oleate > palmitate > stearate > linoleate; these four FAs contributed around 95% of all the FAs. In sebaceous wax esters, the pattern was similar although behenic acid was now the fourth most abundant FA. After linoleate, linolenate was the second most abundant polyunsaturated FA in both fractions. The FA composition of epidermal lipids, detected as free FAs, as well as those integrated into ceramides and cholesterol esters, was similar to that of sebum, although it contained significantly more hydroxy-FAs and had a greater contribution of FAs with more than 20C. Techniques used herein could be useful to better understand how changes in diet, microbiology and disease could interplay with the two primary lipid components that contribute to canine skin health and function.

Source of funding
This study was funded by Waltham

Conflict of interest
All the authors are employees of Waltham
SHORT COMMUNICATIONS

Proof-of-concept for the use of spectrophotometry to describe coat colour in dogs

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Coat colour in dogs is controlled by multiple factors that include genetics and nutrition. It is described using a variety of terms, and it would be valuable to have an objective means of expressing variations in hair colour. The Spectro Guide 45/0 (BYK-Gardner, Geretsried, Germany) is a handheld spectrophotometer reading in the 400-700nm spectrum. It provides quantitative colour information in three spectral ranges: white to black (L; 100 to 0); red-green (a; +120 to -120); and yellow-blue (b; -120 to -120). We tested the device for its ability to detect differences in three unpatterned canine coat colours: white, mid-brown and black (three dogs each). The spectrophotometer consistently discriminated between the three colours: there were distinct values for ‘L’ and ‘b’, and although ‘a’ overlapped between white and black, it clearly discriminated brown. Individual and all-colour coefficients of variation (CV) were lowest for the ‘L’ parameter (<5%), followed by ‘b’ and ‘a’ (both between 10 and 20%). Brown was detected with the lowest variation among all three parameters (CV < 10%), followed by white and black. Body site repeatability gave a CV < 10% across five distinct sites, with the exception of ‘a’ for the sternum, possibly due to reduced hair density. Hair length did not have a significant effect on measurements. The Spectro Guide 45/0 appears to be a valuable tool for objectively defining coat colour in dogs; larger studies with dogs of different coats and colours are now required.

Source of funding
Royal Canin

Conflict of interest
The authors are employees of Royal Canin
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Cephalexin is the principle that gives less resistance! Ideal not only for dermatological infections but also for the treatment of infections of the respiratory system, urogenital system and bone infection! Without "palatable" coat that could give intolerance and/or allergy! More effective*!

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**References:**

1. *S. pseudintermedius*, *Porphyromonas*, *Prevotella multocida*, *Escherichia coli*, *Staphylococcus intermedius*. 
2. *S. pseudintermedius*, *Porphyromonas*, *Prevotella multocida*, *Escherichia coli*, *Staphylococcus intermedius*.
3. *S. pseudintermedius*, *Porphyromonas*, *Prevotella multocida*, *Escherichia coli*, *Staphylococcus intermedius*.
4. *S. pseudintermedius*, *Porphyromonas*, *Prevotella multocida*, *Escherichia coli*, *Staphylococcus intermedius*.
5. *S. pseudintermedius*, *Porphyromonas*, *Prevotella multocida*, *Escherichia coli*, *Staphylococcus intermedius*.

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- **Contraindications:** S. pseudintermedius

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