AUSTIN

TEXAS

32ND PROCEEDINGS OF

NAVDF

North American Veterinary Dermatology Forum®

April 10-13

HILTON AUSTIN

2021

NAVDF

North American Veterinary Dermatology Forum®

NEW ORLEANS

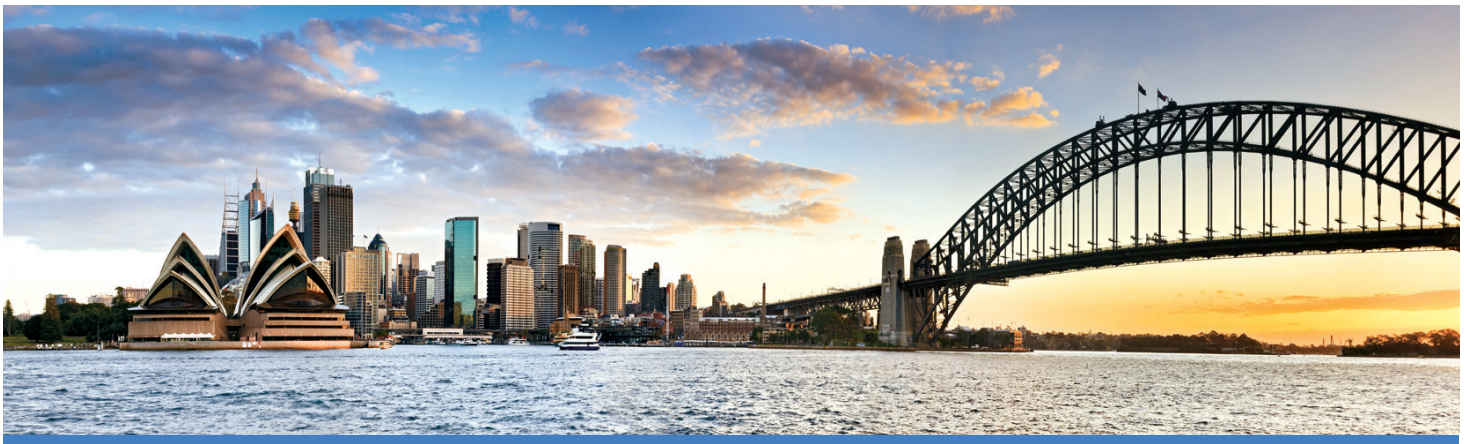
APRIL 21-24

SHERATON NEW ORLEANS HOTEL

SYDNEY

9 WORLD CONGRESS OF VETERINARY DERMATOLOGY

20–24 OCTOBER 2020



Sydney is closer than you think.

Follow us for updates
vetdermsydney.com

Principal Sponsors

WAVD World Association for Veterinary Dermatology · Virbac · ceva · zoetis · ROYAL CANIN · PURINA Institute Advancing Science for Pet Health · BAYER

Major Sponsors

Hill's Transforming Lives · vetoquinol Achieve More Together · Boehringer Ingelheim · STALLERGENES GREER Life beyond allergy

TABLE OF CONTENTS

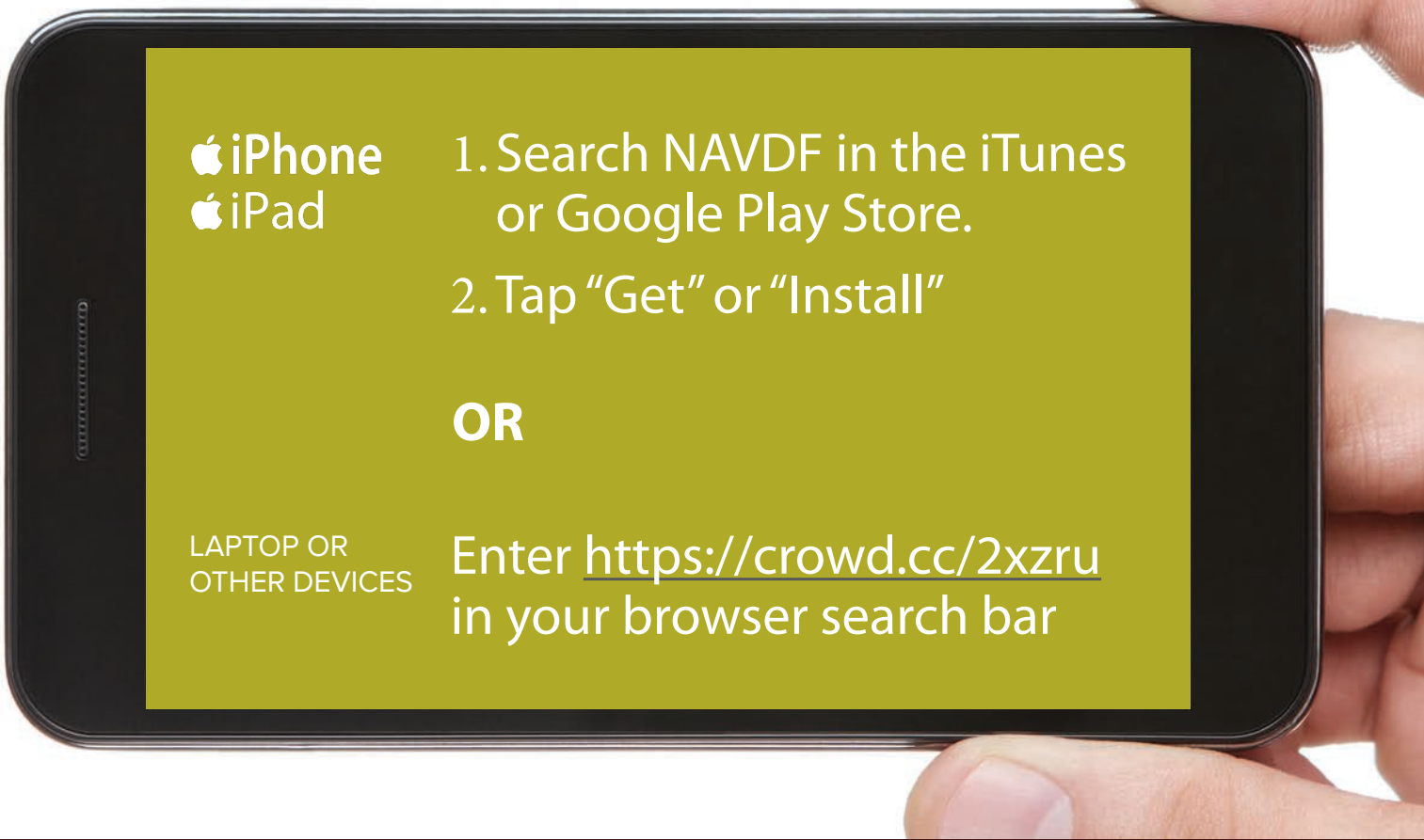
HELP US KEEP TRACK OF DEX!

Dex is ready to explore Austin. While we'd love for him to sample some BBQ and jam out on South Sixth Street, we want to make sure he's not getting into any trouble. Help us keep track of him during the conference.

If you spot him make sure to snap a photo and share it on the app using your Instagram account. **Remember to tag NAVDF (@navdf) and use the hashtags #detectDex, #NAVDF, and #NAVDF2019.**

Once your photo is shared, return Dex to his dog house at NAVDF registration and claim your reward!

APP DOWNLOAD INSTRUCTIONS



iPhone
iPad

1. Search NAVDF in the iTunes or Google Play Store.
2. Tap "Get" or "Install"

OR

LAPTOP OR OTHER DEVICES

Enter https://crowd.cc/2xzru in your browser search bar

HOTEL MEETING SPACE

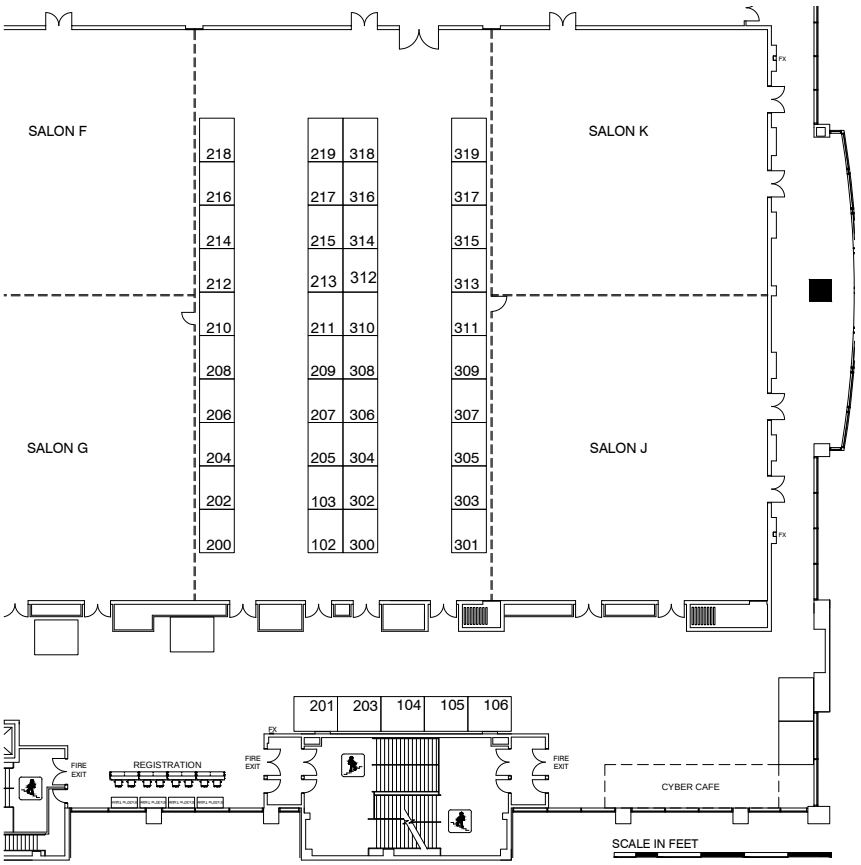
REGISTRATION & EXHIBIT HALL HOURS

REGISTRATION INFORMATION

Wednesday, April 10	5:00pm to 7:00pm
Thursday, April 11	7:00am to 5:30pm
Friday, April 12	7:00am to 5:30pm
Saturday, April 13	7:00am to 5:30pm

EXHIBIT HALL & POSTER HOURS

Thursday, April 11	8:30am to 4:30pm
Friday, April 12	8:30am to 4:30pm
Saturday, April 13	8:30am to 11:30am



SPONSORS

GOLD

STALLERGENES GREER

ROYAL CANIN®
INCREDIBLE IN EVERY DETAIL™

Dechra
Veterinary Products

BLUE
THE BLUE BUFFALO CO.

SILVER

ALK

Hill's®

BRONZE

VETBIOTEK
Innovative Solutions®

Elanco™

ceva

EXHIBITORS

- Aesculight
- ALK
- ANTECH
- Aventix
- Bayer Animal Health
- BLUE BUFFALO COMPANY
- Boehringer Ingelheim
- Ceva Animal Health
- CryoProbe
- Dechra Veterinary Products
- Delmont Laboratories, Inc.
- Dental Focus, LLC
- Elanco Animal Health
- Elsevier, Inc.
- Heska
- Hill's Pet Nutrition, Inc.
- KARL STORZ Veterinary Endoscopy of America
- MDS Incorporated
- MedVet
- Merck Animal Health
- NorthStar VETS
- Purina Pro Plan Veterinary Diets
- Rayne Clinical Nutrition
- Royal Canin
- Serenegy, LLC
- Stallergenes Greer
- Taylors Pharmacy
- VARL
- VetBiotek, Inc.
- Vetericyn Animal Wellness
- Veterinary Specialty and Emergency Care
- VetMatrix
- Vetoquinol
- VetriMax Veterinary Products
- VIRBAC CORPORATION
- Zoetis Petcare
- Zymox & Oratene by PKB Animal Health
- 9th World Congress of Veterinary Dermatology

COMPLETE SCHEDULE

WEDNESDAY, APRIL 10

8:00 AM – 4:30 PM	**ACVD Resident Forum**
8:30 am – 10:00 am	Dr. Colleen Mendelsohn – Wound Healing
10:00 am – 10:30 am	Break
10:30 am – 12:00 pm	Dr. Heide Newton – Keratinization and Cornification
12:00 pm – 1:00 pm	Lunch
1:00 pm – 2:30 pm	Dr. Craig Griffin - Ears
2:30 pm – 3:00 pm	Break
3:00 pm – 4:30 pm	Dr. Karen Campbell - Endocrinology
	Sponsored by Zoetis Petcare
	● LOCATION: AUSTIN BALLROOM J
8:00 AM – 5:00 PM	ACVD Exam Committee Meeting
	● LOCATION: ROOM 614
8:00 AM – 12:00 PM	WCVD9 EOC Meeting
	● LOCATION: ROOM 619
8:30 AM – 12:30 PM	ADVT VTS - Board Examination
	● LOCATION: ROOM 616 A/B
9:00 AM – 12:00 PM	NAVDF Organizing Committee Meeting
	● LOCATION: ROOM 615 B
12:00 PM – 5:00 PM	AAVD Executive Board Meeting
	●LOCATION: ROOM 615 B
12:00 PM – 5:00 PM	ACVD Executive Board Meeting
	●LOCATION: ROOM 615 A
1:00 PM – 5:00 PM	NAVDF Program Committee Meeting
	●LOCATION: ROOM 613
2:00 PM – 5:00 PM	ACVD QW Committee Meeting
	● LOCATION: ROOM 617
2:00 PM – 6:00 PM	ISVD Board Meeting
	● LOCATION: ROOM 619
2:00 PM – 6:00 PM	Exhibitors Move-In
	● LOCATION: AUSTIN GRAND BALLROOM – SALON H & PREFUNCTION
5:00 PM – 7:00 PM	Registration
	● LOCATION: AUSTIN GRAND BALLROOM PREFUNCTION
5:00 PM – 7:00 PM	Welcome Reception
	● LOCATION: AUSTIN GRAND BALLROOM SALON J/K
	Sponsored by Blue Buffalo

COMPLETE SCHEDULE

THURSDAY, APRIL 11

6:00 AM – 7:00 AM	ICADA Meeting ● LOCATION: ROOM 403	
6:00 AM – 8:00 PM	Cyber Café ***Sponsored by Veterinary Information Network*** ● LOCATION: AUSTIN GRAND BALLROOM PREFUNCTION	
7:00 AM - 5:30 PM	Registration Open ● LOCATION: AUSTIN GRAND BALLROOM PREFUNCTION	
7:00 AM - 8:30 AM	Weed Walk ***Sponsored by Stallergenes Greer***	
7:00 AM - 8:30 AM	Roundtable Breakfast Buffet (registration required) ● LOCATION: OUTSIDE ROOM 616	
7:30 AM – 8:45 AM	ROUNDTABLE 1	Dermatology In Academia: Developing A Collaborative Spirit For Teaching And Research | Dr. Jim Noxon ● LOCATION: ROOM 602
	ROUNDTABLE 2	Adverse Effects of Apoquel | Dr. Jennifer Bentley ● LOCATION: ROOM 617
	ROUNDTABLE 3	Treatment of Canine Pseudomonas Otitis | Dr. Marcia Murphy ● LOCATION: ROOM 616 A
	ROUNDTABLE 4	Treatment of Cutaneous and Oral Papillomatosis in Dogs | Dr. Carine Laporte ● LOCATION: ROOM 616 B
	ROUNDTABLE 5	Laser Therapy | Dr. David Duclos ● LOCATION: ROOM 615 A
	ROUNDTABLE 6	Treatment of Canine Hyperadrenocorticism | Dr. Katharine Lunn ● LOCATION: ROOM 615 B
	ROUNDTABLE 7	Treatment of Feline Allergies | Dr. Diana Simões ● LOCATION: ROOM 614
	ROUNDTABLE 8	Cytopoint Updates | Dr. Ben Tham ● LOCATION: ROOM 619
7:30 AM – 8:45 AM	ADVT Regents Meeting ● LOCATION: ROOM 613	
8:30 AM – 4:30 PM	Exhibits & Posters ● LOCATION: AUSTIN GRAND BALLROOM – SALON H & PREFUNCTION	

COMPLETE SCHEDULE

THURSDAY, APRIL 11

	ISVD SESSION ● LOCATION: AUSTIN BALLROOM F	CONCURRENT SESSION ● LOCATION: AUSTIN BALLROOM G	ABSTRACT SESSION ● LOCATION: AUSTIN BALLROOM J/K
9:00 AM – 10:00 AM	Inflammation vs Lymphoma Animals Stefan Keller, DVM	Client Communications Lidiya Alaverdova, DVM ***Sponsored by Hill's***	**Resident Abstracts** 8:55 AM | Announcement 9:00 AM | Dr. Allison Cox 9:15 AM | Dr. Katherine Backel 9:30 AM | Dr. Allison Inga 9:45 AM | Dr. Zijin Zhou
10:00 AM – 11:00 AM	The Immunopathogenesis and Treatment of Human Cutaneous T-Cell Lymphoma: Contrast with the Dog Alain Rook, MD		10:00 AM | Dr. Britt Levy 10:15 AM | Dr. Sara Ramos 10:30 AM | Dr. Alexandra Gould 10:45 AM | Dr. Julie Soohoo
11:00 AM – 11:30 AM	BREAK/ Visit Exhibits & Posters		
11:30 AM – 12:00 PM	The Immunopathogenesis and Treatment of Human Cutaneous T-Cell Lymphoma: Contrast with the Dog Alain Rook, MD	Allergen Immunotherapy: Mechanism of Action Douglas DeBoer, DVM	**Resident Abstracts** 11:30 AM | Dr. Erin Aufox 11:45 AM | Dr. Michelle Piccione
12:00 PM – 12:30 PM	ISVD Mystery Slides Neoplasia 12:00 pm-12:15 pm Verena K Affolter, DVM 12:15 pm-12:30 pm Danielle Desjardins, DVM		12:00 PM | Dr. Danielle Cobiella 12:15 PM | Dr. Teresa Boehm
12:30 PM – 2:00 PM	LUNCH on Your Own		
12:30 pm – 2:00 pm	ACVD Residency Mentors Meeting ***Sponsored by Stallergenes Greer*** ●LOCATION: ROOM 616 A/B		
2:00 pm – 3:00 pm	Mast Cell Tumors Douglas Thamm, VMD	Allergen Immunotherapy: Controversies Jon Plant, DVM	**Resident Abstracts** 2:00 PM | Dr. Julia Miller 2:15 PM | Dr. Endya High 2:30 PM | Dr. Amanda Young 2:45 PM | Dr. Sarah Flanagan
3:00 pm – 4:00 pm	Follicular Neoplasia Dominique Wiener, PhD-DVM, DECVP	Allergen Immunotherapy: Controversies Craig Griffin, DVM	3:00 PM | Dr. Chris Hudec 3:15 PM | Dr. Lara Tomich 3:30 PM | Dr. Christina Yamazaki 3:45 PM | Dr. Brittany Lancellotti
4:00 pm – 4:30 pm	BREAK/ Visit Exhibits & Posters		

COMPLETE SCHEDULE

THURSDAY, APRIL 11

	ISVD SESSION	CONCURRENT SESSION	ABSTRACT SESSION
	● LOCATION: AUSTIN BALLROOM F	● LOCATION: AUSTIN BALLROOM G	● LOCATION: AUSTIN BALLROOM J/K
4:30 pm – 5:00 pm	ISVD Mystery Slides Neoplasia 4:30 pm -4:45 pm Chanran Ganta, BVSc 4:45 pm – 5:00 pm Elisa Salas, DVM	Allergen Immunotherapy Controversies - Panel Discussion Douglas DeBoer, DVM Jon Plant, DVM Craig Griffin, DVM	**Resident Abstracts** 4:30 PM | Sarrah Hoppers 4:45 PM | Dr. Sarah Hoff
5:00 pm – 5:30 pm	ISVD Annual General Meeting (AGM)		5:00 PM | Dr. Carolyn Emery 5:15 PM | Dr. Christina Mazulis
5:45 pm – 7:15 pm	ACVD Diplomates Business Meeting ● LOCATION: AUSTIN BALLROOM J/K		
6:00 pm – 10:00 pm	ACVD Residents' Dinner | ***Sponsored by Dechra Veterinary Products*** ● LOCATION: STUBB'S BBQ		
7:15 pm – 10:30 pm	ACVD Diplomates' Dinner | ***Sponsored by CEVA | by invitation only*** ● LOCATION: BRAZOS HALL – 204 EAST 4TH STREET		
7:30 pm – 9:30 pm	Veterinary Technician Specialists Reception | ***Sponsored by CEVA*** ● LOCATION: MOONSHINE GRILL		

COMPLETE SCHEDULE

FRIDAY APRIL 12

7:00 AM –8:30 PM	Roundtable Breakfast Buffet (registration required) ● **LOCATION: OUTSIDE ROOM 616**	
7:30 AM –5:30 PM	Registration ● **LOCATION: AUSTIN GRAND BALLROOM PREFUNCTION**	
7:30 AM – 8:45 AM	**ROUNDTABLE 9**	Allergy Testing | Dr. Jon Plant ● **LOCATION: ROOM 602**
	ROUNDTABLE 10	Sublingual Immunotherapy | Dr. Douglas J. DeBoer ● **LOCATION: ROOM 617**
	ROUNDTABLE 11	Business Topic | Dr. Andrew Rosenberg ● **LOCATION: ROOM 616 A**
	ROUNDTABLE 12	Work-Life Integration | Dr. Lidiya Alaverdova ***Sponsored by Hill's*** ● **LOCATION: ROOM 616 B**
	ROUNDTABLE 13	Nutritional Nuggets Q&A | Dr. Julie Churchill ● **LOCATION: ROOM 615 A**
	ROUNDTABLE 14	Anatomy of a Multiple-choice Item and Common Flaws | Dr. Jared Danielson ● **LOCATION: ROOM 615 B**
	ROUNDTABLE 15	Alternatives to Antibiotic Therapy | Dr. Sheila Torres ● **LOCATION: ROOM 614**
	ROUNDTABLE 16	Immunosupressive Therapies | Dr. Elizabeth Layne ● **LOCATION: ROOM 619**
7:30 AM – 8:45 AM	ADVT Membership Meeting ***Sponsored by Stallergenes Greer*** ● **LOCATION: ROOM 613**	
8:00 AM – 8:00 PM	Cyber Café | ***Sponsored by Veterinary Information Network*** ● **LOCATION: AUSTIN GRAND BALLROOM PREFUNCTION**	
8:30 AM – 4:30 PM	Exhibits & Posters ● **LOCATION: AUSTIN GRAND BALLROOM – SALON H & PREFUNCTION**	
9:00 AM – 9:15 PM	Resident Research Awards presentation | ***Sponsored by Bayer*** ● **LOCATION: AUSTIN BALLROOM J/K**	

	SCIENTIFIC SESSION	**CONCURRENT SESSION**	**ABSTRACT SESSION**
	● **LOCATION: AUSTIN BALLROOM F**	● **LOCATION: AUSTIN BALLROOM G**	● **LOCATION: AUSTIN BALLROOM J/K**
9:00 AM – 10:00 AM	Updates on Immunopathogenesis of Human Atopic Dermatitis Anna De Benedetto, MD	Cutaneous Lupus Erythematosus Hilary Jackson, BVM&S	**Original Abstracts** 9:15 AM | Dr. Kathy Tater 9:30 AM | Dr. Kathy Tater 9:45 AM | Dr. Cherie Pucheu-Haston

COMPLETE SCHEDULE

FRIDAY APRIL 12

	SCIENTIFIC SESSION	CONCURRENT SESSION	ABSTRACT SESSION
	●LOCATION: AUSTIN F	●LOCATION: AUSTIN G	●LOCATION: AUSTIN J/K
10:00 AM – 11:00 AM	Updates on Skin Barrier of Human Atopic Dermatitis Anna De Benedetto, MD	Cutaneous Lupus Erythematosus: Treatment Hilary Jackson, BVM&S	10:00 AM | Dr. Rosanna Marsella 10:15 AM | Dr. Rosanna Marsella 10:30 AM | Dr. Rosanna Marsella 10:45 AM | Dr. Caitlin Older
11:00 AM – 11:30 AM	BREAK/ Visit Exhibits & Posters		
11:30 AM – 12:30 PM	Current Treatments in Human Atopic Dermatitis Anna De Benedetto, MD	Liver Diagnostic Tests Interpretation for Dermatologist Katharine Lunn, BVMS, MS	11:30 AM | Dr. Gary Bammert 11:45 AM | Dr. Steve Dunham 12:00 PM | Dr. Galia Sheinberg 12:15 PM | Dr. Galia Sheinberg
12:30 PM – 2:00 PM	LUNCH on Your Own		
12:30 PM – 1:30 PM	Sponsor Lunch Symposium | ***Sponsored by ALK*** ● LOCATION: AUSTIN BALLROOM J/K		
12:30 PM – 2:00 PM	ACVD Resident Lunch | ***Sponsored by Stallergenes Greer*** ● LOCATION: ROOM 616 A/B		
12:45 PM – 2:00 PM	ACVD Ethics Committee Meeting ● LOCATION: ROOM 615 B		
12:45 PM – 1:45 PM	ACVD Website Committee Meeting ● LOCATION: ROOM 614		
2:00 pm – 3:00 pm	Canine Atopic Dermatitis: Pathogenesis Thierry Olivry, DrVet, PhD	Renal Panel and Urinalysis Interpretation for Dermatologist Katharine Lunn, BVMS, MS	**Original Abstracts** 2:00 PM | Dr. Elizabeth Layne 2:15 PM | Dr. Brennan McKinney 2:30 PM | Dr. Katje Baumann 2:45 PM | Dr. Natalie Gedon
3:00 pm – 4:00 pm	Atopic Itch: From Pathogenesis to Targeted Therapy Thierry Olivry, DrVet, PhD	Dealing with Side-Effects of Immunosuppression: Case-Based Approach Hilary Jackson, BVM&S Katharine Lunn, BVMS, MS	3:00 PM | Dr. Kenneth Lee 3:15 PM | Dr. Petra Bizikova 3:30 PM | Dr. Michael Canfield 3:45 PM | Dr. Darren Berger
4:00 pm – 4:30 pm	BREAK/ Visit Exhibits & Posters		
4:30 pm – 5:30 pm	Atopic Itch: From Pathogenesis to Targeted Therapy Thierry Olivry, DrVet, PhD	Dealing with Side-Effects of Immunosuppression: Case-Based Approach Hilary Jackson, BVM&S Katharine Lunn, BVMS, MS	**Clinical Abstracts** 4:30 PM | Dr. Lynette Cole 4:45 PM | Dr. Alberto Cordero 5:00 PM | Dr. Darren Berger 5:15 PM | Dr. Diana Di Mattia
6:00 pm – 10:00 pm	Reception| ***Sponsored by Royal Canin Veterinary Diet*** ● LOCATION: THE BELMONT		

COMPLETE SCHEDULE

SATURDAY, APRIL 13

7:00 AM –8:30 PM	Rountable Breakfast Buffet (registration required) ● **LOCATION: OUTSIDE ROOM 616**	
7:30 AM –5:30 PM	Registration ● **LOCATION: AUSTIN GRAND BALLROOM PREFUNCTION**	
7:30 AM – 8:45 AM	**ROUNDTABLE 17**	Tech - Defining the Roles and Responsibilities of the Veterinary Technician in a Dermatology Practice | Juliann London ● **LOCATION: ROOM 602**
	ROUNDTABLE 19	Extra Label Uses of Apoquel | Dr. Elizabeth Falk ● **LOCATION: ROOM 617**
	ROUNDTABLE 20	Cytopoint Updates | Dr. Alberto M Cordero ● **LOCATION: ROOM 615 A**
	ROUNDTABLE 21	Management of Equine Allergies | Dr. Sandra Koch ● **LOCATION: ROOM 615 B**
	ROUNDTABLE 22	Video-otoscopy | Dr. Martha Friedman ● **LOCATION: ROOM 614**
	ROUNDTABLE 23	What Could Veterinary Profession Learn from Startups | Dr. Lidiya Alaverdova ***Sponsored by Hill's*** ● **LOCATION: ROOM 619**
7:30 AM – 8:45 AM	ACVD Residents' Roundtable ● **LOCATION: 616 A/B**	
8:00 AM – 4:00 PM	Cyber Café ***Sponsored by Veterinary Information Network*** ● **LOCATION: AUSTIN GRAND BALLROOM PREFUNCTION**	
8:30 AM – 11:30 AM	Exhibits & Posters ● **LOCATION: AUSTIN GRAND BALLROOM – SALON H & PREFUNCTION**	

	SCIENTIFIC SESSION	**CONCURRENT SESSION**	**ABSTRACT SESSION**	**ADVT SESSION**
	● **LOCATION: AUSTIN BALLROOM F**	● **LOCATION: AUSTIN BALLROOM G**	● **LOCATION: AUSTIN BALLROOM J**	● **LOCATION: AUSTIN BALLROOM K**
9:00 AM – 10:00 AM	Principles of Genetic Investigations and The Era of Personalized Medicine Tosso Leeb, PhD	Food Allergies in Dogs and Cats: Selected Topics Thierry Olivry, DrVet, PhD	**Clinical Abstracts** 9:00 AM | Announcements 9:15 AM | Dr. Dawn Logas 9:30 AM | Dr. Alberto Cordero 9:45 AM | Dr. Andrew Simpson	Otitis - The Dermatology Technician's Role John Angus, DVM
10:00 AM – 11:00 AM	Update on Genodermatoses and New Genetic Tests in Veterinary Dermatology Tosso Leeb, PhD		10:00 AM | Dr. Jennifer Schissler 10:15 AM | Dr. Jennifer Schissler 10:30 AM | Dr. Jeanine Peters-Kennedy 10:45 AM | Dr. Tom Lewis	
11:00 AM -11:30 AM	BREAK/ Visit Exhibits & Posters			

COMPLETE SCHEDULE

SATURDAY, APRIL 13

	SCIENTIFIC SESSION	CONCURRENT SESSION	ABSTRACT SESSION	ADVT SESSION
	● LOCATION: AUSTIN BALLROOM F	● LOCATION: AUSTIN BALLROOM G	● LOCATION: AUSTIN BALLROOM J	● LOCATION: AUSTIN BALLROOM K
11:30 AM – 12:30 PM	Unconventional Alternatives to Conventional Antibiotics Sheila Torres, DVM	IBD and Food Sensitivies Katharine Lunn, BVMS, MS	11:30 AM | Dr. Mona Boord 11:45 AM | Dr. Jennifer Schissler 12:00 PM | Dr. Linda Frank 12:15 PM | Dr. Christoph Klinger	Otitis - The Dermatology Technician's Role John Angus, DVM
12:30 PM – 1:30 PM	AAVD Business Meeting ● LOCATION: AUSTIN BALLROOM G			
12:30 PM – 2:00 PM	LUNCH on Your Own			
2:00 PM – 3:00 PM	Picking the Wolves from the Sheep: Prognostic Markers in Canine Cutaneous Mast Cell Tumors Michael Childress, DVM	Feeding for Life- Meeting Optimal Needs Through All Life Stages to Set Pets Up For Success Julie Churchill, DVM		ACVD Exam Writing Workshop: How to Write Questions that Work Jared Danielson, PhD.
3:00 PM – 4:00 PM	Choosing the Right Treatment for Dogs with Cutaneous Mast Cell Tumors Michael Childress, DVM	Food Fads- Fact or Fiction? Assessing the Claims Behind Benefits of Grain Free, Raw, Homemade or Other Diet Trends. Julie Churchill, DVM		
4:00 PM – 4:30 PM	BREAK			
4:30 PM – 5:30 PM	Managing Cutaneous Lymphomas in Dogs Michael Childress, DVM	"Eliminate" The Pitfalls When Considering A Food Trial Julie Churchill, DVM		ACVD Exam Writing Workshop: How to Write Questions that Work Jared Danielson, PhD.

ROUNDTABLE SCHEDULE

THURSDAY, APRIL 11, 2019

1	**Dermatology In Academia: Developing A Collaborative Spirit For Teaching** | Dr. Jim Noxon	LOCATION: ROOM 602
2	**Adverse Effects of Apoquel** | Dr. Jennifer Bentley	LOCATION: ROOM 617
3	**Treatment of Canine Pseudomonas Otitis** | Dr. Marcia Murphy	LOCATION: ROOM 616 A
4	**Treatment of Cutaneous and Oral Papillomatosis in Dogs** | Dr. Carine Laporte	LOCATION: ROOM 616 B
5	**Laser Therapy** | Dr. David Duclos	LOCATION: ROOM 615 A
6	**Treatment of Canine Hyperadrenocorticism** | Dr. Katharine Lunn	LOCATION: ROOM 615 B
7	**Treatment of Feline Allergies** | Dr. Diana Simões	LOCATION: ROOM 614
8	**Cytopoint Updates** | Dr. Ben Tham	LOCATION: ROOM 619

FRIDAY, APRIL 12, 2019

9	**Allergy Testing** | Dr. Jon Plant	LOCATION: ROOM 602
10	**Sublingual Immunotherapy** | Dr. Douglas J. DeBoer	LOCATION: ROOM 617
11	**Business Topic** | Dr. Andrew Rosenberg	LOCATION: ROOM 616 A
12	**Work-Life Integration** | Dr. Lidiya Alaverdova	LOCATION: ROOM 616 B
13	**Nutritional Nuggets Q&A** | Dr. Julie Churchill	LOCATION: ROOM 615 A
14	**Anatomy of a Multiple-choice Item and Common Flaws** | Dr. Jared Danielson	LOCATION: ROOM 615 B
15	**Alternatives to Antibiotic Therapy** | Dr. Sheila Torres	LOCATION: ROOM 614
16	**Immunosupressive Therapies** | Dr. Elizabeth Layne	LOCATION: ROOM 619

SATURDAY, APRIL 13, 2019

17	**Tech - Defining the Roles and Responsibilities of the Veterinary Technician in a Dermatology Practice** | Juliann London	LOCATION: ROOM 602
18	**ACVD Residents' Roundtable**	LOCATION: ROOM 616 A/B
19	**Extra Label Uses of Apoquel** | Dr. Elizabeth Falk	LOCATION: ROOM 617
20	**Cytopoint Updates** | Dr. Alberto M Cordero	LOCATION: ROOM 615 A
21	**Management of Equine Allergies** | Dr. Sandra Koch	LOCATION: ROOM 615 B
22	**Video-otoscopy** | Dr. Martha Friedman	LOCATION: ROOM 614
23	**What Could Veterinary Profession Learn from Startups** | Dr. Lidiya Alaverdova	LOCATION: ROOM 619

ABSTRACT SCHEDULE

THURSDAY, APRIL 11

Abstract Session: Resident Abstracts

9:00 AM	**Dr. Allison Cox:** Detection of DNA from undeclared animal species in commercial canine and feline raw meat diets using qPCR	21
9:15 AM	**Dr. Katherine Backel:** Canine ischemic dermatopathy: a retrospective study of 177 cases (2005–2016)	22
9:30 AM	**Dr. Allison Inga:** Sterile granulomatous dermatitis and lymphadenitis (juvenile cellulitis) in adult dogs: a retrospective analysis of 90 cases (2004-2018)	23
9:45 AM	**Dr. Zijin Zhou:** A retrospective evaluation of pemphigus foliaceus in dogs with and without vasculopathic changes on histopathology	24
10:00 AM	**Dr. Britt Levy:** Detection of circulating antikeratinocyte IgG autoantibodies in feline pemphigus foliaceus	25
10:15 AM	**Dr. Sara Ramos:** Residual antibacterial activity of canine hair treated with five mousse products against *Staphylococcus pseudintermedius in vitro*	26
10:30 AM	**Dr. Alexandra Gould:** Recovery of meticillin-resistant *Staphylococcus* spp. from pet grooming salons	27
10:45 AM	**Dr. Julie Soohoo:** Efficacy of disinfectant formulations and a hydrogen peroxide and silver fogging system against meticillin-resistant *Staphyloccus pseudintermedius* (MRSP)	28
11:00 AM -11:30 AM	**BREAK**	
11:30 AM	**Dr. Erin Aufox:** The prevalence of *Dermatophilus congolensis* in horses with pastern dermatitis using PCR to diagnose infection in a population of horses in southern USA	29
11:45 AM	**Dr. Michelle Piccione:** A comparative study of serum IgE against cross-reactive carbohydrate determinants (CCD) in atopic and healthy dogs	30
12:00 PM	**Dr. Danielle Cobiella:** Evaluation of vascular endothelial growth factor (VEGF) in the stratum corneum and serum of healthy and atopic dogs: a pilot study	31
12:15 PM	**Dr. Teresa Boehm:** Clinical effects of two commercially available essential fatty acid-enriched veterinary diets on canine atopic dermatitis	32
12:30 PM - 2:00 PM	**LUNCH**	
2:00 PM	**Dr. Julia Miller:** Comparison of three clinical scoring systems for insect bite hypersensitivity in a herd of Icelandic horses	33
2:15 PM	**Dr. Endya High:** Development and validation of a graphic two-dimensional Investigator's Global Assessment instrument for assessing the overall severity of atopic dermatitis in dogs	34
2:30 PM	**Dr. Amanda Young:** Canine pruritus visual analogue scale: How does it capture owners' perception of their pet's pruritus level?	35
2:45 PM	**Dr. Sarah Flanagan:** An assessment of barriers and perceptions of general practice veterinarians for referral and allergen-specific immunotherapy in the management of atopic dermatitis – a pilot study	36
3:00 PM	**Dr. Chris Hudec:** Changes in the stress markers cortisol and glucose before and during intradermal testing in cats after single administration of pre-appointment gabapentin	37
3:15 PM	**Dr. Lara Tomich:** Effect of topical lidocaine on intradermal allergy testing in dogs with atopic dermatitis	38
3:30 PM	**Dr. Christina Yamazaki:** Pilot evaluation of *Enterococcus faecium* SF68 as adjunctive therapy for adult atopic dogs responsive to oclacitinib	39

ABSTRACT SCHEDULE

THURSDAY, APRIL 11

Abstract Session: Resident Abstracts

3:45 PM	**Dr. Brittany Lancellotti:** Comparison of malignancies and nonmalignant skin masses in 339 allergic dogs receiving long-term (> 6 months) oclacitinib with age and breed matched control population	40
4:00 PM - 4:30 PM	**BREAK**	
4:30 PM	**Dr. Sarrah Hoppers:** Prevalence of bilateral feline inflammatory polyps: a retrospective analysis	41
4:45 PM	**Dr. Sarah Hoff:** Stability of diluted ceftazidime in three otic preparations under different storage conditions over a 28-day period	42
5:00 PM	**Dr. Carolyn Emery:** Stability of dexamethasone when added to commercial veterinary ear cleaners over a 90 day period	43
5:15 PM	**Dr. Christina Mazulis:** Ear cytology and resident flora of clinically normal alpacas (*Vicugna pacos*)	44

Dermatopathology Session (ISVD) Presentations

9:00 AM - 10:00 AM	**Stefan Keller, DVM:** Inflammation vs Lymphoma Animals	45
10:00 AM - 11:00 AM	**Alain Rook, MD:** The Immunopathogenesis and Treatment of Human Cutaneous T-Cell Lymphoma: Contrast with the Dog	50
11:30 AM - 12:00 PM	**Alain Rook, MD:** The Immunopathogenesis and Treatment of Human Cutaneous T-Cell Lymphoma: Contrast with the Dog	50
12:00 PM - 12:30 PM	**ISVD Mystery Slides Neoplasia**: Verena K Affolter, DVM, Danielle Desjardins, DVM	
2:00 PM - 3:00 PM	**Douglas Thamm, VMD:** Mast Cell Tumors	53
3:00 PM - 4:00 PM	**Dominique Wiener, PhD-DVM, DECVP:** Follicular Neoplasia	58
4:30 PM - 5:30 PM	**ISVD Mystery Slides Neoplasia:** Chanran Ganta, BVSc, 4Elisa Salas, DVM	

Concurrent Session Presentations

9:00 AM - 11:00 AM	**Lidiya Alaverdova, DVM:** The Communication Cure	63
11:30 AM - 12:30 PM	**Douglas DeBoer, DVM:** Allergen Immunotherapy: Mechanism of Action	67
2:00 PM - 3:00 PM	**Jon Plant, DVM:** Allergen Immunotherapy: Controversies	72
3:00 PM - 4:00 PM	**Craig Griffin, DVM:** Allergen Immunotherapy: Controversies	76
4:30 PM - 5:30 PM	**Allergen Immunotherapy Controversies - Panel Discussion:** Douglas DeBoer, DVM, Jon Plant, DVM, Craig Griffin, DVM	

Detection of DNA from undeclared animal species in commercial canine and feline raw meat diets using qPCR

A. COX*, V. DEFALQUE*, T. UDENBERG*, S. BARNUM†, C. WADEMAN†

**North West Veterinary Dermatology Services, Vancouver, BC, Canada*
†Department of Medicine and Epidemiology, University of California Davis, Davis, CA, USA

Abstract: Currently, the best diagnostic procedure to identify cutaneous adverse food reactions (CAFRs) in small animals remains an elimination diet (ED) with subsequent provocation trials. There are many commercial diets containing novel protein ingredients often selected for an ED. Raw meat-based diets (RMBDs) have historically been fed to racing greyhounds and sled dogs, and this feeding practice began extending to household pets in the nineties. Several commercial RMBDs are now available, and despite evidence for potential individual and zoonotic health risks, these diets have continued to gain popularity. Many pet owners desire to feed RMBDs as EDs despite contrary recommendations. The reliability of RMBDs for this purpose has not been investigated. The aim of this study was to analyze commercial RMBDs for DNA of animal origin other than that declared on the label. Nine canine and nine feline commercial RMBDs were tested for species-specific DNA of animal origin (chicken, duck, turkey, pork, rabbit, lamb, beef, salmon, kangaroo) using quantitative PCR. Two separate batches of each diet were sampled to assess content consistency. DNA of one or more unlisted animal species was identified in 89% of canine, and 61% of feline batches. Discrepancy between batches was noted in 78% of canine, and 56% of feline batches. The unlisted DNA most frequently detected was lamb (n=12) in canine diets, and turkey (n=7) in feline diets. Therefore, if the selected diets are representative of commercially available RMBDs, use of these diets cannot be recommended in the clinical diagnosis of CAFRs in dogs and cats.

Sources of funding: Self-funded

Conflict of Interest: None declared

Canine ischemic dermatopathy: a retrospective study of 177 cases (2005–2016)

K. A. BACKEL*, C.W. BRADLEY*, C. L. CAIN*, D.O. MORRIS*, K.H. GOLDSCHMIDT†, E. A. MAULDIN*,

**School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
† Operations and supply chain management, University of St. Thomas, Minneapolis, MN, USA

Abstract: Ischemic dermatopathy encompasses a poorly understood subset of canine diseases that share similar clinical and histologic features. The study objective was to investigate clinical features, treatment and outcome of patients diagnosed with ischemic dermatopathy, excluding cases of hereditary dermatomyositis. This retrospective study identified 177 dogs with a histopathologic diagnosis of ischemic dermatopathy between 2005 and 2016. Medical records were reviewed for patient signalment, body weight, lesion characteristics, vaccine history and medical therapy. A scoring system was created to subjectively quantify outcome and likelihood of a vaccine association. Vaccine scores (0 – 5) were based on vaccine history, lesions, and histologic findings. Toy and miniature poodles, Chihuahuas, Jack Russell and Yorkshire terriers were overrepresented. Of 93 dogs for which historical data was obtained, the median age at biopsy was 5 years (0.42 – 13 years) and the median body weight was 7.3 kg (range 1.32 - 50.3 kg). The condition in 45 dogs (48.3%) was found likely to be associated with vaccination (vaccine score > 2). Younger dogs (P =0.011) and higher body weights (P =0.003) were positively correlated with vaccine scores. Lower vaccine scores (P =0.035), body weights under 10 kg (P =0.016) and older ages (P =0.007) were significantly associated with worse outcomes. Similar to previous reports, this study highlights vaccines as a likely trigger for ischemic dermatopathy, and additionally provides support for breed predispositions and identifies potential prognostic factors. Importantly, over half of the cases were considered unlikely to be vaccine associated, demonstrating the need to investigate other underlying causes.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Sterile granulomatous dermatitis and lymphadenitis (juvenile cellulitis) in adult dogs: a retrospective analysis of 90 cases (2004-2018)

A. J. INGA*, G.C. GRIFFETH*, E.A. MAULDIN†

**Red Bank Veterinary Hospital, Tinton Falls, NJ, USA*
†School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract: Previously described in juvenile dogs, here we describe sterile granulomatous dermatitis and lymphadenitis in adult dogs. Included were 90 dogs ≥12 months old with clinical signs and histopathologic features consistent with the disease. Inclusion criteria were facially-oriented lesions (pustules/swelling on muzzle, pinnae or eyelids), non follicularly-oriented dermal pyogranulomatous inflammation; and negative histochemical stains for pathogens. Signalment, lesion distribution and histopathologic features were analyzed for 90 cases. Median age at onset was 3.54 years. Frequently represented pure breeds included Labrador retrievers, dachshunds, bichons frise, and various setters. Lesions were predominantly located as follows: periocular (65/90), muzzle (50/90), lips (35/90), pinnae (34/90), lymph nodes (32/90) nose (31/90), chin (23/90), and perianal/genital (15/90). Additional histopathologic features included folliculitis/furunculosis (70/90), adnexal effacement (22/90) and ulcers (13/90). All submitted lymph nodes (7/90) displayed pyogranulomatous inflammation. Of 20 aerobic cultures, 19/20 were surface/swab and 1/20 was deep/tissue. Nine yielded no growth (including the sole tissue culture) and 11/20 isolated bacterial species considered secondary invaders. Nine fungal, five anaerobic and four mycobacterial cultures were negative. Medical records were analyzed for 35/90 cases. Glucocorticoid monotherapy was prescribed in eight dogs. In others, glucocorticoids were combined with: antibiotics (18/35), ciclosporin (5/35), doxycycline and niacinamide (3/35), or mycophenolate (1/35). Median treatment length was 60 days. Median time to remission was 28 days. Nineteen cases maintained remission. Recrudescence occurred in 11 cases (follow up 20-3649 days). Remission status was not established in five cases. Sterile granulomatous dermatitis and lymphadenitis should be considered as a differential diagnosis in dogs of all ages.

Funding: Self funded.

Conflict of Interest: None declared.

A retrospective evaluation of pemphigus foliaceus in dogs with and without vasculopathic changes on histopathology

Z. ZHOU*, E. NOLAND†, A. PETERSEN* and E. ROSSER*

**Veterinary Medical Center, Michigan State University, East Lansing, MI, USA*
†Veterinary Diagnostic Laboratory, Michigan State University, East Lansing, MI, USA

Abstract: The study objective was to compare clinical features and outcome of pemphigus foliaceus in dogs with and without vasculopathic changes on histopathology. The study included 46 dogs with a diagnosis of pemphigus foliaceus based on history, clinical signs and histopathology: 27 spayed females, 18 neutered males, and two intact males. New slides were made from embedded paraffin blocks of associated dogs and evaluated for vasculopathic changes. Dogs were grouped based on vasculopathic changes. Group 1 included dogs without vasculopathic changes. Group 2 included dogs with vasculopathic changes (excessive erythrocyte spilling and/or obscuring of vessel walls) but not vasculitis. Group 3 included dogs with vasculitis (fibrin replacing and/or neutrophils infiltrating vessel walls). There were 23 dogs (50%) in group 1, seven dogs (15%) in group 2, and 16 dogs (35%) in group 3. Only one case (group 3) had clinical signs unique to cutaneous vasculitis (pitting limb edema, non-blanching erythema). All others had signs that could be attributable to pemphigus foliaceus. There were no significant differences in rates of remission, recurrence, corticosteroid dosage, and need for secondary agent between groups. Time to remission was longer for dogs in group 3 (85.5 days) compared to group 1 (37.3 days) (P=0.026). Dogs in group 3 were more likely to have systemic signs of illness (P=0.049), have adverse effects associated with treatment (P=0.01), and be euthanized due to pemphigus foliaceus (P=0.045) compared to group 1. Results suggest that dogs with pemphigus foliaceus were more challenging to manage when concurrent vasculitis was observed on histopathology.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Detection of circulating antikeratinocyte IgG autoantibodies in feline pemphigus foliaceus

B. LEVY, L. MAMO, P. BIZIKOVA†

**Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA.*
† Comparative Medicine Institute, North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA

Abstract: Pathogenic, circulating antikeratinocyte IgG targeting desmosomal proteins have been identified in both humans and dogs with pemphigus foliaceus (PF). Though similar pathomechanisms have been proposed for feline PF, circulating antikeratinocyte IgG have not been documented. The goal of this study was to detect circulating, membrane-bound, antikeratinocyte IgG in PF-affected cats diagnosed based on compatible clinical and microscopic findings; specific-pathogen-free (SPF), healthy and allergic cats served as controls. Sera from 30 PF-affected, 11 SPF, 15 healthy and 31 allergic cats were screened, using indirect immunofluorescence on canine footpad and buccal mucosa tissues, for membrane-bound, antikeratinocyte IgG with classic intercellular immunofluorescence patterns described in PF-affected humans and dogs. Circulating antikeratinocyte IgG were detected in the majority of PF-affected cats (23/30, 77%), in some allergic cats (6/31, 19%) and in a single healthy cat (7%). An intercellular IgG pattern was limited to the footpad substrate in two PF-affected, five allergic, and one healthy cat. The remaining sera showed an intercellular immunofluorescence pattern on both substrates. Reciprocal IgG titers were significantly higher in PF-affected cats, compared to healthy, SPF and allergic cats (Dunn's post-test, $P < 0.0001$). These results confirm the presence of circulating antikeratinocyte IgG in a majority of cats with PF and in a small percentage of healthy and allergic cats. Though the molecular target and pathogenic nature of these antibodies remains unknown, the positive immunoreactivity of PF sera on both footpad and buccal mucosa suggests that the major target antigen of feline PF differs from that identified in dogs.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Residual antibacterial activity of canine hair treated with five mousse products against *Staphylococcus pseudintermedius in vitro*

S.J. RAMOS*, M. WOODWARD*, S.M. HOPPERS†, C. LIU*, C. M. PUCHEU-HASTON*, M.S. MITCHELL‡

**Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, 1909 Skip Bertman Drive, Baton Rouge, LA 70803 USA*
†University of Tennessee Knoxville College of Veterinary Medicine, 2407 River Dr. Knoxville,TN 37996 USA
‡Louisiana Animal Disease Diagnostic Laboratory, Department of Microbiology, River Road #1043 Baton Rouge LA, 70803 USA

Abstract: Topical therapy alone can be effective in the treatment of canine pyoderma. To date, no studies have evaluated the efficacy of commercially available mousse products in the treatment of canine pyoderma. The objective of this study was to determine the residual antibacterial activity on canine hairs treated with five mousse products containing different active ingredients. Fifteen client-owned dogs with no history of dermatologic disease were enrolled. Dogs were treated once with following mousse products: (1) 2% chlorhexidine and 1% ketoconazole, (2) 2% chlorhexidine and 2% miconazole, (3) 3% chlorhexidine and 0.5% climbazole, (4) 2% salicylic acid and 10% ethyl lactate, and (5) phytosphingosine HCl 0.05% (control). Hair samples were collected from each treatment area prior to product application, 1 h after product application, and on days 2, 4, 7, 10 and 14 post-treatment. Collected hairs were weighed and plated on Mueller-Hinton agar plates streaked with a *Staphylococcus pseudintermedius* isolate showing no antimicrobial resistance. Plates were incubated for 24 h and bacterial growth inhibition zones around the hairs were measured. Mousse 3 had significantly larger inhibition zones than all other products at all time points ($P < 0.0012$), except on Day 0 (1h post) and 2. The inhibition zones for Mousses 4 and 5 were significantly lower than those for all other mousses through day 10 ($P < 0.0001$). These results suggest that three of the mousse products are effective in inhibiting *S. pseudintermedius* growth in vitro for at least 10 days, supporting their use for the management of canine pyoderma.

Sources of funding: This study was funded by Louisiana State University VCS Corp Grant.

Conflict of Interest: None declared

Recovery of meticillin-resistant *Staphylococcus* spp. from pet grooming salons

A. GOULD*§, K. COYNER*, A. TRIMMER§, J.S. WEESE¶ and C. BUDKE**

**Dermatology Clinic for Animals, Lacey, WA, USA*
§Animal Allergy and Dermatology Specialists, Las Vegas, NV, USA
¶Department of Pathobiology, Ontario Veterinary College, University of Guelph, Ontario, Canada
***Texas A&M College of Veterinary Medicine, College Station, TX, USA*

Abstract: Meticillin-resistant staphylococcal species have been cultured from veterinary hospital environments and veterinary tools. Pet grooming salon environments and tools may pose a risk for dissemination of meticillin-resistant staphylococcal bacteria into the pet population. The objective of this study was to evaluate the prevalence of meticillin-resistant staphylococcal contamination of pet grooming establishments and to collect information regarding cleaning procedures in grooming salons. Nineteen salons in the Seattle, WA metropolitan area were sampled; salons connected to veterinary hospitals were excluded. Sampled sites included clipper blades and handles, leashes, rims of bathtub drains, insides of shampoo caps, and bathtub spray hoses or faucet handles. Selective culture for meticillin-resistant *Staphylococcus* species was performed on 112 samples. Coagulase positive isolates were speciated by MALDI-TOF. Meticillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus pseudintermedius* (MRSP), and *Staphylococcus schleiferi* (MRSS) subsp. *coagulans* were characterized by spa or dru typing. Each grooming salon received a survey on cleaning practices and 12/19 surveys were returned. Meticillin-resistant coagulase positive staphylococci were isolated from 12/19 salons. Overall, 11/112 samples were positive for MRSP, 4/112 samples were positive for MRSA, and 10/112 samples were positive for MRSS. Meticillin-resistant Staphylococcus species of any type were found on 7/19 clipper blades, 7/19 clipper handles, and 6/19 leashes and grooming leads. The number of recruited salons was too low to allow statistically significant correlations between cleaning practices and culture results. Clipper blades, clipper handles, and leashes can serve as potential fomites for meticillin-resistant staphylococci in a grooming salon environment; increased disinfection practices for these items are indicated.

Sources of funding: This study was funded by the American College of Veterinary Dermatology.

Conflict of Interest: None declared

Efficacy of disinfectant formulations and a hydrogen peroxide and silver fogging system against meticillin-resistant *Staphyloccus pseudintermedius* (MRSP)

J. SOOHOO*, J.R. SCHISSLER*, J.B. DANIELS*, S.A. BRAULT*, and R.A.W. ROSYCHUK*

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

Abstract: The increasing prevalence of meticillin-resistant Staphylococcus pseudintermedius (MRSP) and its environmental persistence necessitates effective disinfection to prevent difficult to treat nosocomial infections. Commercial disinfectants have label claims for meticillin-resistant *Staphylococcus aureus*, however, there are no claims nor peer-reviewed studies evaluating efficacy against MRSP. A quaternary ammonium product (QAC; Virex® II 256), an accelerated hydrogen peroxide product (AHP; Oxivir® Five 16), an accelerated hydrogen peroxide and silver product (AHPS; HaloMist™), and a commercial AHPS fogging system (FOG; HaloFoggerTM) were evaluated for efficacy against MRSP. For all experiments, approximately 1.8x106 MRSP colony forming units (CFU) were inoculated onto a sterile plastic surface. Controls included sterile uninoculated and untreated inoculated plastic surfaces. In each QAC, AHP, and AHPS experiment, 14 inoculated surfaces were saturated with the disinfectant for the recommended contact time. To evaluate FOG, a single inoculated surface was placed in eight locations in a closed exam room. Recommended fogging and contact times were used. Least squares mean reduction (log10) in CFU was 3.55 log for QAC ($P < 0.0001$), 3.60 log for AHP ($P < 0.0001$), 1.66 log for AHPS ($P < 0.0001$), and 0.32 log for FOG ($P = 0.004$). In this high inoculum model, QAC, AHP, and AHPS reduced MRSP CFU by 99.97%, 99.98%, and 97.81%, respectively. FOG reduced CFU by 52.14%. We conclude that QAC, AHP, and AHPS are appropriate for MRSP disinfection. FOG as a sole means of MRSP disinfection is not presently supported.

Sources of funding: 2018 Colorado State University College of Veterinary Medicine and Biomedical Sciences College Research Council Interdisciplinary Award.

Conflict of Interest: None declared

The prevalence of *Dermatophilus congolensis* in horses with pastern dermatitis using PCR to diagnose infection in a population of horses in southern USA

E.E. AUFOX*, L.A. FRANK*, E.R. MAY* and S.A. KANIA†

**Department of Small Animal Clinical Sciences, University of Tennessee, Knoxville, TN, USA*
†Department of Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN, USA

Abstract: *Dermatophilus congolensis* is a facultative anaerobic actinomycete that causes papular to exudative dermatitis with crusting in horses. This organism is frequently implicated as a cause of pastern dermatitis, but little data is available validating the organism's association with this disease. The aim of this study was to evaluate if *D. congolensis* is associated with pastern dermatitis in horses utilizing RT-qPCR. Fifteen client-owned horses diagnosed with pastern dermatitis and eight client-owned unaffected control horses were included in this cross-sectional study. History and physical examination findings were recorded, and samples were collected and tested for *D. congolensis* utilizing cytology and RT-qPCR. Dermatophyte culture and superficial skin scrapings were also performed. Ten of 15 horses with pastern dermatitis had feathered pasterns. *Dermatophilus congolensis* was identified by RT-qPCR from one horse with non-feathered pasterns and from no horses with feathered pasterns. Cytology identified bacteria in all horses but failed to identify organisms resembling *D. congolensis* in any horse. Four of 15 horses, all with feathered pasterns, were positive for *Chorioptes* mites. Fungal culture was negative for dermatophytes in all horses. All test results were negative for the eight control horses. *Dermatophilus congolensis* was uncommonly associated with pastern dermatitis in horses in this population. Chorioptic mange was commonly associated with pastern dermatitis in feathered horses and represented an important differential diagnosis for this clinical presentation.

Sources of funding: University of Tennessee's Companion Animal Fund.

Conflict of Interest: None declared

A comparative study of serum IgE against cross-reactive carbohydrate determinants (CCD) in atopic and healthy dogs

M. L. PICCIONE and D. J. DEBOER

School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

Abstract: Cross-reactive carbohydrate determinants are IgE-binding carbohydrate structures common to plant and insect species. In people, anti-CCD IgE is thought to be clinically irrelevant, but to have potential to confound serologic IgE test interpretation. Previous studies reported detection of anti-CCD IgE in 24-73% of atopic dog sera; prevalence in healthy dogs is not reported. We sought to compare the prevalence of anti-CCD IgE in a group of atopic dogs compared with a group of healthy dogs. Sera from 101 dogs with a clinical diagnosis of atopic dermatitis and from 48 healthy dogs were analyzed for IgE against CCD and environmental allergens with a commercial multiplex allergen-specific IgE assay. Anti-CCD IgE was detected in 17/101 (16.8%) of atopic dog sera and 7/48 (14.5%) of healthy dog sera ($P = 0.8$, Fisher's exact test). All healthy and atopic dogs with anti-CCD IgE were positive to both grass pollens and environmental mites. Estimated median anti-CCD concentrations in atopic and healthy dogs were 2.4 kU/L and 0.38 kU/L, respectively ($P = 0.07$, Mann-Whitney *U* test). We conclude that both atopic and healthy dogs can have serum anti-CCD IgE. Differences in prevalence between our findings and prior reports likely represent differing criteria for serum selection, as well as in test methodology. More investigation is warranted to determine the clinical significance of anti-CCD IgE antibodies in dogs, how they are best detected, and if blocking these antibodies during diagnostic testing has clinical value.

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

Conflict of Interest: One of the authors (DJD) supplies test components to Biocheck GmbH.

Evaluation of vascular endothelial growth factor (VEGF) in the stratum corneum and serum of healthy and atopic dogs: a pilot study

D. COBIELLA*, D. SANTORO*, D. GRAM*

**Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA*

Abstract: Vascular endothelial growth factor (VEGF) is a cytokine primarily involved in angiogenesis. Its expression has also been detected in the stratum corneum of humans with inflammatory skin disease such as atopic dermatitis (AD). The aim of this study was to evaluate VEGF expression in the serum and stratum corneum of 15 privately-owned atopic and 15 healthy dogs, and correlate it with clinical severity of AD in dogs. The severity of AD was evaluated via the Canine Atopic Dermatitis Extent and Severity Index (CADESI)-04. For all dogs, a single blood draw was performed via venipuncture and serum collected. Tape stripping (15 times) was performed to obtain stratum corneum samples from the concave surface of the right pinna and on the dorsolumbar area. A commercially-available canine-specific VEGF enzyme-linked immunosorbent assay was performed on the serum and stratum corneum samples. VEGF was detectable in the serum, but not in the stratum corneum of healthy or atopic dogs. In the serum, a significant difference in VEGF levels was not present between healthy (median: 280.6 pg/ml; range: 274.3-300.3 pg/ml) and atopic dogs (median: 284.1 pg/ml; range: 276.4-522.9 pg/ml) ($P = 0.3$). There was no correlation between serum VEGF levels and CADESI-04 scores. More sensitive molecular technology is required to disprove or confirm these results. Because a high variability was seen in the atopic group, a higher number of dogs is needed to accurately evaluate the role of circulating and cutaneous VEGF in AD.

Sources of funding:University of Florida Foundation Research Grant.

Conflict of Interest: None declared

Clinical effects of two commercially available essential fatty acid-enriched veterinary diets on canine atopic dermatitis

T. M. S. A. BOEHM*, C. J. KLINGER†, L. UDRAITE*, R. S. MUELLER*

**Centre for Clinical Veterinary Medicine, Ludwig Maximilian University, Munich, Germany*
†Tierklinik Stuttgart Plieningen, Veterinary Specialist Hospital, Stuttgart, Germany

Abstract: Canine atopic dermatitis (CAD) is a common skin disease typically associated with IgE antibodies to environmental allergens. Studies have shown beneficial effects in the treatment of CAD with essential fatty acids (EFA). The aim of this study was to compare the effect of a newly designed, EFA-enriched diet on the clinical signs of canine atopic dermatitis to those of another EFA-enriched canine diet. In this prospective, randomised, double-blinded, placebo-controlled study, 31 privately owned dogs with atopic dermatitis received the control diet A (*d/d Salmon*®, Hill's, n=17) or diet B (*Dermatology support*®, Virbac, n=14) for 12 weeks. At each of the four monthly study visits, lesions and pruritus were evaluated with validated scores and a per protocol analysis as well as an intention to treat analysis with the last value carried forward. Coat quality was also assessed. Four dogs were excluded due to disease exacerbation or dietary adverse effects (exacerbation of CAD lesions, increased pruritus, diarrhea and vomiting). One dog refused to eat diet A. Six dogs were excluded because of a change of concomitant therapy and six further patients due to compliance issues. After 12 weeks, there was no significant difference in Canine Atopic Dermatitis Lesion Index (CADLI) ($P = 0.93$) or Pruritus Visual Analogue Scale ($P = 0.91$) scores between the two diets and over time with both analyses. Coat quality improved significantly with diet B ($P = 0.029$). In this study, although no differences in CADLI and pruritus were noted with either diet, diet B improved coat quality.

Sources of funding: This study was funded by Virbac, France.

Conflict of Interest: This study was funded by Virbac, France.

Comparison of three clinical scoring systems for insect bite hypersensitivity in a herd of Icelandic horses

J MILLER*, S MANN†, B WAGNER†

**Department of Clinical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA*

† Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Abstract: Insect bite hypersensitivity (IBH) is one of the most common equine allergic conditions affecting horses worldwide. Definitive diagnostics and treatments for this condition are lacking, therefore numerous research efforts are being undertaken to address this problem. To date, no universally accepted clinical scoring system that helps to differentiate horses with IBH from unaffected horses exists. Such a scoring system would provide an additional objective tool to harmonize reporting of research efforts, and allow for additional guidance for clinical practice. In this study, we compared three IBH scoring systems which were based on systems developed for IBH research by Drs. Bettina Wagner, Antonia Fettelschoss-Gabriel, and Tanja Geiben. Objectives were a) to determine the best allergic cut-off for each scoring system, b) to test the accuracy of the system when compared to the clinical diagnosis of an experienced veterinarian, and c) to assess agreement with one another. Icelandic horses (n=20) in a single herd, including eight with IBH, were scored by a single observer initially blinded to the horses' IBH status every two weeks from April until September (predicted *Culicoides* midges activity). Separate logistic regression models were performed for each time point and scoring method. Receiver operating characteristic analysis was used to determine the best allergic cut-off for each time point. Discriminatory ability of each scoring system was high and the systems agreed well with each other. Our results support the use of these scoring systems as a simple, objective measure that can be used as an adjunct tool in diagnosing IBH.

Sources of funding: The research horses in this study were partially supported by funding from the Harry M. Zweig Memorial Fund for Equine Research at Cornell University.

Conflict of Interest: None declared

Development and validation of a graphic two-dimensional Investigator's Global Assessment instrument for assessing the overall severity of atopic dermatitis in dogs

E. J. HIGH* and T. OLIVRY*

**Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA*

Abstract: Clinical trials enrolling dogs with atopic dermatitis (AD) have utilized validated instruments to grade both extent and severity of skin lesions; none of these tools provide global assessment of disease severity, however. We aimed to develop and validate an Investigator Global Assessment (IGA) tool to evaluate the overall severity of AD in dogs. A two-dimensional graphic IGA (2D-IGA) instrument was created to score, with a single dot, both extent and severity of AD lesions. This tool was tested for its validity (content, construct, criterion), reliability (inter-/intra-observer) and sensitivity to change. Twenty-six atopic dogs were evaluated with 2D-IGA. This instrument's content was validated by a supportive vote from the International Committee on Allergic Diseases of Animals. Its construct was verified by positive correlations existing between scores of the 2D-IGA and those of Canine Atopic Dermatitis Extent and Severity Index-4 and Canine Atopic Dermatitis Lesion Index (Spearman, $P < 0.0001$). Positive correlation ($P < 0.0002$) between 2D-IGA values and those of an Owner Global Assessment of Disease Severity satisfied this instrument's criterion. Values scored by the same investigator hours apart and those between investigators were also positively correlated ($P < 0.0001$), thereby validating this scale's intra- and inter-observer reliabilities. Finally, the changes in 2D-IGA values during treatment were positively correlated with scores of an Owner Global Assessment of Treatment Efficacy ($P < 0.0001$), showing its sensitivity to change. In summary, the 2D-IGA is a simple graphic tool that could be useful for clinical trials testing the efficacy of interventions for canine AD.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Canine pruritus visual analogue scale: How does it capture owners' perception of their pet's pruritus level?

A.J. YOUNG*, S.M. TORRES*, S.N. KOCH*, M.N.C. EISENSCHENK†, A.K. RENDAHL‡

** Veterinary Clinical Sciences, University of Minnesota, St. Paul, MN, USA*
† Pet Dermatology Clinic, Maple grove, MN, USA
‡Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA

Abstract: Accurate measurement of pruritus severity is difficult in veterinary medicine. Our objective was to determine how the change in pruritus visual analogue scale (PVAS) scores at follow-up visits correlates with the owner's perception of improvement of their pet's pruritus. Prospectively, 190 pruritic dogs were randomly assigned into five groups and PVAS scores were recorded during two consecutive visits. Group A: previous scores were shown before completing the PVAS; group B: PVAS was completed then owners were shown previous scores and asked to repeat the PVAS; group C: PVAS was completed as previously reported; group D: PVAS and a 0-10 verbal scale (VS) were completed. Retrospectively, PVAS scores were analyzed on 553 pruritic dogs during at least three consecutive visits. Average percent and kappa agreements between PVAS and VS and owner's perception of their pet's pruritus were calculated. PVAS and VS scores were compared in group D. Average percent and kappa agreements were higher in group A (96%; 0.81) and lower in groups B [(80%; 0.54), (82%; 0.59)] and C (79%; 0.37). PVAS and VS scores were not significantly different (P = 0.56). The average percent and kappa agreements for the retrospective study were 50.8% and 0.25, respectively. The highest values (63%; 0.355) were noted at 30-60 day visit intervals. Showing owners previous scores could improve how PVAS captures the change in pruritus at follow-up visits. PVAS and VS scores were similar. Further studies are needed to corroborate our findings.

Sources of funding: Self-funded.

Conflict of Interest: None declared

An assessment of barriers and perceptions of general practice veterinarians for referral and allergen-specific immunotherapy in the management of atopic dermatitis – a pilot study

S. FLANAGAN, A. SCHICK and T. LEWIS

Dermatology for Animals, Gilbert, AZ, USA

Abstract: Allergen-specific immunotherapy (ASIT) has been demonstrated to decrease clinical signs and potential neosensitization in patients with atopic dermatitis (AD). The primary objective of this pilot study was to identify barriers to allergy testing and ASIT. A secondary objective was to identify barriers to dermatology referral for patients with AD. An online survey was administered to small animal veterinarians solicited through several state AVMA chapters. Participants were asked to rate barriers and motivating factors using Likert-scale answers. Survey questions were derived from preliminary interviews and referral satisfaction surveys. Of the 60 responses, 18 (30%) rarely or never recommend ASIT. The top three barriers to recommending ASIT included success utilizing pharmaceutical management of AD (100%), clients' concern for cost (94.4%), and the perception that immunotherapy is unable to reduce pharmacologic dependency (83.3%). The top three motivating factors for referral included concerns for glucocorticoid side effects (83.3%), for patient/owner quality of life (77.8%), and for risk of antibiotic resistant infection (55.6%). The results of the secondary objective revealed a total of 19/60 (31.7%) responders rarely or never refer atopic patients to a dermatologist, with the cost of referral, availability of serology testing, and poor perceived benefit indicated as the largest barriers to specialty referral. Further evaluation of a larger population size may give further insight in ways to overcome barriers to recommending ASIT and referring patients to dermatologists.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Changes in the stress markers cortisol and glucose before and during intradermal testing in cats after single administration of pre-appointment gabapentin

C. HUDEC*, C. GRIFFIN*

**Animal Dermatology Clinic, San Diego, CA, USA*

Abstract: Intradermal allergy testing (IDT) can be difficult to interpret in cats. Studies have shown IDT leads to elevations in serum cortisol which may be an explanation for weak wheal reactions in cats. The primary study objective was to determine if utilizing pre-appointment gabapentin would alter stress before and during IDT, as determined by serum cortisol and glucose concentrations. This was a single-blinded, randomized, cross-over clinical trial of 16 healthy cats. Cats were scheduled two veterinary visits and assigned to receive gabapentin orally (25.0-30.5 mg/kg) or no treatment prior to the first visit and the opposite at the next. Blood samples were obtained directly after physical examination, directly after sedation, and 10 min after the second blood sampling. IDT was performed after the second blood sampling. The primary author also recorded which visit they believed gabapentin was administered. Mean cortisol concentrations were 0.30 µg/dL lower in the gabapentin group albeit not significant ($P = 0.3729$). Mean glucose concentrations were 18 mg/dL higher in the gabapentin group ($P = 0.0014$). Increasing the number of venipuncture attempts ($P = 0.0103$) or time out of carrier ($P = 0.0006$) led to a significant increase in cortisol concentrations when cats did not receive gabapentin but did not significantly influence the gabapentin group. Gabapentin had no effect on intradermal histamine readings. The author was able to correctly identify when 14/16 cats received gabapentin. This study demonstrated that gabapentin administration can diminish outward signs of stress in cats but did not significantly decrease cortisol or glucose concentrations.

Sources of funding: American College of Veterinary Dermatology Research Grant.

Conflict of Interest: None declared

Effect of topical lidocaine on intradermal allergy testing in dogs with atopic dermatitis

L. M. TOMICH*, J. B. PIEPER*, S. C. J. KEATING*, M. C. ALLENDER*

**Department of Clinical Medicine, University of Illinois Veterinary Teaching Hospital, 1008 West Hazelwood Drive, Urbana, IL 61802, USA*

Abstract: Intradermal allergy testing (IDT) is the gold standard for developing allergen-specific immunotherapy and often requires sedation to control pain and patient movement. Sedative drugs create increased risk with some health conditions. Topical lidocaine offers an alternative or adjunct to sedation that has not yet been evaluated for use in IDT. The study objective was to determine whether a 5% lidocaine cream would affect IDT results in dogs with atopic dermatitis. Fifteen dogs with presumed atopic dermatitis based on Favrot's criteria and exclusion of other causes of pruritus were enrolled in this study. A topical 5% lidocaine cream was randomly applied to either the left or right thorax 30 minutes prior to testing. A full IDT was performed on each side of the thorax. Each test was read at 15 and 30 min after injection by a blinded observer (JBP). Photographs were also taken at 15 and 30 min for measurement of wheal diameter at a later date using image processing and analysis software (ImageJ). Results for histamine and the four most commonly positive allergens were analyzed using paired samples t-test or Wilcoxon signed rank test for normally and non-normally distributed data, respectively. There were no significant differences in any diameter measured from photographs at any time point between the control and lidocaine treatments, however there was poor agreement between the control and lidocaine treatments when assessing the subjective score. Due to poor subjective scoring agreement, lidocaine cream as an adjunctive analgesic to sedation during IDT in dogs is not recommended.

Sources of funding: ACVD Stallergenes Greer Residents' Research Grant.

Conflict of Interest: None declared

Pilot evaluation of *Enterococcus faecium* SF68 as adjunctive therapy for adult atopic dogs responsive to oclacitinib

C. YAMAZAKI*; W. ROSENKRANTZ*; C. GRIFFIN†

**Animal Dermatology Clinic, 2965 Edinger Ave, Tustin, CA 92780, USA*
†Animal Dermatology Clinic, 5610 Kearny Mesa Rd. Suite B-1, San Diego, CA 92111, USA

Abstract: Probiotics have potential to alleviate atopic dermatitis (AD) clinical signs given their immunomodulatory effects (increasing interleukin [IL]-12, IL-10, transforming growth factor-beta, interferon-gamma, decreasing IL-4, inducing types 1, 3 and regulatory T helper cell cytokines), as well as modulating gut microbiota and strengthening barrier function. Previous studies of *Lactobacillus rhamnosus* GG in purpose-bred dogs with AD were inconclusive. *In vivo* studies evaluating probiotic supplementation licensed for use in dogs as adjunctive therapy in management of AD in adult dogs is lacking. Supplementation with a veterinary-licensed *Enterococcus faecium* SF68 product (FortiFlora®) was shown to have immunomodulatory effects in as few as 4 weeks. The current randomized double-blind placebo-controlled 12-week study evaluated the effect of SF68 on reducing oclacitinib (Apoquel ®) dosing, while maintaining or reducing Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4 and Pruritus Visual Analog Scale (PVAS) in 21 client-owned AD dogs exhibiting control on oclacitinib for at least the previous 6 months, continuing throughout the study. Enrolled dogs had adverse food reaction ruled out with elimination diet trial(s). Supplementation over 12 weeks with SF68 (1x10^8 CFU/g orally twice daily) versus placebo revealed no difference in oclacitinib dose reduction. PVAS and CADESI-4 scores were not different between groups at study completion. Based on these results, SF68 supplementation had no effect on oclacitinib dosing, while attempting to maintain or reduce PVAS and CADESI-4 scoring throughout the study. Further larger-scale studies are warranted to evaluate optimal strain(s), dosing and duration of probiotic supplementation as an adjunctive strategy in canine AD management.

Sources of funding: Supplies and funding were provided by Nestlé Purina Pet Care.

Conflict of Interest: C. Griffin has lectured for and received research support from Nestlé Purina Pet Care.

Comparison of malignancies and nonmalignant skin masses in 339 allergic dogs receiving long-term (> 6 months) oclacitinib with age and breed matched control population

B. LANCELLOTTI*, J. ANGUS*, H. EDGINTON*, W. ROSENKRANTZ†

**Animal Dermatology Clinic, Pasadena, California, USA*
†Animal Dermatology Clinic, Tustin, California, USA

Abstract: This study aimed to compare the incidence of malignancies and nonmalignant skin masses in canines receiving long-term oclacitinib (Apoquel®) with age and breed matched canines with allergic dermatitis. This age and breed matched retrospective cohort study included 339 dogs receiving oclacitinib and 321 age and breed matched dogs treated for allergic dermatitis without oclacitinib with 112 breeds and/or mixes represented. Electronic medical records from four dermatology specialty clinics were reviewed for allergic dogs receiving oclacitinib for >6 months. Age and breed matched controls consisted of dogs diagnosed and treated for any hypersensitivity dermatitis for which oclacitinib is indicated prior to the introduction of oclacitinib in 2013. Both groups required a minimum of 2 years of follow up or death/euthanasia within 2 years. Incidence of malignancies, nonmalignant skin masses and age of death/euthanasia was compared. The effect of oclacitinib dose on incidence of malignancies and masses was evaluated. Mean and median duration of oclacitinib treatment was 35.5 months and 36 months respectively (range 6-58 months). The incidence of malignancies and skin masses in the oclacitinib group (16.2%, 57.2%, respectively) versus controls (12.5%, 60.1%, respectively) was not statistically different ($P = 0.1687$, $P = 0.4500$, respectively). The age of death in the oclacitinib group (11.2 years) versus controls (11.8 years) was not statistically different ($P = 0.2077$). There was no significant effect of oclacitinib dose on incidence of malignancy or masses. Treatment with oclacitinib for > 6 months does not significantly increase incidence of malignancies compared to best available therapy.

Sources of funding: Self-funded.

Conflict of Interest: Dr. Lancellotti has no conflicts of interest. Dr. Angus, Dr. Rosenkrantz and Dr. Edginton have previously received financial support for participation in continuing education lectures and clinical research from Zoetis. Dr. Angus and Dr. Rosenkrantz personally own Zoetis Inc. stock.

Prevalence of bilateral feline inflammatory polyps: a retrospective analysis

S. HOPPERS*, E. DRAKE*, L.A. FRANK*

**Department of Small Animal Clinical Sciences, University of Tennessee, Knoxville, TN USA*

Abstract: Feline inflammatory polyps (IP) are benign growths that may originate from the epithelium of the nasopharynx, middle ear, or auditory tube of cats. They have been reported as solitary growths, but bilateral polyps may occur more commonly than previously described. The study objective was to identify the prevalence of and risk factors associated with bilateral feline IP within a population of cats seen at a veterinary teaching hospital from 2005-2015. A medical records search identified 28 cases of histologically confirmed IP. The cases were separated into groups based on unilateral versus bilateral disease. Eight of twenty-eight (28.5%) cases featured bilateral IP. Computed tomography was performed in 12 cats (4/8 with bilateral IP; 8/20 unilateral IP). Potential risk factors such as history of prior upper respiratory infection ($P = 0.2$) and age ($P = 1$) were compared between groups, with no significant differences identified. Twenty-two of twenty-five cats had history of otitis externa, but it is unknown if otitis externa preceded or was concurrent with IP. Clinical signs ($P = 1$) and post-treatment complications ($P = 0.46$) were also compared between groups, and no significant differences were identified. In 3/8 cases with bilateral disease, the second polyp was only identified with advanced imaging performed at the time of referral. These findings suggest that occurrence of bilateral IP is more common than previously reported. It is possible that the prevalence reported here may underestimate the frequency of bilateral IP, because not all cats had advanced imaging performed.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Stability of diluted ceftazidime in three otic preparations under different storage conditions over a 28-day period

S.E HOFF*, D.J. BERGER*, D. SCHRUNK†, A.K. VIALL‡, J.O. NOXON*

** Department of Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA*
† Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA.
‡ Department of Veterinary Pathology, Iowa State University, Ames, IA, USA

Abstract: *Pseudomonas aeruginosa* is commonly isolated in cases of canine otitis externa and is intrinsically resistant to certain antimicrobial classes. Ceftazidime is a third-generation cephalosporin with demonstrated anti-pseudomonal activity that has anecdotally been used in compounded otic preparations when multi-drug resistant strains are present. This study aimed to determine the stability of three compounded ceftazidime preparations stored at different temperatures (room-temperature [25ºC], refrigerated [4ºC] and frozen [-20ºC]). Commercially available 1-gram vials of ceftazidime (TAZICEF®) were reconstituted to create stock solutions: 1) Ceftazidime + 100 mLs 0.9% NaCl (NA), 2) Ceftazidime + 118 mLs tris-EDTA (TrizEDTA® Aqueous Flush) (TE), 3) Ceftazidime + 125 mLs 0.02% phytosphingosine HCl (DOUXO® Micellar Solution) (MI) that were stored in 1.0 mL aliquots under the three storage conditions. Stock solutions were made in duplicate while all samples were quantitated in triplicate. Ceftazidime recovery was performed by solvent dilutions and concentration measured via high-performance liquid chromatography at days 0, 7, 14, 21 and 28. Storage temperature, storage length, and diluent each had independent significant effects on the stability of ceftazidime ($P < 0.001$). Storage length was associated with significantly decreased ceftazidime concentrations ($P < 0.001$). Room-temperature stored samples showed increased degradation of ceftazidime over time in each diluent compared to refrigerated and frozen samples ($P < 0.001$). The NA solution demonstrated the best stability of ceftazidime over time, while the TE solution showed the least stability ($P < 0.001$). In vivo studies are warranted to evaluate the clinical efficacy of compounded ceftazidime solutions against *Pseudomonas* otitis externa.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Stability of dexamethasone when added to commercial veterinary ear cleaners over a 90 day period

C. B. EMERY*, C. A. OUTERBRIDGE†, H. K. KNYCH‡, A.T.H. LAM†, J. P. GOMEZ-VAZQUEZ§, and S. D. WHITE†

**William R. Pritchard Veterinary Medical Teaching Hospital, University of California Davis, Davis, CA, USA*
†Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, Davis, CA, USA
‡Department of Molecular Biosciences, School of Veterinary Medicine, University of California Davis, Davis, CA, USA
§Center for Animal Disease Modeling and Surveillance, University of California Davis, Davis, CA, USA

Abstract: Topical corticosteroids are commonly used in management of allergic otitis externa to diminish inflammation and may include adding dexamethasone to ear cleaners. The objective of this study was to determine the stability of 2 mg/ml dexamethasone when added to four commercial ear cleaners; designated ear cleaner (ec)A, ecB, ecC, and ecD. Two concentrations (0.1 mg/ml and 0.25 mg/ml) were formulated for each cleaner with solutions stored in manufacturers' bottles at two temperatures: ambient (22˚C) and refrigerated (4˚C). Samples, evaluated in triplicate, used liquid chromatography-tandem mass spectrometry at 10 time points over 90 days. Separate mixed effects regression models were used to evaluate the formulated concentrations. A solution was considered stable if the dexamethasone value remained above 90% of original concentration. Statistical differences showed the 0.25 mg/ml solutions had lower measured concentrations 4˚C ($P = 0.04$) and that concentrations of dexamethasone in both ecA solutions (0.1 mg/ml $P = 0.039$ and 0.25 mg/ml $P = 0.005$) and the 0.1 mg/ml ecC ($P = 0.001$) were statistically lower than other solutions. However, the 0.1 mg/ml ecC and 0.1 mg/ml ecA remained above 90% at 90 days whereas 0.25 mg/ml ecA solution did not. The results showed dexamethasone less stable in ecA (0.25 mg/ml) than in other cleaners, being stable to 14 days (22˚C) and 21 days (4˚C). All other dexamethasone solution values were stable to 90 days. These results establish pharmacologic stability data for the above compounded solutions at the noted concentrations and temperatures.

Sources of funding: The Center of Companion Animal Health Resident Grant, School of Veterinary Medicine, University of California Davis.

Conflict of Interest: None declared

Ear cytology and resident flora of clinically normal alpacas (*Vicugna pacos*)

C. A. MAZULIS*, H. N. ERB†, A. THACHIL†, and W. H. MILLER*

**Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
†Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

Abstract: Otitis is common in alpacas. Suppurative otitis media/interna can be an extension from the external ear canal or from a respiratory infection. Cytology provides rapid and inexpensive information to assist in therapeutic decision; to date there is no published information regarding the normal cytology and flora of the alpaca external ear canal. To describe normal resident cytology and flora at two different months, cytologies and cultures of clinically normal alpaca ears were examined in August 2017 and January 2018. Fifty privately owned, healthy alpacas of different ages and sexes in two northeastern United States flocks were sampled. One ear per alpaca was sterilely swabbed (bacterial and fungal cultures) and then swabbed from ectoparasites and cytology (inflammatory cells, epithelial cells, bacteria, and yeast). Most alpacas had mild ceruminous debris. No ectoparasites or inflammatory cells were noted. Yeast organisms were noted cytologically in 4% (one per high power field [HPF]) and 2% (1/HPF) in August and January, respectively. Fungal growth was noted in 6% in August and 30% in January. Cytologically, rod-shaped bacteria (1-10/HPF) were seen in 50% in August and 26% in January. Coccal bacteria (1-10/HPF) were seen in 32% in August and 16% in January. No statistically significant findings were noted between sampling months. Common bacterial genera isolated in August were *Bacillus* (44%), *Arthrobacter* (40%), and non-hemolytic *Staphylococcus* (26%) and in January were *Bacillus* (42%), *Pantoea* (38%), and hemolytic *Staphylococcus (*18%). This information may be useful when evaluating alpaca external ear canal samples, which subsequently may help dictate empirical therapy.

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

Conflict of Interest: None declared

INFLAMMATION VS. LYMPHOMA IN ANIMALS

Stefan M. Keller, DVM, Dr.med.vet., PhD, DECVP

Department of Pathobiology, Ontario Veterinary College, Guelph, Canada

INTRODUCTION

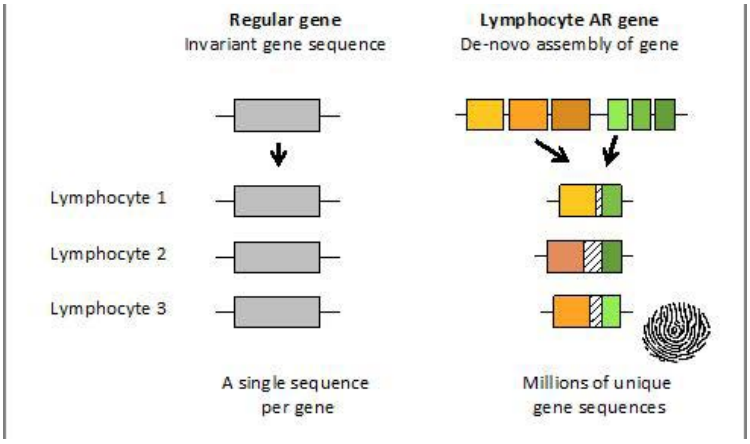
Differentiating inflammation from lymphoma can be challenging - clinically as well as by histopathology. Clonality testing or PCR for Antigen Receptor Rearrangement (PARR) is a molecular method that can be used to help diagnose lymphoproliferative diseases when traditional methods yield equivocal results. The method harnesses the unique feature of lymphocytes to generate an almost infinite number of lymphocyte antigen receptor gene variants that can be used to differentiate or track lymphocyte clones. This talk will discuss the working principle of clonality testing and illustrate its application and limitations in veterinary dermatopathology.

OBJECTIVE

The objective of the talk is to illustrate the utility of clonality testing for differentiating inflammation from lymphoma in veterinary dermatopathology.

IMMUNOLOGY PRIMER

Lymphocytes are a pivotal part of the adaptive immune system and can recognize an almost unlimited number of antigens through their lymphocyte antigen receptor (LAR). These receptors are more commonly known as antibodies or immunoglobulins (B cells) or T cell receptors (T cells). In contrast to other genes, LAR genes do not have a conserved nucleotide sequence but a unique LAR gene is generated in every lymphocyte (Fig. 1). This happens early in lymphocyte development, for B cells in the bone marrow, for T cells in the thymus. Different types of LAR gene segments are randomly recombined (Fig. 1 colored segments) and nucleotides are inserted between the gene segments (Fig. 1 striped segments). This creates a 'junctional region' that differs in size and nucleotide composition between lymphocytes and defines the specificity of a lymphocyte.



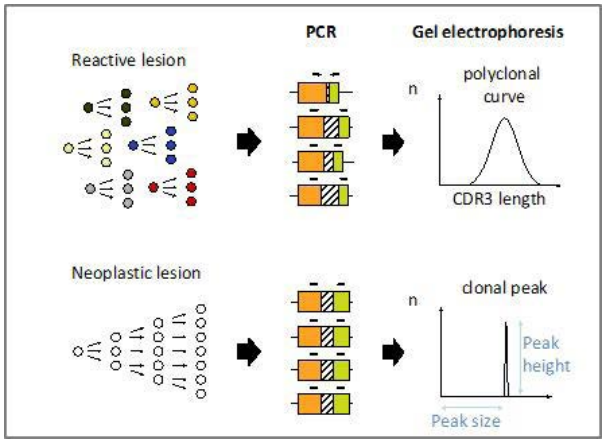


Fig. 1. With few exceptions, every cell in an individual carries the same genetic information. In contrast, lymphocytes have highly variable LAR genes that are created by genetic recombination and that can be used as a molecular fingerprint to identify clones.

Fig. 2. Clonality testing amplifies LAR gene sequences by PCR and separates amplicons by gel electrophoresis resulting in distinct and divergent profiles for reactive and neoplastic processes.

PRINCIPLE OF CLONALITY TESTING

Clonality testing visualizes the clonal diversity of a lymphocyte population through PCR-based amplification of LAR genes followed by size separation of amplicons by gel electrophoresis (Fig. 2). First LAR genes are amplified using primers that are located upstream and downstream of the junctional region. Subsequently, PCR products are size

separated by slab gel electrophoresis or capillary electrophoresis. In reactive processes, many lymphocyte clones proliferate, which results in a heterogeneous lymphocyte population with antigen receptor genes of various sizes. This will result in a 'polyclonal' smear or normally distributed curve on gel electrophoresis (Fig. 2 – top). In lymphoma, all lymphocytes have the same LAR gene sequence and all PCR products are hence of equal size. This will result in a 'clonal' band or peak when visualized by electrophoresis (Fig. 2 – bottom). Consequently, polyclonal results are suggestive of a reactive/inflammatory process while clonal results are indicative of lymphoma. Exceptions to this rule are discussed in the next paragraph. In addition to differentiating between reactive and neoplastic processes, clonality testing allows tracking of neoplastic lymphocyte clones across different time points or locations by the size of the amplified LAR product.

LIMITATIONS OF CLONALITY TESTING

A limitation of clonality testing is that false negative results can lead to lymphoma being diagnosed as inflammation. A false negative result in the context of clonality testing refers to the failure to recognize a neoplastic clone. Awareness of the circumstances in which false negative results can occur is important because the likelihood of a false negative result might modulate the interpreter's confidence in a diagnosis. Causes for false negative results are as follows: 1) The use of primer sets that do not recognize all rearranged gene segments; This cause will eventually become obsolete as primer sets are being improved; 2) Reactive lymphocytes that quench a clonal signal (Fig. 3); this is a general problem in disease processes that are associated with a significant degree of inflammation. Thorough histopathologic assessment of a lesion will identify cases where abundant polyclonal background might be a problem; 3) Mutation or primer sites due to somatic hypermutation (Fig. 4); B cells mutate their LAR genes to increase the affinity of antibodies. This might negatively affect primer binding and amplification.

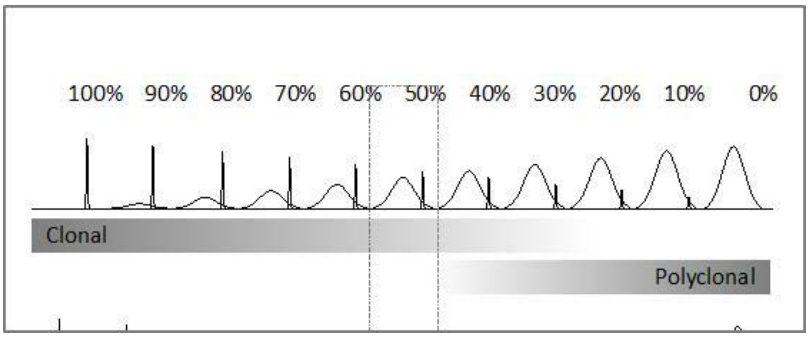


Fig. 3. Dilution of clonal into polyclonal DNA. With increasing 'polyclonal background, the neoplastic clone becomes increasingly obscured.

Fig.4. Schematic of a primer binding to a LAR gene sequence without (top) and with (bottom) somatic hupermutation. The required complementarity of primer and target is lost (bottom) and binding abrogated resulting in no amplification, i.e. a false negative result (red letters = mutated bases).

False positive results, i.e. the presence of a non-neoplastic clone in an inflammatory process, have been described less often and are more difficult to identify. Non-neoplastic or 'benign' clones have been described in response to infectious organisms or drugs.[1-3]

Finally, mixtures of the two archetypal electrophoresis profiles 'clonal' and 'polyclonal' occur. In these cases it might not be possible to reliably differentiate between lymphoma with concomitant inflammation versus an immune response with limited LAR repertoire.

BEST PRACTICES

In human medicine, clonality testing has been standardized by a large multi-institutional consortium, which has created reagents and defined interpretational guidelines.[4-6] In veterinary medicine, no similar efforts have been undertaken and reagents and opinions diverge across institutions. One of the less controversial points is that knowledge of the immunophenotype of the presumed neoplastic cells is important for 1) deciding if a B or T cell primer set should be run and 2) to aid in the interpretation of the results. For cytology samples, immunophenotyping

is often not feasible and both B and T cell assays are commonly run simultaneously. However, for histopathology samples, performing immunohistochemistry before clonality testing will greatly aid the interpretation of clonality results and, in some instances, may give a definite diagnosis.

CLONALITY TESTING IN VETERINARY DERMATOLOGY

Overt cutaneous lymphomas do not require clonality testing. However, quite often the line between lymphoma and inflammation is blurred and histopathology and immunohistochemistry might not yield a definite result. By investigating the clonal composition of lesional lymphocytes, clonality testing can add another facet to a multi-pronged diagnostic work-up.[3]

EPITHELIOTROPIC LYMPHOMA

Analogous to humans, three forms of cutaneous epitheliotropic lymphoma have been recognized in animals: 1) Mycosis fungoides (MF), the most common form spanning the epidermal/dermal junction; 2) Pagetoid reticulosis, in which neoplastic cells are confined to the epidermis and adnexa; and 3) Sézary syndrome, in which neoplastic cells are found in the blood in addition to the skin.[7] Epitheliotropic lymphoma has to be distinguished from inflammatory skin diseases at the interface of epidermis and dermis. Moore and colleagues found a clonally rearranged T cell receptor gene in 80% of dogs with MF.[7] Subsequently, Caubert et al. investigated clonality in canine epitheliotropic T cell lymphoma.[8] Since then, additional sequence data have become available and primer sets have been improved further.[9]

NON-EPITHELIOTROPIC LYMPHOMA

Non-epitheliotropic lymphoma may pose a diagnostic challenge if accompanied by abundant inflammation.

In the dog, dermal inflamed lymphomas might be confused with histiocytic disorders such as reactive histiocytosis or histiocytic tumors due to the frequent involvement of abundant histiocytes. Moore et al. described a series of dogs with inflamed, non-epitheliotropic cutaneous T cell lymphoma.[10] Twenty-three out of 24 dogs had a clonally rearranged T cell receptor gene. Because neoplastic cells were frequently outnumbered by inflammatory cells, careful assessment of histopathology and immunohistochemistry in conjunction with histopathology was required for a correct diagnosis. Noland et al. described a small case series of subcutaneous nonepitheliotropic T-cell lymphomas.[11] Heavy infiltration of histiocytes masked the neoplastic infiltrate in some sections and a clonally rearranged T cell receptor gene was found in 4/5 cases.

In the cat, cutaneous lymphomas are more commonly non-epitheliotropic and small cell and fewer studies have investigated their clonality. One study examined 17 primary cutaneous lymphomas with a history of vaccine injection at the site of tumor development.[12] These tumors shared clinical and pathological features with feline injection site sarcomas and with lymphomas developing in the setting of subacute to chronic inflammation reported in human beings. Given the presence of inflammation, these cases might be confused with inflammation. In this study 8/17 (47%) cases had a clonally rearranged B cell receptor locus (IGH) and 3/17 (18%) had a clonally rearranged T cell receptor. The failure to detect a clonal rearrangement was attributed to either concomitant inflammation obscuring a neoplastic clone, a primer set that failed to detect the neoplastic clone or a natural killer cell origin.

CUTANEOUS LYMPHOCYTOSIS

In humans, cutaneous lymphocytosis, also known as cutaneous pseudolymphoma, cutaneous lymphoid hyperplasia, lymphocytoma cutis and lymphoid dyscrasia, refers to a group of lymphoproliferative skin diseases that are either self-limiting or slowly progressive.[13] In veterinary medicine, the term cutaneous lymphocytosis simply refers to an accumulation of lymphocytes in the dermis, regardless of cause or biological behavior.[14] Using clonality testing, it has been shown that some cases of cutaneous lymphocytosis are in fact indolent lymphomas. Affolter et al. investigated 8 cases of cutaneous lymphocytosis in dogs and found that all cases were of T cell lineage and either had a clonally rearranged T cell receptor gene (7/8) or yielded a pseudoclonal result, which in combination with a T cell phenotype, is consistent with a neoplastic lymphocyte population. In cats, cutaneous lymphocytosis is also primarily composed of T cells with occasional B cell infiltrates and a clonally rearranged antigen receptor gene has been found in the majority of cases. [14-16]

PLASMA CELL TUMORS

Cutaneous plasma cell tumors are often straightforward to diagnose but can exhibit morphologic overlap with canine

cutaneous histiocytoma or T cell lymphomas. Only few reports have investigated the use of molecular clonality testing for cutaneous plasma cell tumors in animals. Takanosu et al. compared the sensitivity of different primer sets for plasmacytoma versus diffuse large B cell lymphoma and found a markedly lower sensitivity for plasma cell tumors.[17] The authors attributed this finding to mutation of primer sites during somatic hypermutation. One case report used clonality testing to illustrate the progression from cutaneous plasmacytoma to plasma cell leukemia.[18] Both, the cutaneous tumor and the neoplastic cells in the blood had immunoglobulin heavy chain gene rearrangements of the same size suggesting that both lesions were derived from the same neoplastic clone.

OUTLOOK – SEQUENCING BASED CLONALITY TESTING

Advancements in sequencing technology are about to revolutionize the methodology of clonality testing and will offer novel opportunities. High throughput sequencing (HTS) will replace gel electrophoresis (GE) as a means to investigate repertoire diversity. 'Clonality' can be characterized much more precisely using HTS than GE because it defines a clone by sequence rather than gene size. The utility of HTS-based clonalty testing is being investigated in human medicine.[19-23] One study showed that NGS-based clonality testing is more diagnostically specific than PCR-based analyses.[19] Moreover, some identically sized peaks detected in concurrent skin and peripheral blood specimens and that were identical by GE turned out to be non-identical by HTS analysis suggesting that the traditional method of minimal residual disease detection might yield false positive results in some cases. As the cost of sequencing is decreasing, HTS-based clonality testing will soon become feasible as the standard method for clonality testing in veterinary medicine.

REFERENCES

1. Burnett, R. C. *et al.* Diagnosis of canine lymphoid neoplasia using clonal rearrangements of antigen receptor genes. *Veterinary pathology* 40, 32-41 (2003).
2. Gentilini, F., Calzolari, C., Turba, M. E., Bettini, G. & Famigli-Bergamini, P. GeneScanning analysis of Ig/TCR gene rearrangements to detect clonality in canine lymphomas. *Veterinary immunology and immunopathology* 127, 47-56, doi:10.1016/j.vetimm.2008.09.014 (2009).
3. Keller, S. M., Vernau, W. & Moore, P. F. Clonality Testing in Veterinary Medicine: A Review With Diagnostic Guidelines. *Veterinary pathology* 53, 711-725, doi:10.1177/0300985815626576 (2016).
4. Langerak, A. W. *et al.* EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia* 26, 2159-2171, doi:10.1038/leu.2012.246 (2012).
5. van Dongen, J. J. *et al.* Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia* 17, 2257-2317, doi:10.1038/sj.leu.2403202[pii] (2003).
6. van Krieken, J. H. *et al.* Improved reliability of lymphoma diagnostics via PCR-based clonality testing: report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia* 21, 201-206, doi:10.1038/sj.leu.2404467 (2007).
7. Moore, P. F., Affolter, V. K., Graham, P. S. & Hirt, B. Canine epitheliotropic cutaneous T-cell lymphoma: an investigation of T-cell receptor immunophenotype, lesion topography and molecular clonality. *Vet Dermatol* 20, 569-576, doi:10.1111/j.1365-3164.2009.00814.x (2009).
8. Chaubert, P. *et al.* Improved polymerase chain reaction-based method to detect early-stage epitheliotropic T-cell lymphoma (mycosis fungoides) in formalin-fixed, paraffin-embedded skin biopsy specimens of the dog. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc* 22, 20-29 (2010).
9. Keller, S. M. & Moore, P. F. A novel clonality assay for the assessment of canine T cell proliferations. *Veterinary immunology and immunopathology* 145, 410-419, doi:10.1016/j.vetimm.2011.12.019 (2012).
10. Moore, P. F., Olivry, T. & Naydan, D. Canine cutaneous epitheliotropic lymphoma (mycosis fungoides) is a proliferative disorder of CD8+ T cells. *The American journal of pathology* 144, 421-429 (1994).
11. Noland, E. L., Keller, S. M. & Kiupel, M. Subcutaneous Panniculitis-Like T-cell Lymphoma in Dogs: Morphologic and Immunohistochemical Classification. *Veterinary pathology* 55, 802-808, doi:10.1177/0300985818789474 (2018).

12. Roccabianca, P. *et al.* Cutaneous Lymphoma at Injection Sites: Pathological, Immunophenotypical, and Molecular Characterization in 17 Cats. *Veterinary pathology* 53, 823-832, doi:10.1177/0300985815623620 (2016).
13. Affolter, V. K., Gross, T. L. & Moore, P. F. Indolent cutaneous T-cell lymphoma presenting as cutaneous lymphocytosis in dogs. *Veterinary dermatology* 20, 577-585, doi:10.1111/j.1365-3164.2009.00833.x (2009).
14. Gilbert, S., Affolter, V. K., Gross, T. L., Moore, P. F. & Ihrke, P. J. Clinical, morphological and immunohistochemical characterization of cutaneous lymphocytosis in 23 cats. *Veterinary dermatology* 15, 3-12 (2004).
15. Gilbert, S., Affolter, V. & Schmidt, P. Clonality studies of feline cutaneous lymphocytosis. *Veterinary dermatology* (2004).
16. Pariser, M. S. & Gram, D. W. Feline cutaneous lymphocytosis: case report and summary of the literature. *J Feline Med Surg* 16, 758-763, doi:10.1177/1098612X14545275 (2014).
17. Takanosu, M., Okada, K. & Kagawa, Y. PCR-based clonality analysis of antigen receptor gene rearrangements in canine cutaneous plasmacytoma. *Vet J* 241, 31-37, doi:10.1016/j.tvjl.2018.09.010 (2018).
18. Rout, E. D. *et al.* Progression of cutaneous plasmacytoma to plasma cell leukemia in a dog. *Veterinary clinical pathology / American Society for Veterinary Clinical Pathology* 46, 77-84, doi:10.1111/vcp.12463 (2017).
19. Rea, B. *et al.* Role of high-throughput sequencing in the diagnosis of cutaneous T-cell lymphoma. *Journal of clinical pathology* 71, 814-820, doi:10.1136/jclinpath-2018-205004 (2018).
20. Weng, W. K. *et al.* Minimal residual disease monitoring with high-throughput sequencing of T cell receptors in cutaneous T cell lymphoma. *Sci Transl Med* 5, 214ra171, doi:10.1126/scitranslmed.3007420 (2013).
21. Kirsch, I. R. *et al.* TCR sequencing facilitates diagnosis and identifies mature T cells as the cell of origin in CTCL. *Sci Transl Med* 7, 308ra158, doi:10.1126/scitranslmed.aaa9122 (2015).
22. Langerak, A. W. *et al.* High-Throughput Immunogenetics for Clinical and Research Applications in Immunohematology: Potential and Challenges. *Journal of immunology* 198, 3765-3774, doi:10.4049/jimmunol.1602050 (2017).
23. Bystry, V. *et al.* ARResT/Interrogate: an interactive immunoprofiler for IG/TR NGS data. *Bioinformatics*, doi:10.1093/bioinformatics/btw634 (2016).

The Immunopathogenesis and Treatment of Human Cutaneous T-Cell Lymphoma: Contrast with the Dog

ALAIN ROOK, MD

Human cutaneous T-cell lymphomas (CTCL) represent a heterogenous group of disorders characterized by skin lesions containing malignant skin-trafficking T-cells. The most common human forms of CTCL are **mycosis fungoides**, typified by patches, plaques, tumors or erythroderma, and **Sezary Syndrome** associated with erythroderma, lymphadenopathy and circulating malignant T-cells (leukemia). The malignant population among the vast majority of human CTCLs consists of CD4+ **alpha/beta T-cell** populations which contrasts with what is observed in dogs where the majority of cutaneous lymphomas are CD8+ or CD3+ **gamma/delta T-cell** malignancies. The malignant cells in man usually lack **CD26** and/or **CD7**, and can be identified in the blood by the absence of these markers on CD4+ T-cells. The prognosis and implications for therapy, described below, are quite significant, as gamma/delta T-cells typically express cytotoxic proteins, including granzyme and perforin, whereas the majority of cases of mycosis fungoides and Sezary syndrome are not cytotoxic cells. A small proportion of CTCLs in humans have a CD8+ phenotype, but most are indolent as well. The exception is **aggressive epidermotropic cytotoxic T-cell lymphoma**. In humans, gamma/delta T-cell lymphomas have a poor prognosis and are considered highly aggressive while CD4+ alpha/beta lymphomas are indolent in about two thirds of cases. The high expression of the adhesion molecule **CLA** (cutaneous lymphoid antigen) and the chemokine receptor **CCR4** are highly relevant to the skin trafficking behavior of the malignant cells infiltrating the skin. CCR4, the receptor for the **chemokine CCL17**, which is produced in the epidermis, is particularly critical for the ability of T-cells to traffic into the skin. This has led to the development of an anti-CCR4 antibody (**Mogamulizumab**) which has recently been FDA approved for human CTCL (see below under therapy). Other targeted therapeutics will be briefly discussed under therapy.

Effects of the Malignant T-Cells on the Host Immune Response

We have known for more than 25 years that the malignant CD4+ T-cells isolated from the blood of patients with human CTCL produce a variety of **soluble factors** which likely permit the cells to circumvent the host immune response. These cells often exhibit a so called **Th2** phenotype characterized by the production of **IL-4 and IL-13** which can suppress the Th1 mediated anti-tumor immune response. Often, there is associated production of **IL-5** by the malignant T-cells which can lead to **hypereosinophilia**. Furthermore, the IL-13 produced may play an important role as an autocrine and paracrine growth factor for the malignant population supporting the perpetuation of growth of these cells. Frequently, the malignant T-cells can produce **IL-10**, and, in some circumstances, **Transforming growth factor beta**, each of which can potently suppress anti-tumor responses.

The expression of a number of different **immune checkpoint molecules**, including PD-1, CTLA-4 and TIGIT, almost certainly plays an important role in immune evasion by the malignant T-cells. This is particularly evident for patients with leukemic CTCL. Nevertheless, malignant T-cells isolated from the skin of non-leukemic CTCL patients also manifest increased expression of immune checkpoint molecules. Recent clinical trials with **immune checkpoint inhibitors**, such the anti-PD-1 antibody Pembrolizumab, have yielded significant responses among patients with advanced refractory CTCL highlighting the relevance of blocking the expression of checkpoint molecules as an important therapeutic approach.

Much less is known regarding the immune phenotype and the nature of factors expressed at the cell surface and the production of soluble molecules by the malignant T-cells in **canine epithiolotropic T-cell lymphoma**. The majority of CTCLs are typified by CD3+ gamma/delta T-cell lymphomas or CD8+ cytotoxic T-cell lymphomas. These cell types are known to contain **cytotoxic molecules**, including granzyme and perforin, which may mediate tissue inflammation and/or damage. They also have the ability to produce interferon gamma and tumor necrosis factor, thus suggesting that interferons should not be used during therapy. The latter findings suggest that anti-inflammatory types of therapeutics, such as cyclosporine, HDAC inhibitors, retinoids or chemotherapeutics are likely to be more effective than pro-inflammatory drugs such as interferons. The exception would be a CD4+ T-cell lymphoma which can respond to interferons or retinoids. Targeted therapies, such as anti-PD-1 or anti-CD30, both of which are likely

to demonstrate efficacy for canine CTCL, are not yet available, although both are currently being used for human advanced CTCL.

Therapy: early stage CTCL

Early stage CTCL is typically considered to encompass **stages IA through IIA** as defined by the **EORTC/ISCL classification**. Approximately 60% of cases at presentation are early stage. **Stage IA** (less than 10% skin involvement with patches or plaques) has in general an excellent prognosis with less than 10% of patients who are treated developing progression of disease. **Stage IB** is defined as patches or plaques involving 10% or more of the skin surface with as many as 20% developing progressive disease with a portion of these patients experiencing fatal disease. Most patients with early stage disease can be effectively treated with skin-directed therapy of which there are numerous choices. Most frequently used are **topical steroids** of mid-potency to high potency, but these often do not produce long term responses and are better used to treat small areas or for pruritus management. Most often used is either **topical chemotherapy**, consisting of topical mechlorethamine (nitrogen mustard) or topical BCNU (carmustine) compounded in ointment or gel. Carmustine appears to be more effective than mechlorethamine for the rare folliculotropic variant as it may penetrate more deeply into the skin where the follicles reside. Topical chemotherapy has a high degree of efficacy and is generally well tolerated. Another common form of skin directed therapy is **phototherapy** consisting or either narrow band UVB or PUVA, the latter being more effective for more infiltrated lesions as UVA can penetrate more deeply into the skin than does UVB. Many patients can have durable responses to PUVA, but it is well known to produce keratinocyte mutations, and, following long term use can be associated with the development of melanoma and non-melanoma skin cancers. Other therapies for early stage include **imiquimod**, a Toll-like receptor agonist and **electron beam therapy**. Multiple new therapies are presently in clinical trial for early stage disease including **topical hypericin** as well as **topical resiquimod** gel. It is noteworthy that in our experience, patients with progressing early stage CTCL can benefit from the use of certain systemic treatments that are considered "biologic" or "immunotherapy". These include **interferon** alpha or gamma injections which can mediate very long term benefit. **Oral retinoids**, including bexarotene, isotretinoin or alitretinoin can also be useful for refractory early stage CTCL.

Treatment: advanced stage CTCL

Advanced stages of CTCL, considered to be **stages IIB through IVB**, typically require systemic therapies to produce clinical improvement. Exceptions may be the occasional good response to either PUVA therapy or to total skin electron beam, both of which can result in complete responses of certain patients with advanced CTCL. **Stage IIB** which consists of one or more tumors, with prognosis related to extent of tumors on the skin and whether cells manifest CD30+ large cell transformation, can be treated with electron beam therapy or with the recently FDA approved antibody, **Brentuximab vedotin**, which targets CD30+ cells and which contains a toxin that is released into the tumor cell following binding. Occasionally, systemically administered histone deacetylase inhibitors (**HDAC inhibitors**), such as romidepsin, are required for extensive tumor stage disease. **Stage IIIA** consists of erythroderma without circulating malignant T-cells. This can often be treated with skin directed therapies, but occasionally requires interferon with or without systemic retinoids. **Stage IIIB**, consisting of erythroderma plus low level circulating malignant T-cells, is often treated with **extracorporeal photopheresis** (ECP) with low dose interferon with or without retinoids. Patients with erythroderma can have severe pruritus which often requires multiple concomitant therapies including topical steroids and antihistamines. **Gabapentin** can be a useful adjunct for pruritus. Recent evidence supports the role of **IL-31** produced by the malignant T-cells in the pathogenesis of pruritus. Anti-IL-31 reagents exist for human pruritus, but none have been tested specifically for CTCL associated pruritus. Treatment of **stage IVA** disease generally, encompassing Sezary syndrome as well as patients with skin and extensive nodal involvement, is treated based upon the degree of blood involvement or the extent of nodal disease and whether the nodal pathology is consistent with large cell transformation. For Sezary syndrome, our initial approach is to utilize a **multimodality** approach with ECP combined with interferon alpha or gamma and bexarotene or another retinoid as tolerated. If patients do not respond, we often add **low dose (12 Gy) total skin electron beam** or proceed to alternative systemic therapies if progression occurs. As mentioned, the anti-CCR4 antibody, **Mogamulizumab,** has been found to be effective for leukemic CTCL patients and is generally well tolerated. Because it eliminates CCR4 positive T-cells by ADCC (antibody dependent cell mediated cytotoxicity), it can at least temporarily remove CCR4+ **regulatory T-cells**

leading to autoreactive phenomenon including dermatitis, thyroiditis, pneumonitis and other inflammatory disorders, but these occur at low frequency. When used just prior to allogeneic transplantation, it is associated with an increased risk of graft versus host disease.

Stage IVB disease is associated with visceral involvement which also includes the oral or tongue mucosa, the rectum or other internal sites other than lymph nodes. Prognosis for these patients is poor and generally requires tumor ablating therapies followed by allotransplantation for a durable response.

For patients with advanced CTCL, stages III and IV, with lymph node disease manifesting large cell transformation, use of **Brentuximab**, which targets CD30+ cells, can be an effective approach when cells have significant expression of CD30 (usually 10% or greater). Use of Brentuximab is associated with **peripheral neuropathy** with risk for this being correlated with numbers of doses.

Romidepsin, a potent HDAC inhibitor, can also be useful for advanced stage CTCL. It is also highly beneficial for pruritus. Adverse effects are most often gastrointestinal with the potential for nausea as well as lethargy and fatigue.

Pralatrexate is a folate antagonist which, when used at a low dose (10 mg/meter squared), can be very useful for maintenance of patients with progressive disease who have few other therapeutic options.

Another antibody which is useful for Sezary syndrome is **Alemtuzumab (Campath)** which targets CD52 present on T-cells and can remove the malignant T-cells from the skin and blood. However, CD52 is also present on B cells, NK cells, monocytes and dendritic cells. Therefore, the prolonged use of Campath can lead to temporary immune deficiency. Therefore, it is typically used at low dose (10 mg three times per week) until the malignant population drops to a low level. But because of the risk of immune deficiency, patients must be prophylaxed with anti-bacterial, anti-fungal and anti-Herpes virus medications until normal lymphocytes can repopulate the blood.

In general, **multidrug chemotherapeutics** are considered as either a bridge to allogeneic transplantation or as palliation when other approaches are not available. Although response rates to chemotherapy are high, responses are typically not durable and are short-lived. Chemotherapy can also be used to rapidly debulk the skin in cases associated with bulky tumors when Brentuximab has not been effective.

Because advanced stage disease is typically not curable, except by **allotransplantation** through the effects of graft versus tumor, this approach is usually discussed with the patient early during their evaluation.

Presently, a number of additional approaches are under investigation. As mentioned above, the **anti-PD-1 antibody**, Pembrolizumab, has been reported in a phase II clinical trial, to produce approximately a 40% response rate when used to treat advanced, refractory CTCL. The use of **immune checkpoint molecule inhibitors**, including anti-PD-1, anti-CTLA 4 and anti-TIGIT, are all likely to have additional testing in the future. Another exciting avenue for therapy includes the potential use of **chimeric antigen receptor T-cells (CAR T-cells)** which are autologous lymphocytes that have their surface receptors genetically manipulated so that they are able to target one or more antigens on tumor cells leading to elimination of the tumor population. Although costly, this approach has proven to be highly successful for B-cell lymphomas and acute lymphocytic leukemia. The approach of using CAR T-cells to treat CTCL is currently under development by multiple groups. Another interesting approach in early clinical trials is the use of antibodies to target **CD47**, the don't eat me molecule expressed at high levels on tumor cells. CD47 inhibits the ability of macrophages to attack and degrade tumor cells. When CD47 is blocked, this can serve as a stimulus so that macrophages can ingest the tumor cells.

Recently, **micro RNAs** have been under study as being pathogenetically related to the genesis of certain diseases, particularly including cancers, such as CTCL. In the case of mycosis fungoides, elevated levels of **Mir 155** can be found in the skin. A recent early trial revealed that an antagonist of Mir 155 resulted in significant clinical improvements. More advanced trials have been announced based upon the the initial results.

References

Larocca C1, Kupper T2.

Mycosis Fungoides and Sézary Syndrome: An Update. Hematol Oncol Clin North Am. 2019 Feb;33(1):103-120. doi: 10.1016/j.hoc.2018.09.001.

(This is an excellent summary of the current literature)

ISVD – MAST CELL TUMORS

Thamm DH

Flint Animal Cancer Center, Colorado State University, 300 W. Drake Rd., Fort Collins, CO 80523 USA

INTRODUCTION

Mast cell tumor (MCT) represents the most common malignant cutaneous tumor in the dog, and is commonly encountered in small animals. There is a large degree of variation in the histologic appearance and biologic behavior of canine MCT, ranging from histologically and behaviorally benign to histologically and behaviorally malignant. However, 65 to 80% of MCT will remain local diseases.

HISTOLOGIC GRADING – IS 2 BETTER THAN 3?

Two equally important pieces of information need to be gleaned from the pathology report: (1) Histologic grade; and (2) Adequacy of surgical margins. Pathologists often utilize a numeric (Patnaik) grading scheme, where "Grade I" is well-differentiated and "Grade III" is poorly differentiated;[1] however, some pathologists will now utilize words such as "low, intermediate or high-grade" or "well, poorly or intermediately differentiated" in place of a numerical scale.

Low or intermediate grade MCT with complete surgical margins usually require no further therapy, as the risk of recurrence or metastasis is only approximately 10%. However, regular rechecks for recurrence, metastasis, or new cutaneous masses is indicated. Low or intermediate grade tumors with *incomplete* surgical margins have a high chance of recurrence, but a low chance for metastasis. Thus, further aggressive local therapy is reasonable. When possible, immediate re-excision of the surgical scar (and an additional 3 cm tissue in all directions and another fascial plane deep) is the most useful treatment. The entire excised tissue should be inked and re-submitted for histopathology. Radiation therapy is an excellent alternative if re-exision is not possible.

High grade MCT with complete surgical margins have a low chance for recurrence, but a high chance for eventual metastasis. Systemic therapy (e.g. prednisone/vinblastine, prednisone/vinblastine/lomustine)[2,3] can be offered in an attempt to delay or prevent this. High grade MCT with incomplete margins have a high likelihood of both recurrence and metastasis: Therapy designed to address both of these possibilities (e.g. additional surgery or radiation therapy, with chemotherapy) is indicated.

The Patnaik grading system may not detect a small percentage of grade I/II MCTs that may behave aggressively, and this is complicated by the fact that there is disagreement in tumor grading schemes among pathologists. In one study, there was significant variation among pathologists in grading a specific set of MCTs, although this was reduced if all pathologists strictly employed the original system described by Patnaik.[1,4,5] Recently, a new grading system has been introduced separates tumors into "high" or "low" grade based on one of four features identified on histopathologic evaluation, in an attempt to minimize inter-pathologist disagreement and still provide useful prognostic information.[6] In this schema, tumors as classified as high grade if they possess 1) at least 7 mitotic figures/10 HPF; 2) at least 3 multinucleated cells/10 HPF; 3) at least 3 bizarre nuclei/10 HPF; or 4) karyomegaly. In a series of dogs evaluated by both systems, the "2-tier" system was somewhat better at predicting which dogs would be more likely to die of disease;[6] at least one follow-up study has confirmed the utility of this 2-tiered system.[7] There remains incomplete information to confirm whether two-tiered system is truly better than the Patnaik system for predicting the biologic behavior of MCT. The author still prefers to receive grades according to both schemes on MCT histopathology reports.

SPECIAL STAINS FOR PROLIFERATION

There is recent information that assessment of mitotic index may be a strong predictor of outcome, identifying intermediate grade tumors at high risk of spread and, potentially, high-grade tumors at lower risk of spread.[8,9]This should be provided on all MCT histopathology reports.

A variety of specialized histochemical or immunohistochemical tests for assessment of proliferation (argyrophilic nucleolar organizer region [AgNOR] Ki67, and proliferating cell nuclear antigen [PCNA]) have been evaluated for their predictive value and have likewise been demonstrated to correlate well with grade and postsurgical outcome.[10-14]

However, it is not clear as yet whether these more cumbersome assessments provide any more prognostic information than that provided by simple assessment of mitotic index.

MAST CELL TUMOR AND KIT

Cancer is a disease characterized by dysregulated growth, abridged cell death, and enhanced cell migration, invasion and angiogenesis. While the molecular mechanisms responsible for this phenotype are very diverse, one class of molecule that has been receiving a great deal of recent attention as a target for therapy are the **receptor tyrosine kinases (RTKs)**. These are cellular receptors for extracellular growth factors that allow communication from the extracellular milieu to the cell interior, mediating functions such as growth, survival, invasion and angiogenesis. Multiple RTKs are expressed by almost all tumor cells, but they can play variable roles in the pathogenesis of the disease.

Most RTKs exist as monomers, which dimerize upon contact with the appropriate ligand, inducing a conformational shift that allows phosphorylation of tyrosine residues in the intracellular domain. This then triggers several different intracellular second messenger cascades, culminating in altered gene expression and, often, a more malignant phenotype (**Fig. 1**).

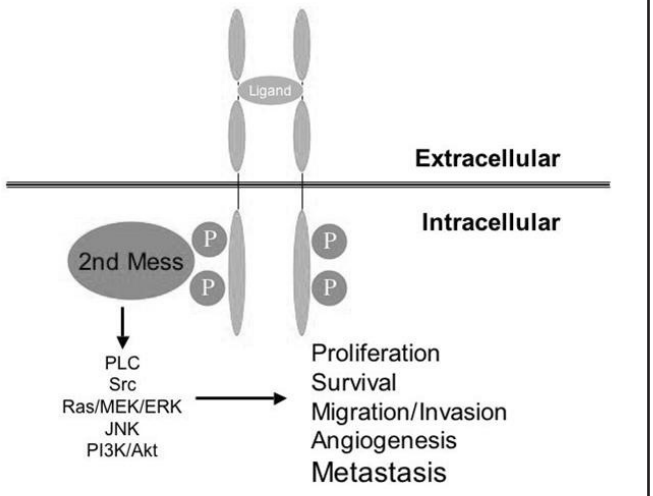


Figure 1. Diagram of a generic receptor tyrosine kinase

The majority of canine (and feline) mast cell neoplasms express the tyrosine kinase growth factor receptor KIT, and a large minority of canine MCT (15-35% depending on the study) possess a mutation in the c-kit gene coding for the KIT protein. (KIT expression and c-kit mutations have also been identified in feline MCT recently). This gene codes for a transmembrane protein that serves as the receptor for the growth factor stem cell factor, important in the maturation of normal mast cells and other hematopoietic cells. Mutations can render KIT active even in the absence of bound stem cell factor. Internal tandem duplications (ITD) in exon 11 are the most commonly observed *c-kit* gene mutations in MCT,[15-17] but deletion mutations have been identified in exon 11, and ITDs and substitutions have been identified in exons 8 and 9. Rare mutations have been identified in exon 17.[15] To date, all mutations that have been characterized *in vitro* have been shown to result in constitutive KIT protein activation.[15]

Prognostic value of *c-kit* gene mutation

Most studies evaluating the association between *c-kit* gene mutations and prognosis have been focused on exon 11 ITDs. Several studies have found increased *c-kit* mutations in higher grade MCTs.[18-20] Additionally, Webster et al. found that dogs with MCTs with *c-kit* mutations had significantly decreased overall survival times and an increased incidence of MCT-related death and recurrence when treated with surgery, and when treated with multimodality therapy.[21,22] Other studies have found associations between mutations and increased cellular proliferation indices.[20,23]

Given its association with both histologic grade and proliferation indices,[18-20,23] it is not clear whether the presence of a *c-kit* mutation represents an independent prognostic factor, or whether it may largely correlate with histologic grade / proliferation, which can be assessed more simply and inexpensively. One recent study suggested that the presence of *c-kit* mutations was predictive of outcome on univariate, but not multivariate analysis.[20]

Prognostic Value of KIT protein localization

Regeura et al. first described variations in KIT expression in canine MCT by immunohistochemistry. In this study, it was noted that Patnaik grade I MCTs had weak KIT labeling scattered in the cytoplasm or on the membrane, while grade II and III tumors tended to have increased cytoplasmic labeling.[24] In three studies, dogs with MCT with focal or diffuse cytoplasmic KIT expression had a worse postsurgical prognosis than MCTs with peri-membrane labeling.[20,21,25,26]

In contrast to the studies described above, Costa Casagrande et al. found no association with KIT staining pattern and histologic grade or survival measures.[27]

As above regarding *c-kit* gene mutation, given the potential correlation between KIT protein localization and other validated prognostic factors (grade, proliferation)[,23-25 20] it is unclear whether KIT localization represents an independent prognostic factor, when taking into account these other features. In one comparatively large retrospective study, KIT localization was significant on univariate analysis but lost prognostic value upon multivariate analysis.[20]

KIT Inhibitors and MCT

New molecules have been developed that inhibit signaling through the KIT tyrosine kinase, and these compounds are able to interfere with the proliferation of canine MCT *in vitro*. The 2 veterinary-approved molecules in this class are toceranib (TOC; Palladia, Zoetis) and masitinib (MAS; Masivet/Kinavet, AB Science).

Following encouraging *in vitro* and early-phase clinical studies with TOC, a multi-center, placebo-controlled, double-blind, randomized study of TOC was performed in dogs with recurrent or metastatic grade II or III MCT. Dogs were randomized to receive oral TOC at 3.25 mg/kg or placebo every other day for 6 weeks in the blinded phase. Thereafter, eligible dogs received open-label TOC. The overall response rate in TOC-treated dogs (n=86) was 37.2% (7 complete response, 25 partial response) versus 7.9% (5 partial response) in placebo-treated dogs. Among the TOC treated responders, the median duration of objective response and time to tumor progression was 12.0 weeks and 18.1 weeks, respectively. The efficacy observed in this study led to the full approval of TOC by the U.S. FDA.

A clinical trial of similar design was completed with MAS in dogs with recurrent or unresectable MCT. MAS was administered at a dose of 12.5 mg/kg daily. This study demonstrated significantly improved time to progression in MAS-treated versus placebo-treated dogs.

Value of *c-kit* mutation status and KIT localization in predicting response to therapy

The potential predictive value of *c-kit* mutation status in predicting outcome following treatment with TOC phosphate and MAS has been evaluated to some degree in the 2 registration trials for these agents. In the TOC registration study as well as in a preliminary study, MCT patients with *c-kit* gene mutations had objective response rates twice as high as those without ¬mutations (60% vs 30%), although effect on long-term outcome was not reported.[28,29] In the MAS registration study, a significant outcome improvement between MAS and placebo arms was observed only in the patients with *c-kit* mutations. Additionally, patients in the MAS arm of this study with c-kit mutations appeared to have longer times to progression (230 d vs 83 d) and maintained higher overall response rates at 6 months (20 vs 10%).[30] In another small study, dogs with *c-kit* exon 11 ITDs were numerically more likely to experience objective responses to imatinib, although long-term outcomes were not reported.[31] However, 2 recent studies have suggested no correlation between response to TOC and *c-kit* mutational status.[32,33]

Interestingly, patients with *c-kit* mutations had significantly **decreased** progression free survival times compared to those without *c-kit* mutations in a study of TOC and hypofractionated radiation therapy, although this was evaluated in a small number of patients,[34] and a recent comparatively large multicenter prospective study suggested a similar **negative** correlation between *c-kit* mutation status and outcome following TOC treatment.[33] These results are important as they suggest that, even if initial response rate may be increased in dogs whose MCT possess ¬*c-kit* mutations, this may not translate into improvements in long-term outcome.

It is worth noting that patients without *c-kit* mutations may to respond to TKIs.[28-30] This may be due to non-mutational activation of KIT (e.g. autocrine or paracrine signaling, amplifications), the presence of activating mutations not screened for as part of testing, or to inhibition of other kinases (e.g. PDGFR, VEGFR2).

The effects of KIT localization on outcome following TKI treatment have been assessed in 2 recent studies.[32,33] Neither study detected a correlation between KIT localization and objective response, but in one of the 2 studies, aberrant KIT localization was associated with an **inferior** progression free and overall survival time following TOC treatment.[33]

Novel Biomarkers: Phosphorylated KIT

Given the possibility that KIT may be pathologically activated by means other than gain-of-function mutations as mentioned above, more direct means to assess activation status of the receptor could provide different and complementary biologic information. Halsey et al validated a pKIT antibody for use in FFPE IHC and demonstrated

modulation of KIT phosphorylation *in vitro* and *in vivo* following TOC treatment.[35] Subsequently, Thompson et al reported that expression of pKIT was predictive of an inferior postsurgical outcome in dogs with MCT.[35]

We recently evaluated pKIT expression by IHC in a prospective cohort of 74 dogs with measurable MCT treated with either vinblastine or TOC. pKIT was significantly correlated with aberrant KIT localization and histologic grade, but not *c-kit* mutation status. On univariate analysis, pKIT did not predict response to TOC, but predicted an inferior progression free interval in TOC treated patients. On multivariate analysis, histologic grade and KIT localization, but not pKIT, were correlated with progression free and overall survival.

Conclusions

In conclusion, reporting of histologic grade, margins and mitotic index continue to provide the most useful information in determining potential outcome and need for additional therapy in dogs with MCT. Additional molecular markers of proliferation, ¬*c-kit* mutation status and KIT/pKIT IHC may be more useful in histologically "ambiguous" tumors. The ability of *c-kit* mutational status to predict benefit from TKI treatment is unclear: while c-kit mutation assessment may have some ability to determine which patients are likely to experience an initial response to single agent TKIs, this many not translate into long-term clinical benefit. Furthermore, while *c-kit* and KIT assessment may predict postsurgical outcome, no studies have demonstrated whether postoperative treatment with TKIs is associated with improvement in outcome in any situation.

References

1. Patnaik AK, Ehler WJ, MacEwen EG. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. Vet Pathol 1984; 21: 469-474.

2. Thamm DH, Turek MM, Vail DM. Outcome and prognostic factors following adjuvant prednisone/vinblastine chemotherapy for high-risk canine mast cell tumour: 61 cases. J Vet Med Sci 2006; 68: 581-587.

3. Cooper M, Tsai X, Bennett P. Combination CCNU and vinblastine chemotherapy for canine mast cell tumours: 57 cases. Vet Comp Oncol 2009; 7: 196-206.

4. Northrup NC, Harmon BG, Gieger TL, et al. Variation among pathologists in histologic grading of canine cutaneous mast cell tumors. J Vet Diagn Invest 2005; 17: 245-248.

5. Northrup NC, Howerth EW, Harmon BG, et al. Variation among pathologists in the histologic grading of canine cutaneous mast cell tumors with uniform use of a single grading reference. J Vet Diagn Invest 2005; 17: 561-564.

6. Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. Vet Pathol 2011; 48: 147-155.

7. Sabattini S, Scarpa F, Berlato D, et al. Histologic grading of canine mast cell tumor: is 2 better than 3? Vet Pathol 2015; 52: 70-73.

8. Elston L, Sueiro FA, Cavalcanti J, et al. The importance of the mitotic index as a prognostic factor for canine cutaneous mast cell tumors - a validation study. Vet Pathol 2009; 46: 362-365.

9. Romansik EM, Reilly CM, Kass PH, et al. Mitotic index is predictive for survival for canine cutaneous mast cell tumors. Vet Pathol 2007; 44: 335-341.

10. Bostock DE, Crocker J, Harris K, et al. Nucleolar organiser regions as indicators of post-surgical prognosis in canine spontaneous mast cell tumours. Br J Cancer 1989; 59: 915-918.

11. Simoes JP, Schoning P, Butine M. Prognosis of canine mast cell tumors: a comparison of three methods. Vet Pathol 1994; 31: 637-647.

12. Kravis LD, Vail DM, Kisseberth WC, et al. Frequency of argyrophilic nucleolar organizer regions in fine-needle aspirates and biopsy specimens from mast cell tumors in dogs. J Am Vet Med Assoc 1996; 209: 1418-1420.

13. Abadie JJ, Amardeilh MA, Delverdier ME. Immunohistochemical detection of proliferating cell nuclear antigen and Ki-67 in mast cell tumors from dogs. J Am Vet Med Assoc 1999; 215: 1629-1634.

14. Scase TJ, Edwards D, Miller J, et al. Canine mast cell tumors: correlation of apoptosis and proliferation markers with prognosis. J Vet Intern Med 2006; 20: 151-158.

15. Letard S, Yang Y, Hanssens K, et al. Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. Mol Cancer Res 2008; 6: 1137-1145.

16. London CA, Galli SJ, Yuuki T, et al. Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. Exp Hematol 1999; 27: 689-697.

17. Ma Y, Longley BJ, Wang X, et al. Clustering of activating mutations in c-KIT's juxtamembrane coding region of canine mast cell neoplasms. J Invest Dermatol 1999; 112: 165-170.

18. Zemke D, Yamini B, Yuzbasiyan-Gurkan V. Mutations in the juxtamembrane domain of c-KIT are associated with higher grade mast cell tumors in dogs. Vet Pathol 2002; 39: 529-535.

19. Downing S, Chien MB, Kass PH, et al. Prevalence and importance of internal tandem duplications in exons 11 and 12 of c-kit in mast cell tumors of dogs. Am J Vet Res 2002; 63: 1718-1723.

20. Horta RS, Lavalle GE, Monteiro LN, et al. Assessment of canine mast cell tumor mortality risk based on clinical, histologic, immunohistochemical, and molecular features. Vet Pathol 2018; 55: 212-223.

21. Webster JD, Yuzbasiyan-Gurkan V, Kaneene JB, et al. The role of c-KIT in tumorigenesis: evaluation in canine cutaneous mast cell tumors. Neoplasia 2006; 8: 104-111.

22. Webster JD, Yuzbasiyan-Gurkan V, Thamm DH, et al. Evaluation of prognostic markers for canine mast cell tumors treated with vinblastine and prednisone. BMC Vet Res 2008; 4: 32.

23. Webster JD, Yuzbasiyan-Gurkan V, Miller RA, et al. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. Vet Pathol 2007; 44: 298-308.

24. Reguera MJ, Rabanal RM, Puigdemont A, et al. Canine mast cell tumors express stem cell factor receptor. Am J Dermatopathol 2000; 22: 49-54.

25. Giantin M, Vascellari M, Morello EM, et al. c-KIT messenger RNA and protein expression and mutations in canine cutaneous mast cell tumors: correlations with post-surgical prognosis. J Vet Diagn Invest 2012; 24: 116-126.

26. Thompson JJ, Yager JA, Best SJ, et al. Canine subcutaneous mast cell tumors: cellular proliferation and KIT expression as prognostic indices. Vet Pathol 2011; 48: 169-181.

27. Costa Casagrande TA, de Oliveira Barros LM, Fukumasu H, et al. The value of molecular expression of KIT and KIT ligand analysed using real-time polymerase chain reaction and immunohistochemistry as a prognostic indicator for canine cutaneous mast cell tumours. Vet Comp Oncol 2015; 13: 1-10.

28. London CA, Malpas PB, Wood-Follis SL, et al. Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. Clin Cancer Res 2009; 15: 3856-3865.

29. London CA, Hannah AL, Zadovoskaya R, et al. Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies. Clin Cancer Res 2003; 9: 2755-2768.

30. Hahn KA, Ogilvie G, Rusk T, et al. Masitinib is safe and effective for the treatment of canine mast cell tumors. J Vet Intern Med 2008; 22: 1301-1309.

31. Isotani M, Ishida N, Tominaga M, et al. Effect of tyrosine kinase inhibition by imatinib mesylate on mast cell tumors in dogs. J Vet Intern Med 2008; 22: 985-988.

32. Horta RDS, Giuliano A, Lavalle GE, et al. Clinical, histological, immunohistochemical and genetic factors associated with measurable response of high-risk canine mast cell tumours to tyrosine kinase inhibitors. Oncol Lett 2018; 15: 129-136.

33. Weishaar KM, Ehrhart EJ, Avery AC, et al. c-Kit mutation and localization status as response predictors in mast cell tumors in dogs treated with prednisone and toceranib or vinblastine. J Vet Intern Med 2018; 32: 394-405.

34. Carlsten KS, London CA, Haney S, et al. Multicenter prospective trial of hypofractionated radiation treatment, toceranib, and prednisone for measurable canine mast cell tumors. J Vet Intern Med 2012; 26: 135-141.

35. Halsey CHC, Thamm DH, Weishaar KM, et al. Expression of phosphorylated KIT in canine mast cell tumor. Vet Pathol 2017; 54: 387-394.

FOLLICULAR NEOPLASIA

Wiener DJ[1]

[1]Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Science, Texas A&M University, 664 Raymond Stotzer Parkway, 4467 TAMU, College Station, TX 77843, USA

Hair follicle (HF) tumors occur in many different species, including humans. They are most common in dogs and rare in cats. Most of the HF tumors are benign with some exceptions (trichoepithelioma and p-lomatricoma). To diagnose HF tumors, a thorough knowledge of follicular anatomy is important as histologically, the follicular tumors are classified according to the differentiation pattern seen in the correspon-ing part of the normal HF.

ANATOMY OF THE HAIR FOLLICLE:

The anagen HF can be divided into three major anatomic regions (Figure 1).

1. The **infundibulum** extends from the opening of the HF to the opening of the sebaceous gland duct. The outer root sheath of the HF joins the epidermis and cannot be differentiated from the epidermis. Keratohyalin granules are present in the stratum granulosum of the epidermis and in the infundulum of the HF.
2. The **isthmus** extends from the entrance of the sebaceous duct to the attachment of the arrector pili muscle and contains the bulge that is the main stem cell bearing region of the HF[1]. No granules are present in the isthmic region of the HF.
3. The **inferior portion e**xtends from the point of insertion of the arrector pili muscle to the base of the follicle and contains the suprabulbar and a bulbar region. Within a concavity of the bulb, underlying the matrical cells of the bulb, and surrounded by a thin basement membrane is the dermal papilla, the only mesenchymal component of the HF. The suprabulbar region shows trichohyalin granules within the Huxley's and Henley's layer of the inner root sheath.

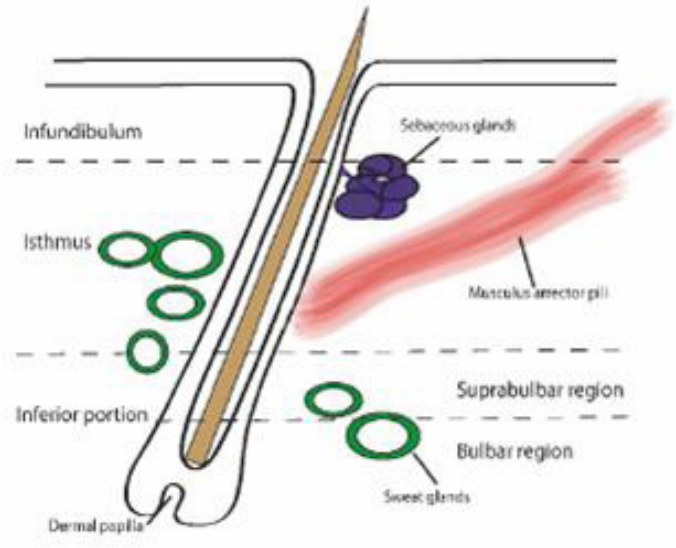


Figure 1. Schematic drawing of an anagen hair follicle illustrating major anatomic regions, namely, the infundibulum, the isthmus and the inferior portion with its suprabulbar and bulbar region. In addition, sebaceous glands and the arrector pili muscle are depicted in this image[2].

The infundibulum and the isthmus constitute the permanent portion of the HF, whereas the inferior segment is transitory and undergoes regression during catagen und is absent during telogen.

HAIR FOLLICLE TUMORS

In the following, characteristics of HF tumors are described with emphasis on diagnostic histologic features (Table 1 and 2). All listed tumors are considered benign with exceptions of rare cases of trichoepitheliomas and pilomatricomas.

Infundibulum

Infundibular keratinizing acanthoma: *Clinically* present as solitary or multiple, partially alopecic nodules. Norwegian Elkhounds have a marked predilection. Spontaneous regression does not occur. *Histopathology* shows a dermal, cup-shaped cyst with a central pore opening to the surface that connects with the epidermis. The cyst wall shows infundibular differentiation with keratohyalin granules. Trabecular, anastomosing pr-jections of the epithelium extend downward from the cyst wall that may form keratinous cysts[3-6].

Isthmus

Tricholemmoma: *Clinically* present as firm, circumscribed nodules, often on the head and neck.

Afghanes may be predisposed, but the tumor is very rare and too few cases have been seen to establish age, breed or sex predilections. *Histopathologically*, there are two types. None of the two types shows kerato- or trichohyalin granules. The isthmus type shows differentiation to the isthmus segment. The neoplastic cells are small and have pale eosinophilic cytoplasm. The inferior type shows cells with clear cytoplasm and there is prominent peripheral palisading. Pallor of cells is due to cytoplasmic glycogen storage that represents differentiation to the outer root sheath cells[3-6].

Hair germ

Trichoblastoma: Mimicks the formation of hair germs as seen in embryonic life or at the lower base of the follicle directly above the dermal papilla in the end-catagen/early telogen phase. Trichoblastomas in dogs showed in one study moderate to strong staining for keratin 15 (recognized as a stem cell marker for the bulge region)[7]. In human trichoblastomas, keratin 15, 17 and 19 expression, as seen in skin stem cells, have been demonstrated[8-9]. *Clinically* present as solitary, form, dome-shaped, alopecic nodules, mostly on the head and neck and on the cranial half of the trunk in cats. Poodles and Cocker Spaniels appear to be predis-posed. *Histopathologically*, there are six types described. The ribbon type/medusoid type is most common in dogs and are composed of small, basaloid keratinocytes arranged in branching and radiating columns. Trabecular trichoblastomas are most common in cats. Spindling of keratinocytes is a common feature in cats. The spindle type frequently has a lima-bean-shaped silhouette with the central indentation at the tumor sur-face. Basaloid epithelial cells arranged in islands, nests and short trabeculae without prominent peripheral palisading. There are large zones of spindeling, producing a whorled configuration. Granular cell type trichoblastomas present similar to ribbon-type tumors but some epithelial aggregates are composed of larger cells with abundant, finely granular or vacuolated cytoplasm. Trichoblastomas with outer root sheath differentiation are composed of multiple lobules and areas of cystic degeneration and anastomosing cords. These cords merge into small islands of cells with glycogenated cytoplasm. The solid type trichoblastomas consists of solid accumulation of keratinocytes arranged in islands surrounded by connective tissue stroma[3-6].

Inferior portion

Pilomatricoma: Arise from the primitive hair matrix. *Clinically* present as solitary, firm, often alopecic and sometimes ulcerated and calcified, dome-shaped to plaque-like nodules, which might be cystic or pigmented. Lesions occur most commonly on the trunk (particularly over the rump and shoulders) or proximal legs. Kerry blue terriers, poodles and Old English sheepdogs may be predisposed. *Histopathologically*, pilomatri-coma may exhibit high mitotic activity, but the tumor is considered benign with rare exceptions. Amyloid may be found in the center of lobules. Small areas of squamous epithelium may be seen. Rare malignant forms of pilomatricomas are described. These tumors are poorly circumscribed and are locally aggressive with frequent invasion into the subcutis. There is an increased ratio of basaloid cells to keratinized ghost cells and nuclear and mitotic atypia. Ulceration and areas of necrosis is common. Reported cases had distant metastasis to a variety of organs, including bone, lung and central nervous system. Differentiation between malignant pilomatricoma and malignant trichoepithelioma may be impossible[3-6].

All three segments of the hair follicle:

- Trichoepithelioma: Can differentiate towards all three of the follicular segments and shows accord-ingly specific structures such as kerato- and trichohyalin granules or matrical differentiation. *Clinically* represent usually as a single (often multiple in bassets), alopecic, firm, white to gray, multilobulated masses that may become ulcerated. Tumors are often located on the trunk and limbs in dogs and on the head, tail and limbs in cats. Among dogs, basset hounds, golden retriever, German shepherds, miniature schnauzers, standard poodles and spaniels may be predisposed. Among cats, Persians may be predisposed. Spayed females, basset hounds and Airedale terriers may be predisposed for malignant trichoepitheliomas. *Histopathologically*, cysts are lined by either squamous epithelium that may show keratohyalin granules or by small, basaloid cells resembling matrical cells that may show trichohyalin granules. Accordingly, keratinization may be gradual or abrupt. Mitotic activity is usually low. Rare malignant forms are described in dogs. These tumors are generally larger, asymmetrical and poorly circumscribed. Nests of tumor cells may be found within stromal lymphatic vessels and there is multifocal epithelial necrosis and atypical mitosis. Differentiation between ma-lignant trichoepithelioma and malignant pilomatricoma may be impossible[3-6].

- Trichofolliculoma: May be a non-neoplastic, hamartoma-like lesion rather than a true neoplasm. *Clinically* represents as a solitary, dome-shaped nodule that may have a central depression or opening that contains hair or sebaceous material. There are no known age, breed or site predilections. *Histopathologically*, it resembles the entire folliculosebaceous unit and shows a well-circumscribed, unencapsulated dermal nodule composed of one or several primary, large, dilated HFs that keratinize through a granular cell layer with keratohyalin granules[3-6].

TUMOR-LIKE LESIONS

True neoplastic lesions of HFs have to be distinguished from tumor-like lesions, which are due to hyperplasia or ectasia. Clinically, they are often difficult to distinguish from true neoplasms.

Follicular hamartoma: Contain one or more clusters of architecturally normal anagen HFs, collagen as wells as mature sebaceous or apocrine sweat glands. HFs are often distinctly larger and extend more deeply than adjacent normal HFs. Fibroadnexal hamartomas do not show hair bulbs and show markedly distorted orientation of the follicles and adnexal glands[3-5].

Follicular cysts (infundibular and panfollicular cysts, isthmus cysts, matrical cysts): Classification of follicular cysts depends on the identification of the lining epithelium[3-5].

- *Infundibular cyst:* Lined by squamous epithelium with keratohyalin granules.
- *Isthmus cyst* (tricholemmal cyst): The lining epithelium and keratinization pattern closely resembles the outer root sheath of the isthmus with absent granules.
- *Matrical cyst*: Lined by small basaloid epithelial cells, occasional with trichohyalin granules. The center of the cyst may contain ghost cells.
- *Hybrid/panfollicular cyst*: Lined by epithelium with features of either two (hybrid) or three (pan-follicular) of the above-mentioned types of follicular cysts.

Dermoid cysts: Congenital lesions found in young animals, often along the dorsal midline. Rhodesian Ridgebacks and Boxers are predisposed. The cysts contain keratin, hair fragments and sometimes sebaceous secretions. HFs are attached to the cyst wall and are radiating downward[3-5].

Dilated pore of Winer: Cup-shaped, dilated, hyperplastic, infundibular epithelium with a cystic cavity filled with keratin that may protrude through a pore, forming a cutaneous horn[3-5].

Warty dyskeratoma: Rare, cystic, cup-shaped dermal mass lined by infundibular epithelium. The base of the structure has many filiform projections. Acantholysis, dyskeratosis and apoptosis of keratinocytes are present[3-5].

Table 1.

	Infundibular keratinizing acanthoma	**Tricholemmoma**	**Trichoblastoma**
Description	Central cyst with pore to the epidermis, lined by infundibular epithelium. Peripherally multiple squamous epithelial islands and cornified cysts connected by anastomosing cords of basaloid cells embedded in mucinous matrix.	*Isthmus type:* Trabeculae of epithelial cells extending between islands of epithelial cells. Islands of clear cells with trichilemmal keratinization *Inferior type:* Islands of small keratinocytes with pale, clear cytoplasm and thick glassy membrane. Central epithelial cells: eosinophilic cytoplasm; peripheral cells: pale vacuolated cytoplasm, palisading.	*Ribbon/medusoid type*: Small, basaloid keratinocytes arranged in branching and radiating columns *Trabecular type:* Islands and broad trabeculae of small keratinocytes with prominent peripheral palisading. *Spindle cell type:* Large zones of spindeling without peripheral palisading, producing a whorled configuration. Ulceration common. *Granular cell type:* Larger cells with abundant, finely granular or vacuolated cytoplasm. *Outer root sheath differentiation type:* Multiple lobules and areas of cystic degeneration and anastomosing cords. Islands of cells with glycogenated cytoplasm. *Solid type:* Solid accumulation of cells surrounded by connective tissue stroma.
Granules	Keratohyalin	No	No
Cartilag./osseous metaplasia	Possible	No	No
Connection with the epidermis	Yes	Only isthmus type	Only spindle cell type
Melanization	No	Yes	Yes (trabecular, spindle cell and outer root sheath differentiation types)
Keratinization	Gradual and abrupt	Abrupt	Keratin microcysts (ribbon, spindle and trabecular type)
Ghost cells	No	No	No

Table 2.

	Pilomatricoma	Trichoepithelioma	Trichofolliculoma
Description	Cystic structures, lined by small, basaloid cells resembling matrical cells. *Malignant type:* Poorly circumscribed, locally aggressive, increased ratio of basaloid cells: keratinized ghost cells, nuclear and mitotic atypia, desmoplastic stroma at the periphery and possible lymphatic invasion.	Epithelial islands and cystic structures, lined by squamous epithelium or small, basaloid cells resembling matrical cells. *Malignant type:* Large, asymmetrical and poorly circumscribed nests of tumor cells with epithelial necrosis, atypical mitosis, desmoplastic stroma and possible lymphatic invasion.	Several primary, large, dilated HFs that may open onto the skin surface and are surrounded by secondary follicular structures (exhibiting various stages of maturization) radiating outward from the primary follicles in an arborizing pattern. Sebaceous glands may be present (= sebaceous trichofolliculoma)
Granules	Trichohyalin	Kerato- and trichohyalin	Kerato- and trichohyalin
Cartilag./osseous metaplasia	Yes	Rare	No
Connection with the epidermis	Yes (only malignant type)	Yes (only malignant type)	No
Melanization	Yes	Yes	Yes
Keratinization	Abrupt	Gradual and abrupt	Gradual
Ghost cells	Yes	Yes	No

Reference list

1. Cotsarelis G, Sun TT & Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 1990: 61: 1329-3
2. Welle MM & Wiener DJ. The Hair Follicle: A Comparative Review of Canine Hair Follicle Anatomy and Physiology. Toxicol Pathol 2016: 44: 564-74
3. Gross TL, Ihrke PJ, Walder EJ, et al. Skin Diseases of the dog and cat: Clinical and Histopathologic Diag-nosis. Oxford, UK: Blackwell Science; 2005. 604-640
4. Goldschmidt MH & Goldschmidt KH. Tumors in domestic animals, Meuten DJ. 5th Edition. Ames, Iowa: Wiley Blackwell; 2017. pp.88-141
5. Mauldin EA and Peters-Kennedy J. Jubb, Kennedy and Palmer's pathology of domestic animals, Grant Maxie M. 6th Edition. St. Louis, Missouri: Elsevier; 2016: 509-736
6. Hnilica KA & Patterson AP. Small Animal Dermatopathology: A Color Atlas and Therapeutic guide,Hair follicle tumors, 4th Edition. St. Louis, Missouri: Elsevier; 2017: 462-463
7. Brachelente C, Porcellato I, Sforna M et al. The contribution of stem cells to epidermal and hair follicle tumours in the dog. Vet Dermatol 2013: 24: 188-94 e41
8. Schirren CF, Ruetten A, Kaudewitz P, et al. Trichoblastoma and basal cell carcinoma are neoplasms with follicular
9. Kim HC, Sohng SH, Choi JS, et al. Immunohistochemical expression of cytokeratin 15, cytokeratin 19, fol-listatin and Bmi-1 in basal cell carcinoma. Int J Dermatol 2016: 55: 36-44

THE COMMUNICATION CURE.

Boost your communication IQ by better getting your point across when communicating on veteri-nary topics with diverse generations and across multiple communication styles.

Lidiya Alaverdova DVM

Senior Brand Manager, Hill's Pet Nutrition Europe

Communication is essential for the effective delivery of veterinary care, and is one of the most powerful tools for you as a veterinary practitioner. Unfortunately, there is often a mismatch between a professional's level of communication and a client's level of comprehension. Patients in human medicine often misinterpret or do not understand much of the information given to them by clinicians. This is also the case in veterinary medicine (supported by AAHA study). Communica-tion between the veterinary healthcare team members also remains critical as we transition into the world of 'everything digital' and often work with different generations in one practice.

THE M(ILLENNIAL) FACTOR & GENERATIONAL GAP

Welcome to life in the new workplace. As people live and work longer than ever before, the mod-ern veterinary office houses multiple wildly different generations, personalities & communication styles under one roof— and this interesting melting pot causes interesting problems.

Baby Boomers (1943 – 1960), Generation X (1961 – 1981), and Generation Y or Millennials (1982-2004) have wide-ranging value sets and often deploy conflicting communication styles. And Generation Z (or whatever the name of the generation that will enter the workplace soon will be) is around the corner! Luckily, there are also CUSPERS - persons born within 3-5 years of a genera-tional divide[1]. These may favor and display characteristics from both relative generations. and of-ten are the people who cement the generations together and function as mediators, translators, and mentors[2].

A generational identity is a state of mind shaped by many events and influences, such as[1]:

- How you are parented (for example: boomers grew up with stay at home moms, generation X grew up in families with both parents busy working or divorced and kids often unsuper-vised, while 'special' Millennials were sheltered and overprotected by both parents, includ-ing much more involved dads)
- Events that occur during your childhood years and society's reactions to those events
- Events that occur as you 'come of age' and your individual reaction to these events

Table 1. G(enerational) snapshot[1,2,3,4,5]

	Baby Boomers (1943 – 1960)	Generation X (1961 – 1981)	Millennials (1982-2004)
Overall	Work-centric, goal-oriented, competitive, free-spirited, relatively optimistic, social cause oriented. Formal. Follow protocol, tend to build processes and procedures around everything. They live to work.	Independent, self-reliant, resilient, risk-taker, cynical, flexible, technologically adept, and resourceful. Challenge the status quo. Value personal time and strive to achieve work-life balance. They appreciate fun in the workplace and have a work hard/play hard mentality.	Deeply aware of social issues, entrepreneurial, comfortable with change and a fast pace, open-minded, confident, self-expressive, upbeat. Task oriented, want options & choices, and expect attention. Think "digital", want to make a difference and value life over work.

My work is…	An exciting adventure	A contract = obligation	A way to make a difference, but first I have to pay those bills.
My Work Ethic	Workaholic	Eliminate the task	What's next?
Words that motivate me at work are…	You are needed/valued	Let's do it your way!	You will work with other brilliant people.
My thoughts on meetings…	I love to have meetings!	Another meeting? Ugh	What's a meeting? In-person?
Job Strength	Service Oriented Team Players	Adaptable and Techno-Literate	Multitaskers and Techno-Savvy
Outlook	Optimistic	Skeptical	Hopeful
View of authority	Love/Hate	Whatever…	Polite
Leadership	By Consensus	By Competence	By Pulling Together
Relationships	Personal Gratification	Reluctant to Commit	Inclusive
Time on the job	Visibility is key "Face Time"	As long as I get the job done, who cares	It's quitting time – I have a real life to live
Diversity	Integration began	Integrated	No majority race
Feedback	Once a year with documentation	Interrupts and asks how they are doing	Wants feedback at the push of a button
Work/Life Balance	Balances everyone else but themselves	Wants balance now	Need flexibility to balance activities

The intricacies of workplace communication—what we say, how we say it and what our choices say about us—have become increasingly complex as each group brings a different set of experi-ences and expectations to the table. Everybody has to adapt their 'style by learning their cowork-ers' preferences and attempting to meet in the middle.

The solution won't come from any one person or generation. The solution (or the cure) is really simple - flexible approach and mutual respect. Being aware of the differences is a good start. More than that - talk about them in your team to demystify what's unknown or misunderstood. Go out of your way to learn from each other. Older workers can lend their vast industry knowledge and expe-rience. Younger workers can shed light on new ways of doing things and technology trends.

Getting along with Boomers[3,4,5]

- Show respect. Acknowledge that you have less experience and can learn from them.
- Choose face-to-face conversations.
- Give people your full attention. Stop multitasking while someone is talking.
- Learn the history. Find out what has gone wrong and right in the past before making suggestions for changes.

Getting along with Xers[3,4,5]

- Get to the point. Avoid jargon and buzzwords that obscure your point.
- Use email. Take advantage of technology and only have face-to-face meetings when required.
- Give them space. Xers crave autonomy.
- Get over the notion of dues paying.
- Lighten up. Remember it's OK for work to be fun.

Getting along with Millennials[3,4,5]

- Challenge them. They want to do work that really matters.

- Ask for their opinion. They love to collaborate and be a team player.
- Encourage finding a mentor and become their champion.
- Provide timely feedback. They are used to getting feedback instantaneously and need positive motivation.
- Lighten up. Remember it's OK for work to be fun
- Be transparent.
- Allow them to experiment and rethink traditional ways of doing things.
- When communicating with millennials – frame everything as a story or even better – visualize it for them.
- Millennials value authenticity. Show them your personality.

Here are a few ideas to consider if you not only work in multi-generational environment, but also tasked to lead and motivate a diverse team:

- Understand what makes each generation tick—offer different options to best meet the needs of a multi-generational workplace. Adapt your attitudes about rewards, work styles, communication preferences and motivators to match generational expectations.
- Make an effort to start conversation. Run educational sessions about generations and con-sider implementing reverse-mentoring program.
- Leverage the strengths of each generation—pursue and encourage a multiplicity of perspectives and ideas. This leads to innovation and will help your clinic be progressive.
- Build bridges —build on strengths and encourage people to become more of who they are rather than pushing them to conform.
- Make a point to ask people about their individual needs, views and preferences.

GETTING YOUR POINT ACROSS

Being a good communicator is very important within the veterinary healthcare team, but even more so with your clients. When communicating with others pay special attention to not only verbal, but also to non-verbal and visual communication. The CURE is to over communicate and yet keep it simple!

To improve communication among your team members, try to observe and discover ways to meet the different communication styles of individuals. Consider group exercises such as: Neurocolor assessment9, DISC or alternative to help you get started. Bonus: it is a great team building activity!

When it comes to client communications, you might be surprised at what they aren't understanding. In dermatology, we use everyday words such as 'growth', 'lesion', 'polyp', 'screening', but to a regular person those words may have a completely different meaning.

One way to increase client understanding is to use plain, nonmedical language, and getting more specific to cut down confusion. Try explaining medical terms by breaking them down based on word's root.

Table 2. Medical terms in plain language

Medical term	Plain language
cardiomyopathy	disease of the heart muscle
febrile	feverish
hyperlipidemia	high fat or high cholesterol
referral	send you to a specialist
monitor	keep an eye on, observe closely
oral	by mouth
topical	on the skin

When you say that patient's test results came back positive, people actually might think that posi-tive is good and negative is bad - watch out for how are you using these terms. Be very specific with prescription label instructions and your recommendations, for example instead of saying 'Twice daily', say 'every 12 hours'.

Make an effort to understand the client's treatment goals and perspectives about their pet's health ('you look uncomfortable today?', 'how do you feel about this plan?'). Be empathetic. Clients are more satisfied and more likely to adhere to recommendations if they feel understood, supported, and a sense of partnership with veterinarian or vet nurse. Make it clear you are on their side ('I cer-tainly understand that you want Fluffy to feel better right away'). Alway confirm agreements and ask clients to repeat back the plan briefly.

Other tips to improve communications:

- Reiterate key points at the start and finish even if it seems redundant to you.
- Explain physical exam findings as you are conducting exam
- Use 'teach back method' to ensure understanding & utilize open ended questions
- Use visual aids to help enhance comprehension (pictures, diagrams, charts).
- You can also explain terms to patients using simple analogies and metaphors.
- Explain procedures and testing before they are ordered or performed ('skin scrub' may sound really scary to people without medical background)
- Slow down & speak as you would to a 6 years old (short & simple sentences)
- Utilise written handouts to supplement verbal information when possible (experience is of-ten stressful for clients, so even if you did a great job communicating, they often forget)
- Give clients a way to record their questions and your answers
- Pay attention to your clients' non-verbal cues and acknowledge emotions. At the same time watch your own body language - don't appear hurried, worried, bored etc. Look directly at clients when speaking to them, but don't forget to show empathy and care for the pet.
- Be comfortable with science - sometimes humans need those 5 secs to process information.

A successful veterinary clinic should be a melting pot of different generations, personalities, com-munication styles and talent (not problems!), all coming together toward a common goal – save and transform the lives of animals. That is the only way to bring fresh perspectives to oftentimes com-mon problems within a multigenerational environment. Every time you interact with clients, make sure you help them understand their pet's main health problem, what do clients need to do and why it is important. You are 100% responsible for everything you say and you are 100% responsible for everything the other person understands. If the person you're speaking to doesn't understand what you've said then the whole point of the communication has been missed. And that is something we simply can't effort when it comes to life, health & death type of conversations.

References

1. Ypulse.com
2. Lancaster L, Stillman D. When Generations Collide. Who they are. Why they Clash. How to solve the gen-erational Puzzle at work. HarperBusiness; Reprint edition, 2003
3. Zemke R, Raines C, Filipczak B. Generations at Work: Managing the Clash of Boomers, Gen Xers, and Gen Yers in the Workplace . AMACOM; Second Edition edition, 2013
4. Howe N, Nadler R. Millennials in the Workplace. LifeCourse Associates, 2010
5. Caraher L. Millennials & Management: The Essential Guide to Making It Work at Work. Bibliomotion, 2014
6. Leadership in the Multi-Generational Workplace. Available at: http://www.cellaconsulting.com/blog/leadership-in-the-multi-generational-workplace/ Accessed Feb 19th, 2016
7. Cross-generational mentoring. Available at: http://www.cellaconsulting.com/blog/cross-generational-mentoring/ Accessed on Jan 4th, 2019
8. How To Communicate In The New Multigenerational Office. Available at: http://www.forbes.com/sites/jennagoudreau/2013/02/14/how-to-communicate-in-the-new-multigenerational-office/#5b19502450d8. Accessed on Jan 4th , 2019
9. Putting neuroscience & personality to work https://inventory.neurocolor.com/

ALLERGEN IMMUNOTHERAPY: Introduction and Mechanisms of Action

Douglas J. DeBoer, DVM

School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin USA

Allergen immunotherapy (AIT) is one of the most common treatments for which owners of allergic pets seek specialty care. With the recent advent of effective new drug and biological treatments for allergy, some have questioned if AIT is as necessary or useful as in the past. The author believes strongly that AIT should remain a foundational and important part of a multimodal treatment approach. It is the only treatment for allergy that can modify, or reverse, at least part of the pathogenesis of this condition as we know it – both alleviating clinical signs and preventing progression in the process. This modification is accomplished without the possible long-term adverse effects of a lifetime of drug treatment, with minimal chance for its own adverse effects, and with the potential of long-lasting effectiveness. There are certainly disadvantages to AIT – including the fact that may take several months or more to begin working, that it does not always work, and that it may be relatively expensive. Nevertheless, if we are to provide optimal management of this lifelong disease, AIT should still be offered to clients as an important option.

OPTIONS AND TESTING

Most current AIT approaches in AD center on either subcutaneous (SCIT) or sublingual (SLIT) administration. In North America, SCIT utilizes aqueous, saline-phenol preserved extracts. Most often, 2- or 3-vial sets of increasing concentration are used, beginning with frequent injections of dilute extract and progressing to less frequent injections of concentrated extract as maintenance treatment. Less commonly, "rush immunotherapy" is accomplished using the same extracts, but with rapid dose-escalation over the course of 24 hours under medical supervision.[1] Rush immunotherapy has the advantage of limiting the number of injections that an owner must give at home, though safety concerns and initial costs may be higher. The major disadvantages of all SCIT protocols include a slight risk of anaphylactic reaction, and owner resistance to giving injections, which can be a substantial factor in compliance.

Administration of SLIT has been a popular method of human AIT for many years in European countries, in some countries being more popular than SCIT. In pets, most commonly, mixtures of glycerinated extracts are administered onto the oral mucosa in very small amounts one or more times daily. SLIT is an effective treatment that has a favorable safety profile compared with SCIT, and in some cases compliance with the oral dosing method (vs. injections) may be greater. It can also be effective even in dogs that have failed SCIT treatment.[2] However, it requires faithful daily administration, which is difficult for some owners.

To date, there have been no studies in pets that directly compare the results of AIT with SLIT vs. SCIT; the very limited evidence available suggests both have approximately equal efficacy. In human beings, the choice is still an ongoing discussion.

Most effects of AIT are thought to be allergen-specific, though it is clear that some of the benefits are nonspecific. Historically, accurate testing (via intradermal or serologic methods) to identify the offending allergens in each patient has been considered very important to successful immunotherapy. More recently, the observation of extreme variability in serologic test results from laboratory to laboratory has prompted some to question our ability to accurately pinpoint "allergen specificity" with serologic tests.[3] If intradermal testing is not available, would it then be better to administer a standard mixture of common allergens rather than to depend on a serologic test? In this case, efficacy would depend on "sheer luck" in picking the right mixture, along with the benefit of the nonspecific effects of AIT. One downside of this approach would be the possible administration of allergens to which the patient is not sensitive, though the consequences of such administration are mostly unknown.

Above all, clinicians must always bear in mind that no type of "allergy test" must ever be used for initial diagnosis of allergy. Initial diagnosis is always a clinical diagnosis, made by careful observation of historical and physical information combined with diagnostic evaluation to rule out other causes of pruritus.

Only after a firm clinical diagnosis of allergy is made should an "allergy test" be considered, and only as a guide to either AIT formulation or less commonly, to allergen avoidance.

MECHANISMS: THE BASICS

Mechanisms of AIT are complex, not completely understood, and even review papers[4-7] can be baffling to the non-immunologist. The goal of AIT is to take advantage of mechanisms within the immune system that induce *peripheral tolerance*, meaning the ability to "tolerate" a specific antigen (and not react to it) that is induced in the immune system "periphery," meaning the lymph nodes, spleen, and other immune tissues outside of the "central" bone marrow and thymus. Tolerance is a basic, important function of the immune system that is important in things like tolerating self-antigens and thus preventing autoimmune disease, and tolerating food allergens that are absorbed across mucosal surfaces. We take advantage of this natural "tolerance" function when attempting AIT.

Tolerance encompasses a variety of mechanisms that occur in a cascade over time, explaining why some benefits of AIT can be seen very quickly and some take much longer. Very early on, exposure to large doses of allergen results in reduction in effector cell activity (i.e., mast cells, basophils, eosinophils). How this occurs is not fully understood. As an example, one early effect is an increase in histamine H2 receptor expression on these cells.[8] When H2 receptors are then activated by histamine released during the allergic response, they suppress the activity of the effector cells. Thus, histamine binds to the increased number of H2 receptors on mast cells, which downregulates their activity. Interestingly, upregulation of H2 receptors can occur amazingly fast – even 6 hours after AIT treatment! This helps explain some of the very rapid response that can occur with, e.g., rush immunotherapy.

As AIT progresses, other mechanisms of tolerance are invoked. There is suppression of helper T-cell responses and a long-term immunologic shift from a Th2 (pro-allergic) to Th1-biased (non-allergic) response.[4-7] These shifts are mediated through an increase in inducible T-regulatory (Treg) cells, and an increase in cytokines such as TGFß and IL-10. There is an increase in allergen-specific IgG (especially IgG4 in people), referred to as "blocking antibody" because it can bind to the allergen and prevent it from interacting with mast cells and dendritic cells. With extended treatment, there is a decrease in allergen-specific IgE production by effector B-cells. In dogs, though much less is known, a shift to Th1, increases in IgG, appearance of more Treg cells, and rising IL-10 have all been demonstrated – thus establishing the parallels to AIT in human beings.[9-11]

At least part of the mechanism of tolerance occurs through IL-10-positive "tolerogenic dendritic cells" – a special type of dendritic cell (antigen presenting cell) that picks up the allergen, processes it, then presents it to T-lymphocytes and instructs them to "NOT REACT" or tolerate the allergen. This process can occur anywhere within the immune system, but has special importance in SLIT, as will be discussed below.[12]

Non-specific effects of immunotherapy may be seen within the first 4 weeks of treatment and are partly mediated by IL-10 produced by dendritic cells and Treg cells. An important observation from study of multiple- versus single-antigen sublingual immunotherapy was that suppression of the allergic response by IL-10 was non-specific; treating a major allergen sensitivity also resulted in some symptomatic benefit even to minor allergens that were not included in the treatment mixture.[13] Thus, AIT is in part "allergen specific" and in part nonspecific." This explains why it may not be necessary to formulate an AIT prescription based on "every single thing" that he is allergic to, in order to obtain a good therapeutic response.

One of the strong benefits of AIT in people is that it can both prevent new sensitizations, and to prevent the "allergic march" of gradually escalating allergic symptoms over the course of the patient's lifetime.[14] It is unknown if such benefits occur in AIT for AD in animals.

In many studies of human allergy, it appears the immunologic effects induced by AIT persist for a very long time, including for long after the treatment has been discontinued.[4,15] The question of how long AIT must be conducted, whether it can be discontinued at some point, and how long the benefits last are completely unexplored in animals. Treatment of pets with AIT is often considered to be lifelong, though it is possible to attempt discontinuation after 2 to 3 years of injections if the animal has responded very well.

SPECIAL NOTES FOR SUBLINGUAL IMMUNOTHERAPY

Over recent years, a great deal of understanding has been gained on the mechanisms of SLIT in human patients.

Sublingual immunotherapy allows specific antigens placed within the oral cavity to induce immunologic tolerance. The mucosal area under and around the tongue is a privileged immunologic site with unique characteristics. It consists of a physical barrier with integrated immunologic elements that allow the uptake of antigens, while preventing invasion by pathogens. Local immune cells must constantly differentiate between harmless antigens and harmful pathogens and must tolerate a broad range of food antigens for normal function. There is a high concentration of dendritic cells and T-cells and a low concentration of mast cells, basophils and eosinophils.[12, 17-18] The "oromucosal dendritic cells" have unique functional properties as well as differences in cell surface markers compared with other dendritic cells, which may explain part of the difference in response between SCIT and SLIT. As with other dendritic cells, they are responsible for allergen uptake, processing, and presentation to T-lymphocytes. However, oromucosal dendritic cells are especially proficient at inducing tolerance. The oromucosal immune system is set to a default of *nonreactivity* to substances placed in the mouth, e.g. foodstuffs. We take advantage of this normal, homeostatic process when attempting to desensitize a patient via the oromucosal route.

EFFECTS OF DOSE, TIMING, SCHEDULE, and CONCURRENT MEDICATIONS

Dose-dependency of AIT efficacy has been long-observed in human trials, and recent studies are increasingly pinpointing these effects using both field studies and animal models.[19,20] In general, low and intermittent doses of allergens tend to enhance sensitivity, but higher and more frequent or continuous doses tend to invoke tolerance. Not only the absolute dose, but dosing intervals have measurable effects on efficacy under some circumstances.

Unfortunately, these effects remain unstudied for AIT in animals. Protocols for AIT in pets are completely unstandardized and subject to enormous variation. Different veterinary dermatologists are likely to use different allergen doses, of unstandardized extracts that vary in composition and potency from manufacturer to manufacturer and from batch to batch, using different schedules of administration, and different formulation rules. In veterinary medicine, we simply don't know the optimal allergen dose for AIT. If we are ever to study this dose effects in pets, the efforts will have to begin with determination of the specific epitopes to which pets are sensitized.[21] In the human allergy world, determining this has been a major effort over the past 10+ years. This information has permitted standardized dosing, preparation of recombinant allergens, determination of T-cell epitopes, use of peptide immunotherapy, and other advances.

As far as is known, concurrent treatments with antihistamines, fatty acid supplements, ciclosporin, or low-dose glucocorticoids will not interfere with response to AIT; clinical experience suggests the same is true for newer drugs such as oclacitinib and lokivetmab. Such treatments are virtually always necessary as part of the overall treatment plan, to provide immediate and short-term relief while waiting for the AIT to work. These treatments can be slowly tapered as response to AIT occurs.

EFFECT OF FORMULATION CHOICES

With the wide variation among veterinary dermatologists in how, and how many, extracts of different types are mixed together, it is no wonder that patient experience varies. Should the number of extracts in a mixture be limited to eight? Ten? Twelve? Or unlimited? We do know there is a dose effect, so if too many allergens are mixed together, might this lower the dose of each to the point that efficacy is compromised? Can protease-containing extracts such as molds be mixed in the same vial with pollens, or should they be administered only by separate injection?

On the human side, these debates have not ended, though recent large-scale studies are providing at least some guidance. Evidence is accumulating that in polysensitized humans, treatment with a single, dominant allergen is as effective as multi-allergen ASIT, even though polysensitization is more prevalent.[22,23] This "less is more" approach – prevalent in Europe - is unpopular in the United States among physician allergists. The evidence for limiting the number of allergen extracts used in treatment stands in contrast to protocols used by most veterinary dermatologists.

Both American and European "best practice" guidelines for human AIT specify that mold extracts should not be mixed in the same vial as pollens, as there is abundant evidence that pollen allergens will be degraded by mold proteases during storage.[24] Despite similar (though less) evidence in dogs,[25] many veterinary dermatologists continue to recommend such mixtures. Clearly, there is a need for further experimentation to solve this dilemma, among many others. Because SLIT formulations generally contain glycerin and/or other stabilizers in the vehicle, they may protect protein allergens from protease degradation; therefore, SLIT may be preferable to aqueous SLIT if protease-containing

extracts are included in the mixture…but once again, this concept is only theoretical!

So many of the things we believe about AIT in animals are either extrapolated from the human situation, or are unstudied, or are myth and legend, or are "all of the above." The difficulty, expense, and long duration of AIT trials clearly hampers our ability to learn more. Until the time that more trials are undertaken, it is wise for us to remember that what we feel certain about may not be true, and to view ourselves and what we have been taught with healthy skepticism.

REFERENCES

1. Mueller RS, Bettenay SV. Evaluation of the safety of an abbreviated course of injections of allergen extracts (rush immunotherapy) for the treatment of dogs with atopic dermatitis. *Am J Vet Res* 2001; 62: 307-310.
2. DeBoer D, Morris M. Multicentre open trial demonstrates efficacy of sublingual immunotherapy in canine atopic dermatitis (abstract). *Vet Dermatol* 2012: 23 (Suppl. 1): 65.
3. Plant JD, Neradelik MB, Polissar NL, et al. Agreement between allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories in the USA. *Vet Dermatol* 2014; 25: 15-e6.
4. Eifan AO, Shamji MH, Durham SR. Long-term clinical and immunological effects of allergen immunotherapy. *Curr Opin Allergy Clin Immunol* 2011; 11: 586-593.
5. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ J* 2015; 8: 17-25.
6. Ozdemir C, Kucuksezer UC, Akdis M, et al. Mechanisms of aeroallergen immunotherapy: subcutaneous immunotherapy and sublingual immunotherapy. *Immunol Allergy Clin N America* 2016; 36: 71-86.
7. Cavkaytar O, Akdis CA, Akdis M. Modulation of immune responses by immunotherapy in allergic diseases. *Curr Opinion Pharmacol* 2014; 17: 30-37.
8. Novak N, Mete N, Bussman C, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. *J Allergy Clin Immunol* 2012; 130: 1153-1158.
9. Shida M, Kadoya M, Park SJ, et al. Allergen-specific immunotherapy induces Th1 shift in dogs with atopic dermatitis. *Vet Immunol Immunopathol* 2004; 102: 19-31.
10. Hou CC, Griffin CE, Hill PB. Dermatophagoides farinae-specific IgG responses in atopic dogs undergoing allergen-specific immunotherapy with aqueous vaccines. *Vet Dermatol* 2008; 19: 215-220.
11. Keppel KE, Campbell KL, Zuckermann FA, et al. Quantitation of canine regulatory T cell populations, serum interleukin-10 and allergen-specific IgE concentrations in healthy control dogs and canine atopic dermatitis patients receiving allergen-specific immunotherapy. *Vet Immunol Immunopathol* 2008; 123: 337-344.
12. Moingeon P. Update on immune mechanisms associated with sublingual immunotherapy: practical implications for the clinician. *J Allergy Clin Immunol: In Practice* 2013; 1: 228-241.
13. Marogna M, Spadolini I, Massolo A, et al. Effects of sublingual immunotherapy for multiple or single allergens in polysensitized patients. *Ann Allergy Asthma Immunol* 2007; 98: 274-80.
14. Porcaro F, Corsello G, Pajno GB. SLIT's prevention of the allergic march. *Curr Allergy Asthma Rep* 2018; 18: 31.
15. James LK, Shamji MH, Walker SM, et al. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immuno*l 2011; 127: 509-516.
16. Moingeon P, Batard T, Fadel R, et al. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006; 61: 151-165.
17. Novak N, Haberstok J, Bieber T, et al. The immune privilege of the oral mucosa. *Trends Molec Med* 2008; 14:191-198.
18. Allam JP, Novak N, Fuchs C, et al. Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcERI expression. *J Allergy Clin Immunol* 2003; 112: 141-148.

19. Pfaar O, van Twuijver E, Boot JD. A randomized DBPC trial to determine the optimal effective and safe dose of SLIT-birch pollen extract for the treatment of allergic rhinitis: results of a phase II study. *Allergy* 2016; 71:99-107.
20. Rask C, Brimnes J, Lund K. Shorter dosing intervals of sublingual immunotherapy leads to more efficacious treatment in a mouse model of allergic inflammation. *Scand J Immunol* 2010; 71: 403-412.
21. Mueller RS, Janda J, Jensen-Jarolim E, et al. Allergens in veterinary medicine. *Allergy* 2016; 71: 27-35.
22. Calderon MA, Cox L, Casale T, et al. Multiple-allergen and single-allergen immunotherapy strategies in polysensitized patients: looking at the published evidence. *J Allergy Clin Immunol* 2012; 129: 929-934.
23. Cipriandi G, Incorvaia C, Puccinelli P, et al. What should drive the choice of allergen for immunotherapy in polysensitized patients? *Ann Allergy Asthma Immunol* 2012; 109: 148-149
24. Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: A practice parameter third update. *J Allergy Clin Immunol* 2013; 127(1 Suppl): S1-55.
25. Rosenbaum MR, Esch RE, Schwartzman RM. Effects of mold proteases on the biological activity of allergenic pollen extracts. *Am J Vet Res* 1996; 57: 1447-1452.

ALLERGEN IMMUNOTHERAPY: CONTROVERSIES

Jon Plant, DVM, DACVD

SkinVet Clinic, Lake Oswego, OR

HOW SPECIFIC IS ALLERGEN IMMUNOTHERAPY?

Many veterinary dermatologists practice allergen immunotherapy (AIT) with the presumption that an ideal allergenic extract prescription exists for any given animal with atopic dermatitis (AD). Following from this concept is the notion that with proper allergen selection, thorough history taking, accurate allergy testing, skilled allergen formulation, and ideal dosing, the efficacy of AIT will be maximized.

Other veterinary dermatologists have questioned, directly or indirectly, the idea that there is an ideal allergenic extract prescription for each patient, or at least whether the current state of the art allows us to consistently formulate one. I'll review some studies that support each side of the controversy and then propose how these seemingly opposed views might be reconciled.

First, imagine that you are given the task of baking a dessert with any number of 100 possible ingredients. The ingredients are familiar to you and your understanding is that some ingredients are more important to include than others. You have a fundamental understanding of how to combine ingredients to make a cake, pie, tart, etc.

You are instructed to make the tastiest, most beautiful dessert that you can make for a judge. At your disposal are a dozen books from which to choose a recipe, but you are not required to follow any one of them. Each recipe claims to be the "best."

Now, if 100 people were given the same task, how many different desserts would we end up with? Most likely there would be 100. There would almost certainly be some very similar cakes, or pies, but each would be a bit different in some respect. I contend that allergen formulation for a given atopic patient is not so different from this thought experiment. There are many steps, each one introducing some degree of variability that compounds upon the last step. Some steps introduce more variability, others less, but each introduces variability.

Let's consider some of the major steps that go into AIT formulation from start to finish and look at how the variability of each step affects the decision to include or exclude an individual allergen from the final formulation.

Step 1. A testing method is chosen – either intradermal test (IDT), serum allergy (IgE) test (SAT), or some combination of these. There seems to be a general consensus that there is imperfect agreement between IDT and SAT.[1,2] Codner found poor agreement between IDT and SAT (kappa = 0.17).[3] With regards to *Malassezia* sensitivity alone, SAT was reportedly 77% sensitive and 89% specific relative to IDT.[4] Foster et al explain that SAT and IDT are measuring different things and conclude that "it would not be surprising if the results of a serological assay did not correlate highly with those of an IDT," which is what they found. Sensitivity for individual allergens, SAT compared to IDT, ranged from 0% (molds and feather) up to 75% (sheep sorrel); specificity ranged from 64.2% (flea) up to 99.3% (Aspergillus).[5] Overall, they found little correlation of SAT with IDT for pollens. From these findings, it is reasonable to conclude that the step of choosing an allergy test method introduces at least 20% variability into the decision whether or not to include an allergen.

Step 2. For IDT, a subset of the commercially available allergens is chosen. Hensel found that among 10 locations within the same geographic area, veterinary dermatologists tested for 115 different allergens. Each tested for 47-84 items, with 28/115 (24%) included at 9 or 10 of the practice locations.[6] In the case of SAT, the commercial laboratory routinely defines the allergens for testing based on geographic areas. Among four SAT laboratories, panels for the same region (Texas) included 110 individual allergens in total (range 40-63 per laboratory). Only 16/110 individual allergens were included in all four assays.[7]

Conservatively, Step 2 introduces at least 30% variability into the decision whether or not to include an allergen.

Step 3. Certain medications (e.g. glucocorticoids) have been shown to suppress the strength of IDT reactions and it is recommended that they be withheld prior to IDT and SAT (by some laboratories). Recommended drug withholding periods, when known, may or may not be adhered to. The recommended withholding times for these medications are

often uncertain.[8] With the availability non-steroidal anti-pruritic medications that may be administered up to the time of testing, this problem is likely less significant than in years past, but may still result in some (perhaps 5%) variability in whether or not to include an allergen.

Step 4. Possible seasonal influences on IDT or SAT are considered or ignored. In humans, skin test reactivity may peak after the end of the patient's allergy season, then decline. This has not been well studied in animals.[9] In one experimental canine model, allergen-specific IgE levels declined quickly without persistent allergen exposure, while IDT reactivity was more persistent. It seems quite possible that the choice of what time of year a seasonally affected patient is tested could introduce 20% variability in the decision to include an allergen or not.

Step 5. Test-retest repeatability and inter-assay agreement variability are introduced when the SAT laboratory or the individual performing an IDT are chosen. The test-retest repeatability of both IDT and SAT are poorly studied. One unpublished report found unacceptable variability between blinded replicate aliquots from 10 dogs when assayed by the same laboratories.[1] For the 3 laboratories included in the study, the percentage of discordant test-retest scores ranged from 9-43%. Patterson et al submitted replicate samples from 42 dogs for SAT and found that the probability of a positive allergen finding in one sample being positive in both samples was 62%.[10] Hubbard and White found a slight level of agreement (kappa = 0.2) between subjective and objective scoring of IDT.[11] In a workshop, Beale described another unpublished study by Ferrer-Canals et al that found that the test-retest repeatability of IDT scoring (n=12 dogs) was fair to moderate for each of the 3 investigators.[12] Inter-assay agreement between clinicians was fair for scores of 0, 2, and 3; good for scores of 4, and poor for scores of 1.

Some SAT assays have been found to have good performance characteristics using sera pooled together form multiple dogs and based on continuous measurement scales of allergen-specific IgE.[13] In contrast, the agreement of four SAT in 10 dogs using positive/negative cutoffs was generally poor.[7] In general, even when tests perform well, scores close to cutoff values for the determination of positive/negative are susceptible to misclassification.

Based on these studies and the lack of proficiency monitoring, it seems reasonable that the step of selecting an individual to perform an IDT or a laboratory for SAT introduces at least 30% variability into the decision to include or exclude an individual allergen in a prescription.

Step 6. Once a test result is obtained, it should be interpreted in relation to the animal's clinical history (seasonality of signs and likely exposure), possible cross-reactivity, and aerobiology of the local environment.14 Aerobiology information from websites or client-provided historical information may or may not be accurate, available, or even considered. Many commonly referenced websites make pollen level predictions based on weather and season, but do not provide actual pollen counts. Clients are often unfamiliar with many of the grasses, trees, and weeds that are included in IDT and SAT panels, limiting their ability to provide accurate exposure information.

When presented with the same test results and history, veterinary dermatologists may disagree on whether or not to include an allergen. In an informal vetderm listerve survey, 11 participants were provided with the same hypothetical patient history and IDT result (33/50 allergens scored 2, 3 or 4) and asked to formulate an allergen vaccine. Among the findings: there were 11 unique formulations, 2/50 (4%) of "tested" allergens were prescribed by all 11, 28/50 (56%) of allergens were prescribed by at least one; and 16/50 (32%) of allergens were prescribed by ≥ 50%. On the other hand, Beale reported that despite the IDT scoring discrepancies in the Ferrer-Canals study cited above, the ensuing allergen mixtures showed little variability, presumably due to formulations being informed by clinical history and local aerobiology.[12] Taken together, it seems likely that the uncertainty associated with an animal's actual exposure to an allergen introduces 20% or more variability.

Step 7. How one combines allergenic extracts into treatment sets is not standardized. The veterinarian must choose, based on custom and experience, how many allergens to include per vial, their ratio, and their concentration. This may lead one veterinarian to exclude a particular allergen, while another would include it in the allergenic mixture. This step may also contribute 20% to the variability regarding including an allergen or not.

Implications: What are we to make of the variability introduced at each step? The estimates or variability provided here do not lend themselves to simple statistical calculations, but it seems intuitive that we are propagating errors at each step of the way in allergen formulation. Analogous to the baking thought experiment, if 100 veterinarians could

independently allergy test the same dog, on the same day, under the same conditions, in the same location, how many unique allergenic extract mixtures would we prescribe? How closely would each come to matching the "ideal" mixture for that dog? And perhaps more importantly, how would the effectiveness of a particular allergy vaccine correlate with how closely it approximates the dog's ideal mixture?

A number of reviews of AIT state that despite the poor agreement between IDT and SAT in dogs, there is no clear difference in effectiveness of AIT based on one or the other test.[2,15,16] How do we account for this? Nearly all of the reports on the effectiveness of AIT in dogs are open and uncontrolled.[14] Perhaps the potential benefit of overall case management is responsible for a substantial portion of the perceived benefit. Few AIT randomized controlled trials (RCTs) in dogs have been reported. Willemse's landmark AIT study reporting ≥ 51% improvement in 59% of 27 dogs, while admirable, had significant limitations.[17] Namely, at the 15-month time point at which statistical significance between the placebo and treatment groups was first seen, the dropout rate was high (59% in the treatment and 79% in the placebo group) and not accounted for with intent-to-treat analysis.[14] Other RCTs have compared AIT protocols such as low versus standard dose,[18] conventional versus rush induction,[19] and custom formulated versus standard extracts. It is difficult to draw firm conclusions from these studies due to small sample sizes and the use of non-validated outcome measures, but in general, significant differences have not been found. In a workshop abstract concerning a study of 30 dogs with AD, Willemse reported that custom-formulated AIT was more effective than a standard mixture of housedust, dog dander, human dander, and grasses.[20] In contrast, Garfield reported in an unpublished abstract that a standard mixture of 32 allergens was effective in 76% of 29 dogs, not significantly different from groups treated with a high dose (85%, n = 27) or low dose (68%, n = 22) allergenic extracts formulated based on IDT.

How can we explain the apparent effectiveness of imperfectly matched allergen mixtures? Perhaps including a limited subset of the "correct" allergens is sufficient to achieve a close to maximal benefit, with diminishing returns once a certain level of match is achieved. Perhaps the non-specific effects of AIT are sufficient to achieve the level of response that we are able to detect with our current methodology (measurement scales and study design). The unpalatable alternative is that we don't consistently detect a difference because the perceived benefit of AIT for AD is a placebo effect. Ultimately, we need adequately powered RCTs to answer these questions.

1. DeBoer DJ, Hillier A. The ACVD task force on canine atopic dermatitis (XVI): laboratory evaluation of dogs with atopic dermatitis with serum-based "allergy" tests. Vet Immunol Immunopathol. 2001;81(3-4):277-87.
2. Hensel P, Santoro D, Favrot C, et al. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. BMC Vet Res. 2015;11:196.
3. Codner EC, Lessard P. Comparison of intradermal allergy test and enzyme-linked immunosorbent assay in dogs with allergic skin disease. J Am Vet Med Assoc. 1993;202(5):739-43.
4. Oldenhoff WE, Frank GR, DeBoer DJ. Comparison of the results of intradermal test reactivity and serum allergen-specific IgE measurement for Malassezia pachydermatis in atopic dogs. Vet Dermatol. 2014;25(6):507-11, e84-5.
5. Foster AP, Littlewood JD, Webb P, et al. Comparison of intradermal and serum testing for allergen-specific IgE using a FcεRIα-based assay in atopic dogs in the UK. Vet Immunol and Immunopathol. 2003;93(1–2):51-60.
6. Hensel P. Differences in allergy skin testing among dermatologists within the same geographical region in the USA. Abstracts of the 7th World Congress of Veterinary Dermatology. July 24-28, 2012. Vancouver, Canada. Vet Dermatol. 2012;23 Suppl 1:60.
7. Plant JD, Neradelik MB, Polissar NL, et al. Agreement between allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories in the USA. Vet Dermatol. 2014;25(1):15-e6.
8. Olivry T, Saridomichelakis M. Evidence-based guidelines for anti-allergic drug withdrawal times before allergen-specific intradermal and IgE serological tests in dogs. Vet Dermatol. 2013;24(2):225-e49.
9. Hillier A, DeBoer DJ. The ACVD task force on canine atopic dermatitis (XVII): intradermal testing. Vet Immunol Immunopathol. 2001;81(3-4):289-304.
10. Patterson AP, Schaeffer DJ, Campbell KL. Reproducibility of a commercial in vitro allergen-specific assay for immunoglobulin E in dogs. Vet Rec. 2005;157(3):81-5.

11. Hubbard TL, White PD. Comparison of subjective and objective intradermal allergy test scoring methods in dogs with atopic dermatitis. J Am Anim Hosp Assoc. 2011;47(6):399-405.
12. Halliwell REW, Gilbert S. Allergy testing revisited. In: Torres SMF, Frank LA, Hargis AM, eds. Advances in Veterinary Dermatology. Vol. 7. Oxford: Wiley-Blackwell; 2013. p. 305-6.
13. Lee KW, Blankenship KD, McCurry ZM, et al. Performance characteristics of a monoclonal antibody cocktail-based ELISA for detection of allergen-specific IgE in dogs and comparison with a high affinity IgE receptor-based ELISA. Vet Dermatol. 2009;20(3):157-64.
14. Griffin CE, Hillier A. The ACVD task force on canine atopic dermatitis (XXIV): allergen-specific immunotherapy. Vet Immunol Immunopathol. 2001;81(3-4):363-83.
15. Schnabl B, Bettenay SV, Dow K, et al. Results of allergen-specific immunotherapy in 117 dogs with atopic dermatitis. Vet Rec. 2006;158(3):81-5.
16. Loewenstein C, Mueller RS. A review of allergen-specific immunotherapy in human and veterinary medicine. Vet Dermatol. 2009;20(2):84-98.
17. Willemse A, Van den Brom WE, Rijnberk A. Effect of hyposensitization on atopic dermatitis in dogs. J Am Vet Med Assoc. 1984;184(10):1277-80.
18. Colombo S, Hill PB, Shaw DJ, et al. Effectiveness of low dose immunotherapy in the treatment of canine atopic dermatitis: a prospective, double-blinded, clinical study. Vet Dermatol. 2005;16(3):162-70.
19. Mueller RS, Fieseler KV, Zabel S, et al. Conventional and rush immunotherapy in the treatment of canine atopic dermatitis. In: Hillier A, Foster AP, Kwochka KW, eds. Advances in Veterinary Dermatology. Vol. 5. Oxford: Blackwell Publishing; 2005. p. 60-69.
20. Anderson R, Sousa C. Workshop report 7. In vivo vs. in vitro testing for canine aotpy. In: Ihrke PJ, Mason I, White SD, eds. Advances in Veterinary Dermatology. Vol 2. New York: Pergamon Press; 1992. p. 425-7.

ALLERGEN IMMUNOTHERAPY- CONTROVERSIES

Griffin, CE

Animal Dermatology Group, San Diego, Ca

I have three topics I wanted to cover in controversies though it is possible they may not rise to the level of a controversy involving strong disagreement but they certainly are topics where there is disagreement and different ways of dealing with the topics. Some of the disagreement stems from a lack of any controlled science on addressing these issues which are primarily opinion based medicine. The purpose of this session will be to ascertain what the audience does in these situations and possible stimulate some studies that take us from opinion based medicine to evidence based medicine as the first step is asking the right questions. So, we will discuss lots of questions.

TOPIC 1

How do you deal with dogs and cats that have adverse reactions to immunotherapy? Adverse reactions to allergen immunotherapy can vary from serious systemic reactions such as anaphylaxis, angioedema and urticaria to the most common reaction, pruritus following an injection. When a pet has these reactions how do you deal with continuation of the injections? Are there variables that would alter how you continue injections? If there are variables what are they, type or seriousness of the reaction, breed of dog, volume or concentration of the injection, how long or point of treatment regimen that the pet reacts at or some other criteria? What is your typical response? Do you lower the dose of allergen or pre-medicate prior to any subsequent allergen administration? If you lower the dose there are different options such as changing the volume or the concentration of the allergen administered. If you pre-medicate what drug to you use, hydroxyzine, loratidine, cetirizine, other antihistamine, glucocorticoid or another drug. Do you ever send epinephrine or injectable antihistamine home? Do you recommend discontinuing allergen immunotherapy for reactions and if so why? If you did lower the dose do you still try to reach your standard protocol maintenance and if so are there cases you cannot or at what point do you stop trying? I can say how I deal with these situations has changed over my career. However, those changes are based on my opinions regarding my experiences. There are no studies comparing responses to allergen immunotherapy reactions but there is one study that showed dogs that did have AE and then had the volume of allergen administered changed to a level of no reaction did have a higher success rate than those dogs that did not react.[1] However, the paper did not compare responses to reactions and so we do not really know was it the response or just the dog having a reaction that improved the success. If a different response such as pre-medicating would have resulted in a different response is not known. Is it possible pre-medicating and following the normal protocol or keeping the allergen dose the same has the same response, better and or faster response. I will show a case example of a dog that had severe reactions that ended up doing very well on subcutaneous allergen immunotherapy but only by being on lower volume (0.3ml) and lower concentration (200PNU/ml) and increased frequency of injections.

TOPIC 2

How many different allergens will you mix into a treatment allergen solution? Over the years the number of allergens tested for has increased by some veterinary allergists but also by some of the commercial serum in vitro testing companies. However even when I started out many years ago I was taught to select the most appropriate 10 items to put into a treatment set. We also know there are various criteria used to decide which allergens may be appropriate and one study has shown that the results of what is recommended treatment by commercial laboratories would vary and the agreement that occurred is only slightly better than by chance.[2] Certainly, what is selected and how those results compare is a topic for discussion but for this controversy I have chosen how many of the positive items will be treated may also vary between companies and veterinary dermatologists. Some recommend treating for all positives and if needed using multiple vials. Others, including myself, limit the number of allergens that will be included in on vial of allergen solution and only use one maintenance vial. For those allergists that limit it to one vial if the case does not respond do you give up on allergen immunotherapy, reformulate the allergen solution mixture, retest and reformulate the allergen solution mixture, or add additional vials with different allergens that the pet was initially positive to? When different sets of allergen solutions are used there is also differences in how they may be given. To my knowledge there are no studies that have evaluated these differences in how immunotherapy is done and what

impact it would have on treatment success and if it is cost effective. I will present a case that was allergic to over 30 allergens and treatment was not as effective as wanted until the dog was put onto three different vials of allergens.

TOPIC 3

It is believed that ASIT is a treatment that may result in a cure or at least some longer-term remission. In dogs, long term remission has been described in 4% - 35% of cases.[1,3-5] However these studies do not always define what remission really meant. In the study showing 4% criteria were described and though 15 clients felt their dog was in remission only 5 met the authors definition of complete remission of atopic dermatitis.3 Do you tell clients to stop ASIT when it has been effective? Is so how effective does it have to be? What do you tell clients about stopping allergen immunotherapy? Do you discuss how to stop such as by increasing time between treatments prior to stopping, probability of staying in remission, what to watch for and for how long, or what to do if allergy signs return?

References

1. Rosser, E. Aqueous hyposensitization in the treatment of canine atopic dermatitis: a retrospective study of 100 cases. Advances in Vet Derm 1998: K. K. Kwochka, T. Willemse and C. vonTscharner. Boston, Butterworth Heinemann. **3**: 169-176.
2. Plant JD, Neradelik MB, Polissar NL, et al. Agreement between allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories in the USA. Vet Dermatol 2014: **25**: 15-e16.
3. Dell DL, Griffin CE, Thompson LA et al. Owner assessment of therapeutic interventions for canine atopic dermatitis: a long-term retrospective analysis. Vet Dermatol 2012:**23**: 228-e247.
4. Park SF, Ohya K, Yamashita K, et al Comparison of response to immunotherapy by intradermal skin test and antigen-specific IgE in canine atopy. J Vet Med Sci 2000:**62**: 983-988.
5. Loewenstein C and Mueller RSA review of allergen-specific immunotherapy in human and veterinary medicine. Vet Dermatol 2009:**20:** 84-98.

ABSTRACT SCHEDULE

FRIDAY, APRIL 12

Abstract Session: Original Abstracts

9:15 AM	**Dr. Kathy Tater:** Eyes instead of ears: canine and feline cases of eye injuries after ocular exposure to topical ear medications	80
9:30 AM	**Dr. Kathy Tater:** Topical minoxidil toxicity to dogs and cats	81
9:45 AM	**Dr. Cherie Pucheu-Haston:** Serum IgE and IgG responses to dietary antigens in dogs with and without cutaneous adverse food reactions: a pilot study	82
10:00 AM	**Dr. Rosanna Marsella:** Investigation of tight junction proteins in Primary cell cultures of normal and atopic keratinocytes and their relationship to transepithelial electrical resistance (TEER)	83
10:15 AM	**Dr. Rosanna Marsella:** Investigation on the effects of allergen exposure on skin pH in atopic dogs and correlation with disease severity	84
10:30 AM	**Dr. Rosanna Marsella:** Investigation of dose, frequency and duration of allergen exposure on development of staphylococcal infections in a model of canine chronic atopic dermatitis	85
10:45 AM	**Dr. Caitlin Older:** The Staphylococcal communities inhabiting healthy and allergic feline skin	86
11:00 AM -11:30 AM	**BREAK**	
11:30 AM	**Dr. Gary Bammert:** Use of *in situ* hybridization to assess the expression of genes associated with peripheral nociception and pruriception in feline neuronal tissues	87
11:45 AM	**Dr. Steve Dunham:** Identification and characterization of monoclonal antibodies targeting feline IL-31	88
12:00 PM	**Dr. Galia Sheinberg:** PCR use for identification of *Malassezia* spp. in canines with otitis externa	89
12:15 PM	**Dr. Galia Sheinberg:** Implementation of a multiplex PCR for ultra-fast diagnosis of *Pseudomonas aeroginosa*, *Malassezia* spp and *Proteus mirabilis* in canine otitis externa	90
12:30 PM - 2:00 PM	**LUNCH**	
2:00 PM	**Dr. Elizabeth Layne:** Intradermal test reactivity to two pollen allergen concentrations in atopic dogs	91
2:15 PM	**Dr. Brennan McKinney:** Consistency monitoring of *Dermatophagoides farinae* allergenic extracts	92
2:30 PM	**Dr. Katje Baumann:** Intra- and inter-assay variability of serum tests for environmental allergen-specific IgE from different laboratories	93
2:45 PM	**Dr. Natalie Gedon:** Agreement of serum allergy test results with unblocked and blocked immunoglobulin E against cross-reactive carbohydrate determinants (CCD) and intradermal test results in atopic dogs	94
3:00 PM	**Dr. Kenneth Lee:** Discovering the pitfall of using horseradish peroxidase in enzyme-linked immunosorbent assays for detection of pollen specific IgE	95
3:15 PM	**Dr. Petra Bizikova:** Detection of anti-desmocollin-1 and anti-desmoglein-1 autoantibodies in dogs with pemphigus foliaceus with or without the classic facial involvement	96
3:30 PM	**Dr. Michael Canfield:** Pruritus and dermatitis scoring in flea-infested dogs before and after administration of flea adulticides	97
3:45 PM	**Dr. Darren Berger:** Efficacy of a 0.02% topical hypochlorous acid containing solution as a sole therapeutic intervention for canine superficial pyoderma: a randomized, blinded, controlled pilot investigation	98
4:00 PM - 4:30 PM	**BREAK**	

ABSTRACT SCHEDULE

FRIDAY, APRIL 12

Abstract Session: Clinical Abstracts

4:30 PM	**Dr. Lynette Cole:** Low-level laser therapy as an adjunctive treatment for canine acral lick dermatitis: a randomized, double-blinded, sham-controlled study	99
4:45 PM	**Dr. Alberto Cordero:** Topical 0.5% timolol maleate for the treatment of incipient cutaneous haemangiomas in dogs: a pilot study	100
5:00 PM	**Dr. Darren Berger:** Meticillin-resistant coagulase-negative staphylococcal pyoderma in a captive African lion (*Panthera leo*)	101
5:15 PM	**Dr. Diana Di Mattia:** Long term use of L-phenylalanine in combination with oral fatty acids and topical sunscreen in the treatment of vitiligo associated with carcinoma in situ involving the planum nasale in a dog	102

Scientific Session Presentations

9:00 AM - 10:00 AM	**Anna De Benedetto, MD:** Updates on Immunopathogenesis of Human Atopic Dermatitis	
10:00 AM - 11:00 AM	**Anna De Benedetto, MD:** Updates on Skin Barrier of Human Atopic Dermatitis	
11:30 AM - 12:30 PM	**Anna De Benedetto, MD:** Current Treatments in Human Atopic Dermatitis	
2:00 PM - 3:00 PM	**Thierry Olivry, DrVet, PhD:** Canine Atopic Dermatitis: Pathogenesis	103
3:00 PM - 4:00 PM	**Thierry Olivry, DrVet, PhD:** Atopic Itch: From Pathogenesis to Targeted Therapy	104
4:30 PM - 5:30 PM	**Thierry Olivry, DrVet, PhD:** Atopic Itch: From Pathogenesis to Targeted Therapy	104

Concurrent Session Presentations

9:00 AM - 10:00 AM	**Hilary Jackson, BVM&S:** Cutaneous Lupus Erythematosus	105
10:00 AM - 11:00 AM	**Hilary Jackson, BVM&S:** Cutaneous Lupus Erythematosus: Treatment	108
11:30 AM - 12:30 PM	**Katharine Lunn, BVMS, MS:** Liver Diagnostic Tests Interpretation for Dermatologist	109
2:00 PM - 3:00 PM	**Katharine Lunn, BVMS, MS:** Renal Panel and Urinalysis Interpretation for Dermatologist	113
3:00 PM - 4:00 PM	**Hilary Jackson, BVM&S, Katharine Lunn, BVMS, MS:** Dealing with Side-Effects of Immunosuppression: Case-Based Approach	
4:30 PM - 5:30 PM	**Hilary Jackson, BVM&S, Katharine Lunn, BVMS, MS:** Dealing with Side-Effects of Immunosuppression: Case-Based Approach	

Eyes instead of ears: canine and feline cases of eye injuries after ocular exposure to topical ear medications

K.C. TATER*, S. GWALTNEY-BRANT*, T. WISMER†

** Veterinary Information Network, Davis, CA, USA*
† ASPCA Animal Poison Control Center, Urbana-Champaign, IL, USA

Abstract: Medication errors are preventable events in the patient care process (i.e. prescription to administration) that lead to inappropriate use and patient harm. An ocular exposure can occur for otic medications because of the similarity between "optic" and "otic" medical terms, "look-alike" medication packaging for ophthalmic and otic products, and the anatomical proximity of the eyes and ears. To determine the epidemiology of ocular exposures and toxicoses in dogs and cats from otic products, 78 dog and cat cases in which there was an ocular exposure to an otic topical were retrieved from the ASPCA Animal Poison Control Center database. Cases involved otic ointment, drop, and flush formulations. Prescription products were involved in 74/78 (95%) of cases. Clinical signs included conjunctivitis, blepharospasm, epiphora, ocular discharge, and corneal ulceration. Case narratives were reviewed and coded to examine ocular exposure-related circumstances for otic medications resulting in clinical signs. Medication error, specifically involving mistaken identification (i.e. otic product was confused with an ophthalmic product), occurred in 67/78 (86%) of cases. In five cases, the mistaken identification occurred when an otic instead of opthalmic medication was dispensed to the pet owner. Unintentional delivery (i.e. accidental ocular exposure in the course of otic application) occurred in 9/78 (12%) of cases. Because mistaken identification was the most common cause of ocular toxicoses from otic products, separate storage and/or distinctive packaging for ophthalmic versus otic products could reduce medication errors. Animal poison control center epidemiological data can be used as a source of information about veterinary medication errors.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Topical minoxidil toxicity to dogs and cats

K.C. TATER*, S. GWALTNEY-BRANT*, T. WISMER†

** Veterinary Information Network, Davis, CA, USA*
† ASPCA Animal Poison Control Center, Urbana-Champaign, IL, USA

Abstract: Topical minoxidil is a medication for hairloss, initially available in the US by prescription only and then since 1996 available as an over-the-counter product. To determine the epidemiology of minoxidil exposures and toxicities to dogs and cats, 249 dog and cat cases with topical minoxidil exposure were retrieved from the ASPCA Animal Poison Control Center database. Clinical signs of toxicosis developed in 25/118 (21%) of dogs and 59/131 (45%) of cats. Severe hypotension and other cardiac and respiratory symptoms were common clinical conditions. Hospitalization occurred in 78 (31%) dog and cat cases. Death was confirmed in eight (6%) cat cases. In the 84 cases with clinical signs of toxicosis, case narratives were reviewed and coded for exposure-related circumstances. Unintentional delivery, especially while pet owners applied minoxidil for his/her own hair loss (e.g. pet licked owner's skin or pillowcase, pet was splashed during a medication spill), was the most common exposure circumstance in clinical cases. Intentional delivery (i.e. direct application to the skin to treat pet's alopecia) occurred in two clinical feline cases. Toxic doses in cats and small dogs were low, such as one drop or one or two licks per pet. In households with human topical minoxidil use, pet owners should be informed of the risk of dog and cat toxicosis from accidental minoxidil exposure. Despite prior publications on topical minoxidil as a treatment for alopecia in dogs and cats, the use of minoxidil to treat alopecic dogs and cats is not recommended due to the risk of toxicosis.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Serum IgE and IgG responses to dietary antigens in dogs with and without cutaneous adverse food reactions: a pilot study

C. M. PUCHEU-HASTON* and I. MOUGEOT†

**Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA*
†CanCog Technologies, Toronto, ON, Canada

Abstract: It is suspected that many canine cutaneous adverse food reactions (CAFR) are true immunologic hypersensitivities, but few specific dietary allergens have been identified. This study's objective was to compare serum IgE and IgG reactivity to specific food antigens in privately-owned dogs with and without CAFR. Eighteen adult dogs with non-seasonal pruritus were fed an extensively hydrolyzed chicken-based diet (Ultamino®) exclusively for 12 weeks. Serum collection was performed at the beginning and end of the diet trial. Immunoblotting was performed to identify IgE and/or IgG binding to specific proteins in beef, egg, milk, chicken, pork, soy and wheat extracts. CAFR (defined as an unequivocal relapse of pruritus after dietary challenge) was diagnosed in 10 dogs, with 60% relapsing when fed chicken-based diets. Lesional and pruritus scores were "mild" or "in remission" in 10 and eight CAFR dogs, respectively, prior to rechallenge. Binding of patient IgG to almost all of the proteins in the extracts was seen regardless of reported dietary history. No protein was consistently bound by IgE or IgG in CAFR versus non-CAFR dogs. However, a poorly defined high molecular weight protein (or proteins) in beef and milk was predominantly bound in CAFR sera. This protein may correspond to aggregated bovine serum albumin and/or IgG. In conclusion, our study demonstrated the successful use of an extensively hydrolyzed diet to identify dogs with CAFR, even in patients with known intolerance to chicken. Almost all extract proteins were bound by IgG in all patients, suggesting extensive prior exposure to unreported foods.

Sources of funding: Royal Canin SAS, Aimargues, France.

Conflict of Interest: Isabelle Mougeot is consultant for Royal Canin.

Investigation of tight junction proteins in primary cell cultures of normal and atopic keratinocytes and their relationship to transepithelial electrical resistance (TEER)

R. MARSELLA* R. WILKES,*K. AHRENS,*

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Transepithelial electrical resistance (TEER) in monolayer cultures is used to assess barrier integrity of epithelia. TEER of primary keratinocytes harvested from normal dogs is significantly higher than TEER of atopic dogs, 6 days after confluence. The cause of this difference is currently unknown. The study aim was to investigate the gene expression of tight junction proteins (claudin, occludin, ZO-1), filaggrin, and filaggrin 2 in keratinocytes harvested from normal (n=6) and atopic (n=6) dogs, at baseline and at day 6 of cell culture and to correlate them to TEER. At baseline, no statically significant difference was detected between normal and atopic keratinocytes for filaggrin 2, claudin, occludin, ZO-1 while filaggrin was higher in atopics compared to normal keratinocytes (P =0.047). A significant increase in gene expression for claudin and occludin was seen in both normal and atopics between day 0 and 6 but no significant difference in the increase was noted between the two groups. When considering correlations with TEER, the only significant positive correlation was with filaggrin (r=0.95; P =0.0003). Western blot was also done for claudin and filaggrin on day 0 and 6 in normal and atopics. On day 0 atopics had higher filaggrin than normals (P =0.046). Normals increased significantly over time (P =0.032) but atopics did not. The correlation with filaggrin is interesting and warrants further investigation. Similar findings had been reported in esophageal barrier studies but not in skin. Larger number of samples are needed to detect more significant differences due to large variability of results.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Investigation on the effects of allergen exposure on skin pH in atopic dogs and correlation with disease severity

R. MARSELLA,* R. WILKES,* K. AHRENS,* A. TRUJILLO*

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Skin pH modulates microbiota and proteolytic enzymes. The effect of allergen exposure on skin pH in atopic dogs and correlation between pH, disease severity and transepidermal water loss (TEWL) are unknown. In this study, a colony of 15 allergic beagles were challenged twice weekly for 2 weeks by epicutaneous application of house dust mite on inguinal and medial thigh. pH and TEWL were monitored during challenge. Severity of dermatitis was scored daily using Canine Atopic Dermatitis Extent and Severity Index (CADESI-03)-03. On days of challenge, dermatitis was scored both before and 4 h after allergen exposure. TEWL was measured daily on both inguinal and medial thigh using a close chamber device (VapoMeter). Two measurements were taken per dog/day with three replicates each. Skin pH of both inguinal and medial thigh was taken daily using the Skin-pH-Meter. Two measurements were taken per dog/day with 10 replicates each. Repeated measures ANOVA showed a significant effect of time with increased pH over the course of allergen exposure ($P < 0.0001$, both sites), increased TEWL ($P < 0.0001$ for both sites) and increased CADESI-03 ($P < 0.0001$). Significant positive correlation was found between CADESI-03 and pH ($r = 0.3556$; $P < 0.0001$), CADESI-03 and TEWL ($r = 0.36$; $P < 0.0001$), pH and TEWL ($r = 0.45$; $P < 0.0001$). We conclude that pH is a marker of disease severity in canine atopic dermatitis. Future studies should evaluate the effect of allergen exposure on normal skin and compare it with atopic skin.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Investigation of dose, frequency and duration of allergen exposure on development of staphylococcal infections in a model of canine chronic atopic dermatitis

R. MARSELLA,* R. WILKES ,* K. AHRENS

** Blanche Saunders Dermatology Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Canine atopic dermatitis (AD) is chronic and frequently complicated by infections. The majority of models describe acute allergen challenges. We aim to describe a model of chronic AD. It is unknown whether the duration of allergen exposure or the dose or both are important factors for staphylococcal infections. Aim 1 was to evaluate the effect of duration of allergen exposure comparing three protocols. The daily dose (25mg/dog/day *Dermatophagoides farinae*) remained the same but *D. farinae* challenge frequency and study duration varied. The one-week study involved three epicutaneous challenges, 3 days in a row. The one-month study involved twice-weekly challenges for 4 weeks. The three-month study involved twice-weekly challenges for 12 weeks. Aim 2 was to evaluate the effect of different daily doses while keeping the total weekly dose and study duration the same. Low-dose involved challenges with 5mg *D. farinae* for 5 days each week for 3 weeks. High-dose involved challenges with 12.5mg *D. farinae* twice weekly for 3 weeks. In both aims, dogs were evaluated clinically each day of the allergen challenge. Diagnosis of pyoderma was based on clinical signs and consistent cytology. In Aim 1, the longer the study, the more pyoderma developed (67% in the three-month study, 25% in the one-month study and none in the one-week study). In Aim 2, low-dose daily exposure caused more infections (60% of dogs) than high-dose infrequent exposure (no dogs). It is concluded that low-grade daily exposure for longer time may be more relevant when studying ways to prevent staphylococcal infections.

Sources of funding: Self-funded.

Conflict of Interest: None declared

The Staphylococcal communities inhabiting healthy and allergic feline skin

C.E. OLDER*, J.M. STARKS*, J. WU*, S.D. LAWHON*, A.B. DIESEL†, and A. RODRIGUES HOFFMANN*

**Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA*
†Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA

Abstract: *Staphylococcus* spp. are important cutaneous commensals and opportunistic pathogens. The relative abundance of these bacteria differs between cats with and without allergic dermatitis, suggesting they may play a role in feline allergic skin disease (FASD). However, the distribution and relevance of specific staphylococcal species on feline skin is unknown. To describe the species-level staphylococcal communities on feline skin, skin swabs were taken from two groups of cats: samples for cohort one were obtained from the axilla and interdigital space of 17 healthy and 10 allergic cats, cohort two samples were obtained from the ear canal and groin of 10 healthy and 10 allergic cats. Extracted DNA from cohort one was used for quantitative PCRs targeting the genus Staphylococcus along with specific species *S. aureus* and *S. pseudintermedius*, while cohort two DNA was used for next-generation sequencing of the 16s rRNA gene. With qPCR, *S. pseudintermedius* was only detected in 4/27 samples and S. aureus was detected in none of the samples. Quantitative PCR revealed *Staphylococcus* spp. to be more prevalent in healthy cats compared to allergic cats. Sequencing data revealed Staphylococcus spp. accounted for 3.03% of all bacterial sequences. *S. epidermidis* (40.3%) and *S. capitis* (30.8%) were the most abundant species in *Staphylococcus* sequences from healthy and allergic samples, respectively. The data from this study indicate that feline skin communities have diverse staphylococcal communities containing multiple species with relative abundances greater than 10% and with a composition that may be shaped in part by health status.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Use of *in situ* hybridization to assess the expression of genes associated with peripheral nociception and pruriception in feline neuronal tissues

C. HEDKE*, S. DUNHAM*, M. ALEO*, J. CURRY*, J. ROSS*, K. ESCH*, A. GONZALES*, G. BAMMERT*

**Veterinary Medicine Research and Development, Zoetis Inc., Kalamazoo, MI USA*

Abstract: Perception of itch occurs through transmission of signals from peripheral nerve endings in the skin to central neuronal compartments of the dorsal root ganglion (DRG). Knowledge of the expression of relevant cytokines and their receptors will benefit the understanding of the disease mechanisms involved with atopic and allergic dermatitis. Atopic dermatitis in dogs is mediated by the dysregulation of the cytokine interleukin-31 (IL-31). To further understand the role of IL-31 and its co-receptors (*IL31RA and OSMR*), we used RNAScope *in situ* hybridization (RNA-ISH) to determine single gene expression patterns in the context of cellular and tissue morphology. This report describes a comparative analysis of the tissue-specific expression of IL-31 and its co-receptors in relation to a panel of genes which encode proteins associated with nociception (*TRPV1, TRPA1, NTRK1*), atopic dermatitis (*IL4R, IL33, IL1RL1*), inflammation (*IL1B*), histaminergic response (*HRH1, HRH4*), and the ionotropic receptors (*GABBR1, GABRA1*). Notably, the expression pattern of *IL31RA* and OSMR was observed within the soma of small to medium-sized neurons throughout the DRG. *IL31RA* was also associated with axons and less commonly with satellite cells. From the panel of transcripts probed, *OSMR, NTRK1, TRPA1*, and *TRPV1* were highly abundant. *IL31RA* presented an equivalent pattern of expression to *IL33* and *IL4R*. Application of this technology will play an important role in the elucidation of spatial and temporal gene regulation in the context of feline atopic and allergic disease.

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

Conflict of Interest: The authors are employed by Zoetis, Inc.

Identification and characterization of monoclonal antibodies targeting feline IL-31

T.J. FLECK*, G. BAMMERT*, *, S. MAHABIR*, C. JAVENS, B. GALVAN, S. STEINIGER, A. GONZALES*, S. DUNHAM*

**Veterinary Medicine Research and Development, Zoetis Inc., Kalamazoo, MI USA*

Abstract: The use of lokivetmab (Cytopoint®), a monoclonal antibody (mAb) targeting canine interleukin-31 (IL-31), for the treatment of canine allergic and atopic dermatitis has proven the therapeutic benefit of targeting IL-31 in canine disease. To evaluate the role of IL-31 in feline allergic and atopic dermatitis we have generated monoclonal antibodies (mAbs) and speciated derivatives targeting feline IL-31. Each mAb was evaluated for its ability to bind feline IL-31 and inhibit IL-31 mediated signaling in a feline cell line. Several mAbs were found to bind and neutralize the function of feline IL-31. Here we present the affinity (sub nanomolar to low picomolar) and *in vitro* potency (IC_{50} = 3.7 – 15 □g/mL) of three progenitor antibodies and several speciated derivatives. We also explored the *in vivo* efficacy of one antibody in a feline model of IL-31-induced pruritus. In this model eight cats per group were treated with either placebo or mAb (2 mg/kg, subcutaneously). Feline IL-31 was intravenously administered (1 □g/kg) prior to mAb administration and 7 days post mAb administration. Pruritic behavior was evaluated using a categorical scoring system in 1 min intervals over the course of 1 h after IL-31 injection. The results of this study demonstrated that a single 2 mg/kg subcutaneous dose resulted in statistically significant reduction of pruritus ($P < 0.0001$) for at least 1 week when compared to a placebo group administered mAb buffer. These data demonstrate the ability of anti-IL-31 antibodies to effectively inhibit IL-31-induced pruritus in a model of feline disease.

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

Conflict of Interest: The authors are employed by Zoetis, Inc.

PCR use for identification of Malassezia spp. in canines with otitis externa

A. FLORES*, C. ROMERO*, G. SHEINBERG†, A. M. CORDERO§

**Centro Universitario UAEM Amecameca, Universidad Autonoma del Estado de México, Amecameca de Juárez, Estado de México, México*
†Centro Veterinario México, Departamento de dermatología, Ciudad de México, México
§VETDERM: Dermatologia Veterinaria Especializada, Guadalajara, Jalisco, México

Abstract: Otitis externa is a common condition in dogs. Malassezia spp. are present in up to 57% of otitis externa cases in the dog. Cytology aids in the diagnosis. Cultures can be performed but growth is slow and sometimes unproductive. PCR tests are highly sensitive and fast with results obtained in 2 h. The study objective was to compare two different diagnostic procedures used for diagnosis of otitis externa: 1) sample cytology and 2) sample for PCR identification of yeast, both from ear exudate. Twenty-four ear swab samples (one from each ear) were obtained from 12 dogs with normal ears and twenty-four samples from 12 dogs with otitis externa. Samples were obtained using sterile swabs, rubbed along the vertical canal wall for 5 sec and stored in Stuart Transport Medium at -80°C until ready for DNA extraction. The samples were prepared for PCR and the amplified fragments were checked in a photocontroller. A comparison was made using a chi-square distribution. In healthy ears, PCR detected Malassezia spp. in statistically more ears (8/24) compared to cytology (2/24) ($P = 0.03$). In samples of ears with otitis, no significant difference was observed between PCR and cytology where 10/24 and 5/24 samples, respectively, were positive for *Malassezia* spp. ($P = 0.10$). PCR is a sensitive test for detection of *Malassezia* spp. in the ear canal of dogs. Future studies to include PCR differentiation of pathogenic from commensal strains of yeast may aid in therapeutic decisions.

Sources of funding: Self-funded.

Conflict of Interest: None declared.

Implementation of a multiplex PCR for ultra-fast diagnosis of *Pseudomonas aeroginosa*, *Malassezia* spp and *Proteus mirabilis* in canine otitis externa

A. FLORES*, C. ROMERO*, G. SHEINBERG†, A. M. CORDERO§

**Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de México, Amecameca de Juárez, Estado de México, México.*
†Centro Veterinario México, Departamento de dermatología, Ciudad de México, México.
§VETDERM: Dermatología Veterinaria Especializada, Guadalajara, Jalisco, México

Abstract: Canine otitis externa can derive from diverse causes, resulting in a secondary infection. Current clinical diagnostic methods of secondary infections are limited to cytology. Microbiological culture with antibiotic susceptibility is also available but results usually take several days. Quick and precise identification of pathogens allows accurate treatment of infection reducing antibiotic resistance. The objective of this study was to develop a multiplex PCR where three common ear pathogens (*Pseudomonas aeruginosa, Proteus mirabilis* and *Malassezia* spp.) are included in one test. A multiplex PCR was standardized using controls from ATCC strains. Three pairs of primers were used (*Pseudomonas aeruginosa, Proteus mirabilis* and *Malassezia* spp.) amplifying segments of 222pb, 325pb and 600pb. Multiplex PCR identifies tested for microorganism(s) in 2 h and if sequencing is performed after PCR, antibiotic resistant genes in 24 h. The multiplex PCR was used in two dogs with otitis externa. In the first patient *Pseudomonas aeuroginosa* and *Malassezia* spp. were identified by PCR; cytology determined only the presence of yeast. In the second patient *Pseudomonas aeuroginosa* and *Proteus mirabilis* were identified by PCR; cytology revealed cocci and bacilli. Bacterial cultures were not performed in these cases. Multiplex PCR with sequencing potentially allows institution of therapy more accurately and sooner compared to cytology and culture, thus decreasing antibiotic resistance and increasing owner/patient satisfaction. Future studies with larger number of dogs are needed to further evaluate multiplex PCR with sequencing in canine otitis externa cases. This ear multiplex PCR technology has wider application such as in skin pathogen identification.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Intradermal test reactivity to two pollen allergen concentrations in atopic dogs

E.A. LAYNE

Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

Abstract: Intradermal test (IDT) is the gold-standard for determination of hypersensitivity to specific allergens. The optimal concentration of allergen extracts used for IDT has not been established in atopic dogs. Inadequate test concentrations can give false-negative results. These false-negative results could lead to treatment failure due to lack of inclusion of important allergens in allergen-specific immunotherapy (ASIT). This retrospective study analyzed IDT reactivity in atopic dogs. Medical records were reviewed for all atopic dogs undergoing IDT over a 3 year period. In addition to the standard IDT concentration of pollen allergens (1000 PNU/ml), five pollen allergens (ragweed mix [*Ambrosia trifida, Ambrosia artemisiifolia*], cocklebur [*Xanthium strumarium*], sheep sorrel/yellow dock mix [*Rumix acetosella, Rumex crispus*], pigweed mix [*Amaranthus hybridus, Amaranthus palmeri, Amaranthus retroflexus*], Kentucky blue/junegrass [*Poa pratensis*]) were duplicated at published 0% irritant threshold concentrations (3000-8000 PNU/ml). Sixty-seven IDT results were analyzed. Subjective scores for the standard test concentration of each allergen were compared to the scores for the higher concentration. Positive reactions occurred much more commonly with the higher concentration for each of the paired allergens ($P < 0.05$, Chi-square). Importantly many of the reactions (37% to 57%) changed from negative to strongly positive, which resulted in those allergens being included in ASIT. The currently recommended standard test concentration of 1000 PNU/ml is likely too low for these pollen allergens. Lack of inclusion of allergens in ASIT based on false-negative results could lead to poor treatment response. Analysis of optimal test concentrations for all pollen allergen extracts in atopic dogs is warranted.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Consistency monitoring of *Dermatophagoides farinae* allergenic extracts

B. MCKINNEY*, K. BLANKENSHIP*, and R. LANKOW*

**Stallergenes Greer, Lenoir, North Carolina, USA*

Abstract: An enzyme-linked immunosorbent assay (ELISA) inhibition procedure, analogous to the procedures currently used for standardizing mite extracts intended for human use, has been adapted and validated for defining lot release potency of *Dermatophagoides farinae* extracts intended for veterinary use. A dog sera pool that is specifically reactive to the allergenic components present in *D. farinae* extracts adequate for use in an inhibition ELISA has been prepared and characterized. A reference *D. farinae* extract used in the inhibition ELISA has been defined and characterized. When reconstituted to 2 mG/mL, this reference is defined to contain 600,000 IgE reactivity units when evaluated using the dog inhibition ELISA. The relative potency of all production lots of *D. farinae* intended for use in veterinary allergy testing and immunotherapy produced over the past four years has been independently evaluated. The protein content of these extracts has averaged 323 µG/mL (range 264 – 416 µG/mL) while the average IgE reactivity has been calculated to be 105,200 specific IgE reactivity units/mL (range-74,400 – 158,400). The 95% confidence range (mean ± 1.96 SD) was calculated to be 51,577 – 158,823. The information derived from this consistency monitoring program can be used as the foundation for "standardization of veterinary mite extracts program" analogous to the standardization program for extracts intended for human use. Until such time as absolute potency units are defined and characterized, the arbitrary relative potency unit based on IgE specific reactivity anchored to the original reference *D. farinae* extract will provide the basis of standardized units.

Sources of funding: Funding for this study was provided by Stallergenes Greer

Conflict of Interest: All authors are employees at Stallergenes Greer

Intra- and inter-assay variability of serum tests for environmental allergen-specific IgE from different laboratories

K. BAUMANN*, N. GEDON*, R.S. MUELLER*

**Centre for Clinical Veterinary Medicine, Faculty of Veterinary Medicine, LMU University, Munich, Germany*

Abstract: Serum testing for allergen-specific IgE is commonly employed to identify allergens used for desensitization, but reliability of results has been a matter of debate. The aim of this study was to evaluate the reproducibility of serum tests for environmental allergens in three different European laboratories. Serum was obtained from 28 client-owned dogs diagnosed with allergies to environmental allergens and then divided into three aliquots and sent to the laboratories under three different names. Two aliquots were sent simultaneously to one of the laboratories on the first day, the third sample was then sent to the same laboratory the subsequent day. The laboratory for each patient was chosen according to a predetermined randomization list. The agreement between different samples from the same dog for each of the laboratories was calculated by using the Cohen's Kappa test. Spearman's rank coefficients (r_s) as well as the coefficients of variation (CV) were additionally assessed. The intra- and inter-assay agreements for laboratory 1 were 0.97 and 0.93, the CVs 14.2% and 17.0% and r_s 0.96 and 0.95, respectively. For laboratory 2, the intra- and inter-assay agreements were 0.93 and 0.88, the CVs 14.6% and 17.5% and r_s 0.81 and 0.77, respectively. Finally, the intra- and inter-assay agreements for the third laboratory (3) were 0.77 and 0.76, the CVs were 19.6% and 23%, and the rs 0.72 and 0.68, respectively. These differences complicate test interpretation and underline the importance of interpreting results of serum testing for allergen-specific IgE in the context of the patient's clinical history.

Sources of funding: German Society for Veterinary Dermatology.

Conflict of Interest: None declared

Agreement of serum allergy test results with unblocked and blocked immunoglobulin E against cross-reactive carbohydrate determinants (CCD) and intradermal test results in atopic dogs

N.K.Y. GEDON*, T.M.S.A. BOEHM*, C.J. KLINGER*, L. UDRAITE VOVK*, R.S. MUELLER*

**Centre for Clinical Veterinary Medicine, Ludwig-Maximilian-University, Munich, Germany*

Abstract: Tests for allergen-specific immunoglobulin (Ig) E are used to select allergens for immunotherapy in atopic dogs. Recently antibodies against cross-reactive carbohydrate determinants (anti-CCD IgE) were identified in serum samples of atopic dogs. Their presence in humans is a known cause of clinically irrelevant polysensitization. This study aimed to compare the results of an intradermal test (IDT) and a serum test for allergen-specific IgE with and without inhibited anti-CCD IgE. Thirty-one privately owned dogs with atopic dermatitis prospectively underwent intradermal allergy testing and had their serum samples analyzed for anti-CCD IgE. An Fc-ε receptor-based serum test for allergen-specific IgE was performed with and without blocking anti-CCD IgE. The agreement between the different tests was analysed with Cohen's Kappa. In dogs with negative anti-CCD IgE samples, the agreement between the results of the serum test and the IDT was substantial ($\kappa = 0.71$). Dogs with positive anti-CCD IgE samples (38.7%) showed no agreement between serum and intradermal testing ($\kappa = -0.35$); blocking anti-CCD IgE in these samples resulted in a fair agreement ($\kappa = 0.43$). Anti-CCD IgE positive sera had multiple positive results for grass and weed allergens; blocking decreased these markedly. These results indicated that intradermal testing correlated best with serum testing in dogs with no detectable anti-CCD IgE. Sera containing anti-CCD IgE had multiple positive reactions on serum testing and no agreement with IDT. This was improved by blocking anti-CCD IgE. Apparent serum test polysensitization to grass and weed allergens was caused by anti-CCD IgE.

Sources of funding: HESKA conducted the serum testing for allergen-specific IgE free of charge. Other aspects of this project were self-funded.

Conflict of Interest: None declared.

Discovering the pitfall of using horseradish peroxidase in enzyme-linked immunosorbent assays for detection of pollen specific IgE

K.W. LEE* AND R. E. ESCH†

**Stallergenes Greer, Lenoir, NC, USA*
†School of Natural Sciences, Lenoir-Rhyne University, Hickory NC, USA

Abstract: Enzyme-linked immunosorbent assays incorporate a functional enzyme conjugate in at least one procedural step: horseradish peroxidase (HRP) and alkaline phosphatase (AP) are the most commonly used. Recent evaluations in our laboratory have shown that some sera samples will react with a streptavidin-HRP (SA-HRP) conjugated secondary tracer even in the absence of a biotinylated primary tracer. A screen of 1008 randomly selected samples, identified 128 samples that reacted in a classically expected profile (CEP) of positive responses when evaluated with an anti-IgE-biotin primary tracer followed by SA-HRP as secondary tracer, and negative responses when tested without anti-IgE-biotin. An additional 96 reactive samples yielded a non-classical response (NCR) profile of equal magnitude with or without the biotin conjugated tracer. Adsorption of IgE from sera pools prepared from CEP samples and heat inactivation of IgE reactivity in these pools readily reduced the signal evident in untreated pools. Similar treatment of NCR pools had no effect on the signal generated. Inhibition evaluations using unconjugated biotin or streptavidin indicates that neither is involved in the aberrant reaction. However, inhibition evaluations with unconjugated HRP eliminates reactivity in the CEP pool and that the reactivity evident in the NCR pool is unaffected by free HRP inhibition. These results are consistent with the hypothesis that IgG specific for an epitope on glycoproteins present in the allergen extracts cross reacts with a carbohydrate component of HRP and binds the secondary tracer molecule without interfering with its enzymatic reactivity; consequently, false positive interpretation for pollen allergens is the outcome.

Sources of funding: Funding for this study was provided by Stallergenes Greer.

Conflict of Interest: Authors are employees at Stallergenes Greer.

Detection of anti-desmocollin-1 and anti-desmoglein-1 autoantibodies in dogs with pemphigus foliaceus with or without the classic facial involvement

P. BIZIKOVA*, L. MAMO*

**North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA*

Abstract: While diagnosis of pemphigus foliaceus (PF) in dogs with classic nasal planum/muzzle involvement is relatively easy to make, cases with trunk-predominant lesion distribution without the nasal planum/muzzle involvement ("atypical" PF) present a diagnostic challenge, and a lengthy diagnostic work-up is often required to differentiate these cases from resembling diseases such as superficial pyoderma. It remains unknown, whether an anti-DSC1 or anti-DSG1 autoantibody assay could assist in the diagnostic approach. Therefore, the goal of the study was to determine the prevalence of anti-DSC1 and/or anti-DSG1 IgG in dogs with classic and atypical PF. Sera from dogs with classic (25) and atypical (31) PF were tested by indirect immunofluorescence for anti-DSC1 and anti-DSG1 IgG using DSC1- and DSG1-transfected 293T cells. Sera from healthy dogs (30), dogs with superficial pyoderma (exfoliative superficial pyoderma (22), impetigo (11)), atopic dermatitis (25) and vector-borne diseases (25) served as controls. Anti-DSC1 IgG were detected in 100% and 53% of sera from dogs with classic and atypical PF, respectively. Anti-DSG1 IgG were detected only in two atypical PF sera (7%) only, which were also anti-DSC1 IgG positive. Anti-DSC1 and anti-DSG1 IgG were not detected in any control sera. Anti-DSC1 IgG immunofluorescence assay, due to its high sensitivity and specificity in dogs with classic PF, presents a reliable diagnostic test for this clinical subset. In atypical PF, this assay is useful because it has a high positive predictive value; however, due to the lower sensitivity, a negative result does not exclude a diagnosis of atypical PF.

Sources of funding: Source of funding: ACVD/AAVD grant.

Conflict of Interest: None declared

Pruritus and dermatitis scoring in flea-infested dogs before and after administration of flea adulticides

M.S. CANFIELD*, M.W. DRYDEN‡, B. H. HERRIN‡, A.J. RUMSCHLAG†, W. G. RYAN§

**Animal Dermatology South, 7741 Congress Str, New Port Richey, FL 34653, USA*
‡Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS 66506, USA
†Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140, USA
§Ryan Mitchell Associates LLC, 16 Stoneleigh Park, Westfield, NJ 07090, USA

Abstract: Data from studies of flea-infested dogs were examined for relationships between flea counts, owner-scoring using the canine pruritus severity scale (CPSS), and dermatologic changes following treatments. In Study 1, dogs received spinosad (n=65) or fipronil/(S)-methoprene (n=63); Study 2, spinosad (n=36) or lotilaner (n=33). In both studies the correlation of CPSS score with flea counts was assessed. In Study 2 the relationships between each CPSS score, scoring of flea allergy dermatitis (FAD) and of canine atopic dermatitis extent and severity index (CADESI)-4 were examined. There was little to no correlation in either study between CPSS score and flea counts. In Study 1, CPSS score reductions were greater in spinosad than in fipronil/(S)-methoprene treated dogs (P <0.0001). In Study 2, there were significant reductions in CPSS, CADESI-4 and FAD scores in spinosad and lotilaner treated dogs (P <0.0001), with strong correlations (coefficient 0.8642; P <0.0001) between CADESI-4 and FAD scores at baseline and at week 4 (0.8155; P <0.0001), declining at week 8 (0.5999; P <0.0001) when flea counts were zero, but only a moderate overall correlation between CPSS and CADESI-4 (0.5442; P <0.0001) and FAD (0.5989; P <0.0001) scores. Although the severity of CPSS scores in flea-infested dogs is not dependent on infestation numbers, effective flea treatment can lower dermatologic scores and help reduce pruritic stimuli to sub-threshold levels. CADESI-4 scoring is appropriate for assessing dermatologic improvement following elimination of fleas; FAD scoring could be utilized where rapid flea allergy assessment is necessary. Further investigations of these relationships are recommended.

Sources of funding: Studies were funded by Elanco Animal Health.

Conflict of Interest: MC has provided lectures sponsored by Elanco; MWD has had research projects funded at Kansas State University and lectures sponsored by Elanco Animal Health. BHH has had projects and lectures funded by Elanco. AJR is an employee of Elanco Animal Health; WGR has acted as a paid consultant for Elanco. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

Efficacy of a 0.02% topical hypochlorous acid containing solution as a sole therapeutic intervention for canine superficial pyoderma: a randomized, blinded, controlled pilot investigation

D.J. BERGER*, J.R. PETERSEN†, A.K. VIALL‡, J.O. NOXON*

** Department of Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA*
† Lakeshore Veterinary Specialists, WI, USA
‡ Department of Veterinary Pathology, Iowa State University, Ames, IA, USA

Abstract: Canine superficial pyoderma associated with meticillin-resistant *Staphylococcus* species is occurring more frequently, resulting in increased efforts to find alternative treatment options. Hypochlorous acid (HOCl) is a reactive oxygen species with demonstrated *in vitro* antimicrobial activity. The purpose of this study was to evaluate the clinical efficacy of a 0.02% HOCl containing solution as a sole therapeutic intervention to determine if further large-scale comparative studies are warranted. Eighteen dogs with a clinical diagnosis of mild superficial pyoderma and *Staphylococcus* spp. recovered on bacterial culture were enrolled and randomized to one of two treatment groups; (i) 0.02% HOCl spray applied three times daily (12/18) or (ii) a previously described topical regimen utilizing 4% chlorhexidine containing products (6/18). Dogs were evaluated by a blinded investigator and their owner on days 0, 14, and 28 for percent body surface affected, clinical score (0-4 severity scale for five parameters), cytology score (0-4 severity scale), and pruritus level via a visual analog scale. Dogs treated with the chlorhexidine protocol showed statistically significant improvement in all measured parameters by day 28 with all six dogs being considered clinically resolved. Dogs treated with the HOCl protocol showed no statistical improvement in any of the measured parameters with only 5/12 dogs completing the 28-day study. Comparisons between the two groups revealed statistically significant differences for all measured variables at days 14 and 28. This pilot investigation suggests the HOCl product applied three times daily is not clinically effective in treating superficial pyoderma when used as a sole therapeutic intervention.

Sources of funding: Veticus, LLC and the Iowa State University Center for Industrial Research and Service.

Conflict of Interest: D.J. Berger has lectured for Vetbiotek. J.O. Noxon has lectured and consulted for Bayer Animal Health.

Low-level laser therapy as an adjunctive treatment for canine acral lick dermatitis: a randomized, double-blinded, sham-controlled study

A. H. SCHNEDEKER*, L. K. COLE†, S. F. DIAZ†, G. LORCH†, P. J. RAJALA-SCHULTZ‡, R. N. JENNINGS†, E. T. HOSTNIK†

**VCA SouthPaws Veterinary Specialists & Emergency, Fairfax, VA, USA*
†Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA
‡ Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland

Abstract: Conventional therapy for canine acral lick dermatitis (ALD) consists of antibiotics and anti-anxiety medications. Low-level laser therapy (LLLT) is a non-invasive therapy used to treat inflammatory and painful conditions. The primary objective was to determine whether LLLT combined with conventional therapy would result in a greater decrease in licking of ALD lesions than conventional therapy alone. We hypothesized that LLLT with conventional therapy would result in >50% reduction in licking visual analog score (LVAS) than conventional therapy alone. Secondary objectives were to assess change in lesion/ulcer size, thickness and hair growth. Dogs were randomly assigned to two groups. All dogs received systemic antibiotics and trazodone. Treatment group (TG) received LLLT (130mW, 2 min) with blue and red light-emitting diodes, while control group (CG) had sham therapy (laser off). Treatments were three times weekly for 2 weeks, then twice-weekly for 2 weeks for a total of 10 visits. Descriptive statistics were performed (mean, median); primary and secondary objectives were assessed with non-parametric ANOVA (Kruskal-Wallis test). Thirteen dogs (CG=six; TG=seven) were enrolled. There were no significant differences in median LVAS, lesion/ulcer size, or thickness of the ALD lesion between TG and CG. There was a significant increase in hair growth in TG ($P = 0.0081$) compared to CG and the median increase in hair growth was 24% greater in TG versus CG. Treatment of ALD requires multimodal therapy. Although combining LLLT with conventional therapy did not result in >50% reduction in LVAS, there was a significant increase in hair growth.

Sources of funding: American Holistic Veterinary Medical Foundation, Ohio State University College of Veterinary Medicine Intramural Canine Research Fund and CTSA Grant number (UL1TR001070).

Conflict of Interest: None declared

Topical 0.5% timolol maleate for the treatment of incipient cutaneous haemangiomas in dogs: a pilot study

A. M. CORDERO*, C. LOPEZ-MARQUEZ*, G. SHEINBERG†, C. ROMEROS§

§Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de México, Amecameca de Juárez, Estado de México, México
†Centro Veterinario México, Departamento de dermatología, Ciudad de México, México
** VETDERM: Dermatologia Veterinaria Especializada, Guadalajara, Jalisco, Mexico*
† VETDERM: Dermatologia Veterinaria Especializada, Guadalajara, Jalisco, Mexico

Abstract: Haemangiomas (angiomas) are uncommon (in dogs) to rare (in cats) benign neoplasms arising from the endothelial cells of blood vessels. Research and clinical observation suggest that chronic solar damage may be the cause of haemangiomas and haemangiosarcomas. Dogs with short hair coats and lightly pigmented skin appear to be predisposed to develop dermal haemangiomas or haemangiosarcomas, especially on the ventrum. Infantile haemangiomas are the most common vascular tumours among children, occurring in 3% to 10% of infants. Topical 0.5% timolol maleate, an unselective beta blocker, has been successfully used in the treatment of infantile haemangiomas in humans. However, no studies treating canine haemangiomas with timolol maleate have been published. Fourteen haemangioma lesions in four patients were treated topically with 0.5% timolol maleate twice daily. Lesion size was evaluated on days 0, 14, 28, 42, 56. Lesion size was significantly reduced by day 14 ($P = 0.03$), however on the day 28 visit no difference was observed ($P = 0.19$) compared to day 14. On days 42 and 56, a marked reduction of lesion size was noted when compared to day 0 ($P = 0.04$). Twice daily 0.5% timolol maleate applied topically resulted in a significant reduction of haemangioma size over a 2 month period of time. Topical therapy with 0.5% timolol maleate could be a beneficial and inexpensive treatment for shrinking haemangiomas prior to surgery and for eliminating incipient haemangiomas. Longer studies with a larger number of dogs are required to evaluate further success of therapy and possible systemic effects.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Meticillin-resistant coagulase-negative staphylococcal pyoderma in a captive African lion (*Panthera leo*)

D.J. BERGERr*, J.O. NOXON*, J.S. HAYNES†, D.L. ARMSTRONG‡, J. NAPIER‡

**Department of Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA*
†Department of Veterinary Pathology, Iowa State University, Ames, IA, USA
‡Henry Doorly Zoo and Aquarium, Omaha, NE, USA

Abstract: The incidence of meticillin-resistant infections in veterinary medicine is increasing, not only in traditional companion animals. *Staphyloccus felis* is a coagulase-negative species that is a common commensal of the skin in cats. An 11-year-old, 180 kg female African lion (*Panthera leo*) was presented for non-resolving skin lesions of 5 months' duration. The lion had a history of recurrent skin lesions that had responded to prior systemic therapy with cefpodoxime. Dermatologic exam revealed multiple circular, hyperpigmented, alopecic patches with erosions, crusts, or occasional punctate draining lesions predominantly along the flanks, abdomen, inguinal region and rear legs. Skin scrapings and trichograms revealed no parasites or fungal agents. Impression smears from exudative lesions revealed neutrophilic inflammation with intracellular coccoid-shaped bacteria that stained Gram-positive. Multiple 6 mm biopsy specimens were acquired for histopathology along with bacterial and fungal tissue culture. Histopathology revealed a mastocytic, eosinophilic, pleocellular perivascular dermatitis with dermal edema, serocellular crust formation and the presence of bacterial colonies. Culture results yielded heavy growth of a meticillin-resistant *Staphylococcus felis* while fungal cultures did not reveal growth of pathogenic species. The patient was prescribed compounded marbofloxacin capsules (Wedgewood Pharmacy) at a dose of 5 mg/kg orally once daily. Treatment was continued for a total of 8 weeks, which included medication administration for 2 weeks past clinical resolution. Following discontinuation of therapy the patient has remained symptom free for 6 months. This case report represents the first documented and successfully treated meticillin-resistant staphylococcal associated pyoderma in a captive lion.

Sources of funding: Self-funded.

Conflict of Interest: None declared

Long term use of L-phenylalanine in combination with oral fatty acids and topical sunscreen in the treatment of vitiligo associated with carcinoma in situ involving the planum nasale in a dog

D. DI MATTIA*, F. DE BELLIS†, P. ROCCABIANCA ‡

** Praxis Veterinary Clinic, Via Nazionale 68, 83013, Avellino, Italy*
† London Vet Specialists 56 Belsize Ln, London NW3 5AR
‡ Department of Pathology, Hygiene and Veterinary Public Health, Università degli studi di Milano, Via Celoria 10, Milan, 20133, Italy

Abstract: Vitiligo is a rare acquired disorder in animals mostly seen in dogs and horses. In humans, numerous therapies have been used, including L-phenylalanine with phototherapy with seemingly good results. Although in veterinary dermatology L-phenylalanine has been reported to be effective in the treatment of vitiligo, long-term studies are lacking. A 3.6-year-old, intact male mixed breed dog presented for progressive depigmentation of the planum nasale associated with moderate crusting without overt architectural damage, except for a small region close to the nasal philtrum. Complete blood count and serum biochemistry findings were unremarkable. Serological titer for *Leishmania infantum* was negative. Histopathological evaluation showed a marked reduction to complete absence of epidermal melanocytes and melanin, pigmentary incontinence, mild perivascular infiltrations of lymphocytes and melanophages and severe epidermal dysplasia. The almost complete loss of epidermal melanin despite the mild dermal inflammation favoured a diagnosis of vitiligo associated with carcinoma in situ, the latter potentially due to pigment loss and chronic solar exposure. Treatment was initiated with oral L-phenylalanine (Fenilalanina 500) 50 mg/kg once daily, oral polyunsaturated fatty acids and topical sunscreen. Photodynamic therapy was declined by the owner. Approximately 6 months later, the dog showed a marked improvement of the nasal planum depigmentation and the L-phenylalanine dose was reduced to every other day. Over the next year remission was almost complete although a tiny area of hypopigmentation associated with an ulcer spanning the nasal philtrum remained. Larger studies are needed to investigate the use of L-phenylalanine for canine vitiligo.

Sources of funding: Self-funded.

Conflict of Interest: None declared

PATHOGENESIS OF CANINE ATOPIC DERMATITIS: SELECTED TOPICS

Olivry T[1,2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, and
[2]Comparative Medicine Institute, North Carolina State University, Raleigh, NC, USA

The pathogenesis of canine atopic dermatitis (AD) is complex and, like that of its human homologue, it involves an inflammatory cascade that is likely initiated, in most patients, by allergens recognized by IgE. In this lecture, I will cover several topics relevant to the pathogenesis of canine AD. We will begin with a section on the characterization of the skin barrier defects that exist in this disease, starting with the concept of transepidermal water loss before describing the existing changes in the complex stratum corneum lipids (e.g., ceramides) and in upper epidermal proteins (filaggrin, claudin-1, corneodesmosin). The available evidence will be presented to suggest that epidermal barrier defects likely occur secondary to—and not before—skin inflammation. Our second topic will be the description of the cellular inflammation present in the skin of dogs with AD, one that mainly involves lymphocytes, dendritic cells and eosinophils. We will then review the role of IgE in canine AD, and will describe how such immunoglobulins are likely important for the capture of allergens and the initiation of the inflammatory cascade. From IgE, we will move onto mast cells and the importance of histamine, which is likely produced in the early phase of inflammation. We will finish with the review of cytokines expressed in the skin of dogs with natural AD, and we will introduce the currently proposed atopic cytokine cascade, which was characterized after allergen challenge in experimentally-sensitized atopic dogs. We will conclude this lecture with a brief synopsis on the similarity of the human and canine AD transcriptomes and the identification of the four main targets for canine AD management: the skin barrier, the inflammation and the associated pruritus as well as the surface microflora.

Note: a pdf-formatted copy of the slides of this presentation will be available from the meeting time till the end of May 2019 at the following location: https://www.dropbox.com/sh/qkwzbnvaj7huaew/AAACKYcg3PL4zPX8vLI7kX8Wa?dl=0

CANINE ATOPIC ITCH: FROM PATHOGENESIS TO TARGETED THERAPY

Olivry T[1,2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, and
[2]Comparative Medicine Institute, North Carolina State University, Raleigh, NC, USA

Itch/pruritus is the most important clinical signs of atopic dematitis (AD) in dogs—as it is in humans—as it profoundly impacts the quality of life of affected patients. In this lecture, we will first review the current knowledge in the neuroanatomy of itch. We will begin with the network of intraepidermal, predominantly unmyelinated C-type, itch-specific sensory neurons whose cell bodies are in the dorsal root (DRG) and trigeminal ganglia. Histamine activates histaminergic neurons expressing the H1R and H4R receptors to induce acute itch. In contrast, non-histaminergic neurons are activated by other mediators (proteases, cytokines/chemokines, amines…) that bind to their respective receptors and are believed to be involved in chronic itch. Neuronal receptors will lead, via different signal transduction intermediates, to the activation and opening of the neuronal transient receptor potential (TRP) channels TRPV1 and/or TRPA1. The propagation of the neuronal depolarization involves the activation of (at least) voltage-gated sodium channels, especially Nav1.7 and perhaps Nav1.9. The itch signal is transmitted from the DRG neurons to at least two dorsal horn spinal cord interneurons, first via the B-natriuretic peptide (BNP) and then the gastrin-releasing peptide (GRP) and their respective receptors NPRA/NPR1 and GRPR. Inhibitory interneurons expressing Bhlhb5 can release GABA, glycine and the kappa-opioid dynorphin to interfere with the propagation of the itch signal in GRPR expressing neurons. In contrast astrocytes can potentiate the itch transmission. Eventually, the itch signal is transferred to the thalamus via propagation neurons in the spino-thalamic tract and to the parabrachial nucleus via the spinoparabrachial pathway before being projected to other areas to induce itch, the main one being the primary and secondary somatosensory cortex. In the next section, we will then review the current knowledge existing on some of the itch-provoking mediators (pruritogens) shown to be released in the skin of dogs with AD and whether or not their therapeutic targeting results in itch improvement in dogs with spontaneous or experimental AD. Among the myriad of pruritogens released in canine AD skin, we will focus on histamine, IL-31, nerve growth factor (NGF) neuromedin B (NMB) and periostin. We will also review several AD-relevant neuronal receptors (TRPM8, MRGPRX2 and the GABAA) whose therapeutic targeting could be clinically beneficial.

The forthcoming years will provide us with many different options to treat the itch that affects our atopic dogs, starting with interventions targeting pruritogens or their receptors on DRG neurons. Other thera-peutic strategies will aim at inhibiting the transmission of the itch signal in the spinal cord interneuronal synapses or by activating or mimicking the action of inhibitory interneurons.

Note: a pdf-formatted copy of the slides of this presentation will be available from the meeting time till the end of May 2019 at the following location: https://www.dropbox.com/sh/rwj1p46zpgzlvok/AADDKgBJ8hKFP9466Ulihdgpa?dl=0

Cutaneous lupus: diagnosis and management

Hilary A Jackson ARPS BVM&S DVD DipACVD DipECVD MRCVS

Dermatology Referral Service, Glasgow, Scotland

Definition of CLE

The diagnosis of canine cutaneous lupus is dependent on the breed, age, signalment and presenting clinical signs. Autoimmune diseases tend to present with symmetry of lesions. Confirmation of the diagnosis relies on cutaneous histopathology which is quantitatively similar in each form of CLE and consists of an interface dermatitis with a mononuclear cell infiltrate effacing the dermo-epidermal junction with single cell apoptosis and satellitosis in the basal layers of the epidermis. Auto-antibody profiles can be useful in the diagnosis of lupus in man and in many cases can be helpful in determining the prognosis. Specific auto-antibody profiles however have not been widely investigated in companion animals and are not routinely available as a diagnostic option. The following discussion details the forms of cutaneous lupus which have been recognised in companion animals.

Vesicular cutaneous lupus erythematosus (VCLE)

Vesicular cutaneous lupus was described in the late 1960s but characterised as a form of CLE in 2001 (Jackson and Olivry, 2001). It is a rare disease affecting Shetland sheepdogs, rough collies, border collies and their crosses. It typically affects adult dogs and in In eight of 11 (73%) dogs with VCLE, clinical signs were reported to first arise in the summer with three cases which were followed relapsing in the following summer (Jackson, 2004). Serpigenous ulceration occurs on the ventral abdomen, axillae, groin and concave aspect of the pinnae. Ulceration at mucocutaneous junctions is also noted. Secondary bacterial colonisation of the ulcerated skin is common. The disease is not typically associated with any other organ dysfunction although affected dogs may be febrile, lethargic and due to the ulceration in considerable pain.

In VCLE the histopathology is typically a lymphocyte rich interface dermatitis with prominent basal cell apoptosis. Clefting at the basement membrane can occur.

Immunophenotyping performed in 11 affected dogs showed a CD3 positive T cell infiltrate at the dermo-epidermal junction. Further characterisation of this infiltrate was performed in 2 dogs only and showed the T cells to be predominantly CD8 and Langerhans cells also to be present. A lupus band (IgG at the basement membrane) was detected in 50% of the dogs. Where sera was available dogs were tested for the presence of circulating antibodies directed at extractable nuclear antigens (ENAs). Autoantibodies were detected in 9/11 cases with the most common antigens detected being Ro/SSA (45% of dogs), La/SSB (45%) and Sm/RNP (45%) (Jackson et al., 2004).

Management

- Affected dogs should be given suitable pain relief.
- Secondary infections should be managed with topical anti-microbials and in severe cases systemic antibiotics
- Since many cases are exacerbated by UV light sun exposure should be limited

The initial case series reported the outcome of treatment in 11 dogs but it should be noted that this predated the advent of licensed ciclosporin for dogs(Jackson, 2004). Six of these dogs required glucocorticoids in combination with other immunosuppressive agents to control the disease and three were euthanased directly as a result of the disease or side effects of therapy. More recent reports suggest that the addition of ciclosporin and/or topical tacrolimus with glucocorticoids for initial treatment has superior results and that long term dogs may be maintained on ciclosporin alone (Banovic et al., 2017).

Exfoliative cutaneous lupus erythematosus

This is a rare disease reported in German shorthaired pointers (GSPs) and Magyar viszlas. A pedigree analysis of GSPs point to an autosomal recessive inheritance associated with a single nucleotide polymorphism on CFA chromosome 18 (Wang et al., 2011). This disease typically has an early age of onset < 12 months and is characterised by generalised scaling, and alopecia. Follicular casting may be present. Less commonly crusting and ulceration can occur and mild pruritus is present in some dogs(Bryden et al., 2005). Granulomatous sebaceous adenitis is an important rule out in this breed. Many dogs have a stiff gait and arched back, infertility and peripheral lymphadenopathy. A fluctuating

thrombocytopaenia is also seen(Mauldin et al., 2010).

Histopathology demonstrates a lymphocyte rich interface dermatitis which also involves the proximal follicular infundibulum. Sebaceous glands may be absent and lymphocyte inflammation around the apocrine glands may be present in around 50% cases. Immunoglobulin deposition (IgG, IgM) at the epidermal and follicular basement membrane can be demonstrated (Bryden et al., 2005). Various immune-modulating agents have been used alone or in combination to treat this disease. A recent review of the longterm outcome concluded that over 50% dogs are euthanased due to a poor response to therapy (Olivry et al., 2018)

Discoid lupus erythematosus

This variant can present localised to the face or in a more generalised condition. A recent review of four publications of facial DLE identified 104 dogs, 31% were GSD or their crosses (Olivry et al., 2018). The more generalised form is less common and thus smaller numbers of dogs have been reported. German Shepherd dogs do not appear to be predisposed.

Lesions in the dog are characterised by erythematous macules and scaling, progressing to depigmentation, atrophy and scarring of affected skin. Facial DLE typically affects the nasal planum and sometimes other facial mucocutaneous membranes. The haired skin of the muzzle may also be affected. In the generalised form discoid (annular) plaques progress through the same cycle.

Histologically this is a cell rich interface dermatitis with keratinocyte vacuolation, basal cell apoptosis and thickening of the basement membrane. In the generalised form the interface reaction extends into the follicular epithelium as far as the isthmus. In cases of facial and generalised DLE a positive "lupus band" i.e immunoglobulins at the basement membrane, can be demonstrated in the majority of cases.

Management

Facial DLE has been reported to respond to the combination of a tetracycline antibiotic and nicotinamide at supra-physiological doses. Whilst this is a safe and well tolerated form of treatment the use of chronic antibiosis is to be discouraged in respect of responsible antibiotic stewardship. Topical tacrolimus 0.03% applied twice daily has been shown to be an effective treatment in dogs. The same treatment at 0.1% in combination with oral steroids is also effective (Griffies et al., 2004). There are smaller number of dogs reported with generalised DLE and a wide range of systemic treatments has been used. Chronic treatment may be required however as lesions recur with tapering doses.

Mucocutaneous lupus erythematosus (MCLE)

Mucocutaneous lupus erythematosus affects the mucocutaneous membranes. It manifests in adult dogs, and of the published cases (n=36 dogs) 50% were German Shepherd dogs represented with a female predominance. Lesions are typically seen around the peri-anal, genital, lips peri-ocular, peri-nasal and peri-oral skin. Facially oriented lesions are reportedly less common(Olivry et al., 2018). Erosions and ulcerations at these body site readily become colonised with bacteria. A major differential diagnosis is mucocutaneous pyoderma which can have a very similar clinical presentation. In the case of mucocutaneous pyoderma the lesions should completely resolve with topical/systemic antibiosis whereas in MCLE only partial resolution will occur. The role of bacterial antigens in the development of MCLE has not been determined.

Histopathology is of a lymphocyte rich interface dermatitis. Suprabasilar apoptosis is reported and differentiation between MCLE and erythema multiforme should be made.

A positive lupus band test is reported in cases where this has been tested (Olivry et al., 2015).

Affected dogs appear to respond best to immunosuppressive doses of glucocorticoids. The addition of nicotinamide with or without tetracyclines may have a beneficial steroid sparing affect.

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus is a chronic relapsing multi-organ disease. The skin may be involved in approximately 60% of cases. Clinical signs are diverse and affect many organs although not necessarily at the same time. Major signs include fever, polyarthritis, renal, haematological and cutaneous involvement. Cutaneous lesions can present as alopecia, scaling, ulceration or erosions.

Where cutaneous lesions are present interface dermatitis may be demonstrated on biopsy material taken from affected areas. However, it is also possible that cutaneous lesions may arise from a lupus induced vasculitis or cryoglobulinaemias.

More than one organ system should be involved and a positive ANA demonstrated. However, a positive ANA is not specific for SLE and has been commonly measured in other inflammatory diseases. ANA titres generally fall with disease improvement.

This disease can typically wax and wane, thus response to therapy may be difficult to determine.

Feline cutaneous lupus

Cutaneous lupus is a rare disease in the cat. Exact analogues of the canine diseases are not reported. Five cats with localised to generalised scaling and an histologically, an interface dermatitis have been reported as DLE or CLE; (Medleau, 1990) Wilhelm et al., 2005, Day et al., 1993)

A major rule out for such cases would be thymoma associated exfoliative skin disease. It should be noted that some cats are reported in which a thymoma cannot be identified (Linek et al., 2015)

References

BANOVIC, F., ROBSON, D., LINEK, M. & OLIVRY, T. 2017. Therapeutic effectiveness of calcineurin inhibitors in canine vesicular cutaneous lupus erythematosus. *Veterinary Dermatology*, 28.

BRYDEN, S., OLIVRY, T., WHITE, S., DUNSTON, S. & BURROWS, A. 2005. Clinical,histopathological and immunological characteristics of exfoliative cutaneous lupus erythematosus in 25 German shorthaired pointers. *Veterinary Dermatology*, 16, 239-252.

DAY, M., HANLON, L. & POWELL, L. 1993. Immune mediated skin disease in the cat. *Journal of Comparative Pathology*, 109, 395-407.

GRIFFIES, J., MENDELSOHN, C., ROSENKRANTZ, W., MUSE, R., BOORD, M. & GRIFFIN, C. 2004. Topical 0.1% tacrolimus for the treatment of discoid lupus erythematosus and pemphigus erythematosus in dogs. *Journal of the American Animal Hospital Association*, 40, 29-41.

JACKSON, H., OLIVRY, T., BERGET, F., DUNSTON, S., BONNEFONT, C. & CHABANNE, L. 2004. Immunopathology of vesicular cutaneous lupus erythematosus in the rough collie and Shetland sheepdog: a canine homologue of subacute cutaneous lupus erythematosus in humans. *Veterinary Dermatology*, 15, 230-239.

JACKSON, H. A. 2004. Eleven cases of vesicular cutaneous lupus erythematosus in Shetland sheepdogs and rough collies: clinical management and prognosis. *Veterinary Dermatology*, 15, 37-41.

JACKSON, H. A. & OLIVRY, T. 2001. Ulcerative dermatosis of the Shetland Sheepdog and Rough Collie may represent a novel vesicular variant of cutaneous lupus erythematosis. Veterinary Dermatology, 12, 19-27.

LINEK, M., RÜFENACHT, S., BRACHELENTE, C., TSCHARNER, C. V., FAVROT, C., WILHELM, S., NETT, C., MUELLER, R., MAYER, U. & WELLE, M. 2015. Nonthymoma-associated exfoliative dermatitis in 18 cats. *Veterinary Dermatology* 26, 40-45.

MAULDIN, E., MORRIS, D., BROWN, D. & CASAL, M. 2010. Exfoliative cutaneous lupus erythematosus in German shorthaired pointer dogs: disease development,progression and evaluation of three immunomodulatory drugs (ciclos porin,hydroxychloroquine, and adalimumab) in a controlled environment. *Veterinary Dermatology*, 21, 373-382.

MEDLEAU, L. 1990. Recently described feline dermatoses. *Veterinary Clinics of North America* 20, 1625-1632.

OLIVRY, T., LINDER, K. & BANOVICH, F. 2018. Cutaneous lupus erythematosus in dogs: a comprehensive review. BMC Veterinary Research, 14, 132.

OLIVRY, T., ROSSI, M., BANOVIC, F. & LINDER, K. 2015. Mucocutaneous lupus erythematosus in dogs (21 cases). . *Veterinary Dermatology* 26, 256-e55.

WANG, P., ZANGERL, B., WERNER, P., MAULDIN, E. & CASAL, M. 2011. Familial cutaneous lupus erythematosus (CLE) in the German shorthaired pointer maps to CFA18, a canine orthologue to human CLE. *Immunogenetics*, 63, 197-207.

WILHELM, S., GREST, P. & FAVROT, C. 2005. Two cases of feline exfoliative dermatitis and folliculitis with histological features of cutaneous lupus erythematosus. *TIERAERZTLICHE PRAXIS AUSGABE KLEINTIERE HEIMTIERE* 33, 364-369.

CASE DISCUSSIONS

Ozzy
4 year MN British Bulldog
Sudden onset multifocal alopecia and crusting. Mild pruritus, lethargy

Ginger
8 year MN DSH diagnosed with Pemphigus foliaceus 2 years previously.
Current treatment:
Prednisolone 10mg BID (4mg/kg)
Ciclosporin 4.2mg/kg
Also had recently received systemic enrofloxacin and Cefovecin and topical aural treatments containing: marbofloxacin, dexamethasone, clotrimazole, fusidic acid, polymyxin B, prednisolone and miconazole.
Physical examination: within normal limits
Dermatological examination: Multifocal crusting nasal planum, pustules footpads and concave pinnae, purulent otic discharge extending down neck.

Molly
6 year Labrador retriever
Presented with dysphagia, halitosis.
Meloxicam prescribed by referring vet prior to referral.

THERAPEUTICS: COMMON SIDE EFFECTS

Azathioprine
Anorexia, GI signs, pancreatitis, hepatotoxicity, bone marrow suppression

Chlorambucil
Anorexia, nausea, vomiting, bone marrow suppression

Ciclosporin
GI upset, gingival hyperplasia, hirsuitism, verrucose growths

Glucocorticoids
PU/PD, polyphagia, weight gain, retarded hair regrowth, calcinosis cutis, demodicosis, behavioural changes, epidermal atrophy

Hydroxychloroquine
Lethargy, gastrointestinal upset

Intravenous immunoglobulins
Anaphylaxis

Mycophenolate mofetil
Nausea, vomiting, diarrhoea, bone marrow suppression

Oclacitinib
Lethargy, GI signs, weight gain, bone marrow suppression, proteinuria, hypercholesterolaemia, demodicosis, histiocytomas

LIVER DIAGNOSTIC TESTS: INTERPRETATION FOR DERMATOLOGISTS

Katharine F Lunn BVMS MS PhD MRCVS DACVIM

Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

SIGNALMENT, HISTORY, PHYSICAL EXAMINATION

Liver disease can be congenital or acquired, acute or chronic, clinical or subclinical, and the causes can fall into many disease categories: infection, inflammation, neoplasia, degeneration, anatomical disorders, drugs/toxins etc. Thus signalment, history, and physical examination findings will very much depend on the underlying cause. In the context of patients with skin disease, we are likely most concerned with liver involvement in a multi-system disorder (e.g. hyperadrenocorticism [HAC]), or the effects of medical therapy on the liver (e.g. azathioprine).

DIAGNOSTIC TESTING

The diagnostic tests that are most readily available, and commonly used, to evaluate the liver are primarily the serum biochemistry profile, measurement of serum bile acids, and several imaging modalities. Coagulation testing and plasma ammonia may be evaluated when loss of liver function is suspected. The results of a complete blood count (CBC) and urinalysis may also provide support for a diagnosis of liver disease or dysfunction. Aspirates and biopsies of liver are often obtained in a more in-depth work-up of a patient with liver disease.

It is important to distinguish between liver *disease* and liver *function*, and understand how testing can evaluate these. A patient may have liver disease that does not significantly impact liver function. In these patients, there may be evidence of hepatocellular damage or cholestasis, but synthetic function may be preserved. However, if acute liver disease causes massive damage, or if chronic liver disease progresses undetected and untreated, these may significantly impact function. Evidence of loss of function may come from diagnostic tests, or from clinical signs, particularly signs of hepatic encephalopathy.

SERUM BIOCHEMISTRY PROFILE TESTS AFFECTED BY LIVER FUNCTION OR LIVER DISEASE

Alanine aminotransferase (ALT)

Previously referred to as serum SGPT (glutamate pyruvate transaminase). This is a hepatocellular (or "leakage") enzyme. It is present in the cytoplasm of hepatocytes and leaks into the extracellular space if the cell membrane is damaged. This is usually a marker of liver disease, not function. Other sources are skeletal muscle (look at CK) and red blood cells (usually not clinically very relevant)

Increased by: primary insult to the liver (e.g. infection, trauma, toxin, inflammation, neoplasia) or secondary to another physiological disturbance, such as anemia, sepsis, or hypoxemia. Increased in cats with hyperthyroidism. Because the liver receives portal blood from the gastrointestinal (GI) tract, ALT can also be increased secondary to GI or pancreatic disease.

Decreased by: significant loss of functional hepatic mass (thus low ALT could potentially be an indirect indicator of loss of function).

Interpretation: magnitude of increase is not necessarily prognostic or indicative of seriousness or reversibility of damage. Can have significant increases with non-hepatic disease (e.g. significant anemia, hypoxemia, GI disease, pancreatitis). Normal ALT does not rule out liver disease, particularly if the disease is associated with significant hepatic atrophy or fibrosis. Increased ALT should not be ignored. The half-life in dogs is 1-3 days, compared to 3-4hours in the cat. Mild increases in the absence of other abnormalities can be rechecked in 2-4 weeks, but if the increase worsens or persists, further investigation is indicated. ALT is commonly increased in patients with HAC or patients treated with exogenous glucocorticoids. In these patients, the magnitude of ALT increase is usually less than that of alkaline phosphatase (ALP). If ALT is higher than ALP in this situation, another cause should be investigated, in addition to glucocorticoid exposure.

Aspartate Aminotransferase (AST)
Previously called serum glutamate oxaloacetate transaminase (SGOT). This is also a "leakage" enzyme (found in cytoplasm and mitochondria). It is not liver specific, but also found in muscle and red blood cells, therefore it can be increased with muscle damage (look at CK) and also hemolysis. A marker of liver disease, not function.

Increased by: primary insult to the liver, as well as muscle disease, or hemolysis.

Decreased by: significant loss of functional hepatic mass (thus low AST could potentially be an indirect indicator of loss of function).

Interpretation: AST is released later than ALT, after hepatic insult, and may reflect more severe liver injury (leading to mitochondrial damage). May also resolve more quickly, due to shorter half-life (12 hours in the dog, 1.5 hours in the cat). Monitoring ALT and AST together can potentially give information about progression of liver disease, however this is not always clinically utilized. Both ALT and AST can also be increased due to regeneration of hepatocytes when the liver is recovering from an insult.

Alkaline Phosphatase (ALP)
This enzyme is located on the membrane of hepatocytes, associated with the canaliculi. Increased serum ALP is the result of increased enzyme production due to either cholestatic disease or enzyme induction by drugs or hormones. Cholestasis can be purely intrahepatic, associated with hepatocyte swelling or disrupted hepatic architecture, or it can result from obstruction at any level of the biliary system. It is important to note that cholestasis does not necessarily only result from gross biliary obstruction, it can be at the level of the canaliculi. Drugs that induce ALP include glucocorticoids and anti-convulsants. Excess of endogenous glucocorticoids also induces ALP. Corticosteroid-induced ALP (CIALP) can be measured and discriminated from cholestasis-induced ALP, but this is not diagnostically useful in patients with increased ALP. Another clinically significant source of ALP is bone. This isoenzyme is increased in young growing animals.

Increased by: cholestatic disease (at any level of the liver and biliary system), glucocorticoids, anticonvulsants, hyperthyroidism in the cat.

Decreased by: nothing significant

Interpretation: because cats have less ALP than dogs and it has a shorter half-life compared to dogs (dogs: 66 hours, cats: 6 hours), any increase in this species is significant. They also rarely have increased ALP in association with glucocorticoid therapy, or spontaneous HAC. The magnitude of increase in ALP caused by endogenous or exogenous glucocorticoids in dogs is not correlated with dose of medication or severity of illness. It is also not uncommon for ALP to remain increased after the source of glucocorticoids has been removed. Increased ALP is a common finding in dogs with HAC, however approximately 5% of dogs with HAC do not have an increase in ALP, and may only have an increase in ALT.

Gamma Glutamyl Transferase (GGT)
This enzyme is located on the membrane of hepatocytes and biliary cells. As for ALP, increased serum GGT results from enzyme induction associated with biliary stasis or glucocorticoids. In the cat, GGT is more sensitive than ALP for detection of cholestasis. Also found on mammary epithelial cells and renal tubular cells. Urine GGT can be measured and used as an indicator of renal tubular injury.

Increased by: cholestatic disease, glucocorticoids.

Decreased by: nothing significant

Interpretation: similar to ALP, however more diagnostically useful in the cat. Can be increased both in cholestatic disease and also induced by glucocorticoids, but less likely to be induced by anti-convulsants. The half-life in dogs is reported to be 3 days.

Bilirubin
This is the product of the breakdown of hemoglobin and myoglobin. The unconjugated form is transported in the blood and the conjugated form is produced by hepatocytes and excreted in the bile. Increased bilirubin is neither sensitive nor specific for liver disease, but when interpreted together with other tests it can be extremely useful.

Increased serum bilirubin is called *icterus*; visible discoloration of membranes and skin is termed *jaundice*.

Increased by: hemolytic disease (pre-hepatic icterus), decreased liver uptake or conjugation (hepatic icterus), biliary obstruction or rupture (post-hepatic icterus). Also, increased in sepsis and other conditions that lead to "paralysis" of bile flow without physical obstruction.

Decreased by: N/A

Interpretation: pre-hepatic icterus is generally easy to rule out as it will be associated with significant anemia. If hemolysis is not present, hyperbilirubinemia is very specific for liver disease, however the type of liver disease cannot be determined from evaluating other biochemistry test results. Blood tests cannot distinguish between hepatic and post-hepatic icterus. In both situations, there are likely to be increases in liver enzymes as well as increased bilirubin, and imaging is necessary to rule out post-hepatic icterus. Drugs that cause induction of ALP or GGT should not also cause increased bilirubin. Mild increased in bilirubin above the reference range, or even increases within the reference range when monitoring a patient, should not be ignored, particularly if monitoring for hepatotoxicity of medications. Measurement of conjugated and unconjugated bilirubin is rarely diagnostically helpful and is not recommended.

Blood Urea Nitrogen (BUN), Albumin, Cholesterol, and Glucose

All of these analytes are synthesized by the liver and decreased serum concentrations occur with loss of functional hepatic mass. When this is detected it typically indicates more severe acute liver disease or long duration of chronic liver disease. These analytes are sometimes referred to as "pseudofunction" tests, because they are affected by function of the liver, but all of them are also affected by many other processes, thus they are neither sensitive nor specific for liver disease. Nonetheless, monitoring of these parameters can alert the clinician to the possibility of loss of liver function before the development of icterus or other more overt clinical signs. Decreases in the pseudofunction parameters do not necessarily imply permanent loss of function or a poor prognosis, but they do warrant aggressive investigation before liver damage becomes irreversible.

BUN is affected by pre-renal, renal, and post-renal factors. Pre-renal factors include liver function, hydration and volume status, blood pressure, diet, and GI bleeding. Renal factors include causes of kidney disease (infection, inflammation, drugs, toxins etc.) and post-renal factors include urinary obstruction or rupture. Decreased serum BUN can result from decrease hepatic production of urea, but can also result from increased glomerular filtration rate, polyuria, and decreased dietary protein intake. These causes can be distinguished by evaluating urine specific gravity, serum creatinine, and dietary history.

Albumin is synthesized in the liver and can be lost through the GI tract, kidneys, or third-spacing in effusions. Renal loss and third-spacing are relatively easy to confirm. Loss through the GI tract is more difficult to prove and is often a diagnosis of exclusion. Reference ranges for albumin can be wide, but serum albumin in most healthy animals should be within or above the middle of the reference range. Monitoring of trends can be useful and prompt the clinician to perform more specific tests of liver function (see below). The only cause of increased serum albumin is dehydration.

Cholesterol is also synthesized in the liver and can be decreased with liver failure. It can also be decreased with GI disease (protein-losing enteropathy) and hypoadrenocorticism. Cholesterol can be increased in cholestatic liver disease, hypothyroidism, HAC, diabetes mellitus, and significant protein-losing nephropathy.

Glucose produced in the liver is the major source of fasting blood glucose, and hypoglycemia is regarded as a significant finding in liver failure, because it implies loss of at least 70% of liver function. There are many other differential diagnoses for hypoglycemia, including iatrogenic (administration of insulin or hypoglycemic), hypoadrenocorticism, insulinoma, other tumors, and sepsis. Causes of hyperglycemia include stress, hyperadrenocorticism, and diabetes mellitus.

As noted above, all of the "pseudofunction" tests are affected by non-hepatic disease or physiology. In a patient with loss of liver function is it therefore possible for these test results to be normal if more than one factor is at play. For example, a patient with severe liver failure could have normal serum albumin and BUN if it is also dehydrated, or cholesterol could be normal in a patient with liver failure due to cholestatic disease, because the two disease processes "cancel out". Because of these limitations, more sensitive and specific liver function tests are indicated if loss of liver function is suspected. These tests are not typically included on a standard serum biochemistry profile.

Serum Bile Acids (SBA)

There primary indication for performing SBA testing is to evaluate liver function. This is typically done for one of three reasons: (a) to evaluate a young animal for evidence of a congenital abnormality such as a porto-systemic shunt (PSS) or portal vein hypoplasia, (b) to assess liver function in a patient with known liver disease, or (c) to determine the cause of a decreased liver synthetic product such as albumin, glucose, or cholesterol or the cause of a vague clinical sign such as weight loss or anorexia. Not every patient with known or suspected liver disease is necessarily a candidate for SBA testing. In the presence of icterus, measurement of SBA does not typically provide additional information if pre-hepatic causes have been ruled out. Measurement of SBA does not differentiate between hepatic and post-hepatic icterus, and magnitude of SBA increase does not necessarily correlate with prognosis.

Serum bile acids measurement tests the ability of the liver to clear bile acids from the blood, the integrity of the biliary tract in delivering the bile acids to the GI tract, and the functional anatomy of the portal venous system. Measuring paired fasting and post-prandial SBAs improves the sensitivity and specificity of this test. There is currently controversy regarding the need to fast before obtaining the first sample, but unless contraindicated (e.g. in a small-breed puppy) this author recommends a 12 hour fast. After fasting a blood sample is collected, the animal is then immediately fed a small amount of high-protein/high-fat food. Canned food is typically given and can be syringe-fed if necessary. Only 1-2 tablespoons of food should be needed to stimulate gallbladder contraction. Larger amounts of food can cause lipemia which affects SBA measurement. The post-prandial sample is collected 2 hours after feeding. Laboratories provide reference ranges for fasting and post-prandial SBAs. Occasionally the fasting SBA value is higher than the post-prandial value. If this occurs, it should be interpreted against the post-prandial reference range. In general, the magnitude of the SBA increase does not correlate tightly with diagnosis or prognosis. However, young animals with PSS typically have SBA values greater than 100 umol/l (typical post-prandial reference range of < 20 umol/l). Random non-fasting "pre-prandial" SBA can be measured. If these are normal, liver dysfunction is not ruled out and the proper paired testing should be performed. If a random SBA is increased above the reference range, this finding is meaningful. Once decreased liver function is confirmed with SBA measurement, following changes in the numerical results over time is of limited value, although a change from normal to increased, or vice versa, would be significant.

Ammonia

Ammonia is produced in the GI tract, absorbed into the portal blood, and converted to urea in the liver. Hyperammonemia can result from loss of 70% of liver function. This test is less sensitive than SBA for the detection of liver failure, and it is not as readily available as the test requires specialized sample handling. The most useful application of measurement of plasma ammonia is in a patient with neurological signs for which hepatic encephalopathy is a differential diagnosis. If performed appropriately, the results of this test can direct therapy and diagnostic testing in a neurological patient.

OTHER BLOOD TESTS AFFECTED BY LIVER DISEASE

CBC findings that support liver disease include microcytosis (decreased RBC MCV) in patients with PSS or acquired chronic liver disease, and the presence of acanthocytes.

Findings on *urinalysis* that are not sensitive or specific for liver disease, but may alert the clinician, include the presence of bilirubinuria, decreased urine specific gravity, or glucosuria. The latter can be associated with copper hepatopathy, which is now recognized in a variety of breeds of dog.

Coagulation tests are affected by significant loss of liver function, because many coagulation factors are synthesized in the liver; specifically, the vitamin K-dependent factors (II, VII, IX, and X) and fibrinogen. Therefore, patients with liver failure may have prolonged PT and APTT, and decreased serum fibrinogen.

IMAGING TESTS

Abdominal radiographs are very useful for determining liver size. Abdominal ultrasound examination gives significantly more information regarding architecture, internal structure, and the biliary tree. Ultrasound examination is particularly indicated to distinguish between hepatic and post-hepatic icterus. Ultrasound guidance is useful for acquiring aspirates of liver parenchyma or bile. Biopsies are more valuable if obtained via laparoscopy or laparotomy. Computed tomography is often used for diagnosis of PSS, planning surgical correction of PSS, or evaluating hepatic tumors prior to resection. Nuclear medicine studies (e.g. colorectal scintigraphy) have high sensitivity and specificity for the diagnosis of PSS.

RENAL DIAGNOSTIC TESTS AND URINALYSIS: AZOTEMIA, PROTEINURIA, AND PUPD: INTERPRETATION FOR DERMATOLOGISTS

Katharine F Lunn BVMS MS PhD MRCVS DACVIM

Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

AZOTEMIA

Azotemia is increased blood urea nitrogen (BUN) or increased creatinine, or both (or increase in other non-proteinaceous nitrogenous waste products). Uremia is defined as the presence of urine constituents in the blood, however we typically think of uremia as a clinical syndrome, with signs of anorexia, nausea, vomiting, weight loss, and uremic breath. Severe azotemia can be associated with oral ulceration and less common signs such as encephalopathy or pneumonitis.

Renal Handling of Blood Urine Nitrogen (BUN) and Creatinine

BUN is a measure of urea in the blood. Although urea is a waste product, it also has a role in normal physiology, specifically in the maintenance of water balance. Urea is the end-product of nitrogen metabolism, and is produced from ammonia in the liver. Things that affect the "production side" of BUN are a high protein diet, upper GI tract bleeding (which is really a form of a high protein meal), corticosteroid therapy, which increases endogenous protein turnover, and fever. The liver produces less BUN if it is failing, or if there is a portosystemic shunt, or if protein intake is significantly low. Hill's u/d is an example of a diet that can generate a low BUN, if fed exclusively.
Creatinine is produced by the normal metabolism of muscle; specifically of creatine and phosphocreatine. Creatinine production is relatively constant and proportional to muscle mass. This means that a poorly-muscled animal may have a normal creatinine at the lower end of the reference range, whereas a large muscular patient (a military working dog, for example) could have a normal creatinine at the high end of the reference range, or even just above it. It is important to think about these subtleties when interpreting chemistry results, rather than focusing only on absolute values and fixed reference ranges. Creatinine may be slightly increased by eating a meat-rich meal, but this is not often clinically significant. Creatinine is not increased by muscle trauma or inflammation. Creatinine is freely filtered by the kidney and is not reabsorbed (although a small amount is secreted). As creatinine production is fairly stable and constant on a day-to-day basis and its handling by the kidney is relatively simple, serum creatinine is a better indicator of glomerular filtration rate (GFR) than BUN, with an inverse relationship between GFR and serum creatinine (GFR goes down, creatinine goes up). In contrast, BUN is affected by many pre-renal factors, such as diet or GI bleeding, which makes it a less accurate indicator of GFR.

In the kidney, urea is also freely filtered; the initial concentration of urea in the renal tubular lumen is the same as in the plasma. About 50% is reabsorbed in the proximal tubule (by following water). A similar amount is secreted back into the lumen in the loop of Henle. Half is reabsorbed again in the medullary collecting duct. The net result is that about half the filtered load is excreted. While urea is moving in and out of the renal tubular lumen, water is also being reabsorbed at several parts of the nephron, and this affects the concentration of urea inside the tubule. The net result is that urea "cycles" between the Loop of Henle and the medullary collecting ducts. The final luminal urea concentration can be 50 times greater than the plasma concentration. Reabsorption of urea in the inner medullary collecting ducts is also under the control of antidiuretic hormone (ADH), due to the presence of specialized urea transport proteins that are sensitive to ADH.

Symmetric Dimethylarginine (SDMA)

The problem of interpretation of serum creatinine in a poorly-muscled animal has been reduced by the availability of measurement of serum SDMA. This analyte is a more sensitive marker of GFR than creatinine, it is not affected by muscle mass, and measurement of SDMA has been shown to allow earlier detection of chronic kidney disease (CKD) in both dogs and cats.[1,2]

Pre-Renal Azotemia

During dehydration, ADH is released, to promote reabsorption of water from the distal nephron. But at the same time ADH binds to the urea transporters and urea is also reabsorbed. This is important because the urea is needed to maintain a high renal medullary osmolality. But what it means for the patient is that less BUN is excreted, and blood concentrations will rise. But they will rise proportionally more than creatinine, because this reabsorption does not occur with creatinine. A simplified way to think of this is that urea and water go together: during dehydration (or hypovolemia), ADH levels rise and both water and urea are reabsorbed. Conversely, in a polyuric patient, less urea is absorbed and BUN concentration will actually fall. Examples include overhydration with fluid therapy, primary polydipsia, diabetes insipidus, or hyperadrenocorticism.

Evaluating a BUN:creatinine ratio can be helpful in a number of circumstances. Rather than focus on the exact value of the ratio, determine if BUN and creatinine are elevated proportionately to each other in an azotemic patient. If BUN is disproportionately high, consider the following (in no particular order): 1. High protein diet, 2. GI bleed, 3. Dehydration or hypovolemia. These are all *pre*-renal factors, and they are important because they can be addressed and potentially corrected.

Pre-renal factors that decrease GFR, and hence may increase both BUN and creatinine, include dehydration, hypovolemia, hypotension, decreased cardiac output, and locally reduced renal perfusion (for example thrombosis of a renal artery). Any factor leading to decreased renal perfusion will eventually lead to renal azotemia as the kidneys become damaged.

Pre-renal azotemia is suspected on the basis of physical examination findings. If an azotemic patient is clinically dehydrated and urine specific gravity shows hypersthenuria (i.e. urine is concentrated) then this implies normal renal function and the azotemia can be attributed to pre-renal causes. However, problems may arise in the interpretation of urine specific gravities that are not concentrated (hyposthenuric, isosthenuric, or minimally concentrated). When the urine is not concentrated in the face of a challenge such as dehydration, this may reflect renal failure, but it may also reflect the inability of the kidney to concentrate urine due to non-renal disease. Examples include the administration of fluid therapy, use of diuretics or glucocorticoids, or the presence of concurrent disease, such as hyper- or hypo-adrenocorticism, hypercalcemia, or diabetes insipidus. In a patient with azotemia and inappropriately low urine specific gravity, the best way to determine how much of the azotemia is pre-renal is to assess the response to fluid therapy. For example, animals with hypoadrenocorticism can present with profound azotemia and non-concentrated urine, and the azotemia will fully and rapidly resolve with appropriate fluid therapy.

Post-Renal Azotemia

Post-renal azotemia arises from either obstruction or rupture of the urinary tract. Rupture of the urinary tract may be suspected from the history, from signs of abdominal pain, or from detecting abdominal effusion. Uroabdomen is confirmed by measuring creatinine or potassium (not BUN) in the effusion and comparing the values to those of the serum.

Obstruction of the urinary tract cannot be ruled out simply because the patient is able to produce urine. It is important to remember that obstruction can occur at any level of the urinary tract, including the renal pelvis or ureter. The only way to rule out obstruction at this level is with imaging techniques. Options include abdominal ultrasound, plain radiographs or contrast radiographs; the latter achieved either by excretory urography or direct injection of contrast into the renal pelvis under ultrasound guidance. Obstruction of the renal pelvis or ureter is a significant problem in feline urology, with the recognition of many cats that form calcium oxalate stones in the upper urinary tract. Therefore, it is strongly recommended that the urinary tract be appropriately imaged in newly diagnosed azotemic patients, and in any previously stable chronic renal failure patient that presents with acutely worsened azotemia. It is also important to recognize that the development of azotemia in this type of patient is dependent on how well the non-obstructed kidney is working. Approximately 66% of GFR must be lost in order to lose concentrating ability, and about 75% must be lost in order for the patient to become azotemic. If the patient originally had 100% of normal renal function and one kidney is acutely obstructed, initially only 50% of GFR is lost, so azotemia will not be detected. Azotemia will only occur if the remaining unobstructed kidney is already abnormal. Therefore, both renal and post-renal disease co-exist in this situation.

Renal Azotemia

Renal azotemia is suspected in a patient with azotemia and concurrent non-concentrated urine. However, as noted above, other conditions may affect urine concentrating ability and thus inability to concentrate the urine for some other reason must be ruled out. This is one reason why it is important to assess urine specific gravity in an azotemic patient *before* starting fluid therapy. Renal azotemia is diagnosed by ruling out pre-renal and post-renal causes and by looking for other supportive evidence. Renal azotemia may be acute, associated with acute kidney injury (AKI) or chronic, due to CKD. Important causes of AKI include infection such as pyelonephritis or leptospirosis, drugs and toxins, pancreatitis, and decreased renal perfusion.

PROTEINURIA

In the healthy urinary tract (kidneys and lower urinary tract) there should be very little protein in the urine. The glomerular capillary wall restricts filtration of most proteins, depending on their weight (> 65,000 daltons) and charge. Smaller proteins may pass through the glomerulus, but are reabsorbed in the healthy proximal tubule. There is also some secretion of protein in the renal tubules, lower urinary tract, and genital tract. When protein is detected in the urine, it is important to determine the source, the amount, and the significance.

Pre-renal proteinuria refers to the production of proteins in the body of a type or quantity that cannot be reabsorbed by the kidneys. Examples include hemoglobin, myoglobin, or Bence Jones protein in myeloma patients. Post-renal proteinuria refers to proteins produced in the lower urinary tract, most likely associated with inflammation. Post-renal proteinuria is suspected when a patient has an "active" urine sediment. Causes include urolithiasis, infection, feline idiopathic cystitis, trauma, or neoplasia. If the inflammation is at the level of the bladder or urethra, signs of pollakiuria or stranguria might be expected. Inflammation at the level of the renal pelvis or ureter can be considered to be "post-renal" but would not typically cause lower urinary tract signs. History, clinical signs, physical examination, microscopic examination of urine sedimentq2, and imaging findings should help to determine if proteinuria is post-renal. Renal proteinuria may be either tubular or glomerular in origin. It can be difficult to distinguish these on the basis of routine testing, but generally tubular proteinuria is of lower magnitude (urine protein:creatinine ratio [UPC]<2)and may be accompanied by other signs of tubulopathy such as casts, glucosuria, or amino aciduria. Glomerular proteinuria is due to altered glomerular function, and typically associated with UPC>2.

If proteinuria is detected on routine dipstick analysis, the first step should be to examine the urine sediment for signs of inflammation. Urine culture is also recommended, even if no bacteria are seen. The second step should be to repeat the urinalysis to determine if proteinuria is persistent. If proteinuria is persistent, there are no clinical signs of lower urinary tract disease, sediment is inactive, and urine culture is negative, a UPC should be obtained. Guidelines for investigation and management of proteinuria are provided elsewhere,[3] but in general proteinuria should be investigated and not ignored.

POLYURIA-POLYDIPSIA (PUPD)

Polyuria and polydipsia (PUPD) are common problems in small animal practice. Polyuria is defined as urine output in excess of 50 ml/kg per day. Polydipsia is defined as fluid intake exceeding 100 ml/kg per day. While it is useful to keep these definitions in mind, most cases of PUPD are evaluated without the need to accurately quantitate water intake. In fact, some individual animals can be PUPD without exceeding the limits defined above. In general, the observations of the pet owner, together with the urine specific gravity are used as a basis for further evaluation of PUPD.

The ability of the kidney to form a concentrated urine depends on the presence of ADH, the ability to respond to ADH, and maintenance of the high osmolarity of the renal medullary interstitial fluid. Thus, polyuria, with compensatory polydipsia can result from reduced or absent ADH synthesis or release, failure of the renal tubule to respond to ADH, or reduction in the osmotic gradient between the filtrate in the distal convoluted tubule and the renal medullary interstitium. The latter can be due to the presence of osmotically active particles in the filtrate, or decreased medullary hypertonicity. Primary polydipsia can result from a psychogenic (behavioral) abnormality in which hypothalamic, pituitary, and renal function are normal. Fever, pain, and hyperthyroidism can also cause polydipsia. In addition, polydipsia can be a normal physiological response to environment changes such as elevated ambient temperature, or the feeding of a dry diet.

Urine Specific Gravity (USG)

Interpretation of USG is confounded in a patient receiving fluid therapy or diuretics. It is not helpful to think in terms of "normal" and "abnormal" USG; the clinician should determine if the USG is "appropriate" or "inappropriate". The following terms are used to describe specific gravity: *Hyposthenuria*: SG < 1.008. *Isosthenuria*: SG of 1.008 - 1.012. *Minimally concentrated urine*: USG of 1.013 - 1.030 in dogs, and 1.013 - 1.040 in cats. *Hypersthenuria:* USG > 1.035 (dogs) and USG > 1.045 (cats). Marked hyposthenuria is most likely in diabetes insipidus (DI) and psychogenic polydipsia, however hyposthenuria can occur with hyperadrenocorticism (HAC), hypercalcemia, and pyometra (examples of secondary nephrogenic DI). The detection of hyposthenuria rules out chronic kidney failure (CKD) as the sole cause of PUPD. In the latter condition, the urine is usually isosthenuric or minimally concentrated. Isosthenuric urine is not really "dilute"; it is neither diluted nor concentrated, but is urine that is at the same osmolality as plasma. Persistent isosthenuria is most suggestive of CKD, but intermittent isosthenuria could occur with many causes of PUPD. A random USG in the hypersthenuric range means that obligate PUPD is unlikely (the kidney is *capable* of producing a concentrated urine), and that further confirmation of PUPD is needed. Table 1 lists causes of PUPD, proposed mechanism, and suggested diagnostic tests.

References

1. Hall JA, Yerramilli M, Obare E, et al. Serum concentrations of symmetric dimethylarginine and creatinine in dogs with naturally occurring chronic kidney disease. J Vet Intern Med 2016;30:794-802
2. Hall JA, Yerramilli M, Obare E, et al. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. J Vet Intern Med 2014;28:1676-1683.
3. Lees GE, Brown GA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM forum consensus statement (small animal). J Vet Intern Med 2005;19:377-385.

Table 1: Causes of PUPD and the Underlying Mechanism(s)

Cause	Mechanism (s)	Specific Diagnostic Test (s)
Diabetes mellitus	Osmotic diuresis	Blood glucose, urinalysis
Primary renal glycosuria	Osmotic diuresis	Urinalysis
Chronic kidney disease (CKD)	Osmotic diuresis	Serum biochemistry profile, urinalysis, measure GFR
Polyuric acute kidney injury (AKI)	Osmotic diuresis	Serum biochemistry profile
Postobstructive diuresis	Osmotic diuresis	History, physical exam (PE)
Renal medullary solute washout	Decreased renal medullary tonicity	Look for underlying cause
Pyometra	Bacterial endotoxin reduces tubular sensitivity to ADH (2° nephrogenic diabetes insipidus [NDI])	History, PE, imaging
Hypercalcemia	Interferes with action of ADH (2°NDI)	Serum biochemistry profile
Liver failure	Loss of medullary hypertonicity Impaired hormone metabolism Psychogenic	Fasting and post-prandial bile acids
Hyperadrenocorticism	Impaired tubule response to ADH (2°NDI) Impaired release of ADH	ACTH stimulation test Low dose dexamethasone suppression test
Pyelonephritis	Bacterial endotoxin reduces tubular sensitivity to ADH (2°NDI) Damaged countercurrent mechanism	Urinalysis, urine culture, abdominal ultrasound, excretory urography
Hypokalemia	Interferes with action of ADH (2°NDI)	Serum biochemistry profile
Hypoadrenocorticism	Loss of medullary hypertonicity Interferes with action of ADH (2°NDI)	ACTH stimulation test
Hyperthyroidism	Loss of medullary hypertonicity Psychogenic	Total thyroxine (T4), free T4, thyroid scintigraphy
Acromegaly	Osmotic diuresis due to diabetes mellitus	History, PE, insulin-like growth factor-1 (IGF-1), brain imaging
Primary hyperaldosteronism	Impaired tubule response to ADH (2°NDI)	Aldosterone levels, ACTH stimulation test
Hyponatremia	Loss of medullary hypertonicity	Serum biochemistry profile
Polycythemia	Action of atrial natriuretic peptide	Complete blood count
Pheochromocytoma	Excessive catecholamines	Imaging, hormonal assays
Leptospirosis	CKD, 2°NDI?	Serology, polymerase chain reaction
Splenomegaly	Psychogenic?	Imaging
Leiomyosarcoma	?	Imaging
Diet, drugs, and toxins	Various	History
Primary nephrogenic diabetes insipidus (1°NDI)	Congenital inability of nephron to respond to ADH	Signalment, water deprivation test (WDT)
Psychogenic polydipsia	Primary polydipsia (behavioral) Gastrointestinal disease	Urine specific gravity, WDT
Central diabetes insipidus	ADH deficiency	Response to desmopressin, WDT

ABSTRACT SCHEDULE

SATURDAY, APRIL 13

Abstract Session: Clinical Abstracts

9:15 AM	**Dr. Dawn Logas:** Treatment outcomes for chronic canine otitis externa in primary care and dermatology specialty practice settings	120
9:30 AM	**Dr. Alberto Cordero:** Non-surgical treatment of canine auricular haematoma with intralesional and systemic corticosteroids, a pilot study	121
9:45 AM	**Dr. Andrew Simpson:** Outcomes of canine chronic tympanic membrane perforation: retrospective study of 34 ears	122
10:00 AM	**Dr. Jennifer Schissler:** Bilateral vestibular syndrome following application of a proprietary long-acting otic topical sans aminoglycoside in a cat with intact tympanic membranes	123
10:15 AM	**Dr. Jennifer Schissler:** Ehlers-Danlos syndrome in a Bombay cat with cutaneous asthenia, vasculopathy, steroid-responsive pruritus, hyperesthesia, and epilepsy	124
10:30 AM	**Dr. Jeanine Peters-Kennedy:** Sterile pyogranuloma/granuloma syndrome involving the skin, testes and epididymides in two dogs with excellent clinical response to mycophenolate mofetil in one dog	125
10:45 AM	**Dr. Tom Lewis:** Evaluation of a new hydrolyzed salmon-based diet in dogs with suspected cutaneous adverse food reaction: a multi-center randomized triple-blinded clinical trial	126
11:00 AM -11:30 AM	**BREAK**	
11:30 AM	**Dr. Mona Boord:** Canine anaphylaxis caused by human albumin contained in honeybee immunotherapy	127
11:45 AM	**Dr. Jennifer Schissler:** Assessment of oclacitinib as a treatment for canine chronic idiopathic rhinitis using a validated rhinitis scoring instrument: a retrospective study	128
12:00 PM	**Dr. Linda Frank:** Results of a pilot clinical trial using a vaccine containing key antigens from *Staphylococcus pseudintermedius* to treat superficial pyoderma in dogs	129
12:15 PM	**Dr. Christoph Klinger:** Various indications for the clinical utilization of cold atmospheric pressure plasma (CAPP) therapy in veterinary dermatology	130
12:30 PM - 2:00 PM	**LUNCH**	

Scientific Session Presentations

9:00 AM - 10:00 AM	**Tosso Leeb, PhD:** Principles of Genetic Investigations and The Era of Personalized Medicine	131
10:00 AM - 11:00 AM	**Tosso Leeb, PhD:** Update on Genodermatoses and New Genetic Tests in Veterinary Dermatology	135
11:30 AM - 12:30 PM	**Sheila Torres, DVM:** Unconventional Alternatives to Conventional Antibiotics: A Glance at the Pipeline	141
2:00 PM - 3:00 PM	**Michael Childress, DVM:** Picking the Wolves from the Sheep: Prognostic Markers in Canine Cutaneous Mast Cell Tumors	145
3:00 PM - 4:00 PM	**Michael Childress, DVM:** Choosing the Right Treatment for Dogs with Cutaneous Mast Cell Tumors	149
4:30 PM - 5:30 PM	**Michael Childress, DVM:** Managing Cutaneous Lymphomas in Dogs	154

ABSTRACT SCHEDULE

SATURDAY, APRIL 13

Concurrent Session Presentations

ADVT Session Presentations

Treatment outcomes for chronic canine otitis externa in primary care and dermatology specialty practice settings

D. B. LOGAS

Veterinary Dermatology Center, Maitland, FL, USA

Abstract: The study objective was to assess the differences in rate of reoccurrence, resolution of clinical signs, and resolution of proliferative otic changes in dogs with chronic otitis being treated by primary care veterinarians (pcDVMs) versus board-certified veterinary dermatologists. This retrospective study included 65 dogs that had been treated for recurring/persistent otitis externa by pcDVMs for at least 3 months before being referred to board-certified veterinary dermatologists. Per the inclusion criteria, dermatologists must have had access to all the pcDVMs records pertinent to the treatment of the otitis. Cases were randomly selected from chronic otitis externa patients seen by dermatologists between September 2006 and March 2017. Dermatologists entered data from both the pcDVMs' and their own records into an online survey platform hosted by an independent market research firm (MarketVision Research). For each dog, otitis externa treatment history including referral timeframe, recurrence rate, clinical signs, and resolution of signs was collected. While under dermatologists' care, dogs had lower median rate of otitis recurrence (171 days) compared to pcDVMs (59 days) [$P = 0.0016$]. More dogs under dermatologists' care experienced complete resolution of otitis externa clinical signs (56% versus 8%) [$P < 0.0001$] and improved resolution of otic proliferative changes (95% versus 30%) [$P < 0.0001$] compared to pcDVMs care. In conclusion, dogs with chronic otitis had better long term outcomes when treated by board-certified veterinary dermatologists in addition to pcDVMs than those treated by pcDVMs alone. Therefore, referral is recommended for otits cases not resolving with treatment after 2-3 months.

Sources of funding: VetSoap.

Conflict of Interest: None declared

Non-surgical treatment of canine auricular haematoma with intralesional and systemic corticosteroids, a pilot study

A. M. CORDERO*, C. LOPEZ-MARQUEZ*, G. SHEINBERG†, C. ROMERO§

§Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de México, Amecameca de Juárez, Estado de México, México
†Centro Veterinario México, Departamento de dermatología, Ciudad de México, México
** VETDERM: Dermatologia Veterinaria Especializada, Guadalajara, Jalisco, Mexico*

Abstract: Aural or auricular haematomas are fluctuant swellings filled with haemorrhagic fluid affecting the concave pinnal surface. Pharmacologic and surgical treatments have been used with various outcomes. This study aimed to evaluate a non-surgical treatment for dogs with auricular haematomas. On day 0, 10 dogs with auricular haematomas were treated with a single injection of 0.4 mL triamcinolone acetonide (6 mg/mL) into the haematoma cavity following drainage of haematoma fluid; the dogs also received a 10-day course of oral prednisolone (1 mg/kg). Follow-up visits were on days 5 and 10. Eight dogs completed the study. Two dogs were lost to follow-up. Haematoma length showed no statistical change from day 0 to day 5, however on day 10 a significant reduction in length was noted from 6.92 cm to 1.17 cm (P = 0.03). Mean haematoma width showed no change at day 5, but on day 10 there was a significant (*P* = 0.03) reduction: day 0 (4.85 cm), day 5 (3.92 cm), day 10 (1.0 cm). Haematoma volume (height x length) was significantly reduced (*P* = 0.04) from day 0 to day 5, however no change in volume was noted from day 5 to day 10. The amount of haematoma fluid collected decreased with each visit (means: day 0 [14.78 mL], day 5 [3.28 mL], day 10 [0.0 mL]). All haematomas were resolved by day 10. The combination of an intralesional triamcinolone injection and oral prednisolone after drainage of haematoma fluid is an effective non-surgical treatment choice for canine auricular haematomas.

Source of funding: Self-funded.

Conflict of Interest: None declared

Outcomes of canine chronic tympanic membrane perforation: retrospective study of 34 ears

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

** VCA Aurora Animal Hospital, Aurora, IL, USA*
† Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

Abstract: A Colorado State University records search for tympanic membrane (TM) perforations observed for ≥ 3 months yielded 29 dogs, 34 ears. Mean follow-up was 26.6 months (range 3-75). TM absence was noted in 20/34, perforation in 14/34. Etiologies included allergic otitis externa/media (OM) in 15/34 (44.1%) and grass awns in 6/34 (17.6%). Hearing was reported as reduced in 12/29 dogs (41%); adequate in 13/29. Nine dogs had neurologic deficits: ipsilateral head tilt (8), facial nerve paralysis (3), Horner's syndrome (2), ataxia (2) and nystagmus (1). Thirty middle ears (ME) were flushed. Cytology and culture confirmed bacterial OM in 14/30 ears; *Malassezia* in 3/30. All ears received topical treatment. There was resolution of ataxia (2/2), nystagmus (1/1), head tilt (4/8), and Horner's syndrome (1/2). Deafness occurred in 4/12 dogs with initial reduced hearing; partial hearing loss in 3/13 with initial adequate hearing. Flushing was repeated in 15/34 ears (44.1%). For dogs followed > 12 months, 15/20 ears required ≥2 flushing events (FE) (range 2-7). Reasons for FE included debris (19), otic pruritus (5), head tilt/ataxia (4), ceruminolith (3), infection (3), head shaking (3), and reduced hearing (2). Bacteria but no *Malassezia* were noted via cytology in the ME and/or horizontal canal (HC) in 14/29 repeat FE. Cytology of the HC and ME demonstrated correlation in 6/14 FE. Surgery was performed in 2/34 ears for suspect cholesteatoma and 2/34 for recurrent OM. In dogs with chronic TM perforation, topicals were generally tolerated, flushing common, neurologic recovery variable, and surgical intervention rare.

Source of funding: Self-funded.

Conflict of Interest: None declared

Bilateral vestibular syndrome following application of a proprietary long-acting otic topical sans aminoglycoside in a cat with intact tympanic membranes

J.R. SCHISSLER

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

Abstract: Vestibulotoxicity occurs as a result of hair cell damage in the vestibular apparatus and has been documented in man with systemic gentamicin administration. Clinical vestibulotoxicity is not well-documented in domestic animals. A 15-year-old spayed female domestic shorthair cat presented with a 4 day history of inappetence with progressive ataxia, third eyelid protrusion, lethargy developing within 3 h of bilateral administration of a 16.6 mg/mL florfenicol, 14.8 mg/mL terbinafine and 2.2 mg/mL mometasone otic product (Claro®). Hearing ability was uncertain. Examination revealed severe ataxia, basewide stance, reluctance to walk, slow circling in both directions, and nearly continuous head sway indicative of bilateral vestibular syndrome. Bilateral Horner's syndrome and intermittent fast-phase left nystagmus were present. Facial nerve and conscious proprioception reflexes were normal. Video-otoscopy revealed bilateral scant cerumen adjacent to a normal tympanic membrane, with no identifiable topical product. An anesthetized saline otic irrigation and esophogostomy tube placement was recommended. The clients opted to have these procedures performed by the primary veterinarian, who was instructed by the author with regard to flushing technique. Flushing was performed, as instructed, on the same day. Ambulation and appetite improvement was noted within 72 h of the flush procedure. Ten days after the procedure, lethargy was significantly reduced, appetite was normal, and deafness was noted. Five months later, deafness remains as well as left-sided intermittent vestibular syndrome. A similar case has not been reported. Idiosyncratic topical ototoxicity may occur with intact tympanic membranes, sans aminoglycoside. Rapid intervention is recommended.

Source of funding: Self-funded.

Conflict of interest: None.

Ehlers-Danlos syndrome in a Bombay cat with cutaneous asthenia, vasculopathy, steroid-responsive pruritus, hyperesthesia, and epilepsy

J.R. SCHISSLER

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

Abstract: Ehlers-Danlos syndrome (EDS) in man has 14 classifications involving cutaneous asthenia, musculoskeletal, vascular, neurologic, and mast cell activation disorders as a result of collagen and extracelluar matrix gene mutations. Cutaneous laxity has been rarely described in Bermese, Himalayan, and domestic shorthair (DSH) cats, with some reports of vasculopathy and joint laxity. Recently a COL5A1 frameshift mutation was discovered in a DSH with EDS. An 8-month-old female spayed Bombay presented with a history of lumbar self-trauma, periaural pruritus, and six successive lumbar wounds with failed prednisolone and gabapentin therapy. Previous histopathology indicated severe leukoclastic vasculitis and superficial collagen calcification. Videos demonstrated cutaneous trunci spasms with reactive licking and biting of the dorsal lumbar skin. Exam findings included a skin extensibility index of 19%, papulocrustous dermatitis and alopecia at the head, and a 7.5 cm diameter lumbar ulcer. Hyperesthesia was successfully managed with 6.25 mg/kg topiramate per os once daily. Pruritus, papules, and crusts resolved with dexamethasone (0.3 mg/kg per os every 72 hr), and relapsed upon dexamethasone discontinuation. Cyclosporine was not tolerated. A 12-week elimination diet had no steroid-sparing effect. Grand mal seizures were observed 3 months after initial presentation, and controlled with oral phenobarbital (1.97 mg/kg twice daily). The patient is presently non-pruritic with no self-trauma, spasms, nor lesions. A protective shirt is worn daily. This patient demonstrates EDS signs observed in man, including hyperesthesia, pruritus, and epilepsy, that have not been reported in cats. Further steroid-sparing therapies will be explored.

Source of funding: Self-funded.

Conflict of interest: None declared.

Sterile pyogranuloma/granuloma syndrome involving the skin, testes and epididymides in two dogs with excellent clinical response to mycophenolate mofetil in one dog

J. PETERS-KENNEDY*, J. E. MILLER †, E. A. DEMETER*

**Department of Biomedical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA*
† Department of Clinical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Abstract: Cutaneous sterile pyogranuloma/granuloma syndrome (SPGS) is an idiopathic skin disorder in dogs characterized by solitary to multiple papules and nodules and a proposed immune-mediated pathogenesis. Mycophenolate mofetil (MM) is an immunosuppressive drug that inhibits lymphocyte proliferation. We describe two intact male dogs, a 3-year old mixed breed dog (case 1) and an 8-year old golden retriever (case 2), with multiple papules and nodules of the skin, testes and epididymides. Histologically, vertically oriented pyogranulomatous infiltrates surrounded pilosebaceous units with multifocal nodules extending into the panniculus. Nodular infiltrates of similar inflammatory cells expanded and partially effaced the epididymis in both cases with similar involvement of the testis in case 1. Histochemical stains for bacteria, acid-fast bacteria, fungi, and protozoa were negative. Tissue cultures for bacteria and fungi were negative in case 1. Case 2 was negative for *Brucella* spp. via polymerase chain reaction. Immunophenotyping in both cases was consistent with a mixed population of predominantly IBA-1 positive histiocytes, with fewer infiltrating CD3 positive T-cells and Pax-5 and CD20 positive B-cells. A clonal population of lymphocytes was not detected in either case. Case 1 had a poor clinical response to doxycycline (5mg/kg orally twice daily) and niacinamide (500mg orally twice daily) but had a 90% improvement with MM (12.5 mg/kg orally twice daily). Case 2 had an excellent clinical response to 2mg/kg/day oral prednisone tapered to 1mg/kg/day every 3 days. This is the first description of SPGS affecting the testes

Source of funding: Self-funded.

Conflict of interest: None declared.

Evaluation of a new hydrolyzed salmon-based diet in dogs with suspected cutaneous adverse food reaction: a multi-center randomized triple-blinded clinical trial

T. LEWIS*, C. LAPORTE†, G. MOORE‡, R. MILLER§, K. COYNER¶, A. SCHICK, D. GENOVESEβ, S. LOCKWOODμ, S. SCHWARTZ*, B. SIMON*, S. MORAR***

**Dermatology for Animals, Gilbert, AZ, USA*
†Dermatology for Animals, Walnut Creek, CA, USA
‡Department of Veterinary Administration, College of Veterinary Medicine, Purdue University, West Lafayette, Indiana, USA
§Dermatology for Animals, Salt Lake City, Utah, USA
¶Dermatology Clinic for Animals, Tacoma, WA, USA
***Dermatology for Animals PC, Scottsdale, AZ, USA*
βDermatology for Animals PC, Campbell, CA, USA
μDermatology for Animals PC, Avondale, AZ, USA

Abstract: Dietary elimination trials and allergen avoidance are mainstays of diagnosis and treatment in dogs with cutaneous adverse food reactions (CAFR). Non-prescription diets may contain undeclared ingredients or contaminants that compromise dietary manipulation. Certain prescription diets have established purity, but some patients still demonstrate sensitivity. Therefore, alternative diets addressing patients' specific requirements are needed. We performed a multi-center, triple-blinded, crossover clinical trial to validate the benefits of a prescription hydrolyzed salmon diet (HSD) with verified purity in dogs with suspected CAFR. We hypothesized that HSD would be equally efficacious and well-tolerated as an established hydrolyzed poultry feather diet (HPFD) for dietary elimination trials. Patients were randomized to receive HSD or HPFD for 8 weeks and then received the other diet for 8 additional weeks. Patients were examined every 4 weeks wherein Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4, owner-scored pruritus visual analog scale (VAS), and adverse events were recorded. Data were compiled from 47 dogs. Only HSD significantly decreased CADESI score (HSD: -7.3 ± 11.6 and HPFD: -3.0 ± 11.1; $P = 0.001$ and $P = 0.090$, respectively), however, the diets did not differ from each other ($P = 0.053$). Both diets lowered VAS scores (HSD: -1.15 ± 2.41 and HPFD: -0.67 ± 2.52; $P < 0.001$) and did not differ from each other ($P = 0.322$). Sixteen dogs had adverse events with HPFD compared to seven dogs with HSD ($P < 0.01$). These results suggest HSD is a valuable option in the diagnosis and treatment of canine CAFR.

Source of funding: Blue Buffalo Company, Ltd.

Conflict of interest: None declared.

Canine anaphylaxis caused by human albumin contained in honeybee immunotherapy

C. HUDEC*, M. BOORD*, C. GRIFFIN*

**Animal Dermatology Clinic, San Diego, CA, USA*

Abstract: This report details a 5-month-old, intact female, Labrador retriever who experienced three anaphylactic reactions nearing the end of the induction protocol to honeybee venom immunotherapy (VIT). Contained within the VIT is honeybee venom and human albumin, used as a stabilizer in buffered saline. To define if the reactions were due to honeybee sensitivity or due to a new sensitization to human albumin, human albumin and canine albumin were obtained for testing from ALK. Canine albumin was to act as a negative control for the human albumin. During the initial intradermal testing (IDT) to honeybee, the patient had a positive reaction at 0.0001%. Upon repeat IDT, honeybee sensitivity remained at 0.0001%, and a positive reaction occurred with the initial testing strength of 0.0003% human albumin. End-point titration yielded positive reactions to human albumin down to a concentration of 0.0000003%. Canine albumin tested negative. Four years later IDT was repeated. Honeybee sensitivity remained at 0.0001%, human albumin tested positive to 0.000003%. This demonstrated a long lasting human albumin and honeybee sensitivity. To further provide evidence of sensitization to human albumin, six healthy dogs, six atopic dogs and four dogs on VIT to honeybee underwent IDT to human albumin. All dogs were negative except one who was being maintained on honeybee VIT. This dog tested positive to an initial dose of 0.0003% and end-point titration was positive to 0.00000003%. In conclusion, utilizing VIT with human albumin can sensitize canine patients to this protein.

Source of Funding: Self-funded.

Conflicts of Interest: None declared.

Assessment of oclacitinib as a treatment for canine chronic idiopathic rhinitis using a validated rhinitis scoring instrument: a retrospective study

T. ANDERSON* and J.R. SCHISSLER*

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

Abstract: Canine chronic rhinitis (CCR) is idiopathic, inflammatory, and unpredictably responsive to anti-inflammatory and antimicrobial therapies. Oclacitinib (Apoquel®) is a janus kinase inhibitor labelled for treatment of canine allergic dermatitis. Oclacitinib's efficacy as a CCR treatment has not been described. Canine records at Colorado State University from 2012-2018 searched for "rhinitis" and "oclacitinib," and yielded 12 individuals. Study inclusion required client completion of a validated Severity of Nasal Inflammatory Disease for Dogs questionnaire to quantify severity (never [1] to extremely often [5]) of 22 criteria (e.g. sneezing, activity interruption) before and during oclacitinib. Six dogs were included. At treatment onset, median rhinitis duration was 5.5 months (range: 2- 9). Three had previous nasal CT; five had nasal histopathology. All previously failed antibiotic, antihistamine, avermectin, and/or glucocorticoid therapies for CCR. Four dogs had concurrent allergic dermatitis. Median oclacitinib maintenance daily dose was 0.44 mg/kg per os (range 0.38- 1.01); median duration was 31.5 months (range 7.5- 45). Antibiotics and/or antihistamines were concurrently administered in four dogs. Mean scores pre- and post-treatment were 2.95 and 2.44 (difference, -17.3%); this was statistically significant (Wilcoxon signed ranks, $P = 0.0350$). Scores were reduced in five, static in one. One dog had severity reduction in 19 criteria (mean pre 4.18, post 1.95, and difference -53.3%). Oclacitinib was discontinued in four dogs for: lack of efficacy (2), cholangiohepatitis (1), and superior efficacy of lokivetmab (1). A larger prospective, placebo-controlled study could be useful to better assess oclacitinib's efficacy in CCR without selection bias for previous treatment failures.

Source of funding: Self-funded.

Conflict of interest: Jennifer Schissler provides continuing education sponsored by Zoetis, the manufacturer of Oclacitinib (Apoquel®).

Results of a pilot clinical trial using a vaccine containing key antigens from *Staphylococcus pseudintermedius* to treat superficial pyoderma in dogs

L. A. FRANK*, M. ABOUELKHAIR†, D. A. BEMIS†, S. A. KANIA†

**Dept of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA*
†Dept of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

Abstract: We developed a vaccine composed of antigens from key meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) immunoevasive proteins secreted and/or exposed on the bacterial surface. Recombinant proteins attenuated with amino acid substitutions and antigenically but not functionally similar to the corresponding native proteins were produced. These included coagulase, protein A, leukotoxin, leukocidin, and adenosine synthase. Eight dogs with pyoderma were recruited. Three injections of the vaccine containing 20 µg of each protein were given subcutaneously 1 week apart. No antimicrobials or immunosuppressive medications were permitted. Serum was collected before vaccination and weeks 1, 2, and 4 post-vaccination for antibody reactivity to vaccine components. Complete blood counts, chemistry panels, and urinalyses were assessed at weeks 0 and 4. MRSP was cultured from 5/8 dogs and meticillin-susceptible *Staphylococcus pseudintermedius* (MSSP) from 3/8 dogs upon enrollment. Two of three dogs with MSSP had complete resolution of pyoderma at week 4 without antibiotics or topical therapies. Three of five dogs initially with MRSP had MSSP cultured at week 4. Of those, two dogs responded to cefalexin and one initially to clindamycin. No abnormalities were noted on bloodwork or urinalyses. Prior to vaccination no dog had substantial antibody reactivity to vaccine components. After two injections, all dogs produced antibodies with strong reactivity to all vaccine antigens except coagulase. Results of the study showed potential benefit of a vaccine containing antigenically modified components of MRSP. Future work includes modification of the vaccine to include other antigenic components and a prospective placebo controlled trial.

Source of funding: University of Tennessee Research Foundation Maturation Grant.

Conflict of interest: None declared.

Various indications for the clinical utilization of cold atmospheric pressure plasma (CAPP) therapy in veterinary dermatology

C. J. KLINGER*, C. THEEL†, R. S. MUELLER‡

** Tierklinik Stuttgart Plieningen, Veterinary Specialist Hospital, Stuttgart, Germany*
† neoplas GmbH, Greifswald, Deutschland
‡ Centre for Clinical Veterinary Medicine, LMU Munich, Germany

Abstract: Cold atmospheric pressure plasma (CAPP) is a painless, well-tolerated intervention in humans with multi-resistant infections, including cases of biofilm formation and wound healing disorders. CAPP stimulates fibroblast DNA, lipids and proteins, antimicrobial peptides, and growth factors. The effects of this physical, gaseous particle mixture of charged carriers with high ionization levels and electrical conductivity is founded on the formation of reactive species, radicals and radiation. Herein we present three cases where CAPP with the plasma jet "*kINPen Vet®*" (neoplas GmbH) was utilized. A 4-year-old female spayed crossbreed dog who was being treated with allopurinol, glucantime, pentoxifylline for leishmaniasis developed pinnal ulcers due to vasculitis which had not responded to 6 months of standard treatments. This dog's pinnae were then treated biweekly with CAPP; the pinnal lesions resolved completely within 28 days. A 5-week-old female Bengal kitten who presented with ulcerations on the head, neck and tarsus due to multidrug-resistant *Staphylococcus aureus* responded completely to three times weekly CAPP (and no antimicrobials) within 15 days. A 3-year-old male castrated German shepherd dog with perianal fistulas was treated with oral ciclosporine, tacrolimus ointment and additionally biweekly CAPP on one-half of the perianal area for comparison of wound healing. At re-evaluation 24 days later, the dog showed more rapid wound healing on the CAPP-treated side compared to the untreated side. These cases showed positive results in the treatment of infectious dermatoses (including resistant infection) and wound healing. Future controlled clinical studies evaluating CAPP therapy are warranted.

Source of funding: neoplas GmbH, Germany provided the plasma jet device free of charge, but was not involved in the choice of patients nor their clinical evaluation and thus had no influence on the outcome of the cases.

Conflict of interest: Christian Theel is an employee of neoplas GmbH.

PRINCIPLES OF GENETIC INVESTIGATIONS AND THE ERA OF PERSONALIZED MEDICINE

Tosso Leeb, PhD

Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland

INTRODUCTION

Technical advances in the last decade have revolutionized the field of veterinary genetics. The costs for obtaining the full genome sequence of an individual mammal have dropped to US$ 1,000 and are thus comparable to the costs for other diagnostic tests such as e.g. magnetic resonance imaging. Genetic investigations may be expected to further gain in importance in veterinary medicine. Therefore, veterinarians should be familiar with the possibilities of genetic analyses. Veterinarians also need to be able to order and interpret genetic analyses appropriately. In humans, genetic variants in about ~500 genes have been shown to cause heritable skin disorders, also termed genodermatoses.[1,2] With genome sequencing, it has become feasible to screen all known candidate genes in individual patients. In this lecture, four examples of genetic investigations on heritable skin traits will be presented to illustrate the power of modern veterinary genetics.

FAMILY-BASED STUDIES – NAKED FOAL SYNDROME IN AKHAL-TEKE HORSES

Naked Foal Syndrome (NFS) is a monogenic autosomal recessive condition in Akhal-Teke horses. Affected foals are born hairless and die early in life, most likely due to unspecific pathologies caused by the lack of hair, such as e.g. an impaired thermoregulation. Anecdotal reports indicate that NFS has existed since at least 1938 in the breed.[3] However, due to a lack of genetic analysis tools and insufficient knowledge about hereditary diseases, the condition was not efficiently dealt with and many affected foals were unintentionally bred for more than 70 years.

We conducted a genetic analysis of NFS.[4] Typical for a lethal hereditary disease, it was very difficult to obtain DNA samples from affected foals. We finally received samples from three affected half-siblings and their four non-affected parents. The seven samples of this small family allowed us to perform linkage analysis, which excluded about 75% of the genome.

In purebred animals from closed populations, monogenic autosomal recessive traits typically are caused by the same genetic variant that originates from a single founder animal. The inbreeding in closed populations greatly increases the probability that such recessive deleterious alleles become homozygous in descendants of the founder. This typical situation can be exploited for the genetic analysis in a so-called homozygosity or autozygosity mapping approach. This mapping technique searches for long homozygous genome segments with shared sequence among the cases. The causative variant for a recessive trait is expected to reside within such a shared homozygous interval that is due to identity by descent (IBD) from a common ancestor. We performed homozygosity mapping in the three NFS foals of our study and found six homozygous segments totalling 19 Mb or 0.79% of the genome.

We obtained the most precise final mapping information for NFS by intersecting the linked genome segments with the shared homozygous segments in the three cases. This combined linkage and homozygosity mapping approach localized the causative genetic defect to 18 Mb or 0.75% of the genome with a large potential segment on chromosome 7 and a much smaller potential segment on chromosome 27. The genome segments that remain after the mapping steps are termed critical interval. Narrowing down the size of the critical interval as much as possible is the goal of all positional cloning methodologies.

To identify the disease causing genetic variant, we sequenced the genomes of two affected foals and two obligate carriers and compared the data to 75 genome sequences from horses of other breeds that were assumed to be clear of the disease allele. While this massive dataset contained millions of genetic variants, only four of them fulfilled all our search criteria: (i) homozygous mutant genotype in the two cases, (ii) heterozygous in the two obligate carriers, (iii) homozygous wildtype in the 75 horses from other breeds, (iv) localized in the critical interval.

The four identified candidate variants consisted of one intergenic, two intronic, and one protein-coding exonic

variant, which then became our primary candidate causative variant. This variant, *ST14*:c.388G>T, introduced a stop codon into the open reading frame of the *ST14* gene encoding "suppression of tumorigenicity 14". The genotypes at *ST14*:c.388G>T were perfectly associated with the NFS phenotypes in a cohort of more than 600 horses. Variants in the human *ST14* gene had previously been shown to cause autosomal recessive congenital ichthyosis 11 (ARCI11) in humans. Taken together, this evidence strongly supports the causality of the *ST14*:c.388G>T variant for NFS.[4] Genetic testing is now available, so that risk matings between carriers can be avoided.[5]

STUDIES USING UNRELATED CASES AND CONTROLS – LETHAL ACRODERMATITIS IN BULL TERRIERS

Lethal acrodermatitis (LAD) is a monogenic autosomal recessive genodermatosis in Bull Terriers and Miniature Bull Terriers. Affected puppies show characteristic skin lesions on the feet and on the face, diarrhea, bronchopneumonia, failure to thrive, and a subtle pigment dilution.[6] LAD clinically resembles acrodermatitis enteropathica in humans and a related disease in cattle, which are caused by genetic variants in the *SLC39A4* gene and defective intestinal zinc uptake. However, in contrast to the human and cattle disease, LAD is not responsive to zinc supplementation and affected puppies die early in life. The first scientific description of LAD dates back to 1986.[6]

We conducted a genetic investigation of LAD.[7] Availability of samples from affected dogs was again limited and we were not able to collect sufficiently large and complete families required for linkage analysis. We finally had samples from 22 LAD cases and 48 controls. Typical for purebred dogs, some of these dogs were closely related, for others the exact relationships were unknown. Such a material is suitable to conduct a genome-wide association study (GWAS), another powerful method that can be used to localize the position of a trait causing genetic variant. GWAS unambiguously mapped the LAD locus to chromosome 14. We additionally performed homozygosity mapping as described above, which refined the critical interval to a 1.11 Mb segment on chromosome 14. This corresponded to 0.05% of the entire dog genome.

Whole genome sequencing of one case and comparison to 188 control genomes from other dog breeds revealed only 5 private variants in the LAD affected dog within the critical interval. They consisted of four intergenic variants and one variant close to an exon/intron boundary, *MKLN1*:c.400+3A>C. Additional experiments confirmed that the *MKLN1*:c.400+3A>C variant indeed causes a splice defect and skipping of exon 4 from the *MKLN1* transcript. Genotypes at the *MKLN1*:c.400+3A>C variant showed perfect association with the LAD phenotype in a cohort of more than 800 dogs, which strongly supports the claimed causality of this variant for LAD.[7] Genetic testing for LAD is now available at several diagnostic laboratories.

TRIO ANALYSIS – ICHTHYOSIS IN A GERMAN SHEPHERD

We investigated a single purebred female German Shepherd with a congenital mild ichthyosis. The affected dog was born in a litter together with six normal littermates. Similar cases had not been reported before by breeders or in the scientific literature. In this situation, the mode of inheritance must be considered unknown. The phenotype could have been inherited as a recessive trait. Alternatively, the phenotype might also have been the consequence of a *de novo* mutation event leading to a dominant deleterious allele.

For the genetic analysis, we performed whole genome sequencing on the affected dog and both parents.[8] Such a design is termed "trio analysis" in medical genetics. We searched for candidate causative genetic variants under a recessive and a dominant inheritance model. In the recessive scenario, the putative candidate variant was expected to be homozygous mutant in the case, heterozygous in both parents, and absent from control dogs of other breeds. In the dominant scenario with a *de novo* mutation event, the case should be heterozygous at the causative variant, while both parents should be homozygous wildtype.

The recessive model did not reveal any convincing candidate causative variants. The dominant model yielded a single *de novo* variant in the affected dog. This variant, *ASPRV1*:c.1052T>C, was predicted to change an evolutionary conserved amino acid next to the functionally important cleavage site of the ASPRV1 protein, p.(Leu351Pro). ASPRV1 is a protease required for proper filaggrin processing in the epidermis. We could prove the causality of the detected variant by demonstrating an abnormal distribution of filaggrin in the epidermis of the affected dog.[8]

FUNCTIONAL MOSAICISM IN X-LINKED TRAITS – CORNIFICATION DISORDER IN DOGS AND CATS

Skin lesions in stripes or specific patterns termed Blaschko's lines in female patients provide a strong clue to a possible

underlying X-linked genetic defect. The mechanism behind these patterns is the random X-chromosome inactivation (also called lyonization) during early embryonic development. In heterozygous females, cells with the inactivated X-chromosome carrying the pathogenic variant give rise to normal skin, whereas inactivation of the wild-type X-chromosome results in skin lesions. This leads to a visible functional mosaicism with patches of normal or lesioned skin following the lines of Blaschko.[9,10]

We investigated a female crossbred dog with striking linear hyperplastic and partially alopecic lesions.[11] The lesions were distributed along Blaschko's lines in a bilateral rather symmetrical fashion, and were more evident on the limbs, the head, the neck, and the dorsal trunk. The abdominal and inguinal skin appeared normal. The attending clinician (Michela De Lucia) found out that the mother of the index patient had similar linear lesions. Moreover, two male siblings of the index patient died perinatally. These findings were strong indicators for an X-linked semidominant mode of inheritance. According to this model, heterozygous females will show linear skin lesions, while hemizygous mutant males die shortly after birth.

We sequenced the genome of the affected daughter and searched for heterozygous variants on the X chromosome. This led to the identification of a large deletion truncating the last three exons of the *NSDHL* gene encoding encoding NAD(P) dependent steroid dehydrogenase-like, a 3β-hydroxysteroid dehydrogenase involved in cholesterol biosynthesis. Genetic variants in the human *NSDHL* gene cause CHILD syndrome (congenital hemidysplasia with ichthyosiform nevus and limb defects, OMIM #308050) in humans. CHILD syndrome shows many clinical similarities with the observed cornification defect in the investigated dogs, which supports the claimed causality of the detected NSDHL deletion.[11]

A very similar phenotype was observed in a cat, which was diagnosed multiple congenital lesions resembling inflammatory linear verrucous epidermal nevi (ILVEN). Whole genome sequencing revealed a missense variant in the *NSDHL* gene affecting a functionally important amino acid of the NSDHL enzyme.[12]

CONCLUSION AND OUTLOOK

Veterinary genetics has become an immensely powerful tool to identify causative genetic variants in genodermatoses. Dozens of targeted genetic tests for skin-related traits exist[13] and a positive test result from any of these tests may help to confirm a suspected diagnosis. However, it is now also possible to quickly identify novel deleterious genetic variants or to screen many candidate genes in parallel. It has become technically feasible to obtain whole genome sequence information from single isolated patients. Whole genome sequencing of a single patient has excellent sensitivity to identify the causative variant for a genodermatosis, if this variant affects the protein-coding sequence of a gene with known function in the skin, which should cover the majority of genodermatosis cases in a dermatological clinic.[1,2] Identification of the causative variant becomes more challenging, if it is a non-coding regulatory variant or if the causative variant affects a gene whose function in skin has not yet been recognized (mostly genes for which no human phenotypes have been reported). In these challenging situations, the success rates are still excellent, if several affected patients and/or complete families are available for positional cloning approaches (linkage mapping, GWAS, homozygosity mapping). For isolated cases with novel heritable disorders, trio analysis is another powerful approach that may allow the identification of the disease causing genetic variant. Occasionally, knowledge of the causative genetic variant opens an opportunity for therapeutic intervention. At the very least, knowledge of the causative variant should enable informed breeding decisions and the implementation of breeding programs that minimize the frequency of puppies with heritable diseases.

The scientific methodology to implement whole genome sequencing in veterinary genetic diagnostics is now in place. To make this more accessible to veterinary clinicians, the turnaround times for whole genome sequencing experiments must become faster, so that results can be reported within a few days rather than several weeks or months. Optimization, standardization and automation of data analysis, interpretation, and reporting are dearly needed. Nonetheless, genetic diagnostics of single gene Mendelian diseases may be one of the first applications of personalized medicine.

References

1. Lemke JR, Kernland-Lang K, Hörtnagel K, et al. Monogenic human skin disorders. *Dermatology* 2014; 229: 55-64.
2. Online Mendelian Inheritance in Man. Available at: https://www.omim.org/
3. Kuznetcova Y, Kozyreva M, Aleksandrova N. The Stavropol Sphynx. *Akhal-Teke Inform* 2006: 144–147 (in Russian).
4. Bauer A, Hiemesch T, Jagannathan V, et al. A nonsense variant in the *ST14* gene in Akhal-Teke horses with naked foal syndrome. *G3 (Bethesda)* 2017; 7: 1315-1321.
5. UC Davis Veterinary Genetics Laboratory - Naked Foal Syndrome in Akhal Teke. Available at https://www.vgl.ucdavis.edu/services/horse/nakedfoalsyndrome.php
6. Jezyk PF, Haskins ME, MacKay-Smith WE, et al. Lethal acrodermatitis in bull terriers. *J Am Vet Med Assoc* 1986; 188: 833–839.
7. Bauer A, Jagannathan V, Högler S, et al. *MKLN1* splicing defect in dogs with lethal acrodermatitis. *PLoS Genet* 2018; 14: e1007264.
8. Bauer A, Waluk DP, Galichet A, et al. A *de novo* variant in the *ASPRV1* gene in a dog with ichthyosis. *PLoS Genet* 2017; 13: e1006651.
9. Happle R. X-chromosome inactivation: role in skin disease expression. *Acta Paediatr Suppl* 2006; 95: 16–23.
10. Happle R. The categories of cutaneous mosaicism: a proposed classification. *Am. J. Med. Genet.* A 2016; 170: 452–459.
11. Bauer A, De Lucia M, Jagannathan V, et al. A large deletion in the *NSDHL* gene in Labrador Retrievers with a congenital cornification disorder. *G3 (Bethesda)* 2017; 7: 3115-3121.
12. De Lucia M, Bauer A, Spycher M, et al. Genetic variant in the *NSDHL* gene in a cat with multiple congenital lesions resembling inflammatory linear verrucous epidermal nevi. *Vet Dermatol* 2018; doi: 10.1111/vde.12699.
13. Online Mendelian Inheritance in Animals. Available at https://omia.org/home/

UPDATE ON GENODERMATOSES AND NEW GENETIC TESTS IN VETERINARY DERMATOLOGY

Tosso Leeb, PhD

Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland

INTRODUCTION

In humans, genetic variants in about ~500 genes have been shown to cause heritable skin disorders, also termed genodermatoses.[1,2] Research progress over the last years has revealed many homologous genodermatoses in domestic animals. Interestingly, the research in domestic animals has also identified a few instances of genodermatoses caused by variants in "novel" genes that had not previously been associated with normal skin function. Veterinary genetics thus provides new candidate genes for human dermatology and indeed there are now several instances where findings in domestic animals have helped to establish the genetic diagnosis in human patients.

Two years ago, we gave recommendations on the use of genetic tests and compiled the known causative variants for genodermatoses in cats, dogs, and horses in a review paper.[3] In this lecture, I will give an update with a special emphasis on newly discovered causative variants. Updated lists of causative genetic variants are given for dogs (Table 1), cats (Table 2), and horses (Table 3).

GENETIC TESTS AS DIAGNOSTIC TOOL IN VETERINARY DERMATOLOGY

A substantial number of genodermatoses have now been associated with specific variants in domestic animals, and the number will further increase as gene discovery becomes more efficient. DNA tests can be used like other diagnostic tests to help establish or eliminate differential diagnoses for a particular presenting case.

For example, if a Greyhound with crusts and possibly infected fissures of the nasal planum is presented, the genetic test for hereditary nasal parakeratosis (HNPK) is clearly indicated.[4,5] If this test is positive, the diagnosis is established and more invasive diagnostic procedures, such as taking a biopsy from the nose can be avoided. Thus, it is recommended that clinical dermatologists should stay up-to-date on the ever-expanding list of known causative genetic variants and available genetic tests. The most comprehensive database on Mendelian traits and the underlying causative genetic variants in domestic animals is the OMIA database.[6] Another valuable resource is the MyBreedData database, from which breed-specific frequencies of specific genetic defects can be obtained.[7]

In humans, there is a high degree of genetic heterogeneity. Consequently, unrelated patients with the same genodermatosis due to the altered function of a single gene are likely to have different independent variants in the causative gene. Genodermatoses in purebred animals are much less heterogeneous than in humans. Thus, for many hereditary diseases we find that all affected individuals of one breed carry the same deleterious genetic variant. However, this is not an absolute rule, and it always has to be kept in mind that the currently offered targeted genetic tests typically only interrogate a single position in the genome. Therefore, a positive test result clearly establishes the diagnosis, while a negative test result only excludes one particular genetic defect, but not other unknown variants, which may very well be located in the same gene. Negative genetic test results must therefore be interpreted with care. For the same reason, if a genetic test has been validated in a particular breed, it should not be generally assumed that the test will also work in other breeds. Again, a positive test result is diagnostic, but a negative test result does not eliminate the possibility of a different genetic variant in the same gene to be causative for the disease in the new breed and thus does not exclude all possible genetic defects. In cases in which targeted genetic testing was negative, but a hereditary disease is nevertheless suspected, more comprehensive approaches, such as whole genome sequencing should be considered. In such instances, it may be valuable to consult with a veterinary geneticist, ideally the one who was involved in the identification of the first causative variant in a given candidate gene.

Table 1 Genodermatoses and hair morphology traits with known causative genetic variants in dogs

Phenotype	Gene	Variant[1]	Breed(s)	Inheritance[2]	OMIA[3]
Ichthyoses / disorders of cornification					
Congenital cornification disorder (CHILD-like syndrome)	NSDHL	14 kb deletion	Labrador Retriever	XSD	002117-9615
Ectodermal dysplasia / skin fragility syndrome	PKP1	c.202+1G>C; r.spl	Chesapeake Bay Retriever Golden Retriever	AR	001864-9615
Footpad hyperkeratosis	FAM83G	c.155G>C; p.R52P	Irish Terrier Kromfohrländer	AR	001327-9615
Hyperkeratosis, epidermolytic	KRT10	IVS5+1G>T; r.spl	Norfolk Terrier	AR	001415-9615
Ichthyosis	ASPRV1	c.1052T>C; p.L351P	German Shepherd	AD	002099-9615
Ichthyosis	NIPAL4	variant identified, but not yet published	American Bulldog	AR	001980-9615
Ichthyosis	PNPLA1	c.1445_1447delinsTACTACTA; p.N482Ifs*11	Golden Retriever	AR	001588-9615
Ichthyosis	SLC27A4	c.1250G>A; p.Arg417Gln / r.spl	Great Dane	AR	001973-9615
Ichthyosis	TGM1	1980 bp LINE-1 insertion	Jack Russell Terrier	AR	000546-9615
Keratoconjunctivitis sicca and ichthyosiform dermatosis	FAM83H	c.977delC; p.P326Hfs*258	Cavalier King Charles Spaniel	AR	001683-9615
Nasal parakeratosis	SUV39H2	c.972T>G; p.N324K	Labrador Retriever	AR	001373-9615
		c.996+3_996+6delAAGT; r.spl	Greyhound	AR	001373-9615
Palmoplantar keratoderma, non-epidermolytic	KRT16	complex genomic variant; p.E392*	Dogue de Bordeaux	AR	002088-9615
Epidermolyses and blistering disorders					
Epidermolysis bullosa, dystrophic	COL7A1	c.5716G>A; p.G1906S	Golden Retriever	AR	000341-9615
Epidermolysis bullosa, junctional	LAMA3	6.5 kb insertion	German Pointer	AR	001677-9615
Hyperkeratosis, epidermolytic	KRT10	c.1125+2G>T; r.spl	Norfolk Terrier	AR	001415-9615
Disorders involving altered pigmentation (only coat color variants that are associated with disease)					
Dilute coat color (predisposing risk factor for color dilution alopecia)	MLPH	c.-22G>A; r.spl(?) c.705G>C; p.Q235H	Many breeds	AR (complex)	000031-9615
Oculocutaneous albinism type II (photophobia)	OCA2	c.-45+2T>G; r.spl	Spitz	AR	002130-9615
Oculocutaneous albinism type IV (photophobia and predisposing risk factor for melanocytic neoplasms)	SLC45A2	4.1 kb deletion	Doberman Pinscher	AR	001821-9615
		c.1287delC; p.M430Cfs*4	Bullmastiff	AR	
		c.1487G>A; pG493D	several breeds	AR	
Merle coat color (predisposes for eye and ear defects)	PMEL	SINE insertion	many breeds	ASD	000211-9615

Developmental disorders including defects of the ectodermal appendages					
Dermoid sinus	FGF3, FGF4, FGF19	133 kb genomic duplication	Rhodesian Ridgeback Thai Ridgeback	AR (complex)	000272-9615
Ectodermal dysplasia, hypohidrotic	EDA	c.910-1G>A; r.spl c.842delT; p.L281Hfs*22 r.385_487del; p.M129Vfs*112	German Shepherd Dachshund crossbred dogs	XR XR XR	000543-9615
Ectodermal dysplasia	FOXI3	c.57_63dup7; p.A23fs*219	Chinese Crested Mexican Hairless Peruvian Hairless	ASD	000323-9615
Hair morphology: Curly hair	KRT71	c.451C>T; p.R151W	Many breeds	AR	000245-9615
Hair morphology: Curly hair & risk for follicular dysplasia	KRT71	c.1266_1273delinsACA; p.S422Rfs*212	Many breeds	AR	000245-9615
Hair morphology: Furnishings (wire hair)	RSPO2	167 bp insertion into 3'-UTR	Many breeds	AD	001531-9615
Hair morphology: Long hair	FGF5	c.284G>T; p.C95F c.556_571del16; p.A186Tfs*69 c.559_560dupGG; p.R188Afs*73 c.578C>T; p.A193V c.362-11T>A; r.spl	Many breeds Eurasier Afghan Hound Akita, Samoyed, Siberian Husky Afghan Hound	AR AR AR AR AR	000439-9615
Hypotrichosis	SGK3	4 bp deletion; p.V96Gfs*50	American Hairless Terrier	AR	001279-9615
Connective tissue defects					
Excessive skin and periodic fever	HAS2	16.5 kb genomic duplication	Shar-Pei	ASD	001561-9615
Musladin-Lueke syndrome (geleophysic dysplasia)	ADAMTSL2	c.661C>T; p.R221C	Beagle	AR	001509-9615
Renal cystadenocarcinoma and nodular dermatofibrosis	FLCN	c.764A>G; p.H255R	German Shepherd	AD	001335-9615
Genodermatoses with unclear aetiology					
Lethal acrodermatitis	MKLN1	c.400+3A>C; r.spl	Bull Terrier, Mini Bull Terrier	AR	003126-9615

[1] A detailed description of genetic variant nomenclature can be found at https://varnomen.hgvs.org/. For some large structural variants a simplified variant designation is given.
[2] AD: autosomal dominant; AR: autosomal recessive; ASD: autosomal semi-dominant; XR: X-chromosomal recessive; XSD: X-chromosomal semi-dominant
[3] Online Mendelian Inheritance in Animals, http://omia.angis.org.au/.

Table 2 Genodermatoses and hair morphology traits with known causative genetic variants in cats

Phenotype	Gene	Variant[1]	Breed(s)	Inheritance[2]	OMIA[3]
Ehlers-Danlos syndrome	COL5A1	c.3420delG; p.L1141Sfs*134	domestic shorthair (not purebred)	AD	not yet available
Hairlessness with short life expectancy	FOXN1	c.1030_1033delCTGT; p.L344Gfs*203	Birman	AR	001949-9685
Hairlessness	KRT71	c.816+1G>A; r.[816+1_816+43ins; 816+1g>u]	Sphynx	AR	001583-9685
Hair morphology: Curly hair (rex phenotype)	KRT71	c.1108-4_1184del81insAGTTGGAG; r.1108_1221del	Devon Rex	AR	001581-9685
Hair morphology: Curly hair (rex phenotype)	KRT71	c.445-1G>C; r.445_464del	Selkirk Rex	AD	001712-9685
Hair morphology: Curly hair (rex phenotype)	LPAR6	c.250_253_delTTTG; pF84Efs*9	Cornish Rex, German Rex	AR	001684-9685
Hair morphology: Long hair	FGF5	c.356insT; p.M119Ifs*43	Maine Coon, Ragdoll	AR	000439-9685
		c.406C>T; p.R136*	Norwegian Forest Cat	AR	
		c.474delT; p.F158Lfs*104	Ragdoll	AR	
		c.475A>C; p.T159P	Many breeds	AR	
Inflammatory linear verrucous epidermal nevi	NSDHL	c.397A>G; p.S133G	domestic shorthair (not purebred)	XSD	not yet available

[1] A detailed description of genetic variant nomenclature can be found at https://varnomen.hgvs.org/. For some large structural variants a simplified variant designation is given.
[2] AD: autosomal dominant; AR: autosomal recessive; XSD: X-chromosomal semi-dominant
[3] Online Mendelian Inheritance in Animals, http://omia.angis.org.au/.

Table 3 Genodermatoses and hair morphology traits with known causative genetic variants in horses

Phenotype	Gene	Variant[1]	Breed(s)	Inheritance[2]	OMIA[3]
Brindle 1	MBTPS2	c.1437+4T>C; r.spl	Quarter Horse and crossbreds	XSD	002021-9796
Coat color, grey (predisposes to benign melanoma)	STX17	4.6 kb intronic duplication	many breeds	ASD	001356-9796
Coat color, leopard complex spotting (predisposes to congenital stationary night blindness)	TRPM1	1.4 kb insertion	many breeds	ASD	002139-9796
Coat color, silver (predisposes to eye anomalies)	PMEL	c.1873C>T; p.R625C	many breeds	ASD	001438-9796
Coat color, white spotting with risk for deafness	MITF	c.519_523del5; p.C174Sfs*20	Quarter horse	AD (ASD)	000214-9796
		c.629A>G; p.N210S	Franches-Montagnes	AD (ASD)	
		Large deletion	American Paint Horse	AD (ASD)	
Coat color, white spotting with risk for deafness	PAX3	c.209G>A; p.C70Y	many breeds	AD (ASD)	001688-9796
		c.95C>G; p.P32R	Appaloosa	AD (ASD)	
Ehlers-Danlos syndrome ("WFFS")	PLOD1	c.2032G>A; p.G678R	Warmblood	AR	001982-9796
Ehlers-Danlos syndrome ("HERDA")	PPIB	c.115G>A; p.39G>R	Quarter Horse	AR	000327-9796
Epidermolysis bullosa, junctional	LAMA3	6.6 kb genomic deletion	American Saddlebred	AR	001677-9796
Epidermolysis bullosa, junctional	LAMC2	c.1368C[5]>[6]; p.R458Pfs*27	Belgian Draft Horse, Trait Breton, Trait Comtois	AR	001678-9796
Hair morphology: Curly hair	KRT25	c.266G>A; p.R89H	Curly Horse	AD	000245-9796
Hoof wall separation syndrome	SERPINB11	c.504_505insC; p.T169Hfs*3	Connemara Pony	AR	001897-9796
Incontinentia pigmenti	IKBKG	c.184C>T; p.R62*	Quarter Horse, Warmblood	XSD	001899-9796
Lavender foal syndrome	MYO5A	single base deletion	Arabian	AR	001501-9796
Naked foal syndrome	ST14	c.388G>T; p.E130*	Akhal-Teke	AR	002096-9796

[1] A detailed description of genetic variant nomenclature can be found at https://varnomen.hgvs.org/. For some large structural variants a simplified variant designation is given.
[2] AD: autosomal dominant; AR: autosomal recessive; ASD: autosomal semi-dominant; XSD: X-chromosomal semi-dominant
[3] Online Mendelian Inheritance in Animals, http://omia.angis.org.au/.

References

1. Lemke JR, Kernland-Lang K, Hörtnagel K, et al. Monogenic human skin disorders. *Dermatology* 2014; 229: 55-64.
2. Online Mendelian Inheritance in Man. Available at: https://www.omim.org/
3. Leeb T, Müller EJ, Roosje P, et al. Genetic testing in veterinary dermatology. *Vet Dermatol* 2017; 28:4-e1.
4. Bauer A, Nimmo J, Newman R, et al. A splice site variant in the *SUV39H2* gene in Greyhounds with nasal parakeratosis. *Anim Genet* 2018; 49: 137-140.
5. Jagannathan V, Bannoehr J, Plattet P, et al. A mutation in the SUV39H2 gene in Labrador Retrievers with hereditary nasal parakeratosis (HNPK) provides insights into the epigenetics of keratinocyte differentiation. *PLoS Genet* 2013; 9: e1003848.
6. Online Mendelian Inheritance in Animals. Available at https://omia.org/home/
7. MyBreedData. Available at https://www.mybreeddata.com/crm/index.html

Unconventional Alternatives to Conventional Antibiotics: A Glance at the Pipeline

Sheila Torre, DVM

The era of antibiotic resistance:
Systemic antibiotic therapy has been used for more than 50 years and has saved many lives. Unfortunately, the non-judicious use of antibiotic has resulted in the widespread bacterial resistance that has seriously impacted patient care around the world. According to the Center of Disease Control and Prevention (CDC) about 2 million people acquire resistant bacterial infections every year and more than 20,000 die. To add to this serious problem, pharmaceutical companies have only developed few new classes of antibiotic since the late 1980s mainly because of the high production costs and the long period of time elapsed before the product reaches the market. In response to this concerning situation, researchers in academia and pharmaceutic companies have worked tirelessly to develop antimicrobial alternatives to chemical antibiotics. The pipeline is full with various new and old compounds at various phases of development and investigation. We will review three alternatives to conventional antibiotics including: (i) antimicrobial peptides; (ii) bacteriophages; and (iii) virolysins.

Antimicrobial peptides (AMP):
About their properties and biologic function: AMP are essential part of the innate immune response of all living organisms. They have been isolated from bacteria, archea, plants, invertebrates and vertebrates including humans and various domestic animals. AMP are small molecules of 100 or less amino acids. Most are cationic and amphipathic, which are important properties for their biologic function. They only target negatively charged membranes that do not contain cholesterol. This selective activity explains why AMP do not attack mammal's cell membranes which have neutral charge and are rich in cholesterol. The antimicrobial spectrum of AMP is broad and include activity against bacteria, fungi, viruses and protozoa. They act before the doubling time of bacteria. An important function of some AMP is the activation of the innate and adaptive immune responses, bringing various inflammatory cells to the site of infection and inducing the secretion of various inflammatory cytokines. For this function, they are known as "host defense peptides". In addition, certain AMP can promote wound healing, inhibit biofilm formation, kill cancer cells and neutralize lipopolysaccharide (LPS) and lipoteichoic acid (LTA) effects.

About their antimicrobial mechanism of action: Briefly, the first step is the electrostatic attachment of the cationic peptide to the anionic phospholipid head of the cell membrane. Following the attachment, the hydrophobic portion of the peptide inserts into the lipid bilayer and interacts with the hydrophobic acyl chains of the phospholipid membrane forming pores which results in membrane depolarization, leakage of cell content and ultimately cell death. Some non-membrane active AMP will penetrate the cell membrane and inhibit various metabolic functions crucial for the bacterial survival.

Obstacles to commercially produce AMP: Pharmaceutical companies have been investigating these molecules for many years and have encountered various obstacles on the way, including: (i) very little pharmacokinetics and pharmacodynamics studies done; (ii) potential for sensitization; (iii) potential for toxicity as physicochemical properties are modified; (iv) short half-life due to proteases destruction; and (v) high production cost.

Solutions to overcome the obstacles: Pharmaceutical companies are developing small molecules with physicochemical and biological properties that mimic the native AMP. These peptidomimetic are more stable because they are not destroyed by proteases and are easier and cheaper to develop. Another solution is the use of microencapsulation of the AMP via various nanoformulations platforms such as, liposome, polymer nanoparticle, nanoemulsion, and micelle. These nanoformulations will provide drug protection against proteases; increase drug availability at the infection site and drug distribution (i.e. increase their concentration at the infection sites). Finally, multiple N-methylation of the molecule has been shown to increase oral bioavailability and metabolic stability of the natural AMP. In addition, exchange of L- to D- amino acid forms will increase stability because proteases are specific for L-amino acid forms.

What is currently available? Niasin has been used as a food preservative for more than 50 years. It is also available as

wipes to help prevent bovine mastitis. Polymixin B is available as topical therapy for eye and ear infections and has been sporadically used systemically to treat multidrug resistant bacterial infections. Gramicidin is currently available to treat eye infections. Ceragenin (Ceragyn™), a peptidomimetic similar in physicochemical properties and biological function to the native AMP, is currently available in various formulations (e.g. spray to treat infected wounds; ear cleanser; eye drops for irritated eyes, etc).

Can bacteria induce resistance against AMP? Considering its non-specific mechanism of action, it is difficult for bacteria to induce resistance against AMP. However, albeit rare, resistance can occur via the following mechanisms: (i) inhibition of AMP by bacterial surface-associated capsular polysaccharide; (ii) degradation of AMP by bacterial proteolytic enzymes (e. g. *staphylokinase*); (iii) sequestration or trapping of AMP by pilus or fimbriae present in certain bacteria; (iv) modification of bacterial cell wall and cell membrane charge and fluidity (v) use of ABC efflux transport systems to expel AMP from bacterial cytoplasm.

Bacteriophages:

What are bacteriophages? Bacteriophages or phages are viruses that infect and kill bacteria by lyses. They are the most abundant bioagent on earth. Bacteriophages were discovered independently by the British bacteriologist, Frederick Twort (1915), and the French Canadian microbiologist, Felix d'Herelle
(1917). However, d'Herelle was the one who saw the value of using bacteriophages to treat bacterial infections. Two years after his discovery, d'Herelle started using bacteriophage to combat dysentery in France and cholera epidemics in India. Thus, phage therapy has been used for 100 years. For a period of time before the discovery of penicillin, bacteriophage was the most important treatment for bacterial infection in the eastern and western countries. However, after the discovery of various chemical antibiotics and the advent of the Cold War, bacteriophage therapy became limited to the eastern world, especially Russia, Georgia and Poland. However, the interest in this treatment modality revitalized in the western countries with the advent of bacterial resistance.

Mechanism of action: The infection process starts with the bacteriophage binding to a specific receptor on the bacterial cell surface. Thereafter, the bacteriophage injects its genetic material into the bacterial cytoplasm. Virulent or lytic bacteriophages will immediately hijack the bacterial replication, transcription, and translation machineries for the production of numerous virions and enzymes (holin and lysine) needed to allow the viral particles to extrude the bacterium host. Once released the bacteriophages will continue to infect bacteria and exponentially replicate until the bacteria are completed eliminated from the site of infection. The infection is very specific, meaning; a certain bacteriophage will only infect a certain bacterial species or strain. If the bacteriophage is temperate or lysogenic, it will incorporate into the bacterial genome and will only replicate when the bacterium host replicates. Virulent or lytic bacteriophages are recommended for therapy not, lysogenic or temperate phages.

What are the pros and cons of phage therapy? **Pros**: (i) narrow host range - prevents damage to the bacterial microbiota; (ii) self-replication - allows for low dose; (iii) easy to isolate – phage are numerous and readily isolated; (iv) easy to manipulate – phages can be modified by a host of techniques (in vivo or in vitro) to generate therapeutically enhanced phage; (v) cheaper to produce than chemical antibiotics; (vi) low risk for development of resistance; safe – phage therapy has been in place for 100 years and has shown to have a very good safety profile. **Cons**: (i) narrow host range – it only infect a specific bacterial specie or strain; this issue can be circumvented by the use of phage cocktails; (ii) need to know the specific disease agent – this issue can be circumvented with the use of phage cocktails; (iii) transfer of virulence factors - temperate phages either encode or mobilize bacterial virulence factors; improper selection of phages could lead to increased virulence of infected bacteria; (iv) release of endotoxins –large scale bacterial lyses, specially gram negative bacteria, could result in the release of significant amounts of toxic cellular components; (v) variable shelf-life – some bacteriophages need to be refrigerated to maintain activity.

What is currently available? Currently in the USA, phages are solely approved for use in the food industry to prevent contamination by bacteria. Examples are: ListShield® for the control of food contamination with *Listeria* spp; EcoShield® for the control of contamination with *E. coli*, and SalmoFresh® for the control of food contamination with *Salmonella* spp. Veterinarians are familiar with a product called Staphage Lysate® which is used to help prevent recurrences of bacterial skin infections caused by *Staphylococcus pseudintermedius*. This product contains Staphylococcus aureus cell lysates and polyvalent virulent bacteriophages specific against *Staphylococcus*

aureus. Because the action of bacteriophages is highly specific, the effect of Staphage Lysate is not related to the bacteriophages present in the compound. The mechanism of action of Staphage Lysate is not known but believed to be related to the stimulation of the immune response by *Staphylococcus aureus* antigens.

Virolysins:

What are virolysins? Virolysins are bacteriophage-encoded peptidoglycan hydrolyses that lyse bacteria. They are synthesized in bacteriophage-infected bacterium at the end of the bacteriophage multiplication cycle. These enzymes usually have two distinct domains: a N-terminal enzymatically-active domain and a C-terminal cell wall-binding domain connected by a short, flexible linker region. The N-terminal enzymatically-active domain is responsible for catalyzing the breakdown of specific peptidoglycan bonds, while the C-terminal cell wall-binding domain recognizes and binds non-covalently to substrate within the cell wall, resulting in the specificity of the lytic enzymes for the target host.

What are the pros and cons of virolysins therapy? **Pros**: (i) narrow spectrum of action – the action is limited to species and genus level which preserves the bacterial microbiota; (ii) fast action of kill – virolysins act within seconds; (iii) difficult to induce resistance - no bacterial resistance to virolysins has been reported likely due to the co-evolution process of billions of years established between bacteriophages and bacteria, leading to the development of a C-terminal binding region highly specific to molecules present on the wall of the bacterial host that are essential to its viability; (iv) good safety profile - a number of pre-clinical trials in vivo have shown that antimicrobial therapy with lysins do not produce concerning side effects; (v) readily available - bacteriophages are the most abundant biological agents on the planet, thus; there is a considerable number of virolysins available for therapeutic applications; (vi) easy to synthesize – lysins have simple synthesis processes and can be synthesized and purified in high quantities at a reduced cost rendering them an excellent and innovative antimicrobial therapeutic strategy; (vii) synergism with antibiotics. **Cons**: (i) narrow spectrum of action - the action of virolysins is limited to a specific bacterial species or genus; however, a mixture of virolysins that have the same binding domain and different catalytic domains can help overcome this issue; (ii) not effective against gram negative bacteria - when virolysins are administered exogenously, they are only active against Gram-positive bacteria because Gram-negative bacterial cells have an outer and impermeable membrane to these enzymes; (iii) immunogenic - unlike chemical antibiotics, which are small and non-immunogenic molecules, virolysins are peptides and, as such, can stimulate the immune response leading to the formation of antibodies that can reduce the activity of the lysin in vivo. However, various studies have shown that the antibodies may delay but, do not neutralize the effects of virolysins.

To increase the spectrum of action and catalytic activity of virolysins, they have been engineered by shuffling and fusing the binding (C-terminal) and catalytic (N-terminal) domains of the peptidoglycan hydrolyzes to create chimeric enzymes. This can be done by adding or switching a heterologous catalytic domain of a specific binding domain or vice-versa. Changes have also been made to increase solubility and subsequently stability of the enzymes. Virolysins have also been engineered to become effective against Gram-negative bacteria.

Selected references

Da Cunha NB, Cobacho NB, Viana JFC et al. The next generation of antimicrobial peptides (AMPs) as molecular therapeutic tools for the treatment of diseases with social and economic impacts. Drug Discovery Today 2017; dx.doi.org/10.1016/j.drudis.2016.10.017

Hawkins C, Harper D, Burch D, et al. Topical treatment of Pseudomonas aeruginosa otitis of dogs with a bacteriophage mixture: A before/after clinical trial. Veterinary Microbiology 2010; doi: 10.1016/j.vetmic.2010.05.014

Kakasis A and Panitsa G. Bacteriophage therapy as an alternative treatment for human infections. A comprehensive review. International Journal of Antimicrobial Agents 2018; doi.org/10.1016/j.ijantimicag.2018.09.004

Kumar P, Kizhakkedathu JN and Straus SK. Antimicrobial peptides: Diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. Biomolecules 2018; doi: 10.3390/biom8010004

Lossner MJ. Bacteriophage endolysins – current state of research and applications. Current Opinion in Microbiology 2005; 8:480-487 Love MJ, Bhandari D, Dobson RCJ et al. Potential of bacteriophage endolysis to supplement or replace antibiotics in food and clinical care. Antibiotics 2018; doi: 10.3390/antibiotics7010017

Moravej H, Morave Z, Yazdanparast M et al. Antimicrobial peptides: Features, action, and their resistance mechanisms in bacteria. Microbial Drug Resistance 2018; doi: 10.1089/mdr.2017.0392

Pastagia M, Euler C, Chahales P, et al. A novel chimeric lysin shows superiority to mupirocin for skin decolonization to methicillin-resistant and –sensitive Staphylococcus aureus strains. Antimicrobial Agents and Chemotherapy 2011; doi: 10.1128/AAC.00890-10

Rios AC, Moutinho CG, Pinto FC, et al. Alternative to overcoming bacterial resistances: State-of-the-art. Microbiology Research 2016; dx.doi.org/10.1016/j.micres.2016.04.008

Rohde C, Wittmann J and Kutter E. Bacteriophages: A therapy concept against multi-drug-resistant bacteria. Surgical Infections 2018; doi: 10.1089/sur.2018.184

Sao-Jose C. Engineering of phage-derived lytic enzymes: Improving their potential as antimicrobials. Antibiotics 2018; doi: 10.3390/antibiotics7030056

Picking the Wolves from the Sheep: Prognostic Markers in Canine Cutaneous Mast Cell Tumors

Michael O. Childress, DVM, MS, DACVIM (Oncology)

Introduction

Mast cell tumors (MCT) are among the most common cutaneous tumors in dogs. They are notoriously diverse in their biological behavior, yet most can be categorized into two clinically distinct groups: 1.) Relatively small, slow-growing, non-invasive tumors that are cured by complete surgical excision, and, 2.) Larger, rapidly growing, invasive, and/or metastatic tumors that are highly unlikely to be surgical curable. Numerous prognostic markers have been proposed as tools for helping clinicians decide to which of the two groups an individual MCT belongs. Of these, the most consistent predictor of a MCT's malignant potential MCT is its *histopathologic grade*.

There are two commonly used systems for specifying the histopathologic grade of a MCT. The first of these, proposed by Patnaik[1] in 1984, was widely used for decades. One of its shortcomings, however, is that it does not accurately predict the clinical behavior of many intermediate-grade ("grade 2") MCT. A two-tiered grading system more recently proposed by Kiupel[2] appears to provide greater clarity in predicting the behavior of these tumors. However, it should be noted that no single feature can predict the behavior of MCT with complete certainty. Therefore, clinicians must be mindful of several clinical and pathological features of MCT when trying to distinguish those tumors that are surgically curable from those that are not. This session will summarize recent research that reshapes our understanding of canine MCT, particularly in how we may better predict the clinical behavior of these tumors, and thereby optimize therapy for them.

Histopathologic Grading Systems for Canine Mast Cell Tumors

Historically the most widely used histopathologic grading system for MCT was that developed by Patnaik.[1] The Patnaik system classifies MCT as either low-grade (grade 1), intermediate-grade (grade 2), or high-grade (grade 3) based upon several histomorphologic features. Higher Patnaik grade is correlated with a more aggressive biological behavior and shorter survival.[1] For example, over 90% of dogs with Patnaik grade 1 MCT survive longer than 1500 days following surgical removal of their tumor, implying that the vast majority of these tumors are surgically curable. In contrast, greater than 90% of dogs with Patnaik grade 3 MCT experience cancer-related death within 6 months following surgical removal of their tumor, implying that surgery alone rarely cures grade 3 tumors. In contrast to Patnaik grade 1 and grade 3 tumors, Patnaik grade 2 tumors have less predictable behavior. Approximately 50% of dogs with Patnaik grade 2 tumors are seemingly cured by surgical tumor removal. However, many of the remaining half of these dogs go on to experience tumor-related death. This implies that some grade 2 tumors behave like grade 1 tumors and some behave like grade 3 tumors. Differentiating which grade 2 tumors will behave in indolent vs. aggressive fashion has historically been challenging, and has confounded the creation of uniform treatment recommendations for dogs with Patnaik grade 2 MCT.

The Kiupel grading system[2] attempts to address this problem by eliminating the "intermediate grade" category altogether, simply categorizing MCT as either "high-grade" or "low-grade." When applied to 95 MCT from a population of dogs treated by surgical excision alone, the Kiupel grade was strongly associated survival: dogs with high-grade tumors experienced a median survival time of less than 4 months, while dogs with low-grade tumors experienced a median survival time of greater than 2 years.[2] Subsequent studies[3,4] have shown that the Kiupel system accurately identifies a proportion of Patnaik grade 2 MCT with a more aggressive biological behavior, and thus helps to clarify the need for additional therapy following surgery in dogs with these tumors. Thus, although many pathologists still report MCT grading according to both the Patnaik and Kiupel systems, the latter appears to have greater relevance to making clinical treatment decisions.

The Importance of Mitotic Index

Historically, tumor grade has been considered the most important predictor of outcome for dogs with cutaneous MCT. More contemporary studies[5,6] have demonstrated that mitotic index is also highly predictive of MCT behavior. *Mitotic index* is defined as the number of mitotic figures observed in 10 high-power (400X) fields (hpf), and is assessed in the

region of the tumor where the greatest mitotic activity is observed at low magnification. In one study, the median survival time for dogs whose tumor had a mitotic index of > 5 mitoses/10 hpf was 2 months, as compared to 70 months for dogs whose tumor had a mitotic index of ≤ 5 mitoses/10 hpf.[5] In another report, MCT were separated into three groups based upon mitotic index. Median survival times were 3 months, 15 months, and not reached (i.e. fewer than 50% of dogs had died) for dogs whose MCT had mitotic indices of > 7 mitoses/10 hpf, 1-7 mitoses/10 hpf, and 0 mitoses/10 hpf, respectively.[6] In both of these reports, mitotic index predicted survival time irrespective of Patnaik grade. Thus, mitotic index may help to discern those Patnaik grade 2 MCT that are surgically curable from those which are not. However, it should be noted that some studies[7,8] show mitotic index to be a specific, but not sensitive, predictor of aggressive mast cell tumor behavior, suggesting that some mast cell tumors with low mitotic indices can still behave aggressively.

This limitation to using mitotic index for prognostication is partially addressed by the Kiupel grading system. Mitotic index is a criterion for grade assignment in this system, using the cut-point of < 7 or ≥ 7 mitoses/10 hpf to classify tumors as low- and high-grade, respectively. However, other histologic features conferring a high grade in the Kiupel system include: 1.) ≥ 3 neoplastic cells with at least 3 nuclei in 10 hpf; 2.) ≥ 3 bizarre nuclei in 10 hpf; and 3.) presence of karyomegaly in the neoplastic cell population, meaning that the nuclei of ≥ 10% of the cells in the tumor vary in size by at least twofold. Any one of these features – including mitotic index ≥ 7 – is sufficient to warrant a high grade in the Kiupel system. Using a combination of these histologic features to assign a tumor grade, rather than relying solely on mitotic index, may help to explain the Kiupel system's greater prognostic relevance when compared to the Patnaik system, as 30% of high-grade tumors classified by the former have a mitotic index < 7.[2] In these cases, which may be incorrectly classified as surgically curable by the Patnaik system, the high histopathologic grade trumps the low mitotic index, prompting the clinician to pursue additional therapy following surgical excision.

Other Clinical Features and Molecular Markers of Tumor Behavior

No single factor can entirely predict the clinical behavior of cutaneous MCT in dogs. However, the behavior of the vast majority of MCT can likely be predicted by a evaluating a combination of clinical and histopathologic characteristics of the tumor. Furthermore, most studies[1,8] suggest that the majority of cutaneous MCT in dogs have follow a benign clinical course, and are cured by surgical excision. Clinical factors associated with a more aggressive MCT include large size, rapid growth rate, surface ulceration, advanced clinical stage (i.e. presence of metastatic disease), recurrence following prior surgical excision, and presence of paraneoplastic syndromes such as gastrointestinal ulceration or coagulopathy. MCT at certain anatomic sites, such as the muzzle, nailbed, prepuce, scrotum, and mucocutaneous or visceral locations also may be more likely to be aggressive. All of these clinical factors should be weighed against the histopathologic findings when attempting to judge whether a MCT will behave aggressively and require additional therapy following surgical removal. The Kiupel grade (or Patnaik grade with evaluation of mitotic index) of the tumor usually provides sufficient complementary information to the clinical evaluation to make this judgement.

The aforementioned clinical and histopathological characteristics can be routinely assessed by most clinicians and diagnostic pathologists. It is the author's belief that a global assessment of these features will accurately predict the behavior of the vast majority of canine MCT. However, there are some MCT for which even such thorough prognostic assessment will fail. For example, one study[8] showed that 13% (11/82) of dogs with Kiupel low-grade MCT still went on to experience tumor-related death. To identify these tumors – the proverbial wolves in sheep's clothing – other molecular diagnostic techniques may be useful. These techniques include counting the proportion of tumor cells expressing cellular proliferation markers (e.g. Ki-67, AgNOR, or MCM7),[8-10] immunohistochemical detection and subcellular localization of the c-kit oncoprotein,[11] and assessment for the presence of activating mutations in the c-kit proto-oncogene.[3,10]

Each of these diagnostic tests has shown some ability to estimate the prognosis for dogs with cutaneous mast cell tumors. What is less clear is how much *value* these tests add to routine prognostic assessment, particularly in cases graded using the Kiupel system. Some studies[8,10] have suggested that assessing several of these prognostic markers in combination allows improved detection of those Kiupel low-grade tumors that will behave aggressively. However, these likely account for only a small proportion of all MCT encountered in dogs, and the diagnostic testing needed to make such refined prognostic assessment is expensive. Accordingly, the author usually reserve such analyses for clients

with a keen desire to collect all possible information about their dog's tumor before making treatment decisions, or for dogs whose tumors display discordant clinical behavior and histopathologic features (e.g. a rapidly-growing, ulcerated mast cell tumor that is interpreted as histologically low-grade (Patnaik or Kiupel)). For more information, interested clinicians are directed to reference laboratories currently offering these additional prognostic tests.[12-14]

Exceptions to the Rule – "Non-gradable" Canine Mast Cell Tumors

A little-recognized nuance in applying Patnaik and Kiupel's grading systems lies in their having been created by reviewing the histopathologic features of MCT originating only in the haired skin.[1,2] Mast cell tumors arising in the subcutis, mucocutaneous tissues (e.g. gingiva), or viscera were not included in these reviews, thus many pathologists feel it is inappropriate to assign a grade to MCT arising at non-cutaneous sites. This becomes particularly important in the clinical management of primary subcutaneous MCT. Contemporary reports have characterized primary subcutaneous MCT, which *originate* in the subcutis, as a biological entity distinct from cutaneous MCT that *invade* the subcutis from the overlying dermis.*15,16* This is an important distinction, as primary subcutaneous MCT appear to have a biologic behavior distinct from that of cutaneous MCT.

Grossly, primary subcutaneous MCT usually present as soft, fluctuant, freely movable masses. These clinical features make them easy to confuse with benign subcutaneous lipomas. Subcutaneous MCT frequently have an indolent clinical behavior. In two studies describing clinical outcome for approximately 350 dogs with primary subcutaneous MCT, long-term survival following surgical excision was the norm. The median survival time was 1,199 days in one of these studies,[15] while in the other, the 5-year survival rate was 86%.[16] Moreover, in each study, over 50% of tumors were excised with microscopically incomplete margins, yet local recurrence rates were less than 10%, suggesting that most primary subcutaneous MCT can be cured by conservative surgery alone.

Although most primary subcutaneous MCT behave in benign fashion, they are life-threatening in a minority of affected dogs. Because these tumors cannot technically be graded using either the Patnaik or Kiupel system, identifying those subcutaneous MCT likely to result in tumor-related death can be challenging. However, high mitotic index (>4 mitoses/10 hpf), infiltrative histologic growth pattern, and presence of multinucleated tumor cells are histopathologic criteria associated with a more aggressive behavior for primary subcutaneous MCT.[16] Tumors displaying these features may be candidates for adjuvant chemotherapy or radiotherapy following surgical removal.

References

1. Patnaik AK et al. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet Pathol* 1984;21:469-474.
2. Kiupel M et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol* 2011;48:147-155.
3. Takeuchi Y et al. Validation of the prognostic value of histopathological grading or c-kit mutation in canine cutaneous mast cell tumors. *Vet J* 2013;196:492-498.
4. Sabattini S et al. Histologic grading of canine mast cell tumor: Is 2 better than 3? *Vet Pathol* 2015;52:70-73
5. Romansik EM et al. Mitotic index is predictive for survival for canine cutaneous mast cell tumors. *Vet Pathol* 2007;44:335-341.
6. Elston LB et al. The importance of the mitotic index as a prognostic factor for survival of canine cutaneous mast cell tumors: a validation study. *Vet Pathol* 2009;46:362-365.
7. Van Lelyveld S et al. Comparison between Ki-67 index and mitotic index for predicting outcome in canine mast cell tumours. *J Small Anim Pract* 2015;56:312-319.
8. Berlato D et al. Comparison of minichromosome maintenance protein 7, Ki67, and mitotic index in the prognosis of intermediate Patnaik grade cutaneous mast cell tumors in dogs. *Vet Comp Oncol* 2018;16:535-543.
9. Berlato D et al. Comparison of mitotic index and Ki67 index in the prognostication of canine cutaneous mast cell tumors. *Vet Comp Oncol* 2015;13:143-150.
10. Webster JD et al. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-kit and its role in prognostication. *Vet Pathol* 2007;44:298-308.

11. Kiupel M et al. The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. *Vet Pathol* 2004;41:371-377.
12. Michigan State University Diagnostic Center for Population and Animal Health. https://cvm.msu.edu/vdl/laboratory-sections/anatomic-surgical-pathology/biopsy-service/prognosis-of-canine-cutaneous-mast-cell-tumors
13. Colorado State University Clinical Immunology Laboratory. http://csu-cvmbs.colostate.edu/academics/mip/ci-lab/Pages/C-KIT-Instructions.aspx
14. Idexx Laboratories, Inc. https://www.idexx.com/files/canine-mct.pdf
15. Newman SJ et al. Canine subcutaneous mast cell tumour: diagnosis and prognosis. *J Comp Path* 2007;136:231-239.
16. Thompson JJ et al. Canine subcutaneous mast cell tumor: characterization and prognostic indices. *Vet Pathol* 2011;48:156-168.

Choosing the Right Treatment for Dogs with Cutaneous Mast Cell Tumors

Michael O. Childress, DVM, MS, DACVIM (Oncology)

Introduction

Developing a sound treatment plan for dogs with cutaneous mast cell tumors (MCT) is sometimes challenging, owing to the tremendous variability in these tumors' clinical behavior. As with all cancers, treatment plans for dogs with MCT should be developed in the context of a complete histopathologic diagnosis and adequate clinical staging data. Assessing dogs for the presence of negative prognostic indicators (reviewed in the previous lecture in this series) is also essential prior to initiating therapy. Surgical removal is the best initial treatment for the majority of canine MCT. However, clinicians must exercise judgement when deciding whether a MCT can be treated effectively with surgery alone, or whether it requires therapy in addition to or as an alternative to surgery. This session will review common treatment options for mast cell tumors and discuss their appropriate use.

The Basics – Getting a Histopathologic Diagnosis and Performing Clinical Staging

There is little doubt that a thorough, well-written histopathology report is indispensable to managing dogs with cutaneous MCT. A complete histopathology report for MCT should always describe *the tumor grade (Patnaik or Kiupel), mitotic index, and, for excisional biopsy specimens, completeness of surgical excision (i.e. surgical margins)*. If all of this information is not conveyed in the histopathology report, the clinician should contact the pathologist to request its reporting.

The extent of clinical staging recommended for dogs with MCT is dependent upon the presence or absence of negative prognostic factors, including information conveyed in the histopathology report. Unlike most cancers, in which thorough clinical staging should precede surgical tumor removal, it is acceptable to perform minimal staging prior to the removal of many MCT (see section on surgery below). Staging tests for dogs with MCT amenable to wide excision and not displaying negative prognostic factors can be as simple as routine hematology/serum chemistry testing and fine needle aspirate cytology of the regional lymph node. In cases where negative prognostic factors are present or the tumor is not amenable to wide excision, more thorough staging is recommended. Additional staging tests for these cases can include thoracic and abdominal imaging, fine needle aspirate cytology of the liver and spleen, and bone marrow aspirate cytology. An important concept to keep in mind is that the thoroughness of staging ultimately should be dictated by the planned aggressiveness of therapy. Dogs for which aggressive, multimodal therapy is planned should undergo the complete battery of tests described above. Conversely, extensive staging is unlikely to meaningfully inform treatment planning for dogs whose owners have elected palliative therapy only.

Surgery for Cutaneous Mast Cell Tumors – How Much is Enough?

The guidelines for surgical removal of mast cell tumors have undergone considerable revision in recent years, and indeed continue to be a topic of debate. Traditional recommendations for curative-intent surgical removal of MCT stipulate excisional margins of at least 3 cm laterally and one fascial plane deep to the tumor.[1] While these excisional margins are likely to effect surgical cure for many MCT, they are not feasible in certain anatomic sites, such as the head, distal limbs, and perineal/inguinal regions. Moreover, they may constitute surgical "overkill" for many MCT, increasing treatment-related morbidity without significantly improving the odds of cure when compared to more conservative surgery.

In 2004 and 2006, two independently conducted studies[2,3] concluded that lateral margins of 2 cm and a deep margin of 1 fascial plane were sufficient to surgically cure the vast majority of Patnaik grade 1 and 2 MCT. A more recent report[4] suggests that lateral margins of ≥ 1cm and deep margins of ≥ 4 mm are adequate to completely excise most Patnaik grade 1 and 2 MCT. Finally, a report[5] in 2013 indicated that excising MCT with lateral margins equivalent to the widest tumor diameter and a deep margin of 1 fascial plane was sufficient to locally control the majority of cutaneous MCT. These reports are particularly relevant when planning surgical removal of small, slowly growing, non-invasive MCT in anatomically challenging locations such as the face, distal limbs, and perineum. In these instances, excision of the tumor with narrower margins that were historically recommended should afford adequate long-term tumor control, while at the same time not compromising functional or cosmetic outcome for the patient. It should be noted

that these narrower margins are NOT recommended for excising invasive or high-grade (Patnaik grade 3) mast cell tumors, which almost always require wider surgical margins to achieve adequate local control.

Interpreting Surgical Margins from Histopathology Reports

One of the greatest challenges to making treatment recommendations for dogs with cutaneous MCT lies in the interpretation of surgical margins from histopathology reports. At the core of this challenge is the fact that an evidence-based histological margin width that assures complete surgical excision has not been defined. Thus, while current guidelines[6] emphasize the importance of reporting surgical margins in quantitative terms (e.g. millimeters of tumor-free tissue at the specimen margin), it is not clear what the critical quantity should be. In fact, two recent studies[7,8] suggest that, for lower grade (Patnaik grade 1 and 2) MCT, *any* measure of tumor-free margin is sufficient to afford a very low risk of tumor recurrence. In these two studies, the rates of recurrence were 2.5% and 4%, respectively. Conversely, the rate of recurrence for similar tumors with histologically incomplete margins in one of these studies[8] was 11%, and recurrence rates as high as 38% have been reported[9] for this population of MCT. Adjuvant chemotherapy or radiation therapy thus should be considered for Patnaik grade 1 and 2 (and presumably Kiupel grade 1) MCT with histologically incomplete margins. Importantly, the likelihood of local recurrence for high-grade (Patnaik grade 3, Kiupel grade 2) MCT can be expected to be much higher than that for lower-grade tumors. One recent study[7] indicated a recurrence rate of 36% for such tumors, *despite histologically complete tumor margins*. Therefore, the choice to pursue surgery for higher-grade MCT should be made carefully. Adjuvant therapy is almost always required for durable control of these tumors, and it is likely some dogs with such tumors derive no benefit from surgery.

Radiation Therapy for Cutaneous Mast Cell Tumors

Radiation therapy plays an important role in the management of dogs with cutaneous mast cell tumors, both as an adjuvant therapy to incomplete surgical excision, and as a palliative treatment for unresectable tumors. As mentioned above, tumor recurrence is relatively common following incomplete surgical removal of cutaneous MCT, even for low-grade tumors. Postoperative radiation therapy appears to significantly reduce the likelihood of recurrence in dogs with Patnaik grade 1 and 2 MCT. The vast majority of dogs treated with radiation in this setting experience prolonged disease-free survival, which is likely tantamount to cure, although the rate of local recurrence may still be in the range of 5-15% for dogs treated in this fashion.[9-11] For dogs with unresectable primary tumors, radiation therapy can be a very effective palliative treatment. When combined with toceranib and/or prednisone, radiation therapy induces measurable remission in approximately 60-90% of macroscopic MCT.[12-14] In dogs with lower-grade MCT, such treatment can result in durable responses, and prolonged progression-free survival.[12] In contrast, progression-free survival times of approximately 3-6 months are more typical for dogs with high-grade, aggressive MCT.[13,14]

Systemic Chemotherapy for Cutaneous Mast Cell Tumors

Among the indications for chemotherapy in dogs with MCT are: 1.) Primary (palliative) treatment of unresectable local or metastatic disease; 2.) Adjuvant treatment for dogs with incompletely excised tumors to prevent/delay local recurrence; and 3.) Adjuvant treatment for dogs with completely excised "high-risk" tumors to prevent/delay metastasis. Although the term "high-risk" has been used to describe a multitude of tumors in the veterinary literature, the author reserves it for high-grade MCT (Patnaik grade 3, Kiupel grade 2) or Patnaik grade 2 MCT with other negative prognostic factors, such as a high mitotic index, high Ki-67 index, or presence of an activating mutation in the *c-kit* proto-oncogene. Although published reports describe the use of a multitude of agents for treating MCT, the evidence of anti-tumor efficacy is strongest for vinblastine, prednisone (or prednisolone), and toceranib. Other agents with reported single-agent activity include lomustine, vincristine, calcitriol, and hydroxyurea.

The author's first choice for primary or adjuvant chemotherapy of MCT is a combination of intravenous vinblastine and oral prednisone first described by Thamm and colleagues.[15] This protocol induces measurable tumor remission in about half of all macroscopic MCT, affording an average response duration of about 5 months. The dose of vinblastine used in this protocol – 2 mg/m^2 IV – has come under scrutiny in recent years as it appears that higher doses (up to 3.5 mg/m^2) are tolerated by most dogs, and that these higher doses are associated with a greater likelihood of tumor response.[16,17] However, since these higher vinblastine doses have to be given with longer inter-treatment intervals, it is still unclear whether dose escalation will improve long-term outcome (i.e. progression-free or overall survival) for dogs with MCT. The most common side effect of vinblastine is myelosuppression, characterized by neutropenia, dictating a need for careful hematologic monitoring in dogs receiving this chemotherapy agent.

Toceranib phosphate (Palladia™) is an orally administered inhibitor of the c-kit oncoprotein, a major molecular driver of tumor progression in approximately 10-40% of canine MCT. C-kit-driven MCT are commonly high-grade tumors.[18,19] Among all mast cell tumors, the likelihood of objective response to toceranib is approximately 40-45%.[20] The likelihood of response to toceranib is greater in tumors bearing activating mutations in the *c-kit* proto-oncogene than in those without this mutation (approximately 60% in tumors with the mutation vs. 30% in tumors without). However, in tumors that do respond to toceranib, there appears to be no association between presence of a *c-kit* mutation and duration of response. Therefore, the author does not consider testing tumors for the presence of *c-kit* mutations to be an absolute prerequisite to starting toceranib therapy. Toceranib is associated with a host of side effects that frequently necessitate dose modification or dose holidays. Gastrointestinal side effects, such as vomiting, diarrhea, or loss of appetite occur in approximately half of all dogs receiving toceranib.[20] Proteinuria with hypertension also occurs commonly, affecting 30-40% of treated dogs.[21] Less common side effects include myelosuppression (usually mild), increased liver enzyme activity, and musculoskeletal pain. Adverse events associated with toceranib are less severe and possibly less frequent in dogs receiving doses ranging from 2.4-2.9 mg/kg PO every other day when compared to dogs receiving the label dose (3.25 mg/kg PO every other day).[22] Importantly, administration of these reduced doses still achieves plasma concentrations of toceranib necessary for c-kit inhibition *in vitro*. Thus, it is likely that such dose reductions do not compromise *in vivo* anti-tumor efficacy.

Because of its potent activity against canine mast cell tumors, toceranib has been combined with cytotoxic agents in recent clinical trials[23,24] with the goal of developing treatment protocols of greater efficacy. Unfortunately, the tolerability of these protocols has been poor, frequently resulting in severe myelosuppression necessitating reductions in the dose of the cytotoxic agent used. Although some evidence of synergistic activity[22] of the combination therapy was evident in these trials, further study is needed before routine use of combination toceranib/cytotoxic therapy can be recommended.

Glucocorticoids were among the first agents used to treat MCT, and remain a cornerstone of many therapeutic protocols today. Although earlier studies[25] report that prednisone induces a modest response rate of 20% in canine MCT, more recent studies[12,26] cite response rates in the range of 70-80%. Glucocorticoids have direct cytotoxic effects against mast cells *in vitro*, but also decrease peritumoral edema, and it is sometimes not clear which effect is predominating when MCT reduce in size following glucocorticoid therapy. Data on the duration of response of MCT to glucocorticoid monotherapy are sparse,[25] although the author believes that such responses are frequently short-lived. However, because their side effect profile does not overlap with that of cytotoxic agents, glucocorticoids can be safely combined with most other drugs that have efficacy against MCT, and in some cases[15] appear to result in synergistic anti-tumor activity.

Most other chemotherapy agents described as treatments for MCT have undergone only limited investigation or are of uncertain efficacy. The author tends to use these agents only when previous attempts at therapy with vinblastine, toceranib, and/or glucocorticoids have failed. One such agent that is used commonly to treat MCT is lomustine (CCNU), a drug the author perceives to be of limited benefit in treating these tumors. An early report[27] describes lomustine inducing an objective response in 42% of canine MCT, affording a median response duration of approximately 2.5 months. Since that time, several reports have documented the use of lomustine in combination with other drugs as a treatment for MCT. Some of these reports suggest additive or synergistic efficacy to these combination protocols. However, a more contemporary report,[28] derived from a well-controlled clinical trial, cites an objective response rate of 1% (1/81 dogs), which is more in keeping with the author's impression of lomustine's efficacy against this cancer. Because of this apparently poor efficacy, the author currently does not recommend the use of lomustine as first-line therapy for MCT, either as monotherapy or as part of a combination drug protocol.

References

1. Thamm DH and Vail DM. Mast cell tumors. In: Withrow SJ and Vail DM, eds: *Small Animal Clinical Oncology* 4th ed., St Louis, 2007, Saunders Elsevier.
2. Simpson AM et al. Evaluation of surgical margins required for complete excision of cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc* 2004;224:236-240.
3. Fulcher RP et al. Evaluation of a two-centimeter lateral surgical margin for excision of grade I and grade II cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc* 2006;228-210-215.

4. Schultheiss PC et al. Association of histologic tumor characteristics and size of surgical margins with clinical outcome after surgical removal of cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc* 2011;238:1464-1469.
5. Pratschke KM et al. Evaluation of a modified proportional margin approach for surgical resection of mast cell tumors in dogs: 40 cases (2008-2012). *J Am Vet Med Assoc* 2013;243:1436-1441.
6. Kamstock DA et al. Recommended guidelines for submission, trimming, margin evaluation, and reporting of tumor biopsy specimens in veterinary surgical pathology. *Vet Pathol* 2011;48:19-31.
7. Donnelly L et al. Evaluation of histological grade and histologically tumour-free margins as predictors of local recurrence in completely excised canine mast cell tumors. *Vet Comp Oncol* 2015;13:70-76.
8. Smith J et al. Recurrence rates and clinical outcomes for dogs with grade II mast cell tumours with a low AgNOR count and Ki67 index treated with surgery alone. *Vet Comp Oncol* 2017;15:36-45.
9. Kry KL et al. Additional local therapy with primary re-excision or radiation therapy improves survival and local control after incomplete or close surgical excision of mast cell tumors in dogs. *Vet Surg* 2014;43:182-189.
10. Chafin K et al. Results of radiation therapy in 19 dogs with cutaneous mast cell tumor and regional lymph node metastasis. *Vet Radiol Ultrasound* 2002;43:392-395.
11. Poirier VJ et al. Radiation therapy for incompletely excised grade II canine mast cell tumors. *J Am Anim Hosp Assoc* 2006;42:430-434.
12. Dobson J et al. Treatment of canine mast cell tumors with prednisolone and radiotherapy. *Vet Comp Oncol* 2004;2:132-141.
13. Carlsten KS et al. Multicenter prospective trial of hypofractionated radiation treatment, toceranib, and prednisone for measurable canine mast cell tumors. *J Vet Intern Med* 2012;26:135-141.
14. Tollett MA et al. Palliative radiation therapy for solid tumors in dogs: 103 cases (2007-2011). *J Am Vet Med Assoc* 2016;248:72-82.
15. Thamm D et al. Prednisone and vinblastine chemotherapy for canine mast cell tumor – 41 cases (1992-1997). *J Vet Intern Med* 1999;13:491-497.
16. Serra Varela JC et al. Tolerability of a rapid-escalation vinblastine-prednisolone protocol in dogs with mast cell tumors. *Vet Med Sci* 2016;2:266-280.
17. Rassnick KM et al. Efficacy of vinblastine for treatment of canine mast cell tumors. *J Vet Intern Med* 2008;22: 1390-1396.
18. Takeuchi Y et al. Validation of the prognostic value of histopathological grading or c-kit mutation in canine cutaneous mast cell tumours: a retrospective cohort study. *Vet J* 2013;196:492-498.
19. Downing S et al. Prevalence and importance of internal tandem duplications in exons 11 and 12 of *c-kit* in mast cell tumors of dogs. *Am J Vet Res* 2002;63:1718-1723.
20. London CA et al. Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. *Clin Cancer Res* 2009;15:3856-3865.
21. Tjostheim SS et al. Effects of toceranib phosphate on systolic blood pressure and proteinuria in dogs. *J Vet Intern* Med 2016;30:951-957.
22. Bernabe LF et al. Evaluation of the adverse event profile and pharmacodynamics of toceranib phosphate administered to dogs with solid tumors at doses below the maximum tolerated dose. *BMC Vet Res* 2013;9:190.
23. Robat C et al. Safety evaluation of combination vinblastine and toceranib phosphate (Palladia) in dogs: a phase I dose-finding study. *Vet Comp Oncol* 2012;10:174-183.
24. Bavcar S et al. Combination toceranib and lomustine shows frequent high grade toxicities when used for treatment of non-resectable or recurrent mast cell tumours in dogs: a European multicentre study. *Vet J* 2017;224:1-6.

25. McCaw DL et al. Response of canine mast cell tumors to treatment with oral prednisone. *J Vet Intern Med* 1994;8:406-408.

26. Stanclift RM et al. Evaluation of neoadjuvant prednisone administration and surgical excision in treatment of cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc* 2008;232:53-62.

27. Rassnick KM et al. Treatment of canine mast cell tumors with CCNU (lomustine). *J Vet Intern Med* 1999;13:601-605.

28. Vail DM et al. A randomized trial investigating the efficacy and safety of water soluble micellar paclitaxel (Paccal Vet) for treatment of nonresectable grade 2 or 3 mast cell tumors in dogs. *J Vet Intern Med* 2012;26:598-607.

Managing Cutaneous Lymphomas in Dogs

Michael O. Childress, DVM, MS, DACVIM (Oncology)

Introduction

Cutaneous lymphomas in dogs are a diverse family of skin neoplasms with an equally diverse spectrum of clinical presentations. These tumors may be classified broadly into epitheliotropic and non-epitheliotropic forms. Epitheliotropic T-cell lymphoma (ETCL) is by far the most common form of cutaneous lymphoma in dogs. Non-epitheliotropic cutaneous lymphomas (NECL), by comparison, are quite rare. Treatment for most forms of cutaneous lymphoma in dogs is challenging, especially for animals with widely disseminated lesions. This session will summarize the presentation, diagnostic features, and treatment options for ETCL. General recommendations for the treatment of NECL, derived from a review of published literature and the author's own experience, also will be provided.

Epitheliotropic T-cell Lymphoma – Clinical and Diagnostic Features

The etiology of ETCL is unknown, although there is some evidence that a history of chronic inflammatory skin disease may be a predisposing factor.[1,2] Likely owing to the uncommonness of the disease, no clear breed predispositions have been identified. However, some sources cite boxers and cocker spaniels as over-represented breeds for this disease.[1] There does not appear to be a sex predilection.

Grossly, lesions of ETCL may be characterized by exfoliative erythroderma, patches and/or plaques, or the formation of frank tumors.[1,3,4] Involvement of mucocutaneous sites such as the oral cavity is common, and may be characterized by depigmentation and ulceration. Lesions may be solitary, locoregional, or generalized. Progression of less advanced lesions (e.g. localized erythroderma) to more advanced lesions (e.g. plaques or tumors) does occur, but is not a defining feature of the disease, as dogs can present with lesions of multiple anatomic types arising concurrently. Lesions of ETCL are variably pruritic, but generally not painful.

As the name suggests, ETCL is characterized by infiltration of the skin by neoplastic lymphocytes demonstrating marked epitheliotropism. Histopathologically, three subtypes of ETCL are recognized. These include the most common "classic" form (sometimes referred to as *mycosis fungoides*) and two far less common forms: pagetoid reticulosis and Sézary syndrome.[1,3] The former is characterized by confinement of the lymphocytic infiltrate to the epidermis and adnexal structures, while the latter is characterized by widespread cutaneous involvement with neoplastic cells in circulation. The lymphocytes comprising ETCL are morphologically and phenotypically diverse, depending upon the type of gross lesion present. Patch and plaque lesions tend to be comprised of small, mature lymphocytes with a low proliferative rate, whereas tumor lesions tend to be comprised of larger, immature lymphocytes with greater mitotic activity.[1,3] Immunophenotypically, the majority of ETCL cases are comprised of CD3+/CD8+/CD4- T-cells bearing the $\gamma\delta$ form of the T-cell receptor.

Epitheliotropic T-cell Lymphoma – Staging, Treatment, and Prognosis

As is true for dogs with any cancer, the purposes of staging for dogs with ETCL are to guide the selection of appropriate therapy, provide a baseline measure of the extent of disease, and estimate the dog's prognosis following therapy. In humans with ETCL, the modified Severity Weighting Assessment Tool (mSWAT) is used for staging and therapeutic response assessment.[5] This system accounts for the type and extent of skin lesions, as well as nodal, visceral, or peripheral blood involvement. No similar staging tool exists for dogs. Application of the World Health Organization staging criteria[6] to cutaneous lymphomas in dogs is unlikely to be helpful, and has not been shown to have prognostic relevance. Staging protocols for dogs with ETCL therefore are typically developed empirically, on a patient-to-patient basis.

All staging tests should be ordered with an eye towards likely sites of tumor dissemination. Extracutaneous spread of ETCL tends to mirror the organ tropism of other lymphomas, with lymph nodes, liver, and spleen being common sites of dissemination. Bone marrow or peripheral blood involvement are also possible. Lymph node involvement is present in approximately 30-40% of dogs with ETCL at the time of diagnosis.[7,8] Visceral involvement appears to be less common, although most dogs described in published reports have not undergone full thoracic and abdominal imaging. A complete staging protocol for dogs with ETCL therefore might include routine hematology/serum

biochemical testing, thoracic radiography, abdominal ultrasonography, and bone marrow aspirate cytology. Fine needle aspirate cytology of regional lymph nodes and visceral organs may also be warranted, depending upon findings from the physical exam and imaging studies.

The need for and thoroughness of staging are largely dictated by the extent of the cutaneous lesions and the planned therapy. Dogs with small and/or localized lesions treatable with local therapies may benefit from thorough staging, particularly if the planned therapy is likely to be expensive or involve significant morbidity. The purpose of thorough staging in these patients is to rule out the presence of more extensive disease that would not be amenable to local therapy. Dogs with widespread cutaneous or mucocutaneous lesions, on the other hand, are not candidates for local therapy, and therefore should receive systemic therapy regardless of the overall extent of their disease. As such, complete staging is likely superfluous for most of these dogs.

A variety of treatment options exist for dogs with ETCL. The choice of treatment depends upon the stage of disease, the animal's overall health, and the owner's financial wherewithal and tolerance for treatment-related side effects. Surgery and radiation therapy (RT) are options for dogs with focal or localized lesions. Published data on the outcomes following surgery or RT for dogs with localized ETCL are sparse. However, ETCL is generally quite responsive to ionizing radiation. One study[9] suggested that some dogs experience durable tumor control (progression-free survival >450 days) following treatment with radiation alone, which is consistent with the author's own experience. It should be noted that approximately 25-50% of dogs presenting with solitary lesions in one study went on to develop distant lesions,[8] suggesting that adjuvant chemotherapy may be needed even for dogs with localized disease.

Whereas the need for chemotherapy is ill-defined in dogs with localized, non-metastatic tumors, systemic chemotherapy is the only therapy likely to be helpful for dogs with extensive cutaneous or metastatic visceral disease. Chemotherapy of ETCL is a notoriously frustrating exercise, with no described treatment clearly affording superior results to all others. The goals of chemotherapy are to produce temporary disease remission, palliate clinical signs such as pruritus, and possibly to extend survival, although it is as yet unclear whether treated dogs live longer than untreated ones. What is clear is that responses to chemotherapy are usually brief, and are inevitably followed by the development of tumoral drug resistance and a need to change course in treatment. The list of medical treatments described for ETCL is long, so only a brief summary of some of the most well-characterized or apparently effective treatments follows.

The drug most frequently cited as the first-line treatment for ETCL is the alkylating agent lomustine (CCNU). This drug is given by mouth at a dose of 50-90 mg/m^2 once every 3 weeks. It induces measurable tumor remission in approximately 80% of dogs with ETCL.[10,11] However, the average duration of remission afforded by lomustine is only approximately 3 months. Potential side effects of lomustine include myelosuppression (characterized by neutropenia and, to a lesser extent, thrombocytopenia) and hepatotoxicosis. Judicious monitoring of hematology and serum biochemistry values, therefore, is absolutely essential for patients receiving this drug. Co-administration of S-adenosylmethionine and silybin appears to lessen both the likelihood and severity of hepatotoxicosis, and is recommended for all dogs receiving lomustine.[12]

Combination chemotherapy protocols for the treatment of ETCL are not commonly described. However, a recent retrospective study[8] provided results for 40 dogs receiving the VELCAP (vincristine, L-asparaginase, cyclophosphamide, doxorubicin, and prednisone) combination protocol to treat this cancer. The overall response rate was 82.5% (33/40; 18 complete and 15 partial remissions). The duration of response for these dogs was not reported, but the median overall survival times were 207 days for dogs with cutaneous disease and 281 days for dogs with mucocutaneous disease. These results compared somewhat favorably to those from a cohort of 13 lomustine-treated dogs described in the same report. In these dogs, the objective response rate was 77% (10/13) and the median overall survival time was 130 days (reported only for dogs with cutaneous disease, not mucocutaneous disease). It is not possible to infer the superiority of VELCAP to lomustine from these retrospectively analyzed data, so further study is needed to determine whether combination protocols such as VELCAP truly offer an advantage over single-agent protocols.

Retinoic acid derivatives, also known as retinoids, are another class of drugs that appear to have single-agent activity against ETCL in dogs. The anticancer activity of retinoids derives from their binding to nuclear receptors where they act as coactivators of gene transcription. The products of this gene transcription cause cell cycle arrest and initiate terminal differentiation in dividing cells. Several isoforms of retinoic acid receptors exist, most of which were shown

to be expressed by a small cohort (n=5) of ETCL lesions.[13] These data lend support to the use of retinoids to treat this cancer, although data on response to such treatment are sparse in the veterinary literature. One study[4] reported that 1 of 3 dogs receiving etretinate experienced a partial cancer remission that was maintained for 8 months until the dog died of unrelated causes. Another describes "successful" (a categorical or quantitative measure for this term was not provided) treatment in 5/11 dogs with ETCL receiving isotretinoin (4 dogs) or etretinate (1 dog) for 5, 10, 11, 13, and 15 months, respectively.[14] The optimal doses of retinoids to use in dogs with ETCL are not well established, but starting doses in the range of 1-2 mg/kg by mouth twice daily are typical. Reported side effects of these drugs include dyslipidemias, joint/bone pain, hepatotoxicity, and periocular or perioral desquamation. Retinoids are also highly teratogenic, so they should not be prescribed to pets whose owners are pregnant or attempting to become pregnant. The teratogenicity of these drugs imposes significant restrictions on access to commercially available formulations. The author has, however, had success obtaining these drugs from veterinary compounding pharmacies.

Finally, prednisone and other glucocorticoids merit discussion as therapies for ETCL. The low cost and predictable side effects of glucocorticoids make them popular treatments for this cancer. While some sources[15] suggest that these drugs have limited single-agent activity against ETCL, one recent study[8] reported a 58% overall response rate (11/19; 4 complete and 7 partial remissions). Median survival times for these dogs were 58.5 days for those with cutaneous disease and 309 days for those with mucocutaneous disease. Because their spectrum of side effects does not overlap with that of other common treatments for ETCL, glucocorticoids are often used in combination with these treatments in an attempt to improve efficacy. Whether this actually results in such improvement has not been established. Even if they do not improve overall therapeutic efficacy, glucocorticoids are still useful as palliative treatment for inflammation and pruritus associated with ETCL.

The prognosis for dogs with ETCL, on the whole, is poor. Most dogs with this cancer do not succumb to the disease naturally, but rather are euthanatized by their owners due to the poor quality of life associated with intractably pruritic and/or infected skin lesions. One study reported an overall median survival time of 6 months.[7] It should be noted, however, that few studies have addressed prognostic factors for this disease in order to allow a better estimation of how individual dogs might benefit from treatment. In a recent study,[8] dogs with solitary lesions and mucocutaneous disease experienced significantly longer survival than those with multiple lesions or cutaneous disease, respectively. For dogs with cutaneous lesions that received chemotherapy, those that attained a complete remission (16/52; 31%) lived significantly longer than those that did not. It is clear, therefore, that although the prognosis for most dogs with ETCL is poor, some individuals do fare much better. This suggests a need to better tailor therapies for ETCL to meet the specific needs of the subsets of dogs with favorable and unfavorable prognoses.

Management of Non-epitheliotropic Cutaneous Lymphomas in Dogs

Compared to ETCL, non-epitheliotropic cutaneous lymphomas are considerably rarer. Information about these tumors can mainly be gleaned from single case reports and small cases series, confounding efforts to make evidence-based treatment recommendations for dogs affected by them. Compounding this problem is the fact that NECL is actually a blanket diagnosis for a family of tumors derived from either B- or T-lymphocytes, each of which likely exhibits a distinct clinical behavior. Therefore, this section will review only some general features of these diseases, with notes on treatments described for them in the veterinary literature.

The gross appearance of NECL tends to be distinct from that of ETCL. NECL are typically characterized by the presence of one or more firm dermal nodules, which may display surface erythema or ulceration.[3,4] Plaques, scaling, pruritus, and mucocutaneous involvement appear to be much less common with NECL than with ETCL. Histologically, NECL are usually comprised of large, immature lymphocytes, as opposed to the smaller, more mature lymphocytes that characterize many lesions of ETCL. The cells of NECL often display a high mitotic rate, which coincides with a more rapid rate of clinical progression and greater predilection for systemic dissemination than is typical of ETCL.[3,4] However, these features are not universal to all NECL, as indolent forms are also described.[16]

There is no clear treatment of choice for dogs with NECL. Dogs with solitary lesions may be best treated by surgical excision, as long-term tumor control or apparent cure has been reported in some cases.[17,18] Radiation therapy also may be a useful treatment for dogs with more locally extensive solitary lesions that are not amenable to surgical excision. Various cytotoxic agents have been used to treat NECL, with responses reported to lomustine, doxorubicin, retinoids, and various drug combinations.[14,18,19] The author typically uses lomustine-based protocols for dogs with

aggressive T-cell NECL and doxorubicin-based protocols for dogs with aggressive B-cell NECL. Dogs with indolent NECL may be treated with glucocorticoids, topical therapies, or watchful waiting. It is not clear if treatment alters the course of disease progression in dogs with indolent tumors.[16]

Overall, the prognosis for dogs with NECL is highly variable, with reported outcomes ranging from outright cure to death within weeks from rapidly progressing disease. No factors that meaningfully impact treatment outcome have been identified.

References

1. Fontaine J et al. Canine cutaneous epitheliotropic T-cell lymphoma: review. Vet Comp Oncol 2009;7:1-14.
2. Santoro D et al. Investigation on the association between atopic dermatitis and the development of mycosis fungoides in dogs: a retrospective case-control study. Vet Dermatol 2007;18:101-106.
3. Moore PF et al. Cutaneous lymphomas in companion animals. Clin Dermatol 1994;12:499-505.
4. Beale KM et al. Canine cutaneous lymphosarcoma: epitheliotropic and non-epitheliotropic, a retrospective study. In: Advances in Veterinary Dermatology, Vol 2. Ihrke PJ, Mason IS, and White SD, eds. New York, Pergamon Press, 1993: 273-284.
5. Olsen EA et al. Clinical end points and response criteria in mycosis fungoides and Sezary syndrome: A consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organization for Research and Treatment of Cancer. J Clin Oncol 2011;29:2598-2607.
6. Owen LN. TNM Classification of Tumors in Domestic Animals. Geneva, World Health Organization Press, 1980.
7. Fontaine J et al. Canine cutaneous epitheliotropic T-cell lymphoma: a review of 30 cases. Vet Dermatol 2010;21:267-275.
8. Chan CM et al. Clinical outcome and prognosis of dogs with histopathological features consistent with epitheliotropic lymphoma: a retrospective study of 148 cases (2003-2015) in Australia. Vet Dermatol 2018;29:154-e59.
9. Berlato D et al. Radiotherapy in the management of localized mucocutaneous oral lymphoma in dogs: 14 cases. Vet Comp Oncol 2012;10:16-23.
10. Risbon RE et al. Response of canine cutaneous epitheliotropic lymphoma to lomustine (CCNU): a retrospective study of 46 cases. J Vet Intern Med 2006;20:1389-1397.
11. Williams LE et al. CCNU in the treatment of canine epitheliotropic lymphoma. J Vet Intern Med 2006;20:136-143.
12. Skorupski KA et al. Prospective randomized trail assessing the efficacy of Denamarin for prevention of CCNU-induced hepatopathy in tumor-bearing dogs. J Vet Intern Med 2011;25:838-845.
13. de Mello Souza CH et al. Immunohistochemical detection of retinoid receptors in tumors from 30 dogs diagnosed with cutaneous lymphoma. J Vet Intern Med 2010;24:1112-1117.
14. White SD et al. Use of isotretinoin and etretinate for the treatment of benign cutaneous neoplasia and cutaneous lymphoma in dogs. J Am Vet Med Assoc 1993;202:387-391.
15. de Lorimier L-P. Updates on the management of canine epitheliotropic cutaneous T-cell lymphoma. Vet Clin North Am Small Anim Pract 2006;36:213-228.
16. Affolter VK et al. Indolent cutaneous T-cell lymphoma presenting as cutaneous lymphocytosis in dogs. Vet Dermatol 2009;20:577-585.
17. Kondo H et al. Canine non-epitheliotropic CD4-positive cutaneous T-cell lymphoma: a case report. Vet Med Sci 2018 Dec 12. doi: 10.1002/vms3.137. [Epub ahead of print.]
18. Moore PF et al. Canine inflamed nonepitheliotropic cutaneous T-cell lymphoma: a diagnostic conundrum. Vet Dermatol 2013;24:204-e45.
19. Noland EL et al. Subcutaneous panniculitis-like T-cell lymphoma in dogs: morphologic and immunohistochemical classification. Vet Pathol 2018;55:802-808.

FOOD ALLERGIES IN DOGS AND CATS: SELECTED TOPICS

Olivry T[1,2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, and
[2]Comparative Medicine Institute, North Carolina State University, Raleigh, NC, USA

In dogs and cats, cutaneous adverse food reactions (CAFRs) are divided among those that are perceived to have an immunologic pathogenesis (i.e., "food allergies" sensu stricto) and those that are not (i.e., "food intolerances"). As we could not find any reports of credible food intolerances with cutaneous manifestations in the veterinary dermatology literature, we will mostly equate CAFRs to food allergies (FAs) with cutaneous manifestations. A large body of knowledge exists about CAFRs/FAs in the literature, but most of it is propagated in an often dogmatic manner usually restricted to the presentation of results of few selected studies. Several years ago, we embarked in the generation of "critically-appraised topics" (CATs) which are short papers aimed at answering clinical questions using evidence-based medicine principles. This lecture will summarize the results of these CATs and expand on several other topics. We will first begin by presenting the data on the prevalence of CAFRs in dogs and cats (CAT3) before highlighting the cutaneous (CAT7 – submitted) and noncutaneous (CAT6) signs of CAFRs in dogs and cats. Then, the issue of diagnosing FAs in companion animals with a cutaneous or serum test will be reviewed (CAT4) before briefly discussing the possible limitations preventing such tests to be reliable for diagnosis; the newly-discovered concept of transcription infidelity will be introduced herein. We will then focus on the duration of elimination diets (CAT1) before presenting the main food allergens diagnosed after dietary provocation (CAT2). Finally, we will highlight two points that are important limiting factors to the specificity of a diagnosis of FAs using elimination diets: the mislabeling of commercial petfoods (CAT5) and the upcoming issue of food allergen cross-reactivity.

Note 1: a pdf-formatted copy of the slides of this presentation will be available from the meeting time till the end of May 2019 at the following location: https://www.dropbox.com/sh/ugxtlph195osjr1/AABzfpo4619ZPV32Y373Dtaia?dl=0

Disclosure: Royal Canin scientists reviewed the CATs proposed and independently written by Thierry Olivry and Ralf Mueller. This company also paid for the publication charge making these articles open access at the following website: https://www.biomedcentral.com/collections/catsfoodreactions

Both authors have lectured and consulted for, and received research funding from this company.

IBD AND FOOD SENSITIVITIES: DIAGNOSTIC APPROACH AND TREATMENT

Katharine F Lunn BVMS MS PhD MRCVS DACVIM

Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

INTRODUCTION

For this brief review we will discuss chronic enteropathy (CE), rather than inflammatory bowel disease (IBD).[1] Inflammatory bowel disease is a fairly specific term in human medicine, and the relevant diseases are usually managed with immunosuppressive therapy. In addition, the diagnosis in dogs and cats should really be restricted to those patients in which inflammation of the gastrointestinal (GI) tract has been confirmed on histopathology. In fact, many dogs and cats with chronic GI disease do not undergo endoscopy or surgery and the diagnosis of IBD is presumptive. For dogs or cats with a confirmed histopathological diagnosis of inflammation in the GI tract, the cause is often not determined. It is presumed that the inflammation results from a combination of genetic factors, the intestinal microbiota, and dietary or other environmental factors.

CLINICAL SIGNS

Signs of chronic enteropathy include chronic vomiting, chronic diarrhea (small or large bowel or mixed bowel), hyporexia or anorexia, and weight loss.

DIAGNOSTIC APPROACH

The goal of the diagnostic work-up of a chronic enteropathy patient is primarily to rule out other diseases that cause chronic GI signs. In addition, evidence gathered during the work-up can help the clinician and client decide how aggressively to pursue a definitive diagnosis. For example, if the patient has a protein-losing enteropathy (PLE) associated with CE, endoscopic (or surgical) biopsies are more likely to be pursued, in order to investigate specific diagnoses such as GI lymphoma or histoplasmosis.

Differential Diagnosis

Non-GI diseases that cause chronic GI signs include liver disease, renal disease, hypoadrenocorticism (dogs > cats), pancreatitis, exocrine pancreatic insufficiency, and hyperthyroidism (cats > dogs). Primary GI diseases that should be ruled out before diagnosing CE include infections (e.g. salmonellosis, campylobacteriosis), parasites, chronic obstruction, and intestinal neoplasia).

Complete Blood Count (CBC)

There are no CBC changes that are sensitive or specific for CE. If blood loss is associated with intestinal inflammation, the CBC may show anemia and decreased total solids. The anemia may initially be regenerative and progress to non-regenerative if chronic blood loss is not detected or treated and leads to iron deficiency. Iron deficiency and GI inflammation may also be associated with thrombocytosis. Depending on the severity of the GI disease, there may be a normal leukogram, or evidence of inflammation. Eosinophilia should increase suspicion for parasitic, allergic disease, or hypoadrenocorticism.

Serum Biochemistry Profile

There are also no biochemistry findings that are specific or sensitive for CE. The primary reason for performing this test is to look for evidence of non-GI disease. Hypoalbuminemia is suggestive of PLE in a dog with CE, however other causes of hypoalbuminemia should be ruled out, because GI protein loss can be more difficult to prove directly. Panhypoproteinemia (decreased albumin and globulins) would be expected in cases of PLE, but in some cases the globulins are normal, presumably because the inflammation in the GI tract stimulates hyperglobulinemia, which is then reduced into the normal range by the GI protein loss. Panhypoproteinemia would not be expected in a patient with liver failure or protein-losing nephropathy. Hypocholesterolemia may also be detected in patients with PLE. Increased liver enzymes (usually mild) can also occur secondary to CE, likely due to the presence of inflammatory mediators in the portal blood that drains the inflamed GI tract.

Urinalysis
This test is performed as part of the overall work-up of a patient with chronic GI signs. In addition, proteinuria should be ruled out in a patient with hypoalbuminemia.

ACTH Stimulation Test
This is recommended in dogs with chronic GI disease, particularly if associated with hypoalbuminemia, hypocholesterolemia, lymphocytosis, or eosinophilia.

Serum Bile Acids
Fasting and post-prandial bile acids should be measured in patients with hypoalbuminemia, to rule out liver failure.

Intestinal and Pancreatic Function Testing
Serum cobalamin, folate, and trypsin-like immunoreactivity (TLI) should be measured in cases of suspected CE. The TLI test is very sensitive and specific for the diagnosis of exocrine pancreatic insufficiency (EPI). Although EPI is uncommon in dogs and rare in cats, cobalamin and folate are often abnormal in patients with this diagnosis, therefore it must be ruled out before the cobalamin and folate results can be interpreted. Cobalamin is absorbed in the ileum and low serum cobalamin (if TLI is normal) is supportive of ileal disease. There is no known significance to a high serum cobalamin concentration. Folate is produced by GI bacteria and can be low with significant proximal small intestinal disease. A high serum folate suggests small intestinal bacterial dysbiosis. Results of these tests can help determine the which regions of the GI tract to biopsy, and results can also guide therapy. Supplementation of cobalamin when serum concentrations are low is recommended. Folate supplementation is also suggested if it is low; this is unlikely to be harmful, although there is less direct evidence to support a beneficial effect. This author recommends submitting samples to the GI lab at Texas A&M University (http://vetmed.tamu.edu/gilab) for intestinal and pancreatic function testing. This lab also provides helpful guidelines for supplementation of cobalamin and folate.

Fecal Parasite Testing
Dogs and cats with chronic diarrhea should always be tested for intestinal parasites, preferably with a direct fecal smear, fecal centrifugation, and sedimentation. Even if these tests are negative, most clinicians will empirically treat these patients with a 5-day course of fenbendazole (50 mg/kg q 24hr).

Fecal Protein Assay
Alpha-1-proteinase inhibitor can be measure in canine fecal samples for detection of GI protein loss. This test is not routinely performed, because protein loss is often indirectly inferred in hypoalbuminemic patients once hepatic failure, renal loss, and hypoadrenocorticism have been ruled out. This test can be useful for screening for PLE in dogs that have a known breed predisposition. The test is performed by the GI lab at Texas A&M University.

Radiography
Abdominal radiographs are more likely to be useful in patients in which vomiting is the primary complaint, or if abnormalities are detected on physical examination. Radiographs can also detect GI stasis or ileus. Thoracic radiographs are recommended in patients in which neoplasia is considered to be an important differential diagnosis.

Abdominal Ultrasound Examination
Ultrasound examination of the abdomen is commonly performed in patients with weight loss, anorexia, vomiting or diarrhea. Unlike plain radiographs, ultrasound examination can determine GI tract wall thickness and the appearance of the layers. It may also reveal lymphadenopathy, GI ulcers, abnormal motility, or obstruction. Ultrasound examination can also facilitate obtaining samples of abnormal tissues for cytopathological examination.

Endoscopy
Endoscopy allows examination of the mucosal surface of the GI tract and collection of multiple samples for histopathological examination. Endoscopy is indicated in any patient with CE, once non-GI diseases have been ruled out and less invasive testing has not revealed a specific diagnosis. The technique is most likely to be performed in patients with more severe clinical signs, patients that are hypoalbuminemic, and patients that have not responded to dietary therapy or a course of antibiotics. In this group of patients, endoscopy is often being performed to ensure that a more serious diagnosis is not missed, such as GI lymphoma or histoplasmosis. For patients with purely large bowel signs, colonoscopy is performed. For patients with small bowel signs, gastroduodenoscopy is performed, followed

by sampling of the ileum by passing the endoscopy through the ileocolic valve from the colon. This is particularly important if hypocobalaminemia has been detected, but ideally ileal biopsies should be obtained in all patients undergoing endoscopy for investigation of small bowel signs. Empirical deworming should always be completed before performing GI endoscopy. Biopsies should always be obtained, even if the GI mucosa appears grossly normal.

Exploratory Surgery
Laparotomy allows all abdominal organs to be examined, and full-thickness biopsies can be obtained from all levels of the GI tract, particularly in patients with small intestinal disease. Full thickness biopsies may be more diagnostically rewarding than small endoscopic biopsies, but this must be balanced with the risk and invasiveness of abdominal surgery. Laparotomy is more likely to be performed in patients with evidence of disease in other abdominal organs, or in patients in which abdominal ultrasound examination has revealed lesions that are not accessible on endoscopy. In patients with severe PLE, healing from surgery can be significantly impaired and therefore endoscopic biopsies are preferred.

THERAPEUTIC APPROACH
Patients with CE are often treated with the stepwise use of a variety of interventions in the absence of a definitive histopathological diagnosis of IBD. This approach is easy to justify as the early steps in the sequence are low risk and relatively inexpensive. In addition, even if biopsies are obtained and IBD is confirmed, dietary and other therapies are often prescribed before prescribing immunosuppressive medications.

Deworming
As mentioned above, fecal parasite testing is always indicated in the work-up of CE, but even if negative, most clinicians will treat the patient with at least a 5-day course of fenbendazole. Endoscopy should never be performed until parasites have been ruled out with effective therapy.

Dietary Therapy
There are a variety of approaches to dietary therapy for CE, and if the response is positive, the enteropathy can be termed FRE (food-responsive enteropathy). The two mainstays of therapy are either highly digestible diets, or elimination diets.[2] The latter may be based on novel antigens or hydrolyzed proteins, and these diets appear to be more effective than the highly digestible diets, in both dogs and cats.[3] Dietary therapy should be prescribed for at least 2 weeks, as most patients appear to respond during that time. If the patient responds fully, the elimination diet can be continued indefinitely if it is a commercial prescription diet, or a balanced and complete homemade diet formulated by a nutritionist. Challenge with another diet, or the original diet, can be tried if CE has been controlled for several months, however many owners will opt to continue the elimination diet if it controlling the clinical signs. If no response is seen to the initial food trial, if the response is incomplete, or if the signs of CE return, antibiotic therapy is often the next step.

Antibiotic Therapy
Empirical antibiotic therapy with tylosin, metronidazole, or oxytetracycline is commonly used in patients with CE.[2] This should be distinguished from specific antibiotic therapy for diagnosed infections such as salmonellosis or campylobacteriosis. This also differs from the use of enrofloxacin for the treatment of granulomatous colitis in Boxer dogs and related breeds. There is little data to guide the duration of therapy with tylosin, metronidazole, or oxytetracycline, but most clinicians will treat for 2 weeks initially. If there is a positive response, treatment is often continued for 4-6 weeks. If there is no response, the antibiotic is discontinued and a different one may be tried. Patients that respond to a course of antibiotics are diagnosed with ARE (antibiotic-responsive enteropathy) and sometimes are maintained on long-term or intermittent therapy with medications such as tylosin or metronidazole, particularly as these medications as perceived to have fewer adverse effects than immunosuppressive medications. For the metronidazole, it is important not to exceed a total daily dose of 30 mg/kg, as neurological side-effects have been reported at higher doses. Adverse effects of tylosin appear rare. For any antibiotic, long-term use may increase the risk of antimicrobial resistance, but that does not appear to have been investigated in the context of dogs with ARE.

Probiotics
The role of probiotics in management of CE has not been extensively investigated, but there is some evidence to support their use. Many clinicians will use these in addition to dietary or antibiotic or immunosuppressive therapy, due to perceived low risk and client preference.

Immunosuppressive Therapy

In patients with CE that is not food- responsive or antibiotic-responsive, the next step in therapy is usually the use of glucocorticoids.[1,2] Options include prednisone, prednisolone, budesonide, or occasionally injectable dexamethasone if poor absorption of oral medications is suspected. These medications are often used in the absence of a histological diagnosis, however having biopsy support for a diagnosis of IBD can be beneficial. Glucocorticoids are not without side-effects, and owners may find it easier to tolerate side-effects, or may be more likely to stay with a long course of therapy, if there is biopsy evidence to indicate that this is the right course of treatment. Patients that respond to glucocorticoids are determined to have steroid-responsive enteropathy (SRE). Additional options for immunosuppression include azathioprine, chlorambucil, leflunomide, and cyclosporine.

Therapy for PLE

Patients with PLE often require more aggressive therapy with immunosuppressive medications, as diet or antibiotics alone may not control the disease. One study suggested that a combination of prednisone and chlorambucil was superior to prednisolone and azathioprine.[4] However dietary therapy can also be very beneficial in patients with PLE, and often involves the feeding of a highly fat-restricted diet.1 Patients with severe PLE are at risk for thromboembolic disease and therefore treatment with platelets inhibitors such as aspirin or clopidogrel is often recommended in these cases.

References

1. Dandrieux JRS. Inflammatory bowel disease versus chronic enteropathy in dogs: are they one and the same? J Small Anim Pract 2016;57:589-599.
2. Makielski K, Cullen J, O'Connor A, et al. Narrative review of therapies for chronic enteropathies in dogs and cats. J Vet Intern Med 2019;33:11-22.
3. Mandingers PJJ, Biourge V, van den Ingh TS, et al. A randomized, open-label, positively-controlled field trial of a hydrolyzed protein diet in dogs with chronic small bowel enteropathy. J Vet Intern Med 2010;24:1350-1357.
4. Dandrieux JR, Noble PJ, Scase TJ, et al. Comparison of a chlorambucil-prednisolone combination with an azathioprine-prednisolone combination for treatment of chronic enteropathy with concurrent protein-losing enteropathy in dogs: 27 cases (2007-2010). J Am Vet Med Assoc 2013;242:1705-1714.

Feeding For Life: Meeting optimal nutritional needs of pets through all life stages

Julie Churchill DVM, PhD DACVN

University of Minnesota College of Veterinary Medicine, Veterinary Clinical Science Department

THE POWER OF FOOD

One of the most important decisions a pet owner makes is what to feed their pet each day. The idea that great or even 'optimal' nutrition positively contributes to an animal's health is fairly well accepted by pet owners and veterinary professionals alike. Increasingly, pet owners are looking to the veterinary health care team to be the nutritional experts offering advice about what to feed their pets. Nutrition can be the cornerstone of preventive care programs in clinical practice. Meeting a pet's optimal nutritional needs requires simple tools to assess each pet and adjust nutritional recommendations at each milestone in their development to prevent diseases and maintain health. Integrating nutrition protocols in the care of small animal patients will help develop a partnership between owner, veterinary healthcare team and lead to healthier pets. In this way, pet owners become bonded to the clinic and feel the power of individualized care.

MAKE NUTRITION A PART OF EVERY PET'S VISIT

The WSAVA Global Nutrition Committee[A] (see Tools below) has developed a suite of practical tools and resources to help the veterinary health care team make nutritional assessments and recommendations more efficiently. The toolkit also contains client education materials for the team to share with pet owners. The Pet Nutrition Alliance (PNA; www.petnutritionalliance.org) was created to help raise awareness about the importance of proper pet nutrition, and the value of nutritional assessments for every pet and every visit. The PNA is also developing practical tools for the entire veterinary healthcare team to assist them in implementing these nutritional guidelines for every pet. One of the first tools, PNA Tips for Implementing Nutrition as a Vital Assessment in Your Practice can be downloaded from the WSAVA Global Nutrition Guidelines[1, 2] page listed in the reference section. Recent evidence suggests this process is most successful when the veterinary team develops easy protocols for each team member to follow.

Make a pet-specific nutrition recommendation for healthy pets. This can be done quickly, by recommending the amount and type of a high quality food that matches the pet's nutritional life stage requirements. Use every visit as an opportunity to educate pet owners about how to check their pet's body condition score (BCS, see tools) and emphasize what the pet owner is doing right. Verify and clearly document the current feeding plan.

RIGHT FROM THE START- PEDIATRIC PATIENTS

The goal of feeding plans for puppies and kittens should be to support healthy immune systems and growth to lead to healthy adults. This is achieved by selecting products that are formulated to meet the needs of growing dogs and cats. In dogs, the length of time and rate of growth differ between small, medium, large and giant breed dogs. Because of this, pet food companies are formulating products differently to meet the needs for the correct period of growth. For example a small breed reaches sexual and physiologic maturity by 6-8 months whereas giant breeds such as Irish wolfhounds may not reach maturity until 18-24 months of age. Pets should eat a product formulated for growing animals until they reach at least 80% of their mature height. Specific objectives of a feeding plan for growing animals are:1) to achieve healthy growth, 2) to optimize immune function and behavioral development (training), 3) minimize developmental orthopedic disease and 4) minimize risk of unhealthy weight gain and obesity.

FEEDING MANAGEMENT

Not only is it important to select a product that meets the need for growing puppies or kittens, it is important to feed the right amount to maintain healthy growth. Optimal growth is controlled rather than feeding for maximal growth. Counsel owners about the importance of preventing unhealthy weight gain while pets are growing to reduce risk of obesity and all the associated health risks. Teach owners to perform a BCS at least every two weeks and make adjustments in the amount of growth food to maintain 4.5-5/9 BCS (9-point scale).[iii] Reinforce the assessment of BCS at every visit and validate when pets are ideal BCS. Reiterate the feeding recommendations at each visit which includes

1) the specific type of food, 2) the amount and frequency of feeding 3) a monitoring plan such as BCS checks. Avoid ad libitum feeding (food

LARGE BREED PUPPIES NEED SPECIAL CARE

Large breed puppies are defined as breeds that will weigh approximately 25 or more kg at adult healthy weight. These breeds are particularly at risk for developmental orthopedic disease (DOD) such as hip dysplasia, osteochondrosis as well as increased risk of osteoarthritis. There are 3 nutritional factors known to increase risk of DOD:

1. **Increased calories and rate of growth.** Feeding puppies excess calories results too rapid growth before the accumulation of body fat. When a growing puppy is even slightly overweight he is growing too quickly which increases his risk of DOD. Teach owners to regularly perform BCS and adjust food to maintain a BCS of 4.5-5/9. This will not affect the ultimate size, but rather slow the growth rate and extend the growing period.
2. **Excess calcium.** Young large reed puppies are unable to regulate the amount of calcium absorbed by the gastrointestinal tract. Thus when excessive calcium is consumed, it is rapidly absorbed and results in abnormal growth in bones and joints. Select foods for large breed puppies that meet the growth requirement but are more modest levels of calcium than many puppy foods.
3. **Nutritional Balance.** Several factors may contribute to nutritional balance when feeding puppies. Commercial pet foods are formulated to meet the nutritional needs for the life stage when fed in the correct amount (to maintain healthy weight) and when fed as the majority (90-95%) of the pet's intake. Many well -meaning pet owners strive to feed the very best foods and will often add human food as top dressing or supplements thought to provide benefit for bone growth. Unfortunately, the effect of these supplements is more likely to imbalance the nutrient profile needed for proper growth and development than it is to provide benefit.

Nutritional Adequacy Statement.

Recently, AAFCO established new regulations for the life stage of large breed . Pet food labels for puppies and all life stages will soon have to have one of two qualifiers for the nutritional adequacy statement (AAFCO statement), which is required on every food to show it is complete and balanced:

- [Pet Food Name] is formulated to meet the nutritional levels established by the AAFCO Dog Food Nutrient Profiles for growth/all life stages ***including*** growth of large-size dogs (70 lbs or more as an adult).
- [Pet Food Name] is formulated to meet the nutritional levels established by the AAFCO Dog Food Nutrient Profiles for growth/all life stages ***except for*** growth of large-size dogs (70 lbs or more as an adult).

Be sure to carefully read these statements so you do not overlook the small, but critical, difference between "except for" and "including"!

TREATS

Another major source of nutritional imbalance may be additional treats. Using food rewards is often an effective way of training puppies. While undergoing training, it is easy to exceed 5-10% of the daily calorie requirement with training treats and foods which can provide excess calories but also interfere with the nutritional balance. This occurs at a time when puppies especially need balanced nutrition to set them up to be healthy adults. Consider using other balanced foods to use as training treats.

GONADECTOMY

Whatever age gonadectomy occurs, it will reduce the caloric requirement by as much as 25%. This is an important time to reassess the pet and adjust the feeding recommendation to meet the new requirements while maintaining ideal BCS. If they are still growing, use a lower calorie high protein growth product and vigilant monitoring of the pet's BCS.

CONSIDER A NEW WAY, AN OPPORTUNITY TO IMPROVE PREVENTIVE CARE

The current conundrum

Based on the conventional first year appointment scheduling strategy, pet owners often view the primary purpose of first year veterinary examinations are to provide vaccinations instead of preventive care advice and early identification of medical and behavioral problems. The schedule for puppies and kittens outline frequent visit between 6 weeks and 4 to 6 months. Initially, to get the vaccine series and then for spay/neutering at the typical 6 months of age. The first

'annual' visit is often scheduled to be 12 months after the rabies vaccine which is given at 16-18 weeks of age. This will create a gap in veterinary care of 10 to 12 months. This gap from ~ 4 months of age until 16 month old is a time of significant development and growth and it occurs without input or oversight of the veterinary health care team. It is during this time of physical growth and social and behavioral development that pet owners get limited advice or advice from non-veterinary professionals. This can lead to irreversible or difficult behavioral problems or physical problems.

Add a new sequence to the First year of preventive care schedule; 2,3,4,6,9,12

The vaccine series occurs approximately monthly 2, 3 and 4 months. If gonadectomy is done at 6 months, see them every 3 months until 1 year of age. These visits should include weight, diet and obesity prevention efforts, behavioral assessment and intervention as necessary, parasite control (heartworm, flea tick and gastrointestinal parasites) and the first annual (12 month) visit. This will allow the health care team to create a proactive approach and an intervention points for nutritional behavioral, life stage and parasite problems. It further establishes veterinary visit habits and ensures proper dosing changes to food and medications (parasite preventions). It also establishes quality and value of preventive care with additional opportunities to bond and establish credibility as the trusted source of pet health information.

MAINTAINING ADULT HEALTH

Once a dog or cat finishes growing their nutritional needs will likely be better met with pet food products formulated for adult maintenance. Once a pet reaches adulthood it is important to complete a nutritional assessment at every visit[Ai]. Prevention, early detection, and early intervention are the first line of defense when talking about disease management and the same is true for nutritional problems. Pets should be considered to have nutritional risk factors when any of the following are present[1];

- Abnormal BCS (or Muscle condition score -MCS)
- Snacks, treats or table foods > 10% of total Calories
- Specific life stage considerations; especially at the time of spay or neuter
- Unconventional diet
- Systemic or dental disease
- Gastrointestinal signs
- Poor skin condition or hair coat
- Inadequate or inappropriate housing

This is a proactive approach as part of every preventive care program. If you identify any nutritional risk factors these should be addressed with a revised nutritional recommendation and follow up.

MEETING UNIQUE NEEDS OF SENIOR YEARS

Senior pets are an increasing percentage of the population visiting veterinary clinics. It is important to remember that old age is not a disease and the goal of pet owners is to maintain health and quality of life for their pet with advancing age. Life span varies between breeds with small breeds of dogs living longer than giant breeds. When considering nutritional needs, consider pets to be senior when they approach the last 25% of their expected life span for their breed.

Despite a somewhat arbitrary categorization of aging, physiologic changes that occur in middle-aged and seniors make them less tolerant of nutritional deficiencies or excesses. Middle aged pets should be considered "at risk" or more vulnerable to age-related health and nutrition-sensitive problems. Middle age is the stage of life when there is an increasing incidence of an onset or progression of chronic diseases, many of which can be influenced by nutritional management. Conduct the same nutritional assessment at every wellness visit that has occurred throughout the lifelong care of the pet, but with advancing age, an extended evaluation is more likely to be needed.

There are many pet food products marketed for aging or senior pets, however there is no established nutrient profile for senior and geriatrics. Instead, an individual assessment must be done and a specific recommendation made for each individual. No changes need be made if the pet is thriving on the adult product they are eating.

NUTRIENTS OF CONCERN FOR SENIOR PETS

Although there isn't an established nutrient profile for seniors, aging can affect nutrient needs of healthy seniors.

ENERGY

Energy requirements to maintain body weight (MER) varies depending on factors such as breed, health, neuter status and age. MER decreases approximately 25% as pets age, with the greatest decline occurring at middle age (7 years)8. Muscle loss also occurs with advancing age further lowering energy requirements and possibly affecting mobility. Unhealthy weight gain exacerbates many other age-related conditions. For this reason, vigilant monitoring of both BCS and MCS of senior pets is vital.

WATER

Age can alter the physiologic control systems such as thirst and appetite/satiety in geriatric humans. Although this is not known in dogs and cats, a similar response is expected[9]. Thus water intake should be monitored or ensured when senior pets are exercising or out in a hot environment. Seniors may also be at risk for inadequate fluid intake if they have subclinical renal insufficiency. When a senior pet's appetite remains good but water intake is suspect, consider offering canned foods or adding water to the food to ensure adequate intake and hydration.

PROTEIN

Contrary to previous recommendations to restrict dietary protein to protect renal function, protein requirements of seniors actually increase with age. Some of the increased protein need arises due to an increase in protein turnover and a reduction in protein synthesis.[10] Dogs and cats vary. Dogs have increased protein requirement is not due to reduced digestibility, but many elder cats have reduced ability to digest fat and protein. Healthy senior pets do not benefit from protein restriction and may show reduced function when dietary protein is limited[7]. As a general rule of thumb for estimating protein needs; consider providing 2.55 gms protein /kg body weight (BW) for dogs and 5 gms protein /kg body weight (BW) for cats as general guide for "minimum" adult daily requirement[7, 11]. This level of protein intake should minimize risk of creating signs of protein deficiency. Senior pets may need up to 50% more than this[13]. Calories will also impact protein intake. Older pets may need fewer calories by middle age, but whit very advanced age, they may not consume as much food or as with cats, they may not digest food well. It is important to match the energy and protein needs to maintain ideal body weight and minimize loss of lean body (LBM) muscle. Assess both BCS and MCS to monitor body weight and LBM.

SUMMARY

Nutrition can play an integral role in maintaining health and preventing diseases. Use nutrition-screening tools to identify changing needs of pets throughout life and address nutritional concerns early to improve the pets quality of life and life span.

TOOLS

A. WSAVA Nutrition toolkit contains Many useful handouts available to use and download and print for clients: http://www.wsava.org/nutrition-toolkit

   i. Nutritional Assessment Checklist: www.wsava.org/sites/default/files/08%20Nutritional%20Assessment%20Checklist%20ESP.pdf

   ii. Diet History Form: http://www.wsava.org/sites/default/files/07%20Short%20Diet%20History%20Form_esp.pdf

   iii. Criteria for How to select a Pet Food: http://www.wsava.org/sites/default/files/15%20Recommendations%20on%20Selecting%20Pet%20Foods%20esp.pdf

   iii. Body Condition Score charts, dogs and cats: http://www.wsava.org/sites/default/files/01%20%28Spanish%29%20Body%20condition%20score%20chart%20dogs.pdf

   http://www.wsava.org/sites/default/files/Body%20condition%20score%20chart%20cats.pdf

   iv. Muscle Condition Score chart-http://www.wsava.org/sites/default/files/03%20%28Spanish%29%20Muscle%20condition%20score%20chart-DOGS.pdf

   http://www.wsava.org/sites/default/files/04%20%28Spanish%29%20Muscle%20condition%20score%20chart-CATS.pdf

Pet Nutrition Alliance Calculator: http://petnutritionalliance.org/calculator/

References and website links

1. Global Nutrition Guidelines. Translation in Spanish available. http://www.wsava.org/guidelines/global-nutrition-guidelines. Accessed January 20, 2019
2. WSAVA Global Nutrition toolkit. http://www.wsava.org/nutrition-toolkit accessed January 27, 2019.
3. Pet Nutrition Alliance. Tips for Implementing Nutrition as a Vital Assessment in Your practice (2012) http://www.wsava.org/sites/default/files/PNA_TipsGuide_WSAVAproof2.pdf accessed January 11, 2019
4. Nutrition: The 5th vital assessment and, Hill's Pet Nutrition, http://www.everypeteverytime.com/nutrition-assessment-support.html
5. Hazewinkel H. Nutritional management of orthopedic diseases. In: Fascetti AJ, Delaney SJ (eds). *Applied Veterinary Clinical Nutrition*. West Sussex: Wiley-Blackwell, 2012: 125-155.
6. Jeusette I, Detilleux J, Cuvelier C, et al. Ad libitum feeding following ovariectomy in female Beagle dogs: effect on maintenance energy requirement and on blood metabolites. J Anim Physiol Anim Nutr (Berl). 2004 Apr;88(3-4):117-21.
7. Churchill J, Nutrition for Senior Dogs.Critical updates on canine and feline health NAVC Proceedings 2015: 19-25.
8. Effect of age on maintenance energy requirements and apparent digestibility of canine diets. Laflamme DP, Martineau B, Jones W, et al. *Comp Cont Educ Small Anima Pract* 22(suppl 9A);113, 2000.
9. Age-related changes in nutrient utilization by companion animals. Fahey GC, Barry KA, Swanson KS. *Annu Rev Nutr.* 28:425-445, 2008.
10. Age-related changes in protein synthesis. Richardson A, Birchenall-Sparks MC. *Rev Biol Res Aging* 1:255–73,1983.
11. Laflamme D, Gunn-Moore D. Nutrition of Aging Cats. *Vet Clin Small Anim* 44 (4) 2014: 761-774.

PET FOOD FADS - FACT OR FICTION: ASSESSING CLAIMS OR BENEFITS OF UNCONVENTIONAL DIET TRENDS

Julie Churchill DVM, PhD DACVN

Julie Churchill DVM, PhD DACVN, University of Minnesota College of Veterinary Medicine

How to decipher information about a pet food to make a diet recommendation

Making a decision about what to feed their pet has become even more complicated for pet owners whose goal is often to feed *the* best food. Clients can bombard the veterinarian and veterinary staff with questions about pet food. With almost 5000 different product labels on the market it is inevitable veterinarians will be asked about a product they are not familiar with. Advice and information recommending *the* best food is readily available almost anywhere; from trainers to pet food retailers, from magazines, internet sources and social media. However these voices can be strongly biased and may compete with the veterinarian healthcare team's advice.

There is no single 'best' food for all pets since optimal nutrition depends on several things such as life stage body condition appetite, activity (or sedentary lifestyle) environment and health status. Pet owners frequently make their decisions based on the marketing claims rather than objective nutritional information. Therefore, veterinary professionals need to be competent and confident in evaluating new or less familiar products in order to make nutritional recommendations for their patients and help owners make sound nutritional decisions for their pet.

Although there are limitations to evaluating a pet food, the label is a good place to start. All pet food labels must include the following: a guaranteed analysis (% nutrient content as fed), a nutritional adequacy statement according to standards required by the Association of American Feed Control Officials (AAFCO), an ingredient list, food name and type, feeding guideline and manufacturer contact information (Zicker, 2008). A systematic approach to evaluating labels is a useful first step in assessing a product for a patient. Of all of these, the two most useful pieces of information on a pet food label are 1) the manufacturer and 2) the nutritional adequacy statement.

What follows is a suggested approach to assessing labels and pet food products for indicators of a product's nutritional value and potential impact on pet health.

Suggested in descending order of importance:

The Manufacturer information

The manufacturer's name and contact information should be provided. Contact the manufacturer whenever you have questions about a product. This can provide you with valuable information as well as an indication of how willing a company is to work with the veterinary profession. The American Animal Hospital Association (AAHA) (Baldwin 2010) and the World Small Animal Veterinary Association (WSAVA, 2011) Nutritional Assessment Guidelines includes an excellent list of questions or considerations to ask of manufacturers.

- The manufacturer should employ at least 1 full-time qualified nutritionist. Appropriate qualifications are a PhD in animal nutrition or board- certification by the American College of Veterinary Nutrition or European College of Veterinary Comparative Nutrition.
- The manufacturer should test its diets with AAFCO feeding trials. If AAFCO feeding trials are not conducted, the manufacturer should, at a minimum, ensure that diets meet AAFCO nutrient profiles through analysis of the finished product.
- The manufacturer should own the plant or plants where the food is manufactured.
- The manufacturer should practice strict quality-control measures. Examples include certification of a manufacturer's procedures (eg, Global Food Safety Initiative, Hazard Analysis and Critical Control Points, or American Feeding Industry Association); testing ingredients and end- products for nutrient content, pathogens, and aflatoxins; materials risk assessments; and supplier audits.
- The manufacturer should be able to provide a complete nutrient analysis for any dog or cat food of interest

(not only the guaranteed analysis, which is listed on the label, but the average [typical] analysis as well). The manufacturer should be able to provide exact values for all nutrients. This should ideally be provided on an energy basis (ie, grams per 100 kilocalories or grams per 1,000 kilocalories), rather than on an as-fed or dry-matter basis, which does not account for the variation in energy density among foods.

- The manufacturer should be able to provide the number of calories for any food on any requested weight or volume basis (eg, per gram, per pound, per cup, or per liter).
- The manufacturer should conduct and publish research in peer-reviewed journals.

Nutritional Adequacy Statement (NAS)

A statement of nutritional adequacy developed by AAFCO is required on all pet food packages. The nutritional adequacy statement confirms 3 important features of that pet food product:

1. **The product is complete and balanced.** This means that the product is intended to provide all the nutrient requirements as the sole source of nutrition. If the statement reads "intended for intermittent or supplemental feeding" it should not be considered complete and balanced and should be avoided for everyday feeding unless overseen by a veterinary professional (as some of the therapeutic diets are labeled).
2. **The life stage.** When a product is complete and balanced, the NAS should identify the life stage for which it is intended. AAFCO defines nutrient profiles and feeding trial requirements for growth, reproduction and adult maintenance life stages only. It is important to remember that there is no AAFCO defined nutrient profile for senior/geriatric life stage and the nutrient content of products marketed for senior pets can vary widely. If a product is formulated to meet AAFCO profiles for "all life stages" it must meet the minimum nutritional requirements for both growth and adult maintenance. Products formulated for "all life stages" may contain excessive amounts of some nutrients, which can result in overfeeding. It is better to feed pets with food designed to match their life stage.
3. **Method for determining nutritional adequacy.** Nutritional adequacy can be established by a pet food company either 1) through animal feeding trials or 2) through formulation tests.

   Feeding trials are conducted with animals to ensure that nutrients in a given food or line of foods are present in sufficient quantities to promote good health and are bio-available to the animal ensuring the nutrients are digested properly.

   a. A product bearing the statement: "Product X" is formulated to meet nutrient profiles established by AAFCO (Species) nutrient profiles for [specified] life stage. These formulated products have had nutrient content confirmed by mathematical calculations (adding nutrient content of ingredients listed in a database) or by analytic testing of the finished product (preferred). The formulation method does not include testing involving animals.

   b. A product bearing the statement: "Animal Feeding tests using AAFCO procedures substantiate "Product X" provides complete and balanced nutrition for [specified] lifestage, has undergone a feeding trail evaluation. Feeding trials allow for an in vivo product evaluation and an indirect measurement of bioavailability of nutrients Feeding trials are preferable to formulations to help test nutritional adequacy but they do not assure the product provides adequate nutrition under all circumstances.

Caloric Content Disclosure

Because of the prevalence of obesity in pets, caloric disclosure and labeling is essential for veterinarians to assess and counsel clients about purchasing pet foods that meet the energy needs of their pets. Fortunately, caloric disclosure has recently required by law and is in the transition phase of manufacturers being required to include on packaging. Owners are still unaware of caloric content variation between foods causing pet owners a risk of over feeding their pets, resulting in obesity and related health problems. Companies that choose to report the caloric content in ways easily found on websites and packaging are preferred. Making calorie content readily accessible greatly helps the veterinary team determine a proper food dose when making a nutritional recommendation and allows consumers to make comparisons between foods and select more appropriate feeding portions.

Ingredients

Evaluation of the ingredient list is often the most controversial aspect when interpreting a label. Evaluating ingredients presents challenges for clients because they are barraged with marketing claims, misinformation and even scare tactics. The belief that the ingredient list is the most important piece of information when judging pet food quality is reinforced when many of the pet food rating systems and pet food reviews are based on judgments about ingredients. Except for patients with adverse reactions or primary food allergies, or a traditional Chinese medicine approach, this is often the least useful information provided. Pets require nutrients, not ingredients, so a food composed of wonderful sounding ingredients may be less nutritious than one with seemingly less appealing (to the pet owner) ingredients. Clients usually want to prioritize ingredients and much of their information is based on misconceptions. The veterinary team must be careful not to discount client concerns yet use the opportunity to educate and guide owners in their decision-making about pet foods.

Evaluation of ingredient lists remains challenging for many veterinary professionals because transparency about ingredients, ingredient sources, and processing methods beyond the minimum of what is legally required is generally difficult to come by in the pet food industry. In addition, the nutrient-based scientific literature is not comprehensive, especially when compared to the research base for human nutrition. Although there is widespread misunderstanding about pet food ingredients, the major ingredients commonly used in pet food (beef, poultry by product, lamb meal etc.) are fairly well regulated and defined by AAFCO. Many fruits, vegetables, and other seemingly healthy ingredients have no AAFCO definition for the ingredient. If an ingredient definition does not exist, AAFCO regulations state that it "shall be identified by the common or usual name." For example, 'Apples' or other fruits may contain seeds, stems, leaves, skins, or pulp. While pulp may contribute nutrients to the food, the generic definition does not clearly exclude any other parts that may not be beneficial to the animal's health. For all of these reasons, reliance on pet food ingredients as the primary way to assess a pet food product would be a poor indicator of a product's overall health impact for a pet. As part of the initiative to consider nutrition the 5th vital assessment, a Nutrition Reference Manual (http://www.everypeteverytime.com/docs/en-us/Pet_Nutrition_Ref_Manual.pdf) provides an excellent description of pet food label requirements and clarifications about ingredient definitions. This is a useful tool for team training about how to talk about ingredients with pet owners.

Another useful tool for discussing nutrition myths about ingredients can be found at http://vetnutrition.tufts.edu/2016/06/why-you-shouldnt-judge-a-pet-food-by-its-ingredient-list/ (accessed January 27, 2019) "you shouldn't judge a pet food by its ingredients". On the other hand foods from boutique or small companies, or marketed as using exotic or grain free, (unusual meats such as venison, bison, alligator, kangaroo, legumes such as chickpea, lentils etc, BEG) have been associated with dog's developing dilated cardiomyopathy. The cause has not been elucidated and is actively being investigated by the FDA.

Nutrition recommendation, a pet-specific process

The final steps of making a nutritional recommendation for a pet food are to use your judgment in evaluating a product and match it closely with life stage, life style and health of the pet. To complete the process, you would continue to monitor the pet's response to make sure you see the expected results, that the patient maintains optimal health.

Recalls

Sadly, pet food safety issues remain a growing concern. We have become more aware of pet food safety issues, most dramatically evident in 2007 with melamine adulteration of wheat gluten which affected many products and led to renal failure in a number of pets. Most recently, at the time of this writing, a number of products have been recalled for excess levels of vitamin D, and several more foods or treats with listeria and salmonella contamination. Pet food safety is now more closely monitored by the FDA, and there are more professional "watch-dogs" sharing information and updates about pet food recall; Veterinary Information Network (VIN), American Veterinary Medical Association(AVMA), and State Veterinary Medical Association etc. There is now a central Food and Drug Administration (FDA) online safety reporting portal for veterinarians and owners to submit reports of concern about pet foods and treats. These can be submitted electronically: http://www.fda.gov/AnimalVeterinary/SafetyHealth/ReportaProblem/default.htm

If the veterinarian has suspicions about the safety of a food, this warrants a thorough diet history. Check the FDA website and contact the pet food manufacturer to alert and confirm your concerns. You will need information from

the product label, so advise client to keep the label with the food until the bag is completely consumed.

Tips for discussing unconventional Diets with Pet Owners

a. Obtain a thorough diet history to include treats, snacks and human foods. Also include any foods used to administer medication. If 90% or more of the intake is composed of a good quality complete and balanced product it is unlikely to adversely affect the nutritional balance for healthy adult (non-reproducing) pets. Pets are higher risk is treats or additional food exceed 10% of the diet and should not contain potentially harmful foods such as chocolate, grapes, raisins, or onions.

b. Identify and discuss the reason for the owner's selection of unconventional foods and their concerns about changing the diet. This can focus your discussion to address their concerns.

c. Use empathy and care when discussing foods, presenting evidence based facts and be aware of determining an owners "readiness to change".

Limited References

Baldwin K, Bartges J, Buffington T, et al. AAHA Nutritional Guidelines for dogs and cats. *J Am Anim Hosp Assoc.*; 46:285-296, 2010.

Chandler, M. Unconventional Diets. Clinician's Brief. February 2014:15-17.

FDA Animal and veterinary safety reporting portal (2010), http://www.fda.gov/AnimalVeterinary/SafetyHealth/ReportaProblem/default.htm

Freeman L, Stern J., Fries R. et. al. Diet-associated dilated cardiomyopathy in dogs: what do we know. Commentary. *JAVMA* 253 (11):1390-94, 2018.

Nutrition reference manual (nd), Hill's Pet Nutrition, http://www.everypeteverytime.com/docs/en-us/Pet_Nutrition_Ref_Manual.pdf

Pet Nutrition Alliance. AAHA Tips for Implementing Nutrition as a Vital Assessment in Your Practice (2012) http://petnutritionalliance.org/PDFS/PNA_TipsGuide_AAHAproof2.pdf

WSASA, Nutritional assessment guidelines (2011) http://www.wsava.org/PDF/Misc/WSAVA_GlobalNutritionalAssessmentGuidelines_2011.pdf

WSAVA nutrition toolkit: http://www.wsava.org/nutrition-toolkit, selecting the best food for your pet. Accessed December 2018

WSAVA nutrition toolkit: http://www.wsava.org/nutrition-toolkit, select the dog/cat owners saavy guide to nutrition on the internet. Accessed December 2018

Zicker, S. Evaluating pet poods: how confident are you when you recommend a commercial pet food?" *Topics in Companion Animal Medicine*. 23 (3).121-126, 2008.

"ELIMINATE" THE PITFALLS WHEN CONSIDERING A FOOD TRIAL

Julie Churchill DVM, PhD DACVN

University of Minnesota College of Veterinary Medicine, Veterinary Clinical Science Department

An elimination diet trial is a common part of the diagnostic plan to cutaneous adverse food reactions and potentially sets the stage for therapeutic management. There are many potential pitfalls along the way, ranging from how to select the best elimination diet, how to support adherence during the trial and then developing a maintenance plan.

When adverse reactions to foods or food allergies are among the rule-out list, a diet trial (food elimination-challenge) is indicated. Before a diet trial is initiated, a nutritional assessment of the patient including a thorough diet history is necessary.

MAKE NUTRITIONAL ASSESSMENTS AND RECOMMENDATIONS A PART OF EVERY PET'S VISIT

The AAHA/WSAVA Nutritional Assessment Guidelines outline the process of performing an assessment specific to the individual patient. By leveraging tasks that the care team already does, the assessment process can be easily integrated into the routine history and physical examination portion of every appointment. In the course of obtaining the history and performing the exam, each patient is screened for nutritional risk factors, established by the pet's breed, life stage and life style, body weight and condition, health history, and underlying comorbidities, medications and diet.

SCREENING FOR NUTRITIONAL RISK FACTORS

Prevention, early detection, and early intervention are the first line of defense when talking about disease management and the same is true for nutritional problems. Pets are considered to have nutritional risk factors when any of the following are present;

- Abnormal BCS (or MCS)
- Snacks, treats or table foods > 10% of total Calories
- Specific life stage considerations; especially at the time of spay or neuter
- Unconventional diet
- Systemic or dental disease
- Gastrointestinal signs
- Poor skin condition or hair coat
- Inadequate or inappropriate housing

When any of these findings, such as potential allergies are discovered by the screening process, it raises the index of suspicion for a nutrition-related problem. Discovery of nutritional risk factors also identifies an opportunity for further evaluation and the potential for nutrition to play an increased role in improving the pet's health. When any of the above risk factors are identified, an extended nutritional evaluation is indicated. The importance of the extended evaluation increases as the number or severity of risk factors increases. The extended nutrition evaluation would include a more in-depth diet history and assessment of the pet's diet. (**See Appendix A** for **WSAVA extended diet history form**). The history should include both past and current foods eaten to establish a dietary "antigen exposure list". The components of a thorough diet history should include:

- The patient's primary diet (brand(s) and specific name(s), amount and frequency)
- Medication list, including chewable forms and method of administration or foods used to medicate
- Dietary supplements, brand, names, forms (chewable?) and method of administration
- Snacks and treats (brands, names and frequency) including human foods
- Dental treats, or toothpastes
- Chewing toys such as rawhides, bully sticks
- Access to other food sources (pets, children, neighbor)

There are three strategies for planning an elimination diet trial.

1. Novel Protein Diets

When an accurate diet history can be collected, a limited and novel protein diet can be identified and incorporated into the trial plan provided the protein and carbohydrate sources are *novel* to *this patient*. For this to be successful, the assumptions made are a) that the diet history is accurate and includes all past and present food antigen exposure and b) the patient is not allergic to the new protein identified. Little information is available regarding cross reactivity between protein ingredients in veterinary patients. There is a wide and expanding number of exotic or rare ingredient sources used in commercial foods (alligator, bison, kangaroo, chickpea lentil etc.) so the healthcare team should not assume these types of proteins are novel for all patients. There are also increasing reports of contamination (or adulteration) of over-the-counter (OTC) diets marketed as limited ingredient.[1-3] Contamination by common dietary antigens can confound the results of the elimination food trial, thus OTC diets should not to be considered to have the quality control measures of a veterinary therapeutic diet and therefore should not be considered as limited antigen for either diagnosis or treatment of food allergies. These foods, boutique exotic ingredient and/or grain free foods also present an additional risk of diet-associated dilated cardiomyopathy which is currently under investigation by the FDA.

2. Hydrolyzed protein diets.

Because dietary proteins initiate the antigenic response, hydrolyzed proteins have become an additional tool in diagnosis and treatment protocols for food allergies. The enzymatic hydrolysis reduces the molecular size of the protein, thus reducing cross linking if IgE and histamine response as well as reducing epitopes for other immune responses. The development and consistent production of high quality hydrolyzed diets was challenging and offered as commercially available therapeutic products. The degree of hydrolysis and molecular size of the proteins varies among these products and none can guarantee complete elimination of an antigenic stimulation. Therefore, pets with a confirmed allergy to a specific protein should probably avoid that protein even when hydrolyzed, or judiciously test tolerance to that product realizing the potential for incomplete hydrolysis.

Hydrolyzed protein therapeutic diets offer several advantages: a) they offer complete and balanced nutrition, b) hydrolyzed diets are a good choice when an accurate and thorough diet history is unavailable or impossible to obtain, and a novel protein cannot be identified, c) when previous dietary protein exposure is excessive and there are no feasible sources for a novel protein, and d) when the pet owner can only consider commercial options for an elimination trial and novel commercial proteins are not an option.

Potential pitfalls arise with hydrolyzed diets as an elimination diet trial as well. Many owners don't hear or understand that hydrolyzed protein diets should be the *exclusive food and treat* for the 8-12 week trial. It is easy to underestimate the need for ways to help the owner administer medications. Additional foods (top dressings), treats, flavored or chewable medications or food used to administer medications are common confounders to an elimination trial. There are a limited number of therapeutic hydrolyzed diet options which are expensive, lower in protein and limited in other nutrient ranges than most other pet foods. For this reason, the food could serve the patient for a short term but not meet the needs long term. Hydrolyzed diets can also be limiting when other comorbidities are present. (See Table 1 for considerations of nutrient ranges when selecting a product for an elimination trial the patient.)

3. Home Cooked diets

A home prepared diet has increased in popularity among pet owners and can be used for a novel protein elimination trial. Some still consider this the gold standard for elimination trials however this is reliant on an accurate diet history to identify a protein novel to the individual patient. Barriers to this have been discussed above and successful protein selection is only as good as the diet history.

Benefits of home cooked foods include the ability to avoid contamination with other proteins and owners can feel more empowered and engaged in the diagnostic and treatment plan. Processing (extrusion, canning) can have variable effects on proteins and thus antigenic structure. This is not well understood but may explain anecdotal reports of success from home cooked foods compared those commercially prepared at higher temperatures. Patients with extensive medical histories or concurrent comorbidities are well suited to home cooked therapeutic diets. These can be custom formulated to manage the other medical conditions and incorporate a novel protein trial. For these reasons, a home cooked diet may offer the most flexible nutrient profile to accommodate multiple medical conditions.

Homemade diets present several potential pitfalls. Unbalanced diets have historically been recommended to limit the number of ingredients. These were considered acceptable because of the short-term nature of a feeding trial. However extended feeding, such as the commonly recommended 8-12 weeks trial of unbalanced diets, can lead to deficiencies of nutrients important for immune function and epidermal barrier. Recommendation of a suboptimal or unbalanced recipe also creates the misperception that it provides balanced nutrition and clients maybe reluctant to change and continue this diet long after the trial is complete. Recipes for long term feeding. Because most nutritional problems do not cause noticeable physical or laboratory abnormalities until the nutritional imbalance has been present for months or years, the link to diet can be hard to identify. Formulating complete and balanced diets requires knowledge of canine and feline nutritional requirements. It is possible to provide complete and balanced nutrition with home prepared foods; however this depends on the training and competence of the person formulating the food as well as the compliance and discipline of the person preparing the food. When readily available canine recipes, obtained from text books, books, or websites were evaluated for the nutritional adequacy of the recipe, very few provided essential nutrients in amounts recommended by the Association of American Feed Control Officials (AAFCO) or the National Resource Council (NRC) unless the recipes were formulated by a Board Certified Veterinary Nutritionist™.

The same cautions must be made to avoid additional proteins, snacks or flavored/chewable medications that could interfere with the elimination trials. Another potential pitfall of homemade and maintaining a nutritionally complete homemade diet is the propensity for owners to substitute ingredients or supplements without checking with the nutritionist first. They may stop (or substitute) a vitamin and mineral supplement because of cost, inconvenience or lack of understanding the importance of these supplements added in the correct amount.

Pet owners will show a wide variety of homemade recipes to their veterinarian that they obtained from a breeder, a trainer, popular press or the internet. Some clients will ask their veterinarian's opinion about whether this would be a good option for their pet or is it ok to change some items. Use the following guideline* to make a quick assessment of a homemade recipe for clients to screen for likelihood of nutritional balance. (Hand 2010, Stockman 2013).

1. Do five food groups appear in the recipe?
   - a carbohydrate/fiber source which is cooked
   - a protein source, preferably animal origin
   - a source of macro minerals, particularly calcium
   - a multi-vitamin and trace mineral source
2. Is the carbohydrate source a cooked cereal and present in equal or higher quantity than the meat?
   - Carbohydrate:protein ratio 1:1 in cats and 2:1 to 3:1 in dogs
   - Sources are cereal grains or potato and should be cooked; corn, rice, wheat, barley or potato
3. What are the type, quality and quantity of the primary protein source?
   - Protein quality can be improved by exchanging vegetable protein with animal based protein.
   - Protein content of various mammalian and avian skeletal muscle tissues is generally equivalent in amino acid profile
   - For amino acid balance, provide some liver as part of the meat portion once a week. This will correct some potential amino acid deficiency and provide fatty acids, vitamin and microminerals.
4. Is the animal protein source lean or fatty?
   - If the protein source is "lean", an additional fat source is required, at least 2% of the formula for dogs and 5% of the formula for cats
5. Is a source of calcium and other minerals provided******* (commonly neglected item)
   - Most homemade formulas require a specific calcium supplement
6. Is a source of vitamins and other micronutrients provided?
   - Supplements providing vitamins, trace minerals, fatty acids and taurine should always be provided (trace minerals zinc, choline copper often deficient, as well as vitamins D and E)

* these guidelines for healthy adult ("maintenance" life stage) animals.

There are many references for home cooked diets for pets. Verify the nutrition credentials and training of people

authoring these diets and use some of the above guidelines to make an assessment of the recipes. Select a recipe that has been formulated by a veterinary nutritionist. Homemade diets for patients with medical problems should always be formulated by a veterinary nutritionist specifically for the individual patient after careful nutritional assessment of the patient. Other resources for complete and balanced homemade diets are www.aavn.org for a list of veterinary nutritionists available for consultation.

Considering the advantages and disadvantages of the three strategies for selecting the diet for an elimination trial, is crucial to matching the best strategy for the patient and client. All dietary diagnostic and interventions should be based on a careful diet history and thorough nutritional assessment of the patient.

Limited Resources and References

Nutritional assessment and nutrition and dermatology

WSASA, Nutritional Toolkit https://www.wsava.org/Guidelines/Global-Nutrition-Guidelines, *accessed Feb. 8, 2019*.

Schmalberg, J. Diets and the Dermis: Nutritional Considerations in Dermatology. TVP. 2017; March/April 33-41.

Novel Protein diets

Willis-Mahn C[1], Remillard R, Tater K. ELISA testing for soy antigens in dry dog foods used in dietary elimination trials. J Am Anim Hosp Assoc. 2014 Nov-Dec;50(6):383-9

Raditic DM[1], Remillard RL, Tater KC. ELISA testing for common food antigens in four dry dog foods used in dietary elimination trials. J Anim Physiol Anim Nutr (Berl). 2011 Feb;95(1):90-7.

Fossati L. Determination of Mammalian DNA in Commercial Canine Diets with Uncommon and Limited Ingredients., AAVN 2018; abstract.

Parr JM[1], Remillard RL. Common confounders of dietary elimination trials contain the antigens soy, pork, and beef. J Am Anim Hosp Assoc. 2014; Sep-Oct;50(5):298-304

Hydrolyzed diets

Olivry T. Bizikova P. A systematic review of the evidence of reduced allergenicity and clinical benefit of food hydrolysates in dogs with cutaneous adverse food reactions. Vet Dermatol 2010; 21:32-41.

Gaschen FP, Merchant SR. Adversefood reactions oin dogs and cats. *Vet Clinic North Am Sm Anim Pract* 2011; 41:361-379.

Homemade diets

Stockman J[1], Fascetti AJ, Kass PH, Larsen JA. Evaluation of recipes of home-prepared maintenance diets for dogs. J Am Vet Med Assoc. 2013 June 1; 242(11):1500-5.

Hand M, et al, eds. *Small animal Clinical Nutrition:* making pet foods at home, 5th ed. Topeka, Mark Morris Institute, 2010.

WSAVA Global Nutrition Committee

Nutritional Assessment Checklist

To be completed by the pet owner. Please answer the following questions about your pet:

Pet's name: _____ Species/breed: _____ Age: _____

Owner's name: _____ Date form completed: _____

1. How active is your pet? **Very active** ☐ **Moderately active** ☐ **Not very active** ☐
2. How would you describe your pet's weight? **Overweight** ☐ **Ideal weight** ☐ **Underweight** ☐
3. Where does your pet spend most of the time **Indoor** ☐ **Outdoor** ☐ **Indoor & Outdoor** ☐

Please list below the brands and product names (if applicable) and amounts of ALL foods, treats, snacks, dental hygiene products, rawhides and any other foods that your pet is currently eating, including foods used to administer medications:

Food	Form	*Amount	Number	Fed since
Examples:				
• Purina Cat Chow	*dry*	*½ cup*	*2x/day*	*Jan 2010*
• 90% lean hamburger	*pan-fried*	*3 oz (85 grams)*	*1x/week*	*May 2011*
• Milk Bone medium	*dry*	*2*	*3/day*	*Aug 2012*
• Greenies Salmon Dental	*treat*	*2*	*daily*	*Jan 2013*

*If you feed by volume, what size measuring device do you use? _____

*If you feed tinned/canned food, what size tins/cans? _____

4. Do you give any dietary supplements to your pet (for example: vitamins, glucosamine, fatty acids, or any other supplements)? **No** ☐ **Yes** ☐

If yes, please list brands and amounts: _____

To be completed by the health care team:

Has the diet history form been reviewed? **No** ☐ If not, please review the diet history form **Yes** ☐ If yes, please continue:

Current body weight: _____ Ideal body weight: _____

Current body condition score* _____/9 or ____/5 *Refer to the body condition scoring chart

Muscle Condition Score: normal ☐ mild wasting ☐ moderate wasting ☐ severe wasting ☐

Screening evaluation checklist

Pets that are healthy and without risk factors need no additional extended evaluation

Nutritional screening risk factors (extended evaluation is OPTIONAL)	***Check ✓if present***
Extremely low or high activity level	☐
Multiple pets in a household	☐
Gestation	☐
Lactation	☐
Growth period	☐
Age of >7 years	☐
Nutritional screening risk factors (extended evaluation is MANDATORY)	
History of altered gastrointestinal function (e.g., vomiting, diarrhea, nausea, flatulence, constipation)	☐
Previous or ongoing medical conditions / disease	☐
Currently receiving medications and/or dietary supplements	☐
Unconventional diet (e.g., raw, homemade, vegetarian, unfamiliar)	☐
Snacks, treats, table food > 10% of total calories	☐
Inadequate or inappropriate housing	☐
Physical examination	
Body condition score less than 4 or greater than 5 (on 9-pt scale)	☐
Muscle condition score: Mild, moderate, or severe muscle wasting	☐
Unexplained weight change	☐
Dental abnormalities or disease	☐
Poor skin or hair coat	☐
New medical conditions / disease	☐

NO CHECKED ITEM(S) ON THIS PAGE? The Nutrional Assessment is complete

CHECKED ITEM(S) ON THIS PAGE? Continue on the next page

wsava.org

Extended evaluation checklist

Changes in food intake or behavior

a. Amount eaten:	increased ☐	decreased ☐
b. Chewing:	normal ☐	abnormal ☐
c. Swallowing:	normal ☐	abnormal ☐
d. Nausea:	yes ☐	no ☐
e. Vomiting:	yes ☐	no ☐
f. Regurgitation:	yes ☐	no ☐

Condition of the integument

a. Easily-plucked hair:	yes ☐	no ☐
b. Thin skin:	yes ☐	no ☐
c. Dry or scaly skin:	yes ☐	no ☐

Abnormalities in serum chemistry profile

a. Glucose:	low ☐	normal ☐	high ☐
b. Albumin:	low ☐	normal ☐	high ☐
c. Total protein:	low ☐	normal ☐	high ☐
d. Electrolytes:			
low_____			
high_____			
e. Urea:	low ☐	normal ☐	high ☐
f. Creatinine:	low ☐	normal ☐	high ☐
g. Total T4:	low ☐	normal ☐	high ☐

Abnormalities in complete blood count

a. Anemia:	yes ☐	no ☐
b. Lymphopenia:	yes ☐	no ☐

Other _____

_____

_____

Abnormalities on fecal flotation / smear / culture: _____

_____

Abnormalities on urinalysis: _____

Abnormalities on other diagnostic tests: _____

_____

Provide the following recommendation(s):

Change in the caloric intake recommended? **No** ☐ **Yes** ☐ **If yes, calculate:**
Current caloric intake** _____ kcal or kJ/day
***Refer to information obtained from the diet history form.*
Recommended caloric intake***_____ kcal or kJ/day
**** Refer to the calorie requirement form.*

Change in the diet recommended? **No** ☐ **Yes** ☐ **If yes, describe:**
New diet recommended_____

Change is the feeding management recommended? **No** ☐ **Yes** ☐ **If yes, describe:**
Amount per serving_____cups_____cans_____grams
Number of servings per day_____
Treat(s) (if applicable); amount(s) and number(s) per day_____
Be sure to specifically discuss **table foods, supplements**, and **medication administration** with the owner.

Change of environmental factors recommended? (i.e., issues with multiple pets, other food providers and sources, extent of enrichment, activity of pet, environmental stressors)

No ☐

Yes ☐ **Describe:**_____

Recommendations for monitoring given to the client?
(i.e., BW, BCS, MCS, food intake, appetite, gastrointestinal clinical signs, activity, overall appearance)

No ☐

Yes ☐ **If yes, please describe:**_____

Did client purchase the recommended food? **No** ☐ **Yes** ☐

Educational information or tools dispensed? **No** ☐ **Yes** ☐

wsava.org

Table 1. Reference for Assessing or Selecting Products with Nutrient Modifications

Nutrient modification	AAFCO* minimum gm or mg/100 kcal	Low gm or mg/100 kcal	Moderate gm or mg/100 kcal	High gm or mg/100 kcal
Protein-dog	4.5 gm/100 kcal	<5 gm/100 kcal	gm/100 kcal	> 9 gm/100 kcal
Protein- cat	6.5 gm/100 kcal	< 7 gm/100 kcal	7-10	> 10 gm/100 kcal
Fat- dog	1.4 gm/100 kcal	< 2 gm/100 kcal ultra-low	2.5-3.5 gm/100 kcal low	> 5
Fat- cat	2.3 gm/100 kcal	< 3 gm/100 kcal		> 5
Phosphorus -dog	100 mg/100 kcal	< 100 mg /100 kcal	<150 mg/100 kcal	>200 mg/100 kcal
Phosphorus -cat	125 mg/100 kcal	< 125 mg /100 kcal ultra-low	<160 mg/100 kcal low	>200 mg/100 kcal
Sodium- dog	20 mg/100 kcal	< 70 mg /100 kcal ultra-low	<100 mg/100 kcal low	>250 mg/100 kcal
Sodium- cat	50 mg/100 kcal	< 70 mg /100 kcal ultra-low	<100 mg/100 kcal low	>250 mg/100 kcal
EPA&DHA-Dog (mg of combined)	NA	NA	~ 10-15 mg/100 kcal	> 15 mg/100 kcal wide variation
EPA&DHA-Cat (mg of combined)	NA	NA		15 mg/100 kcal wide variation

2016 *AAFCO adult maintenance minimums
AAFCO minimum levels and low/moderate and high descriptors are general comparisons to commercial pet foods.

Otitis - The Dermatology Technician's Role

John C. Angus, DVM, DACVD

Animal Dermatology Clinic, Pasadena, CA

Clinically significant otitis externa occurs in 10-20% of the dog and cat population. The majority of cases result from common underlying problems, such as atopy, food allergy, or ectoparasites. Less commonly foreign objects, neoplasia, non-neoplastic masses, endocrinopathies, immune-mediated disorders are the primary cause. Bacteria and Malassezia overgrowth occur secondarily when normal local microenvironment is altered and passive immunity is disrupted. Overgrowth of resident organisms contribute directly to inflammation and perpetuation of clinical signs. Over time infection may extend to the middle ear or chronic physical changes alter the external canal, resulting in recurrence and increased severity of disease. Under appropriate conditions, opportunistic organisms such as *Pseudomonas aeruginosa, Proteus mirabilis, E. coli* and others replace resident organisms. Repeated exposure to antibiotics selects for resistant organisms. Although not the most common bacterial infection, *Pseudomonas aeruginosa* stands out as the most painful, smelly, frustrating, and difficult to manage. Frustration with *Pseudomonas* results from (1) severity of inflammatory response, (2) unpredictable antimicrobial susceptibility patterns, and (3) frequent treatment failure.

The plan for this session is to use the example of chronic, severe Pseudomonas otitis externa/media to illustrate the key points of ear anatomy, disease process, diagnostic procedures, therapy (short and long term) all through the lens of the technician's role in doctor and patient management.

Role of Pseudomonas in ear disease

Pseudomonas is an opportunistic bacteria found primarily in water, decaying vegetation, and only occasionally on or in animals. In order to colonize the ear canal or other animal tissues, *Pseudomonas* must first establish firm adhesion to epithelial cells. Under normal circumstances the adhesion points are occupied by the normal microflora, protected by passive immunity in the form of immunoglobulins and other antimicrobial peptides found in normal cerumen. For *Pseudomonas* to colonize the canine ear, two events are required: (1) presence of organism (water) and (2) disruption of normal microflora and healthy epithelial barrier. Once Pseudomonas has a foothold it is an excellent competitor, suppressing other bacteria and yeast, producing collagenases, protease, and exotoxins; which cause further disruption, eventually resulting in ulceration and tissue breakdown, including the tympanic membrane. To make matters worse the gram negative cell wall and extracellular slime resists immune response. Vast numbers of neutrophils and macrophages are attracted to *Pseudomonas* infections. Although only partially effective against the bacteria, these WBC produce collagenases and proteases, which worsens tissue damage. In fact, serine proteases produced by neutrophils may actually enhance the ability of Pseudomonas to invade tissue. Clinically the result is an external ear canal characterized by severe inflammation, erythema, ulceration, pain, and large quantity of purulent exudate.

DIAGNOSIS

History: The first thing we need to know is (1) First Episode, (2) Relapsing/Recurrent, (3) or failure to respond. That is because the goal changes. For first episode, typically the goal is simply resolution of the current infection and general understanding of cause to get an idea if likely to relapse. If multiple episodes that resolve with therapy that relapse later, then the goal is to treat the current infection AND start diagnosis and management of underlying disease. Very likely, some ongoing maintenance care will also be needed. If not responding to therapy, then the goal is to understand why treatment is failing so that the approach can change.

Ask "age of onset" of very first episode; this is more important than "how long has this been going on?" If, less than 1 year, parasites are most likely. Food also possible. If between 1-4 years, this is consistent with Atopy, but any cause is possible. If greater than >6 years, atopy is less likely, but any cause is possible. Seasonality strongly suggests Atopy. Non-seasonal disease can be atopy or food. If household affected or new puppy or kitten was recently introduced, always consider parasites hypersensitivity, even if none are found on examination. Ask about prior medications. If the patient initially got better then suddenly worsened think about contact reactions. If current infection is of long duration (> 6 months) or more than 3 episodes a year then concurrent otitis media is very likely. General health

questions might include a history of sensitive stomach, borborygmus, increased frequency of bowel movements, or observed flare with new diet. Failure to improve to previous diet changes tells us almost nothing about food allergy; getting worse on a diet tells us much more. As a technician you also want to find out how the owner does administering ear medications at home.

Physical Examination characterizes current state of infection and helps develop likely differential diagnoses for primary cause. A whole body dermatologic examination should be performed to find evidence of concurrent disease such as Atopy, Food, Parasites, Pemphigus, Hypothyroidism, etc. Begin with palpation of external canal and parotid region Record findings such as pain, edema or soft-tissue swelling. Is the canal flexible or rigid due to fibrosis, mineralization? Is cerumen present on pinna? Normal clearance of debris should deposit this debris on the pinna. Absence of debris could indicate failure of epithelial migration. Excessive erythema, erosions, pain could indicate contact reaction or more generalized dermatologic diseases. Finally, check palpebral reflex. Reflex is diminished in some cases of otitis media cases or end-stage disease.

Otoscopy may be futile during initial examination of awake patient. Canal is often too painful, too ulcerated, or too full of pus to permit a satisfactory evaluation. However, otoscopy should be attempted to satisfy the owner's expectations and to evaluate for obvious problems such as a mass in the vertical canal. Recommend diagnostic evaluation under anesthesia, combine with therapeutic flush to remove debris. If the canal is too edematous and stenotic to advance the scope into the vertical canal, then postpone. Treat with oral and topical steroids (prednisone 1 – 2 mg/kg/day) until edema is diminished. This will add value to the procedure.

Otic Cytology is a mandatory diagnostic test for every patient presenting with otitis. Cytology is necessary to characterize type and number of organisms seen. Mixed infections are common: >3 species present in 30-60% of cases. Cytology allows you to identify current organisms, characterize the severity of disease, better interpret culture and susceptibility results, and make rational decisions regarding therapy. Use cytology to differentiate overgrowth from infection. Overgrowth occurs when native organisms take advantage of changes in the microenvironment. Overgrowth contributes to severity of disease, but is more easily managed with topical therapy alone. True infection results from either native or opportunistic organisms penetrating tissue, extending into middle ear, or creating severe inflammatory response resulting in purulent exudate. The presence of white blood cells almost always indicates true infection rather than overgrowth. Cytology is performed by direct smear and in-house stain, such as Diff-Quik. Examine on all objectives including oil immersion 100x. Be sure to record findings in the medical record for comparison with later cytology during re-evaluation: (1) Malassezia: presence/absence, estimated numbers (2) Bacteria: presence/absence, rod or coccoid morphology, estimated number, phagocytosis by neutrophils, (3) White blood cells: presence/absence; which organisms are they targeting, and (4) Parasites: presence/absence.

Culture and Susceptibility testing of bacteria in otitis externa is of questionable value. Susceptibility breakpoints used by the laboratory don't correlate to achievable concentrations in external canal. Organisms present in the external ear canal are usually very different than those found in the tympanic cavity (89.5% of cases had different species or susceptibility patterns in one study). Laboratory results are not repeatable even when obtained from same ear canal collected submitted the same time. Variability between commercial laboratories methodology and reporting can yield very different results. Do not think of antibiotic susceptibility results as rock solid fact. Culture should never be used to monitor response to therapy. Culture only tells you presence or absence of organism, but provides little information regarding response to therapy, changing numbers, changing presence of white blood cells, etc. Culture and sensitivity is only indicated for antibiotic selection against bacteria with unpredictable susceptibility patterns, or if poor response to appropriate therapy.

Other diagnostic procedures

- Food trial – 8 weeks with provocative challenge at the end
- Parasite treatment trial – Eliminate the possibility of parasites with simple therapy.
  - Selemectin every 2 weeks for 3 treatments (95-100% effective)
  - Isoxazaline class of flea/tick prevention
- Allergy testing – Intradermal Allergy Testing, Allergy serology
- Thyroid profile – T4, free T4, TSH is much better screen than T4 alone
- Discontinue all prior topical or systemic therapies in case of contact or adverse drug reaction

Biofilm: Biofilm is a frequent cause of treatment failure in Pseudomonas otitis, but also mixed infections and even Malassezia otitis alone. Biofilm occurs when single cell organisms attach to the epithelial lining to form complex colonies, sometimes containing multiple species. The colony produces a mucopolysacchride slime that covers the entire colony, protecting it from topical antibiotics and breakdown. The colony will form channels that allow it to resist washing off even with brisk flushing. Biofilm excludes antimicrobial therapy, has oxygen and nutrient gradients and often hosts the most resistant, even inert sessile microbes at the center that can rapidly recolonize if the biofilm is damaged or removed in completely.

THERAPY

Specific therapy for the underlying primary disease is essential for long term success. Simply chasing bacteria will ultimately result in treatment failure and progression to more severe disease. That said, anesthesia for otoscopy and deep ear flush is the most valuable diagnostic test and indispensable therapeutic procedure for Pseudomonas otitis. Flushing of mucopurulent, septic exudates from the middle ear is necessary for relief. Even the most spectacular antibiotic in the world cannot exceed the value of physical removal.

Preparation: You may need to postpone until decrease inflammation in the canal. Prednisone 1.0-2.0mg/kg/day for 4-7 days with a topical corticosteroid, such as flucinolone is very useful prior to deep ear flush under anesthesia. The day of the procedure, be sure to collect a sample for cytology before flushing. Always always always use an endotracheal tube with a good cuff! Anesthestized or heavily sedated dogs lack a gag reflex and may aspirate irrigation fluid with microorganism and debris. The fluid can easily run through the ruptured tympanum, through the tympanic bulla, down the Eustachian tube, into the oropharynx, down the trachea and into the lower respiratory tract. Also, be sure to protect the eyes with excessive amounts of sterile lubricant. Tilt the head to prevent irritating irrigation fluids with debris, bacteria, and bacterial proteases from running over the eye and damaging the cornea. Finally, be sure to warm the sterile saline or other flush solution prior to cleaning. Large volumes of room temperature fluid should not to be used! You are very close to the brain and the brain is at body temperature. Cool or room temperature fluid will be very painful over time.

Procedure: Collect samples for cytology and culture prior to flush. If debris is thick and tenacious use ceruminolytic to break up debris. Never leave ceruminolytics in the tympanic bulla, since they can damage the more sensitive respiratory epithelial lining. Only use ceruminolytics at the beginning to do the heavy lifting, then irrigate with warm water or sterile saline for the detail work and large volume flushing. You will finish with a drying agent so don't worry about leaving a wet canal at this stage. Bulb syringes, catheters and 12ml syringes, or mechanical flush/suction devices are all useful. Use a lot of fluid, keep flushing until debris is cleared. Use buck curette to remove any adherent debris. Do not use cotton-tipped swabs.

Advantages of video-otoscopy vs. standard operating head scopes: (1) Superior optics provide significantly higher degree of magnification and detail resolution, (2) permits continuous viewing during irrigation, (3) avoids light reflecting off fluid interface, (4) decreases problem of blocking view by instrumentation, (5) increases precision, (6) decreases risk of injury to fragile structures, and (7) creates photographic record.

Assess the tympanum: Otitis media may be present even if the tympanum appears intact. In one study, the tympanum appeared intact with standard hand-held operating head otoscope in 27 of 38 cases of otitis media. Video-otoscopy enhances opportunity for accurate diagnosis by increasing detail of image and permitting visualization under fluid. Hand held otoscopes require suctioning of the ear canal. Typically the tympanum will appear opaque, sclerotic or discolored if the tympanic cavity contains fluid, mucus, or pus rather than air, or if healing from a prior rupture. Suspect otitis media if: (1) large numbers of leukocytes on cytology, (2) characteristic changes in color or consistency of membrane, (3) vestibular signs, deafness, or Horner's syndrome, (4) poor palpebral or corneal reflex, (5) clinical signs of otitis externa greater than 6-months, (6) tympanum may be entirely absent, or (7)notice flush fluid emerging from nostrils.

Flushing of the tympanic cavity: If the tympanum is ruptured and otitis media is present the cavity is often filled with purulent exudates. Any debris or infection is very irritating to the respiratory epithelium, which responds by producing more mucus and pus. Failure to adequately remove material during the flush will result in persistent otitis media, inability of the tympanic membrane to heal, and ultimately treatment failure regardless of follow-up.

Warm sterile saline is excellent for breaking up mucus and is not ototoxic. Do not use ceruminolytics, chlorhexidine, or other ototoxic substances in this region. If using a catheter in the tympanic bulla, aim caudoventrally to avoid fragile structures such as the auditory ossicles, the corda tympani (a branch of the facial nerve), the round window and the oval window. Also, the largest portion of the tympanic bulla is ventral to the opening from the external canal. Be sure to avoid excessive pressure. Potential complications include: pain, vestibular signs, facial nerve injury, deafness, Horner's syndrome (cats in particular are predisposed). When flushing is complete suction the tympanic cavity as dry as possible. Rinse with drying agent or astringent that is "safe" in the tympanic cavity. (e.g. Oticalm, Epiotic, Burotic-HC). Instill 50:50 mixture of Baytril injectable and Dexamethasone SP directly into the tympanic bulla. Steroid suppresses inflammation of respiratory epithelium and the injectable antibiotic should have a high enough concentration to overcome antibiotic resistance mechanisms. In very rare cases I have used Amikacin and Dexamethasone in this manner, without observing ototoxic reactions; however, aminoglycosides are reported as ototoxic agents, so proceed with caution unless peer reviewed publications support usage of Amikacin in this manner.

To go home therapy: Prescibe awake ear flush at home after patient has recovered. The goal is to remove debris, dry the canal, and kill microorganisms. Avoid irritating, ototoxic, or wet solutions. If the patient is too painful to permit at home ear cleaning, treat with steroids and medicated drops only until able to tolerate cleaning. Product selection can be challenging. Recommend acidifying flushes for yeast infections to inhibit growth; however, in bacterial infections these products may decrease efficacy of topical antibiotics. Both fluoroquinolones and aminoglycosides function better at a neutral pH than acidic pH. Virtually any Pseudomonas can adapt to a constant acidic environment. TrisEDTA containing product are the preferred solution for Pseudomonas or resistant bacteria. pH = 8.0 is ideal for fluoroquinolones or aminoglycosides. The EDTA punches holes in bacterial cell wall by chelating calcium and magnesium. In high concentrations and prolonged contact TrisEDTA is directly bactericidal. At a minimum, it is synergistic with antibiotics and may help to overcome many resistance mechanisms. Soak ear 10 minutes before topical antibiotic.

Topical medication is typically prescribed to follow ear cleaning. In general thin liquids preferred over thick ointments. Several choices exist. Zymox is a gentle triple enzyme formulation that is very well tolerated by patients, even those with ulceration. Recent study in human burn patients demonstrated that lactoferrin inhibited binding of Pseudomonas to epithelium. This drug is my first choice if patient is too painful to tolerate anything else. Antibiotic/Antifungal/Steroid combinations are also a good choice. Fluoroquinolones are best if there is a lot of debris building up in the ear canal. Veterinary Baytril otic™: 0.5% Baytril + 1% Silver Sulfadiazine or the human product Cipro HC otic can be used. A marbofloxacin otic formulation is available in Europe (Aurizon). A home-made solution of Baytril inj + + Dexamethasone SP in TrisEDTA-Chlorhexidine base in a 1:1:4 ratio can be useful. Aminoglycosides have excellent anti-Pseudomonas activity, but are inactive when large amounts of organic debris or in low oxygen tension environment. Use only when after exudates are resolved. A home-made Tobramycin solution combinines two 5ml bottles of Tobramycin ophthalmic + 4ml Dexamethasone injectable. There is no commercial amikacin product, however, injectable Amikacin + TrisEDTA or Sterile saline with a target concentration of 30-50mg/ml has been recommended. Gentamicin is available in many commercial ointments, but thickness of ointment may prevent penetration to deep canal. Ticarcillin, Ticarcillin/Clavunate have great anti-Pseudomonal activity. Unfortunately stability after reconstitution minimizes usefulness. If sending home, reconstitute with sterile saline, divide into aliquots, and keep frozen. The owner then thaws each aliquot prior to application. Ceftazidime has brilliant anti-pseudomonal activity, but internists will throttle anyone using Ceftazidime for Pseudomonas otitis.

Systemic steroids are ESSENTIAL for managing severe Pseudomonas otitis. You must shut down neutrophils proteases, edema, and stenosis. Recommend Prednisone 1-2 mg/kg/day until recheck in 7-14 days combined with topical dexamethasone or flucinolone.

Systemic antibiotics are controversial. Resistance is high, concentration in the external canal is low. Use systemic antibiotic only for otitis media. There is limited selection of effective antibiotics available for oral administration. SQ or IV administration of injectable antibiotics may not provide any substantial benefit and may select for resistance to drugs that should be preserved for use in dogs with life-threatening systemic infections (i.e. don't use imipenem to treat ear disease). If using systemic antibiotics, ears require higher doses than other body systems because of difficulty

penetrating into external and middle ear canal. As a general guideline use a dose and duration as if treating an osteomyelitis. Fluoroquinolones remain the best empirical choice for Pseudomonas however, resistance is on the rise! enrofloxacin 10-50% of isolates are susceptible, ciprofloxacin fairs better at 75-90% susceptibility, marbofloxacin >90% susceptible. If using a fluoroquinolone aim for the highest achievable dosage: enrofloxacin – minimum acceptable dose is 15mg/kg SID, Ciprofloxacin – limited studies in dogs. Suggested dose 20mg/kg BID, Marbofloxacin – 5mg/kg SID. Combination therapy may be needed, since mixed infections with highly fluoroquinolone resistant streptococcus, enterococcus, corynebacteria, or anaerobes are common. Recommend marbofloxacin with mlindamycin or Clavamox for mixed bacterial infections. Note: enrofloxacin and clindamycin have been shown to concentrate in WBC and therefore may be more effective in purulent otitis than other antibiotics. Treat for 6-12 weeks depending on severity of disease, the organism present, response to therapy, and healing of the ruptured tympanum.

Pain management is appropriate, since ear pain can be extreme in Pseudomonas otitis. Steroids decrease pain causing events, but do not provide direct analgesic effects. NSAIDS provide the best analgesia for ear pain, but cannot be used in conjunction with high dose prednisone. Opiod analgesics such as Tramadol can be used in conjunction with steroids, but have more variable pain control.

Follow- up to assess progress with PE and cytology. Cytology is the best method for monitoring response to therapy. Evaluate for decreasing numbers of organism, change in dominant organism, and change in WBC. Ideally at two weeks there are no WBC and substantial reduction of bacterial numbers. Repeat deep ear flush if significant mucopurulent exudate and debris remains. If responding continue with current course of therapy for additional 4-6 weeks, then recheck prior to discontinuation of therapy.

Long term plan. Outline ideal management of primary disease (avoidance of food allergy, treating parasites, allergen-specific immunotherapy, cyclosporin, etc). Keep ears dry. Flush with astringent ear solutions once or twice weekly; especially after swimming or bath. Avoid using water, dilute vinegar, or hydrogen peroxide. Monitor frequently for recurrence and treat all infections aggressively at the earliest sign. Surgical intervention with total ear canal ablation is appropriate for end-stage ears: chronic pain, no chance for response to medical management. Lateral ear canal resections except are of limited benefit and are not recommended.

Suggested reading

1. Angus JC. Diseases of the Ear. In: Campbell KL, editor. Small Animal Dermatology Secrets. Philadelphia: Hanley & Belfus; 2004. p. 364-384.
2. Gotthelf LN Small Animal Ear Diseases: An Illustrated Guide. 2nd Ed, 2005.
3. Matousek JL (ed) Ear Disease. Vet Clin North Amer Sm Anim Pract March 2004
4. Scott DW. External ear diseases. In Scott DW, Miller WH, Griffen CE (eds) Muller and Kirk's Small Animal Dermatology, 6th edition, Philadelphia, W.B. Saunders, 2001, pp 1203-1232.

POSTERS

Linear eosinophilic collagenolytic granuloma in a quarter horse mare

B. T. SPONSELLER*, D.J. BERGER*, S. J. HOSTETTER†, A. J. FALES-WILLIAMS† and J.O. NOXON*

**Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, USA*
†Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

Abstract: Eosinophilic granulomas are common cutaneous lesions in horses that are normally observed in the spring and summer and are often located along the neck, back, and withers. They typically present as single or multiple non-painful, non-pruritic, firm, raised, round, nodular swellings of various sizes. A 28-year-old quarter horse mare presented with acute onset of a raised, firm, irregular, linear skin lesion extending from the caudodorsal base of the left pinna to the lateroventral base of the neck. The overlying skin was intact and palpation of the lesion did not elicit a pain response. In addition, a firm 2.0 cm ulcerated plaque was present at the inner, cranioproximal aspect of the left pinna that was painful on palpation. Fine needle aspirates were taken of both lesions and revealed neutrophilic inflammation with a lesser number of eosinophils from the ear lesion, while atypical spindle cell proliferation and mild macrophagic inflammation were seen in the sample acquired from the linear mass. Biopsies of both lesions were taken and submitted due to concern of potential soft-tissue sarcoma. Histopathology yielded hyalinized collagen within the superficial to mid-dermis with eosinophilic granulomatous dermatitis, consistent with an eosinophilic collagenolytic granuloma. Treatment with an oral dose of ivermectin (0.2 mg/kg) and a tapering dose of intramuscular dexamethasone (0.04 mg/kg to 0.01 mg/kg) over several weeks resulted in complete resolution of both lesions with no relapse of the condition. This case represents a unique clinical presentation of an eosinophilic granuloma in the horse that has not previously been described.

Source of funding: Self-funded.

Conflict of interest: None declared.

Incidence of IgE reactivity to house dust and storage mites in enzyme linked immunosorbent assay during a ten year timespan

K. BLANKENSHIP*, B. MCKINNEY*, and R. LANKOW*

**Stallergenes Greer, Lenoir, North Carolina, USA*

Abstract: Mite reactivity evident in individual serum samples from 74,044 presumed atopic dogs was evaluated using Stallergenes Greer enzyme-linked immunosorbent assay for detection of allergen specific IgE. All samples were tested for reactivity to *Dermatophagoides farinae*, *D. pteronyssinus*, and *Tyrophagus putrescentiae*. A subpopulation of 58,633 samples was also evaluated for reactivity to *Acarus siro*, while reactivity to *Blomia tropicalis* reactivity was assessed in 9983 of these samples; reactivity to all mites was evaluated in 7426 sera samples. When considering individual mite reactivity, approximately 42.2% of the samples were reactive to *D. farinae*, 42.5% were reactive to *T. putrescentiae*, 43.4% were reactive to *A. siro*, 19.4% were reactive to *D. pteronyssinus*, and 22.3% were reactive to *B. tropicalis*. Overall, approximately 54% of the samples were reactive to at least one of the mites. Approximately 20% of 74,004 samples were reactive to *D. farinae, D. pteronyssinus*, and *T. putrescentiae*. Likewise, approximately 20% of the 58,633 samples were reactive to A. siro as well as *D. farinae, D. pteronyssinus*, and *T. putrescentiae*. Approximately 15% of the tested samples were reactive to all five mites. No geographic related reactivity profile was readily distinguished. Collectively these results demonstrate that a large proportion of the dog population is reactive to the various house dust and storage mites and that simultaneous reaction with multiple mites is common.

Source of Funding: Funding for this study was provided by Stallergenes Greer

Conflict of Interest: All authors are employees at Stallergenes Greer

Evaluation of N-acetylcysteine combined with Natural extracts against *Staphylococcus pseudintermedius*, *Malassezia pachydermatis* and *Pseudomonas aeruginosa* biofilms: final data from an in vitro study

DR. LIONEL FABRIES

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

C. FEUILLOLAY*, E. BENSIGNOR†, L. FABRIÈS‡, R. ANDRIANTSALAMA§ and C. ROQUES¶
*Fonderephar, Faculté des Sciences Pharmaceutiques, Toulouse, France
†Dermatology Referral Service, Clinique Vétérinaire, Rennes-Cesson, France
‡Clinique vétérinaire Croix du Sud, Saint-Orens de Gameville, France
§Laboratoire de Dermo-Cosmétique Animale (LDCA), 81100 Castres, France
¶Laboratoire de Génie Chimique (UMR 5503), Université Paul Sabatier, Toulouse, France

Abstract: With the emergence of microbial resistance to conventional treatments and the increasingly suspected role of biofilms in this phenomenon, this in vitro study investigated the effect of two topical formulations containing N-acetylcysteine combined with natural extracts (propolis, honey and essential oils) against biofilm formed from three strains commonly involved in skin infections and otitis externa. The spray formulation (Dermoscent® PYOclean® Spray) was tested on Staphylococcus pseudintermedius and the ear cleanser (Dermoscent® PYOclean® Oto) on Malassezia pachydermatis and Pseudomonas aeruginosa. Biofilms were cultivated in microtiter plates; their formation was assessed by counting adhered microbial colonies at 24, 30, 48, 54 and 72 h, versus control. Effects on preformed biofilms were evaluated by counting adhered and planktonic colonies after 10, 20, 30, 120 and 240 min. Numbers were expressed as log CFU/mL or log CFU/well and biofilm structures were visualized through a confocal microscope. There was significant inhibition of biofilm formation from S. pseudintermedius (6.9 log, 72 h) after applying the spray and from M. pachydermatis (4.3 log, 48 h) and P. aeruginosa (7.25 log, 72 h) after applying the ear cleanser during all test periods ($P < 0.05$). Results on mature biofilms from P. aeruginosa indicate a significant disruptive effect with a 3.7 log reduction of adhered microbial colonies ($P < 0.05$, 120 min). Combining the anti-biofilm properties of N-acetylcysteine with the antimicrobial efficacy of certain natural extracts provides potentially synergistic activity against microbial growth and biofilm formation which could be useful in the management of skin and ear infections.

Source of funding: The study was funded by Laboratoire de Dermo-Cosmétique Animale (LDCA, France).

Conflict of interest: E. Bensignor is a consultant for LDCA. L. Fabriès and R. Andriantsalama are employees of LDCA.

A sodium hypochlorite/salicylic acid shampoo is effective in resolving canine superficial pyoderma, including those infections caused by meticillin-resistant staphylococcal strains in a prospective open-label pilot study

V. FADOK*, K. IRWINt

Gulf Coast Veterinary Speciasts, Houston, TX, USA
**Currently Zoetis, Parsippany, NJ USA*
tCurrently Dermatology for Animals, Omaha, NE USA

Abstract: Emergence of meticillin-resistance in canine staphylococcal isolates has inspired increased interest in topical therapy for treatment and control of pyoderma. Chlorhexidine shampoos followed by dilute bleach rinses are often recommended, but household bleach can dry the skin and is unpleasant to use. A shampoo formulated with sodium hypochlorite and salicylic acid was evaluated as sole therapy on client-owned dogs with generalized superficial pyoderma cultured positive for *Staphylococcus pseudintermedius*, including meticillin-resistant strains (MRSP). This prospective, open-label pilot study examined efficacy of the shampoo when used three times weekly for 4 weeks for the treatment of canine superficial pyoderma. Seventeen of nineteen dogs completed the study. Bacterial counts assessed cytologically were significantly decreased at 2 (0.59 +/- 0.51, $P < 0.0001$)) and 4 weeks (0.18 +/- 0.39, $P < 0.0001$), compared to baseline (2.59 +/- 0.94). At week 2, 5/17 dogs had negative cytologies; at week 4, 14/17 had negative cytologies. Clinical severity scores decreased from 10.95 (+/- 2.92) at baseline to 5.8 (+/- 2.7, $P < 0.01$) and 3.65 (+/- 1.85, $P < 0.01$) at weeks 2 and 4, respectively. Client assessment scores decreased significantly from 12 to 7.3 (2 weeks, $P < 0.004$) to 6.2 (4 weeks, $P < 0.004$). Clients reported excellent lathering and dispersion, reduced malodor, and brightening of white and light coats. No owners reported skin dryness or other adverse events. This shampoo containing sodium hypochlorite in a non-drying vehicle is an effective aid in the treatment for canine superficial pyoderma.

Source of funding: TopVET Dermacare provided shampoo (currently licensed to Vetrimax®_as CommandTM), and generated the client questionnaire.

Conflict of interest: None declared.

In vitro antibacterial activity of the manuka essential oil from *Leptospermum scoparium* combined with Tris-EDTA against gram-negative otitis isolates from dogs

S.Y. SONG*, J.E. HYUN*, J.H. KANG*, C.Y. HWANG*

**Laboratory of Veterinary Dermatology and the Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea*

Abstract: Manuka oil, an essential oil from *Leptospermum scoparium*, is well known for its antimicrobial action, however, gram-negative bacteria have resistance to several essential oils because of the impermeability of their outer membrane. The purpose of this study was to examine the antibacterial effects of manuka oil combined with Tris-EDTA against gram-negative bacteria isolated from dogs with otitis externa. A total of 53 clinical isolates including *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* ssp. *pneumoniae*, and *Proteus mirabilis* were collected from dogs with otitis externa. Nineteen (36%) isolates were identified as multidrug-resistant in the antimicrobial susceptibility tests. The minimal inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of manuka essential oil solely or combined with Tris-EDTA were investigated. Positive and negative controls were included. There was no bactericidal effect at the final Tris-EDTA concentration (1.125:0.3 mg/ml) in this study. The MICs and MBCs of manuka oil solely were > 1% (v/v) and > 2% (v/v), respectively. However, combination with Tris-EDTA significantly decreased MICs (ranged from 2^{-4}% to 2^{-1}%, v/v; $P < 0.001$) and MBCs (ranged from 2^{-4}% to 1%, v/v; $P < 0.001$). There was no significant difference between multidrug-resistant and non-resistant bacterial isolates in antimicrobial activity of manuka oils with Tris-EDTA ($P > 0.05$). These findings suggest that manuka oil combined with Tris-EDTA may be considered as an alternative treatment option for the canine ear infections caused by gram-negative bacteria.

Source of funding: Self-funded.

Conflict of interest: None declared.

Investigating the effects of an autophagy inducer on atopic dermatitis in vitro model using canine primary epithelial keratinocytes

H. J. KIM*, S. H. LEE†, S. K. JEONG‡, S.I. LEE*, H. Y. KO*, H. J. KIM‡, H. J. CHUNG‡, C. H. YANG§, G. J. LEE¶, W. K. PARK**

**Department of Internal Medicine, College of Veterinary Medicine, Chonnam National University, Gwangju, Korea*
†Asan Institute for Life Sciences, College of Medicine, University of Ulsan, Seoul, Korea
‡Incospharm. Corp.Daejeon, Korea
§TIMES Animal Medical Center, Suwon, Korea
¶Royal Animal Medical Center, Seoul, Korea
***Yong-Gang Animal Hospital, Seoul, Korea*

Abstract: Autophagy, an intracellular degradation system that is associated with the maintenance of cellular homeostasis, plays a key role in inflammasome inactivation. Some human studies have reported that autoimmune or oncologic diseases are closely related to autophagy activation. However, there is not enough data regarding the association between autophagy and canine atopic dermatitis (AD). This study aimed to investigate the effects of an autophagy inducer (Aquatide™) on the mediators of allergic inflammation *in vitro* using canine primary epithelial keratinocytes (CPEKs). CPEKs were cultured with lipopolysaccharide (LPS) and/or an autophagy inducer for 4 h, 6 h, and 24 h. The cells were collected, and RNA was extracted. mRNA levels of binding immunoglobulin protein (BiP), IL-4, IL-13, and glutathione S-transferase P (GSTP1) were quantified by real-time PCR. Expression of Bip, the endoplasmic reticulum stress marker, and GSTP1, the reactive oxygen species (ROS)-related enzyme, increased significantly after treatment with the autophagy inducer and LPS for 4 h. On the other hand, pre-treatment of the cells with the autophagy inducer and LPS significantly suppressed the levels of these mediators over time. Under inflammatory conditions induced by LPS, the levels of Th2 cytokines (IL-4 and IL-13) were significantly suppressed after 4 h of treatment with the autophagy inducer. These findings suggest that the autophagy inducer could suppress allergic inflammation, including the levels of ROS mediators, in CPEKs. Therefore, autophagy inducers could possibly be helpful for the management of canine AD. Further investigation of their action on atopic keratinocytes *in vitro* and *in vivo* is warranted.

Source of funding: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2016R1D1A3B04934798).

Conflict of interest: None declared.

Evaluation of a diet with a non-conventional source of protein (rabbit) and carbohydrate (cassava) in dogs with adverse food reaction

R. LUCAS*, J. LOPES *, D. LUCAS*, C. PELEGRINI*, T. ALBUQUERQUE*, M. MASCARENHAS†

** Dermatoclinica, São Paulo, S.P., Brazil.*
† Promove and The Pet from Ipanema, Rio de Janeiro, R.J., Brazil.

Abstract: The diagnosis of adverse food reaction (AFR) is based on an 8 week elimination diet (ED) and is confirmed by relapse upon re-challenge with the previously fed diet. Home-cooked diets have been reported to be superior for the diagnosis of AFR, however, such diets are labour-intensive for owners and ingredients novel to the dog may not be readily available. The object of this study was to evaluate the performance of a diet with a non-conventional source of protein (rabbit) and carbohydrate (cassava) (ElevenChimps) in animals with AFR. Thirty-two non-seasonally pruritic dogs were assessed with a Visual Analog Scale (VAS), with the Canine Atopic Dermatitis Lesions Index (CADESI)-4 and for quality of life with a validated questionnaire on days 0, 30 and 60. In case of bacterial or yeast infection, only topical therapy with ointments, creams and shampoos was applied during the ED. Dogs showing at least 50% improvement in pruritus were re-challenged with their prior diet. Thirty-two dogs completed the elimination diet. Of these, 22 did not improve whereas 10 were considered to have improved by VAS, CADESI-4 and the validated questionnaire. These ten dogs reacted to their prior diets and were diagnosed with AFR although in two the pruritus was statistically significantly reduced but not eliminated ($P < 0.001$). This diet containing a non-conventional source of protein (rabbit) and carbohydrate (cassava) appeared to be a good option of an ED for the identification of AFR in select dogs.

Source of funding: None declared.

Conflict of interest: ElevenChimps helped the authors to formulate the diet and kindly funded the analysis of the data.

Allergen-specific IL-31 transcription in equine leukocytes: a pilot study

N. CRAIG*, R.S. WILKES and R. MARSELLA****

**Department of Gastroenterology, College of Medicine, University of Florida, Gainesville, FL, USA*
*** Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Abstract: Pruritic allergic skin diseases are common in horses. Interleukin-31 (IL-31) is important in allergic pruritus in dogs and humans. IL-31 production by dog and human leukocytes is induced by allergens including house dust mite allergen and tree pollens, respectively. This pilot study aimed to identify allergen-specific IL-31 transcription in leukocytes from an allergic horse. This horse had a history of recurrent pruritus in the summer and was severely pruritic at the time of the study. Blood (10ml) was collected from this horse which had demonstrated positive intradermal skin test (IDST) responses (≥2) to a variety of insect, grass, tree, weed and fungal allergens. After centrifugation the buffy coat was aspirated with 1ml autologous plasma, re-suspended in 10.5ml RPMI media with 10% heat-inactivated fetal bovine serum. The leukocytes were stimulated overnight with saline control, allergens used for IDST (10µl/well), or phytohemagglutinin-M. Allergens chosen included the insect, grass, tree, weed and fungal allergens which produced high and low IDST scores, grain mill dust and oats. After TRIzol™ RNA extraction, IL-31 mRNA was quantified using a SYBR™ Green qPCR assay with GAPDH as a reference gene. Data analysis was conducted using the Pfaffl method with the saline control as the calibrator, and Spearman's rank correlation analysis. Increase in IL-31 transcription was observed following stimulation with several allergens, in particular *Culicoides*, mosquito, alfalfa and grain mill dust. No significant correlation with IDST score was observed. This study provides the foundation for further work investigating equine IL-31 responses to allergens.

Source of funding: University of Florida College of Veterinary Medicine Spring 2017-18 Research Grant.

Conflict of interest: None declared.

Safety of the ingredient A97614A1 in a model of reconstructed canine epidermis

E. OLLIVIER*, N. AMALRIC§, V. RAHOUL§, MC. CADIERGUES†, N. MAUBERT*, C. ZEMIRLINE*

**Ceva Santé Animale, Libourne, France*
§Synelvia, Labège, France
† Université de Toulouse, ENVT, Toulouse, France

Abstract: *In vitro* approaches are part of the development of human dermatology products and are of growing interest in veterinary dermatology, due to regulatory and ethical considerations. A new *in vitro* model of Reconstructed Canine Epidermis (RCE) was developed and used to evaluate the new ingredient coded A97614A1 which was shown to have beneficial effects on the skin barrier in an *in vitro* model of Reconstructed Human Epidermis. Primary canine keratinocytes obtained from normal dog skin were cultured on polycarbonate filters in a specific medium and incubated at 37°C under 5% CO2. The medium was changed every two days. After 12 days, 3-dimensional RCE were produced. RCE were treated with A97614A1 and compared to non-treated RCE. Two doses (400 and 600 µg/mL) of A97614A1 were used. RCE morphology (hemalun eosin staining) and cell viability (MTT assay) were assessed. The structure of developed RCE was similar as *in vivo* canine epidermis, with 4 typical layers and keratohyaline granules in differentiated keratinocytes of the *stratum granulosum*. Treatment with A97614A1 did not impact RCE morphology at either dose. The cell viability was 80% at the low dose and 91% at the high dose (an ingredient is considered toxic when the cell viability is <75%). Under our *in vitro* conditions, RCE model was developed in which A97614A1 was shown to be well-tolerated. These tolerance results should be confirmed by *in vivo* safety study in dogs.

Source of funding: Ceva.

Conflict of interest: E. Ollivier, N. Maubert and C. Zemirline are employees of Ceva.

Efficacy of a phytosphingosine-concentrated sprayable gel in dogs with non-seasonal allergic pododermatitis

E. OLLIVIER*, D. HUFF§, N. REYMOND#, E. WAKEM†, C. JOHNSON†

**Ceva Santé Animale, Libourne, France*
§Mountain View Animal Hospital, Redmond, OR, USA
#Villeneuve Loubet, France
†Ceva Animal Heath. LLC, Lenexa, KS, USA

Abstract: The efficacy of a phytosphingosine-concentrated sprayable gel (DOUXO® Calm Gel) in dogs with non-seasonal allergic pododermatitis was evaluated in a prospective, non-controlled, unblinded, muticenter field study. Privately-owned dogs respecting Favrot's criteria for confirmation of atopic dermatitis and with mild to moderate pedal pruritus and interdigital erythema were enrolled after exclusion of other pruritic skin conditions. Steroids, antihistamines, lokivetmab and antibiotics were forbidden; no changes in other treatments were allowed. The product was applied on interdigital skin of the affected paws daily for 1 week then every other day for another week. Dogs were evaluated by veterinarians on days 0, 3, 7 and 14. Owners recorded in diaries daily. Pedal pruritus using a pruritus visual analogue scale (PVAS), interdigital erythema, overall assessment of the pododermatitis evolution and owner satisfaction were assessed. Ten dogs were included in the analysis. Compared to baseline, pedal pruritus significantly improved throughout the study (median PVAS score: 4.1, 3.6 and 2.4 on days 3, 7 and 14, respectively, *versus* 5.4 at baseline; $P < 0.05$ at all timepoints). A progressive improvement of interdigital erythema was also observed. At the end of the study, 90% of owners stated there was an overall improvement of their dog's pododermatitis. The product was considered easy to use, efficacious, and improved their dog's quality of life by 100%, 90% and 60% of owners, respectively. Finally, the product was well-tolerated and seemed to quickly improve the pododermatitis. A blinded placebo-controlled study would be needed to confirm these results.

Source of funding: Ceva.

Conflict of interest: E. Ollivier, E. Wakem and C. Johnson are employees of Ceva. N. Reymond is consultant for Ceva.

Wound healing effects of polydeoxyribonucleotide sodium in an experimental dog model

M.H. KANG*, D.W. SONG*, W.B. RO*, S.G. KIM * and H.M. PARK*

**College of Veterinary Medicine, Konkuk University, Seoul, South Korea*

Abstract: Polydeoxyribonucleotides (PDRN) activate adenosine A2A receptors and have effects on cell growth and tissue regeneration. This study investigated the effects of PDRN and autologous platelet-rich plasma (PRP) injections on experimentally induced skin wounds by clinical and histopathologic evaluation. Four healthy male beagle dogs were used. Skin wounds were created under general anesthesia via punch-biopsy bilaterally on the trunk. Intralesional injections of PDRN and PRP were made on days 0, 2, and 4. Saline injections were used as negative controls. The healing process of the wounds was evaluated clinically daily and histopathologically weekly by semi-quantitative and quantitative methods for 4 weeks. Compared with baseline values, skin wounds treated with PDRN and PRP injections exhibited rapid clinical and histological improvements when compared to controls (granulation tissue formation, $P = 0.014$; amount of inflammation, $P = 0.004$; fibroblasts, $P = 0.004$; angiogenesis, $P = 0.041$). In addition, wound contraction and epithelialization were rapid in the PDRN injected wounds when compared to the PRP injected wounds ($P = 0.016$). Histopathological tissue granulation, fibroblast formation and angiogenesis were marked at week 1 and collagen organization and amounts were marked at week 2 in the PDRN injected wounds. At week 3, the evaluated healing parameters were similar in both the PDRN and PRP injected wounds. In conclusion, intralesional injection of PDRN was effective in the early stages of the wound healing when compared to the PRP injected wounds, however the overall healing process was not significantly different between the PDRN and PRP injected wounds.

Source of funding: Self-funded.

Conflict of interest: None declared.

Successful treatment of cutaneous *Curvularia geniculata*, *Nocardia niigatensis* and viral papillomatosis in an immunocompromised dog during the therapeutic management of immune-mediated hemolytic anemia

E. STRZOK*, C. SIEPKER†, E. HOWERTH†, J. SMITH* and F. BANOVIC*

**Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA*
†Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA

Abstract: Opportunistic infections represent a major cause of mortality in immunocompromised patients. We report a case of cutaneous opportunistic bacterial, fungal and viral infection in a 7.5-year-old female spayed great dane treated for immune-mediated hemolytic anemia (IMHA) with twice daily oral prednisone (0.5 mg/kg), ciclosporine (5 mg/kg) and mycophenolate mofetil (MMF; 10 mg/kg). The patient developed diffuse right forelimb swelling with deep draining tracts 6 weeks into immunosuppressive treatment; histopathology revealed pyogranulomatous fungal dermatitis with *Curvularia geniculata* growth. Oral once daily terbinafine (32 mg/kg) and itraconazole (4.8 mg/kg) were initiated; ciclosporine was immediately discontinued and the MMF/prednisone administration frequency was reduced to once daily. The right forelimb skin lesions resolved after 4 weeks, but the patient presented with a diffuse left forelimb edema and multifocal draining tracts. Histopathology revealed severe neutrophilic dermatitis with branching bacilli; 16S rRNA sequencing identified *Nocardia niigatensis*. Cutaneous nocardiosis was treated with oral enrofloxacin (once daily) and doxycycline (twice daily) each at 5 mg/kg; systemic immunosuppressives were continued for IMHA control. One month later, the left forelimb lesions completely resolved but the patient developed several multifocal, exophytic warts; the histopathology revealed irregular epidermal hyperplasia with intranuclear keratinocyte inclusion bodies characteristic of viral papillomas. Within the following 4 weeks, the patient developed severe diffuse papillomatosis of the left forelimb which was successfully treated with every other day topical imiquimod administration. In this case, successful treatment of cutaneous opportunistic bacterial, fungal and viral infection was possible with proper treatment even though the immunosuppressive drug treatments could not be discontinued.

Source of funding: Self-funded.

Conflict of interest: None declared.

Efficacy of topical use of florfenicol-terbinafine-betamethasone acetate otic gel in dogs with acute otitis externa or recrudescence in Brazil

R. LUCAS*, B. S. TADINI†, K. P. DOUCETTE‡, T. SHIGEMOTO† and C. RECUPERO†.

**Dermatoclínica, Sao Paulo, SP, Brazil*
†Elanco Animal Heath, Sao Paulo, SP, Brazil
‡Elanco Animal Health, Greenfield, Indiana, USA

Abstract: Otitis externa, a common condition diagnosed in canines, can be difficult to treat with conventional owner-administered otic products influencing overall treatment success. The study objective was to evaluate efficacy of an adaptable otic gel, containing florfenicol, terbinafine and betamethasone acetate (Osurnia™) for the treatment of acute or recrudescent otitis externa cases in dermatology practices in Brazil, including the veterinary and pet owner experience. Sixty-nine client-owned dogs were treated twice, one week apart, after a thorough ear canal cleaning only before the first application. Otitis externa was evaluated by veterinarians on Days 0 and 28±2 with a qualitative and quantitative cytology score, and a total clinical score (TCS) comprised of four otic clinical signs (erythema, exudate, edema, and erosion/excoriation) on Days 0, 7±2 and 28±2. A satisfaction questionnaire evaluating veterinarian and pet owner experience was completed. Fifty-nine cases were included in the efficacy dataset which were compliant with inclusion and exclusion criteria. Cytological presence and enumeration of microorganism and neutrophils decreased (70-86% and 89%, respectively) at the Day 28±2 assessment when compared with Day 0. All individual clinical parameters and the TCS significantly decreased ($P < 0.05$) at Days 7±2 and 28±2 compared with Day 0. Veterinarians and pet owners recognized improvement of clinical signs and its superiority in ease of treatment compared to previous products. Ear hypersensitivity was reported in one dog with complete recovery. The two dose adaptable otic gel is an option for treatment of canine acute or recrudescent otitis in Brazil, which may improve owner compliance.

Source of funding: Elanco Animal Heath.

Conflict of interest: BST, KPD, TS and CR are Elanco Animal Health employees.

AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks

The American Academy of Veterinary Dermatology

as a 2018

Principal Sponsor

of the ACVD Research Foundation

AMERICAN COLLEGE OF VETERINARY DERMATOLOGY

ACVD

RESEARCH FOUNDATION

AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks

ALK

as a 2018

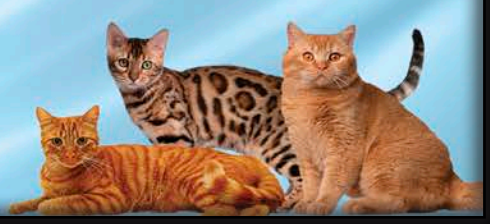
Principal Sponsor

of the ACVD Research Foundation

AMERICAN COLLEGE OF VETERINARY DERMATOLOGY

ACVD

RESEARCH FOUNDATION



AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks

Royal Canin

as a 2018

Principal Sponsor

of the ACVD Research Foundation

AMERICAN COLLEGE OF VETERINARY DERMATOLOGY

ACVD

RESEARCH FOUNDATION

AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks

Stallergenes Greer

as a 2018

Principal Sponsor

of the ACVD Research Foundation

AMERICAN COLLEGE OF VETERINARY DERMATOLOGY

ACVD

RESEARCH FOUNDATION

AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks

VARL

as a 2018

Principal Sponsor

of the ACVD Research Foundation

AMERICAN COLLEGE OF VETERINARY DERMATOLOGY

ACVD

RESEARCH FOUNDATION

AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks

BLUE Natural Veterinary Diet

Boehringer Ingelheim

Heska

Animal Dermatology Clinic

(Southern California & multiple locations)

as 2018

Major Sponsors

of the ACVD Research Foundation

AMERICAN COLLEGE OF VETERINARY DERMATOLOGY

ACVD

RESEARCH FOUNDATION

AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks our 2018

Supporting Sponsors

Rayne Clinical Nutrition

Animal Allergy & Dermatology Specialists- Las Vegas NV

Animal Skin & Allergy Clinic- Lynnwood WA

Sponsors

Vetoquinol

Vetrimax

Animal Allergy & Dermatology Center - Indianapolis IN

Lehigh Valley Veterinary Dermatology - Allentown PA

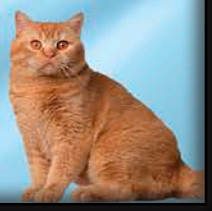
Southeast Veterinary Dermatology & Ear Clinic - Multiple locations

Veterinary Dermatology Center - Maitland FL

Veterinary Dermatology Center of Baltimore - Baltimore MD

Contributors

Animal Allergy & Dermatology Clinic- Kingston NH
Animal Allergy & Dermatology Service of Connecticut- Plantsville CT
Animal Dermatology & Allergy- Rocklin CA
Animal Dermatology Referral Clinic- Grapevine TX
Guelph Veterinary Dermatology- Guelph, Ontario Canada
Hope Veterinary Specialists- Malvern PA
Long Green Animal Dermatology- Baldwin MD
Midwest Veterinary Dermatology Center- Buffalo Grove IL
Peninsula Animal Dermatology- San Carlos CA
Pet Dermatology of South Florida- Miami FL
Pittsburgh Veterinary Dermatology- Pittsburgh PA
Riverdale Veterinary Dermatology- Riverdale NJ
SacDerm Vet- Sacramento CA
University of California, Davis- Davis CA
VCA South Shore Animal Hospital- Weymouth MA
Veterinary Allergy & Dermatology- Springfield OR
Veterinary Dermatology of Richmond- Richmond VA
Veterinary Dermatology Service- Golden Valley MN
Veterinary Healing Arts- East Greenwich RI
VRCC Dermatology- Englewood CO
Kimmel Arn
Carol Caspillo
Joslyne Caspillo
Dr. Allison Diesel
Nancy Easton
Dr. Dana Liska
Andrea Radvon
Julien Schlotnick
Debbie Stricklin
Marilyn Taylor
Dr. Ursula Thomas
Jeffrey and Jo Tyson
Donald Workman



of the ACVD Research Foundation

AMERICAN COLLEGE OF

ACVD

VETERINARY DERMATOLOGY

The American College of Veterinary Dermatology

recognizes and thanks

ACVD Past Presidents Dinner

ROYAL CANIN

ACVD Diplomates Dinner

Ceva — Together, beyond animal health

ACVD Award of Excellence

Bayer HealthCare

Peter J. Ihrke Resident Research Awards

Bayer HealthCare

ACVD Residents Dinner

Dechra
Veterinary Products

ACVD Resident Education Forum

ZOETIS PETCARE
WE TAKE CARE OF PETS LIKE PETS TAKE CARE OF YOU.™

for their generous support in 2019