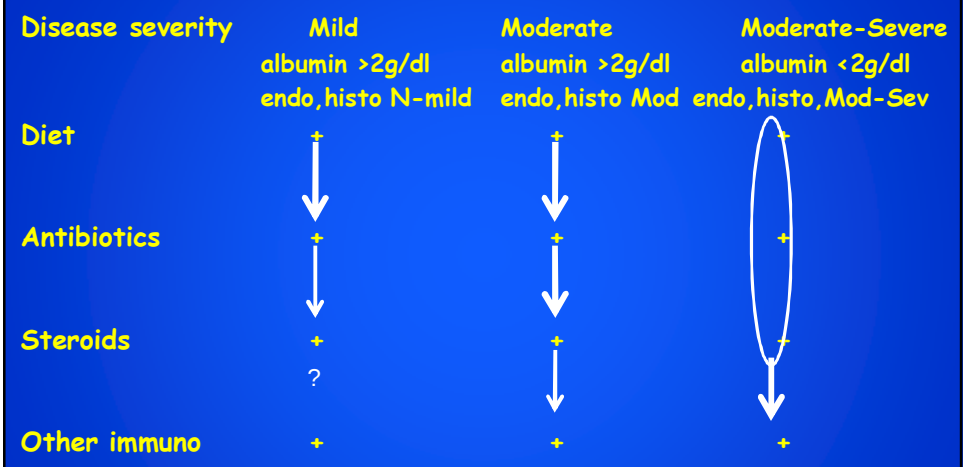
PERHAPS IT's WHAT is NOT in THESE DIETS?

"ABSENCE MAKES THE GUT DO WON

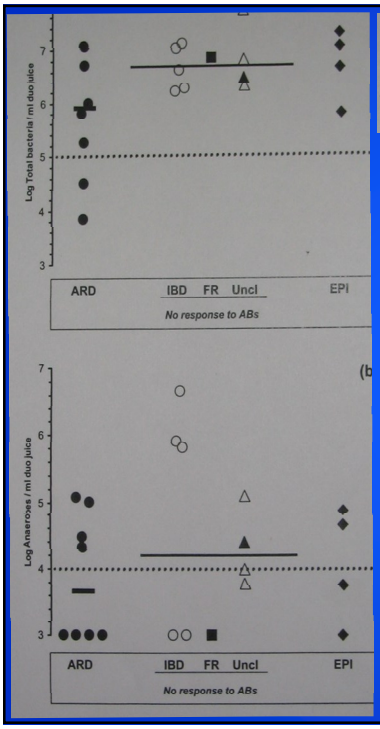
Treatment by Therapeutic Trial



Antibiotic Responsive Enteropathy

- Diarrhea, wt loss, failure to gain wt.
- Absence of a stagnant loop
- Absence of severe mucosal inflammation and EPI
- Breed predisposition: GSD
- Bacterial numbers > (10^5 aerobes, 10^4anaerobes)
- May have high folate, low cbl
- Variable mucosal IgA
- Oral antibiotic treatment was effective in 77% (23/30 dogs), but prolonged treatment (> 4 weeks) was required to control signs and prevent recurrence in 50% (15/30)

Res Vet Sci. 1983,35(1):42-6., Gut. 1984,25(8):816-23 J Am Vet Med Assoc. 1995, 15;206,187-93,Gastroenterology. 1987,93:986-93



J Vet Intern Med 2003;17:33–43

Comparison of Direct and Indirect Tests for Small Intestinal Bacterial Overgrowth and Antibiotic-Responsive Diarrhea in Dogs

A.J. German, M.J. Day, C.G. Ruaux, J.M. Steiner, D.A. Williams, and E.J. Hall

?"SIBO" = 10^5 total or 10^4 anaerobes?

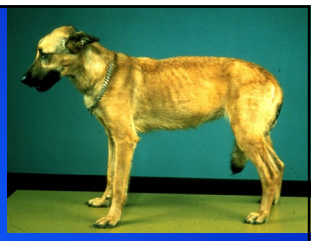
Antibiotic Responsive Enteropathy

- Loss of tolerance to normal flora?
- Innate or adaptive immune defects? IgA deficiency?
- Dysbiosis?- predominant cultivable bacteria were *E.coli, Enterococcus*
- Genetic Predisposition?

Vet Microbiol. 2010 Dec 15;146(3-4):326-35. Epub 2010 May 27.

Evaluation of mucosal bacteria and histopathology, clinical disease activity and expression of Toll-like receptors in German shepherd dogs with chronic enteropathies.

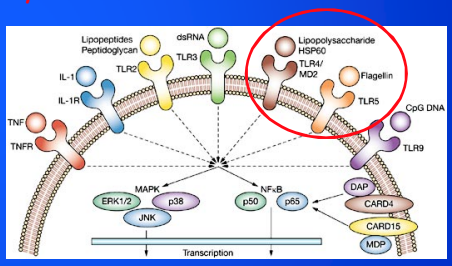
Allenspach K, et al



- Duodenum, colon and ileum of 13 affected GSD and 10 healthy greyhounds.
- 16S rRNA gene libraries.

Bacilli, and Erysipelotrichi, and to the orders of Lactobacillales, Actinomycetales and Erysipelotrichales

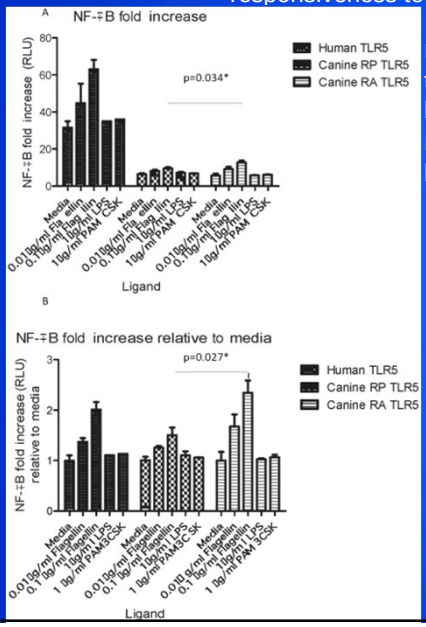
TLR4 expression was > in GSD
TLR5 expression was < in GSD



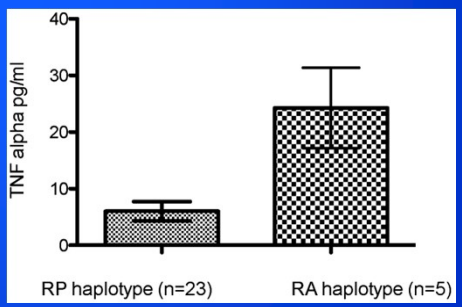
- PLoS One. 2010 Dec 23;5(12):e15740.
- Polymorphisms in the TLR4 and TLR5 gene are significantly associated with inflammatory bowel disease in German shepherd dogs.

Kathrani A, et al

PLoS One. 2012;7(1):e30117. 2012 Jan 18.TLR5 risk-associated haplotype for canine inflammatory bowel disease confers hyper-responsiveness to flagellin.Kathrani A, et al



- increase in NF-kB activity when cells transfected with the risk-associated TLR5 haplotype were stimulated with flagellin
- whole blood taken from risk-associated TLR5 haplotype produced significantly more TNF



Tylosin-responsive enteropathy

J Vet Intern Med. 2005 Mar-Apr;19(2):177-86. **Westermarck E** et al.

Tylosin-responsive chronic diarrhea in dogs.

Tylosin-responsive diarrhea (TRD) affects typically middle-aged, large-breed dogs with both small and large intestinal signs. Tylosin eliminated diarrhea in all dogs within 3 days, most within 24 hours.

J Vet Intern Med. 2005 Nov-Dec;19(6):822-7. **Westermarck E** et al.

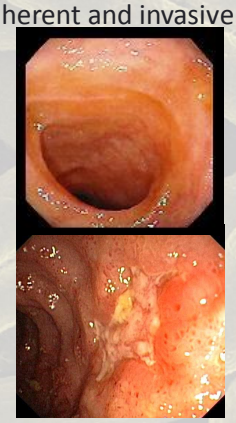
Effect of diet and tylosin on chronic diarrhea in beagles

Seven beagles in a colony of dogs had chronic diarrhea for at least 30 days reponded to tylosin 20 mg/kg BW q24h PO for 10 days.

Dysbiosis:

- Altered balance of beneficial/protective and harmful bacteria

- Functional change in resident flora, e.g. adherent and invasive *E. coli* – AIEC



Mel Craven

Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. Xenoulis et al. FEMS Microbiol Ecol. 2008 Jul 21

- Duodenal brush cytology samples from 10 IBD and 9 healthy
- 16S rRNA gene was amplified using universal bacterial primers.
- Species richness was significantly lower in the IBD group (P=0.038).
- Enrichment in Enterobacteriaceae in IBD

Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. Suchodolski JS et al. Vet Microbiol. 2010 May 19;142(3-4):394-400. Epub 2009 Nov 10.

- Duodenal biopsies from 7 IBD and 7 healthy
- IBD: Cairn terrier, Yorkshire terrier, Basset hound, Afghan hound, miniature Dachshund, Shih Tzu, and Golden Retriever.

Controls:2 Beagles and 5 were mixed-breed Hound dogs

Dogs with IBD had higher abundance of Alpha-, Beta-, and Gamma-proteobacteria ($p<0.0001$ for all classes), and lower abundance of Clostridia ($p<0.0001$).

PLoS One. 2016 Feb 3;11(2):e0147321. doi: 10.1371/journal.pone.0147321. eCollection 2016.

Alterations of the Ileal and Colonic Mucosal Microbiota in Canine Chronic Enteropathies.

Cassmann E[1], White R[1], Atherly T[2], Wang C[3], Sun Y[3], Khoda S[1], Mosher C[4], Ackermann M[5], Jergens A[1].

Author information

Abstract

BACKGROUND: The intestinal microbiota is increasingly linked to the pathogenesis of chronic enteropathies (CE) in dogs. While imbalances in duodenal and fecal microbial communities have been associated with mucosal inflammation, relatively little is known about alterations in mucosal bacteria seen with CE involving the ileum and colon.

AIM: To investigate the composition and spatial organization of mucosal microbiota in dogs with CE and controls.

METHODS: Tissue sections from endoscopic biopsies of the ileum and colon from 19 dogs with inflammatory bowel disease (IBD), 6 dogs with granulomatous colitis (GC), 12 dogs with intestinal neoplasia, and 15 controls were studied by fluorescence in situ hybridization (FISH) on a quantifiable basis.

RESULTS: The ileal and colonic mucosa of healthy dogs and dogs with CE is predominantly colonized by bacteria localized to free and adherent mucus compartments. CE dogs harbored more ($P < 0.05$) mucosal bacteria belonging to the Clostridium-coccoides/Eubacterium rectale group, Bacteroides, Enterobacteriaceae, and Escherichia coli versus controls. Within the CE group, IBD dogs had increased ($P < 0.05$) Enterobacteriaceae and E. coli bacteria attached onto surface epithelia or invading within the intestinal mucosa. Bacterial invasion with E. coli was observed in the ileal and colonic mucosa of dogs with GC ($P < 0.05$). Dogs with intestinal neoplasia had increased ($P < 0.05$) adherent (total bacteria, Enterobacteriaceae, E. coli) and invasive (Enterobacteriaceae, E. coli, and Bacteroides) bacteria in biopsy specimens. Increased numbers of total bacteria adherent to the colonic mucosa were associated with clinical disease severity in IBD dogs ($P < 0.05$).

CONCLUSION: Pathogenic events in canine CE are associated with different populations of the ileal and colonic mucosal microbiota.

Dysbiosis associated with enrichment in Proteobacteria and depletion of Firmicutes appears to be a common end point of Intestinal Inflammation across species

Let's just give them antibiotics …

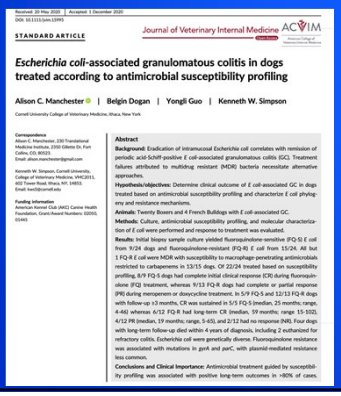
Dysbiosis is present in dogs with Food Responsive Disease

- Treat the underlying cause

Antimicrobials may amplify dysbiosis

- Metronidazole and *E.coli* and *Enterococcus*

Antimicrobial Resistance is RAMPANT



Received: 20 May 2020 | Accepted: 1 December 2020
DOI: 10.1111/jvim.15995

STANDARD ARTICLE

Journal of Veterinary Internal Medicine ACVIM

***Escherichia coli*-associated granulomatous colitis in dogs treated according to antimicrobial susceptibility profiling**

Alison C. Manchester | Belgin Dogan | Yongli Guo | Kenneth W. Simpson

Cornell University College of Veterinary Medicine, Ithaca, New York

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Email: kws5@cornell.edu

Funding Information
American Kennel Club (AKC) Canine Health Foundation, Grant/Award Numbers: 02010, 01443

Abstract

Background: Eradication of intramucosal *Escherichia coli* correlates with remission of periodic acid-Schiff-positive *E coli*-associated granulomatous colitis (GC). Treatment failures attributed to multidrug resistant (MDR) bacteria necessitate alternative approaches.

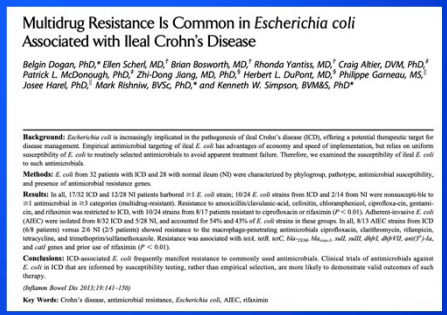
Hypothesis/objectives: Determine clinical outcome of *E coli*-associated GC in dogs treated based on antimicrobial susceptibility profiling and characterize *E coli* phylogeny and resistance mechanisms.

Animals: Twenty Boxers and 4 French Bulldogs with *E coli*-associated GC.

Methods: Culture, antimicrobial susceptibility profiling, and molecular characterization of *E coli* were performed and response to treatment was evaluated.

Results: Initial biopsy sample culture yielded fluoroquinolone-sensitive (FQ-S) *E coli* from 9/24 dogs and fluoroquinolone-resistant (FQ-R) *E coli* from 15/24. All but 1 FQ-R *E coli* were MDR with susceptibility to macrophage-penetrating antimicrobials restricted to carbapenems in 13/15 dogs. Of 22/24 treated based on susceptibility profiling, 8/9 FQ-S dogs had complete initial clinical response (CR) during fluoroquinolone (FQ) treatment, whereas 9/13 FQ-R dogs had complete or partial response (PR) during meropenem or doxycycline treatment. In 5/9 FQ-S and 12/13 FQ-R dogs with follow-up ±3 months, CR was sustained in 5/5 FQ-S (median, 25 months; range, 4-46) whereas 6/12 FQ-R had long-term CR (median, 59 months; range 13-502). 4/12 PR (median, 19 months; range, 5-65), and 2/12 had no response (NR). Four dogs with long-term follow-up died within 4 years of diagnosis, including 2 euthanized for refractory colitis. *Escherichia coli* were genetically diverse. Fluoroquinolone resistance was associated with mutations in *gyrA* and *parC*, with plasmid-mediated resistance less common.

Conclusions and Clinical Importance: Antimicrobial treatment guided by susceptibility profiling was associated with positive long-term outcomes in >80% of cases.



Multidrug Resistance Is Common in *Escherichia coli* Associated with Ileal Crohn's Disease

Belgin Dogan, PhD, Ellen Scherl, MD,† Brian Bosworth, MD,† Rhonda Yantiss, MD,† Craig Altier, DVM, PhD,‡ Patrick L. McDonough, PhD,‡ Zhi-Dong Jiang, MD, PhD,§ Herbert L. DuPont, MD,§ Philippe Garneau, MS,|| Josee Harel, PhD,|| Mark Rishniw, BVSc, PhD,* and Kenneth W. Simpson, BVM&S, PhD**

Background: *Escherichia coli* is increasingly implicated in the pathogenesis of ileal Crohn's disease (ICD), offering a potential therapeutic target for disease management. Empirical antimicrobial targeting of ileal *E. coli* has advantages of economy and speed of implementation, but relies on uniform susceptibility of *E. coli* to routinely selected antimicrobials to avoid apparent treatment failure. Therefore, we examined the susceptibility of ileal *E. coli* to such antimicrobials.

Methods: *E. coli* from 32 patients with ICD and 28 with normal ileum (NI) were characterized by phylogroup, pathotype, antimicrobial susceptibility, and presence of antimicrobial resistance genes.

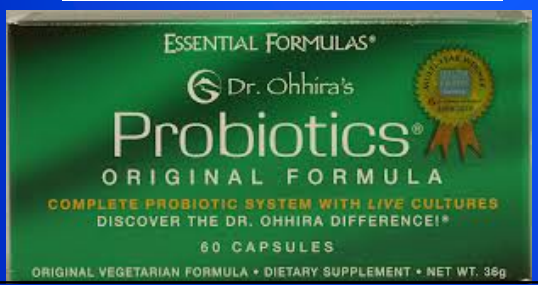
Results: In all, 17/32 ICD and 12/28 NI patients harbored ≥1 *E. coli* strain; 10/24 *E. coli* strains from ICD and 2/4 from NI were nonsusceptible to ≥1 antimicrobial in ≥3 categories (multidrug-resistant). Resistance to amoxicillin/clavulanic-acid, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, and rifaximin was restricted to ICD, with 10/24 strains from 8/17 patients resistant to ciprofloxacin or rifaximin ($P < 0.01$). Adherent-invasive *E. coli* (AIEC) were isolated from 8/32 ICD and 5/28 NI, and accounted for 54% and 43% of *E. coli* strains in these groups. In all, 8/13 AIEC strains from ICD (6/8 patients) versus 2/6 NI (2/5 patients) showed resistance to the macrophage-penetrating antimicrobials ciprofloxacin, clarithromycin, rifampicin, tetracycline, and trimethoprim/sulfamethoxazole. Resistance was associated with *tetA, tetB, tetC, bla*$_{TEM}$, *bla*$_{OXA-1}$, *sulI, sulII, dhfrI, dhfrVII, ant(3")-Ia*, and *catI* genes and prior use of rifaximin ($P < 0.01$).

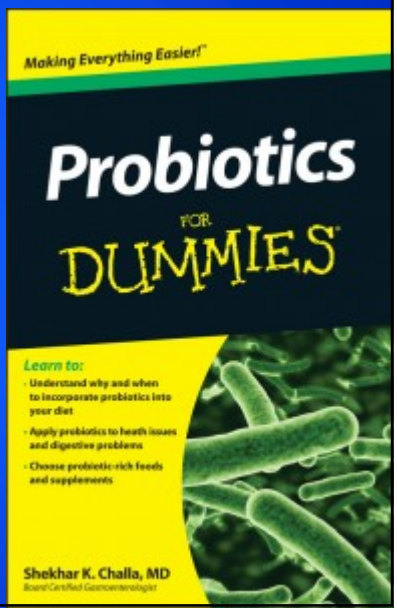
Conclusions: ICD-associated *E. coli* frequently manifest resistance to commonly used antimicrobials. Clinical trials of antimicrobials against *E. coli* in ICD that are informed by susceptibility testing, rather than empirical selection, are more likely to demonstrate valid outcomes of such therapy.

(Inflamm Bowel Dis 2013;19:141–150)

Key Words: Crohn's disease, antimicrobial resistance, *Escherichia coli*, AIEC, rifaximin

HOPE !





Probiotics:

- May increase in feces of dogs and cats being supplemented
- May impact immune responses in healthy dogs and cats
- May improve fecal consistency and /or shorten duration of acute diarrhea/gastroenteritis in dogs and cats
- May improve fecal consistency in chronic diarrhea in cats
- May decrease the frequency of diarrhea in cats in Shelters
- May have a protective effect against racing induced diarrhea in dogs
- No clinical trials in IBD
- Do not maintain remission for tylosin responsive enteropathy

Treatment by Therapeutic Trial

Disease severity	Mild albumin >2g/dl endo,histo N-mild	Moderate albumin >2g/dl endo,histo Mod	Moderate-Severe albumin <2g/dl endo,histo,Mod-Sev
Diet	+	+	+
Antibiotics	+	+	+
Steroids	+ ?	+	+
Other immuno	+	+	+

Laboratory Evaluation

- Hypoalbuminemia
- *Albumin <2mg/dl poorer prognosis, OR 11.4
- ↑ liver enzymes
- Hypocholesterolemia
- Hyperkalemia / hyponatremia

Profile

Protein losing enteropathies	
Infectious	Parvo,Salm.,Histo.
Endoparasites	Giardia, Ancylost.
Lymphangiectasia	
Neoplasia	Lymphosarcoma
IBD	LPE, eos., granulom.
HGE	
G.I. haem.	Neoplasia, ulcers
Structural	Intussusception

*JSAP 2004, 45,336-343, Craven et al 80 dogs retrospective
*JVIM 2007,21,703-708, Allenspach et al 70 dogs prospective

Immunosuppression

- **Prednisolone**

  @ 2 mg / kg / day PO q 10-21d then taper

- **Azathioprine**

  @ 2mg / kg PO SID-EOD - dog

- **Cyclosporine**

5mg/kg PO q24hrs 10 wks JVIM 2006,20,239-244

Pharmacokinetics and clinical efficacy of cyclosporine treatment of dogs with steroid-refractory inflammatory bowel disease

J Vet Intern Med. 2006 Mar-Apr;20(2):239-44..Allenspach K,

- Pharmacokinetics and clinical efficacy of PO cyA treatment in dogs with steroid-refractory IBD (n = 14).
- cyA 5 mg/kg PO q24h for a period of 10 weeks.
- Improvement of clinical signs was observed in 12 of 14 dogs with IBD.
- Median clinical activity score was significantly reduced from a median score of 9 to a median score of 5 (P = 0.001).
- T cell numbers in decreased after treatment : in the villous region 28 (19-30) cells/10,000 microm2 to 7 (0-10)/10,000 microm2, P = 0.01; crypt region 15 (6-23) cells/10,000 microm2 before versus 4 (0-9)/10,000 microm2 P = 0.02,
- implies T cell lysis as a possible mechanism of action.
- cyA may be an alternative drug in dogs with IBD that are refractory to immunosuppressive doses of steroids.

Does Immunosuppression work?

- Prednisolone
  - @ 2 mg / kg / day PO q 10d then taper
  - 10/21 diet unresponsive dogs responded to prednisolone, no relapse after taper for up to 3 years
  - 3 euthanased after steroids JVIM 2007 21,703-708
- Cyclosporine
  - 5mg/kg PO q24hrs 10 wks JVIM 2006,20,239-244
  - 2/8 steroid refractory responded to cyclosporine, 6/8 euthanased
  - 7/10 hypoalbuminemia and ascites responded to cyclosporine

61/70 (87%) and 31/41 (76%) dogs with IBD have had a positive response to sequential therapy

5yo MN Yorkie

Presenting complaint:

Wight loss

Distended abdomen

Occasional diarrhea

Hx: no response to diet alteration, deworming

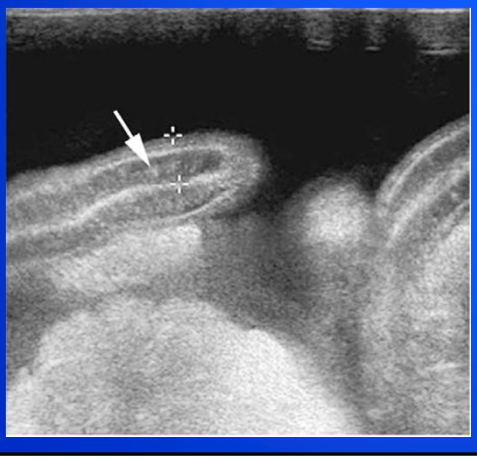
PE: poor body condition, ascites

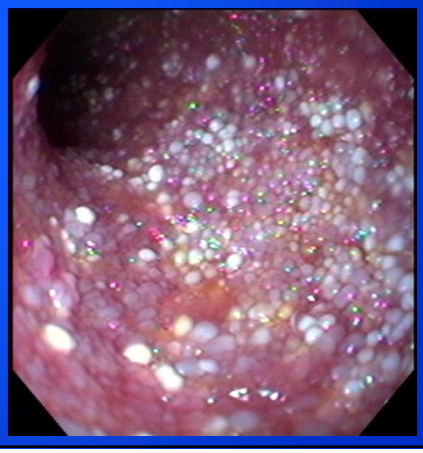
• PCV	.39	• Na	145
• MCV	67	• K	3.8
• retics	0.0		
•		• urea	25 (8.9)
• WBC	11.0	• creat	0.9 (80)
• Neut	10.0		
• Band	0.0	• TP	4.2
• Lymph	0.6	• ALB	2.0
• Mono	0.2	• GLOB	2.2
• Eos	0.2		
• PLT	900,000	• GLUC	99 (5.5)
• TP	68	• ALT	120
		• AST	75
• UA: 1.03, pH 7.0, no protein		• ALP	111

Imaging

Ultrasound: ascites, hyperechoic speckles and linear striations in SI.

Endoscopy: dilated lymphatics





YORKSHIRE TERRIER ENTEROPATHY M Craven, et al

- 14 YT with PLE, median age 96mo.
- Vomiting (7), diarrhea (6) and inappetance (6). Bicavity effusions in 5 dogs, and ascites alone in 3.
- Hypoalbuminemia (< 3.1g/dl) in all 12 dogs (median 1.6g/dl), and hypoglobulinemia (<1.9g/dl) in 7 (median 1.7g/dl).
- Duodenal biopsies from all affected YT contained cystic intestinal crypts.
- Lymphangiectasia, crypt hyperplasia and villus blunting were less consistent features. Mucosal infiltration of lymphocytes and plasma cells and eosinophils was common.
- Empirical therapy with corticosteroids (11/12), azathioprine (2/12), antibiotics, plasma and diuretics had a poor outcome.
- 7/12 cases died or were euthanased within 3m of diagnosis.
- Long-term survival in 3 dogs, (36, 24, and 8m), and 2 are alive at 3m and 4m after diagnosis.
- FISH showed no evidence of a bacterial association

J Vet Intern Med. 2014 Mar-Apr;28(2):331-7. Zimmerson et al.
Clinical features, intestinal histopathology, and outcome in protein-losing enteropathy in Yorkshire Terrier dogs.

ANIMALS:Thirty client-owned Yorkshire Terrier dogs with PLE.

- Females outnumbered males (20/30). Median age was 7 years (range 1-12).
- Common clinical signs were diarrhea (20/30), vomiting (11), ascites and abdominal distension (11), and respiratory difficulty (8).
- Histopathology : villous lymphatic dilatation, crypt lesions, villous stunting, and variable increases in cellularity of the lamina propria.
- All dogs were treated with glucocorticoids.
- Of 23 dogs with long-term follow-up, 9 had complete, and 3 had partial, resolution of signs, and 11 failed to respond to treatment.
- Median survival of responders was 44 months and of nonresponders was 12 months, with 4 dogs experiencing peracute death.
- Vomiting, monocytosis, severity of hypoalbuminemia, low blood urea nitrogen concentration, and villous blunting were predictive of survival <4 months.

Lymphangiectasia Treatment

- Fat restricted, high quality protein diet
- MCT oil @ 1-2ml/kg (or diet compounded with it)
- Prednisolone @ 1mg/kg/day PO
  - Injectable pred, Dexamethasone?
  - Cyclosporine 5mg/kg PO q24hrs 10 wks

    JVIM 2006,20,239-244
- Diuretics
  - lasix 0.5mg/k po bid 2-3d
  - Spironolactone0.5-1mg/kg po bid
- Aspirin 0.5mg/kg po sid
- ± Antibiotics e.g. tylosin

Prognosis is Unpredictable

J Small Anim Pract. 2017 Feb;58(2):103-108. doi: 10.1111/jsap.12625.

Dietary management of presumptive protein-losing enteropathy in Yorkshire terriers.

Rudinsky AJ[1], Howard JP[1], Bishop MA[2], Sherding RG[1], Parker VJ[1], Gilor C[1].

Author information

Abstract

OBJECTIVES: To describe the clinical outcome of dietary management of Yorkshire terriers with protein-losing enteropathy without immunosuppressive/anti-inflammatory medications.

METHODS: Records were searched for Yorkshire terriers with hypoalbuminaemia and a clinical diagnosis of protein-losing enteropathy that were managed with diet and without immunosuppressive/anti-inflammatory medications. Serum albumin changes were compared using a one-way repeated measures ANOVA. Canine chronic enteropathy clinical activity index scores were compared using a Wilcoxon signed-rank test.

RESULTS: Eleven cases were identified. Clinical signs were variable including: diarrhoea, respiratory signs, vomiting, lethargy and weight loss. Diets fed included home cooked (n=5); Royal Canin Gastrointestinal Low Fat (n=4); Hill's Prescription Diet i/d Low Fat (n=1); or Purina HA Hypoallergenic (n=1). Clinical signs resolved completely in eight dogs, partially resolved in two dogs and failed to respond in one dog. In dogs that responded, albumin significantly improved from baseline (mean 14·9 g/L, sd ±3·7), at 2 to 4 weeks (mean 24·2 g/L, sd ±5·5, P=0·01), and at 3 to 4 months (mean 27·0 g/dL, sd ±5·9, P=0·01).

CLINICAL SIGNIFICANCE: These results indicate that dietary management of protein-losing enteropathy is a potential management strategy in Yorkshire terriers. Randomised clinical trials in Yorkshire terriers with protein-losing enteropathy are necessary to compare success rate, survival and quality of life with dietary management versus combined dietary and immunosuppressive/anti-inflammatory therapy.

Nutritional management of chronic enteropathies in dogs and cats

Adam J. Rudinsky John C. Rowe, Valerie J. Parker JAVMA , 2018, Vol. 253, No. 5, Pages 570-578

Table 2— Summation of studies conducted to evaluate nutritional management of dogs and cats with chronic enteropathies.

Dietary strategy	Species	Indication	Evidence level†	Reference
Hydrolyzed diet	Canine	Chronic enteropathy	4	34,bb
	Canine	Chronic enteropathy	2	35
	Canine	Chronic enteropathy	3	36
	Canine	Chronic enteropathy	3	37
	Feline	Chronic enteropathy	4	38
Limited-ingredient diet	Canine	Chronic enteropathy	2	39
	Canine	Chronic enteropathy	2	40
	Canine	Chronic enteropathy	2	41
	Canine	Chronic enteropathy	2	37
	Feline	Chronic enteropathy	2	42
	Feline	Colitis	4	43
Fiber modification	Canine	Colitis	4	44
	Canine	Colitis	4	45
	Feline	Colitis	3	46
Highly digestible diet	Canine	Chronic enteropathy	2	35
	Canine	Colitis	4	47
	Feline	Chronic enteropathy	2	48
	Feline	Chronic enteropathy	2	56
Fat restriction	Canine	PLE	4	28
	Canine	PLE	3	29
	Canine	PLE	4 or 5	49–52,cc

†Evidence-based medicine levels are as follows: 1, high-quality randomized trial; 2, lesser-quality randomized trial or prospective comparative study; 3, case-control study or retrospective comparative study; 4, case series; and 5, expert opinion.

"Dietary modification can induce clinical remission in quite a lot of dogs with chronic enteropathy"

PLACEBO CONTROLLED TRIAL OF HYDROLYZED FISH DIETS IN DOGS WITH CHRONIC ENTEROPATHY

PLE

2 wks → 12wks → 26wks

ADDITIONAL MEDICATIONS ARE ALLOWED

- HYDROLYSATE
- HYDROLYSATE +

- Palatability
- Weight Gain
- CCCEAI
- Fecal Scores
- Cobalamin /Folate

CONTINUE IF POSITIVE RESPONSE

CROSSOVER IF NEGATIVE RESPONSE

HYDROLYSATE → HYDROLYSATE +

HYDROLYSATE + → HYDROLYSATE

PATIENT CHARACTERISTICS

PLE n=8

Breed distribution:

- 3 Mix, 2GSD, ShiTzu, Frenchie, Boston
- 5/8 less than 15kg

Sonography :

8/8: 5 Hyperechoic mucosa / enteropathy, 4 ascites, 3 mural thickening, 2 hyperechoic striations, 2 hyper echoic mesentery, 1LN , 1 NSF

Endoscopic biopsy and histopathology:

4/8 biopsy (GSD, Boston, Frenchie, Mix)

3 moderate to severe LP, eos, crypt cysts, 2 with dilated lymphatics. Some histiocytes and villus atrophy in 1 case each. Frenchie : Hx resected lipogranulomatous lymphangitis

OUTCOME

PLE n = 8

Diet	HF n = 5	HF+ n = 3
Response		
Initial	5/5	1/3
cross-over	2	
Final	6/7	1/3
Sustained	6/6	1/1

Albumin		Cholesterol		Weight (kg)		Δ Weight
0	12	0	12	0	12	kg
2.4 (1.5-2.9)	3.0 (2.3-3.7)	104 (91-197)	176 (109-324)	13.3 (2.2-55)	14 (3.6-59)	4.2 (0.7-6.8)

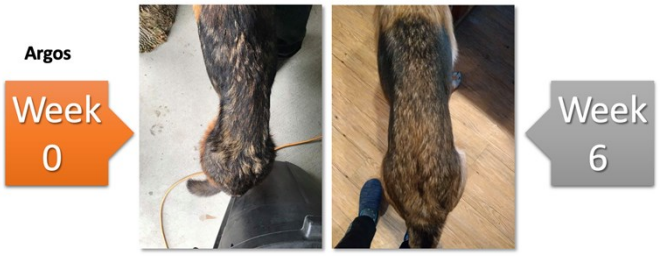
Median (range)

PLACEBO CONTROLLED TRIAL OF HYDROLYZED FISH DIETS IN DOGS WITH CHRONIC ENTEROPATHY

PLE 2 DOGS REQUIRED ONLY DIET TO ACHIEVE LASTING REMISSION

		CCEAI				FECAL SCORE				Albumin		Cholesterol		Cobalamin		Weight (kg)	
DIET		0	6	12	26	0	6	12	26	0	12	0	12	0	12	0	12
HF	Buddy	9	0	0	0	5	2.5	2.5	2.5	2.4	2.6	118	191	275	580	28.4	35.4
HF+	Argos	8	2	0	0	4	3	2	2	2.9	3.6	101	175	149	1512	24.4	31.2

Argos



SUMMARY AND CONCLUSIONS

- Changing diet, independent of antigen restriction or supplementation, was associated with long-term clinical remission in dogs with chronic enteropathy, and a subset of PLE.
- Further study is required to determine the basis of this clinical response and decrease in serum folate.
- It is important to consider possibilities other than hypersensitivity to intact proteins and cereals.
- Hydrolyzed fish diets were palatable and supported weight gain in dogs with PLE, and serum concentrations of B_{12} in CE.

Neutrophilic or Granulomatous Inflammation

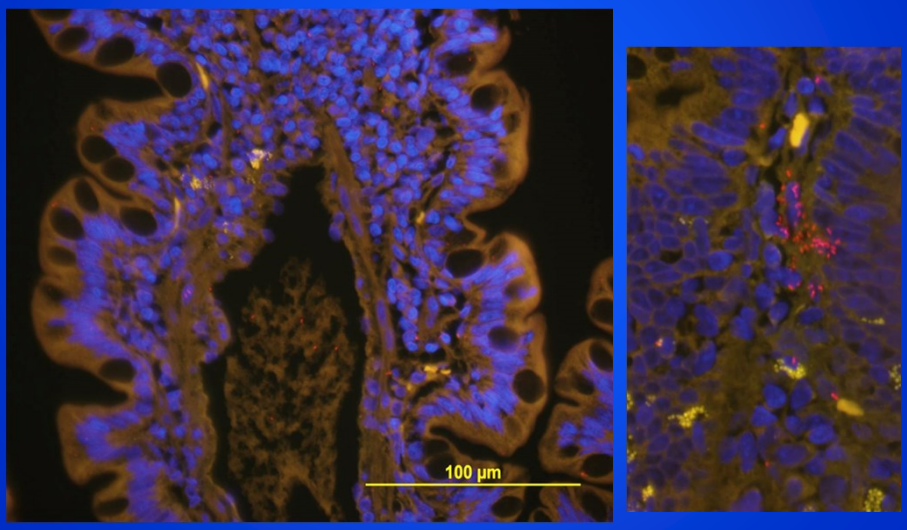
- Much less common than lymphocytic plasmacytic
- May be secondary to infection
  - Bacterial
  - Fungal
- Additional diagnostics warranted
  - Special stains / FISH analysis
  - Radiographs
  - Titers
- Prognosis
  - Depends on underlying cause
  - Typically guarded to poor

'Lucy'

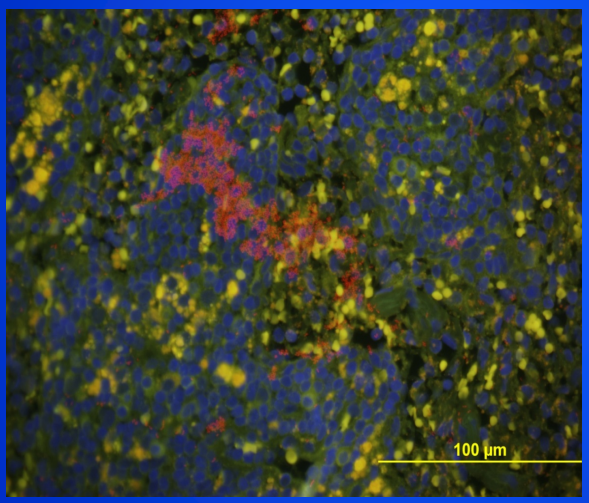
- 7 yo FN Bichon Frise
- Hx: chronic vomiting, reduced appetite
- rDVM biopsies (surgical):
  - Duodenum: moderate LPE, mild nos. eos & neuts, mild lymphatic dilation
  - Ileum: mild LP & neutrophilic inflammation. TRANSMURAL. Occasional granulomas
  - Mesenteric node: 'unremarkable'
  - PAS, Gram and acid-fast stains negative

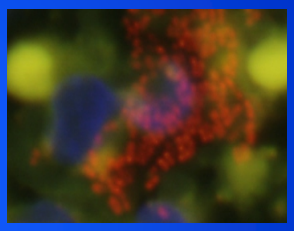
Lucy – ileal FISH

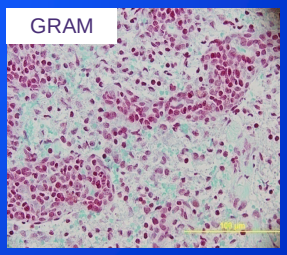
EUB338 Cy3 (red), non-EUB-6FAM (green)



Lucy: mesenteric node FISH





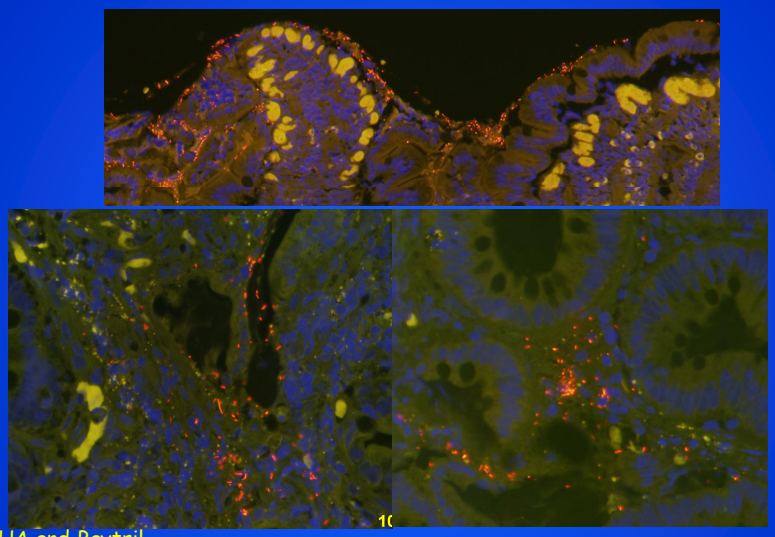


2yo FS Shar-Pei Sukie

- Hx: 3 months chronic diarrhea, 2 llb weight loss
- rDVM diagnostics:
  - CBC, Chem WNL
  - TLI WNL
  - Cobalamin low
  - Folate WNL
  - Feces negative PCR for Clostridium Toxin,
  - NO response to cbl weekly, diet changes, flagyl, tylan, panacur

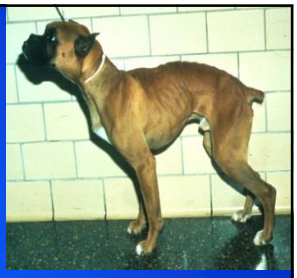
Surgical biopsies: moderate to severe LP enteritis

2yo FS Shar-Pei Sukie



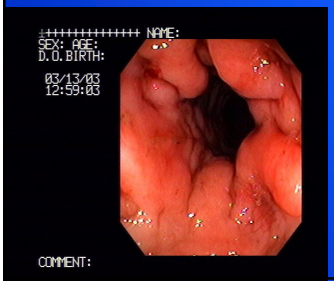
- Rx HA and Baytril
- Lost 6# since September
- CBC and chem normal with exception of 2.6 albumin. B12, folate and TLI pending.

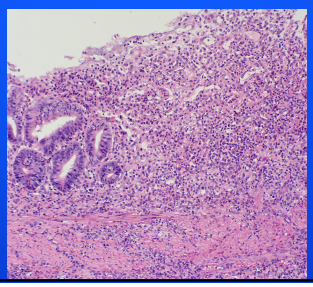
Granulomatous Colitis of Boxer Dogs

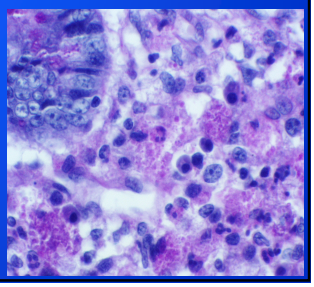


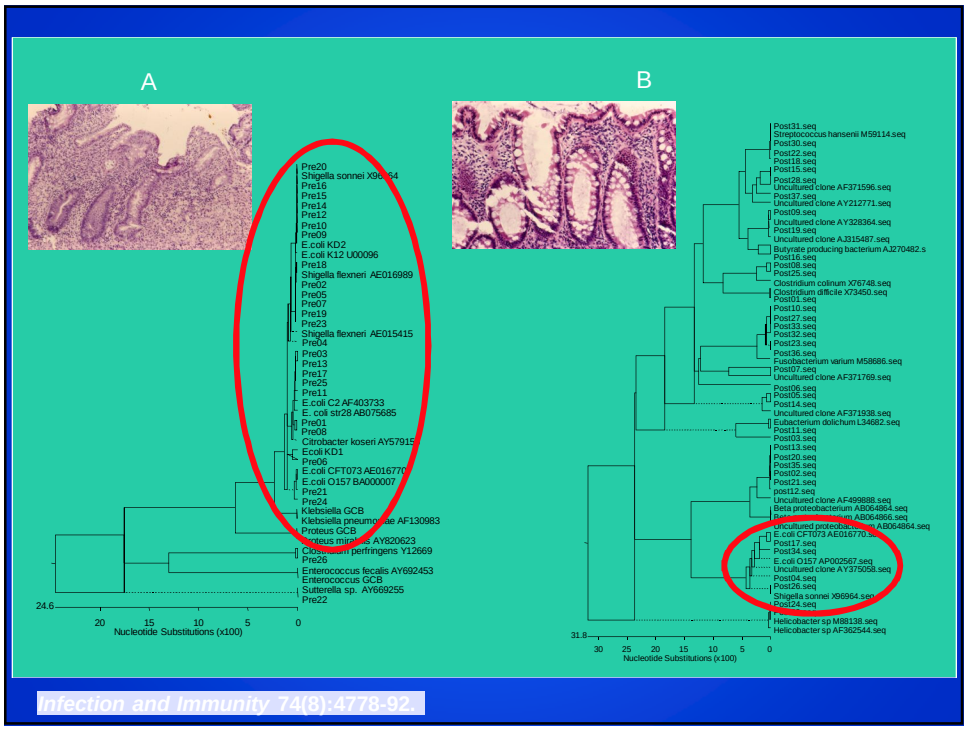
Van Kruiningen 1965

- Colony of Boxer dogs
- 9/30 dogs affected (8@ 5-18mo, 1@4y)
- Bacteria visualized in ulcerated mucosa and clinical response of 6/9 dogs to Chloramphenicol
- No infectious agent consistently identified
- Bacteria in ulcerated mucosa considered secondary invaders
- Categorized as idiopathic immune mediated





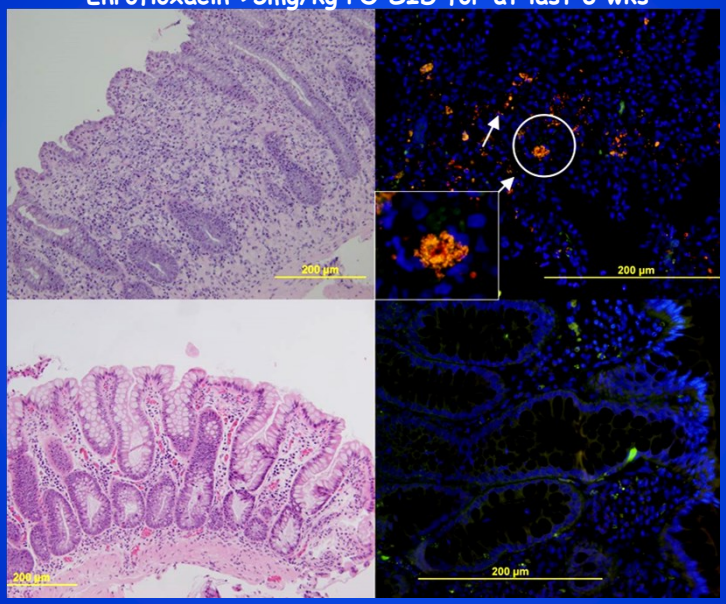




Infection and Immunity 74(8):4778-92.

Remission of granulomatous colitis in Boxer dogs correlates with eradication of invasive intramucosal *E. coli*

Enrofloxacin >5mg/kg PO SID for at last 6 wks



***E.coli* / *Shigella* (red), nonEUB-338 (green) and DAPI (blue).**

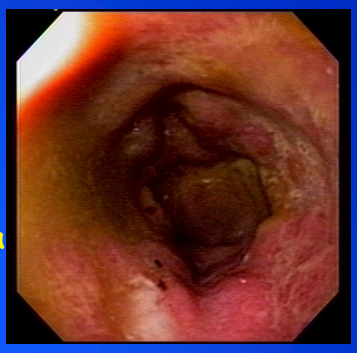
Bullet

8 mo M French Bulldog

Hx: chronic large bowel diarrhea, hematochezia

KW Simpson 2011

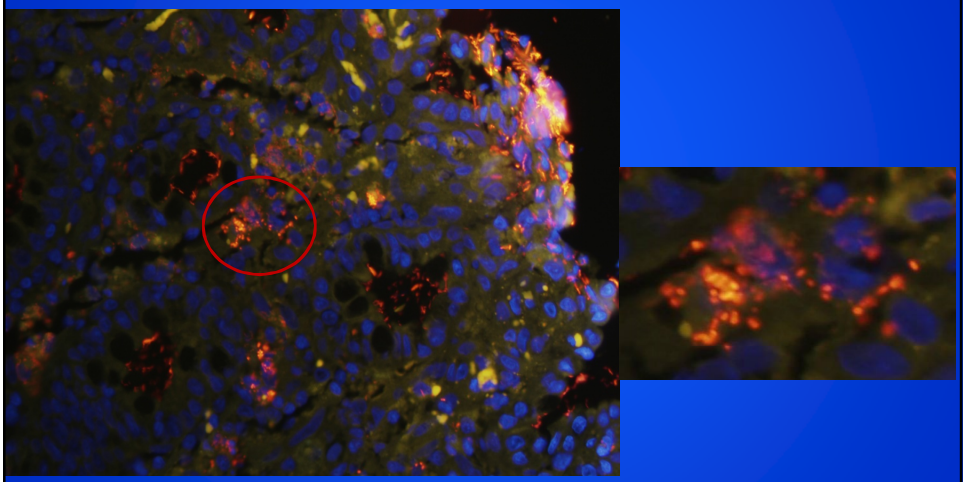
'Bullet'



- 8 mo M French Bulldog
- Hx: chronic large bowel diarrhea hematochezia
- Diagnostics:
  - CBC, chem, fecal parasitology unremarkable.
  - Colonoscopic mucosal biopsy: moderate inflammation (macrophages, L-P, eos).
  - PAS positive: granulomatous colitis

Bullet

E coli-Cy3 (red)
EUB338-6FAM (green)



100 % clinical response to 8 weeks enrofloxacin

KW Simpson 2011

J Vet Intern Med 2013;27:56–61

Association between Granulomatous Colitis in French Bulldogs and Invasive *Escherichia coli* and Response to Fluoroquinolone Antimicrobials

A.C. Manchester, S. Hill, B. Sabatino, R. Armentano, M. Carroll, B. Kessler, M. Miller, B. Dogan, S.P. McDonough, and K.W. Simpson

Background: French Bulldogs develop a form of granulomatous colitis (GC) with histopathological resemblance to GC of Boxer dogs (GCB). GCB is associated with mucosally invasive *Escherichia coli* whose eradication correlates with clinical remission.

Hypothesis/Objectives: To characterize the clinical and histopathological features, presence or absence of invasive colonic bacteria, and response to fluoroquinolones in French Bulldogs with GC.

Animals: A total of 6 French Bulldogs with a histological diagnosis of GC.

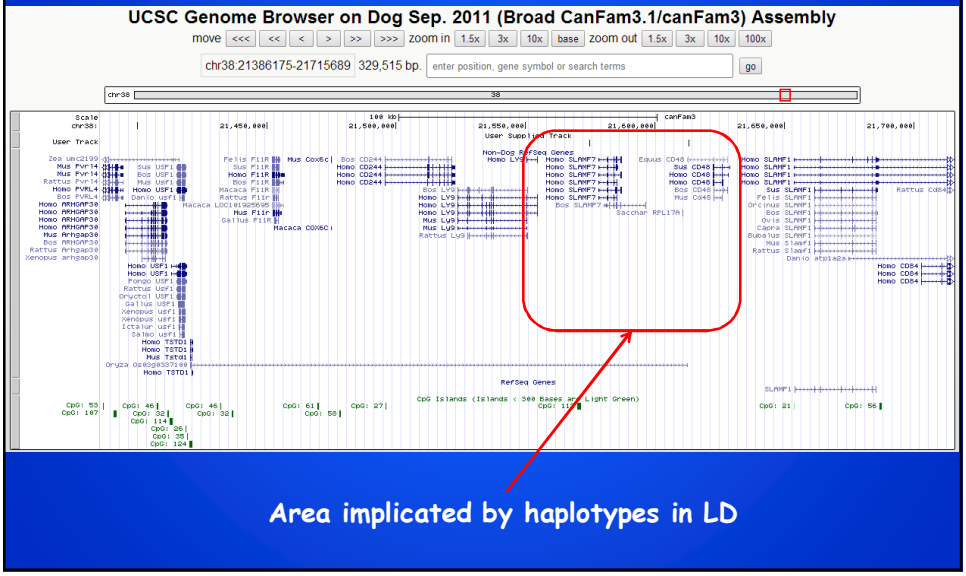
Methods: Retrospective study of medical records. Bacterial colonization was evaluated using 16S rRNA probes for eubacteria and *E. coli*. Biopsy specimens from 3 dogs were cultured for bacteria. Clinical response to fluoroquinolone antimicrobials was determined.

Results: All dogs were ≤1 year of age with hematochezia that was refractory to empirical therapy. Clinicopathologic and fecal analysis did not reveal abnormalities. Abdominal ultrasound revealed patchy thickening of the colon in 4/5 dogs and regional lymphadenopathy in 5/5. Colonoscopic abnormalities included irregularly thickened and ulcerated mucosa, hyperemia, and overt bleeding in 4/6 cases. Multifocal accumulations of PAS-positive macrophages and intramucosal *E. coli* were present in colonic biopsies of all 6 dogs. Administration of enrofloxacin (5/6) or marbofloxacin (1/6) at 4.4–10 mg/kg (median 10 mg/kg) PO q24h for 6–10 weeks was associated with clinical improvement within 5–14 days. All dogs remained in remission over a 3–30 month follow-up period.

Conclusions: Granulomatous colitis in young French Bulldogs is associated with the presence of invasive *E. coli* and closely parallels GCB. Treatment with fluoroquinolone antimicrobials can induce lasting clinical remission.

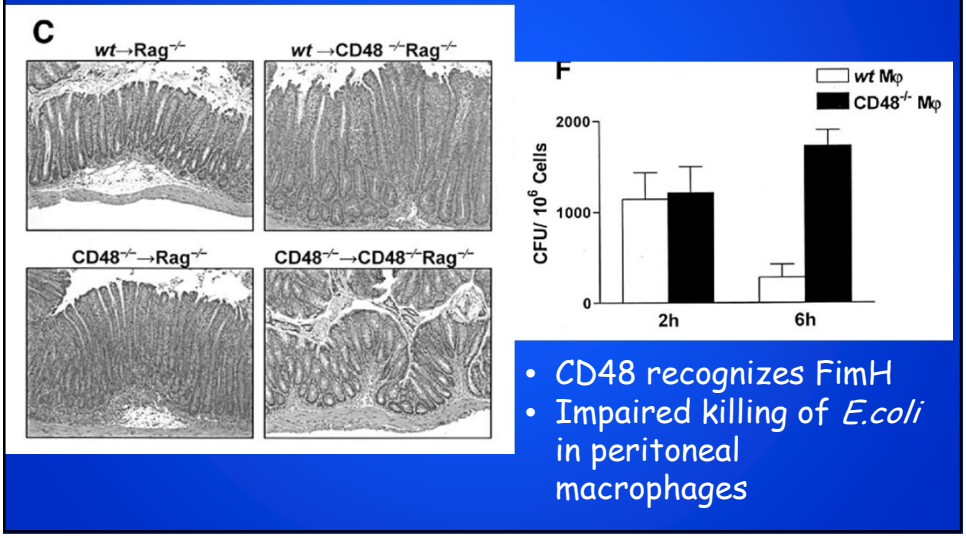
Key words: Chronic diarrhea; Endoscopy; Fluorescence in-situ hybridization; Inflammatory bowel disease.

Candidate region of interest



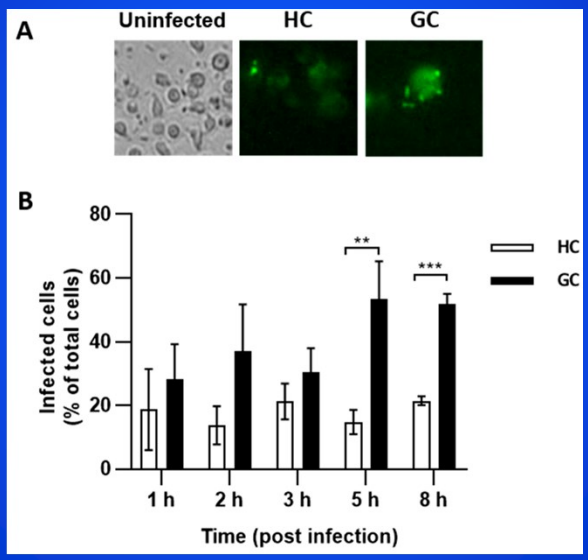
Area implicated by haplotypes in LD

Gastroenterology. 2006 Feb;130(2):424-34.
CD48 controls T-cell and antigen-presenting cell functions in experimental colitis.
Abadía-Molina AC……. Terhorst C.

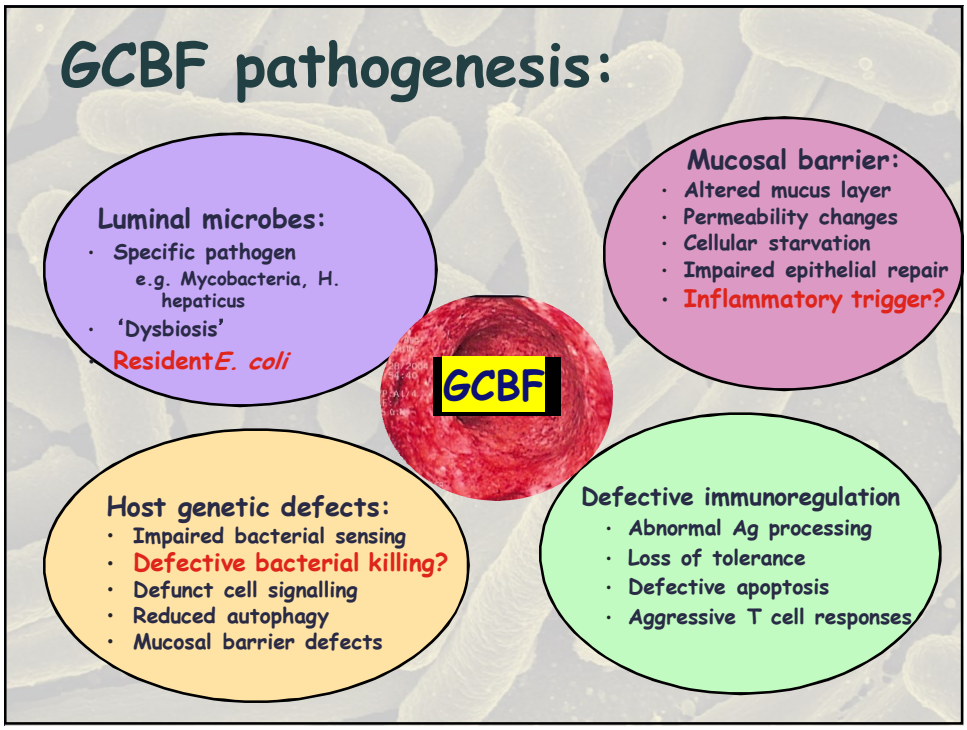


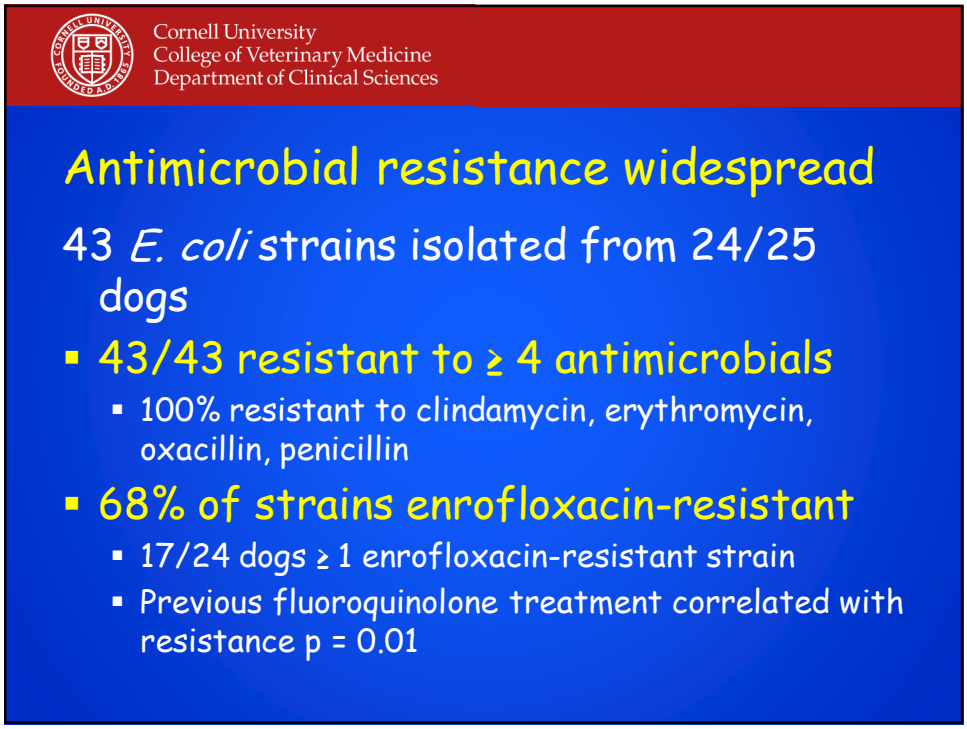
- CD48 recognizes FimH
- Impaired killing of *E.coli* in peritoneal macrophages

AIEC replicate in monocyte derived macrophages of GC Boxer with CD48/SLAM risk haplotype but not the HC.



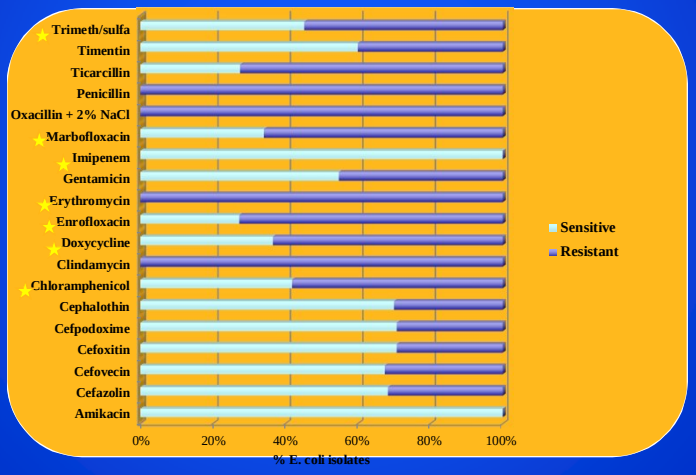
Antibiotics **2020**, *9*(9), 540; **https://doi.org/10.3390/antibiotics9090540**


GCBF pathogenesis:
Luminal microbes:
Specific pathogen
e.g. Mycobacteria, H. hepaticus
'Dysbiosis'
Resident E. coli
Mucosal barrier:
Altered mucus layer
Permeability changes
Cellular starvation
Impaired epithelial repair
Inflammatory trigger?
GCBF
Host genetic defects:
Impaired bacterial sensing
Defective bacterial killing?
Defunct cell signalling
Reduced autophagy
Mucosal barrier defects
Defective immunoregulation
Abnormal Ag processing
Loss of tolerance
Defective apoptosis
Aggressive T cell responses


Cornell University
College of Veterinary Medicine
Department of Clinical Sciences
Antimicrobial resistance widespread
43 E. coli strains isolated from 24/25 dogs
43/43 resistant to ≥ 4 antimicrobials
100% resistant to clindamycin, erythromycin, oxacillin, penicillin
68% of strains enrofloxacin-resistant
17/24 dogs ≥ 1 enrofloxacin-resistant strain
Previous fluoroquinolone treatment correlated with resistance p = 0.01

Cornell University
College of Veterinary Medicine
Department of Clinical Sciences

Susceptibility profiles of 43 strains



★Macrophage-penetrating antimicrobial

Received: 20 May 2020 | Accepted: 1 December 2020
DOI: 10.1111/jvim.15995

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access ACVIM American College of Veterinary Internal Medicine

Escherichia coli-associated granulomatous colitis in dogs treated according to antimicrobial susceptibility profiling

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Funding information
American Kennel Club (AKC) Canine Health Foundation, Grant/Award Numbers: 02050, 01445

Abstract

Background: Eradication of intramucosal *Escherichia coli* correlates with remission of periodic acid-Schiff-positive *E coli*-associated granulomatous colitis (GC). Treatment failures attributed to multidrug resistant (MDR) bacteria necessitate alternative approaches.

Hypothesis/objectives: Determine clinical outcome of *E coli*-associated GC in dogs treated based on antimicrobial susceptibility profiling and characterize *E coli* phylogeny and resistance mechanisms.

Animals: Twenty Boxers and 4 French Bulldogs with *E coli*-associated GC.

Methods: Culture, antimicrobial susceptibility profiling, and molecular characterization of *E coli* were performed and response to treatment was evaluated.

Results: Initial biopsy sample culture yielded fluoroquinolone-sensitive (FQ-S) *E coli* from 9/24 dogs and fluoroquinolone-resistant (FQ-R) *E coli* from 15/24. All but 1 FQ-R *E coli* were MDR with susceptibility to macrophage-penetrating antimicrobials restricted to carbapenems in 13/15 dogs. Of 22/24 treated based on susceptibility profiling, 8/9 FQ-S dogs had complete initial clinical response (CR) during fluoroquinolone (FQ) treatment, whereas 9/13 FQ-R dogs had complete or partial response (PR) during meropenem or doxycycline treatment. In 5/9 FQ-S and 12/13 FQ-R dogs with follow-up ≥3 months, CR was sustained in 5/5 FQ-S (median, 25 months; range, 4-46) whereas 6/12 FQ-R had long-term CR (median, 59 months; range 15-102), 4/12 PR (median, 19 months; range, 5-65), and 2/12 had no response (NR). Four dogs with long-term follow-up died within 4 years of diagnosis, including 2 euthanized for refractory colitis. *Escherichia coli* were genetically diverse. Fluoroquinolone resistance was associated with mutations in *gyrA* and *parC*, with plasmid-mediated resistance less common.

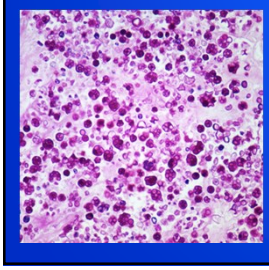
Conclusions and Clinical Importance: Antimicrobial treatment guided by susceptibility profiling was associated with positive long-term outcomes in >80% of cases.

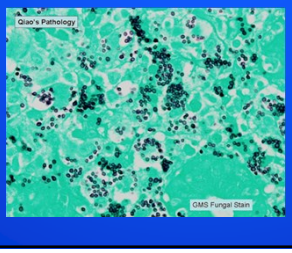
Antimicrobial therapy in GC should be guided by:

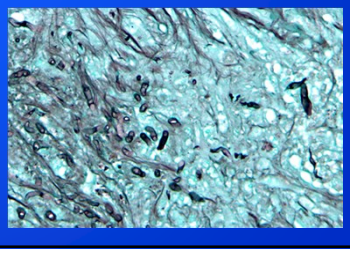
- FISH analysis for invasive *E.coli*
- Antimcrobial susceptibility of mucosal *E.coli*

FISH negative GC

- How many biopsies were taken?
- Screen (PCR/culture) for Enteropathogens
- Special stains for Prototheca, Histoplasma, Pythium







Med Mycol. 2007 May;45(3):249-66.
Protothecosis in 17 Australian dogs and a review of the canine literature.Stenner VJ1et al

- Systemic protothecosis was diagnosed in 17 Oz dogs between 1988 and 2005.
- **Young-adult (median 4 years), medium- to large-breed dogs. Females (12/17) and Boxer dogs (7 cases, including 6 purebreds and one Boxer cross) were over-represented.**
- 16 of 17 dogs died, with a median survival of four months.
- In most patients, first signs were referable to colitis (11/17 cases), which was often present for many months before other symptoms developed.
- Subsequent to dissemination, signs were mostly ocular (12 cases) and/or neurologic (8 cases).
- Microscopic examination and culture of urine (5 cases), cerebrospinal fluid (CSF;1 case), rectal scrapings (4 cases), aspirates or biopsies of eyes (5 cases) and histology of colonic biopsies (6 cases) as well as skin and lymph nodes (2 cases) helped secure a diagnosis.

The Future…… **Individualized care**

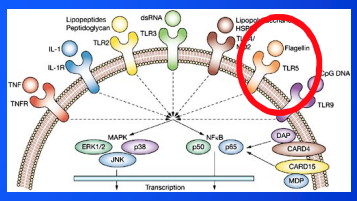
Phenotype:

- **Breed**
- **Type and location of IBD**
- **Microbiome**
- **Metabolome**

Genotype:

- **TLR5?**
- **NOD2?**
- **NCF?**

• PLoS One. 2010 Dec 23;5(12):e15740.
•Polymorphisms in the TLR4 and TLR5 gene are significantly associated with inflammatory bowel disease in German shepherd dogs.
•Kathrani A, et al



The microbiota in the duodenum of GSDs belongs to the classes of Bacilli, and Erysipelotrichi, and to the orders of Lactobacillales, Actinomycetales and Erysipelotrichales……

Questions?

KWS5@CORNELL.EDU

http://www.vet.cornell.edu/labs/simpson

Boxers

Yorkies

Clinical trials

FISH analysis

Inflammatory Bowel Disease: Histopathology

Chronic Enteropathy: Clinical Phenotype

The Biopsy Line

Non-PLE Lymphocytic Plasmacytic Enteritis

- **Corticosteroids (High)**
- "Intestinal diet"
- Metronidazole

(1980s)

- **Antibiotics**
- Corticosteroids (High)
- Intestinal Diet

(1983: iSIBO espy. GSD)
(1994: **Antibiotic Responsive, ARD**)
(2003: **RIP iSIBO :** NSD bacteria, cbl, folate, TUBA, FRD/EPI/ Uncl / IBD/ARD)

- **Wheat free diet**
- Irish Setters
- (1990)
- **Exclusion diet**
- Antibiotics (SIBO)
- Corticosteroids (High)

(1995: espy GLDR permeability)

Sequential therapy
- **Antigen restricted diet**
- Corticosteroids (High)
- Cyclosporine

(2007)

Sequential therapy
- **Hydrolyzed diet**
- Tylosin (ARD)
- Corticosteroids (High)
- Other immuno.

(2007-11)

Diet modification
- **Hydrolyzed diet vs "Intestinal diet"**
- Increased relapse on intestinal diet

(2010)

Clinical Phenotype
- Exclude non-GI causes of signs
- Characterize GI dz:
  - Breed, age
  - Small / Large / Mix

Clinical pathology:
- Is EPI likely? TLI
- PLE vs non-PLE
- Cobalamin / folate
- iCa^{2+} / [Vit.D(EA)K]
- Thrombotic potential

Sonography:
- WNL
- Ascites
- Masses
- Thickening/Loss of layering
- Striations
- LN

± Endoscopy / Surgery

- ❑ **Breed**
- ❑ **Response to therapy**
  - Diet: **Food first**
  - Cobalamin / folate
  - Antibiotic
  - Steroid
  - Other immunosuppression
  - Fiber (psyllium)
  - Probiotic/ prebiotic/synbiotic
  - FMT
  - Non-responsive

PLE LP IBD, Lymphangiectasia (R/O Lymphoma, neoplasia, granulomatous, infectious)

Corticosteroids (High)
- Low fat cott. chz. +rice
- Metronidazole

(1980s)

Immunosuppression for PLE
- Corticosteroids (High)
- **Cyclosporine**
- Diet

(2007)

Sequential, low dose steroid, breed
- Response of some PLE to diet alone
- Anti-inflammatory steroids
- Lymphangiectasia / crypt cysts, YT
- Aspirin

(2007-11)

Food first and Second
- Response to ULF or Hydro / AgR
- ± Anti-inflammatory steroids
- Transition to higher fat / other diets

(2014-2021)