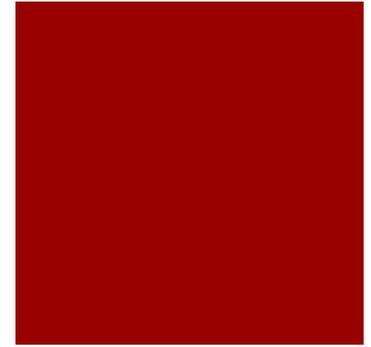


Rational antimicrobial treatment in GI disease

Karin Allenspach
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Iowa State University
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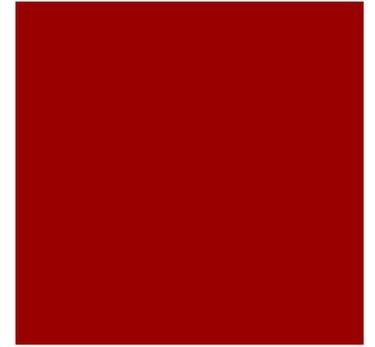
Drugs used in diarrhea cases in UK



- **First-choice therapy for dogs presenting with diarrhoea in clinical practice in UK: Vet record 2014 (German A et al)**
- Computerized referral histories were reviewed for dogs admitted to the University of Liverpool Small Animal Teaching Hospital between January 2000 and December 2008 with diarrhoea among the clinical signs

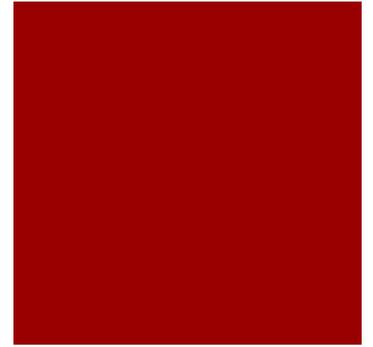
Drugs used in diarrhea cases

- A total of 371 cases presenting to the referring veterinary surgeon were included in the study
 - **Antibiotics in 71% of cases**
 - Steroids 19%
 - Miscellaneous antidiarrheal products (including probiotics, prebiotics, adsorbents and antimotility drugs) 13%
 - Antiemetics 13%
 - Gastric protectants 10%
 - Sulfasalazine 7%

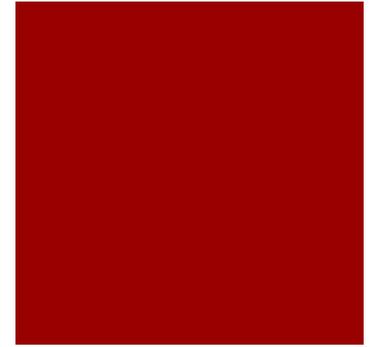


Evidence for bacterial causes of diarrhea in dogs and cats?

- Usefulness of culture/indications for AB treatment?
 - Acute diarrhea
 - Chronic diarrhea



Bacterial culture/other detection methods - useful?

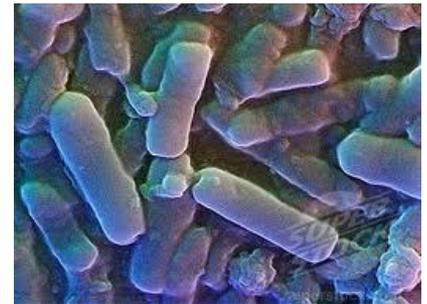


- Pathogenic bacteria:
 - Clostridia perfringens, difficile
 - Salmonella
 - Campylobacter

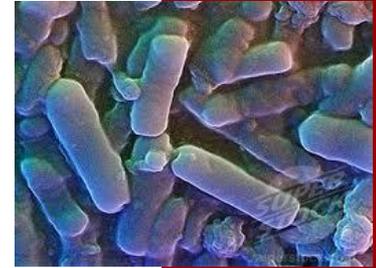
Clostridium perfringens



- Gram pos anaerobic spore-forming bacillus
- 5 Biotypes A-E
- 4 major toxin genes: $\alpha, \beta, \iota, \epsilon$
- Enterotoxigenic *C. perfringens* type A causes food poisoning and sporadic diarrhea in people



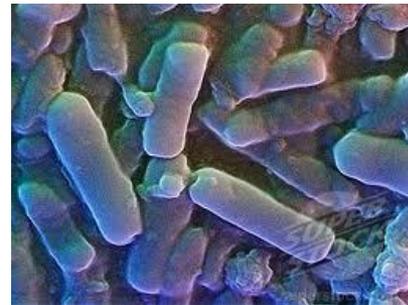
Clostridium perfringens



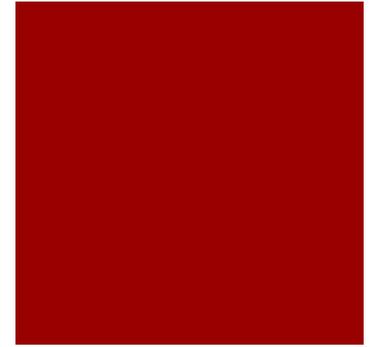
- Prevalence in dogs
 - Can be cultured from up to 80% of dogs with dx
 - Enterotoxigenic strains can be cultured from up to 34% of dogs with dx but also in up to 14% of dogs without dx
 - One study in dogs with AHDS: 70% of dogs have enterotoxigenic C. perfringens
- Prevalence in cats: 4% of cats with dx, 2% of cats without dx

Clostridium perfringens

- Clinical presentation: Most cases are mild, self-limiting, some can present with acute colitis and some with severe HGE



Clostridium perfringens



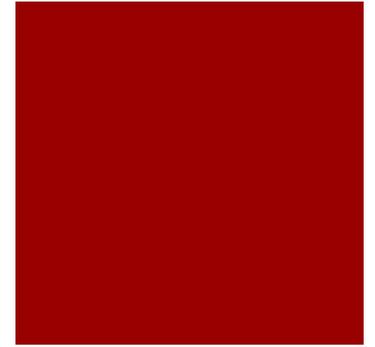
- Diagnosis:
 - Fecal bacterial culture/counting of spores in fecal smear: very low sensitivity
 - ELISA for toxin detection; both low Sensitivity/Specificity
 - PCR alone also difficult as many healthy animals are carriers
 - Recommendation: ELISA to detect enterotoxinogenic C.perfringens and PCR to detect enterotoxinogenic strains

AHDS and C.perfringens

- Histology in acute cases: C.perfringens in deeper mucosal areas
- However: Same bacteria also found in feces
- Problem: C. perfringens is normal commensal in many dogs
- Hypothesis: “overgrowth” of C.perfringens when mucosa is severely damaged
- AB: Not indicated as bacterial translocation extremely rare and AB use not associated with faster recovery or longer survival

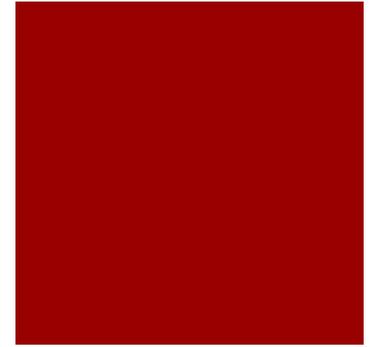


Clostridium perfringens



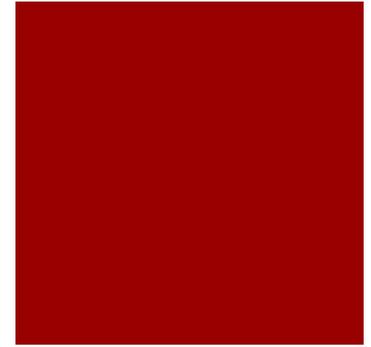
- Treatment
 - If severe clinical signs:
 - Ampicillin, erythromycin, metronidazole, tylosin, tetracyclin
- New study out of Switzerland: Up to 54% of *C. perfringens* are resistant to metronidazole

AHDS and antibiotics

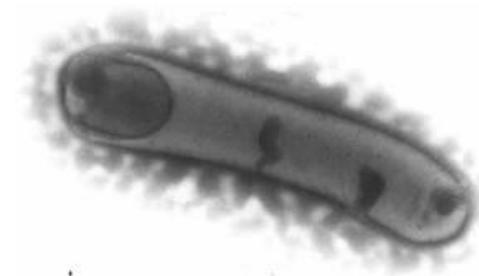


- Treatment of Aseptic Dogs with Hemorrhagic Gastroenteritis with Amoxicillin /Clavulanic Acid: A Prospective Blinded Study (Unterer et al JVIM 2011)
- Patients were randomly divided into treatment (amoxicillin/clavulanic acid for 7 days) and placebo groups
- 53 dogs: No significant difference between treatment groups concerning mortality rate, dropout rate, duration of hospitalization, or severity of clinical signs

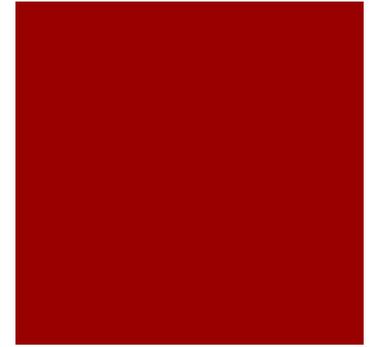
Clostridium (Clostridiodes) difficile



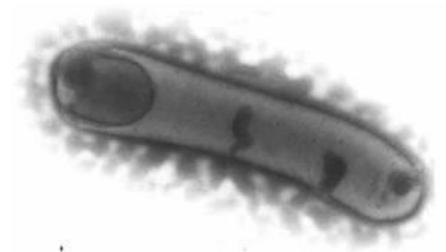
- Anaerobic spore forming bacillus
- Vegetative cells cause disease in intestines, spores very resistant and survive for years, responsible for transmission
- Toxin production in intestines: Toxin A and B most important
- Causes pseudomembranous colitis in humans
- Hospital-associated infection
- Recently frequency and mortality has increased
- “hypervirulent” strains: ribotype 027 has recently been isolated from dogs!



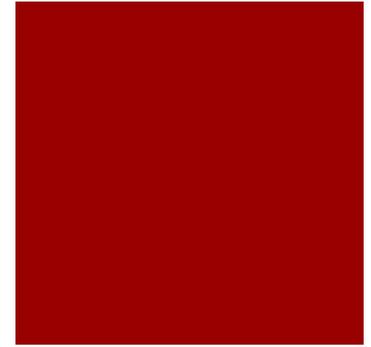
Clostridium difficile



- Cause of diarrhea in dogs?
 - Prevalence 0-58% in healthy dogs and cats
 - Young dogs, dogs that visit hospitals
 - Clinical signs: subclinical carriage to AHDS



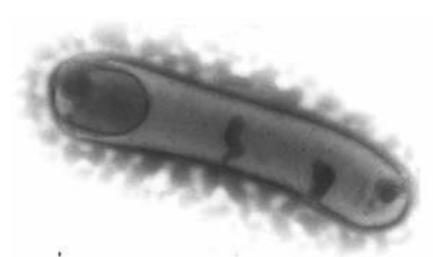
Clostridium difficile



- C. Difficile: 60% of dogs that visit hospitals are positive, probably human - dog transmission
- In humans: fecal transplants is the mainstay of treatment! restores levels of 2nd bile acids
- Dogs are unresponsive to metronidazole, better only with dietary change
- One case report in French: C. Difficile and diarrhea, received FMT and resolved

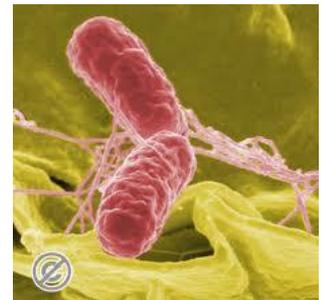
Clostridium difficile

- Diagnosis: Need to detect toxin
- ELISA poor sensitivity/specificity
- Recommendation: If suspicion, do ELISA to detect both A and B toxins and combine with real-time PCR to detect enterotoxigenic strains
- Zoonosis: recent publications show that dogs and cats can be carriers for human infection
 - Huge AB resistance problems in human beings (especially vancomycin):
 - Treatment in small animals is being discouraged

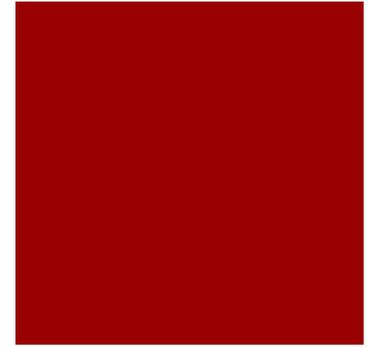


Salmonella

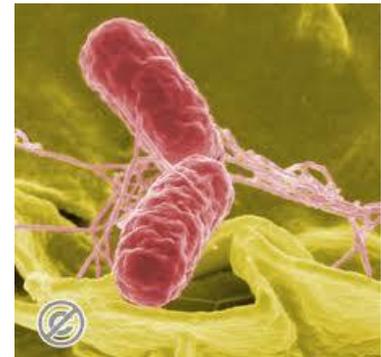
- Gram neg motile facultative anaerobe bacilli
- Ubiquitous: mammals, birds, reptiles, insects..
- Prevalence in healthy dogs and cats with dx similar to that of dogs and cats without dx
- Study at UC Davis 2010: 1.2% of cats and dogs with diarrhoea were culture positive
- **Sled dogs or greyhounds who are fed raw meet: up to 70% are carriers!**



Salmonella



- Is a pathogen in dogs and cats, but very uncommon!!
- Clinical signs variable, mostly mild disease or subclinical
- If sick, looks like food poisoning in people
 - 3-5 days after infection: fever, abdominal pain, hemorrhagic gastroenteritis

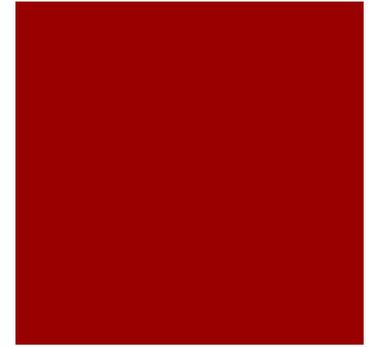


Salmonella

- **Diagnosis:**
- Culture/qPCR
- qPCR available and more sensitive than culture, **but:**
 - New study in 5 countries > 7000 samples:
 - prevalence in diarrhea cases between 0-5%
 - Prevalence in controls: 0-3% (not stat sign)



Salmonella



- **Treatment:**

- AB not needed in most cases
- If severe disease ampicillin or enrofloxacin
- Recommendation: Culture (for sensitivity) and qPCR, if suspicious case: call local hospital to get recommendation for AB treatment
- Educate owners (zoonosis, raw food..)

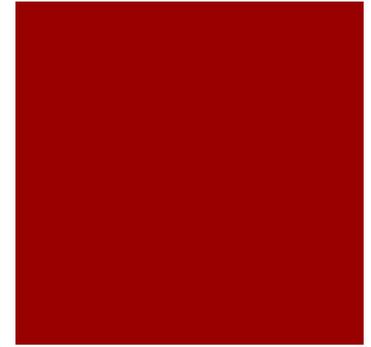


Campylobacter

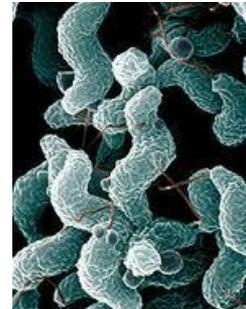
- Gram neg micro-aerophilic curved motile rods
- 37 species and subspecies
- Most non-pathogenic
- Pathogenic strains: *C. jejuni*, *C. coli*
- Most common in dogs: *C. helveticus* and *upsaliensis*



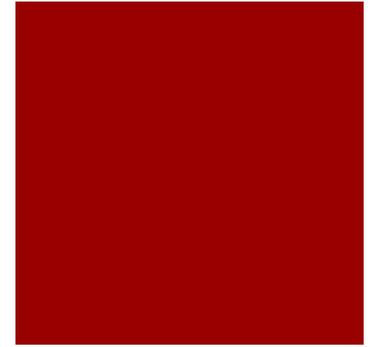
Campylobacter



- Campylobacter commonly found in feces of cats and dogs with/without diarrhea
- <12 mths old dogs: *C.jejuni* and *upsaliensis* 2x more common in dogs with dx than those without
- More common if feeding home cooked diet/table scraps/raw food
- cats: Prevalence highly variable, also *helveticus/upsaliensis* most common

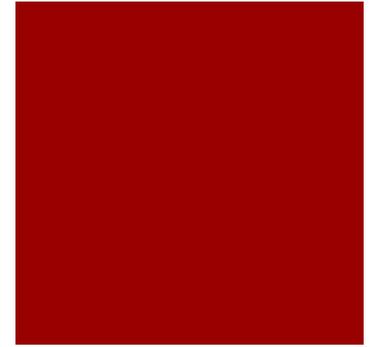


Campylobacter



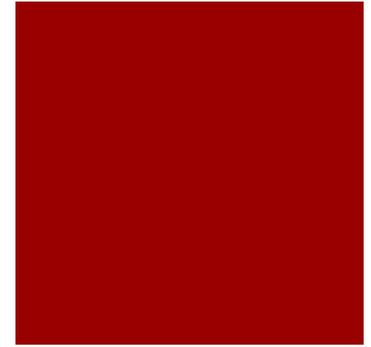
- C.jenuni in young animals possibly pathogenic, other species probably not
- Clinical signs vary, mild enteritis to severe haemorrhagic gastro-enteritis
- Treatment with enrofloxacin or macrolides if severe clinical signs and culture positive and/or PCR positive or if puppies
- Is a zoonosis! Has been shown to be transmitted to humans by dogs or food

Bacterial infections causing **chronic** diarrhea in small animals



- Evidence is sparse
 - Similar to acute cases: Prevalence of culture positive cases is similar to healthy dogs and cats
 - Clostridia, Campylobacter and Salmonella cultures positive in about 5% of cases of chronic diarrhoea at the QMH
- Considered secondary “overgrowth”
- We don’t routinely perform faecal culture anymore in our chronic diarrhoea cases.....

Definition of Antibiotic Responsive Diarrhea



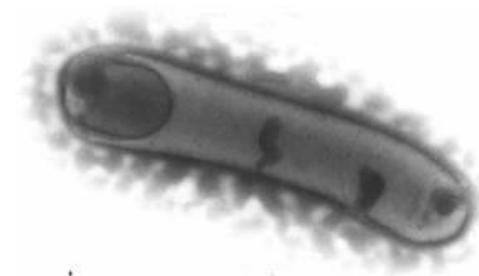
- antibiotic-responsive chronic diarrhea without an identifiable underlying etiology
- previously termed idiopathic small intestinal bacterial overgrowth (SIBO). This term is not used anymore - based on quantitative culture of bacteria in the upper GI tract
- Pathogenesis? German Shepherd Dogs: mutations in pattern recognition receptors?



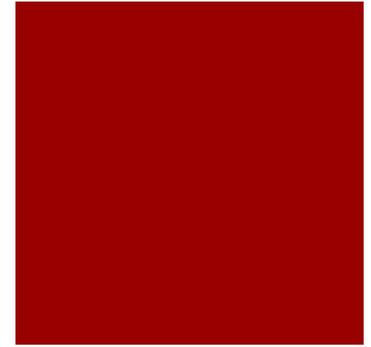
Treatment

- Metronidazole 15mg/kg bid for 4 weeks
- Tylosin 20 mg/kg PO q8–12h for 4 weeks
- Amoxicillin-clavulanic acid, others...
- Long-term management with tylosin or low dose metronidazole

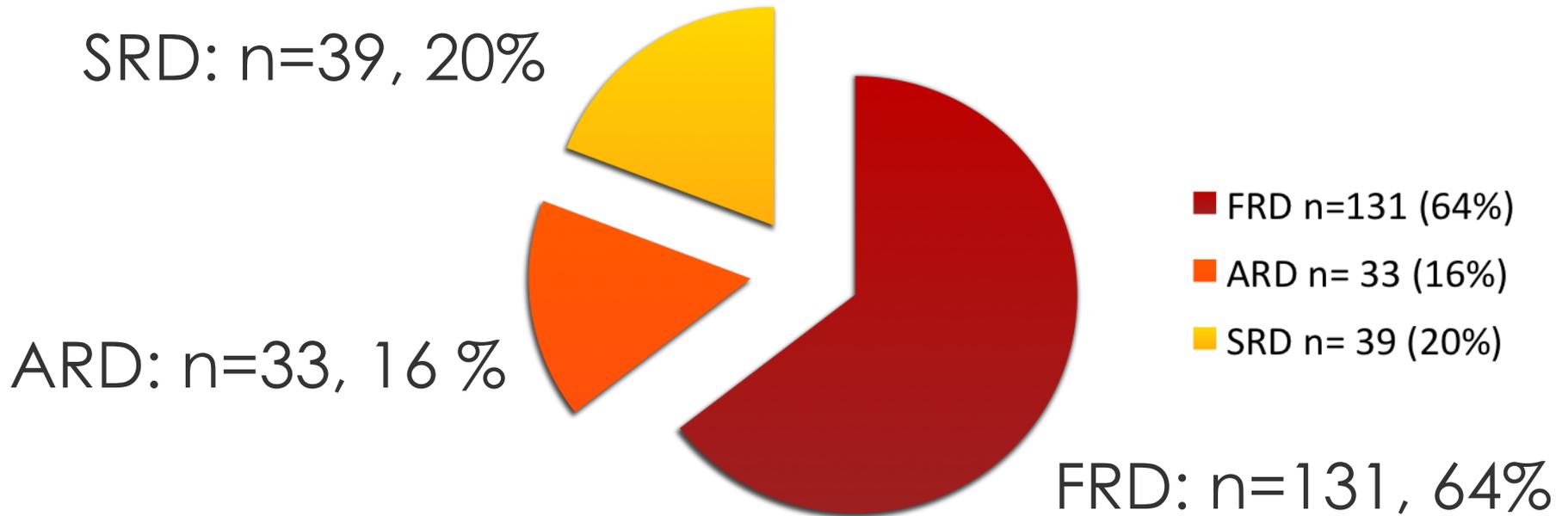
Kilpinen et al 2011, 2014, 2015; Hall et al 2011



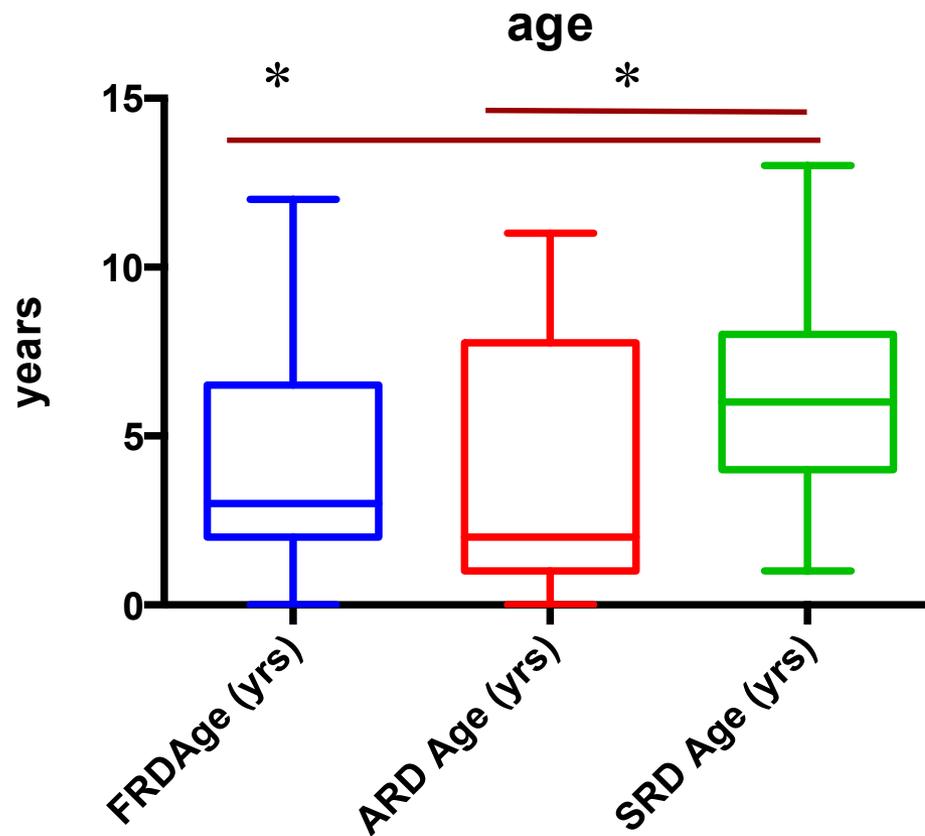
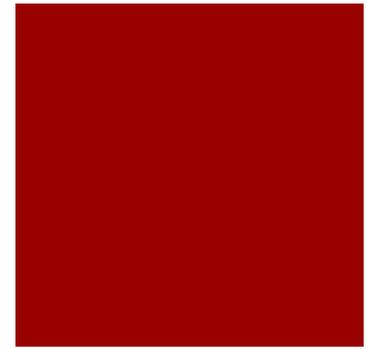
Retrospective study RVC: 203 dogs



Chronic enteropathies

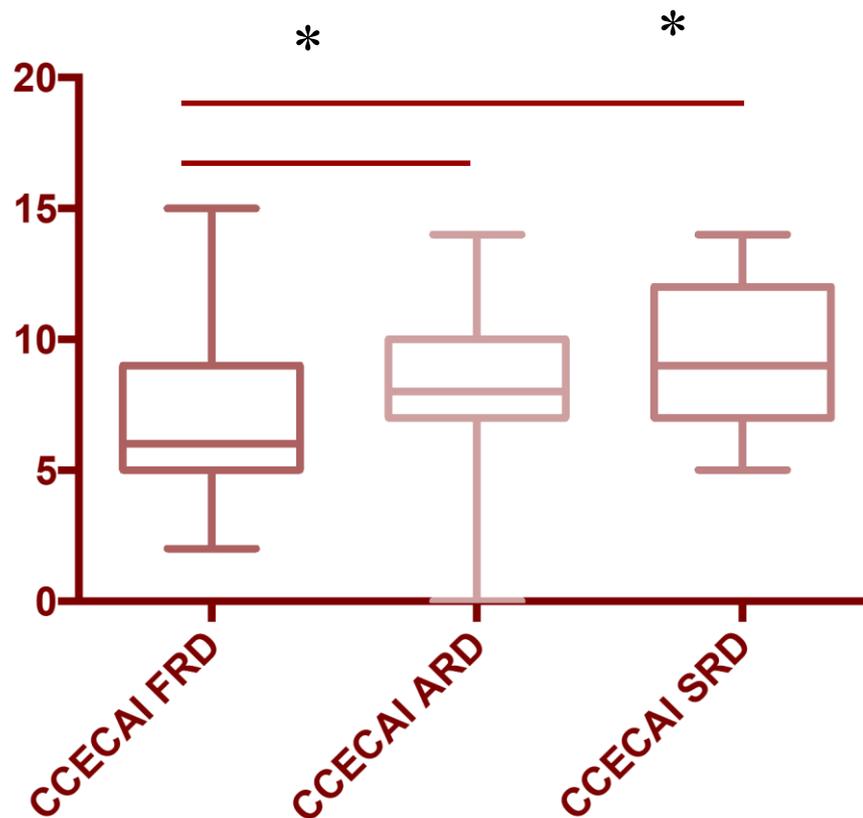
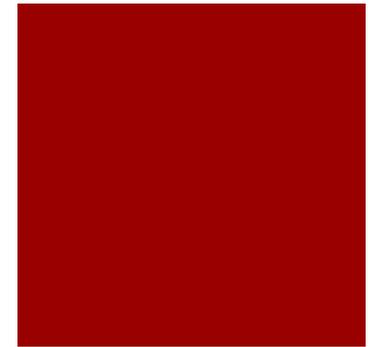


Age



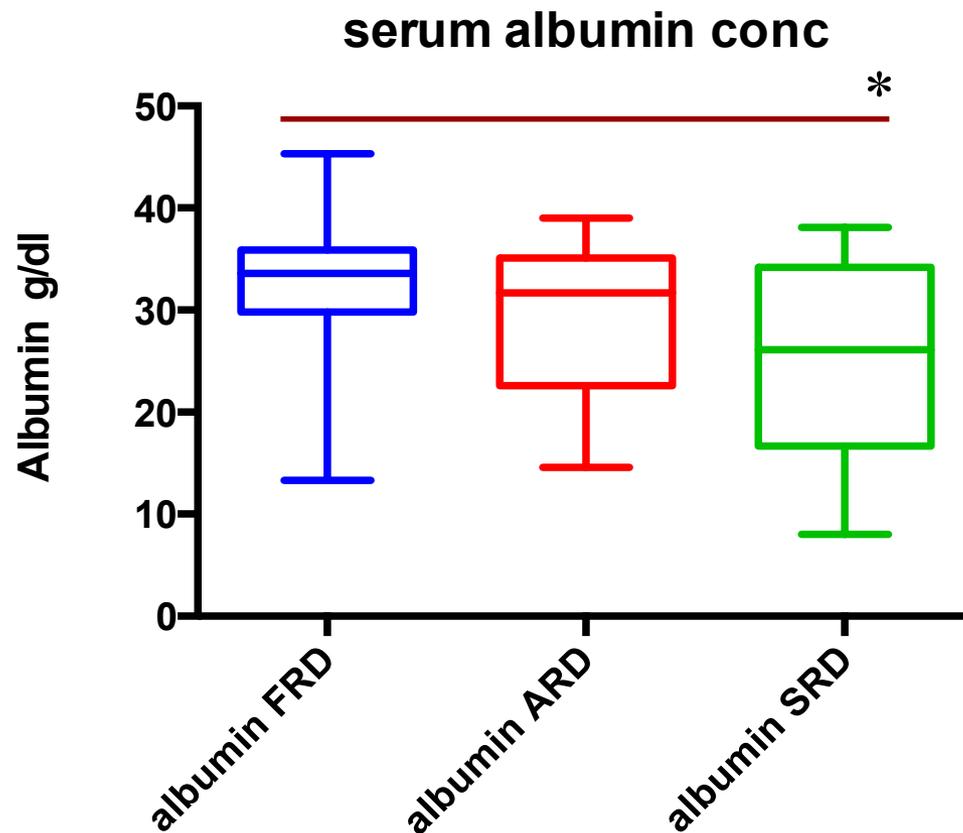
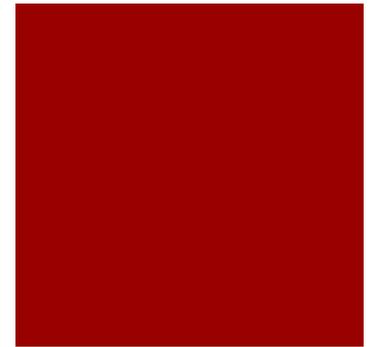
- ARD median 2 yrs,
- range 0-11

Clinical activity index (CCECAI)



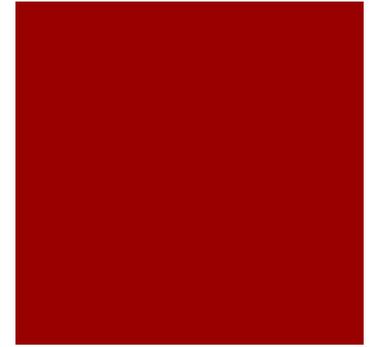
- median FRD 6
range 2-12
- median ARD 8
range 0-14
- median SRD 9
range 5-14

Serum albumin concentration



- mean FRD 32g/dL SD 5.8
- mean ARD 29 g/dl SD 7.3
- mean SRD 26 g/dL SD 9.0
- $p = <0.001$

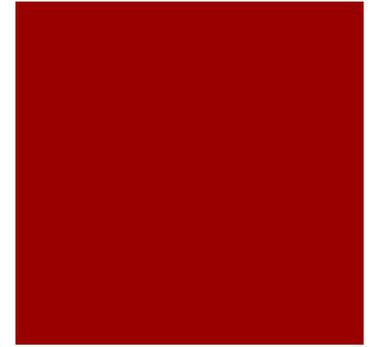
Long-term outcome



- 6 months–1 year after discharge
 - FRD : very good
 - **ARD : none of the dogs improved: worst outcome!**
 - SRD : moderately good

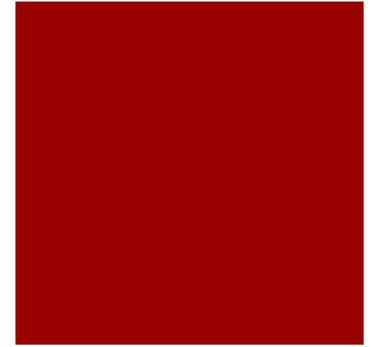
- 6 months–1 year, outcome was significantly better for FRD vs ARD and for FRD vs SRD (Chi² p<0.001, and p= 0.002).

Summary: 203 dogs with IBD: ARD



- GSD or other large breed dogs
- Young! Median 2 years
- Respond QUICKLY to Metronidazole or Tylosin
- Have a relapse when antibiotics are discontinued
- ARD dogs consistently have the worst outcome after 1 year of follow-up!

Deviation from diet/medication



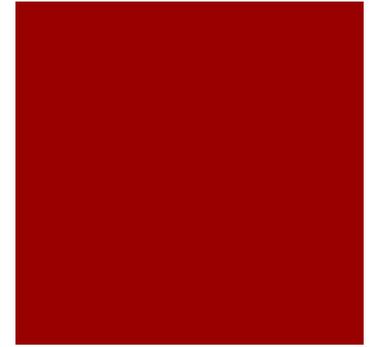
FRD dogs- deviation from prescribed diet



- Daily deviation: 43%
- Weekly deviation: 24%
- Monthly deviation: 5%
- Never: 28%

- ARD and SRD: Owners reported no deviation from either antibiotic or immunosuppressive treatment

Relapse rate



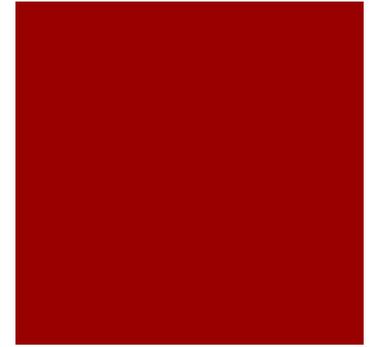
relapse rate CE 1-3 years after diagnosis



Highest rate for ARD,
sign different from FRD and SRD, $p=0.004$

Microbiome in dogs with diarrhea: Dysbiosis!!

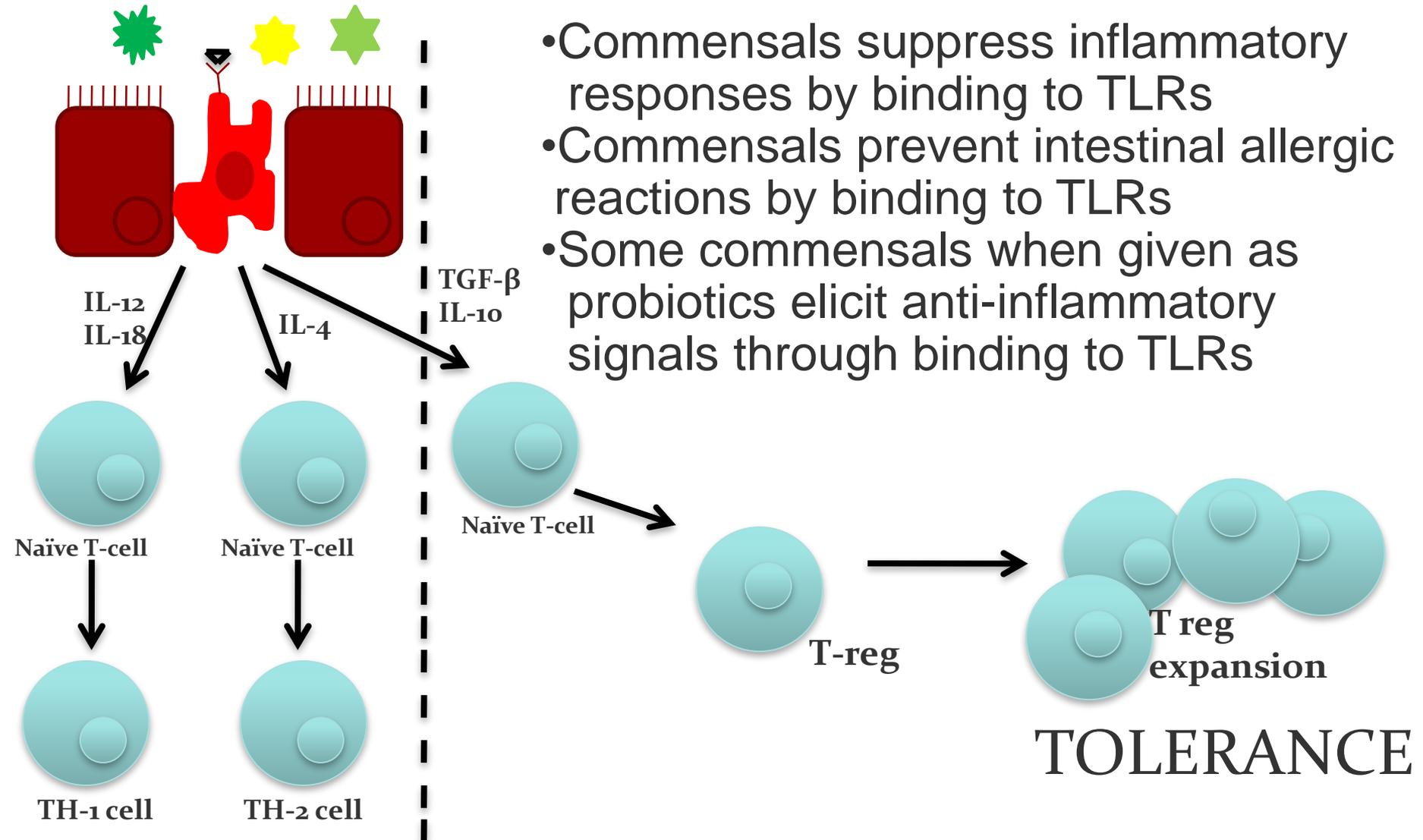
- CE and acute diarrhea:
- Increase in Proteobacteria (E.coli)
- Decrease in Fusobacteria, Bacteroides and Firmicutes (Faecalibacterium, Ruminococcaceae, Turicibacter, Blautia)
- Decreased diversity





Why is microbial dysbiosis bad?

- Commensals suppress inflammatory responses by binding to TLRs
- Commensals prevent intestinal allergic reactions by binding to TLRs
- Some commensals when given as probiotics elicit anti-inflammatory signals through binding to TLRs



IL-12
IL-18

IL-4

TGF- β
IL-10

Naïve T-cell

Naïve T-cell

Naïve T-cell

T-reg

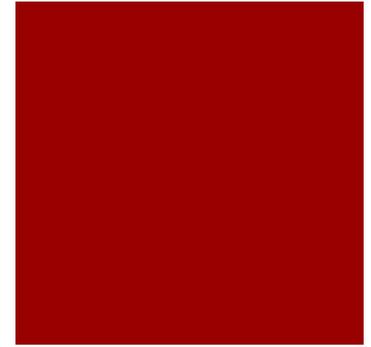
T reg
expansion

TH-1 cell

TH-2 cell

TOLERANCE

Effect of AB on microbiome

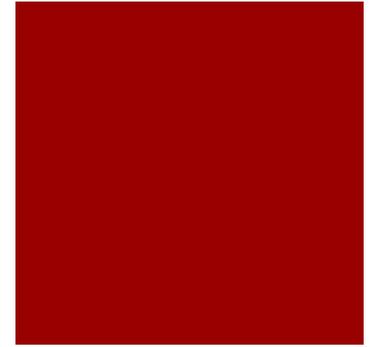


- Metronidazole causes a huge shift in the normal microbiome
- Illustrated by dysbiosis index
- **C. Hiranonis is key species:** is important for conversion of primary to secondary bile acids

DYSBIOSIS index

- AlShaWAQfe et al. FEMS Microbiology Ecology 2017
- **qPCR of:**
Bifidobacterium spp., Blautia spp., Faecalibacterium spp., Turicibacter spp., Lactobacillus spp., Clostridium perfringens, C. hiranonis and E. coli, combined in an index
- Less than 0: normal
- Higher than 0: abnormal

Effect of AB on metabolome

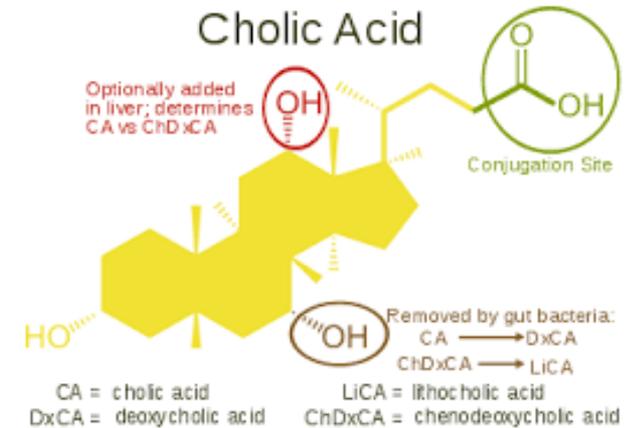


- Metronidazole for 14 days in healthy dogs:
- Disruption in major metabolic pathways
- Particularly **secondary bile acids**

- **Important for:**
- Immune system
- Hepatic function
- Intestinal function

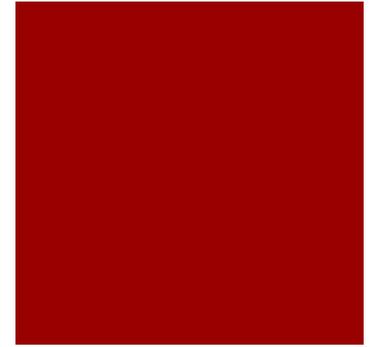
Bile acids

- Produced in liver from cholesterol
- Secreted into bile -> duodenum
- Important for absorption of fat and fat-soluble vitamins
- **Intestinal microbiome: converts primary BA into secondary BA...**
- Re-absorbed in ileal enterocytes, recirculated to liver
- **Small amount escapes into periphery and exerts important effects...**



Bile acids in intestine

- BA act as important antimicrobial molecules
- Many bacteria will metabolize them to be able to survive
- If Dysbiosis present- this effect is gone -> results in pro-inflammatory changes in the intestine with permeability changes -> leads to chronic inflammation in intestine



Antimicrobial resistance

- Recent evolution of antibiotic resistance in anaerobes



Boyanova 2015

Evidence of efficacy for Probiotics in dogs and cats?

- Sparse but better than for
Antibiotics!



Indications for probiotics.. ACUTE DIARRHEA



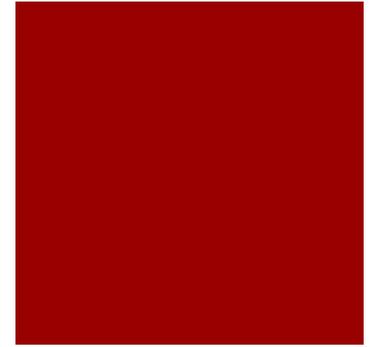
Published, placebo-controlled studies

- 6 trials show mild to moderate efficacy in acute diarrhea
- Chronic diarrhea: No evidence...

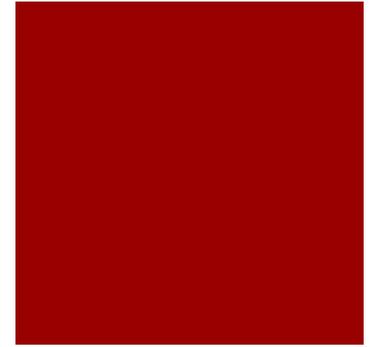
Fecal microbiome transplant: evidence

- Parvovirus infection
- AHDS and acute diarrhea
- Post weaning diarrhea
- Refractory Giardia
- FMT in 16 dogs with refractory IBD

- FMT in cats: not much...



Summary and Discussion: AB in dogs and cats with diarrhea



- We have a problem!
- Is it still indicated to treat these dogs with antimicrobials?
 - Do not respond long-term
 - Become dependent on AB
 - May cause long-term dysbiosis that cannot be reversed, leads to chronic enteropathy
- Rising AMR rates in Europe and USA..

Questions?

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