Allen D. Leman Swine Conference

Volume 39
2012

Published by: Veterinary Continuing Education

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Deciphering diarrhea: What's important

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Diarrhea, scours, and looseness are interchangeable terms we are familiar with in the pig industry. What's causing the diarrhea is sometimes not as easy to understand and can be downright messy (pun intended). The intent of this paper is to highlight the highly complex interactions of the pig digestive tract, but more importantly provide key factors (and some nuances) for diagnosis.

The pig's normal gastrointestinal flora, or microbiome, is extensive. It is estimated to be in the neighborhood of 10^{14} bacteria.[2] The microbiome has diverse functions, varies by intestinal location and age, but it ultimately important for the animals health and growth. The microbiome provides further break-down of feedstuffs (metabolic benefits), is active in water transport and provides immunity. This diverse population is also important in preventing or restricting the growth of harmful, pathogenic infections.[6] In short, pigs need a healthy microbiome. The problem is we are only starting to elucidate its significance and interrelationships as it pertains to pig production.

The reason for starting with the pig microbiome is to emphasize that primary causes of diarrhea are not always infectious; they can be related to alterations in normal physiology. For example, large amounts of fermentable ingesta delivered to the colon can cause osmotic diarrhea.[6] This example can result in microbiome population changes. Different protein sources, fiber, and probiotics also alter microbiome population; not always in a negative direction. Probiotics are beneficial and believed to act be a competitive exclusion method. Diarrhea is complex and often multifactorial, and we sometimes need to dive deeper to solve the primary reason the clinical entity. Non-infectious causes of diarrhea may disrupt the microbiome and subsequently result in infectious pathogens promoting enhanced or sustained diarrhea.

Understanding the mechanism of diarrhea is noteworthy. Inflammation, hypersecretory actions, malabsorption, increased intestinal permeability, and osmotic pull are pathophysiologic mechanisms resulting in diarrhea.[3,6] Malabsorptive diarrhea, due to enterocyte destruction, is the primary mechanism small intestinal viral infections or *Isospora suis*. In general terms, bacterial toxins are associated with hypersecretory diarrhea, and inflammatory diarrhea is more often cause by bacterial invasion. Osmotic diarrhea is correlated with diet (water quality and/or feed). These mechanisms are not exclusive to the above; multiple mechanisms are frequently occurring at the same time.

Determining a definitive diagnosis can therefore be difficult when investigating diarrhea. Infectious diarrhea is not all that problematic to determine. Culture, PCR, electron microscopic (EM), immunohistochemistry (IHC) and other tests are available to aid in detection or association with clinical signs. Albeit, correct samples from acutely affected and non-treated animals are submitted. Interventions for infectious diarrhea are relatively strait forward. However, is this actually the inciting cause (food for thought)? Non-infectious diarrhea may be implied by exclusion of infectious causes or noting specific correlation with water quality, stress, or feed delivery. The following are non-odiferous helpful hints in when diagnosing diarrhea.

Suckling diarrhea

- Infectious diarrhea is common, but can be associated with management issues.
- *Clostridium perfringens* type A and *Escherichia coli*
  - Normal inhabitants of the small intestine and colon.
  - Diarrhea associated with these bacteria can be primary when pure and heavy populations are cultured from the small intestine. *Isolation is not causal.*
  - Intestinal flora is not established, and a bacterial race to form niches/colonization is taking place. Inadequate sanitation, colostral intake, or stress (chilling) can be associated with bacterial overgrowth and diarrhea.
  - Culture small intestine and submit multiple sections of fixed small intestine are desired for histopathology.

- ▸ PCR genotyping is available for both bacteria; identification of fimbriae and/or toxin genes. PCR is done on isolated colonies; not direct from contents.
- ▸ Gross lesions are minimal; watery intestinal and colon contents; lacteals generally contain chyle; villi intact by subgross examination; pH alkaline

- Rotavirus and TGE
  - ▸ Thin-walled intestine because of villous atrophy; lacteals do not generally contain chyle. Colon can by distended and contain copious amounts of fluid or poorly digested/absorbed milk in early stages of infection; pH more likely to be acidic.
  - ▸ Feces, intestinal or colonic contents for PCR or EM.
  - ▸ Microscopic lesions are segmental; 4-6 sections are desired to confirm lesions. IHC is also available, but is less sensitive than PCR.
  - ▸ Rotavirus infection and endemic TGE can be clinically silent or have few microscopic lesions with PCR on feces the diagnostic method of choice in these cases.
  - ▸ All rotavirus serogroups (A, B, and C) are capable of cause severe diarrhea in neonatal piglets.
- *Clostridium difficile*
  - ▸ Normal inhabitant of the large intestine.
  - ▸ Disease is related to toxin production of certain strains.
  - ▸ Mesocolonic edema can be a gross lesion, but not all piglets will develop (~50%).
  - ▸ Feces or large intestinal content for toxin ELISA; the bacterium is difficult to growth and can take several weeks. Culture is not routinely performed.
  - ▸ Colon and cecum for histopathology; ulcerations and suppurative inflammation are consistent with disease.
  - ▸ Diagnosis based on clinical diarrhea with microscopic lesions or elevated toxin detection.
- Isospora suis
  - ▸ Diarrhea presents after 5-7 days of age
  - ▸ Pasty white diarrhea is typically noted.
  - ▸ Small intestine is affected; atrophic enteritis. Fibrinonecrotic (pseudomembranous) enteritis can be observed in severe cases.
  - ▸ Histopathology and fecal floatation can be used for diagnosis.

Points of emphasis in working-up and diagnosis suckling diarrhea are to collect both small and large intestinal contents, submit multiple sections of the entire gastrointestinal tract (both fresh and fixed), and not to over-interpret test results. Pathogen detection is not causal unless there is supporting ancillary information (microscopic lesions, heavy pure bacterial growth, etc.)

Post-weaning and grow-finish diarrhea

- Causes of diarrhea are more often multifactorial: infectious, diet related, stress and/or the possibility of water quality issues.
- Diet changes (solid food) post-weaning changes the microbiome, can be associated with inflammation, and changes the physiology of the gut. Often about 1 week is needed for the intestinal tract to adjust. The adjust period allows for pathogenic bacteria to sometimes establish colonization and be associated with diarrhea. Diets changes involving the addition or increased amounts of a particular feedstuff can also cause intestinal disturbances resulting transient diarrhea.
- *Escherichia coli*
  - ▸ Similar to the above. Heavy growth of a hemolytic colony from the small intestine is preferable for a diagnosis. Some non-hemolytic isolates also carry pathogenic genes. Fecal swabs can be used for culture, but the colonic population maybe different than what is present in the small intestine. Cautious interpretation of colonic *Escherichia coli* infection is warranted.
  - ▸ Isolate genotyping for toxin or fimbriae genes is not always straight-forward. The bacteria must first be isolated, pure isolation, and then colonies are "picked" for PCR testing. Re-streaking colonies and picking a colony for isolation is somewhat problematic. The isolate that is picked may not be the one causing disease. Multiple submissions are sometimes needed for a diagnosis.
  - ▸ Pigs up to 15-16 weeks of age are generally considered to be susceptible to associated disease.
  - ▸ Congested and hyperemic small intestinal sections are good gross indicators of disease. Bacterial adhesion to villous enterocytes can be seen microscopically, but not in all cases.

Deciphering diarrhea: What's important

- Salmonella sp.
  - *Salmonella choleraesuis* is generally a septicemic pathogen; diarrhea can occur during or after clinical signs of septicemia. *Salmonella choleraesuis* is in serogroup C1.
  - *Salmonella typhimurium* is associated with enterocolitis; cecum, colon, and sometimes the ileum. Fibrinonecrotic lesions, referred to as button ulcers, can be seen on the mucosal surface. Affected intestinal tract is usually thickened. *Salmonella typhimurium* is in serogroup B.
  - Other strains and serogroups need to be interpreted with caution. Infections with these isolates are generally transient, and most have not been experimentally reproducible. Diarrhea can occur with other strains, but they are generally not considered primary pathogens (transient infection)
  - *Brachyspira sp* and *Lawsonia intracellularis* infection can cause similar colonic gross lesions.
- *Lawsonia intracellularis*
  - Multiple forms/clinical presentations exist; chronic proliferative enteritis and acute hemorrhagic proliferative enteritis.
  - The bacterium is within enterocytes and is associated with proliferation of immature enterocytes.
  - Chronic proliferative enteritis is associated with varying degrees of diarrhea. Golden yellow diarrhea is often seen in the early stages. The ileum is thicken; cobblestone appearance of the mucosa. Cecum and spiral colon can also be affected. Fibrinonecrotic exudate can also be adhered to the mucosa.
  - Acute hemorrhagic proliferative enteritis is more common in older (late finishing and adult) pigs. Affected pigs often die suddenly, are pale, and have frank hemorrhage within the ileum, cecum and colon. A luminal ileal blood clot is a key gross finding.
  - PCR on feces, IHC, or special stains of affected sections of intestinal are used for diagnosis. PCR is more sensitive, and can pick up infection within the herd early than other tests. Isolation is possible, but only in research settings.
- *Brachyspira sp.*
  - *Brachyspira hyodysenteriae* is the classical cause of swine dysentery
  - *Brachyspira pilosicoli* is associated with a milder colitis and termed colonic spirochetosis.
  - There are multiple other species; some are considered weak pathogens and others commensals. New/different strains have recently emerged and can cause clinical signs and lesions consistent with swine dysentery.
  - Recent information suggested strongly hemolytic isolates cause swine dysentery.
  - Dysentery generally manifests 7 – 14 day after initial infection / may occur secondary to stress
  - Diarrhea with blood and mucus occurs as disease progresses.
  - Direct fecal PCR is not a particularly sensitive diagnostic test at this time. Colon, feces or mucosal scrapings culture is preferred. If strongly hemolytic isolates are isolated, PCR can be used for species identification. Weakly hemolytic isolates are not typically associated with clinical disease.
- Rotavirus and TGE
  - Similar to above; suckling diarrhea. PCR on feces or colon contents is preferred.
- Porcine circovirus type 2 (PCV2)
  - Enteritis/diarrhea can be a sole outcome of infection or in combination with systemic disease.
  - Associated changes can be seen within the small and large intestine. Mucosa can be thickened from inflammatory cell infiltrate and resemble *Lawsonia intracellularis* infection.
  - Immunosuppressive effects of viral infection can cause colonization or infection of other pathogenic organisms; some that normally not associated with diarrhea in a healthy pigs (eg. Fungal or protozoal).
  - Diagnosis is more than detecting by fecal PCR; most viremic animals will shed virus in feces. Histopathology in conjunction with IHC testing is preferred.
- Non-infectious diarrhea
  - Non-specific colitis is the term that is often coined when there an absence (lack of detection) of a pathogen with no distinct pathological changes.[5] Dysbacterosis is another term that is interchangeable and used by some diagnosticians.
  - Duration of diarrhea, immunity (vaccination) or treatment can mask infectious pathogens resulting in a non-specific colitis diagnosis.

- Increased non-starch polysaccharides (NSPs) in the diet are known to cause non-specific diarrhea. This results in elevating total fiber content within the colon, and ultimately changing the microbiome. Interestingly, feeding meal diets controls non-specific diarrhea better then pelleted feed.
- Water quality is thought to contribute to osmotic diarrhea. Total dissolved solids (TDS), including sulfates, are the main areas of interest. Sulfate salts have a laxative effect. There are different maximum recommended levels, depending on the source, for swine. Greater than 3000 mg/L TDS can cause temporary diarrhea, and >7,000 is not recommended.[1] Greater than 7000 ppm sulfates results in diarrhea; mainly in weaned pigs not adapted to water. Growth performance is generally not affected by water quality as measured by TDS and sulfates.[4]
- Toxic levels of some minerals and mycotoxins (ochratoxin and DON) can cause diarrhea.

What is important when deciphering diarrhea? Hopefully some of your questions/concerns have been answered above, but it is important to realize that there are a multitude of factors that can be associated with diarrhea. Neonatal diarrhea is often infectious, but can be linked to environmental issues and the developing/stabilization of the microbiome. Nursery-grow/finish diarrhea tends to be more problematic because the interaction of stress, diet, and infectious pathogens. In my experience, ruling out infectious pathogens is the first step followed by gathering a complete history of the situation with known changes that may have occurred. Careful interpretation is advised when accumulating diagnostic information as not to assign causality if there lack of supporting information. Non-infectious diarrhea is more complex as new information about the pig microbiome is discovered. As a final note, communicate with your diagnostic laboratory to aid in proper submission and understanding discoveries as you investigate causes of diarrhea.

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