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Piglet diarrhea: Diagnostic approach

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Diagnosing the cause of piglet diarrhea is challenging, not because it's difficult to identify a causative agent, but rather because there are so many to choose from. With the exception of TGE virus (in most herds), the usual assortment of infectious enteric agents is present on nearly all farms and they are capable of causing enteric disease in baby pigs under the right circumstances.

A pathogram, or inventory of the enteric pathogens resident in baby pigs reveals the assortment of pathogens involved in piglet diarrhea. In a well-done prospective, case-control study of piglet diarrhea reported by Yeager and others[1], 10 pathogens were detected at varying rates among the pigs examined. Interestingly, the highest rate of detection was for a toxin from *Clostridium difficile* at a 42% level among case pigs. Even more interesting was the finding that the level was nearly two times higher in the control pigs (Table 1). This clearly illustrates the point that agent does not always equal disease. Further, the data show that mixed infections involving multiple pathogens are common.

Lingering effects of enteric disease

Part of the story of neonatal diarrhea is the impact the disease process has on piglet survival and weaning weights. These performance effects are relatively straightforward. Given the recent renewed interest in postweaning or periweaning performance issues, it's worth considering another impact of enteric disease – reduced ability to absorb nutrients and increased susceptibility to enteric and other pathogens postweaning. McOrst and Mellits[2] recently editorialized on this, so further discussion will not be included here other than to point out that we are likely underestimating the biologic and financial impact of piglet enteric disease if we don't consider the additional effects of impaired gut architecture and function in weaned pigs.

How to succeed as a pathogen

Getting to the bottom of the causes of piglet diarrhea requires an understanding of the pathogenesis and behavior

Table 1: Survey of C difficile toxins (TCd) and pathogens identified in case and control pigs.

Agent	Control pigs (n = 29)	Case pigs (n = 100)	Totals (n = 129)
TCd	23	42*	65
C perfringens type A	0	21†	21
Rotavirus	0	14‡	14
PRRSV	0	9	9
TGE	0	3	3
E coli	0	5	5
E durans	3 §	0	3
C perfringens type C	0	6 ¶	6
A suis sept	0	1	1
Coccidia	0	1	1
No pathogens identified	6	5	11

* 1 TCd-positive pig also had peritonitis and another had a strangulate hernia.

† 9 pigs were positive for both TCd and *C perfringens* type A.

‡ 4 pigs positive for rota and TCd.

§ 3 pigs positive for both *E durans* and TCd.

¶ 3 pigs positive for both *C perfingens* type C and PRRSV and 1 pig positive for *C perfringens* and TCd.

Yaeger et al. 2007

of the various infectious agents with respect to piglet and environmental conditions. "Know your enemy" is often quoted as a guiding rule from Sun Tzu's ancient military treatise "The Art of War." Another less cited quote is "All warfare is based on deception." Both have applications in our understanding of how enteric pathogens are able to cause disease.

An introduction to a recently published book[3] provides a helpful outline of steps that a pathogen must follow in order to cause disease. At first reading these steps may seem simplistic, but a review of these steps provides a framework for diagnostic and prevention strategies for these diseases. The steps for succeeding as a pathogen were listed as follows:

1. Enter and/or attach to body
2. Evade host defenses
3. Multiply
4. Damage the host
5. Transmit to the next body

A brief discussion on a few examples may help illustrate the point. For example, the first step – to enter and attach to the gut – involves varying degrees of specificity by species and age. TGE virus is quite species specific to pigs. In contrast, *Salmonella sp.* can readily cross multiple species barriers. In terms of age specificity, TGE virus can infect and cause disease in pigs of all ages. In contrast, *Clostridium perfringens* is susceptible to enteric enzymes that develop with age, so only young pigs are typically affected by this bacteria. Conversely, the receptors in the pig gut for F18 *E coli* don't develop until around weaning age, so young pigs are not susceptible. Additionally, the receptor for the F18 fimbria has been bred out of some lines of pigs, so that even in older pigs, the *E coli* can't adhere to the gut and cause disease. Likewise for other *E. coli* fimbrial receptors.

There are plenty of examples of the second step – evading host defenses – as well. Some pathogens use genetic variation as a form of camouflage to evade host defenses. These would include rotavirus, PRRS virus and *Salmonella spp.* Some also have means of modulating the host immune response, as is the case with PRRS virus and porcine type 2 circovirus. Some pathogens also have the ability to hide from the host immune system, as with porcine type 2 circovirus and *Salmonella spp.*

The third step – to multiply – tells the tale of the numbers game. Some pathogens are able to reproduce at very high rates, virtually guaranteeing exposure at some point and at some level. Rotavirus is a good example of this incredible capacity to multiply. In the first place, rotavirus multiplies rapidly within the cells that are first infected (mature enterocytes), so the virus capitalizes on the previous point of evading host defenses. Then the virus multiplies at very high levels, with as many as 100 billion virus particles present per gram of feces, and with 30% of patients shedding for up to 57 days based on human studies[4]. To put that in perspective, a nickel weighs five grams. A nickel's worth of feces could contain half a trillion rotavirus particles and the theoretical infectious dose for a pig is one virion[5] – daunting math to say the least.

The fourth step for pathogenesis is actually causing pathology. Most bacteria in the gut are important for digestion and other functions and do not cause disease. A few can cause disease locally or systemically. In the case of *E coli* that are able to adhere to the gut, the bacteria must have toxin genes in order to produce toxins that cause disease. A list of toxins is also included in the table (Table 2). Viruses and parasites also have mechanisms for damaging the host to their advantage.

The fifth step is transmission to the next host. With fecal-oral transmission as the primary mechanism this doesn't need further explanation.

Diagnostic approach

A few basic guidelines for investigating enteric disease in piglets are helpful in light of the function of the gut and the pathogenesis of enteric disease:

1. **Autolysis** - The function of the gastrointestinal tract is to digest, so the gut undergoes rapid postmortem autolysis relative to other tissues once blood circulation stops. Structural integrity is lost quickly, limiting histologic interpretation of lesions, and bacterial populations can be altered by overgrowth of cadaver bacteria. Autolysis begins within 15-20 minutes of death. If the primary diagnostic concern is enteric disease, it is important to collect enteric tissue samples first and immediately place appropriately sized samples intended for histology in buffered formalin. Despite broad application of sophisticated diagnostic tests, histopathology remains one of the most important diagnostic tools for evaluating piglet diarrhea. Every effort to provide high quality samples for this piece of the diagnostic effort is beneficial. Rapid chilling of unfixed tissues reduces the likelihood of significant cadaver bacteria overgrowth. Attention to the separation of the unfixed tissues is also important to avoid contamination.
2. **Segmental infections** – several agents primarily affect certain portions of the GI tract. For example, in pigs, *Clostridium difficile* almost exclusively affects the cecum and proximal colon[6]. If these tissues are not included in the submission, detection of the toxin for *C difficile* is not possible. Rotavirus can infect variable portions of the small intestine, starting proximally and progressing distally. If only one or two sections of the 10 feet of jejunum from a baby pig are sampled, it is possible to miss the section(s) with the characteristic

Table 2: *E coli* virulence genes included in the testing panel at the University of Minnesota Veterinary Diagnostic Laboratory.

Escherichia coli virulence factor genes		Description of virulence gene	Clinical effect*
Fimbrial	F4 (K88)	Adhesion to intestinal mucosa	2
	F5 (K99)		1
	F6 (987P)		1
	F18		2
	F41		1
Adhesin	Paa	Porcine attaching and effacing-associated factor	2
	Eae	Attaching and effacing factor	2
	AIDA-I	Adhesin involved in diffuse adherence	2
Toxin	LT	Heat labile toxin (secretory diarrhea)	1
	STa	Heat stable toxin (secretory diarrhea)	1
	STb	Heat stable toxin (secretory diarrhea)	1
	EAST1	Enteroaggregative heat-stable toxin 1	2
	STx2e	Shiga toxin (edema disease principle)	3

* Diseases of Swine 9th edition; Vet Microbiol 2007;123:145-52.

1 = Neonatal diarrhea; 2 = Postweaning diarrhea; 3 = Edema disease

lesions. Taking six to eight samples at different locations reduces the chances of missing affected sections. More broadly, including samples from all portions of the GI tract allows for more complete testing for pathogens and evaluation for lesions.

3. **Multiple infections** – infections with multiple enteric agents are common, so defining the initiating or root cause of disease can be challenging, especially after pigs have been treated. The old mantra of sampling acutely affected, untreated pigs is especially true for piglet diarrhea cases which can progress rapidly once the gut integrity is disrupted by disease and after treatment has been initiated.
4. **Location-specific infections** – just as weaned pigs coming from different sow farms can be infected with different pathogens, pigs from different farrowing rooms and even farrowing crates can have different agents involved in causing disease. Carefully assessing what clinical scenario is in need of investigation and selecting multiple pigs that are representative of that scenario for diagnostic evaluation will provide a more complete picture of significant disease agents and processes present.

There are instances where the single diagnostic objective is to determine presence or absence of a particular disease agent such as TGE. In these instances there may not be the need for an extensive submission of multiple tissues from multiple pigs. However, if a thorough characterization of a complex enteric disease condition is needed, more comprehensive sampling is required.

Sampling protocol

General guidelines for tissue submission for piglet diarrhea cases are detailed in table 3. For most investigations, sampling 3 euthanized pigs with typical signs that are acutely affected and untreated (if available) OR 3 freshly dead pigs will be sufficient. If clinical signs are present in pigs of different ages, submitting a sample set separately for each age group is recommended.

Individual diagnostic laboratories may have different specific preferences for sampling and submissions, so consult with your diagnostic laboratory personnel to understand potential differences. The preference at the University of Minnesota Veterinary Diagnostic Laboratory is to have tissues labeled according to individual pigs rather than submitting pooled tissues from multiple pigs.

A complete history, including the age of pigs, clinical course, treatment and vaccine history and other relevant information is also important to include at the time of submission. This information helps guide the diagnostic investigation and is also helpful for later review of case summaries.

Table 3: Piglet diarrhea sampling protocol.

Tissue/sample	Unfixed† (chilled – not frozen)	Fixed (10% buffered formalin)
Serum	5 ml	
Brain	Cut brain in half longitudinally, slightly off the midline. Submit smaller half fresh/chilled.	Fix the larger half in formalin.
Tonsil	Half of the tonsil	Half of the tonsil
Lung	5 × 5 × 5 cm piece	2 × 2 × 1 cm
Liver	5 × 5 × 5 cm piece	2 × 2 × 0.5 cm
Kidney	Half of a kidney	0.5 cm slice, including cortex and medulla.
Spleen	5 cm section	1 cm section
Stomach*	2 × 3 cm section of pylorus	2 × 3 cm section of pylorus
Jejunum*	6 sections, 5 cm long	6 sections, 2 cm long, unopened
Ileum*	1 section, 10 cm long	2 sections, 2 cm long, unopened
Mesenteric lymph node	Entire lymph node	1 cm section
Spiral Colon*	Approximately ¼ to ½ of colon	2 sections, 2 cm long, unopened
Fluid contents from cecum or colon	In leakproof container	

† Submit unfixed tissues separately as indicated by line separations.

* Fix samples within 15 minutes of death if possible.

Modified from Gramer et al, 2005[9]

Diagnostic trends

Diagnostic trends for piglet diarrhea will be presented. Two figures follow that represent distributions of enteric pathogens for recent cases submitted to the University of Minnesota Veterinary Diagnostic Laboratory. Figure 1 illustrates rotavirus activity by pig age and rotavirus group (Group A, B or C) for cases in 2009 and 2010[10]. Figure 2 illustrates the distribution of toxin genes in *E coli* isolates cultured from clinical cases from 2006 to 2011[11].

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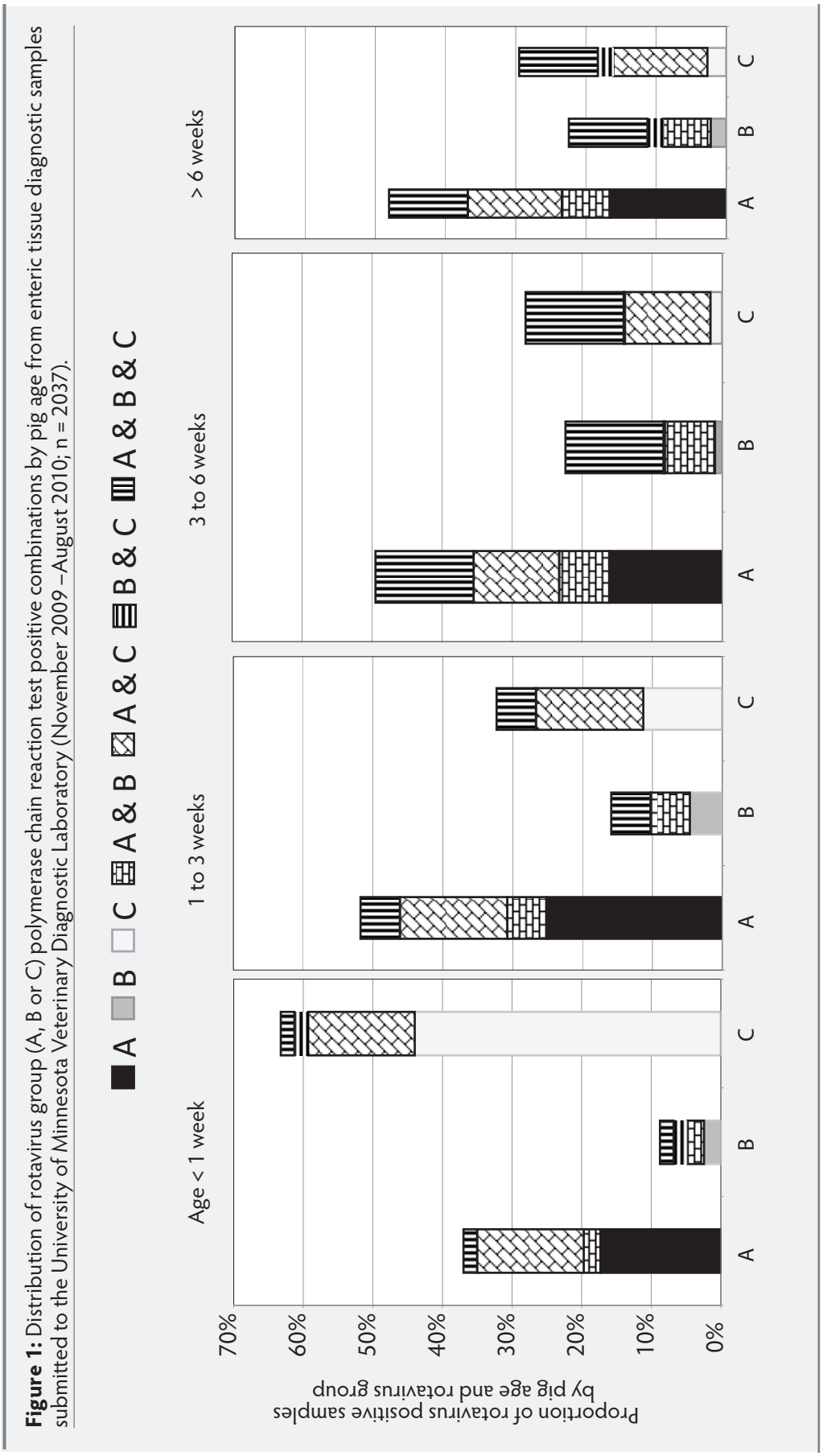

Figure 1: Distribution of rotavirus group (A, B or C) polymerase chain reaction test positive combinations by pig age from enteric tissue diagnostic samples submitted to the University of Minnesota Veterinary Diagnostic Laboratory (November 2009 –August 2010; n = 2037).

Figure 2: Distribution of virulence genes by age among β-hemolytic *E coli* isolates cultured from swine diagnostic cases submitted to the University of Minnesota Veterinary Diagnostic Laboratory (2006 – 2011; n = 2911).