Pitfalls of laboratory diagnosis of piglet enteritis

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Baby pig scours is still a major cause of morbidity and mortality in the farrowing house. Fortunately, an etiologic diagnosis can be achieved in a high percentage of these cases through your diagnostic laboratory. In fact, the diagnostic success rate for baby pig enteritis workups is probably the highest of all the syndromes that the diagnostic lab has to deal with. In contrast, the diagnostic success rate for reproductive failure is probably the lowest of all the syndromes the diagnostic lab handles. Why are diagnostic labs so successful in determining the pathogenic cause of piglet enteritis? There are several reasons:

- the vast majority of piglet enteritis cases are caused by a handful of infectious agents and most diagnostic labs are well prepared to routinely test for and identify these agents;
- it is economically practical for the swine producer to allow the veterinarian to sacrifice one to three piglets for test purposes, enabling the veterinarian to select optimal animals for testing and to collect optimal tissue samples; and
- since baby pig scours is so common, the practitioner and the lab have considerable and on-going experience in handling these types of cases and as everyone knows, "practice makes perfect."

For these reasons, I would estimate that under the conditions of optimal animal and tissue selection, the laboratory diagnostic success rate for baby pig enteritis can be ≥ 80%. In fact, some of my practitioner clients are so adept at selecting the right pigs and collecting the right tissues that they rarely get a report back saying, "sorry, no diagnosis from this submission." This article will review the laboratory submission basics for baby pig scours to help you avoid some common diagnostic pitfalls and improve your diagnostic success.

Animal selection

Selecting the right animal for testing is critical, since everything done subsequently can be fruitless if the test animals are not properly selected. Using only dead animals for test purposes will dramatically decrease your diagnostic success rate for the vast majority of enteritis problems. I encourage practitioners to open two or three of the most recently dead piglets available to them for gross observation, but only collect tissues if the piglets have died within the last 3 hours and were kept in a cool place. Failing to open any dead pigs may cause you to miss an easy diagnosis of acute *Clostridium perfringens* type C. Many cases of fatal acute type C infection will show extensive necrosis of the small intestine with intense serosal hemorrhages and often subserosal gas bubbles. However, most of the time, the only gross change in dead scouring piglets is nonspecific loose-to-watery intestinal contents.

The optimal animal to select for a diagnostic workup is a live, untreated piglet with acute clinical diarrhea (diarrhea less than 72 hours). Selecting two or three piglets instead of one will increase the likelihood of selecting an animal with significant active disease. Selecting an acute case is important so that we can determine the initiating pathogenic cause of scours and not merely identify secondary infections. Chronic cases (piglets scouring for more than a week) seldom yield helpful information. Picking acute cases is important, but avoid being overzealous in this regard because sometimes during an effort to find acute cases, clinically normal animals are selected and submitted by mistake.

Specimen collection

Once the piglet has been selected, killed, and the carcass opened, specimen collection is the next important task. Table 1 contains a list of recommended specimens to collect. Most lesions with piglet enteritis occur in the ileum and lower jejunum. I prefer to start my collection procedure by locating the cecum and the ileum running along side it. Next, I sever the ileum at its union with the colon, and then collect ileum and proceed proximally. This habit ensures that I have collected the most important tissues first (and prevents forgetting where I was and then leaving out the ileum). Because of their small size, it's often tempting to remove the entire piglet GI tract, snip out a few pieces of gut for the formalin jar, and then place the entire remaining tract in a bag and send it to the lab. However, entire GI tracts decompose much more quickly in transit than selected loops of intestine. Loops of intestine about one inch long are adequate.

If short loops of intestine are collected from small animals such as baby pigs, it is not necessary to open or "run" the intestine with your scissors. Most pathologists prefer to look at complete loops of intestine under the microscope for optimal evaluation of

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intestinal villi. Furthermore, in small animals the scissors can create considerable mechanical artifact and hinder pathological interpretation. If you want to visually examine the mucosal surface, do so on portions of the gut that you do not intend to submit to the lab.

Be gentle with the tissues and do not tie off the ends of fresh or fixed intestinal loops. If the loops of intestine are short enough, formalin will enter the lumen from both ends and adequately fix the tissue. If the intestinal content does not readily run out of your loop (most often it will), then formalin can be gently flushed through the lumen to remove the contents prior to placing the tissue in formalin. Do not flush the fresh loops.

Always submit formalin-fixed tissues as well as fresh tissues. Diagnostic histological lesions are common with baby pig enteritis and may be the only means of achieving a definitive diagnosis if the fresh tissues decompose in transit. There is a synergistic relationship between histopathology and microbiology during combined diagnostic efforts. Lesion evaluation is crucial for interpreting the significance of potential pathogens identified by microbiological procedures. Furthermore, histopathology can sort out what the most important or only problem is if more than one potential pathogen is identified.

Specimen protection

Equally important to the entire diagnostic procedure, the specimens must be properly packaged for their trip to the diagnostic lab. Doing everything right up to this point will be worthless if the specimens rot or are physically damaged in transit. Most decomposition during transit is not due to slow delivery by carriers, but is due to improper packaging procedures. A properly packaged case should be able to withstand a 48-hour trip and still arrive at the lab cold. Whirltop bags are ideal for both fresh and formalinized tissues and must be whirled to form a leakproof seal. Double bag all fluids, such as formalin and gut contents. Whenever possible, chill your fresh tissues in the refrigerator prior to packing the shipping carton. Send the minimal amount of fresh tissue needed. Sending extra tissue enhances bacterial proliferation and decomposition and takes up extra space in the carton. Use only styrofoam shipping cartons, preferably with an outer cardboard lining. The box must be large enough to hold all of the following: your specimen bags; crumpled newspaper for padding and extra insulation; and enough ice packs to keep things cool for at least 48 hours.

To pack a typical box: place two to four ice packs in the bottom of the box; then a layer of crumpled newspaper over the ice packs; next place your bagged specimens; and finally fill all the remaining space with more crumpled newspaper. Do not place your specimens directly against the ice packs or they will freeze and/or become squashed.

Specimens that arrive at the lab decomposed or traumatized lower the diagnostic success rate considerably by hindering:

- pathological interpretation (especially if not fixed);
- the fluorescent antibody test procedures; and
- bacterial and viral cultures.

Closely following the above procedures should result in an etiologic diagnosis a high percentage of the time. Hopefully, this article will aid your diagnostic efforts and help you avoid some common diagnostic pitfalls of piglet enteritis.

Table 1

Recommended specimens for laboratory diagnosis of neonatal swine enteritis

1. Fresh tissue* (chilled, not frozen)
   - Two loops ileum
   - Two loops jejunum
   - One loop duodenum
   - One loop spiral colon
2. Formalinized tissue* (in 10% neutral, buffered formalin)
   - Two loops ileum
   - Two loops jejunum
   - One loop duodenum
   - One loop spiral colon
3. Intestinal contents (chilled)
   - 5 to 10 mL of lower small intestinal or colonic contents

* Not ligated, not opened, approximately 1 inch long intestinal loops.