Update on epidemiology and diagnosis of porcine proliferative enteropathy

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Proliferative enteropathy (PE) is an enteric disease that occurs in pigs[1] and a number of other species.[2] The etiologic agent of PE in swine is the obligatory intracellular bacterium *Lawsonia intracellularis.*[3–6] The disease in pigs, commonly referred to as ileitis, occurs in two major clinical forms. Acute hemorrhagic diarrhea and sudden death of replacement animals and finishing pigs close to market age is known as proliferative hemorrhagic enteropathy (PHE); and chronic, mild diarrhea with poor growth performance in grower-finisher pigs (Figure 1) is known as porcine intestinal adenomatosis (PIA).[1,7]

Proliferative enteropathy is widespread among swine herds (30 to 50% are infected) in different types of production systems and in all parts of the world.[8–13] It was the most common disease problem in grower-finisher pigs reported in the 2000 National Animal Health Monitoring System survey, occurring on more than a third of all sites and reported on 75% of large sites (10,000 or more total inventory).[14] Serologic studies have shown that the prevalence of PE-positive herds ranges from 60 to 90% in different countries.[7,15–19] The economic impact of PE on the swine industry is estimated to be very high. It was estimated to cost the industry US$20 per sow annually in Australia,[20] and US$20 million annually in the United States.[21]

The main impact of the disease has been due to increased use of antibiotics and mortality related to the acute form of the disease (PHE). The chronic form (PIA) is seldom detected and diagnosed. In this article, we will discuss some aspects of the epidemiology of the disease, importance of the subclinical-chronic form, and diagnosis of PE.

Figure 1: A group of uneven pigs, 3 weeks after intragastric inoculation with an intestinal mucosal homogenate harvested from pigs with proliferative enteropathy. The chronic form of proliferative enteropathy was confirmed in these pigs by identification of gross and histologic lesions.

Transmission

Feces from infected pigs are the main source of new infections in susceptible animals.[1] A serologic study performed in 184

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This article is available online at http://www.aasv.org/shap.html.

Guedes R. Update on epidemiology and diagnosis of porcine proliferative enteropathy. *J Swine Health Prod.* 2004;12(3):134-138.

herds showed that seropositivity in the breeding herd is an important risk factor for new PIA cases in grower-finisher pigs.[22] In addition, seropositivity in grower-finisher animals was a risk factor for PHE in replacement animals.[22] In a study of the prevalence of *L intracellularis* detected in fecal samples by polymerase chain reaction (PCR) in an endemically infected herd in Europe, the highest proportion of positives occurred 10 to 24 days after weaning.[23] Only 12.9% of the grower-finisher animals and 0.9% of the mature animals were positive. In this study, the possible epidemiological importance of transmission from breeding sows to young suckling piglets was proposed. Fecal shedding has been reported in pigs as young as 3 weeks of age[24] and in pigs 25 and 42 days of age.[25] However, despite evidence suggestive of sow-to-piglet transmission, this has yet to be studied and proven.

On the other hand, pig-to-pig contact is an important route of transmission. Rowland and Rowntree[26] found an association between a PHE outbreak in young breeding stock and the onset of chronic diarrhea in in-contact weaned pigs a few weeks later. *Lawsonia intracellularis* infection was transmitted between breeding stock and young adult pigs in a natural PHE outbreak, where movement of sows and boars between units was permitted.[27] In an experimental trial, sentinel pigs became infected when housed in contact with pigs experimentally inoculated with a pure culture of *L intracellularis*.[28]

Results from a questionnaire survey among British farm owners indicated that slatted and meshed flooring were important risk factors for PE. They suggested that such floors, commonly found in postweaning facilities, are often insufficiently cleaned.[29] However, the findings in this report were based on the owners' opinions, and no diagnostic testing was performed to support the results of the survey. Another study, which included a questionnaire survey, production records, and fecal PCR analysis, reported that the use of new buildings and recent mixing of pigs were associated with PE.[30] These findings support a hypothesis that subclinically infected pigs shed *L intracellularis* in the feces, particularly after stress.

Due to the difficulty in isolating, culturing, and maintaining *L intracellularis* in vitro, the isolation or re-isolation of viable organisms from diseased intestines is very difficult. Therefore, information about survival and resistance of *L intracellularis* in the environment is scarce. A unique investigation into this area[31] found that intestinal colonization of pigs by *L intracellularis* was detected after they had been orally inoculated with feces from positive animals. Infected feces had been stored for up to 2 weeks at temperatures between 5 and 15°C. In this same study, pure cultures of *L intracellularis* were fully susceptible to a quaternary ammonium disinfectant (3% cetrimide), less so to 1% povidone-iodine, but not susceptible to 1% potassium peroxymonosulfate or a 0.33% phenolic mixture.

Other possible mechanisms of transmission that need to be considered in future studies are transmission by mechanical vectors (eg, rubber boots) and biological vectors (eg, mice, small birds, and insects). As a broad range of animal species may be affected by PE,[32] interspecies transmission is a real possibility. Proliferative enteropathy was reproduced in hamsters[33] and mice[34] using homogenized mucosa from PE-affected pigs, and in mice using pure *L intracellularis* culture extracted from pigs.[35] Recently, a natural outbreak of PE was reported in a colony of conventional mice in a University of Missouri research unit.[36] Natural PE cases have been reported in ratite birds (eg, emu[37] and ostrich[32,38,39]), but there are no similar reports concerning other bird species.

It seems reasonable to conclude that pig-to-pig contact is probably the main mechanism of transmission, with subclinically infected animals being key elements of this transmission. Future efforts are necessary to obtain further information about sow-to-piglet transmission, resistance of the organism in the environment, and possible mechanical and biological vectors, which will help to explain the high prevalence of the disease among herds worldwide.

Importance of subclinical proliferative enteropathy

In a field study[40] and in a controlled experiment,[41] intermittent fecal shedding of *L intracellularis,* as assessed by PCR, has been detected for a period of up to 12 weeks. In the field study, no clinical disease was observed among either PCR-negative or PCR-positive pigs. In the experimental study,[41] diarrhea occurred in challenged pigs only from the second week to the fifth week postinoculation. Growth performance was not evaluated in these studies.

In a recent study,[42] clinical, morphological, and microbial findings in animals from good and poor performance herds were compared. The authors concluded that clinically healthy animals from infected herds were often infected with *L intracellularis* (detected by PCR in fecal samples), and that growth performance in these animals was poor compared to that in uninfected herds (Table 1). Three pigs with diarrhea (case pigs) and three pigs with no signs of clinical disease (control pigs) were selected from each of nine poor performance herds and compared to three control pigs from each of four good performance herds, with pigs matched by age in each case. The average age at which pigs reached a body weight of 25 kg was 85.6 ± 3.6 days in poor performance herds, and 64.7 ± 2.6 days in good performance herds. In most case pigs, gross and microscopic lesions were identified that were consistent with PE, colonic spirochaetosis, or both. The most frequently diagnosed enteropathogenic agent was *L intracellularis,* followed by *Brachyspira pilosicoli*. An interesting result from this study was the high percentage of control pigs from poor performance herds that were PCR-positive for *L intracellularis* (41%).

These studies show that growth performance of pigs subclinically infected with a pathogenic isolate of *L intracellularis* is poor, and that they shed the organism into the environment, resulting in infection of susceptible penmates. It appears that subclinical infection with *L intracellularis* may result in poor growth performance, unthriftiness, and lost homogeneity in a batch of grower-finisher pigs. These problems have been associated with *Mycoplasma hyopneumoniae* or circovirus infection, but now an additional possible culprit, *L intracellularis,* should be considered.

Diagnosis

Diagnostic tools for detecting exposure or infection to *L intracellularis* (eg, serology, fecal PCR, and immunohistochemistry in tissue samples) have become more available in the last few years. Each diagnostic method evaluates a different epidemiological aspect of PE. Serology, for instance,

Table 1: Comparison of clinical, morphological, and microbial findings in animals from good and poor performance herds infected with *Lawsonia intracellularis**

Parameter	No. of pigs in poor performance herds (%)		No. of pigs in good performance herds (%)
	Case pigs (n=27)	Control pigs (n=27)	Control pigs (n=12)
Clinical signs	27 (100)	0 (0)	0 (0)
Fluid gut content	21 (78)	5 (19)	0 (0)
Gross lesions	21 (78)	5 (19)	0 (0)
Microscopic lesions	22 (81)	17 (63)	0 (0)
L intracellularis†	18 (67)	11 (41)	0 (0)
Brachyspira pilosicoli†	13 (48)	7 (26)	0 (0)
Escherichia coli†	8 (30)	6 (22)	1 (8)

* Case pigs had diarrhea, control pigs did not. Table adapted from Jacobson et al, 2003.[42]

† Agents identified.

provides historical information on exposure to the bacteria, while fecal PCR and immunohistochemistry are measures of current infection.

Lawsonia intracellularis is an obligate intracellular organism that infects intestinal epithelial cells. Therefore serum, IgG is not likely to be protective against infection, while secretory IgA and cell-mediated immune responses may play more important roles.[41] Nevertheless, detection of serum IgG is a useful tool to evaluate exposure to *L intracellularis.* Optimization and validation studies of serologic tests for PE have been carried out in recent years, creating new opportunities for a better understanding of the immune response induced by *L intracellularis* infection.[43–45] Indirect immunofluorescent antibody (IFA)[44,46] and immunoperoxidase monolayer assay (IPMA)[45] are serologic tests that detect *L intracellularis*-specific serum IgG. Each has a sensitivity of approximately 90% and a specificity of approximately 100%, determined in controlled experimental infection studies. No cross-reactivity was observed when these serologic tests were used on convalescent sera from pigs infected with several *Campylobacter* species, *Salmonella* serovar choleraesuis, *Salmonella* serovar typhimurium, *Escherichia coli* K88, *Brachyspira hyodysenteriae*, *B pilosicoli*, or porcine reproductive and respiratory syndrome virus.[45] Serum IgG is first detected in the second week postinfection and persists for 3 to 13 weeks, depending on the form of the disease (PHE or PIA) and its severity.[40] Serum IgG was detectable for up to 12 weeks in gilts after a natural outbreak of the acute form of PE, and in 5-week-old pigs infected with high doses of pathogenic *L intracellularis*. Conversely, seropositivity in grower-finisher pigs in field conditions usually persisted for only 2 to 3 weeks and was detected mainly in 18- to 26-week-old pigs.[40] However, age at seroconversion in grower-finisher pigs may vary depending on the feed medication program, pig flow, and type of flooring.[30] Although we were unable to demonstrate a statistically significant association between severity of gross lesions and serum titers in pigs 3 weeks after experimental infection,[43] we believe that the level of infection correlates with serum titers. Serum IgG titer decays gradually after reaching its peak; therefore, the higher the peak serum titer, the longer detectable serum IgG persists.

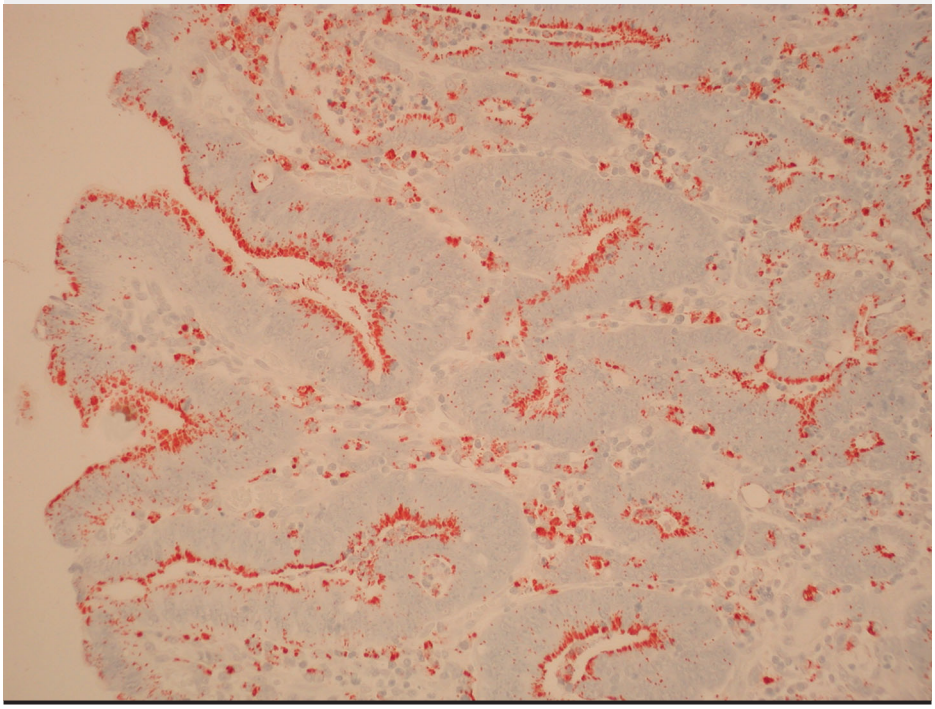
Although the specificity of PCR for detection of *L intracellularis* DNA in fecal samples is virtually 100%,[47] the sensitivity of the technique ranges between 39 and 72% in experimentally infected pigs.[43,46] In field and controlled experimental studies, animals became positive by fecal PCR 1 to 2 weeks before they seroconverted.[41,43] When animals are PCR-positive and seronegative, either they are in the early stage of infection and have not yet had time to seroconvert, or the level of infection is not sufficient to induce a systemic humoral immune response detectable by the serologic test. When animals are PCR-negative and seropositive, either they have been previously exposed to *L intracellularis* and are no longer shedding the organism, or detection of fecal shedding was limited by the low sensitivity of the PCR technique in fecal samples. Nonetheless, stage of infection with *L intracellularis*, based on the percentage of seropositive pigs or the percentage of PCR-positive pigs (representing fecal shedding), or both, and observation of a clinical problem represented by diarrhea or poor growth performance, must be evaluated as a whole.

Immunohistochemistry in histologic sections of ileum, using antibodies specific for *L intracellularis* antigens,[48,49] has a sensitivity of 87%, compared to histological examination using hematoxylin and eosin staining (sensitivity 37%) and or Warthin-Starry silver stain (sensitivity 50%).[43] *Lawsonia intracellularis* can be detected by immunohistochemistry in just a few intestinal crypts early in infection and in the cytoplasm of macrophages in the lamina propria late in the course of the disease (Figure 2). Those two stages of the disease cannot be differentiated in sections stained with hematoxylin and eosin or Warthin-Starry silver stains.

The PE status of a herd should be evaluated in two common field situations.[50] The first one is observation of poor performance, diarrhea, or both in a group of grower-finisher pigs. It is recommended that two or three gaunt pigs with diarrhea be selected from the most severely affected pens for euthanasia, necropsy, and submission of samples of large and small intestine to a veterinary diagnostic laboratory. In addition to the standard bacteriologic and histologic tests, specifically request immunohistochemistry for *L intracellularis* and bacteriology for *Brachyspira* (species identification). Collect fecal samples (at least a pea-sized amount of feces) from 20 pigs with loose stools and submit refrigerated samples for PCR testing. Although pooling of fecal samples from two to three animals is acceptable to reduce costs of testing, this does inherently reduce the sensitivity of the test.

The second field situation is the necessity for knowing the probable time of infection in order to determine the optimum time for strategic medication of grower-finisher

Figure 2: Immunohistochemical staining of ileal sections from a pig experimentally infected with *Lawsonia intracellularis*, using a polyclonal antibody specific against *L intracellularis* antigens. Note the antigen labeling in the cytoplasm of mononuclear cells in the lamina propria. 200 × magnification.



pigs with growth performance problems, which might be related to *L intracellularis* infection. Serological testing is recommended in this situation, as it costs less than PCR testing, is suitable for testing large numbers of samples, and provides an estimate of time of exposure. Serum samples from several groups of at least 20 grower-finisher pigs with 3-week age differences should be submitted for serologic testing. Strategic medication, vaccination, or both, are usually recommended 2 to 3 weeks before the age of seroconversion.

Further research focusing on the epidemiology of *L intracellularis* infection and transmission is imperative in order to design eradication protocols for PE. The presence of subclinically-chronically infected pigs seems to be the major factor contributing to the economic impact of poor growth performance and dissemination of the disease in the herd. Careful interpretation of appropriate diagnostic tests, which may be used to determine the time for treatment or vaccination to be most effective, will help to minimize losses due to PE.

Acknowledgment

I thank Dr Connie Gebhart for the critical review of the manuscript.

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