Subclinical ileitis: Diagnostic and performance parameters in a multi-dose mucosal homogenate challenge model

Marie-Anne Paradis, DVM; Connie J. Gebhart, MSc, PhD; Denise Toole, BSc Agr; Gordon Vessie, Diplomate APM; Nathan L. Winkelman, DVM; Sharon A. Bauer, MSc; Jeff B. Wilson, DVM, DVSc, PhD; Carol A. McClure, DVM, MSc, PhD

Summary
A dose titration study was conducted using a *Lawsonia intracellularis* mucosal homogenate model in weaned pigs. Significant negative effects on performance were observed in the absence of positive diagnostic indicators or clinical signs. The results obtained showed that subclinical infection can have a detrimental economic impact on swine herds.

Received: October 27, 2010
Accepted: September 19, 2011

Porcine proliferative enteropathy (PPE or ileitis) caused by *Lawsonia intracellularis*, an obligate intracellular bacterium, primarily affects the mucosa of the distal ileum. Ileitis has a worldwide distribution, affecting 57% to 100% of herds. Overall, prevalence is underestimated, as some mild or subclinically affected farms go undetected and undiagnosed. Mucosal crypt cells in the ileum, jejunum, and sometimes large intestine. This chronic condition can result in diarrhea, decreased feed intake, and a slower rate of weight gain. Acute infection occurs primarily in mature pigs (>4 months old) causing proliferative hemorrhagic enteropathy (PHE), which is also characterized by proliferation of the crypt cells. Clinical consequences of PHE include weakness, lethargy, anorexia with bloody diarrhea from intestinal hemorrhage, and sudden death. In addition to the prominent clinical manifestations of ileitis in swine, a subclinical form of the disease has more recently been recognized and identified both in natural and challenge infection studies. Subclinical disease caused by *L intracellularis* infection remains incompletely characterized, but results in enterocyte hyperplasia and reduced performance with few or no clinical signs. The objective of this study was to further define subclinical ileitis by measuring the impact of varying doses of *L intracellularis* on clinical signs, *L intracellularis* shedding and seropositivity, performance, and gross and histopathological intestinal changes in weaned pigs.

Materials and methods

Study animals and protocol
The protocol for this study was approved by the Nutreco Agresearch Animal Care and Use Committee.

A total of 144 two-week-old pigs originating from one farrowing unit were weighed and randomly assigned to 24 pens of six pigs each at the beginning of the study (Day -14). Pigs were acclimated for a period of 14 days (Days -14 to 0), challenged with an *L intracellularis* inoculum on Day 0, and observed for a period of 21 to 22 days, at which time each pig was euthanized and necropsied. The pigs to be euthanized (three pigs per pen) on Day 21 or 22 were randomly pre-assigned on Day -14.

Ante mortem diagnostic testing
Blood and fecal samples were collected from two randomly selected pigs in each pen.
on Day -14, Day 14, and at the end of the study (Day 21 or 22). Serum samples were analyzed for antibody to *L. intracellularis* by serum immunoperoxidase monolayer assay (IPMA), and fecal swabs were tested for *L. intracellularis* by polymerase chain reaction (PCR) using previously described methods.

Statistical analysis

All analyses were conducted using analysis of variance (ANOVA) with the pen as the experimental unit and using a statistical significance level of *P* < .05, unless otherwise noted. Tukey’s pairwise comparisons were used for significant differences between treatment groups. Categorical data were transformed into arcsine of the square root of proportions per pen and then analyzed using ANOVA.

Results

**Ante mortem diagnostic testing and inoculum**

All tested animals were negative for *L. intracellularis* both by fecal PCR and serological testing on Day -14 (Table 1). By Day 14, approximately 15% of pigs in Treatments B and C were PCR-positive, while the other treatment groups remained PCR-negative. On Days 21 and 22, fecal samples from 12.5% to 83.6% of the challenged groups were PCR-positive, and 12.5% to 98.0% of challenged pigs were seropositive for *L. intracellularis* antibodies. More pigs in Treatments C and D were PCR-positive for *L. intracellularis* than in Treatment A (nonchallenged controls) (*P* < .01), and more pigs in Treatments B, C, and D were seropositive than in either Treatment E or the nonchallenged controls (*P* < .01). No animals in the nonchallenged control group became positive either by fecal PCR or by IPMA. No contaminating viruses or parasites were identified in the inoculum. Bacterial culture was negative for *Brachyspira* species, *Salmonella* serovar Choleraesuis, and *β*-hemolytic *Escherichia coli*. Control pigs were treated first with pure sucrose phosphate glutamate (SPG), and the five challenged groups were treated beginning with the most dilute dose and progressing up to higher doses.

**Housing, feeding, clinical scoring, and performance measurements**

Each pen contained a feeder and a nipple drinker and had completely slatted floors and solid PVC partitions. Feed and water were provided ad libitum. A commercial, nonmedicated diet was fed during the entire study. Clinical scores for attitude, abdominal appearance, fecal consistency, and fecal blood were recorded for each pig three times per week, from Day 7 to Day 21.

All pigs were individually weighed on Days -14, 0, 7, 14, and 21 or 22. Feed intake was calculated on each weigh day.

**Postmortem diagnostics**

Postmortem examination was conducted by a pathologist who was blinded to treatments for every pig that died or was euthanized during the study. In each pig, the jejunum, ileum, cecum, and colon were opened and scored for consistency of content and presence of blood, mucosal thickness and necrosis, gross diagnosis of ileitis, and length of lesions. All sections were stained with hematoxylin and eosin (H&E). Sections of ileum were stained by the Warthin-Starry (WS) technique to identify intracellular organisms and by immunohistochemistry (IHC) for *L. intracellularis* using an immunoperoxidase staining technique incorporating a specific polyclonal antibody. Sections were scored for mucosal epithelial proliferation, presence of intraepithelial bacteria, proprial inflammation, mucosal necrosis, and crypt abscessation.

**Mortality**

Mortality was distributed across all treatment groups, with no significant difference between treatments (*P* > .05) (data not shown). Thirteen of the 17 pigs that died during the study were diagnosed with K88 *E. coli* septicemia during the early postchallenge period (Days 2 to 5); affected pigs were reported in each treatment group, including the nonchallenged control group. Two pigs (one from Treatment F on Day 14 and one from Treatment C on Day 19) died of nonspecific intestinal disease. On Days 20 and 22, respectively, one pig in Treatment B and one in Treatment C died of ileitis.
Table 1: Mean proportions of animals positive for *Lawsonia intracellularis* (LI) by fecal PCR and serum IPMA testing in nursery pigs challenged on Day 0* with a mucosal homogenate containing LI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of LI organisms per pig</th>
<th>Fecal PCR</th>
<th>Serum IPMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>$2.4 \times 10^8$</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>C</td>
<td>$7.2 \times 10^7$</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>D</td>
<td>$2.2 \times 10^6$</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>E</td>
<td>$3.8 \times 10^5$</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>F</td>
<td>$3.2 \times 10^4$</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>SEM</td>
<td>NA</td>
<td>0.073</td>
<td>0.144</td>
</tr>
<tr>
<td>$P$</td>
<td>NA</td>
<td>.49</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

* Each treatment group included four pens of six pigs (total 24 pigs). Pigs were weaned at 14 days of age (Day -14), challenged with varying doses of LI at 28 days of age (Day 0), and euthanized on Day 21 or 22 (half of each group on each day). Fecal and blood samples were collected from two pigs per pen for fecal PCR and serological testing for LI (total eight pigs per treatment). Sera were tested for antibodies to LI by IPMA. An effort was made to collect samples from the same two pigs per pen on each occasion. The unit of analysis was the pen.

abc Values in a column with no common superscript are significantly different ($P < .01$; ANOVA).

PCR = polymerase chain reaction; IPMA = immunoperoxidase monolayer assay; NA = not applicable.

Table 2: Mean fecal consistency scores in groups of pigs weaned at 14 days of age and inoculated 14 days later (Day 0) with varying doses of a mucosal homogenate containing *Lawsonia intracellularis*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 9</th>
<th>Day 11</th>
<th>Day 14</th>
<th>Day 16</th>
<th>Day 18</th>
<th>Days 21-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.10</td>
<td>0.20</td>
<td>0.08</td>
<td>0.08a</td>
<td>0.00a</td>
<td>0.00a</td>
<td>0.17</td>
</tr>
<tr>
<td>B</td>
<td>0.53</td>
<td>0.57</td>
<td>0.70</td>
<td>1.34b</td>
<td>1.02b</td>
<td>0.94b</td>
<td>0.68</td>
</tr>
<tr>
<td>C</td>
<td>0.70</td>
<td>0.64</td>
<td>0.56</td>
<td>0.93b</td>
<td>0.89b</td>
<td>0.83b</td>
<td>0.81</td>
</tr>
<tr>
<td>D</td>
<td>0.42</td>
<td>0.52</td>
<td>0.45</td>
<td>0.37a</td>
<td>0.33b</td>
<td>0.65b</td>
<td>0.83</td>
</tr>
<tr>
<td>E</td>
<td>0.78</td>
<td>0.59</td>
<td>0.88</td>
<td>0.43a</td>
<td>0.42b</td>
<td>0.29a</td>
<td>0.33</td>
</tr>
<tr>
<td>F</td>
<td>0.29</td>
<td>0.43</td>
<td>0.38</td>
<td>0.18a</td>
<td>0.28b</td>
<td>0.29a</td>
<td>0.85</td>
</tr>
<tr>
<td>SEM</td>
<td>0.22</td>
<td>0.27</td>
<td>0.22</td>
<td>0.20</td>
<td>0.19</td>
<td>0.12</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* Each treatment group included four pens of six pigs (total 24 pigs). Score of 0, normal feces; 1, moderate diarrhea; 2, liquid feces. Inoculum doses for Treatments B through F shown in Table 1. Treatment A pigs were nonchallenged controls. Pen was the unit of analysis.

ab Values in a column with no common superscript are significantly different ($P < .01$; ANOVA).

Discussion

The present study demonstrates both induction of subclinical ileitis after inoculation with a mucosal homogenate containing *L. intracellularis* and a dose response to the number of organisms in the inoculum, creating a spectrum of illness from subclinical disease at the lowest inoculum dosage to clinical disease at higher doses and even death of some infected pigs. By the end of the study, impaired performance was observed at all doses of *Lawsonia* organisms (Treatments B to F). The consistency of poor performance in the challenged groups made performance parameters the most sensitive indicators to identify the disease process experienced by these pigs. The impact of the subclinical form of ileitis on growth was the most remarkable observation of this study. Even at the lowest inoculum dose (Treatment F), average daily gain during the trial period was 37% lower than that of the nonchallenged pigs, and feed conversion was 27% higher. There was no clinical difference in mean fecal consistency scores between Treatments E and F (the two lowest doses of inoculum administered) and the nonchallenged control groups except for one day (Day 16). In a field situation, such an observation could easily go undetected. This is consistent with an observational study in which it was concluded that infection with *L. intracellularis* can result in subclinical disease, demonstrated by poor performance of pigs that did not have diarrhea. Fecal shedding of *L. intracellularis* was detected in some pigs, many of these subclinically infected. Although no clinical signs of disease were observed, histopathological evidence of *L. intracellularis* infection was found. This may be extremely important
for the economics of swine farming. Not only do subclinically infected pigs perform suboptimally, but their feces may infect other pigs in the herd.\(^\text{15}\)

Histological evidence of \textit{L. intracellularis} infection concurrent with minimal clinical effects suggests that the inoculum resulted in subclinical infection for many pigs in this study. Severity of ileal lesions, observed grossly and histologically, increased with dose of \textit{L. intracellularis}. However, peak gross pathology scores (consistency of contents, mucosal thickness, mucosal necrosis, and length of lesions) and peak histopathology scores (H&E, WS, and IHC) occurred unexpectedly in Treatments C and D rather than in Treatment B, which received the highest dose of organisms. This could be explained by the fact that in Treatment B, there was a shorter incubation period and course of disease so that lesions were resolving and appeared less severe at necropsy. Another study\(^\text{16}\) noted that detecting resolving lesions was difficult, as the \textit{L. intracellularis} were no longer found in the enterocytes.

Most deaths in this study were caused by \textit{E. coli} K88 infection in the first 5 days post-inoculation. During Days 2 to 5, 13 pigs died of K88 \textit{Escherichia coli} septicemia, evenly distributed among the treatment groups; one treatment C pig and one treatment F pig died of nonspecific gastrointestinal disease on Days 14 and 19, respectively; and one treatment B pig and one treatment C pig died of ileitis on Days 20 and 22, respectively. Inoculum doses for Treatments B through F are shown in Table 1. Treatment A pigs were nonchallenged controls. The unit of analysis was the pen.
inoculation. As E coli K88 was not cultured from the inocula and the nonchallenged control group was also affected, it is likely that the source of this pathogen was the pigs themselves. Little information was contributed to the study by these pigs, which died before most of the study measurements were made. The end result is that there may have been a decrease in statistical power, but that does not change the effects observed.

Although subclinical ileitis was induced in previous studies, the range of inoculum doses, the number of pigs included, and the measurements were more extensive in the current study. The results confirm that subclinical infection can have a significant impact on growth performance, and that subclinically infected pigs can serve as a source of infection for other animals in the herd. Moreover, a significantly smaller proportion of these subclinically affected animals are positive by routine diagnostic testing methods such as soroconversion and fecal PCR.

**Implications**
- Subclinical infection caused by *L intracellularis* can have a significant impact on growth performance.
- Subclinically infected pigs can serve as a source of infection for other pigs in the herd.
- Routine diagnostic methods such as soroconversion and fecal PCR may be negative in subclinically infected pigs.

**References**