Efficacy study for ileitis protection by single intramuscular vaccination with Porcilis™ Ileitis

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Summary

■ Porcilis™ Ileitis is effective for at least 20 weeks after vaccination and was found to:
  – Induce *Lawsonia intracellularis* antibody titers
  – Control ileitis
  – Reduce colonization of *L. intracellularis*
  – Reduce duration and concentration of fecal shedding

■ Porcilis Ileitis offers 20 full weeks of control for protection all the way to market:
  – The only one-dose, ready-to-use injectable vaccine to control ileitis
  – With its 20-week DOI, Porcilis Ileitis delivers 13 more weeks of protection than the water-administered vaccine*
  – No worrying about removing feed-grade antibiotics during vaccination

*Frozen formulation; non-frozen formulation does not include DOI

Introduction

In today’s modern swine production systems, ileitis continues to be a problem and its impact in reducing pig performance can be a tremendous drain on profitability. Merck Animal Health now offers the first injectable option in your fight against ileitis. Porcilis Ileitis, which contains the Microsol Diluvac Forte® adjuvant system, provides ileitis control for at least 20 weeks after vaccination.

This *L. intracellularis* challenge study demonstrates that a single intramuscular vaccination with Porcilis Ileitis significantly controls ileitis in naïve vaccinated pigs challenged with *L. intracellularis* 20 weeks following vaccination. Porcilis Ileitis aids in the control of ileitis caused by *L. intracellularis*, aids in the reduction of colonization by *L. intracellularis* and aids in the reduction of fecal shedding for at least 20 weeks.

Materials and methods

Eighty-four mixed-breed pigs, with low (< 1:160) *L. intracellularis* immunofluorescent antibody (IFA) titers at the time of vaccination, were utilized. The pigs were allowed to acclimate to their surroundings for three days prior to vaccination. Pigs were allocated to equalize treatments within each pen and were distributed so both vaccinates and placebo-injected controls were represented within littermates. At 22-26 days of age (24 days median), 40 pigs were given a single 2-mL Porcilis Ileitis vaccination intramuscularly in the neck. Another 40 pigs were injected with 2-mL of a placebo containing all the same ingredients as the vaccine but without the *L. intracellularis* antigen. Four pigs were left as unvaccinated sentinels. Pigs were individually observed and palpated for seven days following vaccination for any evidence of adverse systemic or injection site reactions.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Sampling Regimen</th>
<th>Number of Animals</th>
<th>Age at Vaccination</th>
<th>Age at Challenge</th>
<th>End of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates (Porcilis Ileitis)</td>
<td>Tissue sampling at necropsy</td>
<td>24</td>
<td>22-26 days</td>
<td>23 Weeks</td>
<td>21 days post-challenge</td>
</tr>
<tr>
<td>Vaccinates (Porcilis Ileitis)</td>
<td>Fecal sampling 3 times per week</td>
<td>14</td>
<td>22-26 days</td>
<td>23 Weeks</td>
<td>56 days post-challenge</td>
</tr>
<tr>
<td>Controls (Placebo)</td>
<td>Tissue sampling at necropsy</td>
<td>25</td>
<td>22-26 days</td>
<td>23 Weeks</td>
<td>21 days post-challenge</td>
</tr>
<tr>
<td>Controls (Placebo)</td>
<td>Fecal sampling 3 times per week</td>
<td>15</td>
<td>22-26 days</td>
<td>23 Weeks</td>
<td>56 days post-challenge</td>
</tr>
</tbody>
</table>

Pigs were housed such that each pen contained approximately the same number of vaccinates and controls throughout the entire study. At the time of the *L. intracellularis* challenge, which was at 23 weeks of age, the four sentinel pigs were necropsied and evaluated for any evidence of *L. intracellularis* exposure prior to challenge. The remaining 78 pigs were individually orally challenged with *L. intracellularis* infected gut homogenate. Each animal was observed daily for clinical signs of ileitis following challenge.

A subset of 24 vaccinates and 25 placebo control pigs were necropsied at 21 days post-challenge (dpc). Their ileal tissues were evaluated for gross ileitis lesion severity (scored 0-4) and length of lesion. Ileal mucosal scrapings were performed to determine the level of *L. intracellularis* in intestinal epithelial cells as measured by quantitative polymerase chain reaction (qPCR). Formalin-fixed tissues were submitted to an
accredited veterinary diagnostic laboratory for evaluation for *L. intracellularis* antigen level by immunohistochemistry (IHC, scored 0-3) and for histopathological examination to evaluate microscopic lesions, mainly proliferation, consistent with *L. intracellularis* (scored 0-2).

Another subset of 14 vaccinates and 15 placebo controls were fecal monitored for *L. intracellularis* shedding by qPCR at 0, 3, 6, 9, 12 and 15 weeks after vaccination, along with three days prior to challenge, and then three times weekly up to 49 dpc and at 56 dpc when the study ended.

Serum samples were collected at vaccination, every 2-3 weeks thereafter and 3 days prior to challenge from all pigs, at 21 dpc (necropsied pigs only) and 56 dpc (fecal-monitored pigs only) to measure antibody responses by IFA following vaccination and *L. intracellularis* challenge. Two-fold dilutions ranging from 1:40 to 1:5120 were performed.

**Results**

No systemic or injection site adverse reactions were observed in any injected pigs. No evidence of *L. intracellularis* infection was noted in sentinel pigs, up to the time of challenge, as evidenced by lack of gross and microscopic lesions and absence of *L. intracellularis* based on IHC and qPCR. 

The following results show that Porcilis Ileitis was found to be effective for 20 weeks following vaccination.

**Induced *L. intracellularis* antibody titer**

Figure 1 represents the IFA RECIPROCAL geometric mean titers (GMT) of vaccine and control pigs during the entire study. By three weeks post-vaccination, titers from pigs in the placebo group declined to less than 1:40 and remained there until challenge. By three weeks post-vaccination, the IFA titers in vaccinates showed a significant increase from 1:43 to 1:1872. Following challenge, vaccinates showed significantly higher IFA titers versus the control group, apparently due to an anamnestic response from exposure to the *L. intracellularis* challenge.

**Figure 1: IFA geometric mean titers against *L. intracellularis* following vaccination and challenge**

<table>
<thead>
<tr>
<th>Weeks (Post-vaccination)</th>
<th>Vaccinates</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Vaccination)</td>
<td>43</td>
<td>&lt;40</td>
</tr>
<tr>
<td>3 Weeks</td>
<td>1872</td>
<td>&lt;40</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>1458</td>
<td>&lt;40</td>
</tr>
<tr>
<td>9 Weeks</td>
<td>829</td>
<td>&lt;40</td>
</tr>
<tr>
<td>12 Weeks</td>
<td>1371</td>
<td>&lt;40</td>
</tr>
<tr>
<td>15 Weeks</td>
<td>1168</td>
<td>&lt;40</td>
</tr>
<tr>
<td>17.5 Weeks</td>
<td>1059</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Prior to challenge</td>
<td>1250</td>
<td>&lt;40</td>
</tr>
<tr>
<td>3 Weeks Post-challenge</td>
<td>5120</td>
<td>1458</td>
</tr>
<tr>
<td>8 Weeks Post-challenge</td>
<td>5547</td>
<td>616</td>
</tr>
</tbody>
</table>

**Figure 1 key point:** Porcilis Ileitis induced a consistent *L. intracellularis* antibody response within three weeks of vaccination with a pronounced anamnestic response after challenge.

**Controlled ileitis**

Figure 2 represents the percentage of pigs diagnosed with ileitis based on gross and microscopic lesions. In this study, a pig was defined as having ileitis if both the gross lesion score was > 1 and the histopathological score was > 0. Three of the 24 vaccinates met both criteria (12.5%), while 16 of 25 control pigs (64.0%) met both criteria for ileitis diagnosis. This represents an 80.5% reduction of disease and demonstrated that the vaccine was effective at aiding in the control of ileitis 20 weeks after vaccination based on gross and microscopic lesions.

**Figure 2: Percent of pigs with ileitis based on gross and histopathological lesion scores**

**Figure 2 key point:**

Porcilis Ileitis controlled ileitis for 20 weeks after vaccination and reduced disease by 80.5% compared to controls.
Reduced colonization of *L. intracellularis*

Figure 3 represents the percentage of pigs colonized with *L. intracellularis*. A pig that presented with an IHC score > 0 or a positive qPCR on ileal mucosal scrapings was classified as colonized by *L. intracellularis*. For this combined variable, 13 of 24 vaccinates (54.2%) were found to be colonized with *L. intracellularis*, while 23 of 25 control pigs (92.0%) were colonized, representing a 41.1% reduction. All pigs that were classified as colonized were qPCR positive. However, the rate of IHC-positive vaccinated pigs was only 8.3%, while the rate in control pigs was 76.0%, representing an 89.0% reduction. In addition, vaccination reduced the amount of *L. intracellularis* in mucosal scrapings by 68.4%. Overall, the vaccine significantly reduced the bacteria’s ability to colonize the small intestine.

Figure 3: Percent of pigs colonized with *L. intracellularis* by IHC and qPCR

![Bar chart showing colonization percentages](image)

**Figure 3 key point:** Porcilis Ileitis significantly reduced *L. intracellularis* ability to colonize the small intestine.

Reduced duration and concentration of fecal shedding

Shedding of *L. intracellularis* after challenge was evaluated by qPCR of fecal samples, which were collected seven times prior to challenge and then collected starting two days after challenge and continued three times per week until seven weeks post-challenge.

All fecal-monitored pigs were *L. intracellularis* negative by qPCR prior to challenge. Both groups started shedding four days post-challenge. The average duration of shedding was 7.9 days for vaccinated pigs and 18.7 days for control pigs. Vaccination reduced the duration of shedding by 57.8%. In the control group, shedding of *L. intracellularis* continued up to 42 days post-challenge, while vaccinates stopped shedding by 28 days post-challenge.

Figure 4 represents the average amount of daily *L. intracellularis* fecal shedding post-challenge between the treatment groups. The vaccinated pigs had a 67.8% reduction in the average daily amount of *L. intracellularis* shed during the seven-week post-challenge period.

Figure 4: Daily amount of *L. intracellularis* fecal shedding post-challenge

![Bar chart showing fecal shedding amounts](image)

**Figure 4 key point:** Porcilis Ileitis reduced the average daily amount of *L. intracellularis* shedding by 68.7% compared to controls.
Discussion

Ileitis remains a constant source of production variation within groups of finishing pigs. While the cost of the disease varies widely based on prevalence of concurrent diseases, sanitation between groups of pigs, and inconsistent feed intake, it is estimated the cost of the disease can vary between $2.73 and $19.76 per pig in the U.S. annually.

Figure 2 shows that Porcilis Ileitis was effective at reducing the ability of *L. intracellularis* to cause gross and microscopic lesions in the small intestine. Figure 3 demonstrates that Porcilis Ileitis was effective at decreasing the ability of *L. intracellularis* to replicate in the target tissue where damage occurs.

In the field, a definitive diagnosis of ileitis is routinely based on a combination of histopathology and IHC staining of ileal tissue. In this study, only 2 of 24 vaccinates (8.3%) had both histopathological evidence of ileitis and IHC-positive *L. intracellularis* staining versus 19 of 25 control pigs (76%). From this field-based viewpoint, the data clearly demonstrates the vaccines’ ability for not only aiding in control of *L. intracellularis* disease, but also aiding in the prevention of *L. intracellularis*’ ability to colonize the intestine.

This study also demonstrates a marked serum antibody response within three weeks of vaccination along with a pronounced anamnestic response after challenge (Figure 1). Based on serum antibody titers, Porcilis Ileitis induces a strong systemic immune response to *L. intracellularis*. More importantly, the level of immunity at the gut level also appears to be strong based on the ability of the vaccine to reduce lesions, colonization and shedding following experimental challenge. Furthermore, this protective immunity was demonstrated at 20 weeks after vaccination, indicating duration of immunity (DOI) of at least 20 weeks.

In conclusion

This study shows that Porcilis Ileitis is effective for at least 20 weeks after vaccination. Specifically, it:

- Induced *L. intracellularis* antibody titers
- Controlled ileitis lesions, both gross and microscopic
- Reduced colonization of *L. intracellularis*
- Reduced duration and concentration of fecal shedding

Porcilis Ileitis offers 20 full weeks of control for protection all the way to market:

- The only one-dose, ready-to-use injectable vaccine to control ileitis
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