ABSTRACT

This double-blind study investigated the efficacy of injectable tylosin (Tylan® 200 injection, Elanco Animal Health, Indianapolis, IN) for the treatment and control of porcine proliferative enteropathy caused by *Lawsonia intracellularis* using a mucosal challenge model. Intramuscular (IM) tylosin was compared with sterile tylosin injection carrier (negative control). IM treatments (1 mL/22.5 kg [50 lb body weight; 4 mg/lb body weight]) were administered to two groups of pigs twice daily for 3 consecutive days, commencing 14 days after *L. intracellularis* challenge. Clinical signs were evaluated in 80 pigs each day for 14 days after treatment was initiated. Necropsies were performed on all pigs 14 days after treatment to evaluate gross and microscopic intestinal lesions. The challenged pigs had a positive treatment response to injectable tylosin compared with negative controls based on improvements in clinical signs, fecal polymerase chain reaction incidence on days 7 and 14, gross and microscopic intestinal lesions, average daily weight gain, and individual pig weight variation.

INTRODUCTION

Porcine proliferative enteropathy is a highly infectious disease of pigs.1,2 It has been recognized in the US swine industry for many years and is prevalent in virtually all swine populations throughout the world.3–8 Proliferative enteropathy is commonly found in growing and young adult swine.9–11 The disease is characterized clinically by diarrhea, anorexia, and various degrees of inflammation of the small intestinal mucosa, especially at the terminal portion of the ileum. Intestinal lesions can also be found in the spiral colon and cecum. Pig death and decreased weight gain can be consequences of these disease...
processes. Bloody diarrhea, black tarry stools, and death are common in the acute form of proliferative enteropathy. Swine farm financial losses due to proliferative enteropathy result from decreased average daily gain (ADG), increased feed conversion ratio, increased disease intervention cost, and pig death. \textsuperscript{12} \textit{Lawsonia intracellularis} was recently reported to be the cause of proliferative enteropathy. \textsuperscript{13–15}

The diagnostic gold standard of proliferative enteropathy has been the Warthin-Starry stain, \textsuperscript{16} allowing microscopic viewing of \textit{L. intracellularis} residing inside the proliferating ileal crypt enterocytes. Immunohistochemistry is a new diagnostic technique using immunofluorescence in conjunction with the \textit{L. intracellularis} antibody that can be used on formalin-fixed intestinal tissue to determine presence or absence of the bacteria. \textsuperscript{1} Also, such new ante-mortem tests as fecal polymerase chain reaction (PCR) and serum indirect fluorescent antibody (IFA) are useful for diagnosing proliferative enteropathy. \textsuperscript{17–20}

No drugs have been approved by the FDA for treating porcine proliferative enteropathy, but Tylan\textsuperscript{®} premix (Elanco Animal Health, Indianapolis, IN) has been approved for the prevention and control of porcine proliferative enteropathy associated with \textit{L. intracellularis} when fed at 100 g/ton for 21 days. \textsuperscript{21,22} Many water-soluble antibiotic studies have investigated treatment and control of porcine proliferative enteropathy; however, studies on injectable antibiotic use for treatment and control of porcine proliferative enteropathy are scarce. \textsuperscript{23–26} Field reports have claimed that IM tylosin is effective against porcine proliferative enteropathy, but no controlled studies have been completed. After IM administration, the tylosin molecule is excreted primarily via the liver into the bile, thus enabling tylosin to be at the site of infection for enteric disease therapy. \textsuperscript{27}

Several proliferative enteropathy models have been used to demonstrate antibiotic efficacy in swine. \textsuperscript{14,28–31} Injectable antibiotic efficacy against porcine proliferative enteropathy has not been evaluated in these models. This double-blind study investigated the efficacy of IM Tylan\textsuperscript{®} 200 injection for the treatment and control of porcine proliferative enteropathy caused by \textit{L. intracellularis} using a well-characterized mucosal challenge model. \textsuperscript{28,29}

\section*{MATERIALS AND METHODS}

All animals selected for the study were from a farm with no history or clinical evidence of \textit{Salmonella} species, \textit{Brachyspira hyodysenteriae}, or \textit{Brachyspira pilosicoli}. Other animals from the source herd had previously been used in proliferative enteropathy challenge studies that were associated with \textit{L. intracellularis}. All pigs were approximately 6 weeks or younger and from the same production group, within 1 week of age. The study was conducted in a facility that allowed the pigs to be housed in pens. The flooring of the facility was plastic-coated woven wire.

This 28-day study had two 14-day periods as shown in the timeline (Figure 1). The first 14-
day period began at the proliferative enteropathy challenge and allowed the pigs to develop clinical signs of proliferative enteropathy. The second 14-day period began at allotment and included 3 days of treatment along with 14 days of monitoring and data collection.

One hundred and ten pigs of mixed gender (55 gilts and 55 barrows), weighing 27 lb, were orally gavaged with an intestinal mucosal homogenate containing $3.4 \times 10^9$ *L. intracellularis* organisms on day –14. The pigs were individually identified with ear tags. The University of Minnesota diagnostic laboratory determined the mucosal homogenate bacteria count.

On day 0, 14 days after challenge with *L. intracellularis* mucosal homogenate, individual pigs were weighed and fecal diarrhea score was recorded. Allotment began by blocking the animals based on diarrhea score. Within a diarrhea score block, animals were ranked by weight and assigned to treatment using weight-pair groups. Forty pairs of pigs were assigned to 10 pens based on diarrhea score and weight rank. The remaining 30 pigs were excluded from the study because they showed no clinical signs and/or were heavier weight pigs. Assignment of the initial individual animal treatment for each pair of pigs was determined by a coin flip. One pig from each pair received a similar treatment based on diarrhea score and weight rank, which equalized diarrhea scores among pens, pig weight between treatment groups, and treatments among pens. Pig gender also was equalized between treatment groups.

Animals received no treatments other than the test articles in feed, water, or by injection during the 28-day study. The double-blind treatment articles included IM tylosin and sterile tylosin injection carrier (negative control; 50% propylene glycol with 4% benzyl alcohol and purified water). The study investigator, monitor, and animal care personnel remained blind to the test articles until completion of the study. Therapy began on day 1 for both blind products. The red article was administered IM to the red treatment group at 1 mL/22.5 kg (50 lb) body weight, twice daily, for 3 consecutive days (days 1, 2, 3). The blue article was administered IM to the blue treatment group at 1 mL/22.5 kg (50 lb) body weight, twice daily, for 3 consecutive days (days 1, 2, 3). Sterile syringes and needles were used for IM injection of the test articles in the neck according to Pork Quality Assurance Level III guidelines. All individual animal treatments were recorded by date, time of day, article used, and quantity.

Diarrhea score was recorded daily on individual pigs for 14 days (days 0 to 14) based on the following scale:

1 = No diarrhea
2 = Semisolid feces with no blood
3 = Watery but not dark or bloody feces
4 = Blood-tinged feces, loose or formed
5 = Profuse diarrhea with frank blood or dark, tarry feces

Clinical impression score was a summation of the attitude score and the abdominal appearance score. The clinical impression score was recorded each day for 14 days (days 0 to 14) on individual pigs based on the following observations:

Clinical impression score = (demeanor score + abdominal appearance score)

*Demeanor:*
1 = Normal
2 = Slightly to moderately depressed, listless, will stand
3 = Severely depressed, recumbent

*Abdominal appearance:*
1 = Normal
2 = Moderately gaunt
3 = Severely gaunt
Serum was collected from individual animals on days –14 and 14. Fecal samples were collected from individual animals on days 0, 7, and 14. The serum and fecal samples were submitted blindly to the University of Minnesota diagnostic laboratory. The serum was evaluated for the presence or absence of IFAs to *L. intracellularis*. The feces were evaluated for the presence or absence of *L. intracellularis* DNA by PCR.

Animals that died during the study were weighed and a necropsy was performed to determine the cause of death. All remaining animals were weighed and a necropsy was performed on day 14. During the necropsy, fixed and fresh tissue samples were collected from the jejunum, ileum, cecum, and colon. Intestinal proliferative enteropathy lesions were evaluated and scored on individual pigs in the following manner: Jejunum: 1, 2, 3, 4, 5; Ileum: 1, 2, 3, 4, 5; Cecum: 1, 2, 3, 4, 5; and Colon: 1, 2, 3, 4, 5. Intestinal lesion score from jejunum, ileum, cecum, and colon were evaluated by the following criteria:

1 = Normal
2 = Mild edema and hyperemia
3 = Edema, hyperemia, reticulated serosa and mucosa
4 = Edema, hyperemia, reticulated serosa and mucosa; gross thickening of the mucosa (necrotic enteritis)
5 = Edema, hyperemia, reticulated serosa and mucosa; gross thickening of the mucosa; and blood or fibrin

The length of any proliferative enteropathy lesion was measured and recorded at each intestinal region. Ileum length was predetermined to be a maximum of 20 cm for this study’s pig age and weight.

Tissue from the necropsies was submitted to the University of Minnesota diagnostic laboratory. Warthin-Starry stain and immunohistochemistry were completed on the formalin-fixed tissue to demonstrate presence of *L. intracellularis*.

Individual animal weight was recorded on days –14, 0, 7, and 14. Individual animal weight was recorded on the day of mortality on any animal(s) that died during the study. ADG was analyzed for each pig for days 0 to 7, 8 to 14, and 0 to 14.

Feeders were located in each pen (n = 10 pens) to allow for *ad libitum* feed consumption during the study. Average daily feed intake was not analyzed because both treatment groups were represented in each pen. Feed composition was designed for animals of the appropriate weight, genetics, and gender.

**ANALYSIS**

PROC GLM in SAS was completed for diarrhea score, clinical impression score, pig weight, pig weight gain, and intestinal lesion length. Diarrhea score was analyzed on a daily basis. Incidence of mortality and incidence of any lesion (jejunum, ileum, colon, cecum; regardless of severity) were compared between treated and control pigs using Fisher’s exact method. ADG and clinical impression scores were regressed on total gut length and adjusted for treatment. For each variable, two models were considered. The first fit a separate slope and intercept for each treatment group allowing for a test of a common slope between the control and treated groups. The second model, the common slope, was constrained to estimate a common regression coefficient for total gut length while treatment-group specific intercepts were fit. The incidence of positive results from the diagnostic tests was analyzed with Fisher’s exact test.

**RESULTS**

Diarrhea scores were similar for the IM ty-
T. Marsteller, N. Winkelman, C. Gebhart, G. Armbruster, W. Weldon, R. Muller, J. Weatherford, and J. Symanowski

Losin group and the negative control group from days 0 to 4. After day 4, daily diarrhea scores were different for the two groups until days 13 and 14 (Figure 2). IM tylosin improved the diarrhea score. Diarrhea scores for the two groups were statistically different at days 0 to 7, 8 to 14, and 0 to 14 (Table 1).

Intramuscular tylosin improved the clinical impression score. Differences between clinical impression scores for the IM tylosin group and the negative control group were statistically significant at days 0 to 7, 8 to 14, and 0 to 14.

Pig weights and group weight variation were numerically similar for the two groups on days –14 and 0 but numerically different on days 7 and 14 (after treatment). Pig weight for the IM tylosin group at day 0 was 18 ± 1.5 kg (40.2 ± 3.35 lb), and weight for the negative control group was 17.9 ± 1.6 kg (39.8 ± 3.63 lb), indicating little difference in weight or weight variation between treatment groups. Weight gain of individual pigs was analyzed during days 0 to 7, 8 to 14, and 0 to 14. Improved weight gain during these periods was a benefit of treating and controlling proliferative enteropathy in swine administered IM tylosin (Table 1 and Figure 4).

Zero of 40 pigs in the IM tylosin group and 4 of 40 (10% mortality) pigs in the negative control group died between days 0 and 14 (P = .116). Although not significant at the .05 level, this numeric trend in an adequately powered study in terms of replications and numbers might show a treatment-related decrease in mortality. Proliferative enteropathy was diagnosed as the cause of death in the four pigs from the negative control group, indicating an effective L. intracellularis challenge. Salmonella species were cultured from intestinal swabs in the four animals but not isolated from their tissue, indicating no systemic Salmonella disease. There was no evidence of other diseases complicating the proliferative enteropathy challenge.

Thirty-six of 40 pigs in the negative control group had at least one grossly visible intestinal lesion, whereas 26 of 40 pigs in the IM tylosin group had proliferative enteropathy lesions (Table 2). These results clearly indicate a treatment-related decrease in the incidence of pigs with proliferative enteropathy–related intestinal lesions. The high percentage of pigs in the negative control group with lesions is suggestive of an effective L. intracellularis challenge model.

Twenty-eight days after L. intracellularis challenge, proliferative enteropathy lesion scores in the jejunum, ileum, and cecum were decreased in the IM tylosin group compared with those of the negative control group (Table 2). Proliferative enteropathy small intestinal lesion lengths were also statistically decreased in the IM tylosin group (Table 2).

A regression analysis of proliferative enteropathy lesion length was conducted for both
ADG (days 0 to 14) and diarrhea score (days 0 to 7, 8 to 14, and 0 to 14). All regression slopes were highly significant \((P < .001)\), and the sign on the slopes followed expectations. The negative sign on the ADG slope indicates that pigs with increased lesion length had decreased performance. The positive signs of the diarrhea score slopes indicate pigs with increased lesion length demonstrated increased (more severe) diarrhea scores. ADG regression can help illustrate the interpretation of the regression slopes. A 1-cm increase in total gut lesion length was associated with a decrease of 0.002 kg (0.005 lb)/day in ADG from days 0 to 14. None of the slope by treatment interactions was significant \((P > .250)\), suggesting that the relationship between the performance parameters (ADG, diarrhea score) and total gut lesion length was similar when adjusted for treatment. Therefore, whether pigs are treated for proliferative enteropathy, when there is evidence of intestinal lesions, ADG will be decreased and diarrhea score will be increased. Table 3 summarizes the results for the common slope models.

Results based on the IFA serology showed no differences between the IM tylosin and negative control groups. All pigs were serologically negative for antibodies to \textit{L. intracellularis} at challenge (day –14). Twenty-eight days later more than 80% of all pigs were serologically positive for \textit{L. intracellularis} antibodies (Table 4). Pigs treated with IM tylosin 14 days after challenge developed serum antibodies to \textit{L. in-
tracellularis 28 days after challenge at a rate similar to that of negative control pigs.

More than 50% of the pigs’ fecal samples were positive for L. intracellularis (determined by PCR) in both treatment groups 14 days after challenge (day 0), indicating an effective proliferative enteropathy challenge. The percentage of pigs with a positive fecal PCR at allotment was similar for both treatment groups (Table 4). The IM tylosin group had a decreased number of pigs with a positive fecal PCR on days 7 and 14 compared with that of the negative control group (Table 4), suggesting that IM tylosin decreases the amount of L. intracellularis shed from pigs after therapy compared with that of untreated controls.

The IM tylosin group had a decreased number of intestinal proliferative enteropathy histopathology lesions compared with those of the negative control group when evaluated with both Warthin-Starry stain and immunohistochemistry 14 days after therapy was initiated (Table 4).

**DISCUSSION**

This study has a potential field application. The L. intracellularis challenge isolate is from a field clinical porcine proliferative enteropathy case. The authors allowed 14 days of proliferative enteropathy development after challenge with L. intracellularis before therapy. Twenty-two of 40 animals (55%) in both groups had evidence of diarrhea on day 0. In most cases, herdsmen and veterinarians administer therapy before most animals have clinical signs. This was a severe challenge as noted by the 10% death loss in the negative control group; however, field conditions can be different from

**TABLE 2. Gross Intestinal Lesions: Incidence and Lesion Scores,* and Lengths (cm)**

<table>
<thead>
<tr>
<th></th>
<th>Intramuscular tylosin</th>
<th>Negative control</th>
<th>Standard Error</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Animals</td>
<td>40</td>
<td>40</td>
<td></td>
<td>.015</td>
</tr>
<tr>
<td>Lesion Incidence</td>
<td>26/40</td>
<td>36/40</td>
<td></td>
<td>&lt; .001</td>
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<tr>
<td>Jejunum Lesion Score</td>
<td>0.03</td>
<td>1.28</td>
<td>0.15</td>
<td>&lt; .001</td>
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<tr>
<td>Ileum Lesion Score</td>
<td>0.85</td>
<td>1.55</td>
<td>0.16</td>
<td>.002</td>
</tr>
<tr>
<td>Cecum Lesion Score</td>
<td>0.00</td>
<td>0.30</td>
<td>0.09</td>
<td>.014</td>
</tr>
<tr>
<td>Colon Lesion Score</td>
<td>0.45</td>
<td>0.63</td>
<td>0.13</td>
<td>.337</td>
</tr>
<tr>
<td>Jejunum Lesion Length</td>
<td>0.80</td>
<td>89.97</td>
<td>10.98</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Ileum Lesion Length</td>
<td>3.87</td>
<td>10.99</td>
<td>1.05</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Total Small Intestine Lesion Length</td>
<td>4.67</td>
<td>99.96</td>
<td>11.75</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Colon Lesion Length</td>
<td>1.95</td>
<td>2.33</td>
<td>0.47</td>
<td>.572</td>
</tr>
<tr>
<td>Cecum Lesion Length</td>
<td>0.00</td>
<td>1.55</td>
<td>0.43</td>
<td>.014</td>
</tr>
<tr>
<td>Small and Large Intestine Lesion Length</td>
<td>6.63</td>
<td>103.84</td>
<td>11.87</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

*Least square means.
research conditions because of disease, animal, and human interactions. Therapy early in the course of any disease is critical for the well-being of the animal and the group performance. Veterinarians may consider using IM tylosin for pigs experiencing proliferative enteropathy caused by *L. intracellularis*. Because IM tylosin has not been approved by the FDA for the treatment of proliferative enteropathy in swine, it is considered extra label and should be used to treat proliferative enteropathy only if the criteria established under the Animal Medicinal Drug Use Clarification Act for extra label drug use are met. The calculated IM tylosin dose in this study was 4 mg/0.45 kg (1 lb) body weight, administered twice daily for 3 days. This is the label dose for other diseases for which IM tylosin is approved. The FDA-approved withdrawal time for this dose is 14 days before slaughter.

Intramuscular tylosin decreased fecal shedding and thus can aid in preventing the spread of *L. intracellularis* in a group of pigs during proliferative enteropathy disease treatment. Immunohistochemistry appeared to be more effective than Warthin-Starry stain for detecting the *L. intracellularis* in tissue sections. Based on this study, further research to determine the most useful diagnostic technique for proliferative enteropathy in the field is warranted.

**CONCLUSION**

Data from this study support field reports of IM tylosin efficacy for the treatment and control of proliferative enteropathy caused by *L. intracellularis* in swine. The lesion length data and tissue diagnostic data strongly support IM tylosin efficacy for treating proliferative enteropathy caused by *L. intracellularis*. The mucosal challenge used in this study is an effective proliferative enteropathy model based on the clinical signs, intestinal lesions, and diagnostic data that indicate infection with *L. intracellularis*.

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**TABLE 3. Regression Analysis of Porcine Proliferative Enteropathy Intestinal Lesion Length (cm) to Average Daily Gain or Diarrhea Score**

<table>
<thead>
<tr>
<th></th>
<th>ADG (days 0 to 14)</th>
<th>Diarrhea Score (days 0 to 7)</th>
<th>Diarrhea Score (days 8 to 14)</th>
<th>Diarrhea Score (days 0 to 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>-0.005</td>
<td>+0.005</td>
<td>+0.006</td>
<td>+0.005</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

*ADG = Average daily gain.*

**TABLE 4. Diagnostic Test Results (Number Positive/Number Tested)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Intramuscular tylosin</th>
<th>Negative control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA serology at challenge (day –14)</td>
<td>0/40</td>
<td>0/40</td>
<td>NV</td>
</tr>
<tr>
<td>IFA serology at necropsy (day 14)</td>
<td>33/40</td>
<td>35/39</td>
<td>.518</td>
</tr>
<tr>
<td>Fecal PCR (day 0)</td>
<td>20/40</td>
<td>26/40</td>
<td>.258</td>
</tr>
<tr>
<td>Fecal PCR (day 7)</td>
<td>11/40</td>
<td>24/40</td>
<td>.007</td>
</tr>
<tr>
<td>Fecal PCR (day 14)</td>
<td>5/38</td>
<td>12/33</td>
<td>.028</td>
</tr>
<tr>
<td>Warthin-Starry Stain of Ileum Tissue</td>
<td>1/40</td>
<td>19/40</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Immunohistochemistry of Ileum Tissue</td>
<td>2/40</td>
<td>33/40</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

*IFA = indirect fluorescent antibody; NV = no value; PCR = polymerase chain reaction.*
Proliferative enteropathy caused decreased weight gain and an increase in weight variation within the negative control cohort group. Decreased weight gain and increased weight variation are two common performance indices affected by proliferative enteropathy. Treating or controlling porcine proliferative enteropathy with Tylan® 200 injection can alleviate the negative impact of the disease on animal performance and subsequent financial losses.

REFERENCES


