

Subclinical ileitis produced by sequential dilutions of *Lawsonia intracellularis* in a mucosal homogenate challenge model

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Introduction

Porcine proliferative enteropathies (PPE, also known as ileitis) are a group of enteric diseases of swine characterized by hyperplasia of crypt enterocytes in the ileum and large intestine.^{1,2} The causative agent is *Lawsonia intracellularis* (LI), a Gram-negative obligate intracellular bacterium.^{1,2} The disease is widespread in pigs raised under various management systems and causes significant economic impact worldwide.^{1,3,4,5} The acute form occurs principally in young adults 4-12 months of age and is characterized by hemorrhage into the distal ileum resulting in bloody diarrhea or sudden death.¹ The chronic form occurs mainly in pigs between 6 and 20 weeks of age and is characterized by thickening of the distal ileum often accompanied by mucosal necrosis. Affected pigs may develop diarrhea and have decreased and variable rate of gain.^{1,2}

A subclinical form of the disease (defined here as infection with insignificant clinical signs but histologically evident enterocyte hyperplasia) has been identified in the field and in challenge studies^{2,6-8} however it remains incompletely characterized. The objective of this study was to determine the impact of varying doses of LI on clinical signs, performance and gross and histopathologic changes in swine, using a mucosal homogenate challenge model.

Materials and methods

A total of 144 unvaccinated 2-week-old piglets were randomly assigned to 6 treatment groups blocked by body weight on day -14. All pigs came from the same farrowing unit and were of high health status. The pigs were negative for LI by serology and fecal PCR. They were fed a commercial non-medicated diet during the entire study (day -14 to day 21/22) and were acclimatized for 14 days (days -14 to 0). Pigs were challenged orally with pure sucrose phosphate glutamine (SPG) or with SPG diluted mucosal homogenate from LI infected pigs on day 0. There were 4 pens of 6 pigs per pen in each treatment group which consisted of the following:

(A) non challenged control; challenged with the following inoculum dilutions: (B) 1:10, (C) 1:100, (D) 1:1000, (E) 1:10,000, and (F) 1:100,000. Fecal consistency, body weight and feed consumption were recorded throughout the study. Half of the pigs from each pen were euthanized on day 21 and the other half on day 22. Gross and histologic lesions of the gastrointestinal tract (including lesions consistent with PPE in H & E, Warthin Starry and immunohistochemically (IHC) stained gut sections) were scored for severity. All analyses were conducted using analysis of variance with pen as the experimental unit.

Results

Results of gross and histopathologic examination of tissues are shown in Table 1. With the exception of treatment D there were no significant difference in the incidence of gross lesions in inoculated pigs compare to uninoculated. However, there was a significant ($P < 0.05$) increase in the percentage of pigs with histologic lesions of PPE (H & E stained sections) and bacteria consistent with LI (Warthin Starry stained sections) at several doses of inoculum compare to uninoculated. All inoculum doses had a significantly ($P < 0.05$) greater proportion of pigs with evidence of LI infection by IHC than controls. Results of analysis of clinical and performance parameters are shown in Table 2. All doses of inoculum resulted in a statistically significant ($P < 0.05$) decrease in average daily gain and increase in feed:gain (Days 0 to 21/22) compared to uninoculated pigs. Diarrheal scores (Day 14) were mild (< 1) in all pigs and did not differ significantly from controls with the exception of those receiving treatments B and C.

Discussion

Varying doses of inoculum in this study resulted in a range of clinical, pathologic and performance responses. All doses - even the lowest- produced significant decreases in performance. Because this was a randomized experiment, this decrease in performance can be attributed to

Table 1: Gross and histopathologic examination of pigs administered varying doses of *L. intracellularis*

Treatment	Inoculum dose ¹	Gross pathology ²	H & E score ³	Warthin Starry score ⁴	IHC ⁵
A	SPG	0.00 ^{a6}	0.00 ^a	0.00 ^a	0.00 ^a
F	3.2 × 10 ⁴	0.08 ^{ab}	0.34 ^{ab}	0.42 ^b	0.67 ^b
E	3.8 × 10 ⁵	0.13 ^{ab}	0.31 ^{ab}	0.38 ^b	0.63 ^b
D	2.2 × 10 ⁶	0.3 ^b	0.61 ^b	0.54 ^b	0.78 ^b
C	7.2 × 10 ⁷	0.25 ^{ab}	0.57 ^b	0.46 ^b	0.66 ^b
B	2.4 × 10 ⁸	0.25 ^{ab}	0.33 ^{ab}	0.33 ^{ab}	0.62 ^b

¹ *L. intracellularis* count per pig

² Proportion of pigs with gross lesions consistent with PPE

³ Proportion of pigs with H & E lesions in small intestine consistent with PPE

⁴ Proportion of pigs with evidence of *L. intracellularis* in Warthin Starry stained sections of ileum

⁵ Proportion of pigs with evidence of *L. intracellularis* by ileal immunohistochemistry

⁶ Values within a column with no common superscripts are significantly different ($P < 0.05$)

Table 2: Clinical and performance parameters in pigs administered varying doses of *L. intracellularis*

Treatment	Inoculum dose ¹	Fecal consistency score day 14 ²	ADG days 0 to 21/22 ³	Feed:gain days 0 to 21/22 ⁴
A	SPG	0.08 ^{a5}	0.40 ^a	1.63 ^a
F	3.2 × 10 ⁴	0.18 ^a	0.25 ^b	2.07 ^b
E	3.8 × 10 ⁵	0.43 ^a	0.23 ^b	2.10 ^b
D	2.2 × 10 ⁶	0.37 ^a	0.24 ^b	2.24 ^{bc}
C	7.2 × 10 ⁷	0.93 ^b	0.19 ^b	2.51 ^{bc}
B	2.4 × 10 ⁸	1.34 ^b	0.16 ^b	2.92 ^c

¹ *L. intracellularis* count per pig

² Scores: 0 : Normal 1: Moderate 2: Severe

³ Average daily gain (kg)

⁴ Kg feed:kg gain

⁵ Values within a column with no common superscripts are significantly different ($P < 0.05$)

inoculum administration. The following evidence suggests that decreased performance was the result of infection with LI in each case: when compared to controls 1) a significantly greater proportion of pigs in treatments C to F had evidence of LI infection in Warthin Starry stained sections of ileum ; 2) a significantly greater proportion of pigs at all inoculum doses had evidence of LI infection by ileal IHC; 3) a significantly greater proportion of pigs in treatments C and D had evidence of LI infection in H & E stained sections of small intestine, and 4) no pigs had histologic or gross lesions consistent with other known enteric infections.

Fecal consistency scores were all low (0.18 to 0.43) for treatments D to F and did not differ significantly from controls. We conclude, therefore that treatments D to F constitute subclinical LI infection in as much as 1) each of these treatments had evidence of LI infection as described above and 2) clinical effects of infection were insignificant.

These results are consistent with previous challenge studies of subclinical LI infection and observations from the field.^{2, 6-9} Because the source of inoculum was naturally infected intestinal mucosa and because the natural route of infection was used, this study parallels

in important respects infection that occurs under field conditions. As a result, it is reasonable to hypothesize that mild and subclinical LI infection in the field may result in performance impacts such as those seen in this investigation.

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