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# Ileitis: What we know about immunity!?

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## Introduction

Proliferative enteropathy is a common enteric disease of pigs caused by the obligate intracellular bacterium, *Lawsonia intracellularis*. The characteristic pathological feature of the disease is the proliferation of immature epithelial cells in the crypts of the ileum, large intestine, or both, leading to macroscopic thickening of the mucosa. Enteric lesions often have *Lawsonia* organisms found in the apical cytoplasm of these proliferative enterocytes. Clinical signs in affected pigs usually occur after weaning and are associated with proliferative lesions of the ileum/colon, which results in diarrhea and uneven weight gain. In older pigs (>12 weeks of age) the acute form of the disease is often expressed as rapid onset of diffuse hemorrhagic lesions resulting in sudden death. Estimates of the annual economic losses attributable to the clinical and subclinical effects of the disease are approximately $100 million/year for the US swine industry alone (1).

In order to understand the immune response associated with *Lawsonia intracellularis*, it is important to first consider the pathogenesis of this very unique organism. Attachment to specific receptors on crypt cells is a necessary prerequisite for entry into the host cell and this is particularly important for an obligate intracellular pathogen. It is likely that with *Lawsonia*, motility and flagella may play a role in the initial association with these receptors. Adhesins and receptors have not been fully characterized to date but it is known that attachment can be blocked with antibodies (2).

Once attached, the bacteria are then internalized through receptor-mediated endocytosis and enter into a vacuole via the actin cytoskeleton. The process is host cell driven and does not depend on bacterial viability. Following invasion, the viable bacteria rapidly (less than 3 hours) escape from the vacuole and then remain free in the apical cytoplasm. Bacterial replication occurs concomitantly with that of the infected host cell and it is generally believed that actively dividing host cells benefit the bacteria's ability to replicate (3). There is also evidence to suggest that *Lawsonia* infection actually increases the rate of cell division which helps spread the bacteria and may partly explain the pathology often noted in infected tissues.

Infected crypt cells then divide and migrate into columns of cells that form finger-like projections or villi that extend into the intestinal lumen. In infected crypt cells, along with the formation of villi, there is also the formation of *Lawsonia*-infected villi. In cell culture, most infections occur in foci suggesting transfer of bacteria is primarily by cell division rather than transfer out of cells and then re-infection. The mechanism by which Lawsonia induces cell proliferation is not precisely known, but infection does lead to hyperplasia and thickening of the gut mucosa, without extensive destruction or cytopathic effects on the host cell. This gut thickening ultimately prevents normal gut function, absorption capabilities, and nutrient transfer.

*Lawsonia* has a specific tropism for crypt cells associated with the gut and requires attachment, invasion, and intracellular replication. Some potential immune responses that would be important in an infected host in the control of *Lawsonia* may include:

- The organism is gut associated and does not induce a systemic infection. A systemic humoral response would be predicted to be low and slow to develop.
- A gut-associated pathogen that requires receptor-mediated attachment and invasion would have the potential to induce a mucosal immune response and potentially induce IgA antibodies that could block or reduce future infections.
- As an obligate intracellular pathogen, it would be hypothesized that a CMI (cell-mediated immune) response would likely be very critical to eliminate intracellular bacteria and infected cells. It would also serve to provide immune memory for subsequent exposures.

## Lawsonia immunity

The amount of information known about the immune response following *Lawsonia* exposure or what defines a protective immune response is extremely limited. At the present time, our knowledge is based primarily on investigations of serological responses, some early studies on CMI, maternal immunity protection studies, and vaccine efficacy trials.

## Humoral immunity

Serum antibody responses to infection have been measured by several labs using different methods. In a serological study using bacteria extracted from diseased tissue in an ELISA format, it was concluded that the serological immune response was primarily IgM and very short lived (4). Subsequently, others used a pure culture, *Lawsonia*-infected mouse enterocyte assay and an IFA technique to measure the IgG response following *Lawsonia* exposure. In this report the onset of the IgG response was first noted at 14 days but it wasn't until 21 days that a detectable immune response was induced in over 80% of the exposed animals. These investigators noted that serologic detection did not correlate with lesion development (animals could be sero-positive and not have gross lesions) (5). Finally, an evaluation of an IMPA diagnostic format in *Lawsonia*-infected rat enterocytes also confirmed this was an effective technique for herd diagnostic screening (6). The IPMA was also defined a serological profile for pigs exposed to both a commercial *Lawsonia* vaccine (Enterisol" Ileitis) as well as a virulent field challenge (Figure 1) (7). Although not extensively studied, it is generally thought that systemic antibody levels begin to decline 4–8 weeks after a single exposure and don't appear to be life-long without subsequent re-exposure or boosting.

Little information is available on mucosal immunity but it is possible that secretory IgA may play a role in blocking pathogen attachment and subsequent invasion. One laboratory had successfully reported the detection of IgA from gut washings in pigs 22 days after exposure to virulent Lawsonia (Gebhart, personal communication) This area needs extensive investigation both on the stimulation of IgA and its role in protection and control.

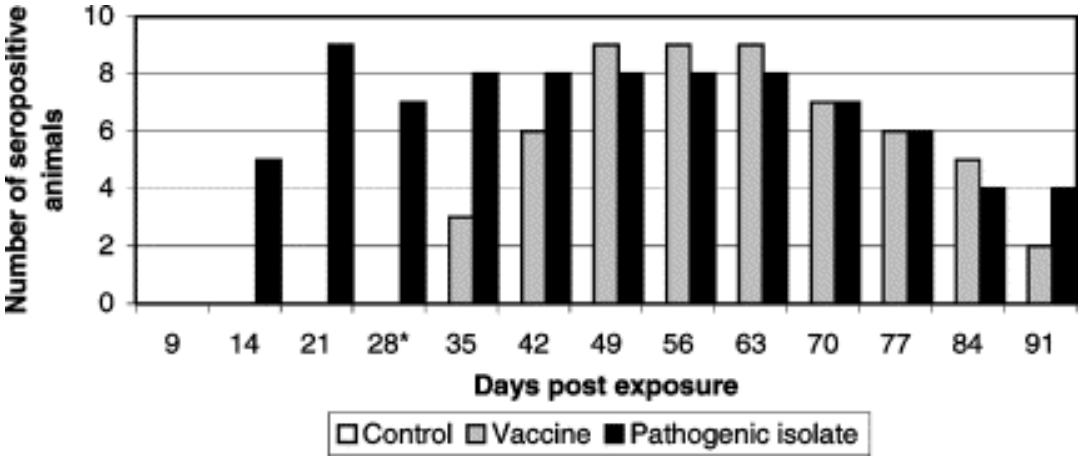
The relevance of a systemic serological response is still unclear, but at the present time there are no data to suggest it is a good indicator of immunity or vaccine efficacy. Nonetheless, monitoring of serology does have some practical implications, including:

- Use of serology on a herd basis can be an extremely valuable tool to determine exposure to *Lawsonia* within a herd. Once a herd profile and peak seroconversion time is defined, you can then predict that *Lawsonia* exposure occurs *at least* 2–4 weeks prior to the onset of seroconversion.
- By defining *Lawsonia* exposure times, this information can help focus efforts on improved management techniques as well as the placement of intervention strategies such as vaccines and antibiotics.
- Currently available *Lawsonia* vaccines do not induce high levels of systemic antibodies and yet have demonstrable efficacy following virulent challenge exposure (8).
- It is important to remember that the presence of antibiotics can significantly impact the development of a humoral immune response to *Lawsonia*. There is evidence to suggest that *Lawsonia* exposure in the face of antibiotics may prevent, reduce, or delay a humoral immune response. This is an important factor to consider if you want to use serology to profile a herd and develop a control strategy (9).
- IgG antibodies to *Lawsonia* appear to decay and decline quickly (i.e., 4–8 weeks) following a mild-moderate disease (PIA) but may be higher and persist longer following an acute disease (PHE).

## Cellular immunity

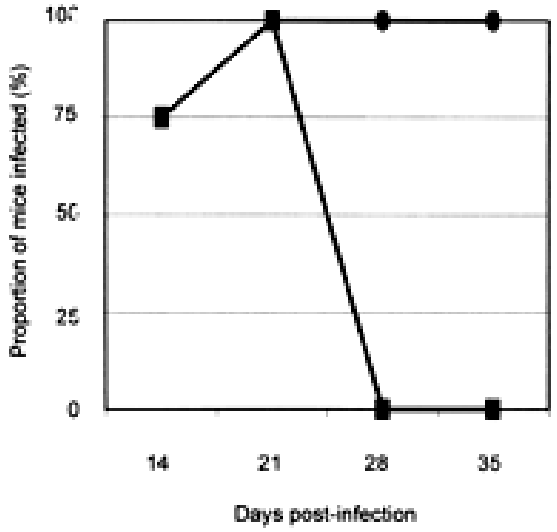
There is relatively little known about the cellular immune response associated with *Lawsonia* exposure. Given that this is an obligate intracellular pathogen, it would seem obvious that a CMI response would be critical, if not required, for solid protective immunity. Interferon-γ is of-

Figure 1: Seroconversion to Lawsonia vaccines and virulent isolates as measured by IPMA (Guedes et al., 2003)



ten a critical component of the immune system in the control of intracellular bacterial and viral pathogens. To evaluate this for the control of *Lawsonia*, a wild-type *L. intracellularis* isolate was inoculated into a normal mouse line and an interferon-γ-deficient mouse line. The results of this study indicated that in the normal mice, *Lawsonia* infection of crypts could be noted in most of the mice up to 21 days post-exposure, but that after this time period, the bacteria were cleared and the mice were normal and healthy. In contrast, when *Lawsonia* was administered to interferon-γ-deficient mice, crypt infection was noted in all of the animals and this infection persisted for the duration of the trial (35 days). Interferon-γ-deficient mice had a higher susceptibility to infection, had a progressive increase in infected crypts, and actually experienced some *Lawsonia*-associated mortality (see Figure 2; note that squares are normal mice and circles are interferon-γ-deficient mice) (10). This study confirms the role of the cellular immune response in the control of *Lawsonia* infection in mice.

Figure 2. Infection levels of *Lawsonia* in normal and interferon-g-deficient mice (Smith et al., 2000)

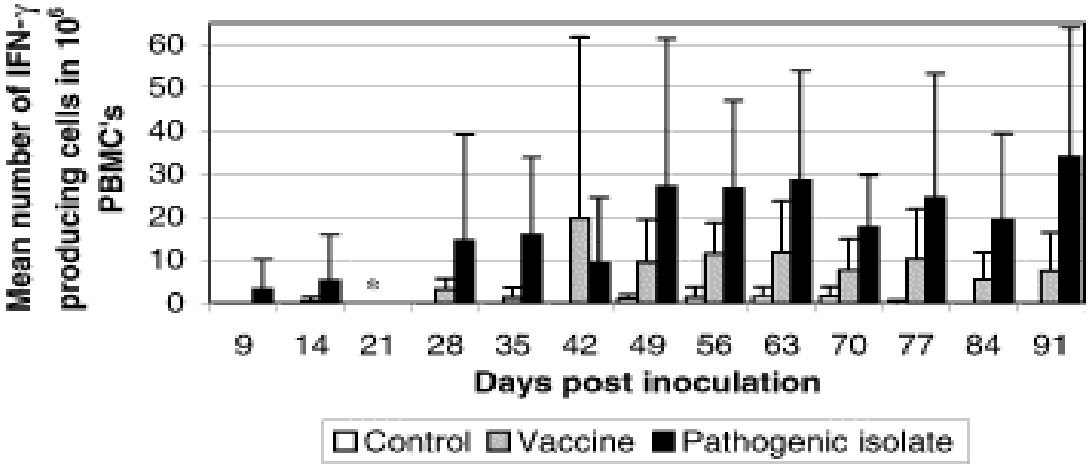



In another investigation, Guedes et al, evaluated the onset and duration of the cell mediated response in the more relevant pig model. This group used ELISPOT to measure interferon-γ production in stimulated T-lymphocytes. The study compared the interferon-γ production in pigs exposed to a control, a commercial vaccine (Enterisol" Ileitis), and a pathogenic *Lawsonia* isolate. It was determined that the initial CMI response was noted as early as 9–14 days of exposure both vaccine and pathogenic isolates had detectable responses by day 28, with peak levels detected in both Lawsonia-exposed groups between days 42–49. There was a detectable CMI response up to 13 weeks after exposure in vaccinates and challenged pigs (Figure 3) (7).

In conclusion, the cell-mediated immune response (as measured by interferon-γ) has been shown to play a significant role in the control of *Lawsonia intracellularis* infection and clearance. Further work is needed in the area of CMI and control of *Lawsonia*, but to date the following information should be considered relative to practical applications of cellular immunity:

- CMI is critical in the control and elimination of *Lawsonia* infections.
- Both virulent isolates and avirulent live commercial vaccines (Enterisol" Ileitis) induce a significant CMI response.
- Peak CMI stimulation did not occur until 42–49 days post-exposure. This should be considered in immune-mediated control programs. It is important to vaccinate animals prior to exposure and early enough to have a peak immune response induced at the onset of virulent challenge exposure.

Figure 3. ELISPOT T-cell assay results for the detection of interferon-γ in pigs exposed to *Lawsonia intracellularis* (Guedes et al., 2003)



- CMI measurements are difficult and not readily available in diagnostic laboratories at this time.

## Maternal immunity

Given that clinical PE often occurs late in the grower/finisher, it could be hypothesized that maternal immunity may play a role in the control of *Lawsonia* infections in the nursery. In fact several labs have noted that antibodies against *Lawsonia* can be found in the colostrum of lactating sows and in the serum of weaned pigs. The level of antibodies in serum from weaned pigs declines quickly in most cases, which supports the idea of passive transfer.

Until now, very little was known about the role of maternal immunity in *Lawsonia* infection, but recently a large study has been completed to address this issue (11). In this study, sows were obtained from a herd with no clinical history of *Lawsonia*. These animals were screened serologically and found to have no detectable antibody titers to *Lawsonia* by IFA. Sows were then split into 2 different treatment groups. Group A was hyperimmunized 3 times with an experimental attenuated live vaccine to induce both a humoral and CMI response. This extreme immunization protocol was designed to ensure maximum levels of maternal antibodies were induced to allow the measurement of passive protection. Group B remained un-exposed to *Lawsonia*. At farrowing, pigs born to these sows were then randomly assigned to treatment groups. The study design is detailed in Table 1, below. Groups 3 and 6 were maintained to ensure that pigs were normal and healthy and there was not a *Lawsonia* infection transferred from sow to pig during the trial. All sows in groups 1–3 were confirmed serologically positive at farrowing. All sows in groups 4–6 were confirmed to be serologically negative at farrowing (11). In this complex study several evaluations were completed.

- Objective 1: Evaluate vaccine efficacy in naive animals by comparing groups 4 and 5.
- Objective 2: Evaluate maternal protection via passive transfer by comparing groups 2 and 5.
- Objective 3: Evaluate vaccine efficacy in the face of maternal immunity by comparing groups 1 and 4 (and 5).

The primary efficacy parameter used in this trial was the evaluation of gross and microscopic lesions associated with *L. intracellularis* infection. Using these parameters and comparing the vaccinated pigs and the non-vaccinated pigs from non-immune sows it was demonstrated that there was significant ($P<.05$) reduction in gross and microscopic lesions associated with *L. intracellularis* infection in both the ileum and colon (Table 2).

To evaluate the effects of maternal immunity, pigs derived from immune sows (group 2) and pigs derived from non-immune sows (group 5) were compared following virulent challenge (Table 3). In this case there was a significant ($P<.05$) reduction of gross and microscopic lesions in the ileum and microscopic lesions in the colon in pigs derived from immune sows. This data confirms that maternal immunity can be passively transferred to offspring and can provide a significant level of protection against proliferative enteritis in pigs born to hyper-immunized sows. It should be carefully noted that these sows were exposed to an extreme vaccination regime to induce this immunity and that the passive protection in pigs was noted only at 6 weeks of age and would be expected to decline similar to field situations, eventually leading to naive and susceptible pigs later in life.

Finally, the data were evaluated to determine if vaccination in the face of maternal immunity could induce pro-

Table 1: Summary of experimental design to evaluate maternal immunity

| *Group* | *# Pigs / Group* | *Sow Immune Status* | *Pig Treatment* | *Route* | *Day of Vacc.* | *Challenge* | *Route* | *Day of Chall.* | *Day of Study Term.* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | Hyper immunized | Enterisol® Ileitis | Oral drench | 21 | Yes | IG | 42 | 63 |
| 2 | 20 | Hyper immunized | Placebo | Oral drench | 21 | Yes | IG | 42 | 63 |
| 3 | 10 | Hyper immunized | None | None | None | None | None | None | 63 |
| 4 | 20 | Negative | Enterisol® Ileitis | Oral drench | 21 | Yes | IG | 42 | 63 |
| 5 | 20 | Negative | Placebo | Oral drench | 21 | Yes | IG | 42 | 63 |
| 6 | 10 | Negative | None | None | None | None | NA | None | 63 |

Table 2: Vaccine efficacy in pigs derived from negative sows

| *Group* | *Sow Immune Status* | *Pig Treatment* | *Average Gross Lesion Scores (Ileum)* | *Average Gross Lesion Scores (colon)* | *Average Micro-Lesion scores (Ileum)* | *Average Micro-Lesion Scores (colon)* |
|---|---|---|---|---|---|---|
| 4 | Negative | Enterisol Ileitis | 0.15[e] | 0.05[e] | 0.15[e] | 0.05[e] |
| 5 | Negative | Non-Vaccinated | 2.35[e] | 0.80[e] | 2.42[e] | 1.35[e] |
| 6 | Negative | Non-Vaccinated Strict Controls | 0.00[ϕ] | 0.00[ϕ] | 0.20[ϕ] | 0.00[ϕ] |

[e] Group 4 and 5 comparison is significantly ($P$<.05) different.
[Φ] Group not included in the statistical analysis as indicated in the protocol.

Table 3: Measurement of maternal immunity in pigs derived from immune sows

| *Group* | *Sow Immune Status* | *Pig Treatment* | *Average Gross Lesion Scores (Ileum)* | *Average Gross Lesion Scores (colon)* | *Average Micro-Lesion scores (Ileum)* | *Average Micro-Lesion Scores (colon)* |
|---|---|---|---|---|---|---|
| 2 | Hyper immunized | Non-vaccinated | 0.85[d] | 0.45 | 0.70[d] | 0.55[d] |
| 5 | Negative | Non-Vaccinated | 2.35[d] | 0.80 | 2.42[d] | 1.35[d] |
| 6 | Negative | Non-Vaccinated Strict Controls | 0.00[ϕ] | 0.00[ϕ] | 0.20[ϕ] | 0.00[ϕ] |

[d] Group 2 and 5 comparison is significantly ($P$<.05) different.
[Φ] Group not included in the statistical analysis as indicated in the protocol.

tection above and beyond that provided by the passive immunity. In order to evaluate this hypothesis, groups 1 (immune sows and vaccinated pigs) and group 2 (immune sows and non-vaccinated pigs) were compared to see if the level of protection induced is measurable and significantly different. The data indicates that pig vaccination significantly ($P$<.05) reduced the ileum scores and confirms that pig vaccination does provide added clinical protection even in the face of maternal immunity (Table 4).

This study conclusively confirms the presence and significance of maternal immunity and the ability of this immunity to be passively transferred from sows to pigs at 6 weeks of age. This may in part explain why the disease in pigs typically occurs in older animals, likely after the decay of the maternal immunity. This decay would likely occur both under field conditions after natural exposure as well as with this sow vaccination protocol.

## Conclusions

The evaluation and understanding of the interaction of *Lawsonia intracellularis* and the immune system is in its infancy. The complexity of an obligate intracellular pathogen and the limited reagents available for cell mediated immune study of the pig all provide huge challenges for the research community. Despite this, we do have a solid starting point from which to begin.

*Lawsonia* does induce a humoral immune response, which provides us with a practical tool to study epidemiology, predict exposure timing, and proper timing to initiate intervention strategies. We also know that CMI is very important in the control of an infection and that animals are able to mount a protective immune response either following virulent challenge exposure and/or vaccination with an attenuated live vaccine. Early indications suggest a mucosal immune response is induced although further work is needed in this area. Lastly, we can confirmed that the transfer of maternal immunity from sow to pig does play some role in the control of *Lawsonia* in young pigs, although it is probably limited to the first few weeks of life.

Table 4: Evaluation of pig vaccination in the face of maternal immunity

| *Group* | *Sow Immune Status* | *Pig Treatment* | *Average Gross Lesion Scores (Ileum)* | *Average Gross Lesion Scores (colon)* | *Average Micro-Lesion scores (Ileum)* | *Average Micro-Lesion Scores (colon)* |
|---|---|---|---|---|---|---|
| 1 | Hyper immunized | Enterisol Ileitis | 0.16[b] | 0.26 | 0.35 | 0.15 |
| 2 | Hyper immunized | Non-vaccinated | 0.85[b] | 0.45 | 0.70 | 0.55 |
| 6 | Negative | Non-Vaccinated Strict Controls | 0.00[ϕ] | 0.00[ϕ] | 0.20[ϕ] | 0.00[ϕ] |

[b] Group 1 and 2 comparison is significantly ($P$<.05) different.
[Φ] Group not included in the statistical analysis as indicated in the protocol.



## References

1. McOrist S, Jansi S, Mackie R et al. Reproduction of porcine proliferative enteropathy with a pure culture of ileal symbiont intracellularis. Infect. Immun 1993;61:4286-4292.
2. McOrist S, Mackie R, Lawson G, and Smith D. In vitro interactions of Lawsonia intracellularis with cultured enterocytes. 1997;Vet Microbiol. 54:385-392.
3. Lawson G, Mackie R, Smith D, and McOrist S. Infections of cultured rat enterocytes by ileal symbiont intracellularis depends on host cell function and actin polymerization. 1995; Vet Microbiol. 45:339-350.
4. Holyoake P, Cutler R, Caple I, and Monkton R. Enzyme-linked immunosorbent assay for measuring ileal symbiont intracellularis-specific immunoglobulin G response in the sera of pigs. 1994; J Clin Microbiol 32:1980-1985.
5. Knittel J, Jordan D, Schwartz K, Janke B, Roof M, McOrist S, and Harris D. Evaluation of antemortem polymerase chain reaction and serological methods for detection of Lawsonia intracellularis exposed pigs. 1998; AJVR 59:722726.
6. Guedes R, Gebhart C, Deen J, and Winkelman N. A comparative study of an IFA test and an IPMA for the diagnosis of porcine proliferative enteropathy. 2002; J Vet Diagn 14:528-530.
7. Guedes R, and Gebhart C. Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic isolate or attenuated vaccine strain of Lawsonia intracellularis. 2003; Vet Microbiol 91:135-145.
8. Kroll J, Roof M, and McOrist S. Evalution of protective immunity in pigs following oral administration of an avirulent live vaccine of Lawsonia intracellularis. 2004; AJVR 65:559-565.
9. Walter D, Knittel J, Schwartz K, Kroll J, and Roof M. Treatment and control of porcine proliferative enteropathy using different tiamulin delivery methods. 2001; SHAP 9:109-115.
10. Smith D, Mitchell S, Nash T, Rhind S. Gamma Interferon influences intestinal epithelial hyperplasia caused by Lawsonia intracellularis infection in mice. 2000; Inf and Immun 68:6737-6743.
11. Kroll, J and Roof M. – Manuscript in progress.