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Vaccinating for ileitis

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Introduction

Porcine proliferative enteropathy (PPE) is a common disease of swine caused by the etiological agent *Lawsonia intracellularis* (*L. intracellularis*). Characteristic features of PPE are crypt epithelial cell proliferation, mucosal thickening of the small intestine (sometimes the colon), and presence of small, curved, intracellular organisms in the apical cytoplasm of proliferating cells. Porcine intestinal adenomatosis (PIA) and proliferative hemorrhagic enteropathy (PHE) are chronic and acute forms of the disease, respectively¹.

Lawsonia intracellularis is an obligate, intracellular pathogen that requires a microaerophilic environment for growth. This makes isolation of the organism very difficult and cultivation requires the use of tissue culture systems. The virulence mechanisms of *L. intracellularis* are poorly understood, but the pathogenesis of the organism has been studied. Bacterial transmission is primarily fecal-oral. Upon arrival in the intestinal tract, bacteria gain entry into the host cell by close association with the cell membrane. The bacteria enter via membrane-bound vacuoles, which soon break down and release the bacteria into the host cell cytoplasm. This infection process is host cell dependent and does not require a viable bacterium for the internalization to occur. This entry seems to be microfilament mediated and may be similar to infection by *Chlamydia* species. Once released from the vacuole, *L. intracellularis* then multiplies within the cytoplasm of the cell. Intracellular growth of the bacteria produces little morphologic change or lysis of the cells in tissue culture systems. Bacterial spread can occur during host cell replication or following cell lysis and attachment/internalization of *L. intracellularis* to new cells. In cell culture systems it appears that host cell replication is critical as actively growing cultures are required and most infection appears as foci derived from growing and actively dividing cells¹.

In reviewing the pathogenesis of *L. intracellularis*, the following would appear to be significant considerations in the “rational” design of a vaccine to control PPE:

- Fecal-oral route of transmission with infection primarily in the intestinal tract—induction of a mucosal

immune response may be important in preventing attachment and invasion.

- *L. intracellularis* is an obligate intracellular pathogen—induction of a cellular immune response may be important in the killing and control of infected cells.

Virulence factors are not well defined and so the potential significance of a systemic immune response is unknown. There is research being conducted to investigate the potential of a hemolysin associated with *L. intracellularis*, which may have systemic immune implications².

In considering the potential options for vaccination against *L. intracellularis* there are four potential options to consider:

- **Killed vaccines.** In general, killed bacterins tend to be injected IM, ID, or SQ and induce primarily a humoral response.
- **Modified live vaccines.** Modified or attenuated live vaccines have the ability to induce a humoral and cellular response. Depending on the route (IN, oral), induction of a mucosal response may be possible as well.
- **Subunit and/or recombinant vaccines.** Subunit and/or recombinant vaccines have the potential to target specific immunogens or virulence factors. Theoretically, they may be designed to induce a specific immune response.
- **DNA vaccines.** DNA vaccines have the potential to target specific immunogens and induce both a cellular and humoral response. At the present time practical applications of DNA vaccination in swine is limited.

Evaluation of potential vaccines requires a standardized and reproducible challenge model. For *L. intracellularis*, there are currently two different models being used to evaluate vaccines as well as antibiotics. The first model is a pure culture challenge model using purified *L. intracellularis*, which is then grown in a tissue culture system. This model using various challenge strains has been previously described^{3,4,5} and typically causes mild

clinical disease, consistent microscopic lesions associated with *L. intracellularis*, and gross lesions that range in prevalence from 5–60%. The advantage of the pure culture model is that the titer of the challenge material can be precisely determined and there are no confounding factors associated with other flora or agents. The second model is a gut homogenate challenge model. This uses intestinal scrapings from gross lesions associated with *L. intracellularis*, which are then collected and frozen as a challenge stock. This model tends to cause more severe clinical disease, with some reports of mortality in challenge control pigs⁶.

The final important considerations in vaccine efficacy are the criteria and parameters to be tested and monitored during the study. The commonly used evaluation parameters in *L. intracellularis* vaccine efficacy trials include clinical scoring, average daily gain (ADG), microscopic lesions, macroscopic lesions, seroconversion, and *L. intracellularis* fecal shedding.

The measurement of ADG is a major factor as *L. intracellularis* causes severe performance problems especially in the finisher. Improvements in feed efficiency are critical so that veterinarians and practitioners can measure the potential benefits associated with vaccination and their return on investment associated with vaccine cost.

The evaluation of microscopic lesions is usually done with H&E or Silver stains. These look for histologic lesions associated with intracellular bacteria. The use of a monoclonal antibody specific to *L. intracellularis* has recently allowed fluorescent antibody (FA) and immunoperoxidase (IP) staining of tissue sections⁷. This has the added advantage of not only identification of histological lesions, but *L. intracellularis* specific detection. Microscopic lesions can then be subjectively scored in terms of relative severity. Gross lesions can also be evaluated. This again has been done both qualitatively and quantitatively. Care must be taken to distinguish between *L. intracellularis* and non-specific enteritis. Most investigators require gross lesions to be confirmed *L. intracellularis*-positive by a second method such as FA, IP, or PCR.

There have been major advances in in vitro techniques to monitor and detect *L. intracellularis* infected pigs. These advances include serological assays and polymerase chain reaction (PCR). Qualitative seroconversion can be measured by using *L. intracellularis*-infected tissue culture plates which are then exposed to swine serum (1:30) followed by a fluorescent or immunoperoxidase conjugate to detect IgG antibody⁸. The PCR assay can be used ante-mortem on fecal samples or on tissue post-mortem to detect the presence of *L. intracellularis* genetic material⁹. Both assays are critical in screening animals to insure susceptibility prior to use in a vaccine trial and secondly to monitor exposure and shedding following vaccination and challenge.

At the present time, there is one commercially available vaccine licensed by the USDA. *Lawsonia Intracellularis* Vaccine, Avirulent Live Culture (Enterisol[®]) Ileitis, Boehringer Ingelheim Vetmedica Inc) is administered orally as a 1 ml dose per pig in the drinking water. It is recommended for the vaccination of healthy, susceptible pigs 3 weeks of age or older as an aid in the prevention and control of porcine proliferative enteropathy (ileitis) caused by *L. intracellularis*.

The most important component of a *L. intracellularis* control program is management. Use of antibiotics is common and vaccine use has recently been initiated. The following section briefly describes studies evaluating a killed bacterin vaccine and a heterologous efficacy study using an avirulent live vaccine.

Data and results

Study #1: Killed bacterin versus avirulent live culture¹⁰

This study evaluated and compared the relative efficacy of an experimental avirulent live culture *L. intracellularis* vaccine (ALC) versus an inactivated *L. intracellularis* bacterin. The study was conducted in healthy 3-week-old pigs and used a homologous virulent challenge (10⁶ per pig). (Table 1)

Table 1

| Group | Number | Day 0 Trt | Day 14 Trt | Day 31 | Day 52 |
|-------|--------|-----------|------------|----------------|----------|
| 1 | 10 | ALC | None | Vir. Chall* | Necropsy |
| 2 | 10 | Bacterin | Bacterin | Vir. Chall* | Necropsy |
| 3 | 10 | None | None | Vir Chall* | Necropsy |
| 4 | 5 | None | None | None | Necropsy |

* virulent challenge strain administer intragastric.

The parameters evaluated in this study included PCR evaluation of the ileum and colon, microscopic lesions, gross lesions (scale of 1–3 with 1 being normal, 2 moderate, 3 severe), and average daily gain post-challenge.

Results

Prior to challenge all of the pigs in groups 1 and 2 had seroconverted to *L. intracellularis* as tested by IFA. All of the control animals remained seronegative. This suggests that seroconversion may be an indicator of exposure but is not an accurate indicator of protection following vaccination or challenge. The avirulent live vaccine had no detection of *L. intracellularis* in the tissues, few gross lesions, and the best ADG. The killed bacterin was not differentiable from the challenge controls and did not provide adequate protection in this trial. (Table 2)

Study 2: Heterologous Efficacy Study¹¹

This study consisted of four experimental groups containing pigs at 3 weeks of age. On day 0, group 1 (n=15) received a single oral dose of a frozen *L. intracellularis* vaccine (Enterisol Ileitis). Group 2 (n=15) received a single oral dose of an experimental lyophilized vaccine. Group 3 (n=10) were challenge controls and received an oral dose of sterile diluent. Group 4 (n=5) were strict controls and received no treatment. On day 21, all pigs in groups 1–3 received a 10 ml dose of a heterologous viru-

lent pure culture challenge (strain N101494). The challenge was delivered via gastric lavage. Pigs were monitored for clinical signs (5 parameters evaluated on a scale of 1–3 with total score of 5=normal), fecal shedding, seroconversion, and average daily gain (ADG). On day 52, pigs were necropsied and examined for gross and microscopic lesions associated with *L. intracellularis*.

Following vaccination, there was no detectable seroconversion or fecal shedding of *L. intracellularis*. After virulent challenge exposure, both vaccine groups had more seropositive animals than did the challenge controls. Fecal shedding of *L. intracellularis* was also lower in both vaccinated groups. Clinical scores for both vaccine groups remained normal (score of 5) post-challenge. The challenge control animals did have diarrhea and significantly ($P<.05$) higher clinical signs were noted.

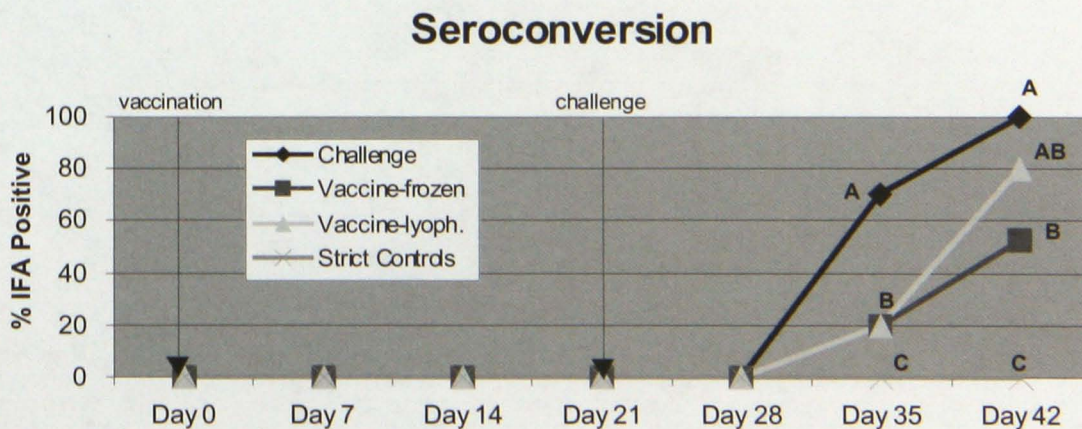
The challenge controls had a post-challenge ADG of 0.33 which was significantly ($P<.05$) lower than both vaccine groups and the strict controls which had ADGs from 1.27–1.48 lb/day.

Gross lesions and *L. intracellularis* colonization of gut tissue was consistent with the clinical and weight data. Challenge controls had significantly ($P<.05$) higher levels of gross lesions and prevalence of colonization (80% positive) when compared to the vaccinated groups (less than 40%).

Table 1

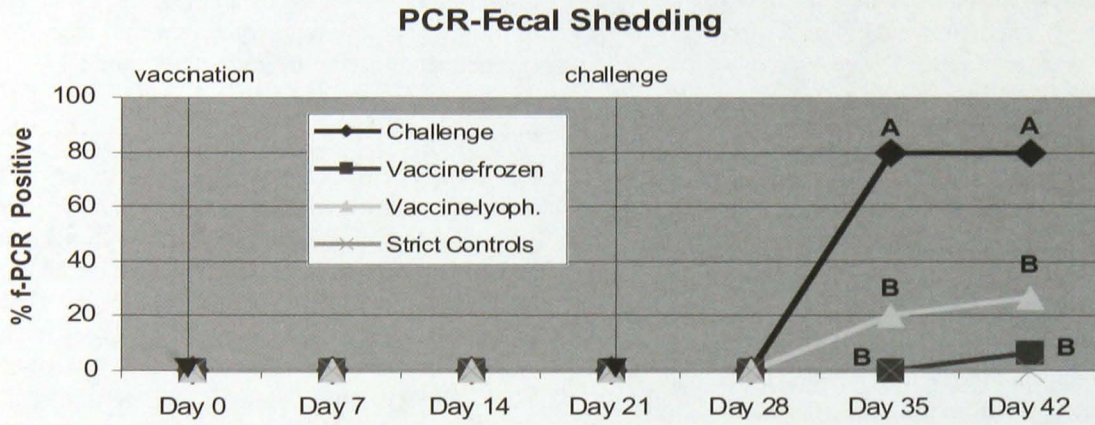
| Group | Ileum PCR | Colon PCR | Micro Lesion | Gross Lesion | ADG-PC |
|--------------|-----------|-----------|--------------|--------------|--------|
| 1 (ALC) | 0/10 | 0/10 | 0/10 | 1.1 | 1.22 |
| 2 (Bact) | 4/10 | 1/10 | 1/10 | 1.9 | 0.97 |
| 3 (Chall C) | 3/10 | 1/10 | 8/10 | 2.6 | 1.18 |
| 4 (Strict C) | 0/5 | 0/5 | 0/5 | 1.0 | 1.05 |

Figure 1. Seroconversion



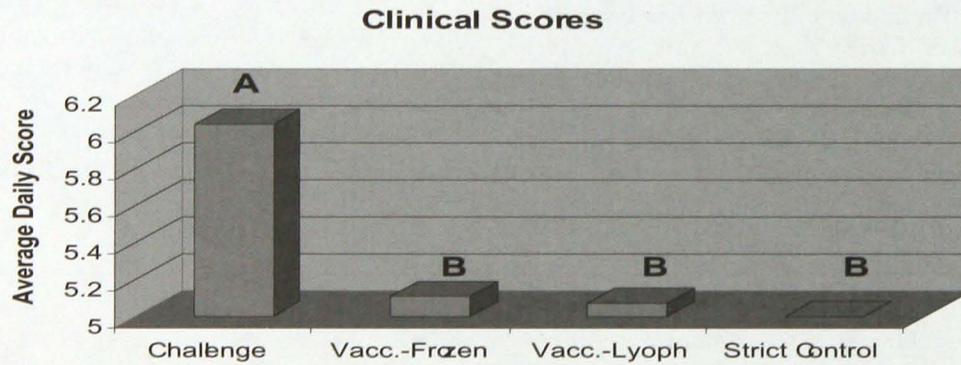
Like letters are not statistically ($P<.05$) different

Figure 2. PCR-fecal shedding



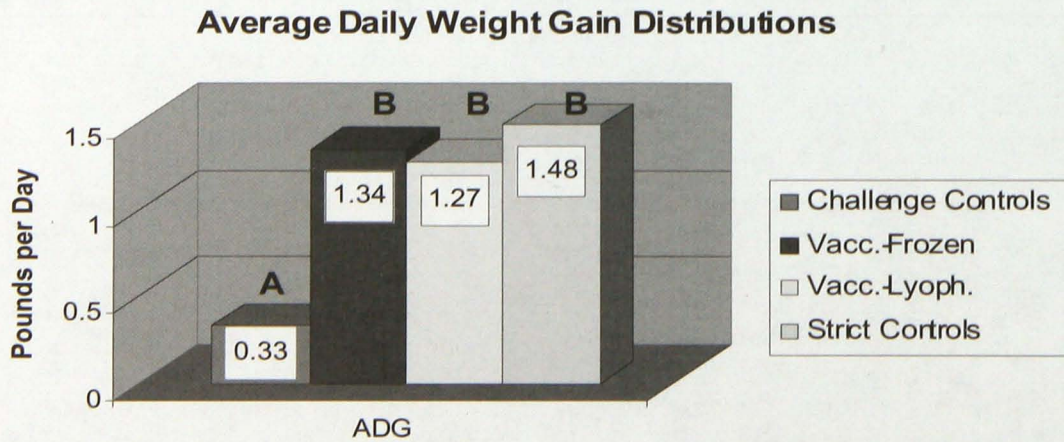
Like letters are not statistically ($P < .05$) different

Figure 3. Clinical scores



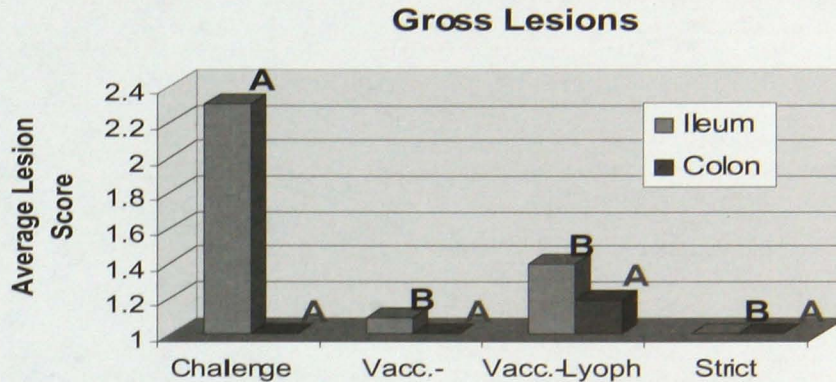
Like letters are not statistically ($P < .05$) different. Normal clinical score = 5.

Figure 4. Average daily weight gain distributions



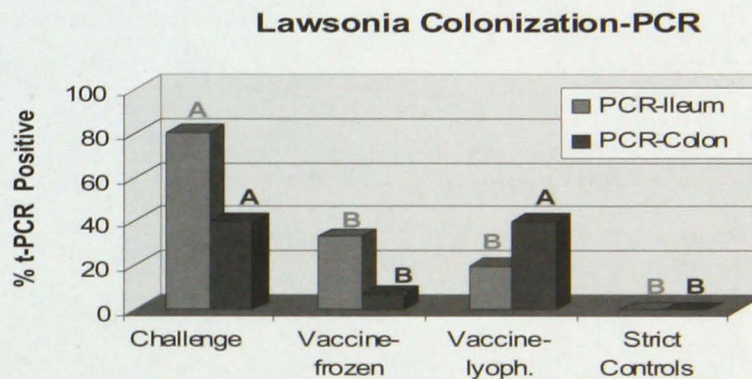
Like letters are not significantly ($P < .05$) different

Figure 5. Gross lesions



Like letters are not significantly ($P < .05$) different

Figure 6. Lawsonia colonization PCR



Like letters are not significantly ($P < .05$) different

Discussion

As this summary shows, avirulent live vaccines can be effective tools in the prevention and control of *L. intracellularis* in a research setting. Practical experiences with applications of vaccines under field conditions are on-going and more information is being obtained daily.

At the present time, the three major issues associated with vaccine use are

- timing,
- antibiotic removal, and
- heterologous protection.

Timing is a critical issue for both vaccines and antibiotics. Most successful field use has involved cross-sectional or longitudinal monitoring to determine when *L. intracellularis* exposure becomes detectable serologically or clinically. Use of fecal PCR and/or serology in the nursery, grower, and finisher have been used to determine when pigs actively shed the organism or seroconvert.

Vaccination is recommended at least 3 weeks prior to this to allow protective immune response to develop.

With the current avirulent live *L. intracellularis* vaccine, vaccination should not be done in the face of antibiotics. Removal of antibiotics 2 days prior to vaccination and then 5 days post-vaccination is recommended to ensure an effective immune response is induced¹².

This study reports experimental data supporting that vaccines can provide protection against heterologous protection. Based on growth characteristics, growth requirements, PCR detection, and protein profiles, the available isolates all appear to be homologous antigenically¹³.

At present many operations are using vaccine in the gilt developer to immunize animals before they enter the breeding herd. The goal is to stop clinical disease and mortality often seen in newly introduced gilts after the stress of shipment from the source farm and arrival at the recipient farm. It is also hoped that stabilization of the sow herd may lead to fewer problems in offspring and the growing pig population.

At the current time, many labs continue to investigate *L. intracellularis* pathogenesis and controls options, including on the following areas of concern:

- role of maternal immunity
- pathogenesis
- immune response associated with *L. intracellularis* exposure (cellular, mucosal, humoral)
- genetic characterization and evaluation of potential virulence factors/immunogens
- improved diagnostic tools
- role of *L. intracellularis* in enteric disease in species other than swine.

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