RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Impact of vaccination on transmission of *Lawsonia intracellularis* in pigs – **NPB #17-131**

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Introduction

Studies determining transmission rates and probability of infection in naïve and vaccinated animals proven to have great value in driving important decisions to control important swine diseases, such as influenza and *Mycoplasma hyopneumoniae* (Romagosa et al 2011 and Roos et al 2016). The broad objective of the present research project is to evaluate differences in growth performance, transmission patterns of PE and quantify the spread of *L. intracellularis* in naïve and vaccinated pigs by estimating the transmission rate and expected probability of infection.

Research objectives

1. Determine the transmission rate for *L. intracellularis* according to the susceptible-infectious model in naïve, orally vaccinated or intramuscularly vaccinated pigs after contact with an infectious animal.

2. Estimate the expected probabilities of a naïve, orally vaccinated or intramuscularly vaccinated animal becoming positive for *L. intracellularis* after contact with an infectious animal.

3. Compare transmission patterns, fecal shedding and clinical signs (diarrhea scoring) of *L. intracellularis* in naïve, orally vaccinated and intramuscularly vaccinated pigs after contact with an infectious animal.

4. Compare growth performance based on average of daily gain and feed conversion in naïve, orally vaccinated and intramuscularly vaccinated pigs after contact with an infectious animal.

Experimental design

A total of 99 healthy crossbred animals were obtained from a known *L. intracellularis* negative source. Twenty-seven pigs per treatment were allocated and divided in 3 pens (9 pigs/pen). Eighteen seeder pigs were housed in a different barn.

- On study day 0, after 2 days of acclimation, the 27 pigs receiving intramuscular vaccine (Porcilis Ileitis) received 2 mL in the right side of the neck. Pigs receiving oral vaccine (Enterisol Ileitis) were orally drenched with 1 mL of life modified vaccine.

- On study day 21, the 18 seeder pigs were orally challenged via intragastric gavage with a 10^8 dose of *L. intracellularis* homogenate diluted into 40 ml of sterile carrier buffer.

- On study day 28, the 9 seeder pigs with lower ct-values, were allocated one in each pen to start the transmission assessment period.

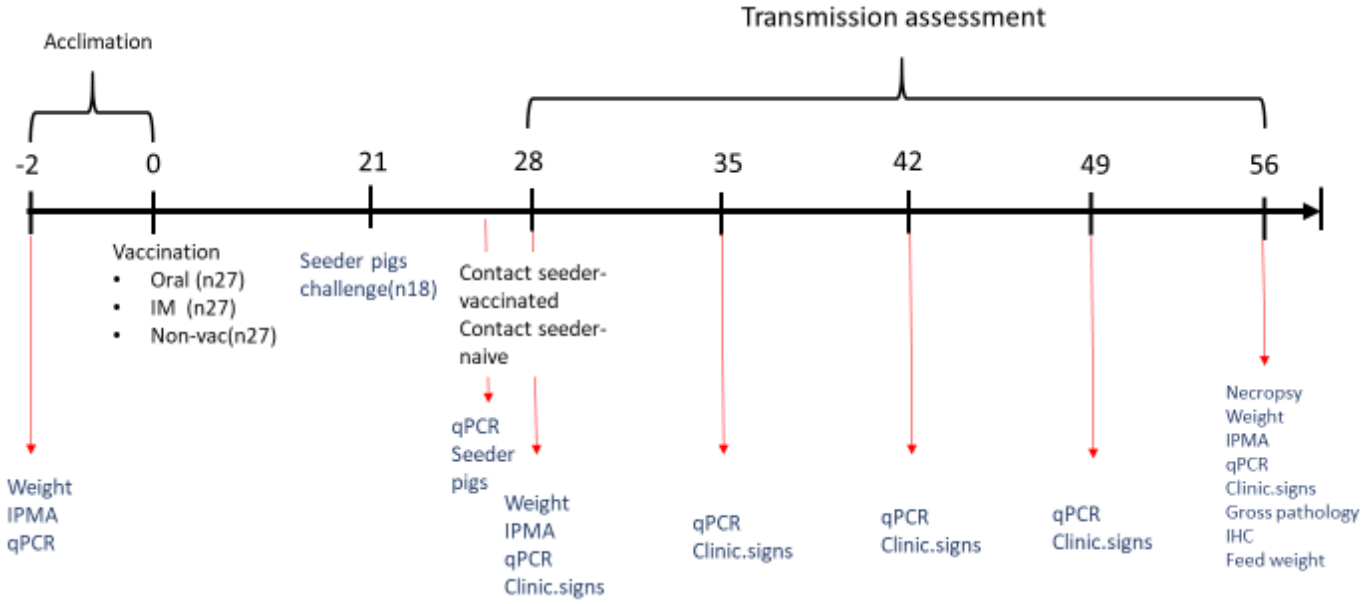

Figure 1 – Representation of the experimental design and timeline (days).

Results

Serology

All the pigs were *L intracellularis* specific antibodies negative at the beginning of the study. On day 28 after vaccination, the intramuscular vaccine showed to induce *L. intracellilaris* specific antibodies in the 62% (17/27) of the pigs, significantly higher than the other 2 treatments ($p=<0.01$), with a mean reciprocal titer between 1:30 to 1:480, the mean *L intracellularis* IPMA titer was 91.15 ±115.56, while the oral vaccine induced *L. intracellularis* antibodies in 33% (1/27) of the pigs, with a mean reciprocal titer of 1:30. In the non-vaccinated group, all pigs were still negative at this time point. At necropsy, the intramuscular vaccinated group showed a robust *L intracellularis* antibodies in the 100% (27/27) of the pigs, significantly higher than the other 2 treatments ($p=<0.01$) with a mean reciprocal titer between 1:30 to 1:7680, the mean *L intracellularis* IPMA titer was 931.11±1803.46. The oral vaccinated group showed *L. intracellularis* specific antibodies in the 50%(13/26) of the pigs, with a mean reciprocal titer between 1:30 to 1:480, the mean *L intracellularis* IPMA

titer was 88.84±123, while the nonvaccinated group showed *L. intracellularis* specific antibodies in the 25%(7/27) of the pigs, with a mean reciprocal titer between 1:30 to 1:480, the mean *L intracellularis* IPMA titer was 52.22±115 (Figure 1)

The serology results from the vaccinated groups demonstrated the booster effect characterized by a robust systemic humoral response more remarkably observed in the group vaccinated with the intramuscular vaccine.

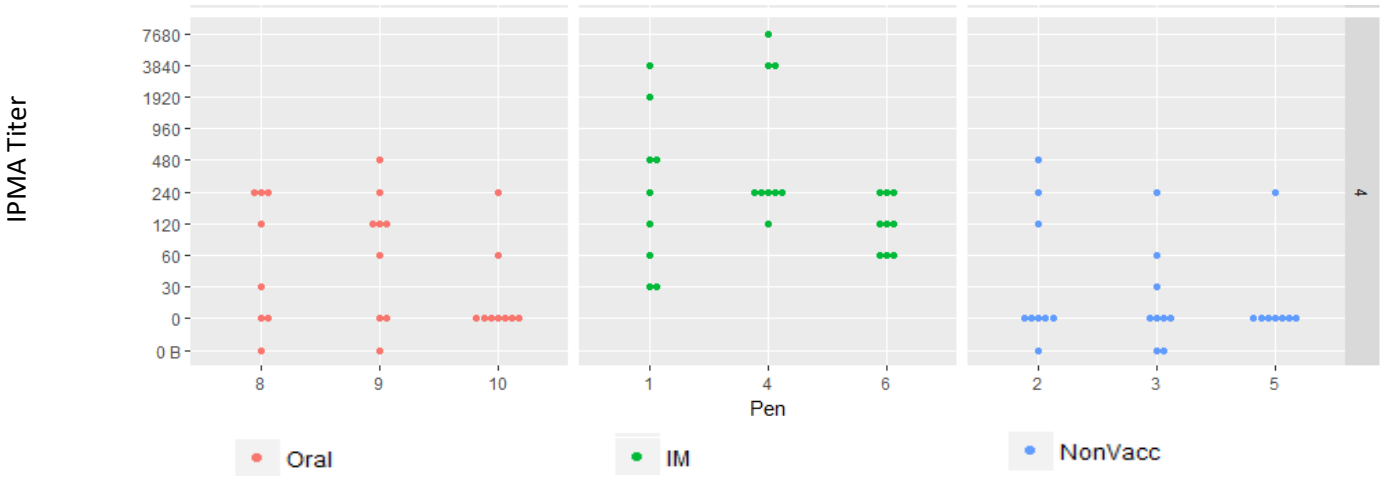

Figure 1 – Serological humoral response 28 days after contacting with seeder-infectious pigs.

Clinical signs

The abdominal appearance and demeanor were normal (score 0), during the entire study period in the 3 treatment groups. One animal was removed from the oral vaccinated group at week 2 of the transmission assessment period. On week 0 of the transmission assessment period, 0.11% (3/27) orally vaccinated pigs, showed fecal score of one, while the oral and non-vaccinated groups remained negative. On week 1 of the transmission assessment period 22%(6/27) intramuscular vaccinated pigs, 11% (3/27) orally and non-vaccinated pigs showed fecal scores between 1 and 2. No statistical difference among the groups. On week 2, 40%(11/27) intramuscular vaccinated, 34%(9/26) orally vaccinated and 29%(8/27) non-vaccinated pigs had fecal scores between 1 and 4. No statistical difference among groups. On week 3 of the transmissions assessment period. On week 3, 55%(15/27) intramuscular vaccinated pigs, 26%(7/26) orally vaccinated and 40%(11/27) non-vaccinated pigs had fecal scores between 1 and 4. No statistical difference among groups. On week 4, 37%(10/27) intramuscular vaccinated, 11%(3/26) orally vaccinated and 25%(7/27) non-vaccinated pigs had fecal scores between 1 and 4. No statistical difference among groups. (Figure 2)

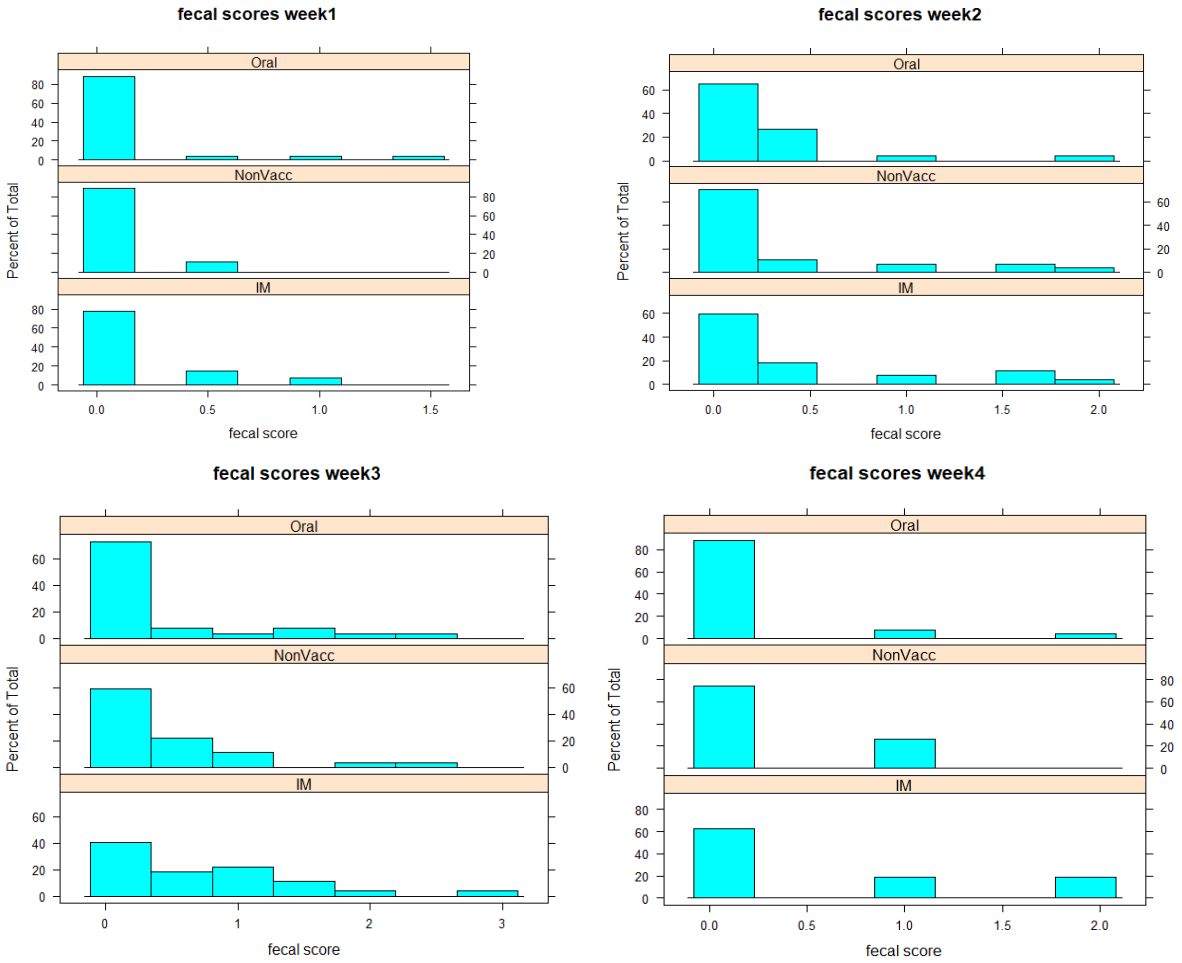

Figure 2 – Fecal score among the experimental groups after contacting with the seeder-infectious pigs.

ADG and FCE

The mean average daily gain was 0.61Kg in the nonvaccinated animals, while in the orally and intramuscular vaccinated was 0.57Kg. There was statistical difference between the intramuscular and non-vaccinated treatments (*p=0.029*) and no statistical difference between the two vaccinated treatments.

The mean feed conversion efficiency in the intramuscular vaccinated group was 2.33±0.39, in the orally vaccinated group was 2.36±0.31, and in the non-vaccinated group was 2.27±0.21. No statistical difference among groups.

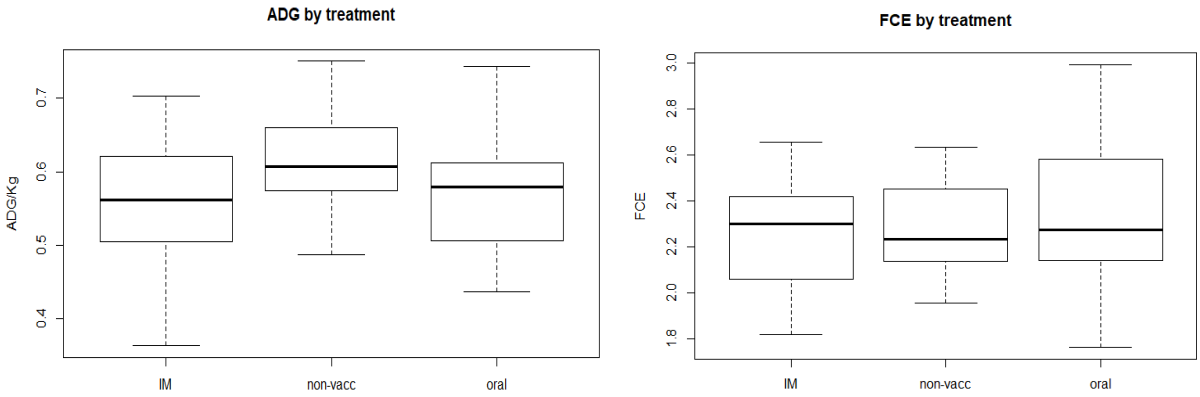

Figure 3 – ADG and FCE among the experimental group after contacting with the seeder-infectious pigs.

Quantitative PCR (qPCR)

The average number of bacteria in the intramuscular vaccinated animals was $5.9x10^3$, in the orally vaccinated animals was $6.4x10^3$ and in the non-vaccinated animals was $1.1x10^3$. There was no statistical difference no statistical difference among group treatments (Figure 4).

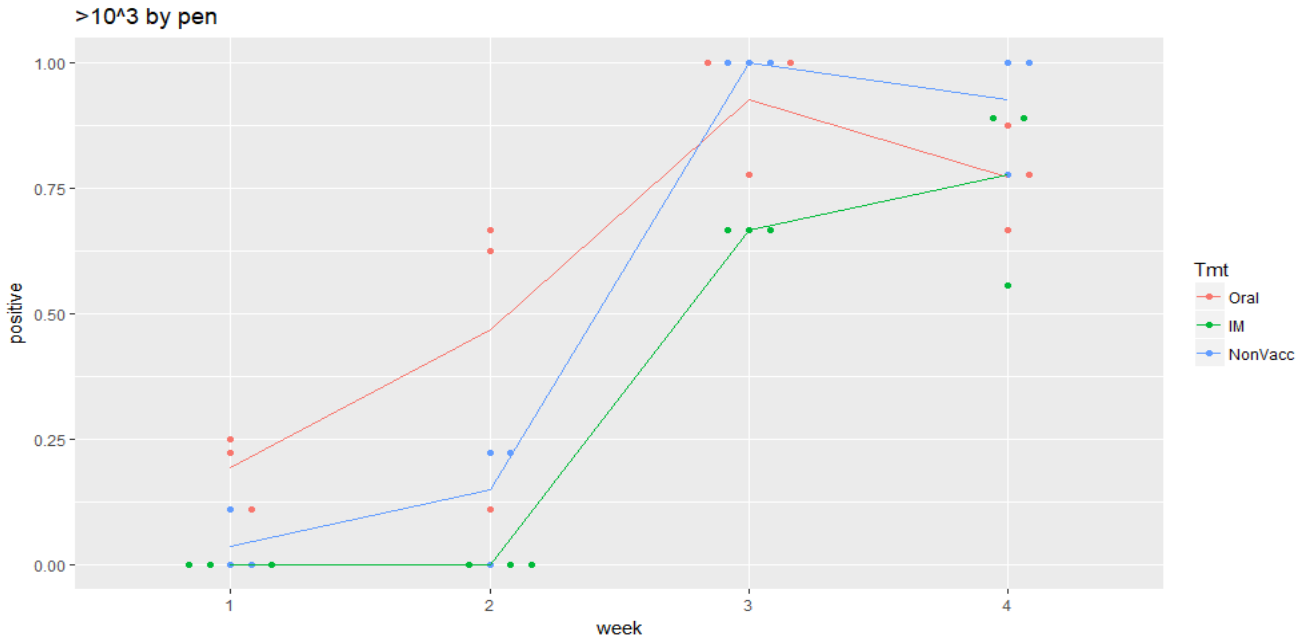

Figure 4 – Fecal shedding ($>10^3$) of *L. intracellularis* based on qPCR after contacting with the seeder-infectious pigs.

Transmission rate and probability of transmission

The transmission rate or incidence rate was calculated based on the minimal infectious dose 10^3 reported by Collins et al (2001). Therefore, animals were considered infectious if fecal PCR was equal or more than 10^3 *L. intracellularis*/g of feces. Using the method described by Velthuis et al. (2003), the transmission rate per week for non-vaccinated animals was 3.6. This indicates that one infectious animal introduced in a susceptible population are able to transmit the infection to 3-4 animals within a week.

In order to evaluate the impact of vaccination on the transmission of *L. intracellularis*, parametric survival analysis with censoring (time from shedding *L. intracellularis* $\geq 10^3$ to shedding below this level) were performed. A Weibull parametric survival analysis with standard errors adjusted for clustering at the pen-level, was conducted to evaluate the association of the length of the time of shedding $\geq 10^3$ according to the experimental groups (oral vaccination, IM vaccination, or no vaccination). The median length of *L. intracelullaris* shedding in the pigs administered the oral vaccine was 43.3% shorter than the median length in those receiving no vaccine. While the median length of *L. intracelullaris* shedding in the pigs administered the IM vaccine was 25.5% shorter than the median length in those receiving no vaccination (Figure 5).

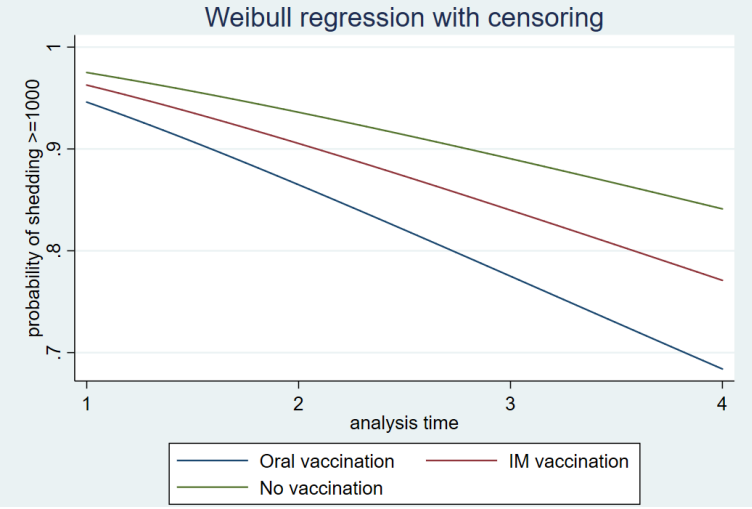

Figure 5 - Proportion of pigs shedding *L. intracellularis* $\geq 10^3$ by length of shedding.

Finally, the probability of transmission was evaluated using a generalized estimating equation (GEE) model. The model performed based on the probability of shedding more than 10^3 *L. intracellularis* per gram of feces as a function of treatment and time of follow-up. The adjusted predicted probability of shedding $\geq 10^3$ *L. intracellularis* was higher in pigs receiving the oral vaccine compared with those receiving the IM vaccine within the 4 weeks of contact with the susceptible population (Figure 6). However, at the week 4 after contacting both vaccinated groups showed similar probabilities, while the non-vaccinated groups showed higher odds of shedding.

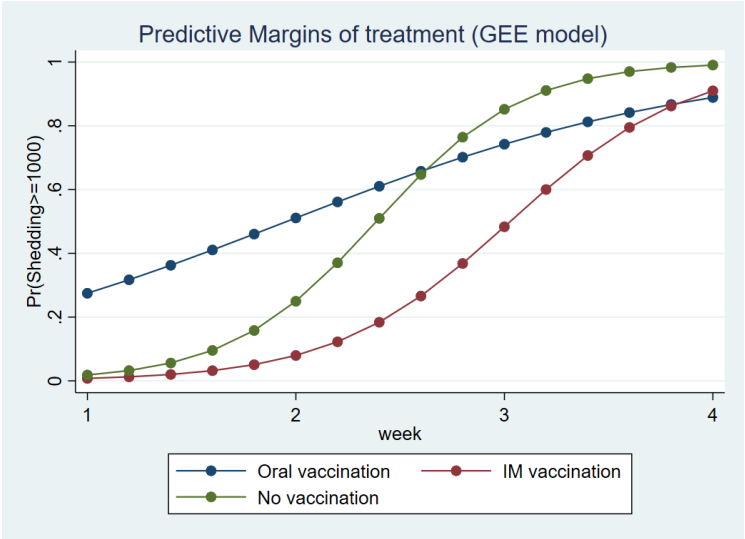

Figure 6 - Adjusted predicted probabilities of shedding $\geq 10^3$ *L. intracellularis* by group and time.

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