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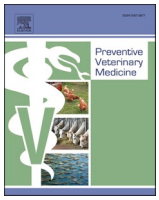

Effect of intramuscular vaccination against *Lawsonia intracellularis* on production parameters, diarrhea occurrence, antimicrobial treatment, bacterial shedding, and lean meat percentage in two Danish naturally infected finisher pig herds

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ABSTRACT

Lawsonia intracellularis (LI) is an economically important enteric pathogen in pigs with a worldwide endemic prevalence. The objective of this study was to evaluate the effect of an intramuscularly administrated LI vaccine (Porcilis®Lawsonia Vet.) in Danish finisher pigs (30–115 kg) measured on key production figures, antimicrobial (AB) treatments, occurrence of diarrhea and LI shedding.

The study was a group-randomized block-trial with parallel groups in two herds, Herd 1 and Herd 2, experiencing a natural subclinical-clinical LI infection in early finisher period. Vaccination occurred at weaning, but the study focused on the first eight weeks in the finisher period. Further, slaughterhouse data were included.

In total, 52 and 50 finisher pens comprising 2184 and 2254 finisher pigs were included in each of two herds, respectively. LI vaccination significantly reduced feed conversion ratio (FCR) by 0.05 and 0.09 FU/kg ($p = 0.007$ *and* $p < 0.001$) alongside a significantly increased average daily weight gain (ADWG) by 31 and 43 gr/day ($p = 0.001$ *and* $p < 0.001$) in each of the herds, respectively. In the vaccinated group, less variation was found in ADWG compared to the control group ($p < 0.001$ in both herds) as an expression of a more uniform growth, which was further confirmed by less variation in lean meat percent in the vaccinated group in one herd ($p = 0.007$). No significant difference between groups were found in mortality and pigs excluded due to welfare reasons. AB flock treatment against diarrhea was significantly reduced in Herd 1 with all pens treated in the control group compared to 30.8 % in the vaccinated group ($p < 0.001$). In Herd 2, the difference was non-significant with 68.0 % in the control group compared to 50.0 % in the vaccination group ($p = 0.252$). Low levels of individual treatments against diarrhea were seen in both herds ($\leq$ 5.0 %) but still significantly reduced in vaccinated pigs compared to control pigs ($p < 0.050$ in both herds). Mean diarrheic blot counts were significantly reduced in vaccinated pens compared to control pens ($p < 0.001$ in both herds). In vaccinated pigs, shedding of LI was reduced in both prevalence ($p < 0.001$ in both herds), excretion level in positive samples ($p < 0.001$ in both herds) and, in one herd, also in duration ($p = 0.003$) when compared to control pigs.

In conclusion, pigs vaccinated with Porcilis®Lawsonia Vet against LI in both of two high-health and high-productive finisher herds had, compared to non-vaccinated pigs, significantly improved key production figures, and reduced AB treatment, occurrence of diarrhea, LI shedding, and growth variation.

Abbreviations: LI, Lawsonia intracellularis; PPE, Proliferative Enteropathy; FCR, Feed Conversion Ratio; ADWG, Average Daily Weight Gain; AB, Antimicrobial usage; SPC, Summary of Product Characteristics; L, Y, D, Danish Landrace, Yorkshire, Duroc (breeds); SPF, Specific Pathogen Free (Danish SPF Health declaration system); FU, Feeding Unit (a measurement of the nutritional value of the feed, 1 FU = 7.38 MJ); PCR, Polymerase Chain Reaction; AUC, Area Under the Curve.

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1. Introduction

Lawsonia intracellularis (LI) is an economically important enteric pathogen in pigs with a worldwide endemic prevalence (Kroll et al., 2005; Lawson and Gebhart, 2000; Vannucci et al., 2019). LI infection may cause porcine proliferative enteropathy (PPE) resulting in increased feed conversion ratio (FCR), reduced average daily weight gain (ADWG) and/or reduced group uniformity despite absence of diarrhea (Kroll et al., 2005; Vannucci et al., 2019). Procedures to control LI to reduce the costs of the infection include management procedures, hygiene measures, rodent and insect control, antimicrobial usage, and vaccination (Kroll et al., 2005; Vannucci et al., 2019). Attempts to eradicate LI have been described but have largely been unsuccessful (McOrist and Gebhart, 2012). With the still increasing demand for reduction of antimicrobial usage, vaccination becomes still more relevant. In 2001, the first live attenuated LI vaccine for oral administration (Enterisol®Ileitis) was marketed. With more than twenty years in the market, several studies have found increased ADWG when introducing the vaccine (Almond and Bilkei, 2006; Bak and Rathkjen, 2009; Hardge et al., 2004; Jacobs et al., 2019, 2020; Kroll et al., 2004; McOrist and Smits, 2007a; Park et al., 2013; Peiponen et al., 2018; Visscher et al., 2018) while only few studies have evaluated the vaccine's effect on FCR ranging from no difference following vaccination (Hardge et al., 2004; Peiponen et al., 2018; Visscher et al., 2018) to diverging results between farms (McOrist and Smits, 2007a). In 2015, a second inactivated LI vaccine for intramuscular administration was marketed in the US (Porcilis®Ileitis) followed by a European version in 2019 (Porcilis®Lawsonia) and an intradermally administered version in 2021 (Porcilis®Lawsonia ID). So far, no concluded field trials evaluating the impact of Porcilis®Lawsonia have been published but under experimental conditions increased ADWG, reduced intestinal lesions, and reduced LI shedding were found (Jacobs et al., 2019). The aim of this study was therefore to evaluate the effect of the intramuscularly administrated LI vaccine in Danish finisher pigs. The effect was measured on production parameters with special focus on the economically important parameter FCR. A field trial was conducted in finisher pigs (30–115 kg) experiencing a subclinical-clinical LI infection. There were three objectives: 1) Comparing FCR, diarrhea occurrence and antimicrobial (AB) usage at pen level; 2) Comparing ADWG, antimicrobial (AB) usage, mortality, and LI shedding (prevalence, excretion level, duration) at pig level; and 3) Comparing variation in lean meat percentage at group level as a measurement of uniformity within treatment groups.

2. Material and methods

2.1. Study design

The study was a group-randomized block trial in herds experiencing a natural LI infection with pigs allocated into parallel treatment groups being either a vaccinated group or a non-vaccinated control group. Pigs in the vaccinated group were vaccinated against LI using Porcilis®Lawsonia Vet., which is an inactivated freeze-dried bacterial antigen one-dose vaccine for intramuscular administration (Intervet International, 2021). The study had a hierarchical order with pigs nested within pens and because several batches were needed to reach the samples size, pens were nested within batches. To account for possible clustering, treatment groups were block randomized within each batch. Treatment group allocation was blinded to herd manager and herd personnel. The study pigs were followed from weaning to slaughter, but only with the finisher period being of interest. However, individual faecal samples were collected also in the weaner period to ensure sampling concurrent with the time of LI infection. All trial procedures, measurements and registrations in the study were performed by the study investigator unless otherwise described. The trial was conducted in two commercial Danish pig herds as two repetitions of the full trial. In both, Herd 1 and Herd 2, consent for being in the trial were given by signed agreements. The trial was approved by the Danish Medicines Agency (2020022825) and was conducted in accordance with Good Clinical Practice Procedures as given by VICH Topic GL 9 (GCP) Guidance on Good Clinical Practices (EMA, 2000).

2.2. Study herds and pre-trial diagnostics

The study herds were included due to a history of LI infection in finisher pigs causing subclinical or clinical PPE and based on being well managed without fluctuations in health declaration. Both herd complexes consisted of separate weaner and finisher herds with a transfer of pigs at around 30 kg equivalent to around 12 weeks of age and with the possibility to measure feed consumption at pen level in the finisher herds. Within each finisher herd, the barn house sections were identical with pens separated by solid walls at ground level. Herd 1 transferred 600 LY/D breed pigs (Danish Landrace-Yorkshire/Duroc) to the finisher unit every two weeks where single pens housed 21 pigs each. The purchased feed was fed as liquid feed in long troughs spacing 33 cm per pig. Herd 2 transferred 460 pigs to the finisher unit every two weeks where single pens housed 23 pigs each. The breed was LY/D but with a minor part of the castrates being L/Y due to own production of replacements gilts in the sow herd. The L/Y breed pigs could be identified from the LY/D breed pigs. The feed was home mixed liquid feed fed in long troughs spacing 26 cm per pig. In both herds, the feed contained 2 ppm organic acid (formic acid) and was fed four times per day and feed formula as well as feeding procedures were kept unchanged during the study period. The density of pigs in pens corresponded to 0.65 m^2 per fully grown slaughter pig assuming full occupancy, pen floor was partly solid concrete and partly slatted tiles, and the pens were washed, disinfected, and kept empty for drying for at least two days between batches. Both herds were part of the Danish SPF Health declaration system and were declared free of *Actinobacillus pleuropneumoniae,* PRRS2, *Brachyspira hyodysenteriae, Pasteurella multocida, Sarcoptes Scabiei var. Suis and Haematopinus suis.* Opposed to Herd 2, Herd 1 was not declared free from PRRS1 but was regarded virus free. This was supported by no seroconversion to PRRS1 found by random sampling of pigs prior to slaughter in any of the finisher batched included in the study. Infections of porcine circovirus type 2 and *Mycoplasma hyopneumoniae* were controlled by piglet vaccination.

Prior to trial inclusion, a series of laboratory diagnostics in consecutive batches were performed in the herds including both faecal sock sampling (Pedersen et al., 2015) and individual blood sampling to determine prevalence and onset of LI infection and to confirm the health status. For both herds, shedding of LI was detected no sooner than in the final week prior to transfer to the finisher unit. The LI excretion levels were above 5.6 log(10) copies/gram of faeces in sock samples in at least one of the samples collected from consecutive batches in the finisher unit. To check for unexpected early LI infection and to confirm health status, diagnostics were performed in terms of faecal sock sampling and blood sampling in every batch during the trial in both weaner and finisher herds.

2.3. Trial procedures

At weaning in each batch, pigs were individually ear tagged and allocated in pens according to weight and sex and in Herd 2 also according to breed. The pens were subsequently block-randomized in trial group blocks accordingly, and decision on which pen to be in the vaccinated group in the trial group block was decided by coin-tossing. At weaning, pigs in the vaccinated group received 2 ml of the combined Porcilis® PCV M Hyo + Porcilis® Lawsonia Vet. vaccine, whereas pigs in the control group received 2 ml of Porcilis® PCV M Hyo vaccine, all administered intramuscularly. Location of pigs in pens or batches, removals or deaths were validated by the individual, electronic PigID read by a handheld scanner (Atid, AT870).

At the day before transfer to the finisher unit, all pigs appointed for

transfer by the weaner herd manager were weighed to account for the weight at entry. At arrival to the finisher unit, the pigs in each batch were reallocated in pens by block-randomization according to treatment group, sex, and transfer weight to account for the confounding effect of these factors on the outcome variables. Equal number of pigs and pens were allocated in each treatment group. Single-sex pens housing only either females or castrates were preferred but to include as many pens as possible in the reallocation procedure, mixed-sex pens with equal distribution of females/castrates between the treatment groups were additionally created. Pigs from the different treatment groups were kept separate from the time of vaccination till end of study period. Following pen allocation, no new pigs entered the pens. Pigs were only removed from their allocated pen during the study due to death or welfare issues, in which case evaluation of cause, weighing and ID registration were done by herd personnel. All pigs in the study were healthy as evaluated by clinical inspection both at vaccination and at reallocation in the finisher herd.

For convenience reasons the study period was concluded eight weeks after entry to the finisher unit when the first pigs in the pens reached slaughter weight. At this time point all pigs were weighed and the pen-wise feed consumption were registered from the feeding computer. Over the following weeks, pigs would be sent for slaughter on a continuous basis as appointed by the herd manager. All pigs were tattooed with a supplier number as per normal routine. However, treatment group level tattooing allowed for group-wise separation of slaughterhouse data.

2.4. Measurements and registrations

Total feed consumption measured as feeding units (FU: a measurement of the nutritional value of the feed; for growing pigs 1 FU equals 7.38 MJ (Tybirk et al., 2006)) was registered in each pen eight weeks post entry. Also pen-wise, clinical assessment of diarrhea was done weekly from entry to eight weeks post entry in accordance with Pedersen and Toft (2011), and Pedersen (2013). All pigs were weighed at entry and eight weeks post entry. Individual LI excretion level in faeces were determined by individual rectal sampling in a subgroup of pigs in each treatment group in every batch. The samples were collected as repeated samplings from the same individually marked pigs every two weeks starting three weeks before transfer to the finisher unit. All pigs contributed with six samples each (two samples collected one and three weeks before transfer to the finisher unit and four samples collected during the study period in the finisher unit in week one, three, five and seven).

AB treatments and removals of pigs were done according to standard herd procedures and registered at individual pig level and at pen level by herd personnel. Dead pigs, or pigs excluded due to welfare reasons, were weighed by herd personnel at the day of their exit. Need of AB treatments were evaluated and initiated by herd personnel in agreement with herd vet instructions and therefore not necessarily in accordance with the clinical assessments of diarrhea as observed by the study investigator. In both herds, in case of flock treatment against diarrhea, the same active compound in the same dosage was used (tiamulin hydrogen fumarate 8 mg/kg in three days).

From the slaughterhouse, treatment group-level data concerning individual weights and lean meat percentage were obtained from each herd. The growth rate of a pig affects the lean meat percent where a slower growth rate usually causes a higher lean meat percentage (Stege et al., 2011). Therefore, the variation in lean meat percent providing an expression of the uniformity of the pig growth in each treatment group were of interest.

2.5. Testing of sampling material

Individual faecal samples were analysed for LI at MSD Animal Health R&D Service Laboratory in Boxmeer, Holland. The faecal samples were tested by real-time quantitative PCR (BactoReal®Kit *Lawsonia intracellularis*, Ingenetix) and reported in log(10) copies/µl of faecal solute with a quantification range from 1 to 6.3 log(10) copies/µl. For data analyses, negative samples were registered as 0 and positive samples below the quantification range as 0.5 log(10) copies/µl.

2.6. Sample size

As FCR was the primary outcome parameter, pen was the statistical unit determining the sample size. To identify a difference of 0.10 FU/kg as significant between vaccination and control group at a standard deviation of 0.10 FU/kg (Nielsen et al., 2017), a confidence level of 95 % and a power of 80 %,16 finisher pens were required per treatment group for a two-sided test (Houe et al., 2004). Note that “a pen” was a double pen with two single pens sharing the same feed chute. To account for the clustering in the hierarchical structure, block-randomization was performed within each batch.

A subgroup of study pigs was randomly chosen for individual faecal testing. To find a difference in LI excretion level by 1.0 log(10) copies/µl at a standard deviation of 1.2 log(10) copies/µl (Pedersen et al., 2012), a confidence level of 95 % and a power of 80 %, 23 pigs were required per treatment group for a two-sided test (Houe et al., 2004). Assuming a maximum within herd prevalence of 50 % (Stege et al., 2004), the sample size of pigs for faecal sampling was increased to around 50 individual pigs per treatment group in each herd. To ensure the required sample size in the finisher unit, the subgroup pigs were selected during the weaner period in a surplus of number randomly selected from each pen in each batch. The subgroup pigs for individual faecal testing were distributed as evenly as possible in pens throughout the finisher unit after reallocation.

The sample size requirements were fulfilled in each of the two herds.

2.7. Data management and statistical analysis

Clustering within pen and batch were to be accounted for in all response variables being FCR, diarrheic blot counts, ADWG, mortality, LI shedding and AB usage but not variance in lean meat percent, that was a treatment group level outcome. As there were only two herds in the study with no predictors at the herd level and the estimated sample sizes were obtained in each herd, the results are presented separately for each herd. Batch and pen were included in the statistical models as random effects; weight at entry, weight deviation in pen at entry, sex, and breed where relevant were included in the statistical models as fixed effect.

Pen-wise FCR were computed based on total weight gain for the corresponding pen (FCR=total feed consumption in pen_i (FU)/total weight gain in pen_i (kg)).

Weight at entry and weight eight weeks post entry computed the individual weight gain and ADWG.

Stocking density was computed pen-wise as an average for the study period (Pen stocking density=total pig days/study days/no. of pigs at entry) and was included as a fixed effect. Weight of dead and excluded pigs were included in the total weight gain for the corresponding pen and pen density were adjusted accordingly for the days each of the exclude pig had been included in the trial.

AB treatment against diarrhea were for both flock treatment in pens and individual pig treatments dichotomized into “treatment” or “no treatment”.

Diarrheic blot counts as predictor variable were categorized. For each week, the pen levels were defined by the following: 0 – 1 diarrheic stool: “low”; 2 – 3 diarrheic stools: “medium”; >3 diarrheic stools: “high”. Outbreaks of diarrhea were typically observed in the weeks immediately following entry being the high-risk period. The study period was therefore divided into two periods and yet a collective categorized score was constructed for each period. The collective score for the first study period (study week 1–4 = Period 1) and latter study period (study week 5–8 = Period 2), respectively, was given “HIGH”, if at least one of the study weeks had a high level; “MEDIUM”, if at least

one of the study weeks had a medium level (and no weeks scored "high") and "LOW" if all study weeks had a low level (predictor variable Diarrhea Period 1 and Diarrhea Period 2, respectively).

Due to the slaughterhouse tattooing, the lean meat percentage from each pig slaughtered was separated by treatment group and mean and variance could be compared at treatment group level. Apart from lean meat percentage, also variation in ADWG and Weight at exit were compared between treatment groups as an expression of treatment group uniformity.

Individual faecal samples were taken at two-week intervals. As a measure of the total shedding over time, Area Under the Curve (AUC) was calculated by the linear trapezoidal rule. To estimate the duration of LI shedding for each individual pig, it was assumed that a pig with two consecutive positive samples also were shedding in the period between the two samplings. Therefore, a positive sample were assumed to cover a shedding period of up to two weeks (one week before and one week after sampling) to fill in the time between the samplings.

In all statistical tests, a value of $p < 0.050$ was considered significant. In multiple regression analysis, interactions between predictors were included and backwards elimination to lowest possible value in Akaikes Information Criterion (AIC) was used to find variables of significance in final models although always retaining effect of vaccination as variable of primary interest. Response variables and statistical methods for analysing are presented in Table 1. The validity of the linear regressions was checked by the homogeneity of the variance and by visual inspection of the residual plots for normality of the residuals. Statistical analysis was performed using R version 4.1.2. (R Core Team, 2021).

3. Results

3.1. Descriptive results

In each herd, the duration of the trial lasting from vaccination of the first pigs to collecting slaughterhouse data of the last pigs was around eight months and the trials were conducted from July 2020 to November 2022. No adverse events were observed during the trial. In Herd 1, 52 finisher pens were included comprising 2184 finisher pigs. In Herd 2, 50 finisher pens were included comprising 2254 finisher pigs. In Herd 1, registration of FCR was mistakenly missing in one pen from the control group but all pig-level information was still available. In Herd 2, one pen from the vaccinated group was excluded (both pen and pigs) due to a mistake in the setting of the feed for the pen causing misleading results. One pig (male, vaccinated) could not be accounted for in Herd 2. Table 2 summarizes the data and premodelling screening results for each herd.

A total number of 137 and 91 individual pigs in Herd 1 and Herd 2, respectively, contributed with a full set of six faecal samples per pig (Table 3).

3.2. FCR

FCR by treatment group is shown in Fig. 1.

Results from multilevel linear regression showed that in both Herd 1 and 2, FCR were significantly reduced in the vaccinated group compared with the control group with an estimate of 0.05 FU/kg and 0.09 FU/ kg, respectively, when taking 'pen-sex' and 'weight at entry' into account ($p = 0.007$ *and* $p < 0.001$, respectively). Further, FCR was influenced by the pen-sex with female pigs having a significantly better FCR (−0.07 FU/kg, $p = 0.002$ and −0.06 FU/kg, $p = 0.004$, Herd 1 and Herd 2, respectively) than pens with male pigs (castrates). In both herds, stocking density, AB flock treatment, weight deviations at entry as well as diarrhea as given by categorized levels in Period 1 and Period 2, were all found non-significant. No interactions were found between the predictors.

Table 1
Response variables, observational level, categorization, and model for analysis.

Response variable				
Variable (units)	Level	Classification (type/scale)	Method	Model
FCR (FU/kg)	Pen-level	Quantitative/ continuous	Multilevel linear regression	FCR ~ LI vaccination + stocking density + AB pen treatment + Diarrhea Period 1 + Diarrhea Period 2 + breed + weight at entry + weight deviations at entry + sex + Ɛ |batch
Diarrheic blot counts (count)	Pen-level	Quantitative/ pseudo-continuous	Fishers exact test and Multilevel Poisson regression	Mean diarrheic blot counts in Period 1 or in Period 2 ~ LI vaccination + pen stocking density + AB pen treatment | batch
			Two-way anova mixed model adjusted for sphericity and with Bonferroni correction	Diarrheic blot counts per week in repetitive sampling ~ LI vaccination + Week post transfer + Ɛ
AB treatment	Pen-level	Qualitative/ dichotomous	Fishers exact test and Multilevel logistic regression	AB pen treatment ~ LI vaccination + pen stocking density + breed + weight at entry + weight deviations at entry + sex | batch
AB treatment	Pig-level	Qualitative/ dichotomous	Fishers exact test	
ADWG (gr/day)	Pig-level	Quantitative/ continuous	Fishers exact test, f-test and Multilevel linear regression	ADWG ~ LI vaccination + pen stocking density + AB pig treatment + breed + weight at entry + sex + Ɛ | batch/pen
Mortality /Preliminary exit	Pig-level	Qualitative/ dichotomous	Fishers exact test	
Lean meat percent (%)	Treatment group-level	Quantitative/ continuous	Fishers exact test and f-test	
LI shedding	Pig-level	Quantitative/ continuous	Fishers exact test	
			Multilevel linear regression	LI shedding (AUC) ~ LI vaccination

(*continued on next page*)

Table 1 (*continued*)

Response variable				
Variable (units)	Level	Classification (type/scale)	Method	Model
				+ AB pig treatment + ε | batch/pen
			Two-way anova mixed model adjusted for sphericity and with Bonferroni correction	LI shedding per week in repetitive sampling ~ LI vaccination + time of sampling + ε
			Poisson regression	Weeks of LI shedding ~ LI vaccination + ε

3.3. Diarrheic counts

Number of diarrheic blots counted in vaccination group pens were significantly lower than in control group pens in several study weeks in both Herd 1 and 2 as can be seen in Fig. 2. In multilevel analysis mean diarrheic blot counts in Period 1 were significantly lower in vaccination group pens compared to control group pens with estimates of −1.4 ($p < 0.001$) and −1.8 ($p < 0.001$) in Herd 1 and Herd 2, respectively, and with LI vaccination being the only significant predictor. Likewise, in Period 2 the mean diarrheic blot counts were reduced by 0.7 ($p < 0.001$) and 0.9 ($p < 0.001$) in Herd 1 and Herd 2, respectively. In both herds, an increased mean weight in pens at entry significantly reduced the mean diarrheic blot counts in Period 2 (est. −0.1, $p < 0.001$ and est. −0.1, $p = 0.001$, Herd 1 and Herd 2 respectively) indicating that the larger the pigs are at entry, the faster they overcome the period at risk for having diarrhea. In Herd 2, the stocking density was of statistical significance in Period 2 ($p = 0.023$) indicating increased mean diarrheic blot counts in pens with reduced stocking density.

3.4. ADWG

In both Herd 1 and Herd 2, ADWG was significantly improved in the vaccinated group compared to the control group by +30.7 gr./day (CI95 %: 13–48 gr./day) and +43.1 gr/day (CI95 %: 27–59), respectively ($p = 0.001$ *and* $p < 0.001$, *respectively*). In contrast to Herd 1, treatment against diarrhea had a positive impact on ADWG in Herd 2 ($p = 0.021$). In Herd 2, breed was also a factor confirming that white breed (LY) that constituted 3.4 and 1.4 of vaccinated and control pigs, respectively, has a reduced growth compared to the traditional finisher pig breed (−102 gr/day, $p < 0.001$). Stocking density and sex was non-significant. Weight distributions on the final study day eight weeks post entry

Table 2
Summary of data from Herd 1 and Herd 2 and results of preliminary univariable screening in each herd.

		Herd 1			Herd 2
	Batches, no.	5			8
		Control group	Vaccination group	Control group.	Vaccination group
Pen level	Pens, no.	26	26	25	24
	Pen-sex, no.	Male: 10 Female: 11 Mix: 4	Male: 11 Female: 11 Mix: 4	Male: 9 Female: 10 Mix: 6	Male: 7 Female: 11 Mix: 6
	Pigs per pen at entry, no.	42	42	46	46
	Pen stocking density, ratio	0.99; 1;	0.99; 1;	0.99; 0.99;	0.99; 1;
	(mean; median; range)	0.95–1	0.97–1	0.96–1	0.96–1
	FCR, FU/kg	2.64; 2.66;	2.59 * ; 2.58;	2.52; 2.53;	2.43 **; 2.42;
	(mean; median; range)	2.39–2.84	2.46–2.76	2.35–2.69	2.28–2.63
	Mean diarrheic blot counts, Period 1, no.	2.4; 2.3;	1 *** ; 0.8;	3.2; 3;	1.4 *** ; 1.3;
	(mean; median; range)	1.3–4	0.3–2.3	1.8–5.5	0.8–2.5
	(HIGH; MEDIUM; LOW)	(18; 8; 0)	(3; 13; 10) ***	(19; 6; 0)	(2; 21; 1) ***
	Mean diarrheic blot counts Period 2, no.	1; 1;	0.3 *** ; 0.3;	1.6; 1.3;	0.6 *** ; 0.3;
	(mean; median; range)	0–2.8	0–1	0.5–3.5	0–1.8
	(HIGH; MEDIUM; LOW)	(5; 15; 6)	(0; 4; 22) ***	(9; 12; 4)	(3; 9; 12) *
	AB pen treatment, no. of pens treated	26	8 ***	17	12 NS
Pig level	Pigs, no.	1092	1092	1150	1103
	Breed	LY: 0 LYD: 1092	LY: 0 LYD: 1092	LY: 16 LYD: 1134	LY: 38 LYD: 1065
	Sex	Male: 558 Female: 534	Male: 558 Female: 534	Male: 585 Female: 565	Male: 584 Female: 519
	Avr. weight at entry, kg	32.2; 32.1;	32.1 NS; 31.8;	34.0; 33.6;	34.2 NS; 34.0;
	(mean; median; range)	22–46.5	22.7–46.8 NS	23.2–47.8	22.9–49.4 NS
	Avr. weight at exit, kg	91.7; 93.0;	93.3 *** ; 94.0;	92.7; 94.0;	95.5 *** ; 96;
	(mean; median; range)	26–118	26–126 ***	30–123	40–120 ***
	ADWG, gram/day	1060; 1086;	1091 *** ; 1104;	1036; 1049;	1080 *** ; 1079;
	(mean; median; range)	-254–1454	-413–1505 ***	1–1439	295–1493 ***
	Mortality, no. of pigs	13	10 NS	13	7 NS
	Excluded pigs, no. of pigs	3	6 NS	11	7 NS
	AB pig treatment against diarrhea, no.	55	17 ***	46	27 *
	Duration of LI shedding in weeks	2.7;	2.4 * ;	3.1;	2.0 *** ;
	(mean; median; range)	2.0; 2.0–6.0	2.0; 0–6.0	3.0; 0–6.0	2.0; 0–6.0
	Slaughterhouse data				
Treatment group level	Pigs, no.(percent of possible)	940 (86.1 %)	961 (88.0 %)	925 (80.4 %)	937 (85.0 %)
	Slaughter weight, kg	91.65;	90.96 * ;	91.88;	91.64 NS;
	(mean, sd)	6.74	6.63	6.18	6.19
	Lean meat percent., %	61.55;	61.55 NS;	61.70;	61.70 NS;
	(mean; sd).	4.92	4.50 ***	2.05	2.05 NS

Statistically significant difference in Fishers exact test and univariable screening of means and in f-test screening of variance: * p < 0,05; **p < 0.01; ***p < 0.001; NS non-significant.

Table 3
Lawsonia intracellularis shedding in faecal samples from each treatment group in each herd in the six consecutive samplings (two samplings prior to transfer and four samplings during study period). Number of sampled pigs having received a treatment against diarrhea (AB) in the sampling period either at pig or at pen level provided.

		Herd 1		Herd 2	
		Control group	Vaccination group	Control group	Vaccination group
	Number of sampled pigs n ($n_{AB\ treatment}$)	69 (69 AB)	68 (27 AB)	47 (36 AB)	44 (21 AB)
	LI shedding, Study week −3[ab]	0.01;0; 0–0.5 (0; 0.0 %)	0.03;0; 0–0.5 (0; 0.0 %)	0.28; 0; 0–4.2 (4; 8.5 %)	0.19; 0; 0–3.8 (3; 6.8 %)
	LI shedding, Study week −1[ab]	0.06; 0; 0–1.5 (1; 1.5 %)	0.04; 0; 0–0.5 (0; 0.0 %)	2.23; 2.1; 0–5.8 (33; 70.2 %)	1.18; 0.5; 0–4.9 (20; 45.5 %)
Individual pig level	LI shedding, Study week 1[ab]	0.43; 0; 0–3.90 (8; 11.6 %)	0.01; 0; 0–0.50 (0; 0.0 %)	2.12; 1.90; 0–5.2 (29; 61.7 %)	1.10; 0.5; 0–4.2 (18; 40.9 %)
	LI shedding, Study week 2[ab]	3.15; 3.35; 0–5.80 (64; 92.8 %)	1.18; 0.50; 0–5.20 (28; 41.2 %)	0.28; 0; 0–4.3 (4; 8.5 %)	0.31; 0; 0–4.7 (4; 9.1 %)
	LI shedding, Study week 3[a,b]	0.82; 0.50; 0–5.00 (22; 31.9 %)	1.83; 1.5; 0–5.80 (41; 60.3 %)	0.05; 0; 0–1.8 (1; 2.1 %)	0.03; 0; 0–1.5 (1; 2.3 %)
	LI shedding, Study week 4[ab]	0.06; 0; 0–1.10 (1; 1.5 %)	0.36; 0; 0–3.70 (9; 13.2 %)	0; 0; 0–0 (0; 0.0 %)	0.01; 0; 0–0.5 (0; 0.0 %)

[a] mean; median, range of all samples in log(10) copies/µl.
[b] (number and % of samples above lower detection limit).

revealed that 4.5 % and 1.6 % of the vaccinated pigs had a weight below 80 kg compared to 9.1 % and 6.3 % of the control pigs in Herd 1 and 2, respectively. This was supported by a statistically significant difference ($p < 0.001$ in both herds) in the variation in both the exit weight and ADWG indicating a more uniform growth in the vaccinated group in both herds.

3.5. Mortality

Pigs dead or excluded due to welfare reasons were registered, but due to lack of veterinary clinical examinations to set or confirm a diagnosis, cause of exit could not be taken into consideration. Number of pigs dead or excluded during the trial did not significantly differ between the groups in neither of the herds (Table 2).

3.6. Antimicrobial treatment

Flock treatment during the trial was in both herds only performed against outbreaks of diarrhea.

In Herd 1, AB pen treatment was performed in all control group pens (26; 100 %) and in barely a third of the vaccination group pens (8; 30.8 %) (OD: *Inf.*; CI95 %: 10.4-*Inf.*; $p < 0.001$). In Herd 2, numerically more pens were treated with AB in the control group pens (17; 68.0 %) compared to the vaccination group pens (12; 50.0 %) (OD: 2.1; CI95 %: 0.6–8.0; $p = 0.252$). These results were not influenced by other predictors.

AB pig treatments against diarrhea were evenly distributed between batches in both herds, but significantly more control pigs were treated than vaccinated pigs (Herd 1: OD: 3.4; CI95 %: 1.9–6.2; $p < 0.001$; Herd 2: OD: 1.7; CI95 %: 1.0–2.8; $p = 0.043$).

3.7. Slaughterhouse data

In both herds more than 80 % of the study pigs contributed with data regarding lean meat percent and slaughter weight. In Herd 1, six control pigs and four vaccinated pigs were reported without lean meat percent. No difference was found in mean lean meat percent in either of the herds. In opposition to Herd 2, a significant difference in variation in lean meat percent was found in Herd 1 (ratio: 0.8, CI95 %: 0.7–0.9; $p = 0.007$) indicating more variation in pig growth in the control group than in the vaccine group.

3.8. LI shedding

The onset of LI infection slightly varied between herds from being at the entrance to the finisher unit in Herd 1 to being in the last week before transfer to the finisher unit in Herd 2. In both herds, a significant difference in the prevalence of pigs not shedding LI in any of the collected samples was found between the vaccination group (20.0 % and 20.5 %, Herd 1 and 2 respectively) and the control group (0.0 % and 4.3 %, Herd 1 and Herd 2 respectively) (OD: 9.4; CI95 %: 2.7–50.1; $p < 0.001$). Further, in both herds the total shedding of LI was significantly reduced in samples from vaccination group pigs ($p < 0.001$ in both herds) compared to samples from control group pigs by 56.2 % and 86.6 % of the AUC, respectively. The calculations were based on the logarithmically transformed values. In Herd 1 and 2, AB treatment

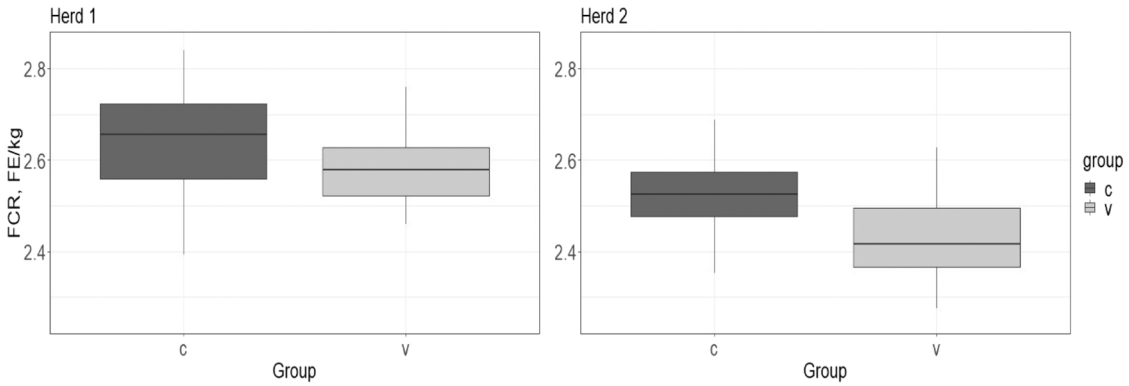

Fig. 1. Feed Conversion Ratio by treatment group in Herd 1 and Herd 2.

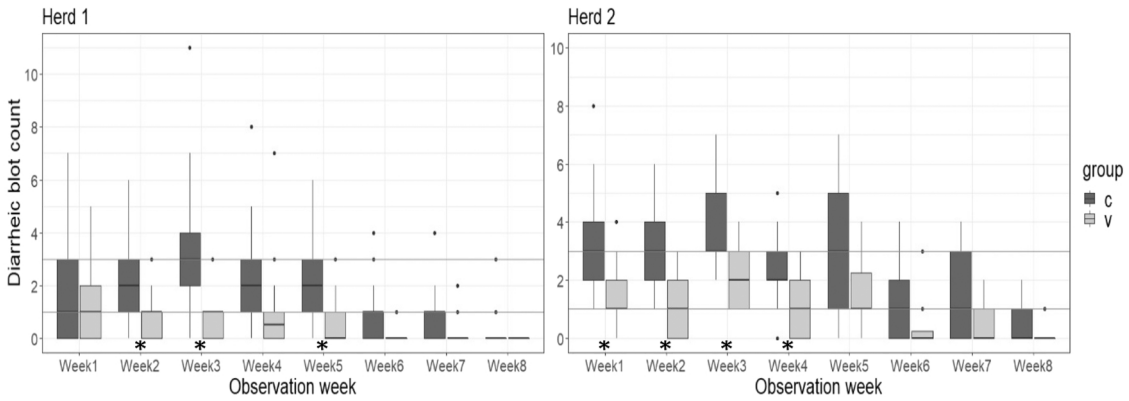

Fig. 2. Diarrheic blot counts in pens as observed each week in the study period (eight repeated measurements) by treatment group and herd. Cut-off lines for categorization and asterisk (*) indicating significant difference between treatment groups are added.

against diarrhea reduced the total LI shedding by 42.8 % ($p = 0.012$) and 28.3 % ($p = 0.061$), respectively. For each week relative to transfer, a significant difference between the group mean was found in Week 1, Week 3, and Week 5 ($p \leq 0.003$) in Herd 1 and in Week −1 ($p = 0.046$) in Herd 2. Mean excretion level of LI during the study period is presented in Fig. 3. The estimated duration of LI shedding was significantly prolonged in control pigs compared to vaccinated pigs in Herd 2 ($p = 0.003$) but not in Herd 1 ($p = 0.196$).

4. Discussion

In both herds, we found that intramuscular vaccination against LI at weaning significantly improved FCR, ADWG, AB pen and pig treatments against diarrhea, pen-wise diarrheic blot counts, and LI shedding (prevalence, excretion level and duration) in the first eight weeks post entry in the finisher herds. Although results differed between the two herds, the results points in the same direction and thereby supports each other. LI vaccination significantly reduced FCR by 0.05 and 0.09 FU/kg alongside a significantly increased ADWG by 31 and 43 gr/day in Herd 1 and Herd 2, respectively. Based on the calculated ADWG, the vaccinated pigs potentially could reach slaughter weight two and three days faster than the control group pigs in Herd 1 and Herd 2, respectively. Antibiotic treatment could be expected to have an improving impact on FCR and ADWG, but despite a higher AB treatment rate among control pigs, vaccinated pigs performed significantly better. The results concerning ADWG confirm or exceed the results found in comparable LI vaccine field trials. When using the oral LI vaccine against PPE an improved ADWG of 20–56 gr./day in finisher pigs have been found in several studies (Bak and Rathkjen, 2009; Hardge et al., 2004; Park et al., 2013; Peiponen et al., 2018; Scholtz et al., 2008). Where FCR was included as an outcome, these studies did not find a concurrent improvement in FCR (Hardge et al., 2004; Park et al., 2013; Peiponen et al., 2018; Scholtz et al., 2008). AB treatment against PPE was included as an outcome in only two of the studies and was found reduced in the one (Bak and Rathkjen, 2009) and without difference in the other (Peiponen et al., 2018).

The AB treatment was initiated on behalf of the herd manager in agreement with normal herd routines. The trial was blinded to the herd manager and the decision for treatment was therefore not based on a predetermined assumption about the impact of the vaccine. But still, the decision for initiating treatment against diarrhea was based on subjective evaluations for each herd and not on veterinary clinical assessment and could therefore have been evaluated incorrectly.

In both herds, the mortality was low and the reduced mortality in the vaccinated group (0.3 % and 0.5 % in Herd 1 and Herd 2, respectively) was non-significant which partly could be due to the sample size not set according to this parameter. To identify if LI was causing mortality, clinical evaluation and autopsies could have been performed on excluded and dead pigs. In alignment with this study, also previous vaccination studies did not find an impact on mortality (Hardge et al., 2004; Park et al., 2013; Peiponen et al., 2018) when performed in herds suffering from subclinical or chronic PPE.

Diarrhea as measured by weekly pen-wise counts of diarrheic blots were significantly reduced in vaccination group pens compared to control group pens confirming results previously seen (Park et al., 2013). The fluctuation in diarrheic blot counts observed in the control group pens coincided with the onset and infection dynamics of LI found in the individual faecal samples supporting that the diarrhea observed most likely was related to LI infection. The increased mean diarrheic blot counts in pens with reduced stocking density, as was the case in Herd 2, could more likely be explained by pigs leaving the pens because of diarrhea rather than diarrhea caused by a lower density. In both herds, the diarrheic counts in the vaccinated group were stable over time at a low level with only minor fluctuations. Changing the periods for

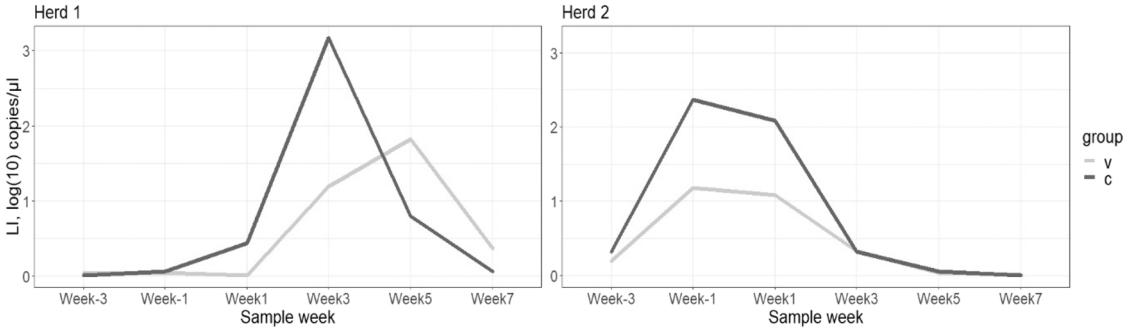

Fig. 3. Mean Lawsonia intracellularis excretion level in faecal samples by treatment group and herd in sampling weeks relative to transfer to finisher unit (three and one week before transfer and one, three, five, and seven weeks post entry).

categorized levels of diarrhea from the first four weeks in Period 1 and the latter four weeks in Period 2 to for instance three and five weeks instead, respectively, did not alter the results. The study was only single blinded posing a bias risk, but all counting's were performed by the study investigator in accordance with previous recommendations (Pedersen, 2013; Pedersen and Toft, 2011) minimizing the risk and uncertainties in the assessment. Although subclinical LI infection does not cause diarrhea (as this would have made the infection clinical), the parameter of diarrheic blot counts was still found of relevance because an LI infection at a given level has a greater impact if it is accompanied by concurrent occurrence of diarrhea (Pedersen et al., 2012) and because evaluating the diarrheic status is included in the herd manager's toolbox for daily assessment of the gut health.

The slaughterhouse data was collected from two different slaughterhouse companies but with separate analysis of data, it was expected to be without influence. For unknown reasons, up to 19.6 % of the study pigs were reported without identifiable group level tattoo and was therefore not included in the data. A previous study has found a negative association between lean meat percentage and ADWG (Stege et al., 2011). Despite higher ADWG in the vaccinated group in both herds, the lean meat percentage was not lowered in this treatment group. Increased variation in ADWG, as was the case in the control groups, would presumably cause a greater variation in the individual lean meat percentages which would be yet another measurement of growth variation within the treatment group. Variation in the lean meat percentage was different between the groups in Herd 1 indicating a more uniform growth in the vaccinated group. No difference was found in Herd 2, despite the greater variation in ADWG in control group pigs and, a greater proportion of runts in the control group compared to the vaccinated group at the final weighing still indicating increased growth variation in control group pigs. Although, one previous study found a "leaner growth" in LI vaccinated pigs as measured by back fat depth (McOrist and Smits, 2007b), apparently only two studies have included information concerning lean meat percent in a LI vaccination study. They found no difference in the mean lean meat percent (Peiponen et al., 2018; Scholtz et al., 2008), as was also the case in this study, but in contrast to the present study, they did not investigate the variation in the parameter.

Shedding of LI was in both herds detected in a greater proportion of samples from control pigs than from vaccinated pigs (80.0 % and 79.5 % vs 100 % and 95.7 %, in Herd 1 and Herd 2, respectively). Further the mean excretion of LI reached an elevated level in samples from control pigs compared to samples from vaccinated pigs. Both observations confirm results found under experimental conditions in a previous study evaluating the same vaccine (Jacobs et al., 2019). In contrast to Herd 1, the duration of shedding was prolonged in the control group compared to the vaccinated group in Herd 2. The difference between herds in duration of LI shedding could be due to the difference in treatment with all non-vaccinated pigs being treated in Herd 1. Duration of shedding in this study was an estimate based on bi-weekly individual sampling. To get a more accurate result of duration, daily samplings would be required.

Both trial herds had pre-trial production results positioned in the better half of the national average of finisher herds (Hansen, 2021) despite being challenged by a subclinical-clinical LI infection in early finisher period. Even so, despite being well-managed and high health, well performing herds, LI infection had a negative impact on the key production figures in both herds and both herds experienced beneficial results of LI vaccination.

The study was concluded eight weeks post entry to the finisher unit which was considered reasonable with LI being introduced in the beginning of the finisher period (or last week of weaner period) and the major impact of the infection were therefore expected to have declined at this time point as indicated by preliminary investigations and by previous studies (McOrist and Gebhart, 2012). When interpreting the results, it must therefore be emphasized that the results only reflect the first eight weeks in the finisher unit and not the full finisher period.

The study was conducted in 2 independent herds, both of which meet the requirement for sample size. In terms of design, it would therefore have been sufficient to conduct the trial in a single herd and describe this case alone. By being able to confirm the results found by repeating the trial with the obvious possibility of no or opposite findings in the subsequent herd, the results stand even stronger despite difference in magnitude. There will always be a variation in effect between herds when performing real-life trials and despite strong results, two herds repetitions are insufficient to transfer the results to LI infected herds in general. Further trials investigated the same vaccine are thus desirable to confirm the results found.

Despite of the advantages in reflecting real-life production, a field trial with parallel groups also has the disadvantage of possible underestimation of the effect measured. The vaccinated pigs and the control pigs were housed in neighbouring pens. Despite of the separating walls between the pens of different groups, cross-contamination between the pens was a risk. It is thus reasonable to assume that the results would have been even more pronounced if all pigs in each individual barn had had the same vaccination status. If so, the infection pressure of LI could be assumed to be lower in fully LI vaccinated barns and higher in fully LI non-vaccinated barns, than was the case in this study.

5. Conclusion

In this field trial it was found that vaccination with an inactivated intramuscularly administered vaccine against LI in both of two high-health and high-productive trial herds suffering from subclinical-clinical LI infection in early finisher period significantly improved FCR and reduced occurrence of diarrhea and AB treatment at pen level; increased ADWG and reduced AB treatment and LI shedding at pig level; and reduced growth variation in both herds at group level as further confirmed in one herd by less variation in lean meat percentage from vaccinated pigs.

Declaration of Competing Interest

Susanne Leth Musse and Gitte Blach Nielsen are employed by MSD Animal Health, which have a commercially interest in selling pharmaceutical products for pigs. The employment did not inflict any bias regarding the study, which has been conducted as part of the first author's enrollment as Industrial PhD student at University of Copenhagen. Nicolai Rosager Weber is employed by Danish Agriculture & Food Council, which manages the interests of primary pig producers and related companies. Hans Houe and Helle Stege are employed by University of Copenhagen which builds on an academic environment based on independence of research.

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