Productivity parameters, antimicrobial consumption, and prevalence of enteric pathogens before and after intramuscular vaccination against Lawsonia intracellularis in naturally infected Danish weaner and finisher pig herds

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Published in:
Preventive Veterinary Medicine

DOI:
10.1016/j.prevetmed.2023.105973

Publication date:
2023

Document version
Publisher's PDF, also known as Version of record

Document license:
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Citation for published version (APA):
Productivity parameters, antimicrobial consumption, and prevalence of enteric pathogens before and after intramuscular vaccination against *Lawsonia intracellularis* in naturally infected Danish weaner and finisher pig herds

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**Abstract**

In Danish pig production, gastro-intestinal diseases account for most of the antimicrobials (AM) used in growing pigs. Diarrhoea is most frequently caused by *Lawsonia intracellularis* (LI), *Brachyspira pilosicoli* (BP), *E. coli* fimbria type F4 (F4) and *E. coli* fimbria type F18 (F18). With a new LI vaccine available from 2019, it was relevant to investigate the effect of this vaccine in a Danish field study including both weaner and finisher sites. The aim was to evaluate the efficacy of Porcilis® Lawsonia Vet. in naturally LI-infected pig herds by comparing of productivity parameters, AM consumption and dynamics of enteric pathogens over two 6-months periods before and after LI vaccination. Further, faecal sock samples were collected from each site before and after vaccination and analysed by qPCR for excretion levels of LI, BP, F18 and F4. In total, 28 weaner and 41 finisher sites were included in the study. Vaccination reduced Feed Conversion Ratio by 0.12 Feed Unit/kg (p = 0.029) and 0.08 Feed Unit/kg (p = 0.005) in weaners and finishers, respectively. Increased Average Daily Weight Gain of 45.6 gr./day (p < 0.001) was found in the finishers. Mortality risk fell by 8.8% in weaners (RR = 0.912; p < 0.001). AM prescriptions for oral group treatments were reduced by 38.8% active compound/kg pig produced (p = 0.005) or 33.3% Weighted Animal Daily Doses per 100 animals per day in finishers (p = 0.004). LI prevalence was reduced in weaners and finishers (both p < 0.001) and BP prevalence was reduced in finishers (p = 0.043). Mean excretion levels of LI and BP decreased at weaner sites (−1.32 and −1.02 log(10) copies/gr faeces, respectively; both p < 0.001) and at finisher sites (–1.04 and −1.16 log(10) copies/gr faeces, respectively; both p < 0.001). Prevalence and excretion levels of F18 and F4 were unaffected by LI vaccination. In conclusion, vaccination against LI using Porcilis® Lawsonia Vet. improved productivity parameters, cut AM consumption, and reduced prevalence and excretion levels of LI and BP in naturally LI-infected pig herds.

**1. Introduction**

*Lawsonia intracellularis* (LI), the cause of Porcine Proliferative Enteropathy (PPE), is an enteric pathogen in pigs with worldwide endemic prevalence (Kroll et al., 2005; Lawson and Gebhart, 2000; Vannucci et al., 2019). In Denmark, LI infection most often causes chronic or subclinical PPE in growing pigs less than 4–5 months of age (Arnold et al., 2019). It is costly due to increased Feed Conversion Ratio (FCR),
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then vaccinated. An implementation period of approximately 5 months was required for all the pigs within each herd complex to be vaccinated. The following hypotheses to be evaluated at both weaner and finisher sites were outlined:

**H1.** LI vaccination improves FCR and ADWG and reduces mortality.

**H2.** LI vaccination reduces AM consumption.

**H3.** LI vaccination reduces diarrhoeic outbreaks and the prevalence and excretion levels of LI, BP, F4 and F18.

### 2. Materials and methods

The study was conducted in twenty-five Danish commercial pig herd complexes holding both weaner and finisher sites and experiencing natural LI infection. Productivity parameters and the amounts of prescribed AM before and after vaccination against LI were compared. Before and after initiation of LI vaccination, faecal sock samples (Pedersen et al., 2014b, 2015) were also collected in batches from each site, at time points both with and without outbreak of diarrhoea. Porcilis® Lawsonia Vet. for intramuscular administration in a single dose of 2 ml. was used for LI vaccination with each pig vaccinated at weaning at approximately 4 weeks of age in all herds. For each herd, the vaccination program was initiated after initial sampling had confirmed that the herd was to be included in the study. Subsequently, all pigs in the herd were then vaccinated. An implementation period of approximately 5 months was required for all the pigs within each herd complex to be vaccinated.

The herds did not start the vaccination program simultaneously but were incorporated into the program over a 8-month period as they were referred to the study. However, all herds were monitored in the same way in accordance with the study protocol for a period of 18 months as outlined in Fig. 1. The study period was June 2019 to June 2021.

### 2.1. Inclusion of study herds

Pig herds planning to implement vaccination against LI at weaning using Porcilis® Lawsonia Vet. were referred to the study by local herd veterinarians. Only herd complexes consisting of one or more weaner and finisher sites, where the pigs could be followed from weaning to slaughter with access to the necessary data, were included. In each herd complex the pigs entered weaner sites at weaning of approximately 4 weeks of age. At approximately 30 kg., 7–8 weeks post-weaning, they were transferred from the weaner site to one or more finisher sites on a weekly or bi-weekly basis. This meant that all pigs within a herd complex originated from the same sow herd. However, the weaner sites and finisher sites within a given herd complex could have the same or different geographical locations and have the same or different owners. In cases of multisite production, a maximum of 3 weaner sites and 3 finisher sites were included from each herd complex to minimise the clustering effect of herd complexes. No herd complexes had the same owner/owners.

In all of the herds all pigs were routinely vaccinated at weaning against Porcine Circovirus type 2 (PCV2). At the same time, if the herd was Mycoplasma hyopneumoniae-positive, the pigs were vaccinated against this pathogen as well. The vaccine used for this purpose was either Porcilis® PCV M Hyo (PCV2 and Mycoplasma hyopneumoniae) or Porcilis® PCV ID (PCV2). According to the herd veterinarians, each herd complex was being managed with a professional and consistent attitude towards the provision of productivity data. The herd complexes had a history of diarrhoea outbreaks. In each herd, LI infection was confirmed through the detection of LI in quantitative Polymerase Chain Reaction (PCR) at excretion levels above 4.8 log(10) copies/gr faeces in at least one sock sample from a batch in either weaner or finisher sites. The cut off level for LI was selected with reference to previous studies indicating that levels above 4.8 log(10) copies/gr faeces are associated with proliferative histological lesions in the intestine (Pedersen et al., 2013) and a negative impact on productivity parameters in terms of ADWG or FCR (Collins and Barchia, 2014; Johansen et al., 2013; McOrist et al., 1997; Paradis et al., 2012; Pedersen et al., 2012). Information provided by the herd veterinarian, and confirmed by the prescriptions of oral AM for group treatment of diarrhoea, showed that at least 25% of the pigs in each herd complex were treated with AM against diarrhoea caused by LI.

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**Fig. 1.** Study design with the timeline of the investigation showing data collection in two 6-month periods (Period 1 and Period 2) before and after Lawsonia intracellularis vaccination.
2.2. Study design and collection of data

The study design is shown in Fig. 1. In every weaner and finisher site, productivity data and AM consumption data were collected over two periods of six months at two time points: immediately before (Period 1) and one year after (Period 2) the LI vaccination intervention. At both time points, information on productivity parameters and AM consumption were obtained for the preceding six months and faecal sock samplings were collected. With this study design, season was identical in the two periods, minimising the effect of seasonal fluctuations and a time gap of six months between the two time periods ensured that all data collected in Period 2 originated only from vaccinated pigs. At both time points, a questionnaire on housing, management and health status was conducted to detect any changes within each herd other than LI vaccination potentially influencing the data collected.

2.3. Productivity data

Productivity parameters including FCR (FU/kg) (Feed unit (FU): a measurement of the nutritional value of the feed; for growing pigs 1 FU equals 7.38 MJ (Tybirk et al., 2006)), ADWG (gr.) and mortality (number of dead relative to total number of pigs) were extracted from the routinely used herd database software packages AgroVision (Agrosoft, 2022) and Cloudfarms (Cloudfarms, 2022).

When productivity data were extracted, each individual report was validated for errors and fluctuations in accordance with the procedures specified and used by Hansen (2021).

2.4. Antimicrobial consumption data

The data used to calculate AM consumption at site level were extracted from the two official databases: VetStat, on which all medicines prescribed for livestock animals in Denmark are registered (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022b) and the Central Husbandry Register, on which the numbers of animals registered at each site are recorded (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022a). It was assumed that the quantity of AM prescribed and delivered to each site in the given periods was utilised within the periods. Only AMs for oral administration, indicating group medication, were included in the study data. Zinc oxide supplements were not included.

2.5. Faecal sock samples

The faecal sock samples were collected by the herd veterinarian or the herd manager over an observation period of approximately two weeks for each sampling. The sampling either targeted batches at a fixed timepoint regardless of diarrhoea status (Fixtime) or targeted batches with outbreaks of diarrhoea occurring before the fixed timepoint (Pre-Fixtime). Hence, each site provided a Fixtime sample that could be collected from batches both with and without diarrhoea. By contrast, Pre-Fixtime samples always were collected at a diarrhoea outbreak during the observation period. This meant that, if no diarrhoea outbreak occurred during the observation period at a particular site, no Pre-Fixtime sample was collected. Diarrhoeic samples were always collected before AM treatment was initiated. An outbreak of diarrhoea was defined as: a situation in a batch of pigs where the presence of diarrhoeic pigs was sufficiently serious, according to normal herd routines and as instructed by the herd veterinarian, to require AM group treatment at batch level.

In the weaner sites, the Fixtime samples were collected from the oldest batch during the last week before transfer to the finisher site, regardless of diarrhoea status (age approximately 7 weeks post-weaning). The first two weeks post-weaning were not included in the study, as its focus was not on E. coli -diarrhoea occurring immediately after weaning (Fairbrother and Nadeau, 2019). Therefore, the diarrhoeic Pre-Fixtime samples were collected from any batch older than two weeks post-weaning but younger than the oldest batch (with a Fixtime sample) experiencing an outbreak of diarrhoea during the observation period (age 3–7 weeks post-weaning).

At the finisher sites, the Fixtime samples were collected from batches 3–4 weeks post-transfer regardless of diarrhoea status. The diarrhoeic Pre-Fixtime samples were collected from batches younger than four weeks post-transfer (1–4 weeks post-transfer).

At sampling, one faecal sock sample included faeces from all pens of the batch, as previously described by Pedersen et al. (2015). Hence, every site, including weaner and finisher sites, contributed at least one Fixtime-sample and in cases of diarrhoea, they further contributed a Pre-Fixtime sample.

Faecal sock sample kits containing all the materials needed for sampling and shipment were supplied by Dianova, National Veterinary Institute, Denmark. Every faecal sock sample was labelled with date of sampling, weeks post-entry, and diarrhoea status. Samples were shipped to the National Veterinary Institute, Technical University of Denmark, where they were prepared and analysed using a high-throughput real-time PCR system to determine the presence and excretion levels of LI, BP, F4 and F18 as previously described by (Goecke et al., 2020b) and Sähl et al. (2011). For all four pathogens, samples were considered positive when the result of the analysis was above the lower detection limit of 3 log(10) copies/gram faeces.

2.6. Sample size

As ADWG is increasingly affected negatively by increasing LI excretion levels (Collins and Barchia, 2014), and as Porcilis® Lawsonia Vet. has been shown to reduce LI shedding (Jacobs et al., 2019), this productivity measure was used to calculate sample size. Based on the identification of a difference in ADWG of 40 gr./day between vaccinated and non-vaccinated groups, and on a standard deviation of 52 gr. between herds (Nielsen et al., 2018) a confidence level of 95% and a power of 80%, the sample size was determined to be 27 sites in each age group (Houe et al., 2004). Assuming that some sites would be excluded during the study due to the potential introduction of new infectious diseases or major changes in the feed/management/housing system over the study period, the aim was to include 50 weaner and finisher sites.

2.7. Statistical analyses

Outcome variables are presented in Table 1.

The outcome variables were evaluated in statistical models as described below for each outcome. In all regression models, backwards elimination to the lowest possible value in Akaike’s Information Criterion (AIC) was performed, although ‘LI vaccination’ was always retained in the models as a fixed effect since it was the variable of primary interest. As two herd complexes had used oral vaccination against LI prior to their inclusion in the study, the variable ‘Use of oral LI vaccination prior to study inclusion’ was also included in the final regression models as a fixed effect. To account for the repetitive sampling, ‘site’ was added as random effect in the multilevel modelling as was ‘owner’ to account for same ownership of some sites. To evaluate the model fit of the linear regression models residuals were visually inspected for normality.

2.8. Productivity data

Data on FCR and ADWG were tested for normality in Shapiro-Wilk test. ADWG in finishers was slightly skewed from a normal distribution by a few outliers (≤2). All observations were kept in the dataset, however, since all observations were confirmed by the site managers and exclusion of the outlying observations did not change the results. Therefore, initial results were evaluated by paired t-test for FCR and paired Wilcoxon test for ADWG. Prior to modelling, the explanatory
### Table 1
Outcome variables

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Level</th>
<th>Variable classification (type and scale)</th>
<th>Variable description</th>
<th>Data acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Conversion Rate (FU/kg)</td>
<td>Site</td>
<td>Quantitative, Continuous (discrete scale)</td>
<td>Extracted value from herd database (database calculation based on total consumption of feed in age group during periods concerned/total pigs produced)</td>
<td>Herd database</td>
</tr>
<tr>
<td>Average Daily Weight Gain (gram)</td>
<td>Site</td>
<td>Quantitative, Continuous (discrete scale)</td>
<td>Extracted value from herd database (database calculation based on total gain in pigs in age group during period concerned/total pigs produced)</td>
<td>Herd database</td>
</tr>
<tr>
<td>Mortality (discrete number of dead relative to total number of pigs)</td>
<td>Site</td>
<td>Quantitative, Counts (discrete scale)</td>
<td>Based on input to herd database regarding number of dead pigs and pigs entered during periods concerned</td>
<td>Herd database</td>
</tr>
<tr>
<td>Antimicrobial consumption (ADD)</td>
<td>Site</td>
<td>Quantitative, Continuous (discrete scale)</td>
<td>Calculated value based on input in VetStat regarding prescribed AM in period concerned and number of animals registered in Central Husbandry Register or kg pig produced based on input to herd database</td>
<td>VetStat, Central Husbandry Register, Herd database</td>
</tr>
<tr>
<td>Bacterial detection in faecal sock samples (Lawsonia intracellularis, Brachyspira pilosicoli, Escherichia coli F4 and F18)</td>
<td>Batch in site</td>
<td>Qualitative, Dichotomous (discrete scale)</td>
<td>Detection of bacterial DNA copies</td>
<td>Commercial rPCR analysis at DTU National Veterinary Institute</td>
</tr>
<tr>
<td>Bacterial excretion level in faecal sock samples (Lawsonia intracellularis)</td>
<td>Batch in site</td>
<td>Quantitative, continuous (discrete scale)</td>
<td>Bacterial DNA copies by pathogen per gram faeces</td>
<td>Commercial rPCR analysis at DTU National Veterinary Institute</td>
</tr>
</tbody>
</table>

Note: 
- a Weaner site and finisher site
- b Feed unit (FU): a measurement of the nutritional value of the feed; for growing pigs 1 FU equals 7.38 MJ (Tybirk et al., 2006)
- c Animal Daily Doses (ADD) is a standardised unit defined by The Danish Veterinary and Food Administration. It indicates the theoretical amount of an AM product provided to a given animal species per kg body weight per day to achieve a standardised daily dose and is weighted in relation to the specific AM group (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022c)
- d (Agrosoft, 2022; Cloudfarms, 2022; Hansen and Grove, 2020)
- e Periods concerned were each of the two 6-months periods of Period 1 and Period 2, respectively, as defined for each site by the herd databases (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022a) (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022b)

Variables 'Weight entry', 'Weight exit', 'Days in production' and 'Number of pigs produced' were tested in univariable linear models. Only variables with $p < 0.2$ were included in the final regression models. Number of pigs produced differed considerably between sites and for a better model fit the variable was included as the logarithmical value of the count number.

Multilevel linear regression was used to evaluate the impact of vaccination on FCR and ADWG. Where weaners were concerned, 'Days in production' and 'Number of pigs produced' were included as fixed effects in the FCR model (Model 1a), and 'Weight exit' was included as a fixed effect in the ADWG model (Model 2a). For finishers, 'Weight exit', 'Days in production' and 'Number of pigs produced' were included as fixed effects in the FCR model (Model 1b) and 'Weight entry' and 'Weight exit' were included as fixed effects in the ADWG model (Model 2b).

Model 1a, weaners: FCR ~ 'LI vaccination' + 'Days in production' + 'Number of pigs produced' + 'Use of oral LI vaccination prior to study inclusion' | owner/site.
Model 2a, weaners: ADWG ~ 'LI vaccination' + 'Weight exit' + 'Use of oral LI vaccination prior to study inclusion' | owner/site.
Model 1b, finishers: FCR ~ 'LI vaccination' + 'Weight exit' + 'Days in production' + 'Number of pigs produced' + .
  'Use of oral LI vaccination prior to study inclusion' | owner/site.
Model 2b, finishers: ADWG ~ 'LI vaccination' + 'Weight entry' + 'Weight exit' + 'Use of oral LI vaccination prior to study inclusion' | owner/site.

Multilevel poisson regression was used to evaluate the impact of vaccination on mortality as indicated by number of dead pigs with offset in the total number of pigs in each period. 'Weight exit' was included in weaner model (Model 3a) and 'Weight exit' and 'Weight entry' were included in finisher model (Model 3b), all as fixed effects.

Model 3a, weaners: Mortality (number of dead) ~ 'LI vaccination' + 'Weight exit' + .
  'Use of oral LI vaccination prior to study inclusion' + offset(log total number of pigs) | owner/site.
Model 3b, finishers: Mortality (number of dead) ~ 'LI vaccination' + 'Weight entry' + 'Weight exit' + .
  'Use of oral LI vaccination prior to study inclusion' + offset(log total number of pigs) | owner/site.
2.9. Antimicrobial consumption data

Multilevel linear regression including ‘LI vaccination’ and ‘Use of oral LI vaccination prior to study inclusion’ as fixed effects was used to evaluate the development in AM consumption for both weaners and finishers (Model 4). The AM consumption was calculated in two ways: as the amount of active AM compound prescribed in the study period relative to the pig production (mg AM/kg pig produced) and, followin the method employed in Denmark, as Weighted Animal Daily Doses (ADD) per 100 animals per day (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022c).

Model 4, weaners and finishers: Oral AM consumption (Weighted ADD per 100 animals per day AND mg active AM/kg pig produced) ~ ‘LI vaccination’ + ‘Use of oral LI vaccination prior to study inclusion’ | owner/site.

2.10. Occurrence of diarrhoea and bacterial detection in faecal samples

Fisher’s exact test was used to evaluate the occurrence of diarrhoeic outbreaks within each observation period. The variables ‘Age’, ‘Diarrhoea’ and the interaction between them were included as fixed effects when the detection of each pathogen was evaluated by logistic regression (Model 5) and the excretion level of each pathogen by linear regression was evaluated (Model 6). In both models, the effect of ‘Age’ was tested both for the actual age in weeks post-weaning and for the sample category Pre-Fixtime vs. Fixtime.

Model 5: LI/BP/F18/F4 detection ~ ‘Li vaccination’ + ‘Diarrhoea’ + ‘Age’ + interaction ‘LI vaccination/diarrhoea’ + ‘Use of oral LI vaccination prior to study inclusion’.

Model 6: LI/BP/F18/F4 excretion level ~ ‘Li vaccination’ + ‘Diarrhoea’ + ‘Age’ + interaction ‘LI vaccination/diarrhoea’ + ‘Use of oral LI vaccination prior to study inclusion’.

3. Results

Initially, 41 herd complexes were referred by veterinarians to the study. Ten herd complexes were never admitted to the study either because they had LI levels below 4.8 log(10) copies/gr. faeces or as a result of the farmer’s reluctance to commit to the study period of at least one year. The remaining 31 herd complexes represented 37 weaner sites and 56 finishing sites. During the study period, 7 weaner sites and 14 finisher sites were excluded due to closure of the production site, deviation from the study protocol (e.g., change in housing or management procedures), the data not being considered valid or an outbreak of infectious disease (Actinobacillus pleuropneumoniae type 2, Porcine Reproductive and Respiratory Syndrome, Swine Influenza Virus, verocytotoxogenic Escherichia coli / Edema disease) as revealed by the data and the questionnaires. This left 25 herd complexes representing 28 weaner sites and 41 finisher sites. The herd complexes, which all were distributed throughout Denmark, had an annual production of pigs within the range 7400–85,000 (mean: 23,000; median: 20,000) and all were included in the study in the period November 2019 to May 2020.

3.1. Productivity data

One herd complex with two weaner sites reported productivity data for both sites in a single report, and therefore only 27 weaner site reports were included.

The productivity parameters for Period 1 and Period 2 are presented in Table 2 along side initial indication of the changes in productivity parameters.

The effects of vaccination on FCR and ADWG resulting from linear regression when Period 2 is compared with Period 1 are presented in Table 3. FCR was significantly reduced by vaccination for weaners by 0.12 FU/kg (p = 0.006) and for finishers by 0.08 FU/kg (p < 0.001). ADWG was significantly improved by vaccination for finishers by 45.6 gr. (p < 0.001). Mortality was reduced for both age groups, but only significantly in weaners (estimate (‘LI vaccination’): –0.073; RR= 0.912; p < 0.001; estimate (‘Weight exit’): 0.068, RR= 1.070; p < 0.001).

3.2. Antimicrobial consumption data

All weaner sites had oral AM prescribed at some point in the study, but two sites were not prescribed any oral AM in Period 2. For finishers, the number of sites without prescription of oral AM rose from 6 in Period 1 to 11 in Period 2.

When AM consumption was evaluated by the amount of mg. active AM compound a reduction in oral AM consumption was found for both weaners (22.1%) and finishers (38.8%), but this was statistically significant only for finishers (p = 0.005).

When oral AM consumption was evaluated by Weighted ADD per 100 animal per day similar results were found. There was a significant reduction for finishers, with estimated treatment with oral AM falling from 1.5% to 1.0% of the animals per day (p = 0.004) and a non-significant reduction for weaners, with estimated treatment with oral AM falling from 7.9% to 6.7% of the animals per day (p = 0.076). These results are presented in Table 4.

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Weaner</th>
<th></th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before LI vaccination</td>
<td>After LI vaccination</td>
<td>Change</td>
</tr>
<tr>
<td>FCR, FU/kg</td>
<td>1.92</td>
<td>1.83</td>
<td>-0.09 *</td>
</tr>
<tr>
<td></td>
<td>1.89</td>
<td>1.83</td>
<td>-0.12</td>
</tr>
<tr>
<td></td>
<td>1.55–2.54</td>
<td>1.6–2.06</td>
<td>-0.67–0.24</td>
</tr>
<tr>
<td></td>
<td>467.3</td>
<td>486.8</td>
<td>19.6 *</td>
</tr>
<tr>
<td></td>
<td>463.0</td>
<td>485.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>379–695</td>
<td>381.5–708.2</td>
<td>-41.0–103.0</td>
</tr>
<tr>
<td>FCR, ADWG, gr./day</td>
<td>2.56</td>
<td>2.66</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>2.47</td>
<td>2.78</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>1.2–6.8</td>
<td>0.89–4.92</td>
<td>-0.42–1.68</td>
</tr>
</tbody>
</table>

* Feed unit (FU): a measurement of the nutritional value of the feed; for growing pigs 1 FU equals 7.38 MJ (Tybirk et al., 2006)

b Mortality as percentage (number of dead/total number of pigs *100)

c Weaners approximately 4–12 weeks of age (7–30 kg); The 28 weaner sites had a total production of 259.166 and 251.873 pigs in Period 1 and Period 2, respectively.

d Finishers approximately 12–23 weeks of age (30-slaughter at 115 kg); The 41 finisher sites had a total production of 137.484 and 136.381 pigs in Period 1 and Period 2, respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before LI vaccination</th>
<th>After LI vaccination</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2022</td>
<td>2022</td>
<td></td>
</tr>
</tbody>
</table>

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against LI was the only variable of significance reducing BP prevalence (p < 0.001). In finisher samples vaccination against LI was the only explanatory variable with a significant impact in both weaner and finisher samples (estimate: = 0.01 (0.01) and p < 0.001). However previous field studies were made using a live, attenuated orally administrated LI vaccine (Jacobs et al., 2019). This was under experimental conditions; the current results confirm these findings in commercial conditions. A significant reduction in AM consumption following vaccination with Porcilis® Lawsonia Vet. was found irrespective of the method used to calculate AM consumption. AM consumption for oral group treatments was reduced by 3.8 mg. active compound/kg. pig produced (p = 0.005) or 0.5 weighted ADD per 100 animals per day in finishers (p = 0.004).

To evaluate the findings in the present study in the right context, it is relevant to review previous studies on the effect of controlling LI by vaccination. However previous field studies were made using a live, attenuated orally administrated LI vaccine (Entersol® Ileitis) as it has been available for about 20 years. Since the vaccines are having different formulations and ways of administration, comparison is only partly meaningful and should be done with caution. Field trials of the oral LI formulations and ways of administration, comparison is only partly meaningful and should be done with caution. Field trials of the oral LI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
<th>Estimate (SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR, FU/kg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Fixed effect</td>
<td>LI vacc.</td>
<td>-0.12 (0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pigs prod.</td>
<td>-0.11 (0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(log10) Days</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td></td>
<td>Random effect (Std.Dev.)</td>
<td>Site</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Owner</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual</td>
<td>0.138</td>
</tr>
<tr>
<td>ADWG, gr./day</td>
<td>Fixed effect</td>
<td>LI vacc.</td>
<td>5.8 (6.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weight exit</td>
<td>9.9 (1.56)</td>
</tr>
<tr>
<td></td>
<td>Random effect (Std.Dev.)</td>
<td>Site</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Owner</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual</td>
<td>23.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Feed unit (FU): a measurement of the nutritional value of the feed; for growing pigs 1 FU equals 7.38 MJ (Tybirk et al., 2006)

### 4. Discussion

In both weaners and finishers, intramuscular vaccination against LI with Porcilis® Lawsonia vet. at weaning significantly improved productivity parameters and reduced AM consumption. Further, the prevalence and excretion levels of both LI and BP were reduced and there were fewer diarrhoeic outbreaks in weaner sites. However, prevalence and excretion levels of F4 and F18 were unaffected by the LI vaccination. In weaners, LI vaccination significantly reduced FCR by 0.12 FU/kg and mortality risk by 8.8%. Although the raw average of weaner mortality seemed to increase numerically (Table 2) probably as a result of a slightly skewed distribution, risk of mortality was significantly when all explanatory factors were taken into account. In finishers, LI vaccination significantly reduced FCR by 0.08 FU/kg and significantly increased ADWG by 45.6 gr./day. The only other published study on Porcilis® Lawsonia Vet. also found increased ADWG for vaccinated pigs (Jacobs et al., 2019). However, this was under experimental conditions; the current results confirm these findings in commercial conditions. A significant reduction in AM consumption following vaccination with Porcilis® Lawsonia Vet. was found irrespective of the method used to calculate AM consumption. AM consumption for oral group treatments was reduced by 3.8 mg. active compound/kg. pig produced (p = 0.005) or 0.5 weighted ADD per 100 animals per day in finishers (p = 0.004). To evaluate the findings in the present study in the right context, it is relevant to review previous studies on the effect of controlling LI by vaccination. However previous field studies were made using a live, attenuated orally administrated LI vaccine (Entersol® Ileitis) as it has been available for about 20 years. Since the vaccines are having different formulations and ways of administration, comparison is only partly meaningful and should be done with caution. Field trials of the oral LI

### 3.3. Diarrhoea occurrence and bacterial detection in faecal sock samples

No sites had more than one Pre-Fixtime sample in addition to the Fixtime sample. One weaner site consisted of two separate buildings at the same location and contributed with two sets of samples. Thus, a total of 29 weaner and 41 finisher faecal sock samples were included.

Before vaccination, three weaner sites experienced no outbreaks of diarrhoea during the observation period in batches later than two weeks post-weaning. This number grew to 13 after vaccination, which was a significant reduction in outbreaks of diarrhoea within the period (p = 0.007). In finishers, the number of sites experiencing no outbreaks of diarrhoea during the observation period was 25 and 26 before and after LI vaccination, respectively. The distribution of faecal sock samples by category, prevalence and excretion levels is presented in Table 5 and Fig. 2.

For detection of LI (Model 5) vaccination against LI was the only explanatory variable with a significant impact in both weaner and finisher samples (p < 0.001 and p = 0.002, respectively). In weaner samples detection of BP did not decrease. In finisher samples vaccination against LI was the only variable of significance reducing BP prevalence (p = 0.043). Only age had a significant impact on the detection of F18 in weaners, with a higher prevalence in younger age groups in Pre-Fixtime samples (p < 0.001). No variables were significant in finishers.

Turning to excretion levels (Model 6), LI vaccination was the only explanatory variable with a significant impact on: LI excretion level, seen in both weaners (estimate: = −1.32; SE: 0.355; p < 0.001) and finishers (estimate: = −1.04; SE: 0.31; p = 0.001), and on BP excretion level, again seen in both weaners (estimate: = −1.02; SE: 0.22; p < 0.001) and finishers (estimate: = −1.16; SE: 0.32; p < 0.001). For F18, none of the explanatory variables were of significance (F18 was detected in fewer than 14 weaner samples and 5 finisher samples). A very low level of prevalence (≤ 5 positive samples), meant that prevalence and excretion level for F4 were not included in the statistical models.
Lawsonia intracellularis and Brachyspira pilosicoli F4 and F18 in the samples collected at different time points during observation periods before and after vaccination against Lawsonia intracellularis samples

<table>
<thead>
<tr>
<th>Vaccination Period</th>
<th>Prevalence (%)</th>
<th>Excretion Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Vac.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After Vac.</td>
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<td>Before Vac.</td>
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<td>After Vac.</td>
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<tr>
<td>Before Vac.</td>
<td></td>
<td></td>
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<tr>
<td>After Vac.</td>
<td></td>
<td></td>
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</tbody>
</table>

**Table 5.**

<table>
<thead>
<tr>
<th>Prevalence (%)</th>
<th>Excretion Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-vaccination</td>
<td></td>
</tr>
<tr>
<td>Post-vaccination</td>
<td></td>
</tr>
</tbody>
</table>

*One-sample t-testing of excretion level in positive samples (copies/gram faeces log(10)).

Vaccination with Porcilis® Lawsonia Vet. significantly reduced LI prevalence in both weaner and finisher sites, and in finisher sites BP prevalence was also significantly reduced. Excretion levels of LI and BP were reduced for weaners by 1.32 and 1.02 log(10) copies/gr faeces, respectively, and for finishers they were reduced by 1.04 and 1.16 log(10) copies/gr faeces, respectively. The reduced prevalence and excretion levels of LI found in this study confirm results for Porcilis® Lawsonia described by Jacobs et al. (2019), and Porcilis® Ileitis described by Roerink et al. (2018), where reduced LI shedding was also demonstrated in trials. A possible correlation between LI and BP excretion levels has been reported (Peiponen et al., 2018) and in finishers, improvements found in 2020, and thus coinciding with the study period, were the largest seen in the past 10 years (Hansen, 2022b), In the same period (2020), total kg. active ingredient of AM prescribed for oral consumption for pigs increased in absolute terms by 4% nationally (Danish Ministry of Health - Statens Serum Institut SSI - DANMAP, 2021), but when adjustments are made for the number of animals produced, consumption remains unchanged (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022b). The improvements found in this study, both in productivity parameters and AM consumption, exceed the improvements seen at a national level for the same period.

Vaccination with Porcilis® Lawsonia Vet. has obtained results for FCR in finishers ranging from non-significant (Hardge et al., 2004; McOrist and Smits, 2007; Peiponen et al., 2018) to improvements of 0.16–0.2 kg/kg (Deitmer et al., 2011; McOrist and Smits, 2007). In weaners improved ADWG by 15 gr./day has been reported (Peiponen et al., 2018) and in finishers, improvements ranging from non-significant to +150 gr./day (McOrist and Smits, 2007) have been reported, with most results within the range of 20–47 gr./day (Bak and Rathkjen, 2009; Deitmer et al., 2011; Hardge et al., 2004; Park et al., 2013; Peiponen et al., 2018). In subclinical LI-infected herds, non-significant differences have been found in mortality (Deitmer et al., 2011; Hardge et al., 2004; McOrist and Smits, 2007; Park et al., 2013; Peiponen et al., 2018). Reductions in AM consumption range from non-significant in both weaners and finishers (Kruse et al., 2017; Peiponen et al., 2018) to a 79% reduction in weaners (Bak and Rathkjen, 2009) and a 50–100% reduction in finishers (Deitmer et al., 2011; Voets and Hardge, 2007).

As the present study compared results obtained in periods being six months apart, ongoing genetic selection for improved productivity must be considered. Annual average national productivity data covering the study period (June 2019 to June 2021) revealed an improved FCR of 0.015 and 0.01 FU/kg, and an increased ADWG of 7.5 and 20.5 gr./day, in weaners and finishers, respectively. In 2021, the respective national averages for weaners and finishers were 1.81 and 2.71 FU/kg for FCR and 463 and 1032 gr./day for ADWG (Hansen, 2022a). The productivity improvements found in 2020, and thus coinciding with the study period, were the largest seen in the past 10 years (Hansen, 2022b). In the same period (2020), total kg. active ingredient of AM prescribed for oral consumption for pigs increased in absolute terms by 4% nationally (Danish Ministry of Health - Statens Serum Institut SSI - DANMAP, 2021), but when adjustments are made for the number of animals produced, consumption remains unchanged (Ministry of Food Agriculture and Fisheries of Denmark - Danish Veterinary and Food Administration, 2022b). The improvements found in this study, both in productivity parameters and AM consumption, exceed the improvements seen at a national level for the same period.

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within sites before and after LI vaccination. Various changes will occur naturally in a pig herd over a period of 19 months. The potential confounding effect of this were mitigated by the inclusion of a large number of herds. Changes in feed, management and health status within herd complexes were also tracked through repeated questionnaires covering Period 1 and Period 2. In addition to the questionnaire, changes in health status were checked by tracking ongoing production data, monthly herd visits by the herd veterinarian and surveillance in accordance with the Danish SPF-status system (21 of the 25 herd complexes were part of the SPF health system and in the remaining 4 herd complexes health status was monitored in clinical inspections and sampling in accordance with the veterinarian’s assessment) (The Danish Agriculture and Food Council, 2023). The decision to initiate LI vaccination, on basis of which the herd was then referred to the study, was made jointly by the herd veterinarian and herd owner without further assistance. No information about productivity level or AM consumption was available at this point nor was it specified by the study protocol in the inclusion criteria. Although each site acted as its own control, the results would have been stronger with a similar group of sites acting as a control group. However, this was not possible owing to 1) the variety of farm characteristics making it impossible to find a perfect control herd for each case herd and 2) the fact that a control herd known by the herd manager to be a “control” would not be unbiased in its use of PPE treatments (which would be a methodologically problem also if making use of different units/sites within each herd as cases and controls over parallel periods of time, as it would not have been possible to extract herd level productivity data by group). As the best possible alternative, national average is therefore included as a comparison enabling the developments that might have occurred over time anyway to be considered.

The interpretation of the results of this study must show due regard for the fact that the herd complexes were selected because they had a history of outbreaks of diarrhoea caused by LI at levels expected to affect productivity figures. This meant, that the vaccination against LI was included in the study if they belonged to a LI-infected herd complex with high levels of LI detected at other sites within the complex. As LI can spread through a herd slowly (Stege et al., 2004) and differences in the onset of infection between pigs within a herd can occur (Musse et al., 2022a), all the sites were found to be at risk. Therefore, both weaner and finisher sites were included from LI-infected herd complexes, even though not all sites could be expected to benefit equally from the LI vaccination given that some sites were more affected by the LI infection than others.

At the point of their inclusion, the sites in this study had average to above-average productivity performance relative to national levels but still experienced subclinical-to-clinical LI infection. The results of the study are therefore relevant for this type of herd, although potentially, an even bigger positive impact might be expected for herds with poor performance (below average) and/or with a massive LI infection causing clinical chronic or acute PPE.

5. Conclusion

In field conditions, through the comparison of several productivity parameters, AM consumption and the dynamics of enteric pathogens, this study evaluated the efficacy of vaccination against LI using an inactivated intra-muscularly administered vaccine in naturally LI-infected pig herds. It was found that vaccination reduced FCR by 0.12 FU/kg and 0.08 FU/kg in weaners and finishers, respectively, and that ADWG increased by 45.6 gr./day in finishers. Further, mortality was reduced in weaners (RR 0912; \( p < 0.001 \)). AM consumption based on AM prescribed for oral group treatments was reduced by 3.8 mg active compound/kg pig produced or 0.5 Weighted ADD per 100 animals per day in finishers. Whereas prevalence and excretion levels of F18 and F4 in faecal sock samples were not affected by vaccination, LI prevalence was reduced in samples from both weaner sites and finisher sites, and in addition BP prevalence in samples from finisher sites decreased. Excretion levels of LI and BP in samples from weaner sites decreased by 1.32 and 1.02 log(10) copies/gr faeces, respectively. In samples from finisher sites they decreased by 1.04 and 1.16 log(10) copies/gr faeces, respectively.

In conclusion, vaccination against LI using Porcilis® Lawsonia Vet. was found to have a significantly beneficial effect on productivity parameters, AM consumption, and the dynamics of enteric pathogens under field conditions in naturally LI-infected pig herds.

**Declaration of Competing Interest**

SLM and GBN are employed by MSD Animal Health, which have a commercial 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 enrolment as Industrial PhD student.
student at University of Copenhagen. NW is employed by Danish Agriculture & Food Council, which manages the interests of primary pig producers and related companies. HH and HS are employed by University of Copenhagen which builds on an academic environment based on independence of research.

Acknowledgements

The authors thank Jeanette Kristensen for her major help in extracting data from the VetStat database and Professor Matt Denwood for assistance in connection with statistical model selection. Further, the authors thank participating herd owners and veterinarians. The study was economically supported by Innovation Fund Denmark (grant number 9065-00097B) and MSD Animal Health Nordics.


