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Efficacy of a novel inactivated Lawsonia intracellularis vaccine in pigs against experimental infection and under field conditions

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ABSTRACT

The efficacy of a novel inactivated *Lawsonia intracellularis* vaccine, Porcilis® Lawsonia, was compared to that of a commercially available live attenuated vaccine in three experimental vaccination-challenge studies in pigs. The efficacy of the new vaccine was further tested under field conditions on a farm with a history of acute ileitis.

The novel inactivated vaccine consists of a freeze-dried antigen fraction that is dissolved just prior to use in either the adjuvant or in Porcilis® PCV M Hyo; an existing combination vaccine against porcine circovirus type 2 and *Mycoplasma hyopneumoniae*.

The three experimental vaccination-challenge trials had a similar design and for each trial 75 piglets were used, randomly allotted to three groups of 25 piglets. The pigs were vaccinated at 4 or 5 weeks of age with either Porcilis® Lawsonia in adjuvant or in associated mixed use with Porcilis® PCV M Hyo (group 1), with the live vaccine (group 2), or left as unvaccinated controls (group 3). The pigs were challenged with virulent *Lawsonia intracellularis* 3, 4 or 17 weeks after vaccination. Post-challenge the pigs were evaluated for clinical signs, average daily weight gain, shedding and macroscopic as well as microscopic immuno-histological ileum lesion scores. In the field study, the mortality and key performance parameters were evaluated over a period of 8 months.

The results of all three experimental vaccination-challenge trials showed that Porcilis® Lawsonia induced statistically significant protection against experimental *Lawsonia intracellularis* infection. This was demonstrated by lower clinical scores, improved weight gain, reduction of *Lawsonia intracellularis* shedding and reduction of macroscopic as well as microscopic ileum lesion scores when compared to the controls. The protection induced was superior to that of the commercially available live vaccine. In the field study, Porcilis® Lawsonia proved to be highly efficacious; reducing Lawsonia associated mortality to zero and improving key production parameters.

1. Introduction

Porcine proliferative enteropathy, also known as ileitis, is an infectious intestinal disease characterised by thickening of the distal small and proximal large intestinal mucosa as a result of enterocyte proliferation associated with the presence of an intracellular bacterium, *Lawsonia intracellularis* [1]. This porcine intestinal pathogen has been identified as one of the main enteric pathogens of fattening pigs worldwide, with prevalence ranging from 48 to 100% in different swine producing countries [2–4]. Clinically affected animals exhibit diarrhoea and reduced growth performance resulting in increased time to market and greater variation in size between pigs. In young adults, the infection can lead to an acute haemorrhagic form of the disease characterised by dark, tarry diarrhoea which may result in death. *Lawsonia intracellularis* also infects pigs subclinically, without clear clinical signs, but still resulting in reduced growth performance [5].

In addition to *Lawsonia intracellularis*, porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (M Hyo) are two other major pathogens affecting the health of fattening pigs [6,7]. Vaccines against these two pathogens are routinely used in pigs worldwide. Several single as well as combination vaccine options are available, for example: the ready to use products Porcilis® PCV M Hyo (MSD-AH) and Suvaxyn® Circo + MH (Zoetis), or the associated mixed use of CircoFLEX® and MycoFLEX® (Boehringer Ingelheim). These vaccines are licensed for use in fattening pigs from 3 weeks of age.

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In the EU, a live attenuated vaccine against Lawsonia is available for oral use in fattening pigs from 3 weeks of age. A disadvantage of this live vaccine is that it is not compatible with the use of antibiotics. A second disadvantage is that it does not have associated use claims for PCV2 and MHyo vaccines that should be administered to animals of the same target age. For animal welfare reasons, as well as to reduce labour, it would be preferred if the vaccines against these three major swine pathogens could be administered at the same time, and ideally if all three vaccine antigens could be administered in one single injection. To address this, a safe and efficacious inactivated Lawsonia vaccine, Porcilis® Lawsonia, which is compatible with Porcilis® PCV M Hyo was developed. This vaccine consists of a freeze-dried antigen fraction and a solvent fraction containing the adjuvant (Emunade®). The freeze-dried Lawsonia antigen is reconstituted just prior to use in Emunade® and administered intramuscularly as a single 2 ml dose to piglets from 3 weeks of age. Alternatively, the freeze-dried fraction can be reconstituted in Porcilis® PCV M Hyo, which also contains Emunade®, and administered in associated mixed use with this combination vaccine.

The aim of this study was to assess the efficacy of the novel inactivated Lawsonia vaccine as standalone in comparison with the commercially available live attenuated vaccine against Lawsonia challenge. Since vaccinations against M Hyo and PCV2 are also required in the field, the associated mixed use of the new vaccine with Porcilis® PCV M Hyo was tested in a challenge study. Besides these experimental vaccination-challenge studies, the vaccine was also tested in a field trial on a farm with Lawsonia associated mortality.

2. Materials and methods

2.1. Design of the experimental vaccination-challenge trials

Three studies were performed, each with a similar design (Table 1).

For each trial 75 piglets, from a farm known to be negative for M Hyo and PRRSV (Porcine Reproductive & Respiratory Syndrome Virus) and no history of Lawsonia infection, were randomly allotted to three groups of 25 piglets each. Group 1 were vaccinated once intramuscularly with 2 ml Porcilis® Lawsonia at 4 weeks of age (studies 1 and 2), or with 2 ml Porcilis® Lawsonia in associated mixed use with Porcilis® PCV M Hyo at 5 weeks of age (study 3). Group 2 were vaccinated orally with 2 ml of the live vaccine at 4 weeks of age (studies 1 and 2), or at 5 weeks of age (study 3). The latter group also received 2 ml of a PCV2-M Hyo vaccine combo intramuscularly at 3 weeks of age. The split vaccination was necessary because the live vaccine has no concurrent use claim with other vaccines. In all three studies group 3 were left as unvaccinated controls. The pigs were challenged with *Lawsonia intracellularis* 4 weeks after vaccination (study 1), 17 weeks after vaccination (study 2), or 3 weeks after the last vaccination (study 3). The challenge was carried out by the oral administration of homogenised *Lawsonia* infected intestinal mucosa. The pigs were observed daily for clinical signs of *Lawsonia* infection and were weighed at weekly intervals throughout the 21-day post-challenge period. Serum samples were collected on the day of vaccination, the day of challenge and the day of necropsy. All pigs were euthanised and necropsied 21 days post-challenge. During necropsy faecal samples were collected from the rectum for testing in a Lawsonia specific qPCR. The intestines, in particular the ileum, were macroscopically checked for *Lawsonia intracellularis* infection and Ileum samples were collected for qPCR and immunohistological scoring (IHC).

The *in-vivo* experiments were conducted according to EU directive 2010 63/EU and the relevant Dutch legislation (Act on Animal Experimentation, permit number AVD221002016561).

2.2. Design of the field trial

The field trial was carried out according to a negative controlled, randomised and masked design in a commercial pig herd in the Netherlands with a history of Lawsonia associated mortality, i.e. acute ileitis occurring from 20 weeks of age onwards. The study involved 2876 pigs of which 1435 were vaccinated once in the fattening unit with vaccinates and controls commingled in pens. At the start of the study all pigs present in the fattening unit, up to 23 weeks of age, were included in the study. Thereafter, during a period of 8 months, all new subsequent batches of pigs arriving in the fattening unit were included in the study at an age of approximately 12 weeks. Half of the pigs were vaccinated with Porcilis® Lawsonia, whereas the control pigs were not vaccinated against Lawsonia. With the exception of a PRRS vaccination at 6 weeks of age no further vaccines were administered and the pigs received no routine medication. The mortality in vaccinates and controls was evaluated until slaughter at approximately 26 weeks of age. Key performance parameters, i.e. overall mortality, average daily weight gain (ADWG) and feed conversion rate were determined for the whole herd and were derived from the farm data management system for historic comparison. Pigs that died or were euthanised were examined by post-mortem to establish the cause of death. Lawsonia infection was determined by the specific signs of proliferative enteropathy and confirmed by immunohistochemistry as described under 2.7. The primary efficacy parameters were the mortality associated with Lawsonia infection as well as the total mortality in vaccinates and controls during the study period. Secondary efficacy parameters such as overall mortality, ADWG and feed conversion rate were determined for the whole herd (derived from the farm data management system) and therefore could only be compared historically.

Table 1
Experimental design of vaccination-challenge trials.

Trial	Group	Number of pigs	Vaccine/age/route/volume	Age at Lawsonia challenge
1	1	25	PLL[a] + Emunade/4w[b]/IM[c]/2 ml	8w
	2	25	Live vaccine/4w/oral/2 ml	8w
	3	25	unvaccinated control	8w
2	1	25	PLL + Emunade/4w/IM/2 ml	21w
	2	25	Live vaccine/4w/oral/2 ml	21w
	3	25	unvaccinated control	21w
3	1	25	PLL + Porcilis PCV M Hyo/5w/IM/2 ml	8w
	2	25	Live vaccine/5w/oral/2ml[d]	8w
	3	25	unvaccinated control	8w

[a] Porcilis Lawsonia lyophilisate to be dissolved in Emunade or in Porcilis PCV M Hyo.
[b] Weeks.
[c] Intramuscularly in the neck.
[d] This group was also vaccinated at 3 weeks of age with a commercially available PCV-M Hyo vaccine combo.

2.3. Vaccines

Porcilis® Lawsonia lyophilisate (three different batches), Emunade® solvent and Porcilis® PCV M Hyo were from MSD Animal Health. Just prior to use, Porcilis® Lawsonia lyophilisate was dissolved in either Emunade® (studies 1, 2 and the field trial) or in Porcilis® PCV M Hyo (study 3).

Two different batches of the commercially available live vaccine were used; one batch in studies 1 and 2 and a second batch in study 3. The vaccine was prepared just before use and administered orally by drenching according to the manufacturers instruction. A PCV2-M Hyo combination vaccine was obtained from the same supplier and used in study 3. This product consists of a vial of PCV vaccine and a vial of M Hyo vaccine that are mixed just prior to use. The mixing and intramuscular use were also in accordance with the manufacturer's instructions.

2.4. Challenge material

Challenge material was prepared from intestinal scrapings derived from Lawsonia infected pigs (field cases). Pigs with clinical signs of Lawsonia infection were transported to MSD-AH and necropsied. Affected parts of the intestines were scraped and the mucosa stored at −70 °C until use. The material was used for challenge only after immunohistological confirmation of the Lawsonia infection and if there was no indication that other pathogens were involved.

Just prior to challenge portions of 500 g infected mucosa were thawed and mixed with 500 ml 0.04 M isotonic PBS. This mixture was homogenised in an omnimixer for one minute at 16,000 rpm on ice. Each pig was orally challenged with 20 ml challenge material.

Different batches of challenge material derived from different sources were used in each of the three studies, but within a study all pigs were challenged with the same batch of challenge material.

2.5. Post-challenge clinical observations

All pigs were clinically observed for signs of Lawsonia infection just prior to challenge, and on a daily basis after challenge. The following scoring system was used: 0 = normal, 1 = mild diarrhea, 2 = moderate diarrhea, 3 = severe diarrhea and/or with incorporation of blood; all other abnormalities were described.

Animals that died or were euthanised because of a severe Lawsonia infection were assigned a score of 3 for each of the remaining observation points to allow the magnitude of the clinical effect to be appropriately recognised in the analysis. In this challenge model clinical signs become apparent in the third week after challenge. The daily clinical scores 13 to 20 days post-challenge were totalled and averaged by group.

2.6. Performance/weighing

The pigs were weighed one day before challenge, and on days 6, 13 and 20 after challenge. In this challenge model the negative effect on growth becomes apparent in the third week after challenge. The ADWG in the third week after challenge (days 13 to 20) was calculated for each individual animal and averaged by group.

2.7. Post-mortem examination

Three weeks after challenge the pigs were euthanised by electric stunning followed by bleeding and a post-mortem examination was carried out. At necropsy the intestines, in particular the ileum (i.e. the last 0.5 m of the small intestine), were examined for lesions indicative of Lawsonia infection. A faecal sample (from the rectum) and an ileum sample (5 cm above the ileo-caecal junction) were collected from each animal for testing in a Lawsonia specific qPCR. In addition, an ileum sample was collected and fixed in 4% buffered formalin and then further processed into slides. These slides were stained with Hematoxylin-Eosin (HE stain) and with an immunohistochemical stain using an anti-*Lawsonia intracellularis* monoclonal antibody (IHC stain) and were examined microscopically.

The ileum mucosa was macroscopically scored using the following scoring system: 0 = normal, 0.5 = slight, 1 = mild, 2 = moderate, 3 = severe thickening and/or reddening; 4 = severe thickening and/or reddening with fibrin and/or necrosis; other abnormalities were described. In addition, the percentage of the ileum affected was estimated as follows: the length of the affected part of the ileum was divided by the length of the ileum and multiplied by 100. The total ileum lesion score was calculated by multiplication of the ileum mucosa score and the % ileum affected. The average total ileum lesion score was calculated for each treatment group.

The histological scoring was performed using the following scoring system for HE stain: 0 = no abnormalities detected, 0.5 = doubtful lesion, 1 = mild lesions, 2 = moderate lesions 3 = severe lesions and for the IHC stain: 0 = no bacteria evident, 0.5 = doubtful presence of bacteria, 1 = presence of single/small numbers of bacteria within the slide, 2 = presence of moderate numbers of bacteria within the slide, 3 = presence of large numbers of bacteria within the slide. The total histological score = HE score × IHC score. The average total histological score was calculated for each group.

2.8. Lawsonia serology

Serum samples were tested in an in-house Lawsonia antibody ELISA. Microtitre plates were coated with Lawsonia antigen. After coating, the plates were washed and serial three-fold dilutions of the test sera were made. Following incubation and subsequent washing, the bound antibodies were quantified by using an anti-pig conjugate and a TMB substrate. Titres were expressed in $\log_2$. In this test, titres < 3.9 are considered negative. For calculation purposes < 3.9 was replaced by 2.9. The intermediate precision and repeatability for this ELISA were established as 0.25 $\log_2$ and 0.14 $\log_2$, respectively.

2.9. PCV serology

In study 3, sera were tested in an in-house PCV2 blocking ELISA as described previously [8]. Serially four-fold diluted serum samples were incubated on microtitre plates coated with baculovirus expressed PCV2 ORF2 antigen. After removing the sera, all wells were incubated with a fixed amount of biotin-labelled PCV2-specific monoclonal antibody. Bound monoclonal antibody was incubated with peroxidase-conjugated streptavidin followed by chromophoric detection. Titres were expressed in $\log_2$. In this test titres < 2.0 are considered negative. For calculation purposes < 2.0 was replaced by 1.0.

2.10. Mycoplasma hyopneumoniae serology

For *Mycoplasma hyopneumoniae* serology (study 3) the "Swine HerdChek M. hyo IDEXX" was performed according to the instructions of the supplier. The results are expressed as S/P ratio and scored as positive (>0.40), inconclusive (0.30–0.40) or negative (<0.30). The serological responses are presented as the average S/P ratio per treatment group.

2.11. Lawsonia PCR

DNA was isolated from 0.2 g faeces and/or mucosa samples using the MagNA Pure 96 robot. Before DNA isolation the faeces and mucosa samples were homogenised. To homogenise faeces, 550 μl STAR buffer was added and subsequently vortexed. To 250 μl homogenate 250 μl lysis buffer and 50 μl proteinase K were added and incubated for 10 min. at 65 °C followed by 10 min. at 95 °C. To homogenise mucosa, the material was transferred to a MagNA Lyser green bead tube, 800 μl lysis buffer was added and subsequently homogenised in the MagNA lyzer for 30 sec. at 7000 rpm. From the homogenised faeces and/or mucosa, 200 μl was processed in the MagNA Pure 96 with the kit DNA/Viral NA SV and protocol Pathogen Universal 200. The MagNA Pure96, MagNA Lyzer, and buffers were from Roche. An in-house quantitative PCR on the AspA gene of *Lawsonia intracellularis* was performed using the Kapa probe fast universal qPCR kit mastermix (Kapabiosystems), 100 nM probe (5′-FAM-TGTACTTGTCCCTG CACCTCC TTGA-BHQ1-3′), 160 nM forward-primer (5′-CTCTGCTG CATGTAATGAAATC-3′), 160 nM reverse-primer (5′-AAGCTCAAGAG CACGATTAC-3′) (Biolegio) with the following program on a CFX real-time machine (Biorad): step 1) 5 min. 95 °C, step 2) 10 sec. 95 °C, step 3) 5 sec. 70 °C, step 4) 10 sec. 55 °C, plate read, ramp rate 2 °C per second, go to step 2 for 49 times. The limit of detection for the PCR on faeces is 10 pg/μl and for the PCR on mucosa samples is 0.1 pg/μl. Values < 10 and < 0.1, respectively, were considered negative and taken as 0.0 for calculation purposes.

2.12. Statistical analysis

The level of significance was set at 0.05 and all tests were two-sided. Statistical analyses were carried out using SAS V9.4 (SAS Institute Inc. Cary NC, USA). Where applicable (ADWG, qPCR faeces and qPCR ileum mucosa), the validity of AN(C)OVA was routinely checked on the homogeneity of the variance and normality of the residuals by visual inspection of the residual plots.

2.12.1. Serology

Antibody titres were evaluated using descriptive statistics. The average values were plotted with the 95% confidence interval where no overlap indicates statistical significance.

2.12.2. Diarrhoea score

In this challenge model, specific clinical signs due to challenge usually become apparent in the third week after challenge. The diarrhoea scores between days 13 and 20 were statistically analysed by a cumulative logit model [9] accounting for the correlation in the repeated measurements using Generalized Estimating Equations (GEE) with p-values based on empirical standard error.

2.12.3 Weight. gain post-challenge

Since the effect of the Lawsonia infection on weight gain is observed mainly in the third week after challenge, the ADWG in this period (days 13 to 20) was calculated and statistically analysed by Analysis of CoVariance (ANCOVA) using the weight at day 13 as covariate and using Tukey's post-hoc test to compare groups.

2.12.4. qPCR: Faeces and ileum mucosa

qPCR data from faeces and ileum mucosa samples were $\log_{10}$ transformed after adding 1 to avoid zeros and statistically analysed by Analysis of Variance (ANOVA) using Tukey's post-hoc test to compare groups.

2.12.5. Gross pathology and histology ileum

The macroscopic total ileum lesion score and the total histology score were statistically analysed by a cumulative logit model [9] with p-values based on Likelihood-Ratio. The odds ratio was defined here as the odds on having lower classes in the vaccine group relative to that in the control group.

2.12.6. Mortality rate in the field trial

The mortality was evaluated by a generalised linear mixed model for binominals using logit as link function and with treatment as fixed effect.

3. Results

3.1. Trial 1

On the day of vaccination, all pigs were either seronegative for Lawsonia or had a low antibody titre. On the day of challenge (4 weeks after vaccination), active seroconversion was evident in group 1, whereas the antibody titres measured in groups 2 and 3 remained comparable to the pre-vaccination levels. All groups had developed an increased antibody response by the day of necropsy, 3 weeks after challenge (Fig. 1).

During the study, three animals were culled before the scheduled day of post-mortem examination. One animal (group 2) was euthanised on the day of challenge after days of increasing locomotory problems. A second animal (group 1) was euthanised one day after challenge because of neurological signs. Necropsy and bacteriological examination confirmed that this animal had been suffering from meningitis caused by *Streptococcus suis*. A third animal (group 3) was euthanised 20 days post-challenge on account of Lawsonia specific disease i.e. several days of diarrhoea, weight loss and deteriorating condition.

In the third week after challenge, the control animals developed clinical signs of Lawsonia infection characterised by diarrhoea, sub-optimal weight gain and shedding of Lawsonia in the faeces (Table 2). Upon necropsy the Lawsonia infection was confirmed by typical macroscopically visible ileum lesions (mucosal reddening and thickening), positive mucosa PCR and immunohistological ileum lesion scores. Pigs vaccinated with Porcilis® Lawsonia showed a statistically significant reduction in clinical signs, weight loss (i.e. ADWG 935 g vs 550 g) and macroscopic as well as microscopic ileum lesion scores when compared with the controls. In addition, the ADWG, the bacterial load in the ileum mucosa (PCR) and macroscopic as well as microscopic ileum lesion scores were significantly improved when compared to group 2. In this latter group, most of the parameters (with the exception of the faeces and ileum mucosa PCR) were also reduced when compared to the controls, but the effects were less pronounced than in the group 1 comparison. The differences between group 2 and the controls were statistically significant for clinical signs and microscopic ileum lesion scores.

3.2. Trial 2

On the day of vaccination all pigs were either seronegative for Lawsonia or had a low antibody titre. On the day of challenge (17 weeks after Lawsonia vaccination), active seroconversion was evident in the pigs of group 1, whereas the antibody titres measured in groups 2 and 3 remained comparable to the pre-vaccination levels. All groups had developed an increased antibody response by the day of necropsy, 3 weeks after challenge (Fig. 2).

During the study four animals were culled before the scheduled day of post-mortem. One animal (group 1) was culled before challenge because of increasing locomotory problems due to kyphosis. Three animals (two in group 2 and one in group 3) were euthanised on day 17 post-challenge on account of Lawsonia specific disease

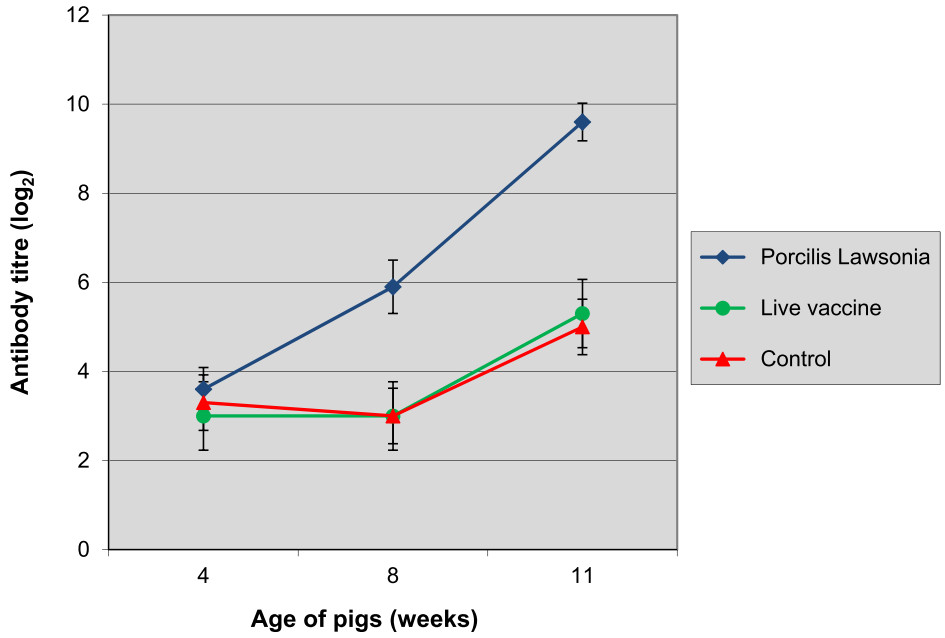

Fig. 1. Time-course of Lawsonia antibody development in trial 1. Group 1 was vaccinated at 4 weeks of age with Porcilis® Lawsonia, group 2 was vaccinated at 4 weeks of age with the live vaccine and group 3 was left unvaccinated. All pigs were challenged with Lawsonia infected gut mucosa at 8 weeks of age. Bars indicate 95% confidence interval.

Table 2
Post-challenge results ± SD of vaccination-challenge trials 1, 2 and 3.

Vaccine group	avg clinical score day 13–20	ADWG g/day day 13–20	PCR faeces avg log pg DNA/μl		PCR mucoosa avg log pgDNA/μl day 21	avg macroscopic ileum score day 21	avg microscopic ileum score (IHC) day 21
			day 0	day 21			
Trial 1: vaccination at 4 weeks of age, challenge at 8 weeks of age, necropsy 21 days after challenge							
PLL[a] + Emunade	0.3 ± 1.2[e]	935 ± 306[e,f]	0	0.23 ± 0.64	0.18 ± 0.43[f]	19 ± 64[e,f]	1.3 ± 2.8[e,f]
Live vaccine[b]	0.9 ± 2.3[e]	655 ± 385	0	0.60 ± 0.82	0.66 ± 0.84	71 ± 127	3.8 ± 3.6[e]
Control	4.4 ± 6.5	550 ± 460	0	0.34 ± 0.62	0.57 ± 0.56	115 ± 164	6.3 ± 3.3
Trial 2: vaccination at 4 weeks of age, challenge at 21 weeks of age, necropsy 21 days after challenge							
PLL + Emunade	3.0 ± 5.5	649 ± 751[e,f]	0	0.27 ± 0.54	0.71 ± 0.76[e]	113 ± 151[e,f]	5.6 ± 3.8[e]
Live vaccine	2.8 ± 5.8	−229 ± 1301	0	0.11 ± 0.38	1.05 ± 0.84	227 ± 194	5.8 ± 4.2[e]
Control	5.7 ± 5.5.	−655 ± 723	0	0.46 ± 0.70	1.36 ± 0.57	288 ± 156	8.5 ± 1.7
Trial 3: vaccination at 3 (PM vaccine) or at 5 weeks of age (PLL + PCV M Hyo and Live Lawsonia vaccine), challenge at 8 weeks of age, necropsy 21 days after challenge							
PLL + PCV Mhyo[c]	1.5 ± 2.6	1012 ± 302[e,f]	0	1.37 ± 1.17[e,f]	1.10 ± 0.42	33 ± 33[e,f]	4.1 ± 3.2[e,f]
PM[d]/Live vaccine	3.9 ± 4.4[e]	549 ± 597	0	2.13 ± 0.98	1.10 ± 0.51	154 ± 130	7.1 ± 3.1
Control	1.0 ± 2.9	537 ± 627	0	2.47 ± 0.78	1.06 ± 0.49	141 ± 122	7.6 ± 2.5

[a] Porcilis Lawsonia lyophilisate.
[b] commercially available live Lawsonia vaccine.
[c] Porcilis PCV M Hyo.
[d] commercially available PCV M Hyo vaccine.
[e] $p < 0.05$ vs control.
[f] $p < 0.05$ vs live vaccine.

i.e. several days of diarrhea, weight loss and deteriorating condition.

In the third week after challenge the control animals developed clinical signs of Lawsonia characterised by diarrhoea, weight loss, and shedding of Lawsonia in the faeces (Table 2). Upon necropsy the Lawsonia infection was confirmed by typical macroscopically visible ileum lesions (mucosal reddening and thickening), positive mucosa PCR and immunohistological ileum lesion scores. Pigs vaccinated with Porcilis® Lawsonia showed a statistically significant reduction in weight loss (i.e. ADWG 649 g vs −655 g), bacterial load in the ileum mucosa (PCR) and macroscopic as well as microscopic ileum lesion scores when compared to the controls. Moreover, the ADWG and macroscopic ileum lesion scores were significantly improved when compared to group 2 (live vaccine). In this latter group, most of the efficacy parameters were also reduced when compared to the controls, but the effects were less pronounced than in the group 1 comparison. With the exception of the microscopic ileum lesion scores, the data did not reach statistical significance (Table 2).

3.3. Trial 3

On the day of vaccination all pigs were either seronegative for Lawsonia or had a low antibody titre. On the day of challenge (3 weeks after vaccination), active seroconversion was evident in group 1, whereas the antibody titres measured in groups 2 and 3 remained comparable to the pre-vaccination levels. All groups had developed an increased antibody response by the day of necropsy, 3 weeks after challenge (Fig. 3).

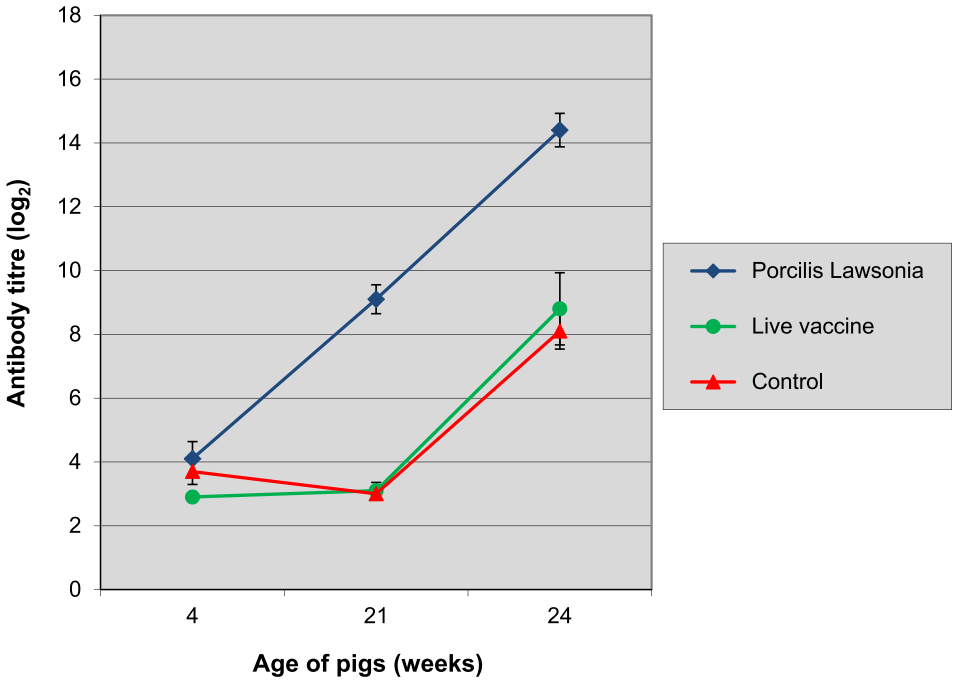

Fig. 2. Time-course of Lawsonia antibody development in trial 2. Group 1 was vaccinated at 4 weeks of age with Porcilis® Lawsonia, group 2 was vaccinated at 4 weeks of age with the live vaccine and group 3 was left unvaccinated. All pigs were challenged with Lawsonia infected gut mucosa at 21 weeks of age. Bars indicate 95% confidence interval.

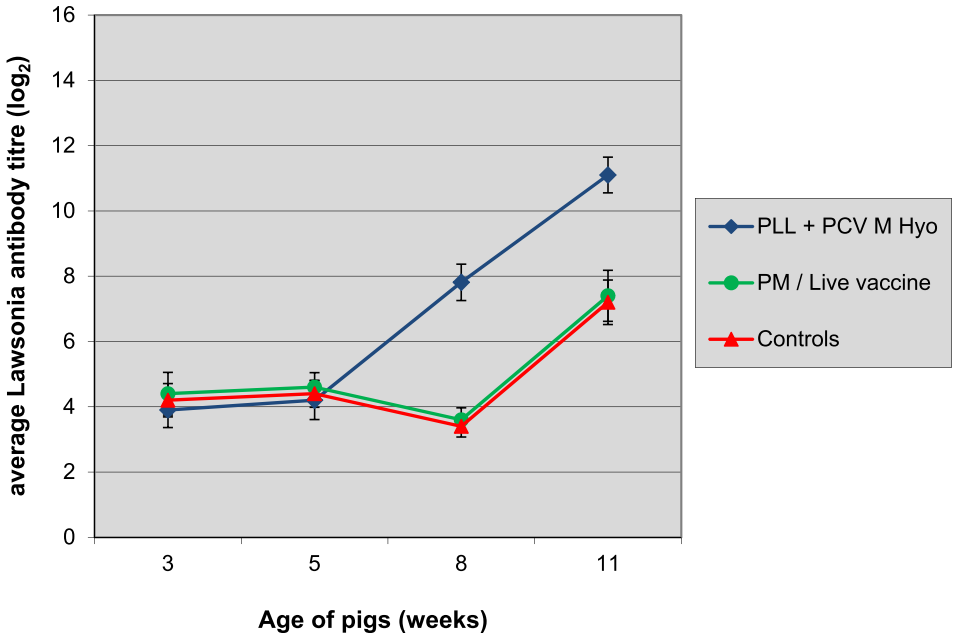

Fig. 3. Time-course of Lawsonia antibody development in trial 3. Group 1 was vaccinated at 5 weeks of age with Porcilis® Lawsonia lyophilisate in associated mixed use with Porcilis® PCV M Hyo (PLL + PCV M Hyo), group 2 was vaccinated at 3 weeks of age with a commercially available PCV-M Hyo combo and at 5 weeks of age with the live Lawsonia vaccine (PM / Live vaccine). Group 3 was left unvaccinated. All pigs were challenged with Lawsonia infected gut mucosa at 8 weeks of age. Bars indicate 95% confidence interval.

At the start of the study the pigs had low to moderate levels of maternally derived antibody against PCV2. The maternal antibody titres in the control animals declined over the course of the study. Group 1 raised a robust response to vaccination with average antibody titres of up to 9 $\log_2$. In contrast, group 2 developed only an intermediate response (Fig. 4).

At 3 weeks of age all animals were seronegative for M Hyo and the control animals (group 3) remained seronegative throughout the study. Group 1 mounted a good serological response to vaccination, whereas group 2 showed virtually no serological response to M Hyo (Fig. 5).

During the study one animal from group 1 was culled on day 7 post-challenge after several days of increasing locomotory problems and deteriorating condition. Necropsy confirmed the arthritis and also revealed fibrinous peritonitis. No further bacteriological examination was performed.

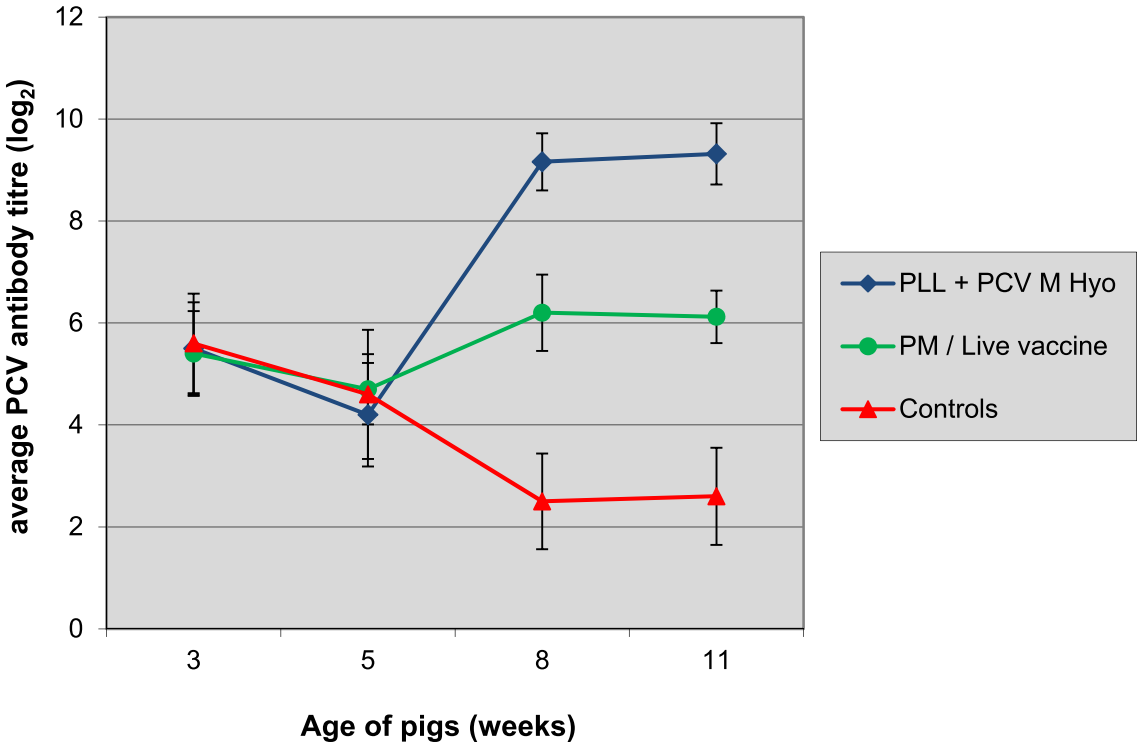

Fig. 4. Time-course of PCV2 antibody development in trial 3. Group 1 was vaccinated at 5 weeks of age with Porcilis® Lawsonia lyophilisate in associated mixed use with Porcilis® PCV M Hyo (PLL + PCV M Hyo), group 2 was vaccinated at 3 weeks of age with a commercially available PCV-M Hyo combo and at 5 weeks of age with the live Lawsonia vaccine (PM / Live vaccine). Group 3 was left unvaccinated. All pigs were challenged with Lawsonia infected gut mucosa at 8 weeks of age. Bars indicate 95% confidence interval.

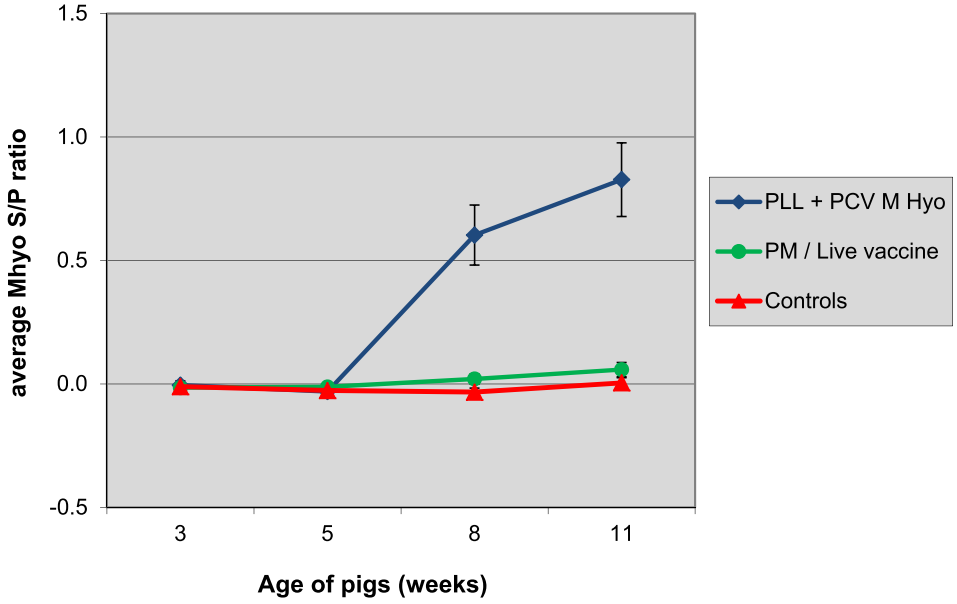

Fig. 5. Time-course of Mycoplasma hyopneumoniae antibody development in trial 3. Group 1 was vaccinated at 5 weeks of age with Porcilis® Lawsonia lyophilisate in associated mixed use with Porcilis® PCV M Hyo (PLL + PCV M Hyo), group 2 was vaccinated at 3 weeks of age with a commercially available PCV-M Hyo combo and at 5 weeks of age with the live Lawsonia vaccine (PM / Live vaccine). Group 3 was left unvaccinated. All pigs were challenged with Lawsonia infected gut mucosa at 8 weeks of age. Bars indicate 95% confidence interval.

In the third week after challenge the control animals developed clinical signs of Lawsonia characterised by diarrhoea, sub-optimal weight gain and shedding of Lawsonia in the faeces (Table 2). Upon necropsy the Lawsonia infection was confirmed by typical macroscopically visible ileum lesions (mucosal reddening and thickening), positive mucosa PCR and immunohistological ileum lesion scores. Pigs vaccinated with Porcilis® Lawsonia showed a statistically significant reduction in weight loss (i.e. ADWG 1012 g vs 537 g), shedding (PCR faeces) and macroscopic as well as microscopic ileum lesion scores when compared to the controls. In addition, these same parameters were also significantly reduced when compared to group 2 (live vaccine). This latter group induced vir-

tually no protection compared to the controls. Moreover, the clinical scores of group 2 were significantly higher when compared to the controls.

3.4. Field trial

After the start of the study, the Lawsonia associated mortality was reduced to zero in the vaccine group whereas 11 animals died or were culled due to acute ileitis in the control group ($p < 0.0001$). Furthermore, the total mortality during the study was significantly reduced in the vaccinates compared to the controls ($p = 0.0335$).

For practical reasons the overall mortality (study pigs and non-study pigs), average daily weight gain and feed conversion rate were determined for the whole herd (derived from the farm data management system) and therefore could only be compared historically. After the start of the study the overall mortality decreased form 3.8% (in the year preceding the study) to 2.3% during the study period. The ADWG gradually increased from 833 g/day in the year preceding the study to 890 g/day at the end of the study, and the feed conversion rate (kg feed/kg body weight) gradually decreased from 2.47 in the year preceding the study to 2.21 in the last 2 months of the study.

4. Discussion

In this study, the efficacy of an inactivated vaccine administered via the intramuscular route was compared to that of a live attenuated vaccine administered orally against intestinal disease caused by *Lawsonia intracellularis*. Based on current knowledge, active mucosal immunity plays a pivotal role in preventing invasion and intracellular proliferation of enteric pathogens such as *Lawsonia intracellularis*. Consequently, it is generally believed that live attenuated vaccines administered locally are more effective in inducing local (mucosal) immune responses than inactivated vaccines administered systemically [10,11]. The latter type of vaccines, in general, are more effective in inducing high concentrations of serum antibodies that can act systemically but that do not reach the gut mucosa [10]. Consistent with this theory, the live vaccine, in contrast to Porcilis® Lawsonia, did not induce a systemic antibody response. This was not unexpected since this live vaccine is believed to induce local immunity rather than a systemic antibody response. This lack of a systemic antibody response is in line with the results of Riber et al [12] who were also unable to detect an antibody response after vaccination with the live attenuated vaccine. In both studies, a clear antibody response was evident after challenge and subsequent infection.

Given the nature of porcine proliferative enteropathy as a local intestinal infection, one would expect that an orally administered live attenuated vaccine would elicit superior efficacy to a systemically administered inactivated vaccine. In this study, however, we demonstrate that an inactivated vaccine administered intramuscularly is highly efficacious against Lawsonia infection. This is in line with the previous results of Roerink et al [13], who also demonstrated good protection against experimental Lawsonia infection using an inactivated whole cell vaccine. Although the exact protective mechanism is not known, and opposite to the general immunological understanding, the inactivated vaccine gave superior protection when compared to a commercially available live vaccine.

The aim of the second vaccination-challenge trial was to compare the duration of immunity of the inactivated vaccine with the live attenuated vaccine. Although Porcilis® Lawsonia has a duration of immunity of at least 21 weeks (data not shown), the animals in this study were challenged 17 weeks after vaccination to align with the licensed duration of immunity for the live vaccine. In this study, the controls appeared to be more severely affected than in studies 1 and 3, with an average weight loss of 655 g per day in the third week after challenge. Despite this severe challenge, the inactivated vaccine induced robust protection. The reason for the difference in the susceptibility of the controls may be related to the age of the animals, i.e. older animals are more susceptible. This is consistent with the situation in the field where notably older animals, a few weeks before slaughter, appear to be affected (4 and this study field trial).

The aim of the third vaccination-challenge trial was to compare the efficacy against Lawsonia following different vaccination regime(s) against three major pathogens of fattening pigs. In this trial, Porcilis® Lawsonia was administered in associated mixed use with Porcilis® PCV M Hyo, as one injection at 5 weeks of age. Since the live vaccine has no concurrent use claim with any PCV or Mycoplasma vaccine, to avoid off-label use, and to administer the group 1 and 2 Lawsonia vaccinations at the same moment in time, the group 2 pigs were vaccinated with a PCV2-M Hyo vaccine combo at 3 weeks of age and the live attenuated Lawsonia vaccine at 5 weeks of age.

Combination vaccines may elicit a lower response to vaccination if compared to either of the single vaccines. This question was not addressed in the third study since to do so would have required an additional vaccine group vaccinated with Porcilis® Lawsonia alone. Despite this, the results obtained in studies 1 and 2 demonstrate a similar level of protection to those obtained in study 3, indicating that the PCV and Mhyo antigens do not have a negative effect on the efficacy of the Lawsonia vaccine.

The field study was performed on a farm with a history of mortality due to acute ileitis occurring a few weeks before slaughter. Economically, this is the worst-case scenario for the farmer since he has maximally invested in the animals by this point. After the start of the vaccinations, the Lawsonia associated mortality was reduced to zero. In addition, although not statistically substantiated, the key production parameters also improved during the course of the study when compared with the historic data: overall mortality (3.8% to 2.3%), ADWG (from 833 to 890 g/day) and feed conversion rate (from 2.47 kg to 2.21 kg feed/kg body weight). It should be kept in mind that these improvements are an underestimation of the real effect because they were calculated for the whole herd, whereas only half of the pigs were vaccinated. If the whole herd had been vaccinated, the improvement of the key production parameters would likely have been even better, not only by the direct effect of vaccination but also by additional indirect effects as described by Knight-Jones et al. [14]. These indirect effects may result from reduced shedding, as shown in the experimental studies, and subsequent reduced infectious pressure resulting in even greater positive effects when a whole herd is vaccinated.

In conclusion, in this study we show that the inactivated vaccine Porcilis® Lawsonia, either as standalone, or in associated mixed use with Porcilis® PCV M Hyo, induced statistically significant protection against experimental *Lawsonia intracellularis* infection. This was demonstrated by lower clinical scores, improved weight gain, reduction of *Lawsonia intracellularis* shedding and reduction of macroscopic as well as microscopic ileum lesion scores, when compared to control animals. In the field trial, the vaccine proved to be highly efficacious; reducing Lawsonia associated mortality to zero and improving key production parameters.

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Conflict of interest

This work was funded by MSD Animal Health and all authors are employed by MSD Animal Health.

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