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Comparison of Protectivity and Safety of Two Vaccines against *Actinobacillus pleuropneumoniae* in a Field Study

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Abstract: *Actinobacillus pleuropneumoniae* causing porcine pleuropneumoniae is responsible for lowered productivity and reduction of performance indicators such as daily weight gain and increase of losses in the swine industry worldwide. To control the disease, vaccination is used to reduce clinical signs and production losses. A randomized, blinded field trail was conducted to compare two licensed *A. pleuropneumoniae* vaccines in 600 finishing pigs in terms of lung lesions, mortality, medication, weight gain and safety, in a farm in northeast Germany. After weaning, pigs were allocated randomly in two groups resulting in group sizes of 300 individuals. Nursery pigs were vaccinated at the age of 7 to 10 weeks either with a *A. pleuropneumoniae* bacterin, containing ApxI-III toxoids (group 1), or with a subunit purified *A. pleuropneumoniae* toxoid vaccine (group 2). Blinded lung lesion scoring at slaughter following the Ceva Lung Program methodology revealed a significantly lower proportion of lungs affected with pleurisy in group 1 compared to group 2. Weighing of the animals did not show a significant difference ($p = 0.092$); however, at the end of finishing animals of group 1 showed a 1.59 kg higher weight (100.40 ± 10.15 kg) compared to animals in group 2 (98.81 kg ± 11.56 kg). Mortality and antimicrobial medication were significantly lower in group 1 compared to group 2 (13 losses and 17 antimicrobial medications in group 2, 4 losses and 1 antimicrobial medications in group 1). Injection site and systemic adverse reactions were recorded on both days of vaccination and did not differ significantly between the groups ($p > 0.05$). In this study, the efficacy of vaccination with a commercially available *A. pleuropneumoniae* bacterin containing ApxI-III toxoids was superior to that of a commercially available *A. pleuropneumoniae* subunit toxoid vaccine in preventing pulmonary lesions associated with *A. pleuropneumoniae* infection.Grzegorz Woźniakowski

Keywords: *Actinobacillus pleuropneumoniae*; Porcine Pleuropneumoniae; PP; Coglapix; Apx; LPS; vaccination

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1. Introduction

Actinobacillus pleuropneumoniae (*A. pleuropneumoniae*), the aetiological agent of porcine pleuropneumonia, is responsible for high morbidity and mortality causes severe reduction of performance indicators such as daily weight gain and increase of losses in the swine industry worldwide. The clinical manifestations can be subclinical, subacute as well as acute and peracute. Animals recovering from acute infections as well as chronically infected can harbour the pathogen in nasal cavities, tonsillar crypts, and chronic lung lesions. Thus, these carrier animals become the source of infection for naïve subpopulations and make it difficult to eradicate the disease from infected herds [1,2].

The acute form of pleuropneumonia features fever, anorexia, coughing, dyspnoea, and/or polypnea and death while the peracute form, per definition, causes sudden death without any clinical signs [3]. Pigs with subacute or subclinical disease show milder symptoms with lower fatality. Together with the chronic form productivity is affected with retarded growth and deteriorated feed conversion and lung lesions, such as pleurisy and adherence together with a fibrino-haemorrhagic and necrotizing pleuropneumonia are commonly seen at the abattoir [3,4]. These lesions are often visible in and on the dorsal and caudal part of the lung and therefore focus is on dorso-caudal pleurisy (DCP) in abattoir evaluations of *A. pleuropneumoniae* induced lesions.

Nineteen *A. pleuropneumoniae* serotypes have been classified worldwide so far with differences in virulence but all able to induce disease [3,5]. Endemic farms in Germany and worldwide are highly prevalent and often infected with more than one serotype. However, between countries and regions inside the countries serotype prevalence varies and can change over time [6]. The pathogenicity between serotypes varies greatly. Some express different virulence factors in different parts of the world [7–11]. Extrinsic virulence factors such as stress-induced increase in host catecholamines, poor air quality, and poor climatic control, and particularly high daily variations in ambient temperature have been demonstrated to increase virulence of also low virulent *A. pleuropneumoniae* strains [12–14].

Various virulence factors have been described for *A. pleuropneumoniae*, among these are the Apx toxins and lipopolysaccharides (LPS) [15]. Apx I-III determine largely by their cytotoxicity and haemolytic activity the virulence of the serotype and one or two of the three are present in all serotypes [16–21]. Via lysis of lung cells including neutrophils and macrophages, they provide nutrients for bacteria growth [16]. Apx IV has a haemolytic activity, but it does not have a cytotoxic activity and is present in all serotypes making it suitable for diagnostic [22]. LPS enhances the effect of Apx I-III and is responsible for adhesion supporting colonization [23–25]. Other metabolic products and cell wall components, particularly the ample group of outer membrane proteins (OMPs) have been indicated and speculated relevant as virulence factors but are still under discussion [15].

Pleurisy, often associated with *A. pleuropneumoniae* [26], has a negative effect on average daily weight gain (ADWG) and feed conversion rate. Slaughter weight in pigs with pleurisy can be reduced by 1.24 kg compared to animals without pleurisy. Studies show that the increase by 10% of affected lung tissue reduces the ADWG by 3.3–4.6% or 375 g [27,28].

Vaccination and antimicrobial treatment are the two major strategies to reduce clinical symptoms and mortality caused by *A. pleuropneumoniae*. Penicillin (in particular aminopenicillins), tetracyclines, florfenicol and trimethoprim-/sulphonamide combinations are most frequently used to control the disease, but antimicrobial resistance has been reported [29–32].

Several commercial vaccines are available which differ in their composition. They are either subunit vaccines containing Apx I-III toxins, killed whole cell vaccines (bacterins) or a combination of both [33]. With distinct differences in efficiency, they all reduce clinical signs, but none can fully prevent infection and colonization [34]. Due to limited cross protection between the serotypes, bacterin vaccines lack in efficacy compared to Apx I-III combined bacterin vaccines [35–38].

The objective of this randomized field study was to compare the efficiency of two commercial vaccines against *A. pleuropneumoniae*, primarily on protective capabilities reflected by weight gain, mortality, antibiotic usage, and lung lesions at slaughter. Secondarily safety parameters measured as injection site and systemic adverse reactions were recorded.

2. Materials and Methods

This study was conducted in a closed two-site farm in North-Eastern Germany with 1200 sows and nursery on one site and a finishers site of 11,000 pen places in an all-in all-out system 70 km away. The farm runs in a 3-week batches system on DanBred × Topigs-Norsvin hybrids (TN70 × Pietrain 408) dams. Both sites were and

still are negative for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Piglets are vaccinated against Porcine Circovirus type 2 and *Mycoplasma hyopneumoniae* at 3 weeks of age (woa).

Before vaccination against *A. pleuropneumoniae*, the farmer reported constant coughing and mortality of up to 4% during fattening. In addition, an increased proportion of pleurisy was observed based on the slaughter evaluations. As there is a correlation between the occurrence of pleurisy and the presence of *A. pleuropneumoniae* in the herds [26], the high prevalence of pleurisy in German slaughterhouses could be related to the high proportion of *A. pleuropneumoniae*-positive farms in Germany. It is estimated that 80–90% of finishing herds in regions with a high density of pigs are serologically positive for *A. pleuropneumoniae* [11,39].

Based on these findings as well as the clinic observed by the veterinarian in the barn and pathological findings, vaccination with a commercial subunit toxoid vaccine against *A. pleuropneumoniae* containing ApxI-III toxoids and outer-membrane-protein-antigen (Porcillis APP®, Intervet Deutschland GmbH, Unterschleißheim, Germany) was introduced six months before the start of the study. One week before the start of the study, serotype 2 of *A. pleuropneumoniae* was confirmed by PCR (species- and serotype-specific real-time PCR (Kylt®APP/species- and serotype-specific PCR + agarose gel electrophoresis)) on dissection of the affected lung tissue after culturing. The presence of this serotype on the farm was not confirmed before this time. The introduction of vaccination against *A. pleuropneumoniae* mentioned above has alleviated the clinical impact of *A. pleuropneumoniae* infections to some extent. However, cough was still observed, and farmers reported systemic reactions after vaccination resulting in loss of appetite, inability to lie down, and fatigue.

The study was conducted from January to August 2018. In total, 600 animals from one batch were included into the study and divided randomly into the 2 equal groups according to their sex and weight at weaning (3 woa). The animals were housed in the nursery nearby while the groups were separated by pens in the same room sharing the same air space. During the whole trial, the groups were blinded. The first vaccinations were done all at the same day; 2 mL intramuscularly at around 7th week of age in group 1 (G1) with a vaccine containing *A. pleuropneumoniae* serotypes 1 and 2 as well as ApxI-III toxoids (Coglapix®, Ceva Sante Animale, Libourne, France) and in group 2 (G2) also 2 mL intramuscularly at about 7 weeks of age with the commercial subunit toxoid vaccine mentioned above. Three weeks later, booster vaccinations were administered, repeating the above schedule, and at the same time pigs were weighed individually again. At the end of the nursery period animals were transported group-wise to the finishing farm at the age of around 11 weeks. Before slaughter pigs were weighed a third time. ADWG was calculated for the time periods: weaning to second vaccination, second vaccination to end of finishing, and the full period from weaning to end of finishing.

Animals vaccinated with the vaccine, containing the *A. pleuropneumoniae* serotypes 1 and 2 as well as ApxI-III toxoids (Coglapix®, Ceva Sante Animale, Libourne, France) in G1 compared to the subunit vaccine (Porcillis APP®, Intervet Deutschland GmbH, Unterschleißheim, Germany)

Lung lesion scoring was performed at the slaughterhouse at three different time points, due to their difference in speed of growth. All scorings were performed by the same two people in a defined, blinded manner: one was tagging the animals of interest (after identifying the animals from the barn by their ear tags, which had already been inserted at piglet age) with a crayon on the carcass while the other, standing at the opposite side, scored the lungs using the Ceva Lung Program® application (Ceva Sante Animale, Libourne, France) [40–42] immediately when hanging on the plucks-chain. Relevant to this trial we focused only on *A. pleuropneumoniae*-like lesions: the dorso-caudal pleurisy (DCP) plus its recalculation into the *A. pleuropneumoniae* index (APPI). The percentage of lungs with different severity of DCP were recorded using the modified slaughterhouse pleurisy evaluation system (SPES). Severity of lesions were recorded as SPES 0 for no lesion, SPES 2 resembling a dorso-caudal mono lateral focal lesion, SPES 3 resembling a dorso-caudal

bilateral focal lesion or extended mono lateral lesion (at least 1/3 of one diaphragmatic lobe), and SPES 4 resembling a severely extended bilateral lesion (at least 1/3 of both diaphragmatic lobes). Score 1 is not used for DCP but for cranial pleurisy in this system. The APPI is calculated by the number of pigs with scores 2, 3, and 4 divided by the total number of pigs examined multiplied by the average score considering only the scores 2, 3 and 4 [40].

Mortality and individual antibiotic treatment were recorded throughout nursery and finishing. Individuals showing respiratory disease were treated by intramuscular administration of 10 mg enrofloxacin (Baytril 10%, Bayer Vital GmbH, Leverkusen, Germany) per kg bw for three consecutive days. These data were collected by the farmer in a blinded manner.

During a period of 24 h (0 h, +6 h, +24 h post vaccination) following both vaccinations safety parameters such as rectal temperature, injection site reaction (ISR) and systemic adverse reaction (SAR) were recorded in 30 (10%) randomly selected animals per group. ISR were scored on a scale from 0 to 3 for redness and swelling (0: absent, 1: $\geq$5 mm, 2: $\geq$20 mm, 3: $\geq$50 mm). SAR which evaluates the general condition and behaviour of the animals were recorded in a similar way (0: normal, 1: mildly disturbed, 2: moderately disturbed 3: severely disturbed).

3. Statistical Analysis

The statistical analysis was computed with IBM SPSS Statistics 23. For the weight, ADWG, and body temperature after vaccination, normal distribution was tested with Kolmogorov–Smirnov (KS) test. Qualitative data were compared with a Chi-squared test. For the parameter weight at weaning and weight at nursery there is no standard deviation. In this case, Kruskal Wallis test was used. Finally, a one-factorial variance analysis with the fixed factor "vaccine" and the variable factor weight at end of finishing and ADWG was performed.

Mortality and treatment index as well as frequencies of scores for injection site and systemic adverse reactions using cross tabling were allocated to the different groups and compared with the Chi-squared test.

Lung lesion scores were evaluated by Chi-squared tests. DCP was tested with a 2 $\times$ 2 table between the groups and for significant differences between the two groups in relation to the severity of the lesions. All models used a confidence interval of 95% to evaluate significance between test groups ($p < 0.05$).

In terms of APPI, the three batches refer to different slaughter dates. All animals come from the same group, but were slaughtered at different times because not all animals reached slaughter maturity at the same time. This is what different batches and different times refer to.

4. Results

4.1. Production Data

Number of animals and data are summarised in Table 1. Starting with 600 animals weighed at weaning, weights from 514 animals were recorded at second vaccination and before slaughter due to mortality (n = 12; G1/G2 = 7/5) and loss of ear tags. None of the weighing's showed significant difference between the groups (Table 1); however, at the end of finishing animals of G1 showed a 1.53 kg higher weight (100.453 $\pm$ 10.00 kg) compared to animals in G2 (99.00 kg $\pm$ 11.53 kg, p = 0.109). The ADWG was calculated for three different time points. From weaning to finishing the ADWG was 705 g in G1 and 694 g in G2, showing a stronger tendency towards significant difference in favour of G1 (p = 0.095) than the difference in weight at the time of slaughter. For the other two time points weaning to second vaccination and from there to end of finishing no statistical difference was observed.

Table 1. Number of animals at the different weighing's.

	Groups	*N*	Average ± Standard Deviation (kg)	*p*-Value
Weight at weaning	Group 1	300	6.00 ± 1.16	0.899
	Group 2	300	6.00 ± 1.16	
Weight at nursery at second vaccination (kg)	Group 1	253	25.80 ± 4.73	0.755
	Group 2	261	25.70 ± 5.05	
Weight at end of finishing (kg)	Group 1	253	100.53 ± 9.99	0.109
	Group 2	261	99.00 ± 11.53	
ADWG weaning to end of finishing (kg)	Group 1	253	0.705 ± 0.07	0.095
	Group 2	261	0.693 ± 0.08	
ADWG weaning to second vaccination (kg)	Group 1	253	0.411 ± 0.09	0.801
	Group 2	261	0.410 ± 0.10	
ADWG from second vaccination to end of finishing (kg)	Group 1	253	0.869 ± 0.11	0.124
	Group 2	261	0.852 ± 0.13	

4.2. Lung Lesion Scoring

In total 407 randomly chosen lungs were scored, turning out to be 230 from group 1 and 177 from group 2. The results of the lung lesion scoring are summarised graphically in Figure 1.

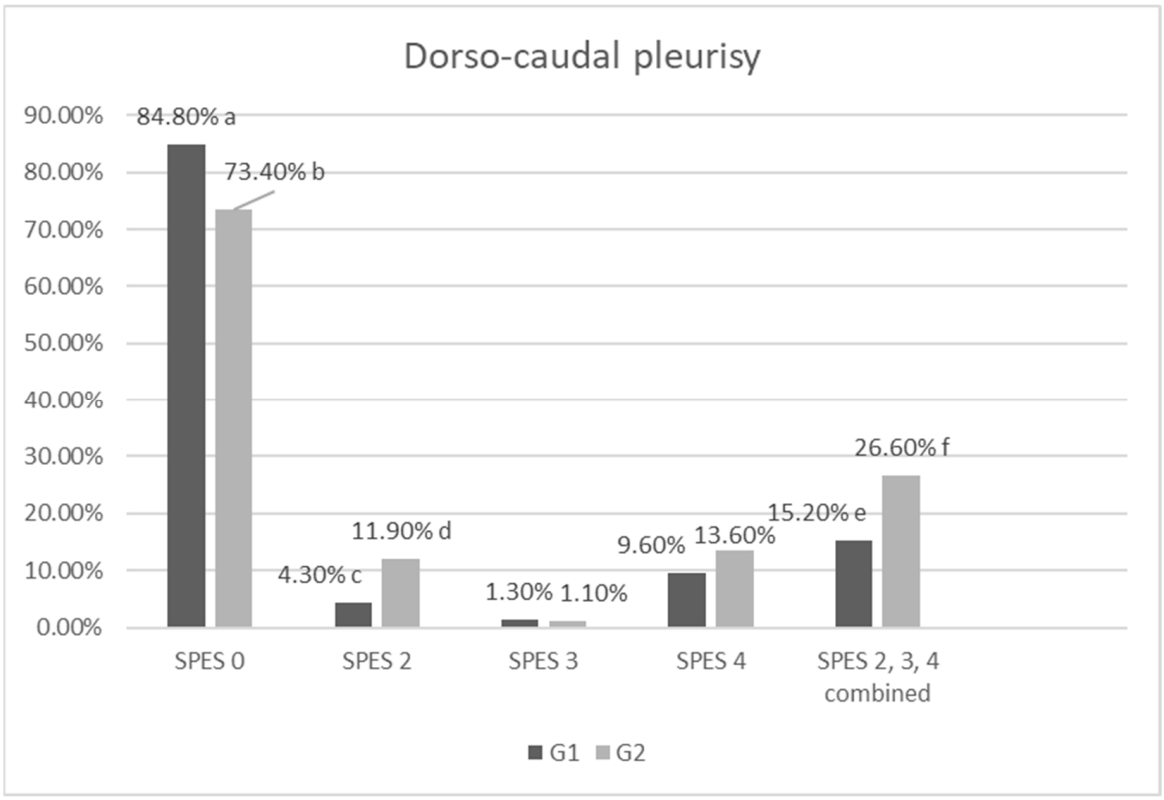

Figure 1. Distribution of pleuropneumonia-like pathological lesions between groups (a:b $p < 0.001$; c:d $p = 0.048$; e:f $p = 0.015$).

The APPI was calculated for each group. For the first two slaughter groups the lungs from group 1 had a lower score than the lungs from group 2 (0.26 vs. 0.78 ($p = 0.038$) and 0.08 vs. 0.37 ($p = 0.003$)), respectively. For the third slaughter group, the slowest growing pigs, the average SPES score was 1.15 and 1.41 for group 1 and group 2, respectively (Table 2).

Table 2. Lung lesion scoring for the different study groups at the different points of time of lung scoring at the slaughterhouse.

Groups	First Slaughter Group			Second Batch			Third Batch			Total		
	APP Index	Number of Evaluated Pigs	*p*-Value	Index	Number of Evaluated Pigs	*p*-Value	Index	Number of Evaluated Pigs	*p*-Value	Index	*n*	*p*-Value
Group 1	0.26	47	0.038	0.08	99	0.003	1.15	84	0.368	0.51	230	0.024
Group 2	0.78	18		0.37	91		1.41	68		0.81	177	

4.3. Mortality

No clinical signs of respiratory disease, including *A. pleuropneumoniae*, were observed during the nursery phase. Within the nursery, before fully immunised by *A. pleuropneumoniae* vaccinations, approximately one week before leaving nursery, seven out of 281 animals died in group 1 while six out of 285 animals died in group 2 ($p = 0.78$). During finishing mortality differed significantly between groups with four dead out of 283 (1.4%) in group 1 and 13 dead out of 283 (4.5%) in group 2 ($p < 0.03$). Necropsy was not performed in these animals, so that the cause of death cannot be determined conclusively.

4.4. Antimicrobial Treatments

During nursery, no antimicrobial treatment related to respiratory symptoms was applied. During finishing, incidents and treatments of respiratory disease were recorded. In total 18/566 animals were treated. A highly significant, lower number of animals were treated against respiratory disease in G1 (1/283, 0.4%) compared to G2 (17/283, 6.0%) respectively ($p < 0.0001$).

4.5. Injection Site and Systemic Adverse Reactions

All animals tolerated both vaccinations well. No significant differences with respect to body temperature, ISR and SAR and were observed between the groups shortly after as well as 6 h and 24 h after both vaccinations ($p > 0.05$, Tables 3 and 4).

Table 3. Temperature measurement at both vaccinations.

		First Vaccination			Second Vaccination		
		Mean Value (°C)	Standard Deviation	*p*-Value	Mean Value (°C)	Standard Deviation	*p*-Value
Temperature before vaccination	Group 1	39.45	0.5	0.316	39.49	0.6	0.329
	Group 2	39.34	0.4		39.64	0.6	
Temperature 6 h after vaccination	Group 1	40.08	0.5	0.977	40.31	1.0	0.928
	Group 2	40.08	0.5		40.34	0.8	
Temperature 24 h after vaccination	Group 1	39.14	0.6	0.954	39.04	0.6	0.714
	Group 2	39.14	0.5		38.99	0.5	

Table 4. ISR and SAR after first and second vaccination.

		First Vaccination				Second Vaccination			
		Score 0	**Score 1**	**Score 2**	***p*-Value**	**Score 0**	**Score 1**	**Score 2**	***p*-Value**
ISR before vaccination	Group 1	99%	1%	0	0.997	99.3%	0.7%	0	0.261
	Group 2	99%	1%	0		98.3%	1.7%	0	
ISR 6 h after vaccination	Group 1	97.7%	2%	0	0.578	96%	4%	0	0.131
	Group 2	98.3%	1.7%	0		95%	3.7%	1.3%	
ISR 24 h after vaccination	Group 1	98%	2%	0	0.761	99%	1%	0	0.606
	Group 2	98.3%	1.7%	0		98.8%	1%	0.1%	
SAR before vaccination	Group 1	99%	1%	0	0.477	99.7%	0.3%	0	0.317
	Group 2	98.3%	1.7%	0		100%	0	0	
SAR 6 h after vaccination	Group 1	98.3%	1.7%	0	0.423	96%	3.7%	0.3%	0.590
	Group 2	97%	2.7%	0.3%		96.7%	3.3%	0	
SAR 24 h after vaccination	Group 1	98.3%	1.7%	0	0.560	99%	1%	0	0.325
	Group 2	97.7%	2.3%	0		99.7%	0.3%	0	

5. Discussion

This field study is a comparison of two vaccines against *A. pleuropneumonia* regarding parameters such as weight gain, the occurrence of App-like lesions, clinical signs of respiratory disease, antimicrobial treatment against respiratory disease and side effects.

A control group was not used in the present study, which carries the risk of misinterpretation of our results, but this is a limitation of some field studies in particular situations.

After randomization, the animals of the two groups were weight a three time points. In retrospect, it would certainly have been interesting to capture the difference between fast, medium, and slow growing pigs. This should be considered in a newer study design. Nevertheless, the differences between the groups were not significant; however, at the end of finishing, animals in group 1 had a 1.53 kg higher weight than animals in group 2 ($p = 0.109$). A higher body weight at the same time of slaughter, gives you a better pen/farm efficacy (cycles per pen place per year) and is indicative for a better feed conversion ratio (FCR).

The proportion of pigs without App-like lesions was significantly higher in group 1 than in group 2 ($p < 0.001$). For the individual SPES scores 2, 3 and 4 a significant difference was visible for SPES 2 ($p = 0.048$). DCP lesions are not pathognomonic for *A. pleuropneumoniae* but strongly indicative [26]. The appearance of DCP at the abattoir can be linked economically to worse efficiency (weight gain, feed conversion ratio, duration of finishing and weight of slaughter) [43]. The existence of *A. pleuropneumoniae* associated disease is a risk factor for pleurisy with a high odds ratio (OR = 8.75), which is supported by previous Danish work [44]. Any reduction of pleurisy will improve weight gain and therefore the economic benefit. Brewster and colleagues [27] were able to show a strong correlation between the appearance of pleurisy and reduction of carcass weight with a reduction of 1.24 kg ($p = 0.038$). These findings are in in line with the results from this trail as the animals in group 1 had a lower prevalence of DCP and tended to show a higher weight at the end of finishing. Therefore, using a highly effective vaccine can be a profitable measure in *A. pleuropneumoniae*-positive farms to reduce the clinical outcome of *A. pleuropneumoniae* and increase the economic benefit for the farm.

Clinical signs of respiratory disease were visible only during the finishing period. Significantly less animals received antimicrobial treatment against respiratory disease in

group 1 than in group 2. This, together with significantly less pigs died during fattening, the period of risk of clinical *A. pleuropneumoniae*, strongly confirms the findings of significantly less App-like lung lesions at time of slaughter, even though necropsy of the animals died was not performed. Here again, the lack of a control group is to be criticised, this would certainly have been advantageous to see whether App vaccination also reduces the mortality rate in positive herds compared to non-vaccinated animals. Furthermore, in a revised study design, the necropsy of deceased animals would also have to be taken into consideration to determine the final cause of death and, if necessary, to include it in the statistical interpretation.

Generally, the decline in maternal antibodies is associated with replication of bacteria on the tonsils and with a higher risk of disease outbreaks from the third month of life onwards [1,45,46]. This is also reflected in this study. A total of 6 out of the 19 animals from group 2 died during the last month of finishing. Both vaccines, Coglapix® in G 1 and Porcillis APP® in group 2, differ in the duration of protective immunity with 5 week less duration of immunity stated for the vaccine used in group 2. It cannot be excluded that differences in duration of immunity was contributing to the differences in losses observed between groups.

In this trial, it was not possible to demonstrate a significant difference between the two groups in terms of ISR and SAR, although the manufacturers described side effects. The adjuvant in the vaccine Porcillis APP® (dl-α-tocopherolacetat) is an oil in water adjuvant. Coglapix® contains aluminium hydroxide gel which is known to be a safe and potent adjuvant [47]. For the procedure of vaccination itself, warming up the vaccines up to body temperature before application and vaccination of fasted pigs might prevent negative side effects. Finally, it would have been interesting to see how a comparison group without vaccination would have performed in comparison to the behaviour of the vaccinated animals after vaccination, as practical experience shows that vaccinated animals often show a change in behaviour (e.g., in the form of reduced activity, lower feed intake) in the first hours after administration of a vaccine.

As mentioned earlier, the lack of a control group is a difficulty in this study. Therefore, this is discussed in more detail in this section.

Regarding the weighing of the animals, it would certainly have been interesting in retrospect to record the difference between fast, medium, and slow growing pigs. This should be considered in a newer study design. Furthermore, it cannot be said in this study that vaccination against *A. pleuropneumoniae* has a positive effect on daily weight gain. However, this is suggested by a Spanish study in which 16,632 animals vaccinated against *A. pleuropneumoniae* were compared with 16,431 vaccinated animals in terms of daily weight gain. A highly significant improvement of 40 g in the daily weight gain of the vaccinated animals from weaning to slaughter was observed [48].

Additionally, a control group would also have been beneficial to see if APP vaccination also reduced mortality in positive herds compared to non-vaccinated animals. Moreover, a revised study design would also have to consider the necropsy of the deceased animals to determine the final cause of death and include it in the statistical analysis if necessary.

Finally, it would have been interesting to see how a comparison group without vaccination would have behaved in comparison to the behaviour of the vaccinated animals after vaccination, as practical experience shows that vaccinated animals often show a change in behaviour (e.g., in the form of reduced activity, lower feed intake) in the first hours after administration of a vaccine.

In the end, in this study, although App infection was confirmed previously to the start of the study was not accompanied by additional laboratory diagnostics and the involvement of other pathogens contributing to the clinical picture cannot be excluded and needs to be considered for interpretation of the findings in this study. However, the significant difference in antimicrobial treatment for respiratory disease between groups, strongly indicate *A. pleuropneumoniae* being of major importance as respiratory pathogen on the farm.

6. Conclusions

Animals vaccinated in group 1 showed better results in the parameters of lung lesion, antimicrobial treatment of respiratory disease and mortality compared to group 2 in this study. Negative side effects were negligible with both vaccines.

Author Contributions: T.W. and S.H. conducted the study design, supervised the study, participated in data collection and corrected the manuscript. K.S. performed the statistics. T.W. and H.G. chose the farm for the study and participated in data collection. P.H., H.G. and I.H.-P. participated in data collection and wrote the manuscript. All authors read and approved the final manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, due to the fact, that only routine measures were performed on the farm. The farmer routinely selected groups for weight control as it was described in this study.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data that support the results of this study are available upon reasonable request from Christoph Wähner.

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Conflicts of Interest: There are no competing interests on the part of the authors.

Ethics Approval and Consent to Participate: The present trial did not include any invasive procedures or treatments to the pigs, therefore animal ethics committee approval was not required. An owner consent was provided by the farmer prior to starting the study.

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