PEER REVIEWED

A Meta-Analysis of the Relationship Between Lung Lesion Scores in Slaughter Pigs and their Daily Weight Gain

Abstract

A meta-analysis was performed on available published articles in which Ceva Lung Program (CLP) method of assessing enzootic pneumonia (EP)-like lesions was utilised and which contained also the record of average daily gain (ADG) data. Eight studies fulfilling the inclusion criteria were analysed using non-parametric tests. All of them were field trials comparing the efficacy of *Mycoplasma hyopneumoniae* vaccines. The results of the analysis showed that in terms of EP scores in different treatment groups the median difference between pairs of values was 0.365 units ($P<0.001$). For ADG the median difference was 39.0 g/day ($P<0.001$). Each point in the EP reduction thus corresponds to 106.8 g of ADG increase. All estimations were significant with $P<0.001$, demonstrating the dependency of the ADG on EP values both for individual subsets calculated separately as well as for all data put together. The relationship between EP-like lesion scores and the growth performance was confirmed, indicating the relevance of lung check providing a predictive value for the growth of pigs.

Enzootic pneumonia, lung scoring, growth performance, meta-analysis

Mycoplasma hyopneumoniae (M. hyopneumoniae) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs, and one of the primary agents involved in the porcine respiratory disease complex (PRDC) (Maes *et al.* 2017). EP is characterised by a chronic, nonproductive cough, decreased growth rate and feed conversion ratio (Sibila *et al.* 2009), typically with no or low mortality. PRDC develops as a consequence of coinfections of both bacterial and viral pathogens, especially porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and porcine circovirus type 2 (PCV2) (Sibila *et al.* 2009). PRDC can result in an increased mortality and severe performance losses. The major threat for the farm economy is represented by the decrease in the daily weight gain and eventual increased medication cost. Infection with *M. hyopneumoniae* often appears to have a subclinical course, where only the growth performance is reduced. It is difficult to assess the economic effect of mycoplasmal pneumonia due to the multifactorial origin of PRDC. It has been reported that enzootic pneumonia can result in a 17% decrease in daily weight gain and a 14% decrease in feed efficiency in affected herds (Straw 1989).

When healthy pigs free from *M. hyo* were mixed into *M. hyo* positive herds and were thus exposed to the natural infection, their performance was lower. The ADG of pigs with a non-complicated mycoplasmal pneumonia compared with those which remained free from *M. hyopneumoniae* was decreased by more than 60 g per day after adjusting for herd, pen, weight and sex (Rautiainen *et al.* 2000).

The presence of the infection is usually confirmed by *M. hyopneumoniae* specific seroconversion or by the detection of germs by PCR in the laryngeal swabs (Sibila *et al.* 2009; Pieters *et al.* 2017). Lung tissue infected with *M. hyopneumoniae* develops consolidation and catarrhal broncho-pneumonia with purple to grey regions of meaty aspect. The consolidation can be observed from 3–12 weeks post infection. The lesions are mainly localised in the apical and cardiac lobes, as well as in the anterior part of the diaphragmatic lobes and in the accessory lobe. Lesions resolve after 12 to 14 weeks with formation of interlobular fissures (Maes *et al.* 2008). Considering the chronic type of such lesions, bronchopneumonia with the cranioventral consolidation of lungs is very indicative for EP also in slaughter pigs. Several methods of identifying and scoring those lung lesions were developed and are implemented to monitor the presence and severity of EP in swine herds (Madec and Kobisch 1982; Straw *et al.* 1986). The aim of this study was to evaluate the original (previously published) data together with data from two other studies being comparable since utilising the same lung scoring method. Those data from multiple studies were analysed for the first time using a new statistical method quantifying the linear relation between lung score results and growth performance, not only the differences between particular treatment groups. The relationship between quantified lung lesions and weight gain will demonstrate whether lung inspection of slaughter pigs can indicate potential losses in weight gain of commercial pigs to the farmer. This may be an important information for justifying the efforts in scoring lung lesions, not only for their diagnostic value but also as an indication of the economic impact of EP in swine herds.

Materials and Methods

We have analysed data describing the impact of vaccines against *M. hyopneumoniae* on respiratory health especially the presence of EP-like lesions in slaughter pigs. For sake of comparability, only articles using the Ceva Lung Program (CLP) scoring methodology (Krejci *et al.* 2013; Cvjetković *et al.* 2018) were considered. The bulk of data originate from our previously published work, however papers of other authors using the same methodology were added to cover all available articles on that topic published at conferences and scientific journals. CLP consists of the modified (Madec *et al.* 1982) method for scoring enzootic pneumonia-like lesions. Cranio-ventral consolidation of lungs is scored from 1–4 for each pulmonary lobe with a maximum possible score of 28 per lung. In this system, enzootic pneumonia index (EP index) is calculated as the sum of all scores per batch of pigs examined divided by the number of lungs scored (Merialdi *et al.* 2012). From the studies using CLP, we focused on the ones in which performance data were also investigated and presented. Finally, we selected published results of comparative trials in which groups of pigs were treated differently and both lung scoring data and growth performance were known, allowing us to compare the differences in achieved average daily weight gain (ADG) in growing-fattening period, corresponding to the differences in their respiratory health.

Inclusion criteria for the publications:

- *Results of comparative field trials (not experimental challenge trials)*
- *The use of CLP for scoring lung lesions in slaughter pigs in those trials*

- *Growth performance data available for both treatment groups*

Eight publications met these criteria, varying in the sample size and degree of data aggregation. The dataset of each study consisted of two subsets of individuals, namely Group 1 (treatment groups vaccinated with Hyogen®, Ceva, Libourne, France) and Group 2 (treatment groups vaccinated with different vaccines in different studies or unvaccinated ones). Each of these sets was characterised by pairs of the Enzootic Pneumonia index (denoted EP) and the corresponding (dependent) average daily weight gain (denoted ADG).

Numbers of individual pigs or groups of pigs in each study and character of the data (primary/aggregated) are listed in Table 1:

values of ADG were selected/repeated. In case the original number of individuals was higher than n (ie. large enough studies with primary data available), random selection of n values of EP and ADG were selected; in case that less than n values were available (ie. small studies with primary data or studies with aggregated data only) random repetition of samples was conducted to generate n new values of EP and ADG. If the original data were recorded in paired design (mutually corresponding values of EP and ADG in publications Cvjetković *et al.* 2018; Espigares *et al.* 2018; Guadagnini *et al.* 2018b and Krejci *et al.* 2017b), these pairs of data were preserved in the selection. The process of random selection/repetition introduces a random error which is reduced by repeating the whole process N times. Also, such selection/repetition may decrease the total variance of the dataset. This implies a deployment of robust non-parametric methods

Publication	Group 1		Group 2	
	EP	ADG	EP	ADG
Costa et al. 2019	n=32 aggregated	n=3 aggregated	n=32 aggregated	n=3 aggregated
Cvjetković et al. 2018	n=280 primary	n=280 primary	n=271 primary	n=271 primary
Espigares et al. 2018	n=14 aggregated	n=14 aggregated	n=13 aggregated	n=13 aggregated
Guadagnini et al. 2018a	n=2 aggregated	n=4 aggregated	n=2 aggregated	n=3 aggregated
Guadagnini et al. 2018b	n=9 aggregated	n=9 aggregated	n=3 aggregated	n=3 aggregated
Krejci et al. 2013	n=38 primary	n=1 aggregated	n=103 primary	n=1 aggregated
Krejci et al. 2017a	n=6 aggregated	n=6 aggregated	n=6 aggregated	n=1 aggregated
Krejci et al. 2017b	n=108 primary	n=108 primary	n=103 primary	n=103 primary

EP = enzootic pneumonia index, ADG = average daily gain

Table 1: Numbers of individuals and character of data in eligible publications

In Table 1 "primary" data consist of one value of EP or ADG for each individual whereas aggregated data characterise a whole group of individuals (cluster) by one value, as a result of selected aggregation function (mean, median). In case of aggregated data there was no information on variance within the cluster.

Data Treatment

Considering all eight publications as having the same relevance, a simulation was performed to align numbers of individuals in all eight studies to the same number n. This simulation prevents larger studies from overweighting the smaller ones. In total n values of EP and corresponding n

for consequent statistical analysis which does not improve significance by repeating the same value multiple times.

The non-parametric Mann-Whitney-Wilcoxon (Mann and Whitney 1947) U test was used for testing both the differences between the set of EP and ADG in the Group 1 and Group 2 subsets with 95% level of confidence. The Theil-Sen linear estimator (Theil *et al.* 1950; Sen *et al.* 1968) was used to quantify the (linear) relation between the EP and ADG in Group 1 and Group 2 with the same level of 95% confidence.

In order to obtain robust results with a 95% confidence interval for the trend, the values of n and N were set to n = 100

values and N = 200 repetitions. This provided a set of 200 results from which the P-value of Mann-Whitney-Wilcoxon test was taken as 95th percentile from the 200 P-values and the trend estimates were characterised as median trend with 95% confidence interval.

Results

Results of the statistical treatment of the dataset are divided into three groups according to the three hypotheses tested; 1. difference in EP values between the Group 1 and Group 2 subsets, 2. difference in ADG values between the Group 1 and Group 2 subsets, 3. linear relationship between EP and ADG.

Hypothesis # 1: EP Group 1 vs. Group 2

The total number of N = 200 generated datasets were used for comparison of EP values in Group 1 and Group 2 subsets of individuals. The median value of EP in the Group 1 subset was 0.560 units and the median value in the Group 2 subset was 1.260 units. Median difference between pairs of values was 0.365 units (higher for Group 1). The difference was statistically significant with the 95th percentile of the P-value less than 0.001. This demonstrates the positive effect of Hyogen® vaccination on the EP index.

Hypothesis # 2: ADG Group 1 vs. Group 2

The same method of comparison of the whole set of N = 200 samples was applied for ADG in Group 1 and Group 2 subsets. The median value in Group 1 subset was 794.0g/day and in the Group 2 subset 707.0g/day. Median difference was 39.0g/day in favour of the Group 1 subset. The difference between both subsets was again statistically significant with 95th percentile of the P-value less than 0.001. The positive effect of Hyogen® vaccination on daily gain of weight was also demonstrable.

Hypothesis # 3: Linear relation between the EP index and ADG

For each of the N = 200 datasets a median daily loss of weight for one unit of EP was computed. All estimations of the Theil-Sen linear regression were significant with $p<0.001$. The median trend in Group 1 was equal to 869.4g/day−86.0g/day/EP unit × EP unit. In Group 2 the dependency had a form of 827.2g/day−30.0g/day/EP unit × EP unit. The 95% slope confidence interval were 77.2g/day/EP−99.7g/day/EP unit in the Group 1 subset and 23.9g/day/EP−37.1g/day/EP in the Group 2 subset. In case both subsets were treated together, the overall median dependency was 859.6g/day −42.7g/day/EP × EP unit.

Results of the Theil-Sen estimator based linear dependency are depicted in Figure 1:

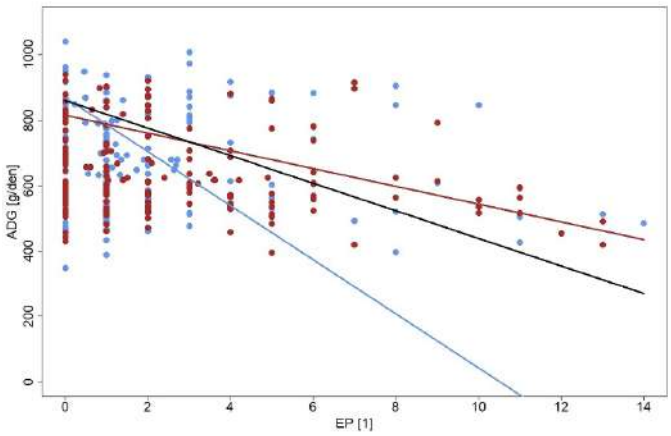

Figure 1: Median linear dependency between EP and ADG based on the set of N = 200 simulations.

The blue points represent pairs of values of EP and ADG for the Group 1 subset of individuals, and the red points represent the Group 2 subset of individuals. The straight lines of corresponding colours show median Theil-Sen regressions in both subsets and the black line shows the overall dependency of all data put together.

Discussion

In our study we analysed data achieved in field trials in which the scoring of cranioventral consolidation of lungs was used as a major indicator of *M. hyopneumoniae* vaccine efficiency. Enzootic pneumonia is characterised by chronic lesions in lung parenchyma which can be easily recognised also in slaughter pigs. *M. hyopneumoniae*-like gross lung lesions consist of purple to grey areas of pulmonary consolidation, mainly located bilaterally in the apical, cardiac, accessory and the cranial parts of the diaphragmatic lobes (Maes *et al.* 2008) often described as cranioventral pulmonary consolidation (CVPC) (Garcia-Morante *et al.* 2016). Quantitative assessment of cranioventral consolidation lesions is recommended as a parameter to measure vaccine efficacy by European Pharmacopoeia (Ph. Eur. 2448). At the slaughterhouse, evaluation of lung lesions is commonly used to estimate the prevalence and severity of respiratory diseases and their impact on carcass market price, risk factor assessment and vaccine efficacy (Sibila *et al.* 2009; Merialdi *et al.* 2012). Even fast scoring on the slaughter line provides relevant information about the presence and extension of those lesions. High correlation was found between the fast and slow-detailed inspection of lungs and it was concluded that Enzootic pneumonia has distinct visual lesions that are easily identified and therefore estimating the extent of the lesion can be done quickly (Hurnik *et al.* 1993). Several scoring methods for EP-like lesions were described (Madec and Kobisch 1982; Straw *et al.* 1986; Christensen *et al.* 1999; Holt *et al.* 2011). In the studies included in this analysis, the CLP was utilised for scoring lungs and processing the data. CLP scoring system is based on modified Madec methodology and utilises a specific software to register, store and calculate the results (Cvjetković *et al.* 2018). The CLP was implemented as a routine tool to assess EP-like lesions in multiple field trials in which vaccine efficacy was measured (Lisgara *et al.* 2018; Palares *et al.* 2018) and large-scale country or continental surveys (Espigares *et al.* 2017; Krejci *et al.* 2018).

We have analysed all reported studies which included both CLP results and growth performance data. The fact that EP affects the growth rate was described extensively (Straw *et al.* 1989; Maes *et al.* 1996). The link between lung lesion scores and corresponding impact on growth performance was described. Market hogs had a mean decrease in final weight of 1.8 kg for each 10% of lung lesion involvement (Morris *et al.* 1995). In addition, it has been measured that for every 10% of the lung with pneumonia, the mean daily gain is reduced by 37 grams (Thacker 2002) with the estimation of the cost per pig for mycoplasmal pneumonia equal to $4.08. The correlation between the lung scores and corresponding impact on the growth performance was estimated previously (Straw *et al.* 1989). However, methodologies were different and sometimes the results were not conclusive (Paisley *et al.* 1993). We were interested to determine whether the improvements in lung lesion scores recorded in several described comparative studies correlated with the decrease in weight gains and, if so, how much. In our analysis a strong negative correlation was found between the decreased EP indexes and higher average weight gains. The efficacy of different vaccination strategies has been compared in field trials and experimental challenge models with different commercial vaccines. Vaccination of pigs against *M. hyopneumoniae* proved to be efficient in reducing lung lesions observed in the abattoir (Sibila

et al. 2007). Moreover, the economic benefit of vaccination against *M. hyopneumoniae* due to higher growth rate was demonstrated and reviewed (Maes *et al.* 2003; Dinslage *et al.* 2008; Maes *et al.* 2008).

Although the positive effect of Hyogen® vaccination on the prevalence and extension of lung lesions (EP index), as well as on the daily weight gain of commercial pigs, is indisputable when using the whole dataset, the analysis of the relationship between the two variables requires a more comprehensive interpretation.

If only the available primary data from the Cvjetković *et al.* 2018 and Krejci *et al.* 2017b studies were used, without the other publications with aggregated values and without the selection/repetition of values, the dependency is still statistically significant. However, due to the Cvjetković *et al.* 2018 study, which significantly outweighs the Krejci *et al.* 2017b study in primary numbers, the dependence is relatively low (approx. 5.4g/day/EP in both Group 1 and Group 2 subsets). Although a comparison of these primary values demonstrates differences in the EP index, the difference in ADG does not show a statistically significant result ($p = 0.213$). This is also mainly due to the very small difference between the median of the Group 1 and Group 2 subsets in the Cvjetković *et al.* 2018 study, which is the largest study in terms of the number of individuals.

In case of the alignment of the individuals' numbers of all eight studies (ie. the use of simulated datasets with $n = 100$ for each study) and repeating the analysis $N = 200$ times, the dependences between EP and ADG are also statistically significant with significantly higher effect on increment per unit of EP index. In case of the Group 1 subset, it is 86g/day/EP unit, whereas in the case of the Group 2 sample it is only 30g/day/EP unit. An additional value of 43g/day/EP unit can be obtained if all values ($n = 1{,}600$) are processed together, regardless of inclusion in the Group 2 or Group 1 sample.

In conclusion, all three established hypotheses can be confirmed. As for quantitative results, we recommend to follow the simulation study results, which represents the distribution of values in different farms better than unaligned analysis of primary/aggregated data.

Conclusion

The results of this meta-analysis bring a confirmation that scoring of lungs in slaughter pigs for typical EP-like lesions provides the quantitative informational value not only concerning the presence and intensity of enzootic pneumonia in the swine herd. It also indicates the losses in weight gain of the pig populations investigated. Following the evolution of the EP index, it can serve as a monitoring tool predicting the improvements or degradation of growth performance. In 8 published field trials using the same scoring methodology and measuring the weight gain, Hyogen® vaccination resulted in a reduction of the EP index by 0.365 units and an increase in ADG by 39.0g. The present results thus demonstrated that the change of each EP-index point corresponds to 106.8g of ADG.

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Roman Krejci

Roman Krejci DVM, is a veterinarian, graduated at the University of Veterinary Medicine in Brno, Czech Rep. He started his professional carrier at the Research Institute of Parasitology, studying I. suis infections in piglets. He then joined Ceva Animal health and has specialised in swine health and reproduction management in the last 20 years, including responsibility for the technical and marketing support of Ceva swine products worldwide. Roman's role is currently Swine Corporate Veterinary Service Manager for both PCV2 and Mycoplasma hyopneumoniae vaccines.

Jiří Kalina

Jiří Kalina Mgr, is a data analyst, leading the data analytical team at RECETOX, Masaryk University in Brno, Czech Republic. He's an reputable expert in statistics, data analysis, mathematical modelling, environment, persistent pollutants, air sampling, mass spectroscopy. He's also teaching at the university. Jiri is an author or co-author of numerous articles published in international scientific journals.

Monika Kuncová

Monika Kuncová is a student in the newly accredited study program Environment and Health, where she learns to comprehensively connect scientific knowledge with statistical data processing. This study program is under the auspices of RECETOX – Research Centre for Toxic Compounds in the Environment, Brno, Czech Republic. In addition to study and research, Monika is also involved in projects for the popularization of science.