Lung Scoring Methodology

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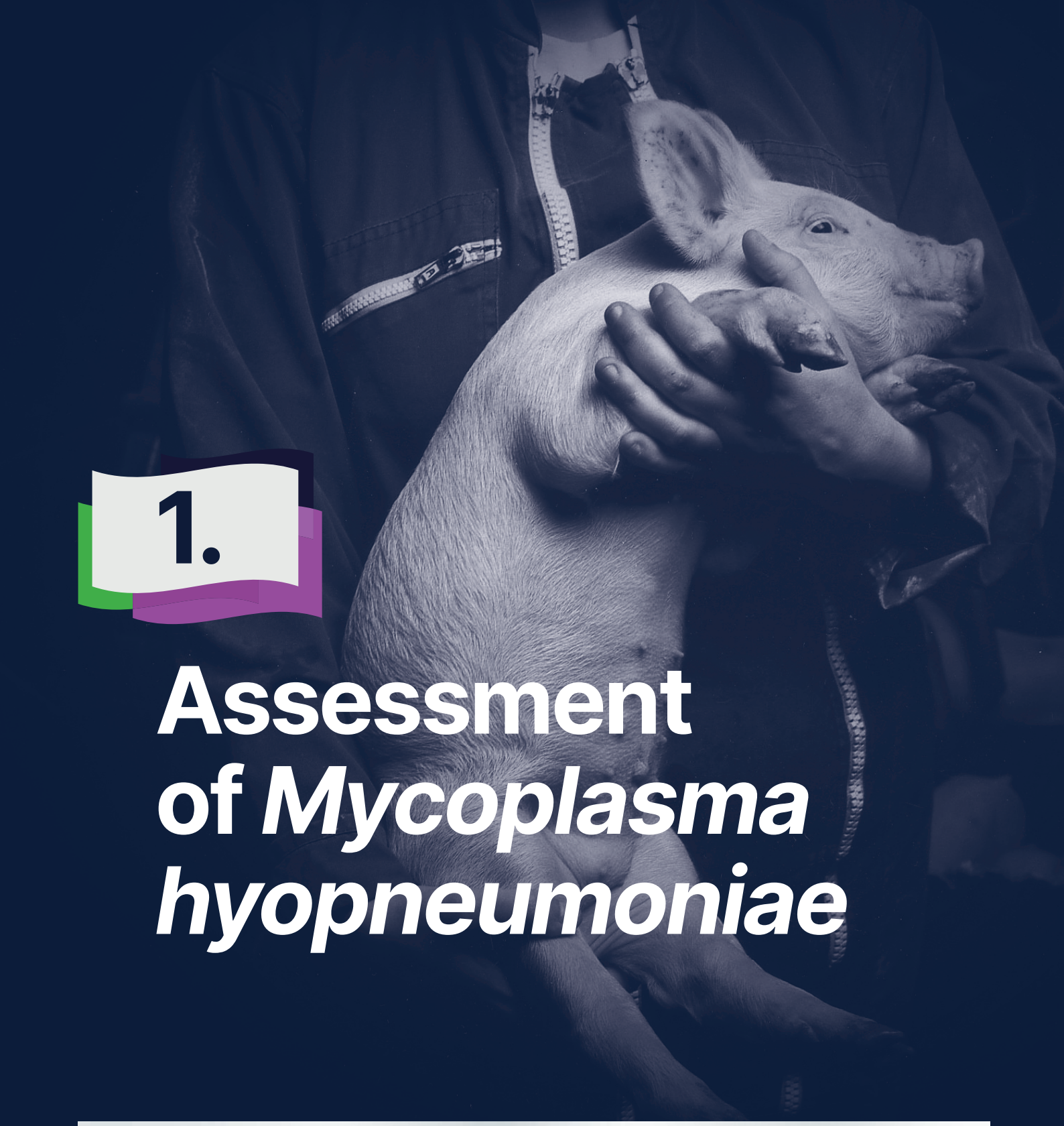
INTRODUCTION

The Ceva Lung Program offers the methodology and guidelines on how to correctly evaluate the presence, incidence, circulation patterns and impact of *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* infections using serological investigation, bacteria detection and lung scoring of slaughter pigs. It is used to determine the appropriate vaccination protocol and monitor the results of vaccination with Hyogen®, DUO™ (Circovac® and Hyogen® Ready to Mix) and Coglapix®. This brochure will bring insight into slaughterhouse lung check in pigs.



1.

Assessment of *Mycoplasma hyopneumoniae*

Mycoplasma hyopneumoniae (M.hyo) 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). EP is characterised by a chronic, nonproductive cough, decreased growth rate and feed efficiency, typically with no or low mortality. PRDC develops as a consequence of coinfections of both bacterial and viral (PCV2, PRRSv, SIV) pathogens and can result in an increased mortality and severe performance losses *(Sibila 2009)*.

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.hyo 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

Decrease in daily weight gain **17%**

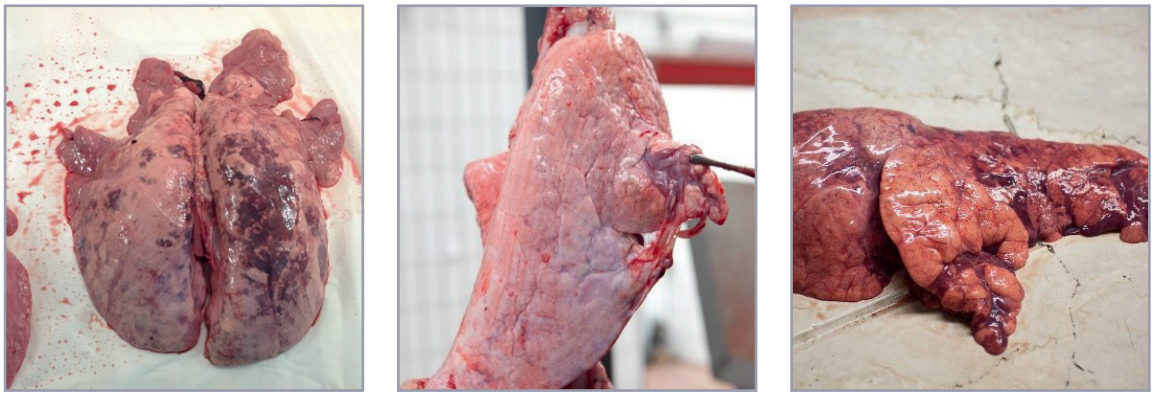
Decrease in feed efficiency in affected herds **14%**

(Straw 1989)

Just the M.hyo subclinical infection can mean a 38 g/day Average Daily Gain (ADG) difference between seropositive and seronegative pigs.

The presence of the infection is usually confirmed by M.hyo specific seroconversion or by the detection of germs by PCR in the laryngeal swabs *(Pieters 2017)*. Lung tissue infected with M.hyo 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 localized in the apical and cardiac lobes, as well as in the anterior part of the diaphragmatic lobes and in the intermediate lobe *(García-Morante, 2016)*. Lesions resolve after 12 to 14 weeks with formation of interlobular fissures scored as scars *(Maes 2008)*. Secondary viral and/or bacterial pathogens associated with primary M.hyo, can also be responsible for the development of pleuritis, which is typically localized in the cranioventral part of the lungs, most often between the apical and cardiac lobes.

Considering the chronic type of such lesions, bronchopneumonia with the cranioventral consolidation of lungs is very indicative for EP also in slaughter pigs. Even fast scoring on the slaughter line provides relevant information about the prevalence and severity of respiratory diseases and their impact on pig performance, risk factor assessment and vaccine efficacy.



/// Fig 1: EP-like lesions on Pig lungs

It was calculated that the improvements in lung scoring due to preventive measures correspond to the increased performance of pigs. Lung inspection of slaughter pigs can thus indicate potential losses in weight gain of pigs to the farmer. The results of a detailed statistical analysis confirmed 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.

Efficient vaccination resulted in a reduction of the EP index by 0,365 units and an increase in ADG by 39,0 g. The results thus demonstrated that **the change of each 0.1 EP-index point corresponds to 11g of ADG** *(Krejci 2022a)*.

This may be important information in 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.

Complementary examination can be performed to describe M.hyo infections in more details using serological and PCR techniques. **ID Screen® *Mycoplasma hyopneumoniae* Competition ELISA is recommended to measure M.hyo specific serological responses in infected pigs,** however this ELISA does not differentiate between post-vaccinal and post-infectious antibodies. Nevertheless, it can be also used to demonstrate the successful vaccination with Hyogen® or DUO™ and to demonstrate high potency to elicit specific M.hyo serological response *(Krejci 2022b)*. **Blood samples of 20 pigs are collected 4 weeks post vaccination (when the natural infection typically does not occur yet)** and tested by this ELISA according to specific instructions.

Detection of early M.hyo infections is possible in case very early circulation is suspected in the farm (weaning, early nursery stage). **Deep laryngeal swabs can be collected from 15 piglets (2 piglets per litter) at weaning and/or 6 weeks of age (3 pigs per pen) and tested by PCR** for the presence of M.hyo individually.

2.

Assessment of *Actinobacillus pleuropneumoniae*

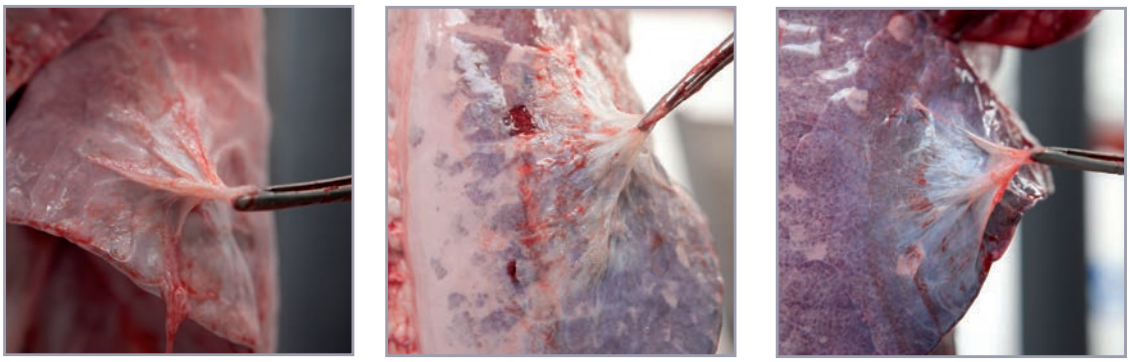
Actinobacillus pleuropneumoniae (Ap) is the etiological agent of porcine pleuropneumonia (PP). *Ap* pneumonic infection leads to inflammation of the lung and the adjacent pleura. It presents as acute or subacute lesions with peracute, acute, subacute, or subclinical signs, all highly likely developing into long-lasting chronic lesions and signs *(Gottschalk 2019, Sassu 2018, Mortensen 2022)*.

Ap induced pleuropneumonic lesions are generally allocated to the two diaphragmatic lobes: at the time of slaughter, clearly visible as chronic fibrotic, dorsocaudal pleurisy (DCP). The evolution of DCP, also named "*Ap* -like lesions", is a long process with a slow resolution easily lasting three months *(DoS)*.

DCP is not pathognomonic to PP and *Ap*. Differential diagnostic considerations are: 1) *Pasteurella multocida* (Pm). This bacterium is a strict secondary pneumonic infection. It is a common complication to *Mycoplasma hyopneumoniae* but then inducing cranioventral pleurisy, not DCP. Pm is also seen as a complication to *Ap*, however in this case: induced by *Ap* and leaving again with *Ap*. So not a complicating factor for CLP evaluations at herd level. 2) Polyserositis inducing bacteria (PSB): *Glaesserella parasuis*, *Streptococcus spp.*, commonly Strep. Suis, and to a less common extent *Mycoplasma hyorhinis* *(Gottschalk 2019)*.

When PSB are involved to any significant extent, in the well-monitored farm the other signs and findings not relevant to *Ap* will be apparent parallel issues to *Ap* induced PP: meningitis, joint-ill, pericarditis, and peritonitis. In such BSP-complicated Ap-endemic farms, actions to lower the impact of these co-infections are necessary to get the full benefit of the Ap vaccination.

On the other hand, under conditions where potential complicating infections are kept under maximal control, as Mhyo controlled for a longer period by Hyogen®, before instituting Ap control by vaccination. **Here Coglapix® can bring DCP% (prevalence) down to 5% and APPI (severity index) down to 0.1** *(Sipos 2021)*.



/// Fig 2: Pig lungs. Cases of fribrous dorsocaudal pleurisy, likely associated with A.p.

Pleurisy induced losses and *Ap* importance in pleurisy.

When reviewing publications on pleurisy prevalence's, and the impact on productivity and profits, large variation between the different studies is seen. Part of the variation may be due to differences in study design (study population, age at slaughter), pathogen prevalence, and scoring of the lesions. The variation between individual farms and/or individual batches of slaughter pigs within each study may even be higher *(Maes 2023)*.

However, *the devasting impact* on productivity of pleurisy and the great importance of *Ap* is demonstrated to be apparent: **Increased days to slaughter up to 6-8 days** *(Hartly 1988, Pagot 2007, Hölzen 2021)*, **reduced carcass weight** *(Mousing 1990, Hölzen 2021)*, a **reduction in average daily weight gain of 5.2-34%** *(Straw 1989, Pagot 2007)*, and a **reduction in feed efficiency of up to 34%** *(Straw 1989)*. Losses in profitability are estimated at €6.0-6.5 per pig produced, when calculating correlated productivity losses to pleurisy at the time of slaughter, including mortality and/or *M.hyo.* coinfection *(Jäger 2009, Ferraz 2020)*. This agrees well with the return on investment (ROI) on Coglapix® vaccination, below.

The *importance of Ap* in pleurisy is confirmed in several studies *(Maes 2023)*. In numbers: a 51% prevalence of pleurisy in Ap-endemic Danish swine farms, with no discrimination to antibiotic strategies and/or vaccination *(Enøe 2002)*, and Ap serotype 2 calculated to be responsible for 44% of all pleurisy lesions at Danish abattoirs *(Mousing 1990)*.

ROI of vaccination with Coglapix®

When looking at the prevented losses from pleuropneumonic lesions, represented by DCP in slaughterhouse investigations, by Coglapix® vaccination, we find return on investment of €6/€ spent *(Catelli 2017)*, €3.3 less production costs per pig *(Cárceles 2019)*, and €3.5 over pigs vaccinate with a well-renown *Ap* subunit-vaccine *(Hölzen 2021)*.

1 *Ap* free or positive herd?

Serological screening using IDEXX APP-Apx IV Ab Test. Sampling is performed according to the schedule below.

Animals to be sampled are selected as follows:

- ✔ Pigs in the last week before slaughter; if not possible at least 20 weeks of age.
- ✔ Careful selection of pigs to sample, well distributed between pens (and rooms).
- ✔ Preferably animals performing below average of the batch.
- ✔ If "post outbreak" sampling: animals recovered from PP started at least 21 days before.

Number of blood samples per sampling are calculated as follows:

10 samples per farm corresponding to a sow herd up to 50 sows.

20 samples per farm corresponding to a sow herd 51 to 2000 sows.

30 samples per farm corresponding to a sow herd of above 2000 sows.

or

10 samples per finishing farm with up to 250 pen places.

20 samples per finishing farm with 251 to 10.000 pen places.

30 samples per finishing farm with >10.000 pen places.

It is ideal to send the sera for testing, but if this is not possible, it may often be acceptable to send whole blood in agreement with the lab.

2 *Ap* free or positive animal?

PCR and/or bacterial isolation from deep tonsillar scraping or biopsies. As *Ap* is lodging in the bottom of the tonsillar crypts, scraping needs to be thoroughly and deep. A laborious and complicated procedure, easily performed to a suboptimal outcome. Performed correctly it remains the only safe method for a definite individual *Ap* "yes/no" diagnosis, and serotype prevalence investigations.

3 Immediate tentative diagnose

In acute outbreaks it should always be considered to perform necropsy on freshly died or euthanised animals typical of the problem. Examination of several dead animals, and in different age categories, is advisable to get a comprehensive impression of the involvement of *Ap*, pre-disposing infections, and/or co-infections involved in farm health issues. Pleuropneumonia is easy to recognize, which can lead to an overestimation of the role of *Ap* on the total losses and expectations of *Ap* vaccination effect, alone.

Relevant differential diagnoses are *Streptococcus spp.*, *Glaesserella parasuis*, and in some countries *Actinobacillus suis*. Common pre-disposing, and/or co-infections are *Mycoplasma hyopneumoniae*, swIAV (flu), PCV2, and PRRSV.

4 Conclusive diagnostics

Necropsy and relevant sampling for pathogen isolation, ideally combined with histology, IF, in-situ hybridisation, or FISH on specimens as in 3), above.

3.

CLP scoring methodology

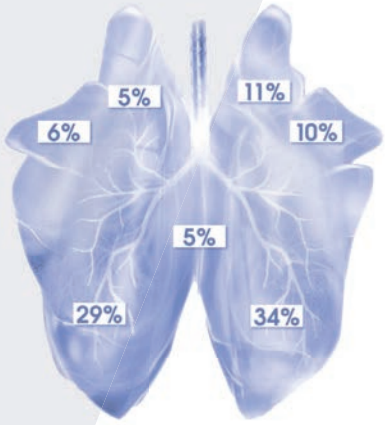
Several methods of identifying and scoring those lung lesions were developed and are implemented to monitor the presence and severity of EP in swine herds.

Pneumonia and pleurisy can be evaluated in a qualitative and quantitative manner. In the Ceva Lung Program pneumonia is scored based on Madec method *(Madec 1982)*, which has been modified considering the contribution of each lobe to the overall capacity of the lungs.

Scoring of EP-like lesions

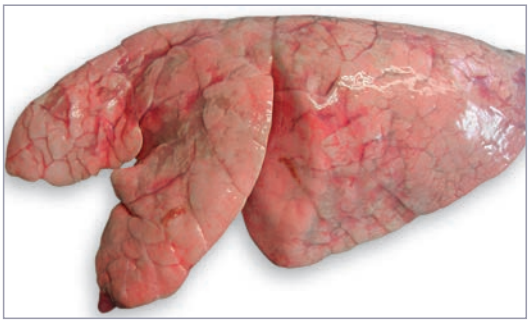
For the EP score, the lesions typical for lung consolidation per each lobe are quantified according to the following:

Since each lobe does not represent an equal proportion of the lung, the following weights were assigned according to Christensen (1999).

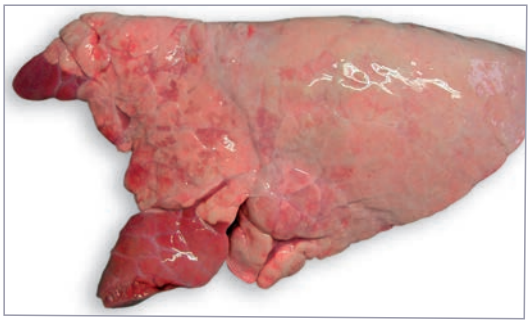


Surface of the lobe affected	
Score 0	No lesions
Score 1	1-25 %
Score 2	26-50 %
Score 3	51-75 %
Score 4	76-100 %

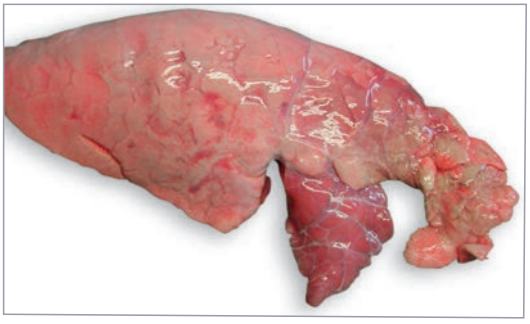
/// Fig 3: Percentage of total lung capacity representated by each lobe



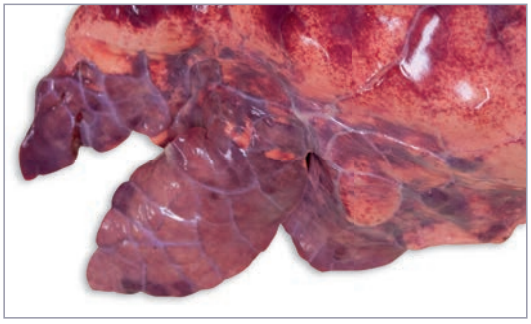
Score 1



Score 2



Score 3



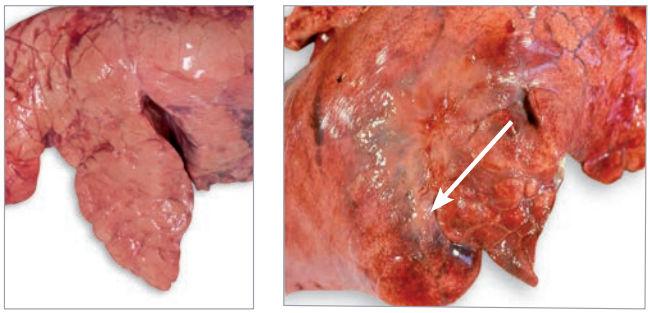
Score 4

/// Fig 4: Scoring of bronchopneumonia lesions typical for EP on the cardiac lobe*

*Courtesy of IZSLER Inst. Reggio Emilia, Italy

Quantification of scarring

The prevalence of fissures, or scarring, is evaluated based on their presence or absence. The incidence of fissures indicates the impact of old M.hyo infections.



Score 0 Score 1

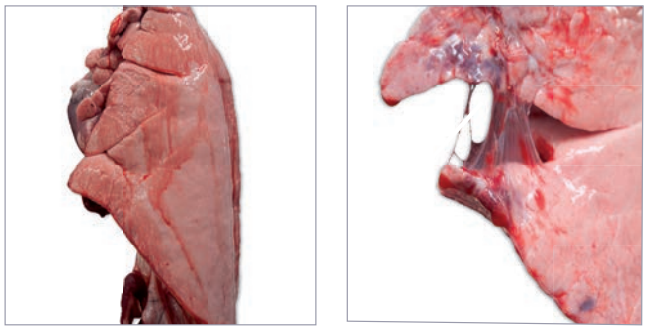
**Courtesy of Pig Research Centre, Denmark

Incidence of fissures	
Score 0	Absence of fissures (scarring)
Score 1	Presence of fissures (scarring)

/// Fig 5: Prevalence of fissures in pig lungs**

Cranial Pleurisy Scoring

Cranial pleurisy can be attributed to pathological processes in this area which are likely related to M. hyo and secondary respiratory pathogens (*Pasteurella multocida*, *Streptococcus suis*...). This cranial pleurisy should be recorded separately from the dorsocaudal pleurisy to allow for the appropriate differential diagnosis. These lesions include pleurisy on the surface of the lung lobes or between lobes as interlobar pleurisy.



Score 0 Score 1

Pleurisy in the apical and cardiac lung lobes are scored as follows	
Score 0	No pleurisy in apical and cardiac lung lobes
Score 1	Pleurisy in apical and cardiac lung lobes

/// Fig 6: Scoring of pleurisy in the apical and cardiac lung lobes

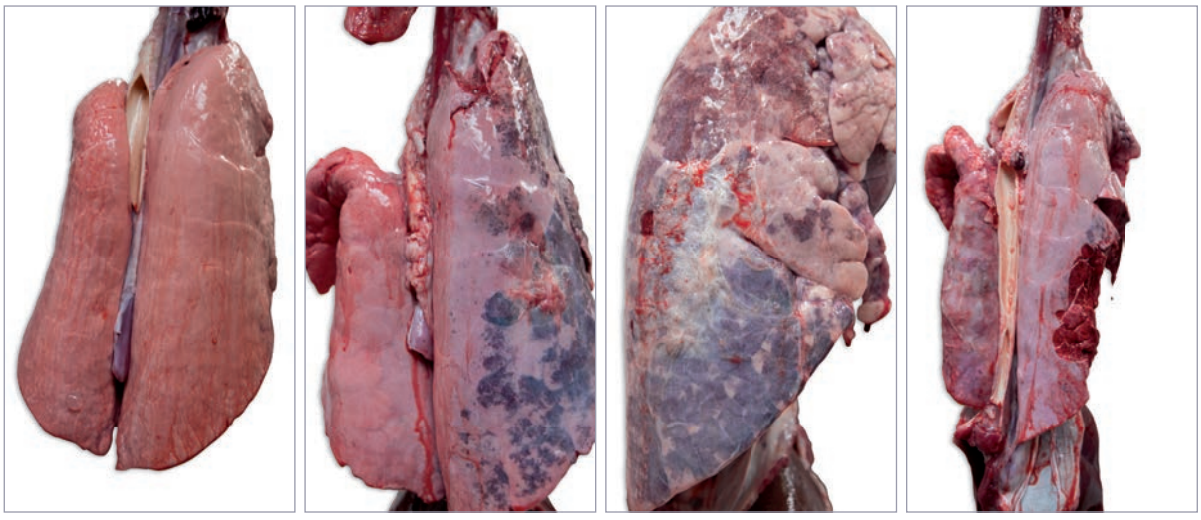
Dorsocaudal pleurisy scoring

Pleurisy is scored using the dorsocaudal pleurisy scoring (DCP) method, although here in the Ceva Lung Program cranial pleurisy is recorded separately.

The DCP method facilitates the assessment of pleural lesions according to their location, appearance and extension. The DCP method is based on a point system from 0 to 4 based on the presence, the extension and position of pleurisy observed on both lungs of each animal directly on the slaughter line.

Dorsocaudal pleurisy grid for chronic pleuritis score	
Score 0	Absence of chronic pleurisy lesions
Score 2	Dorsocaudal monolateral focal lesion
Score 3	Bilateral lesion of type 2 or extended monolateral lesion (at least 1/3 of one diaphragmatic lobe)
Score 4	Severely extended bilateral lesion (at least 1/3 of both diaphragmatic lobes)

Note: Cranio-ventral lesions previously associated with DCP score 1 is now recorded as Cranial pleurisy



Score 0 Score 2 Score 3 Score 4

/// Fig 7: Pig lungs on the slaughter line. Score 2, 3 and 4 lesions

Note the characteristic "stripping" in score 4 due to the tenacious adhesions between the parietal and visceral pleura with resulting laceration of the pulmonary tissue during the slaughter operations.

4.

Processing the scores

Through statistical processing of recorded data, the Ceva Lung Program enables not only measurement of the incidence of the injury, but also identification of the distribution by classes within the sample and restoration of the data in easily legible graphic displays.

The Ceva Lung Program report is capable of providing information about the frequency, severity, and suspected origin of pathological changes most likely due to M. hyo and A.p. This information includes:

Enzootic pneumonia like lesions

1 Average Percent of affected surface out of all lungs:

Using scores assigned to each lobe, after taking into account the weight of that lung lobe in comparison to the entire lung, the percentage of the total lung surface affected for each animal is calculated. These values are then summed and divided by the total number of lungs scored to determine the average percent of affected surface out of all lungs evaluated. This gives the actual value of the lungs that are damaged by Enzootic pneumonia on average in the group of evaluated animals.

$$\text{Average Percent of affected surface out of all lungs} = \frac{\text{Sum of individual \% of affected lung surface}}{\text{N° of all lungs}}$$

2 Average Percent of affected surface out of pneumonic lungs:

This gives us the information about the extent of the lesions and thus severity of the infection in the sick animals. It is possible that a severe infection in a small number of animals can be diluted due to a large number of health animals within the group. This situation will can be revealed with this calculation.

$$\text{Average Percent of affected surface out of pneumonic lungs} = \frac{\text{Sum of individual \% of affected lung surface}}{\text{N° of sick lungs}}$$

3 EP index:

A simplified version of expressing both prevalence and extension of EP-like lesions is also used. EP index is calculated as the average score in a group of examined lungs.

$$\text{EP index} = \frac{\text{Total sum of all scores}}{\text{N° of all lungs}}$$

4 Average percent of scarring in pneumonic lungs:

This parameter reveals the percentage of resolved lesions attributed to Enzootic pneumonia. This indicates old M.hyo infections in the herd.

$$\text{Average percent of scarring in pneumonic lungs} = \frac{\text{Number of lungs with scarring}}{\text{Number of affected lungs}} * 100$$

5 Cranial pleurisy

Cranial pleurisy is scored based on its presence or absence. The percent of cranial pleurisy in the evaluated group comments the prevalence of those lesions besides parenchymal lesions.

$$\text{Percent of cranial pleurisy} = \frac{\text{Sum of scores 1}}{\text{N° of all lungs}} * 100$$

Ap-like lesions

The *Actinobacillus pleuropneumoniae* Index (APPI) provides information regarding the prevalence and seriousness of the dorsocaudal pleurisy, highly indicative of prior pleuropneumonia due to *Ap*.

APPI = frequency of the lesions attributable to *Ap* (number of lungs with scores 2, 3, and 4/total number of lungs submitted examined) * average score (considering score 2,3,4) attributable to *Ap*.

For example: in the case where 10 lungs were evaluated with the following scores: 0,0,0,0,1,1,1,2,3,4,

The frequency of the lesions attributable to *Ap* is thus obtained by applying the previous formula is (3/10*(2+3+4)/3) = 0.9.

According to the original scoring method, a high level of correlation was found between the *Ap* seropositivity of herds and the score. This confirms the specificity of recording of the dorsocaudal pleurisy for previous *Ap* infections.

5.

Interest of Ceva Lung Program implementation

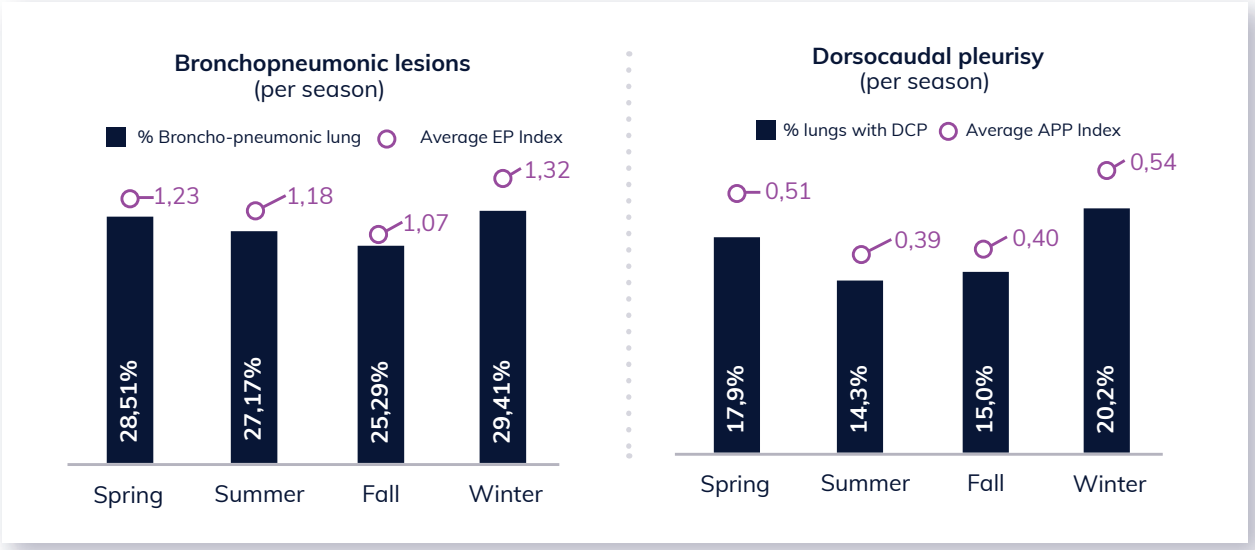
The Ceva Lung Program was designed to assist in identifying the correct diagnosis of respiratory disease through the evaluation of lungs at slaughter especially those which are the most relevant in finishing period – EP and pleuropneumonia. It enables to survey a large group of animals, not just sick or dead individuals and discover also subclinical infections that were not noted during the growing period. It can be repeated and compared with other farms or with retrospective data. It can also be used to check on efficacy of for instance vaccination programs. Very strong relation between CLP results and pig performance makes it also indicative for prospective economic gains if respiratory health will be improved.

Seasonal Effect

When used routinely, the Ceva Lung Program can aid in the recognition of dynamic of those infections in a defined period, of seasonal or regional differences and obviously of the efficacy of the control measures against M. hyo and A.p .

A seasonal effect was studies in Portugal in batches of pigs scored in spring (172 batches), summer (234 batches), fall (161 batches) and winter (148 batches). A variation among seasons was observed for both APPI and EP index *(Costa 2021)*.

Therefore it is recommended to score the same farm several times at different seasons



Regional Effect

Differences in lung health in various regions were described in Germany *(Waehner 2021)*. In total 36.080 lungs of pigs from four geographical regions were assessed and the median values compared: North Western Germany (NWG), n=21.422; Eastern Germany (EG), n=4.123; Southern Germany (SG), n=6.574; Austria (A), n = 3.961

EP Index distribution

Region	n	Mean value
NWG	157	1,32[b]
EG	27	0,90[b]
SG	56	2,02[a]
A	41	2,29[a]
Overall	281	1,56

a:b, p<0.01

APP Index distribution

Region	n	Mean value
NWG	157	0,70[a]
EG	26	0,59
SG	56	0,45[b]
A	41	0,54
Overall	280	0,62

a:b, p<0.01

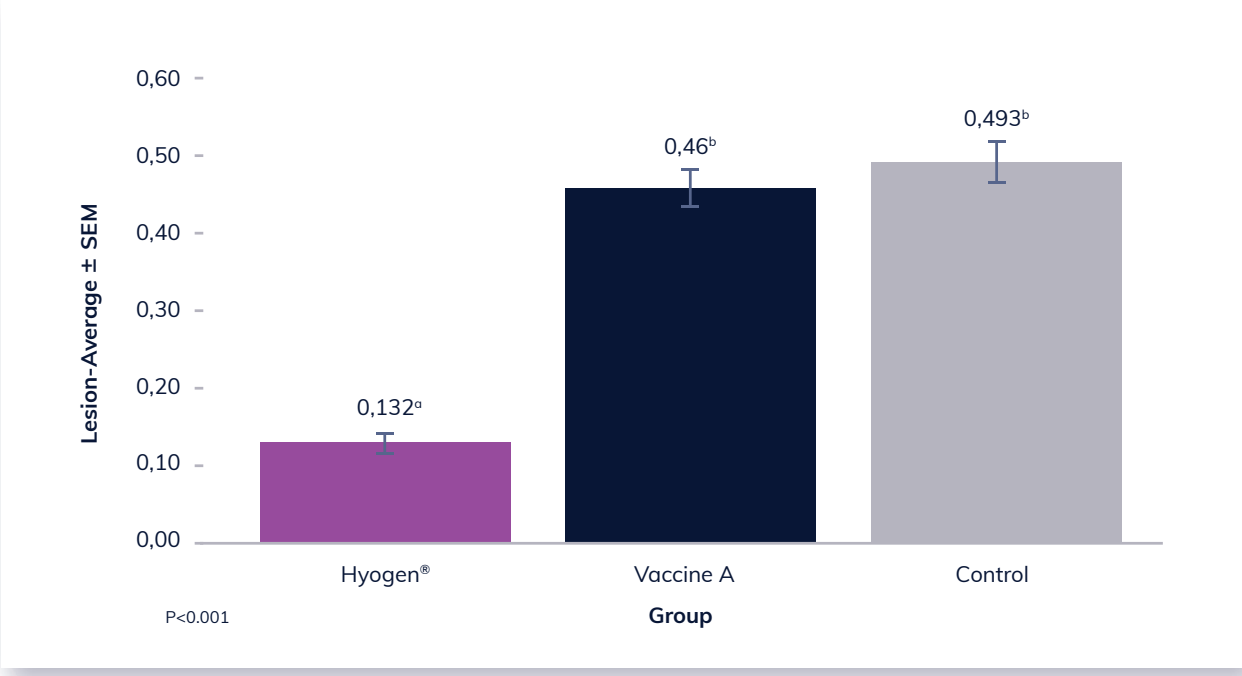
Typology of farms and / or farm density may influence scoring outcomes

Comparison of vaccination programmes

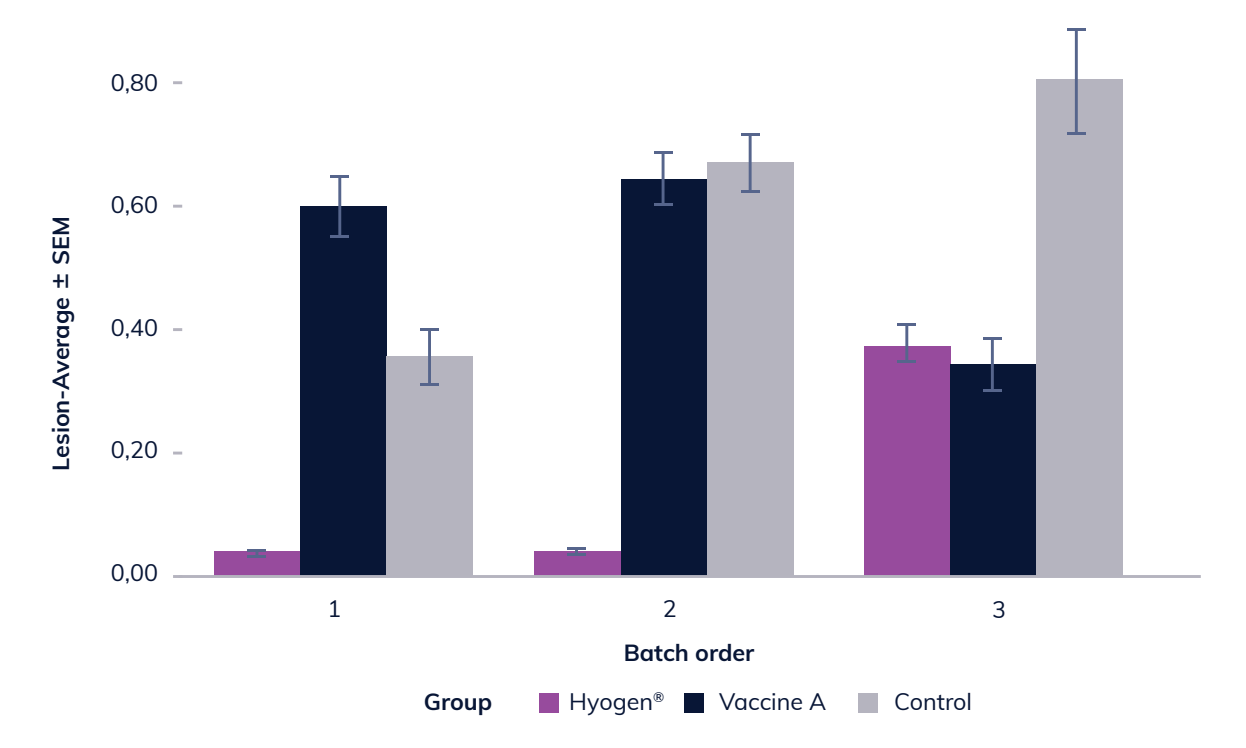
The CLP was implemented as a routine tool to assess EP-like lesions in multiple field trials in which vaccine efficacy was measured.

An extensive field trial was performed in Spain in which pigs were vaccinated with Hyogen® (n=1607) or Vaccine A (n=1008) or remained as non-vaccinated control (n= 1007). Lungs from the same treatment group were examined in three different batches (three rounds) according to the order of being sent to the slaughterhouse *(Pallares 2018)*.

EP Index distribution



EP index per batch sent to the slaughterhouse



Distribution of values related to EP-like lesions in the EU survey *(Krejci 2023)*

Large-scale country or continental surveys evaluating respiratory health and/or efficacy of different vaccines are periodically performed and published.

	% of lungs with EP-like lesions (%BP)		% of affected lung parenchyma	
	2021	2022	2021	2022
Q1 (25% best batches)	12%	11%	2%	0%
Median	24%	30%	5%	3%
Q3 (25% worst batches)	43%	51%	7%	6%

Summary of results (mean and standard deviation) of the groups: Hyogen® (Vaccine H), Monovalent Bi-dose, Other Monovalent-One-dose and Bivalent One-dose M.hyo vaccines in Spain *(Lasierra 2023)*

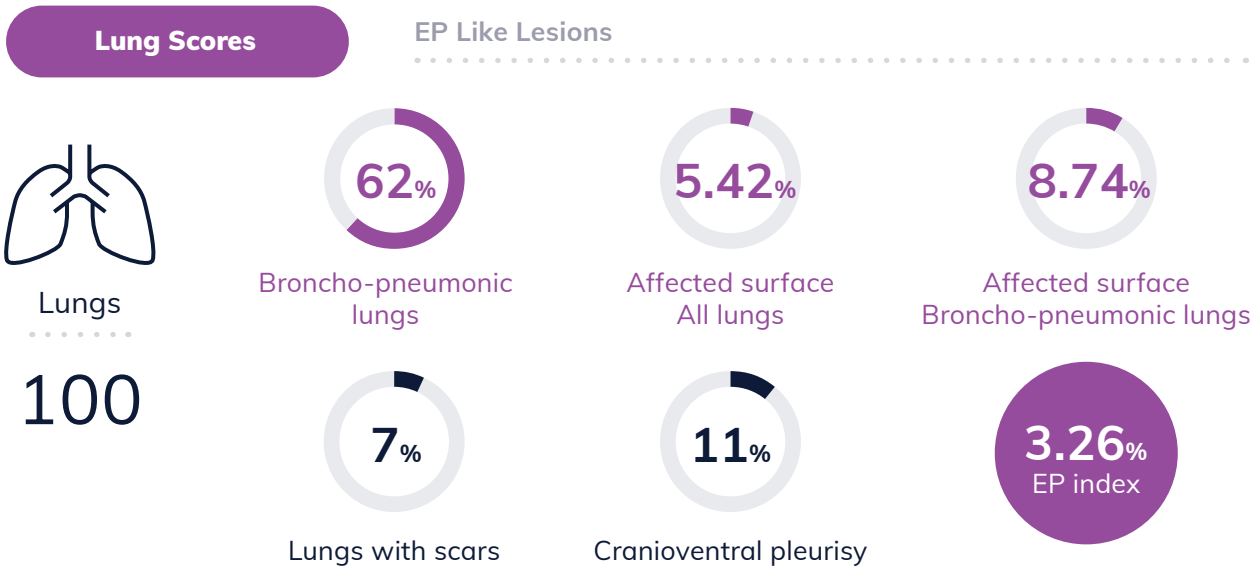
	Percent of affected lungs with Ep-like lesions	Average percent of affected surface out of the lungs	Average percent of affected surface of pneumonics lungs	Percent of scarring lungs	Percent of lungs with cranial pleurisy
Vaccine_H	34.85 ± 21.38	2.09 ± 2.13	4.89 ± 3.08	9.92 ± 11.17	13.59 ± 10.39
Other Monovalent One-Dose	46.4 ± 23.33	3.52 ± 3.14	6.56 ± 3.83	13.99 ± 13.10	16.52 ± 11.32
Monovalent Bi-Dose	39.61 ± 27.88	2.84 ± 3.35	5.2 ± 3.72	9.99 ± 10.37	20.32 ± 13.05
Bivalent One-Dose	47.87 ± 24.25	3.81 ± 3.51	6.58 ± 3.97	13.33 ± 13.16	16.82 ± 10.95

Carrying out lung scoring at slaughter can help pig producers better monitor the respiratory health of their herds and also improve welfare. Lung lesions may be indicative of welfare issues and should be considered as a surveillance tool for pig health and welfare in the context of the abattoir-based measures (EFSA report, 2022).

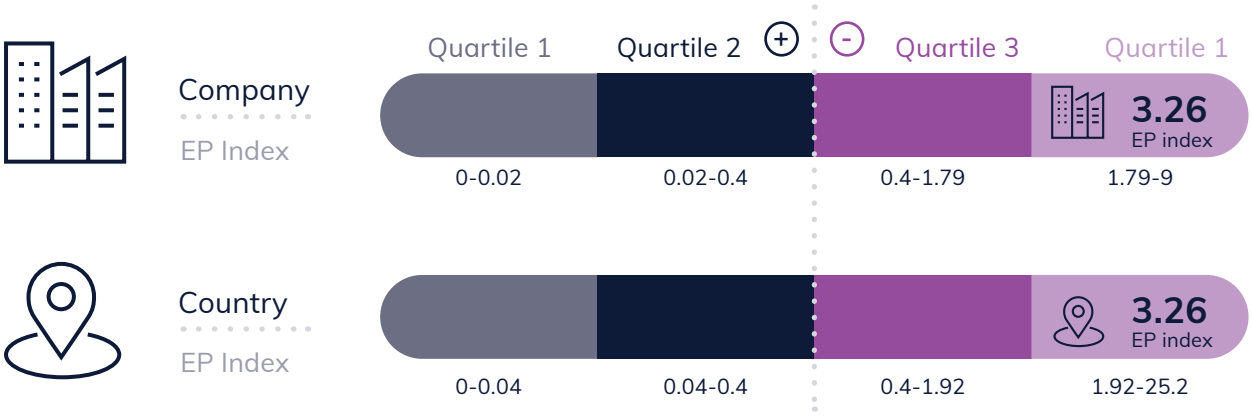
All data collected using CLP application are synchronized in a global data base collecting data from **more than 1 Million pigs per year**.

Dashboards and reports are visible on our web portal **PHP : Precision Health Program**.

This unique database allows Ceva's colleagues and clients to record lung health status and **analyze its evolution over the time** but as well **to benchmark the health status with the farms belonging to the same organization or to the same country.**

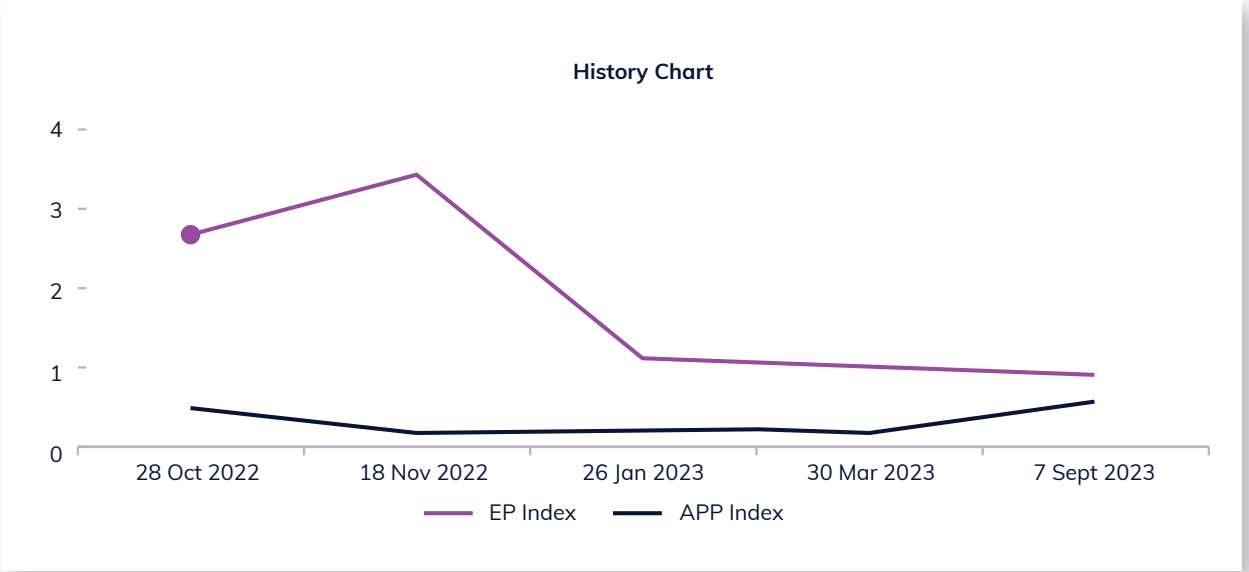


EP Index Benchmark



Example of Enzootic like lesions report from PHP.

Session gate	Bronchio-pneumonic lungs	Infected surface All lungs	EP index	Dorsocaudal pleurisy	App index
2023-09-07	35.98%	1.48%	0.90	21.64%	0.57
2023-03-30	28.04%	1.92%	0.97	6.88%	0.18
2023-01-26	25.13%	2.32%	1.10	7.49%	0.22
2022-11-18	65.22%	7.23%	3.46	6.29%	0.18
2022-10-28	72.79%	4.69%	2.68	15.19%	0.46



6.

Conclusion

The Ceva Lung Program Scoring Methodology is designed to assist in identifying the correct diagnosis of respiratory disease through the evaluation of lungs at slaughter. It enables the discovery of even subclinical infections that were not noted during the growing period.

The calculated values allow the estimation of both the incidence and the severity of enzootic pneumonia and pleuropneumonia. It can also be used to evaluate the efficacy of the control measures against M. hyo and A.p. when used both prior to and after their implementation.

Finally, when used routinely, the Ceva Lung Program can aid in the recognition of the dynamics of those infections in a defined period or seasonal differences.

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