

Case Report - Diagnostic limits of lung lesions scoring at slaughter for the evaluation of dynamics of *Mycoplasma hyopneumoniae* infection

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SUMMARY

Enzootic pneumonia is a major health and economic issue in a number of French farms, despite the widespread use of vaccination. To assess the efficacy of a control program, vet practitioner can use different diagnostic methods like observation of clinical symptoms, lung lesion scoring at slaughter and laboratory tests. On a 200-sow farrow-to-finish farm with important respiratory signs caused by *Mycoplasma hyopneumoniae*, a medication protocol has been implemented on two batches. The treated batches were compared to two non-treated control batches regarding infection dynamic of *M. hyopneumoniae*. In the four batches, we performed clinical evaluation, serological screening and lung examination at slaughter. The pigs from the treated batches have developed disease later than the control pigs, seroprevalence to *M. hyopneumoniae* was lower among finishers from treated batches than those from control batches. However, lung scoring showed statistical differences between the treated and untreated batches with more lesions in pigs from the treated batches and more lungs with scarre tissue in pigs from the untreated batches. These results question about interpretation of lung lesions scoring diagnosis method.

Keywords : Enzootic pneumonia, serology, vaccine, slaughter check

RESUME

Limites diagnostiques de la notation des lésions pulmonaires à l'abattoir pour l'évaluation de la dynamique d'infection par *Mycoplasma hyopneumoniae*

La pneumonie enzootique est un problème économique et sanitaire majeur dans de nombreux élevages en France malgré une vaccination massivement pratiquée. Afin de vérifier l'efficacité de mesures de contrôle de la maladie, le vétérinaire praticien a à sa disposition différents outils de diagnostic tels que l'examen clinique, la notation des lésions pulmonaires à l'abattoir et différentes analyses de laboratoire. Dans un élevage naisseur-engraisseur de 200 truies touché par des troubles respiratoires importants d'origine mycoplasémique, un protocole de traitement a été mis en place sur deux bandes. Les bandes traitées ont été comparées à deux bandes non traitées. Dans les quatre bandes, la dynamique d'infection par le Mycoplasme a été évaluée par des examens sérologiques et des contrôles lésionnels sur les poumons à l'abattoir. Les porcs des bandes traitées ont développé la maladie plus tardivement que les porcs des bandes contrôle, la séroprévalence vis-à-vis de *M. hyopneumoniae* a été plus faible chez les porcs des bandes traitées que chez ceux des bandes non traitées. Cependant, les contrôles pulmonaires ont montré des différences statistiquement significatives entre les bandes traitées et non traitées avec davantage de lésions chez les porcs des bandes traitées mais plus de poumons présentant des lésions cicatricielles chez les porcs des bandes non traitées. Ces résultats posent question concernant l'interprétation à faire des résultats de contrôles lésionnels pulmonaires.

Mots-clés : Pneumonie enzootique, sérologie, contrôle abattoir, vaccin

Introduction

Enzootic pneumonia (EP) is one of the major respiratory diseases in the swine industry of countries that have not eradicated its primary agent, *Mycoplasma hyopneumoniae*. The prevalence of the disease is high in affected countries, as recently highlighted for Europe [13, 14]. The pathogenicity of *M. hyopneumoniae* has been described as the conjunction of a direct action on the cilia of the respiratory tract epithelium, leading to its destruction, and a local immunomodulating effect that potentiates the invasiveness of other pathogens (*Pasteurella* sp. [1], *Actinobacillus pleuropneumoniae* [19], PRRSV [16]), although this effect might be strain-dependant [5]. At farm level, the outcome of EP is the expression of clinical signs among growers, occurring most of the time around 18 weeks of age [17]. *M. hyopneumoniae* is presently

considered as a major component of Porcine Respiratory Disease Complex (PRDC) in all pig producing areas [6, 18].

Although several *M. hyopneumoniae* vaccines have been commercially available in most countries for over a decade, the recent compilation of lung scoring at the slaughterhouse in France showed that the prevalence of EP lesions has not been reduced [14]. Recent investigation in Denmark showed that *M. hyopneumoniae* is among the most frequent pathogen incriminated in lung lesions at slaughter [6]. Also, *M. hyopneumoniae* was the only pathogen detected at slaughter from lungs of finishing pigs in all five farrow-to-finish herds investigated in a French longitudinal study on respiratory infection patterns [4]. A similar finding has also been reported in the US in the mid-90s, where "*M. hyopneumoniae* appeared to be a significant bacterium despite the wide use

of commercial vaccines on swine farms" [3]. In addition of the use of vaccines, other ways to control the disease are well known as management practices, improvement of housing conditions and strategic treatment with antibiotics [11]. Most of the time, the control of the disease at the herd level is accomplished with a combination of such measures.

To assess the efficacy of a control program, vet practitioners can use different diagnostic tools like clinical scoring with cough/sneeze counts, lung examination at slaughter, serology and bacteriological or PCR tests in lungs or nasal or tracheal swabs.

The objective of our work was to investigate how a combination of diagnostic tools (serology and slaughter check) can describe the dynamic of *Mycoplasma hyopneumoniae* in the context of the implementation of various control method. The on-farm study consisted in comparing serological profiles and lung notation at slaughter between batches which were vaccinated and/or treated with antibiotics.

Case description

FARM DESCRIPTION AND MANAGEMENT

The production farm where the trial took place is a 200-sow farrow-to-finish farm located in a high pig-density area in Brittany (western France). It is family-owned and managed all-in all-out, with 50-sow batches (farrowing takes place every 5 weeks). The farm counts two farrowing and three post-weaning rooms in one building and four fattening, one gestating and one quarantine different buildings, all on the same site. Buildings are arranged in a way allowing to start a visit in quarantine and end it in the fattening units. Compliance to biosecurity rules is one of the assets of the farmers, who respect the quarantine-to-fatteners flow on a routine basis and change boots and coveralls when going from one batch of pigs to a younger one, and between buildings. After moving pigs, the ground and walls of the corridors are cleaned and disinfected. Every room is managed in an all-in all-out manner, and is cleaned and disinfected after every batch departure; a 3-day down time is applied.

In the post-weaning rooms, the slatted-floor is in plastic and it is concrete in all finishing pens. All the ventilation system in the buildings is dynamic and the manure handling is separate between rooms. The correct management of the ventilation system by the farmer has been validated before the beginning of the trial by a specialized technician. There is dry feed in post-weaning pens and wet-feed in finishings.

Upon arrival, gilts are isolated in the quarantine unit for 5 weeks, and vaccinated against PRRSV (live attenuated vaccine), *M. hyopneumoniae* (one-dose Ingelvac[®] M. hyo vaccine (Boehringer Ingelheim France)), atrophic rhinitis, Swine influenza viruses H1N1 and H3N2 (European strains), *E. coli*, parvovirus and erysipelas. Multiparous sows

are vaccinated against PRRSV (live attenuated vaccine), atrophic rhinitis, parvovirus, erysipelas, SIVs and *E. coli*. The herd PRRSV status is considered as stable, without virus circulation post-weaning onwards. Piglets were vaccinated at weaning (3 weeks of age) with the one-dose Ingelvac[®] M. hyo vaccine (Boehringer Ingelheim France).

Since the second half of 2008, this farm was experiencing unusually severe respiratory disease either at post-weaning or at the start of fattening, depending on the batches. The pigs began to cough severely; these episodes were difficult to control through medication, and strongly increased the overall antibiotic consumption, which the farmer as well as the practitioner were dissatisfied with. They were feeling that health management was getting out of hand. A number of decisions were taken: an extensive diagnostic investigation, including a detailed follow up of batches and the possibility of a more structured treatment protocol were accepted by the farmer. Lung checks were performed at the slaughterhouse, that evidenced lesions compatible with EP: 110 lungs were checked (average lung score of 4/28); 18% of which had severe pneumonia lesions (as defined by the French Swine Technical Institute, IFIP) and 1.7% had pleuritis. Under the lung lesion scoring method used, a score of 5/28 or more (equivalent to 4/24 or more) is indicative of a severe pneumonia [7]. Serological profiles were performed in January 2009, aiming at detecting SIV (IHA), PRRSV (IDEXX HerdChek PRRS 3XR) and *M. hyopneumoniae* (IDEXX HerdCheck *Mycoplasma hyopneumoniae*). These profiles confirmed the absence of SIV and PRRSV circulation in piglets. *M. hyopneumoniae* serological profiles revealed the active circulation of *M. hyopneumoniae* in sows. Twenty per cent of the gilts that were in the gestation unit were positive with high titres (> 1.5). Pigs were seroconverting at 13 weeks of age.

In order to modulate the infection dynamics of *M. hyopneumoniae* in grower pigs, it was decided to implement a medication of sows around farrowing and piglets after weaning. Four batches were successively included in the trial: one untreated (batch 1), one treated (batch 2), one untreated (batch 3), one treated (batch 4) and no management routines were modified during the trial period. This trial was performed in accordance with the Good Clinical Practices VICH recommendations (EMA).

Sows in the treated batches received, via the oral route, 50 mg/kg bodyweight of oxytetracycline (OTC 50[®] poudre orale, Franvet) and 6 mg/kg bw of tiamulin (Denagard[®] solution buvable, Novartis Santé Animale, SAS), on a daily basis, from 4 days before the expected day of farrowing to 2 days post-partum. On the day of farrowing, the sows were injected with 12 mg/kg bw of tiamulin (Denagard[®] injectable, Novartis Santé Animale, SAS) in order to maintain treatment on the day when feed intake is minimal. Piglets were vaccinated against *M. hyopneumoniae* at weaning, as previously performed, and received an oral supplementation of oxytetracycline and tiamulin at the same dosage, for 8 days, starting 2 weeks post-weaning (5 weeks old).

Sows and piglets in the untreated batches were left untreated; all piglets were vaccinated at weaning, as previously performed.

Any other antibiotic treatment was recorded. As a rule, such treatments were implemented when 5% or more of the pigs were coughing in a given room.

At weaning, 15 piglets in 15 litters from each batch were randomly selected for sampling, and were individually ear-tagged. Blood sampling was performed at 3, 12 and 17 weeks of age. Blood sampling was performed at the jugular vein. Blood was tested for the presence of anti-*M. hyopneumoniae* antibodies (Herdcheck *Mycoplasma hyopneumoniae* ELISA; Idexx Laboratories, France).

The coughing frequency was measured by two operators, who benchmarked their relative expertise before the start of the trial and during the trial, and obtained comparable results. Briefly, coughing and sneezing were counted in each room for 2 minutes (after the animals had all awakened and their activity had gone back to normal), 3 times, and the average of the 3 counts was then expressed as a number of coughs/sneezings per 100 pigs per 2 minutes. Measurements were performed at 2 weeks of age (in the farrowing unit), 7 weeks of age (post-weaning unit), 12 weeks of age (transfer to fattening unit), 17 and 22 weeks of age (fattening unit).

The pigs were sent to slaughter around 24 weeks of age, and lung lesion scoring was performed. The lung lesion scoring was performed on 30 to 40% of each batch (195, 166, 225 and 190 pigs from batches 1-4, respectively), according to the French 28-points notation [10]. In this scoring method, each lobe is scored between 0 (absence of lesion) to 4 (lesion covers over 75% of the lobe surface) and the final score is the sum of the score of each lobe. In this system, a score of 5/28 and over is considered as reflecting a severe pneumonia [7, 8]. In this scoring method, it has been demonstrated that there is a significant correlation between a higher lung lesion score and lower growth performances [14].

The farm performances were recorded by the farmer on a routine basis in a dedicated programme (GTE, Ifip). These data were used to evaluate the impact of the treatment on the performances of pigs in batches 1-3 as compared to those in batches 2-4.

Data were read into the statistical software SAS[®] (SAS[®] 9.1.3 Help and Documentation, SAS Institute Inc., Cary, NC, USA) for analysis. SAS[®] procedure *npar1way* was used to perform two-tailed Mann-Whitney tests. Fisher's Exact test was performed with procedure *freq*. Summary statistics were

calculated with SAS[®] procedure *univariate*. SAS[®] procedure *mixed* was used to perform the analyses of variances and co-variances for weight gain and comparison of treatment groups thereof. The level of significance of the tests is $\alpha = 5\%$; all tests were performed two-sided.

Results

ANIMALS

All piglets from sows on week 10 of 2009 were included in the study, and were considered as batch 1. Since a group of about 50 sows farrowed every 5 weeks, piglets born on week 15 were included as batch 2; piglets born on week 20 were included as batch 3 and piglets born on week 25 were included as batch 4. Each batch contained more than 600 piglets (see table I), and no mixing of pigs between batches occurred since the farm is observant of all-in all-out management for sows as their progeny.

REPRODUCTIVE PERFORMANCES

The data from batches 1 and 3 were pooled together (control), as were those from batches 2 and 4 (treated). No significant difference was evidenced between the treated and untreated batches as far as total born per sow ($p=0.321$), number of liveborn per sow ($p=0.312$) and number of piglets weaned per sow ($p=0.596$) are concerned.

LOSSES AND MORTALITY

The losses that occurred in the farrowing unit were expressed with the number of liveborn piglets as a reference. Although this parameter was strongly variable according to the batches (batch 1: 16.4%; batch 2: 9.1%; batch 3: 10.0%; batch 4: 12.9%), there was no difference once the treated and untreated batches were pooled (11.0 and 13.3% respectively, $p=0.885$).

There was no difference in wean-to-finish mortality between both groups (6.2% in untreated batches, 6.4% in treated batches, $p=0.94$).

GROWTH PERFORMANCES

ADG during post-weaning phase was higher in batches 1 and 4 (517 and 514 g/d, respectively) than in batches 2 and 3 (491 and 489 g/d, respectively), with no statistical difference when data from treated and untreated batches were pooled (503 g/d in each group). Also, the age at 30 kg was the same in both groups (71.1 d).

	Batch 1	Batch 2	Batch 3	Batch 4	Total
Liveborn	672	668	669	620	2,629
Weaned	562	607	602	540	2,311

TABLE I: Number of piglets included in each batch during the on-farm study, in 2009.

ADG during fattening was not significantly different between both groups either (921 g/d in the untreated group; 926 g/d in the treated group, $p=0.87$). The standardized age at 115 kg liveweight was also nearly similar (169.6 d in the untreated group; 170.2 d in the treated group; $p=0.98$). There was no significant difference between groups in lean meat percentage ($p=0.79$).

CLINICAL MONITORING

The coughing counts did not significantly differ between treated and non-treated batches at each measurement time, nor when they were pooled as a whole ($p=0.37$). On the contrary, the weighted mean of sneezing counts were significantly different between treated and untreated batches both at 12 ($p=0.045$) and 17 weeks of age ($p=0.03$), with more sneezing in the untreated batches. This was mostly due to high sneezing counts in batch 3. When data were plotted across all ages (controls at 2, 7, 12, 17 and 22 weeks of age), analysis of variance applied on the log-transformed sneeze percentages reveals a highly significant two-way interaction of "batch" by "age", with more sneezing among untreated pigs ($p=0.0001$).

Several complementary antibiotic treatments have been implemented during the study period, because the threshold of 5% of coughing pigs had been reached.

SUPPLEMENTARY ANTIBIOTIC TREATMENTS

In the non treated batches, the pigs received a seven days treatment with 50 mg/kg bodyweight of oxytetracycline and 6 mg/kg bw of tiamulin at 9 and 17 weeks of age in batch 1 and at 12 and 19 weeks of age in batch 3. In the treated batches, only the pigs of the batch 1 were treated one time with 50 mg/kg bodyweight of oxytetracycline during seven days at 22 weeks. These treatment was only done with oxytetracyclin due to the age of the pigs and the delay before slaughtering.

Although there was no extra antibiotic treatment in batch 4, the number of treatments was not significantly different when the data from treated and untreated batches were pooled, although a tendency was clearly evidenced to a higher number of extra-treatments in the untreated batches ($p=0.09$).

SLAUGHTER CHECKS: (SEE TABLE II)

The proportion of lungs with no lesion was more important in the untreated group than in the treated group (45.2 and 28.5% respectively, $p=0.00002$). Also, the average pneumonia score was much higher in the treated batches (3.8) than in the non-treated ones (2.2, $p=0.0000003$). This was also true when only lung with severe pneumonia were taken into account (35.8% in treated batches, vs 19.8% in untreated batches, $p=0.0000006$). Conversely, the proportion of lungs with scar tissue was lower in the treated batches than in the untreated ones (5.3 and 24.8%, respectively, $p=1.3 \cdot 10^{-13}$). There was only a tendency to a higher proportion of pleuritis in the treated batches as compared to the untreated batches ($p=0.06$).

ANCILLARY TESTING

There was no statistical difference in the proportion of seropositive piglets between treated and untreated batches at the three sampling dates; however, the proportion of seropositive pigs at 17 weeks of age was nearly twice as high in the untreated group than in the treated one (60 vs 35+, $p=0.45$). When only the pigs with a serological titer over 1.5 were considered, no statistical difference was evidenced either, but the proportion was again twice higher in the untreated compared to the treated group. At week 17, the average titer was significantly higher in the untreated group as compared to the treated group (0.95 and 0.60 respectively, $p=0.02$, (see figure 1).

Discussion

Our results show that the treatment did not induce any change in reproductive and zootechnical performances in the sow unit. It is to be noted that a strong between-batch variation was observed for all parameters. This variation was more important between batches for a given category (either treated or untreated) than for treated batches as compared to untreated batches. This result is not surprising since other studies have evidenced there is no direct health or zootechnical effect of *M. hyopneumoniae* in performances in the breeding herd (see [15] for a review).

	Control batches	Treated batches	p value
% of lungs with no lesion	45,2	28,5	$p=0,00002$
Average pneumonia score	2,2/28	3,8/28	$p=0,0000003$
% of lungs with severe pneumonia	19,8	35,8	$p=0,0000006$
% of lungs with scar tissue	24,8	5,3	$p=1,3 \cdot 10^{-13}$

TABLE II: Results of the lung lesion scoring and statistical significance

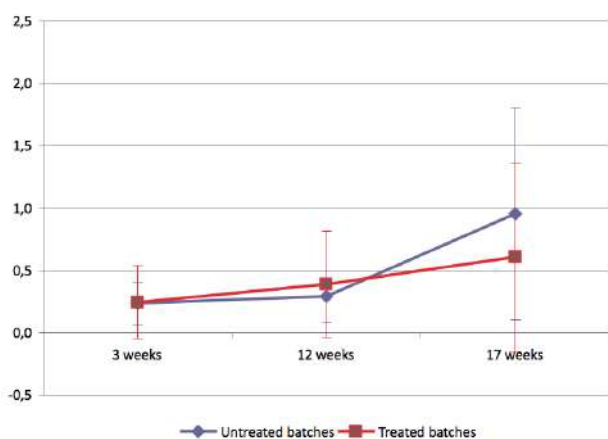


Figure 1: Results of the IDEXX M. hyo serology testing of piglets over their rearing period; the average titre within pooled batches (either treated or untreated) are presented, with SD. All piglets were vaccinated against *Mycoplasma hyopneumoniae* with a single shot inactivated vaccine at three weeks of age.

The main findings of our study is that the interpretation of clinical examination, lung scoring at slaughter and serological screening has to be carried out with caution.

Coughing counts did not reveal statistical differences between treated and untreated batches. However, pigs from untreated batches had to be extra-treated due to coughing outbreaks, which could have influenced the result of the scorings. On the contrary, sneezing counts were significantly lower in treated batches. This suggests that the antimicrobial treatment had an impact on bacterial contamination of the noses.

Regarding serologic tests, neither the percentage of seropositive animals nor the proportion of pigs with high titre statistically differed between the groups even if a tendency was seen. Only the mean serologic titre at 17 weeks of age was higher in untreated batches. We can assume that seroconversion in untreated batches at this age is not linked to vaccination because all the piglets were vaccinated at weaning and serologic titres at 12 weeks were similar between groups. It would confirm that the *Mycoplasma hyopneumoniae* infection was delayed in treated animals.

Slaughter checks and serology concur in showing that piglet infection took place later in treated batches as compared to untreated batches. The lungs of pigs from untreated batches exhibited scar lesions, whereas lung lesions of pigs from treated batches were not healed at the age of slaughter. This suggests a more recent infectious process, since it takes 5 to 6 weeks for pneumonia lesions to heal and turn into scars in the absence of concurrent opportunistic lung infections [2, 9]. Also, the threshold for considering lung lesions as severe that were used in our study (5/28) can be considered as low, since other authors rather consider a cut-off of 10/28

[8], but we remained with the 5/28 threshold because it is the only consensus value published for this lesion scoring grid in France [7]. The observation of scar tissues is not systematically done in practice and it has to be underlined if the objective of slaughter examination is also to give an overview of *Mycoplasma hyopneumoniae* dynamic in the growers.

Regarding lung scoring, in contradiction with a previous study [14], we did not observe any difference in the pig growth during the fattening period at the batch level. Our study was not powerful enough to demonstrate such a difference. It would be interesting to design a ad hoc study to compare growth rate of individual pigs with the different type of lesions (pneumonia score, pleuritis) taking into account scar tissue.

Recent demonstration [12] showed that vaccination alone does not significantly reduce *Mycoplasma hyopneumoniae* transmission among pigs. Antibiotic use on sows around farrowing in order to reduce sow shedding to their offspring and consequently *Mycoplasma hyopneumoniae* circulation among issues is common in practice. Our study underline in some aspects the difficulties for vet practitioners to assess the efficacy of such protocols and the necessity to choose the best tools between all at disposal.

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