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PAPER

# Autogenous vaccination reduces antimicrobial usage and mortality rates in a herd facing severe exudative epidermitis outbreaks in weaned pigs

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This study was conducted in a commercial 1000-sow herd facing recurrent exudative epidermitis (EE) outbreaks during the nursery period and assessed the efficacy of autogenous vaccination in controlling such outbreaks. The vaccine was produced using three *Staphylococcus hyicus* isolates recovered from affected pigs shortly before the onset of the study. All of those isolates were positive for the *exh*B gene, which encodes the exfoliative toxin type B (ExhB). From four consecutive farrowing batches of sows, two batches were vaccinated (V) against *S hyicus* at five and two weeks before farrowing, and two sow batches remained non-vaccinated (NV). Vaccination efficacy was primarily determined by the levels of metaphylactic antimicrobial usage, and the morbidity and mortality data for the pigs of the V and NV sows. The total amount of antimicrobials used metaphylactically against EE in pigs among the V and NV farrowing batches was 39,600 and 88,550 mg, respectively. The used daily dose pig to animal daily dose pig ratio for the V and NV batches were 1.31 and 1.79, respectively (a ratio of 0.8 to 1.2 is indicative of correct dosing). The morbidity and mortality rates were V=6.50 and NV=14.36 (P=0.008), and V=2.59 and NV=5.02 (P=0.000), respectively. To conclude, autogenous vaccination of the sows with a vaccine based on *exh*B-positive *S hyicus* isolates reduced metaphylactic treatment with antimicrobials as well as the morbidity and mortality rates in weaned pigs compared with pigs from NV sow batches.

## Introduction

Exudative epidermitis (EE) is one of the most common skin diseases encountered by pigs.[1] The primary cause of the disease is deemed to be virulent strains of *Staphylococcus hyicus* that produce exfoliative toxins. So far, a total of six toxins have been discovered, namely ExhA, ExhB, ExhC and ExhD,[2 3] and SHETA and SHETB.[4] The lesions can be generalised or localised in specific parts of the body such as the head and the neck, and are characterised by sebaceous exudation, which then develops into epidermal erosions and crusts.[5] The most common ages affected are suckling and weaned pigs up to six weeks of age. In herds facing severe outbreaks of the generalised form of EE, progressive dehydration and wasting of the affected pigs can be observed, together with high mortality rates.[5 6]

A very common finding in herds that are affected by EE outbreaks is the presence of *S hyicus* isolates that exhibit broad-spectrum resistance to antimicrobials.[7] Reports from several countries have indicated frequent resistance to ceftiofur, penicillin, amoxicillin, ampicillin, erythromycin, streptomycin and tetracyclines.[8–14] The aforementioned presence of resistant *S hyicus* isolates together with the fact that EE can reach morbidity rates of up to 90 per cent often complicate the treatment of affected pigs, and make it a laborious and economically impractical task.[9 15]

The pig industry is one of the food production sectors with high use of antimicrobials, favouring spread of antimicrobial resistance.[16 17] Over the years, in several major pig-producing countries, measures have been taken to preserve the efficacy of the currently available antimicrobials and to prevent further development of antimicrobial resistance.[18–20] In this context, there is an urgent need to investigate and improve the efficacy of alternative solutions for the control of EE under field conditions. Such a solution might be vaccination.

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**Table 1: Herd description and health management practices**

| | |
|---|---|
| Number of sows | 1000 |
| Breed of sows | Topigs 20 |
| Breed of boars for artificial insemination | Piétrain |
| Vaccination of sows | |
| Atrophic rhinitis | Rhiniseng (Hipra) four weeks before farrowing |
| *Escherichia coli* + *Clostridium novyi/C perfringens* type C | Suiseng (Hipra) three weeks before farrowing |
| PRRS(V) | Ingelvac PRRS MLV (Boehringer) eight weeks before farrowing |
| Swine influenza (type A) | Gripovac 3 (Merial) six weeks before farrowing |
| Vaccination of gilts in quarantine unit | |
| *Mycoplasma hyopneumoniae* | Ingelvac MycoFLEX (Boehringer) upon entering the unit |
| Parvovirus + *Erysipelothrix rhusiopathiae* | Parvosuin-MR (Hipra) two times four weeks apart |
| PCV2 | Ingelvac CircoFLEX (Boehringer) two times three weeks apart |
| PRRS(V) | Ingelvac PRRS MLV (Boehringer) once four weeks before breeding |
| Swine influenza (type A) | Gripovac 3 (Merial) two times three weeks apart |
| Medication of suckling pigs | Iron (Prolongal 200, Bayer) on day 3<br>Long-action ceftiofur (Readycef, Lab Calier) on day 3 |
| Vaccination in the nursery unit (days 21–70) | |
| PRRS(V) | Ingelvac PRRS MLV (Boehringer) on day 23 |
| Medication in the nursery unit (days 21–70), before the initiation of the study | In-feed apramycin (Apralan, Elanco) after weaning for 14 days<br>In-feed doxycycline (Doxyral 10%, Emdoka) and amoxicillin (Suramox 5%, Virbac) for the entire nursery period |

PCV2, porcine circovirus type 2; PRRS(V), porcine reproductive and respiratory syndrome virus.

However, currently no commercial vaccine against *S hyicus* infections is available. Thus, autogenous vaccines might be able to fill this gap and contribute to the reduction of antimicrobial usage. To the authors' knowledge, there are no published studies investigating whether autogenous vaccines against *S hyicus* can reduce antimicrobial usage during severe EE outbreaks.

The objective of this study was to investigate the efficacy of vaccinating gestating sows of a Belgian breeding herd facing recurrent outbreaks of EE with an autogenous vaccine against *S hyicus*, to control EE in the offspring of those sows. Antimicrobial usage data, morbidity and mortality constituted the main efficacy parameters. Weight gain and average daily weight gain (ADG) were also investigated as secondary efficacy parameters.

## Materials and methods

### Herd and outbreaks description

The study was conducted between October 2015 and June 2016 in a commercial Belgian breeding herd with 1000 sows. The herd operated a four-week batch production system for the sows (Table 1). At three weeks of age, the pigs were weaned and transferred to two different nursery units located on the same site. The first nursery unit (N1) consisted of one compartment with eight pens (120 pigs per pen), while the second nursery unit (N2) consisted of seven compartments of 12 pens each (40 pigs per pen). N1 had fully slatted floors, a conventional mechanical ventilation system (combining vent doors and ceiling fans) and a stocking density of 0.26 $m^2$/pig, whereas each compartment of N2 had fully slatted floors, channel ventilation and a stocking density of 0.24 $m^2$/pig. At 10 weeks of age, all pigs were sold to different fattening herds.

Severe and recurrent outbreaks of EE have been reported since June 2014. Clinical signs of EE appeared approximately one week after weaning and the severity of the lesions increased towards the third week of the nursery period. No clinical problems requiring treatment or any other intervention occurred during the suckling period, before or during the study. In October 2015, during a visit to both N1 and N2, almost 30 per cent of the pigs had clinical signs of EE, while the herd owner reported that 100 pigs were culled or had died during the previous production batch. These losses contributed to an increase of mortality rate from approximately 2.5 to 6.7 per cent during the nursery period. Moreover, the selling price of the nursery pigs was reduced due to the EE-related problems.

From June 2014 until October 2015, the herd veterinarian attempted to treat affected pigs individually *via* intramuscular injections of ceftiofur (Readycef, Lab Calier) or sulphadiazine-trimethoprim (Duphatroxim, Zoetis), together with spraying and washing with a 5 per cent weight per volume (w/v) chlorhexidine product (Ecutan, Ecuphar). Nevertheless, the response to the antimicrobial treatments was poor as EE morbidity and the observed mortality rates in the nursery remained above 20 and 6 per cent, respectively. In October 2015, blood samples were collected from pigs at weaning and at seven weeks of age (five for each age group). The serum from the blood samples collected at weaning was pooled and tested by real-time PCR for porcine reproductive and respiratory syndrome virus (PRRS(V))-specific RNA (LSI VetMAX PRRSV EU/NA Real-Time PCR Kit, Life Technologies) and by a quantitative PCR for porcine circovirus type 2 (PCV2)-specific DNA (ViroReal PCV2, Ingenetix). No positive results were obtained for any of the above-mentioned viral pathogens. Individual analysis of the blood samples collected at seven weeks of age showed that 1/5 pigs was seropositive for PRRS(V) (HerdCheck PRRS ELISA, IDEXX), while 0/5 pigs was seropositive for PCV2 (IgM and IgG, Ingezim PCV2 ELISA, Ingenasa).

In October 2015, the herd was visited for the first time by the Unit of Porcine Health Management of the Faculty of Veterinary Medicine of Ghent University and bacteriological sampling and antimicrobial susceptibility testing (AST) were performed for the first time. During that visit and in four follow-up visits up to November 2015, skin swabs from the dermis layer (after crusts were removed) of 13 affected and non-treated nursery

**Table 2:** Antimicrobial susceptibility profiles for *Staphylococcus hyicus* isolates from non-treated pigs with clinical signs of exudative epidermitis, before the initiation (n=9) and during the study (n=16)

| Number (%) of resistant *S hyicus* isolates | | |
|---|---|---|
| Antimicrobials tested | Before the study | During the study |
| Ampicillin | 2/9 | 2/16 |
| Cephalexin | 0/9 | 0/16 |
| Cefquinome | 0/9 | 0/16 |
| Ceftiofur | 0/9 | 0/16 |
| Clindamycin | 9/9 | 3/16 |
| Doxycycline | 2/9 | 2/16 |
| Erythromycin | 3/9 | 2/16 |
| Florfenicol | 0/9 | 0/16 |
| Neomycin | 1/9 | 1/16 |
| Penicillin | 2/9 | 2/16 |
| Tetracycline | 2/9 | 3/16 |
| Sulphadiazine-trimethoprim | 2/9 | 1/16 |
| Tylosin | 0/9 | 0/16 |

pigs of one age group were collected and sent for bacteriological culture and AST. Swabs were collected between days 10 and 15 of the nursery period, and culture was performed within hours of sampling using blood agar (5 per cent volume per volume (v/v) sheep blood) and a selective/indicative medium for *S hyicus*.[21 22] AST was performed using Iso-Sensitest agar (Oxoid, UK) according to the Kirby-Bauer disk diffusion method. The antimicrobials (Rosco, Denmark) tested are presented in Table 2. After 18 hours of aerobic incubation at 37°C, inhibition zones were read and interpreted according to the manufacturer's guidelines.[23] According to the bacteriological culture results, *S hyicus* was isolated from 9/13 pigs sampled in total, while *Staphylococcus chromogenes* was isolated from 1/13 pigs. The differentiation between the *S hyicus* and *S chromogenes* isolates was performed as described by Andresen *et al*.[24] The AST results of all *S hyicus* isolates obtained are presented in Table 2. Concerning the single *S chromogenes* isolate obtained, no resistance to any of the antimicrobials tested was observed.

Following the aforementioned bacteriological investigations, the following interventions for the reduction of antimicrobial usage were applied: (1) the prophylactic administration of antimicrobials in pigs housed in both nursery units (as described in Table 1) was ceased; and (2) the metaphylactic treatment of the nursery pigs showing clinical signs of EE was continued, however only with ceftiofur (intramuscular injection; Readycef, Lab Calier). In addition to the above interventions, it was decided to proceed with the production of an autogenous vaccine against *S hyicus*. Five different isolates of *S hyicus* (5/9 obtained during bacteriological culture, from different sampling points) were sent to Denmark (DTU Vet, National Veterinary Institute) for the typing of the toxins produced. There were no obvious phenotypical differences among those isolates. Typing was performed according to Andresen and Ahrens,[3] and no detection of the *exh*A, *exh*C and *exh*D genes occurred in any of the aforementioned isolates. In contrast, all isolates were found to be positive for the *exh*B gene, which encodes the exfoliative toxin ExhB (type B). Three of those isolates were randomly selected and sent to France (Biovac Santé Animale) to produce a formaldehyde-inactivated autogenous vaccine against *S hyicus*. The vaccine was produced using only the whole-cell preparations and not the culture supernatants, and was oil-adjuvanted. Importation of the vaccine to Belgium was 'off-label', under the cascade regimen defined in the European Directive 2001/82/EC as amended by Directive 2004/28/EC and by a prescription signed by the herd veterinarian.

## Experimental design

From four consecutive farrowing batches of sows, two batches were vaccinated (V) against *S hyicus* and two remained non-vaccinated (NV). Vaccination of the batches was applied in an alternating way, so that each non-vaccinated batch was followed by a vaccinated batch. All vaccinations were performed by the first author. In the V batches, all gilts and sows received 5 ml of the autogenous vaccine twice, at five and two weeks before the expected farrowing date. Vaccinations were applied *via* an intramuscular injection on the lateral side of the neck and behind the ear. The different farrowing batches participating in the study were named as follows: batch A (first batch, NV), batch B (second batch, V), batch C (third batch, NV) and batch D (fourth batch, V). There were no obvious clinical adverse effects observed in the vaccinated sows during the period that the study was conducted, such as oedema and/or erythematous skin lesions, reduction of feed intake and vomiting. In addition, neither the numbers of total and liveborn piglets/litter were affected, nor abortions were recorded.

Throughout the gestation and suckling period, the feed (all in the form of commercial pellet), the feeding schedule and feeding level, the management and housing factors, and the remaining vaccinations were the same for all batches of sows that participated in the study. Upon transfer to the nursery units, pigs were partly regrouped according to the farrowing compartment that they originated from and their weight. Table 3 presents the distribution of the pigs between N1 and N2. Both N1 and N2 were managed all-in/all-out by compartment, and during the entire study period water

**Table 3:** Distribution of the weaned pigs that originated from the different farrowing batches (A–D) in each nursery unit

| Farrowing batch | N1 | N2 |
|---|---|---|
| A | Whole unit filled | 2 |
| B | No pigs placed | 4 |
| C | Whole unit filled | 2 |
| D | No pigs placed | 4 |

The total capacity of N1 was 960 pigs, while for N2 it was 3360 pigs. In N2 the numbers represent the number of compartments that were fully filled with pigs from each farrowing batch.

and commercial feed (meal) were supplied *ad libitum* to the pigs. Management factors, sex distribution and metaphylactic treatment were kept the same in both N1 and N2.

## Parameters of comparison

For the pigs of each farrowing batch, the following five parameters were investigated.

### Antimicrobial consumption and morbidity rates

Correctness of metaphylactic antimicrobial dosing against EE in nursery pigs was quantified by the used daily dose pig ($UDD_{pig}$) to animal daily dose pig ($ADD_{pig}$) ratio ($UDD_{pig}/ADD_{pig}$). A ratio of 0.8 to 1.2 is considered to be indicative of correct dosing.[25] The $UDD_{pig}$ is defined as the actual administered dose per day per kg pig of an antimicrobial. The average bodyweights at three and ten weeks of age and the ADG calculated by the weighing of 180 randomly selected pigs per batch (see *Performance parameters*) were used to create a standard growth table for a given age of the pigs of each batch. The $ADD_{pig}$ is the nationally defined average maintenance dose per day per kg pig of an antimicrobial.[18]

The pigs treated against EE within each batch, together with the dates, the quantity of ceftiofur used and the duration of treatment, were recorded. The herd owner and the animal caretakers remained blinded during the study with respect to whether the piglets originated from NV or V sow batches. The number of pigs injected with ceftiofur was also used to calculate morbidity rates. More specifically, pigs were considered to be EE-affected on receiving their first intramuscular injection. The standard procedure followed by the herd owner to detect EE-affected pigs was applied, that is, inspection of all nursery pens twice a day. Pigs that were only lightly affected (minor crusts in the head area, with no generalised exfoliation of the skin) were not treated, and thus they were not considered to be affected. Before the initiation of the study, the herd owner and the animal caretakers were instructed by the first author and the herd veterinarian to treat only animals that presented extensive generalised or localised skin lesions characterised by sebaceous exudation, epidermal erosions and crusts.

### Mortality rates

The number of pigs that died during the nursery period showing clinical signs of EE together with the total number (all possible causes) were recorded. Mortality rates were calculated for the entire batch.

### Bacteriological culture and AST

Throughout the nursery period, a subset of pigs that died was necropsied in order to assess the possible cause of death. Skin swabs were collected from necropsied pigs as well as non-treated live pigs with clinical signs of EE and submitted for bacteriological culture and AST. Both were performed using the same method as before the initiation of the study (as described in the Herd and outbreaks description section). A subset of the *S hyicus* isolates obtained from those pigs were typed in order to determine whether any of the exfoliative toxin-encoding genes were present (as described in the Herd and outbreaks description section).

### Performance parameters

From each batch, 180 sows were randomly selected. The parity distribution of the selected sows from each farrowing batch (A–D) is presented in online supplementary appendix 1. On the day of weaning, 180 pigs were randomly selected from each sow batch (one pig per selected litter). All randomisations were performed by the random procedure of the Excel program (Excel 2007, Microsoft Office, Washington, USA). All selected pigs were ear-tagged, so that they could be identified at the nursery units. This method of randomisation was performed to avoid differences in the number of pigs that originated from the different sow batches, and to ensure that the average parity distribution of the selected sows in each farrowing batch will closely resemble the parity distribution of the whole batch. Within each nursery unit, between four and six of the ear-tagged pigs were allocated per nursery pen in order to ensure that all nursery pens were filled with an equal number of ear-tagged pigs.

In order to obtain the ADG (g/pig/day), ear-tagged pigs were individually weighed at weaning (three weeks of age) and at the end of the nursery period (10 weeks of age). The ADG was computed as the difference between starting and final bodyweights (weight gain) divided by the number of days during the period. All pigs that died during the nursery period were weighed and their age was recorded. In this way, the individual ADG values of the dead pigs were considered to calculate the ADG of each batch.

## Statistical analysis

With regard to the nursery mortality rates, the inclusion of a minimum of 1800 pigs per farrowing batch allowed to detect with 95 per cent certainty and 80 per cent statistical power a difference of 1.7 per cent points between pigs of the NV and V sows batches. The number of ear-tagged pigs in each farrowing batch (180) allowed to assess a difference of 19 g (sd=65) in ADG with 95 per cent certainty and 80 per cent statistical power (IBM SPSS Sample Power V.3, Illinois, USA). The pig was considered as statistical unit. Morbidity and mortality constituted the primary outcome parameters, whereas weight gain and ADG constituted secondary parameters. Due to the allocation of the pigs originating from the NV sow farrowing batches in both N1 and N2, all the statistical analyses of the primary and secondary outcome parameters were first carried out by comparing batches A and C (separately and combined) between N1

| Table 5: Per cent (number) of dead pigs with clinical signs of exudative epidermitis together with the total number of pigs that died from all causes | | | | | | |
|---|---|---|---|---|---|---|
| Whole batch level/pigs with EE signs | | | | Whole batch level/overall mortality | | |
| Nursery unit | NV | V | P value | NV | V | P* value |
| Both units | 5.02 (183/3643)$^{A}$ | 2.59 (105/4061)$^{B}$ | 0.000 | 7.05 (257/3643)$^{A}$ | 3.94 (160/4061)$^{B}$ | 0.000 |
| N1 | 2.47 (90/3643) | n/a | n/a | 3.29 (120/3643) | n/a | n/a |
| N2 | 2.55 (93/3643) | 2.59 (105/4061) | 0.928 | 3.76 (137/3643) | 3.94 (160/4061) | 0.730 |

The contribution of each nursery unit (N1 and N2) on the combined (both units) mortality rates between three and ten weeks of age is presented separately. All mortality rates were calculated according to the total number of animals included in the non-vaccinated (NV; batches A and C) and vaccinated (V; batches B and D) farrowing batches upon transfer to the nursery units.
Vaccination status: NV (pigs originating from non-vaccinated sow farrowing batches) and V (pigs originating from vaccinated sow farrowing batches). Values with different superscripts within a row are significantly different ($P<0.05$ and $P^*<0.05$). The P value refers to the comparisons between the summaries of the NV (A–C) and V (B–D) batches concerning the pigs that died showing signs of EE and the P* value to the comparisons between the summaries of the NV (A–C) and V (B–D) batches concerning the pigs that died from all causes.
n/a, non-applicable.

and N2. In case there were no statistically significant differences between N1 and N2, the nursery unit was not included as a factor in the final models (see online supplementary appendix 2). Morbidity and mortality rates were analysed using binary logistic regression with vaccination status and farrowing batch as predictors for the model. General linear mixed models were used to analyse bodyweights, weight gain and ADG with vaccination status (NV or V), and farrowing batch included as fixed factors and pen as a random variable.[26] Bodyweight at weaning was used as a covariate in the analysis. Statistical results were considered significant when P values were ≤0.05 (two-sided test). The statistical package SPSS V.23.0 was used to analyse the data.

## Results

### Antimicrobial consumption and morbidity rates

The $UDD_{pig}/ADD_{pig}$ ratios for the NV and V farrowing batches were 1.79 and 1.31, respectively. When taking into account each of the farrowing batches A, B, C and D individually, the $UDD_{pig}/ADD_{pig}$ ratios were 1.79, 1.60, 1.78 and 0.99, respectively. The total amount of ceftiofur used to treat the EE-affected pigs among the NV and V farrowing batches was 88,550 and 39,600 mg, respectively. For each of the farrowing batches A, B, C and D, this amount was 45,250, 22,480, 43,300 and 17,120 mg, respectively.

In N2, comparisons between the NV and V batches revealed that there were no significant differences regarding their contribution to the morbidity rates (P=0.139; Table 4). When the morbidity rates across N1 and N2 were compared, the V batches reached significantly lower morbidity rates compared with the NV batches (P=0.008; Table 4). Comparisons between individual batches (data not shown) revealed that each of batches B and D had significantly less EE-affected pigs compared with batches A and C (6.25 and 6.74 per cent *versus* 12.81 and 15.92 per cent, respectively; overall P=0.000).

| Table 4: Per cent (number) of pigs with clinical signs of exudative epidermitis that received metaphylactic antimicrobial treatment (also representing morbidity rates) | | | |
|---|---|---|---|
| Nursery unit | NV | V | P value |
| Both units | 14.36 (523/3643)$^{A}$ | 6.50 (264/4061)$^{B}$ | 0.008 |
| N1 | 7.00 (255/3643) | n/a | n/a |
| N2 | 7.36 (268/3643) | 6.50 (264/4061) | 0.139 |

Rates were calculated according to the total number of animals included in the non-vaccinated (batches A and C) and vaccinated (batches B and D) farrowing batches upon transfer to the nursery units.
Vaccination status: NV (pigs originating from non-vaccinated sow farrowing batches) and V (pigs originating from vaccinated sow farrowing batches). Values with different superscripts within a row are significantly different ($P<0.05$).
n/a, non-applicable.

### Mortality rates

The percentages and numbers of dead pigs according to their vaccination status are presented in Table 5. In N2 there were no significant differences between the NV and the V farrowing batches in their contribution to the number of dead pigs with clinical signs of EE, neither to the total number of dead pigs (P=0.928 and P=0.730, respectively). When mortality data from both N1 and N2 were combined, the V batches exhibited significantly less pigs dying with clinical signs of EE and also total mortality than the NV batches (both P=0.000). Comparisons between individual batches (data not shown) revealed that each of batches B and D had significantly less dead pigs with clinical signs of EE compared with batches A and C (2.50 and 2.67 per cent *versus* 6.00 and 4.04 per cent, respectively; overall P=0.007). The same trend was observed for the total mortality rates, where each of batches B and D had significantly lower rates than batches A and C (3.41 and 4.46 per cent *versus* 7.25 and 6.88 per cent, respectively; overall P=0.000).

### Bacteriological culture and AST

Eight out of 257 dead pigs from the NV batches (4/133 from batch A and 4/124 from batch C) and 8/160 dead pigs from the V batches (5/68 from batch B and 3/92 from batch D) were necropsied. Pathological findings showed pneumonia in the apical lung lobes (1/16), rhinitis (3/16), fibrinopurulent pericarditis and endocarditis (2/16), enteritis (4/16) and EE (7/16). The following bacteria were isolated from various organ sites: *Pasteurella multocida* from the lungs and the nasal conchae (3/16), *Trueperella pyogenes* from the lungs (1/16), *Streptococcus suis* from the heart (2/16), *Escherichia coli* from the spleen and the jejunum (4/16), and *S hyicus* and *S chromogenes* from the skin (7/16 and 2/16, respectively).

**Table 6** Performance parameters from three to ten weeks of age for the selected pigs originating from the non-vaccinated (NV; batches A and C) and vaccinated (V; batches B and D) farrowing batches (average±sd)

| Performance parameters† | Age (weeks) | A | B | C | D | P value | A–C (NV) | B–D (V) | P* value |
|---|---|---|---|---|---|---|---|---|---|
| | | n=180 | n=180 | n=180 | n=180 | | n=360 | n=360 | |
| Average bodyweight (kg) | 3 | 5.03±1.12 | 5.46±1.09 | 5.12±1.08 | 5.50±1.16 | 0.673 | 5.09±1.10$^{A}$ | 5.48±1.13$^{B}$ | 0.000 |
| | 10 | 23.91±4.24 | 25.81±4.42 | 24.18±4.45 | 24.71±4.84 | 0.051 | 24.07±4.36 | 25.15±4.70 | 0.643 |
| Weight gain (kg) | 3–10 | 18.89±3.79 | 20.32±3.84 | 19.06±3.98 | 19.21±4.25 | 0.055 | 18.99±3.90 | 19.66±4.12 | 0.628 |
| ADG (g/pig/day) | 3–10 | 356±72 | 376±71 | 353±74 | 356±79 | 0.321 | 354±73 | 364±76 | 0.131 |

Data from batches A and C were summarised together, taking into account both nursery units (N1 and N2). Data from batches B and D were also summarised together (for N2).
Vaccination status: NV (pigs originating from non-vaccinated sow farrowing batches) and V (pigs originating from vaccinated sow farrowing batches). Values with different superscripts within a row are significantly different ($P<0.05$ and $P^*<0.05$). The P value refers to the comparisons between the individual farrowing batches and the P* value to the comparisons between the summaries of the NV (A–C) and V (B–D) batches.
†Average daily gain (ADG).

In addition to the necropsies, skin swabs were collected from 15 live pigs (eight from the NV batches and seven from the V batches). Then, *S hyicus* together with *S chromogenes* were isolated from 9/14 and 2/14 of those swabs, respectively. Table 2 presents the AST results performed on all *S hyicus* isolates from necropsied and live pigs (n=16). When compared with the isolates before the initiation of the study, the isolates obtained during the study were resistant to the same antimicrobials; nevertheless, the percentages of resistant isolates declined. Typing was performed in 8/16 of those isolates. The results showed that 2/8 (from batches C and D), 1/8 (from batch C) and 4/8 (from batches B and C) isolates were positive for the exfoliative toxin-encoding genes *exh*A, *exh*B and *exh*C, respectively. In 1/8 (from batch A) isolates, no toxin-encoding genes were detected.

### Performance parameters

Table 6 summarises all performance parameters, taking into account both nursery units. Over the entire nursery period, there were no significant differences between the pigs from the NV sow batches and the pigs of the V batches in terms of their weight gain and ADG (P=0.628 and P=0.131, respectively).

## Discussion

The present study investigated whether autogenous vaccination of sows against *S hyicus* at the end of gestation was efficacious to control EE outbreaks in their offspring during the nursery period. Pigs originating from the V batches of sows had significantly lower morbidity and mortality rates than those from the NV batches. Apart from having lower percentages of pigs treated with antimicrobials against EE, when taking into account the $UDD_{pig}/ADD_{pig}$ ratios, batches B and D exhibited reduced overdosing with ceftiofur compared with batches A and C. Given that after the initiation of the study all prophylactic antimicrobial treatments were ceased, those results suggest that metaphylactic antimicrobial treatments are not sufficient to resolve such severe EE outbreaks without any additional control measures. Current results suggest that an autogenous vaccination of the sows can help in decreasing morbidity, mortality and antimicrobial use during EE outbreaks.

Nevertheless, the autogenous vaccination of the sows was not able to fully protect against EE in the pigs of the V sow batches, neither to fully prevent mortality in pigs with clinical signs of EE. The average mortality rate of 2.59 per cent in the V batches, attributed to pigs with clinical signs of EE, is considered to be higher than published average nursery mortality rates from herds with no apparent disease outbreaks (ranging between 0.54 and 2.1 per cent).[27 28] Possible explanations may include the insufficient duration of maternal immunity, the presence of different *S hyicus* strains producing different types of exfoliative toxins and the cocirculation of *S chromogenes*.

In the present study, the first clinical signs of EE occurred approximately one week after weaning and the severity of the disease increased towards the third week of the nursery period. The rationale of vaccinating the gestating sows twice in the V batches was that maternal antibody transfer to their piglets would be further enhanced so that protection against the EE outbreaks is extended towards the first three weeks of the nursery period. This approach was based on the fact that, in general, the half-life of maternal antibodies induced by natural infection in pigs is considered to be between 11.3 and 20 days.[29] However, the half-life of maternal anti-*S hyicus* antibodies has not yet been elucidated, and thus it is possible that in the present herd passively acquired immunity against *S hyicus* had already waned towards the third week of the nursery period. To further investigate the optimal vaccination scheme in field cases with similar EE manifestation and severity, it would be interesting to compare autogenous vaccination efficacy in farrowing batches where only sow vaccination is applied *versus* farrowing batches where only suckling pigs are vaccinated.

During the present study, the different *S hyicus* isolates obtained were found to be positive for three different exfoliative toxin-encoding genes (*exh*A, *exh*B and *exh*C). Since the vaccine needed approximately two months to be produced and delivered to the herd, it was not feasible to utilise isolates from the farrowing batches included in the study for its production. Instead, isolates obtained before the initiation of the study were used. Given that all isolates sent for typing before the initiation of the study were *exh*B-positive

(5/5), it was decided to use three of those isolates for the production of the autogenous vaccine. The fact that *exh*A-positive and *exh*C-positive isolates appeared to circulate in batches B, C and D of the study shows the difficulty of keeping autogenous vaccines updated according to the different *S hyicus* strains circulating in a herd during different time periods. In case there was a possibility to continue following this herd over a longer period of time, then the aforementioned *exh*A-positive and *exh*C-positive isolates could be used to produce a more holistic vaccine in terms of the toxigenic isolates included. In this way, the protective efficacy of autogenous vaccination could be further enhanced.

At this point it should be mentioned that apart from *S hyicus*, *S chromogenes* was also isolated from different farrowing batches both before and during the study. So far, there is no published clinical study mentioning the use of an autogenous vaccine against *S chromogenes* in cases of EE. Andresen *et al*[24] showed that pigs inoculated with an *exh*B-positive *S chromogenes* strain developed clinical signs of EE. The currently available literature describes *S hyicus* as the primary pathogen provoking severe EE outbreaks and *S chromogenes* as a secondary pathogen.[30 31] The above-mentioned factors, together with the fact that most of the swabs taken from live pigs before the initiation of the study yielded *S hyicus* (9/13) and not *S chromogenes* (1/13), supported the decision to create an autogenous vaccine by solely using the *S hyicus* isolates obtained. Nevertheless, it is not known to what extent the cocirculation of *S chromogenes* isolates exacerbated the severity of the EE outbreaks. In future studies conducted in herds facing EE outbreaks where both *S hyicus* and *S chromogenes* are isolated, it would be useful to investigate the efficacy of autogenous vaccines combining isolates from both species.

In the current study, the vaccine was produced using only the bacterial cell preparations and not the culture supernatants. According to Wegener and Skov-Jensen[32] exfoliative toxins can be purified from the culture supernatant and serve as a single protective antigen. It is possible that if the culture supernatant is additionally utilised in the production of autogenous vaccines against *S hyicus*, then improved vaccination results can be obtained. However, such choice should be considered with caution as a vaccine with a high toxoid content could lead to severe allergic reactions.[33] Such side effects can be particularly devastating when gestating sows are vaccinated and have already been described by other authors.[34] In the current study, there were no obvious clinical adverse effects in the vaccinated sows, as observed by the investigators and reported by the herd owner and the herd veterinarian. The use of veterinary vaccines under the cascade regimen is a risk-based decision; nevertheless, the current European legislation does not oblige to formally measure possible vaccination-related adverse effects. Although every new commercial vaccine needs to be tested for efficacy and safety, there is not such requirement for the autogenous vaccines.[35] For that reason, literature suggests that new batches of autogenous vaccines should be first tried in a limited number of animals before being used on a larger scale.[35 36]

The reason behind the dose imprecision differences in the metaphylactic ceftiofur treatment between the pigs of the NV and V sow batches might be attributed to the behaviour of the herd owner towards batches with higher morbidity and mortality rates. Similar behaviours have already been observed in other studies due to the perception that overdosing of antimicrobials in batches with high morbidity and mortality rates would achieve better results in a shorter period of time.[18] Under the new legislations applied in countries such as Denmark, where the amount of antimicrobials prescribed is monitored in order to calculate herd $ADD_{pig}$ for their 'yellow card' system,[16] the further optimisation of the efficacy of vaccines against EE under field conditions is of undoubted importance.

To conclude, significantly reduced morbidity and mortality rates were observed in the pigs of the V sow batches compared with those from the NV batches. Additionally, lower quantities of metaphylactic antimicrobial treatment were used in the pigs of the V batches than in the NV batches, implying that autogenous vaccination could serve as a non-antimicrobial way to control severe EE outbreaks. Nevertheless, additional studies are necessary to investigate whether the efficacy of autogenous vaccines to achieve further reductions in morbidity and mortality rates can be enhanced. During the optimisation of autogenous vaccines, it should be taken into account that vaccine performance depends also on many other predisposing factors such as management practices, hygiene and housing conditions. The inherent disadvantages of the autogenous vaccines such as the long time period needed for their production, the need to be kept updated according to the different strains that can circulate during different time periods and the risk of side effects occurring in the vaccinated population should also be considered.

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