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Effect of the vaccination against Shiga toxin 2e in a farm with history of oedema disease, caused by atypical *Escherichia coli* producing Shiga toxin (STEC)

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Abstract: Oedema disease of weaned piglets is caused by shigatoxigenic *Escherichia coli* (STEC), typically harbouring the *stx2e* gene and F18 adhesins. The aim of this study was to assess the effect of a commercially available oedema disease vaccine on the zootechnical performance, mortality and individual antibiotic treatment in a herd, in which non-typical STEC strains without F18 adhesin have been identified. The zootechnical performance (average daily gain, total weight gain), mortality and individual antibiotic treatment were compared between vaccinated and non-vaccinated control piglets in a monocentric field efficacy study, which was performed using two groups in a parallel, randomised design. A significantly higher average daily gain and total weight gain were recorded in the vaccinated piglets in comparison to the controls. The lower morbidity, mortality and antibiotic treatment in piglets in the vaccine group were not statistically significant. As a conclusion, the positive effect of the vaccination was confirmed in the herd with prevalent STEC not harbouring F18 adhesin. The vaccine was, therefore, also effective against oedema disease caused by such unusual STEC isolates, under the conditions of this study.

Keywords: adhesins; pig; STEC; toxoid; whole-genome sequencing (WGS)

Oedema disease (OD) is one of the major diseases in pigs during nursing causing significant losses in affected farms (Fricke et al. 2015). OD is caused by *Escherichia coli* producing Shiga toxin (STEC),

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specifically Shiga toxin 2 subtype e (Stx2e) (Linggood and Thompson 1987). These strains are mostly assigned to the serogroups O138, O139, O141 and O147 and express fimbrial adhesins F18ab (Wittig et al. 1995). However, isolation of STEC strains belonging to different serogroups or without the F18 adhesin has been described (Hornich et al. 1973; Alexa et al. 2002).

OD usually appears in pigs shortly after weaning. OD develops as a toxaemia with clinical manifestation including swelling of the eyelids and other predilection sites, staggering gait followed by paralysis and death. In addition to the well described effect on the reduction in the mortality and clinical signs, OD negatively affects the productive parameters in piglets with subclinical infection (Creach et al. 2015). A correct OD diagnosis is important in order to prevent disease on farms.

Vaccination with products based on the Stx2e toxoid is the most successful protocol preventing clinical OD followed by a decrease in mortality. Vaccines, with market authorisation in the European Union (EU) and several other countries, claim that the active immunisation of piglets in the sensitive post-weaning period reduces the mortality and clinical signs caused by OD. This significant effect of vaccination was described in registration studies as well in field trials (https://www.ema.europa.eu/en/medicines/veterinary/EPAR/ecoporc-shiga; Fricke et al. 2015). Vaccination results in a decreased demand for antibiotic therapy and/or zinc oxide treatments at farms with enzootic OD. The use of colistin sulfate, which is now considered an antibiotic with limited use, is significantly decreased in pig production due to vaccination against OD (Lillie-Jaschniski et al. 2013). However, though there are numerous reports on the efficacy of vaccination in preventing mortality due to the OD caused by prototype porcine STEC expressing both Stx2e and F18 adhesin, less is known about the efficacy of the vaccine against atypical STEC not expressing F18 adhesin and its effect on the zootechnical performance. In this study, we used the opportunity where we had earlier identified a farm with sporadic OD caused by this atypical STEC strain. The aim of this study, therefore, was to test the efficacy of a commercially available vaccine against the OD disease at a farm with a confirmed OD history caused by shigatoxigenic *E. coli* carrying the *stx2e* gene, but without fimbrial adhesins F18.

MATERIAL AND METHODS

Study population

The study was conducted between May 2020 and July 2020 on a commercial farrow-to-finish herd farm housing 1 100 sows in the Czech Republic. The farm was selected based on the known OD history. The post-weaning mortality rate was 2% on average, increasing up to 7% during irregular outbreaks of OD. During routine diagnostic screening and outbreaks of clinical OD at the farm within one year before the vaccination trial was confirmed, 16 *E. coli* strains were characterised by polymerase chain reaction (PCR) as *stx2e*+/F18 negative. No additional adhesins were detected. The presence of OD in this farm before the vaccination trial was, therefore, confirmed.

The sows were previously vaccinated against parvovirus, swine erysipelas, swine influenza, colibacillosis and *Clostridium perfringens* type A. Metaphylactic medication with amoxicillin (Vetrimoxin 50 plv.; Ceva Sante Animale, Libourne, France) in the drinking water was implemented for the control of *Streptococcus suis* infections. The presence of STEC-2e was confirmed in a batch preceding the start of the vaccine trial based on typical lesions at predilection sites, on isolation of *E. coli* followed by PCR confirmation of the *stx2e* gene and F4 (K88), F5 (K99), F6 (P987), F18 and F41 adhesins. The presence of specific antibodies was confirmed by a serum neutralisation test (SNT) at the end of the nursery period (Leneveu et al. 2017).

The ethical review and approval were waived for this study, due to using a commercially available approved vaccine and treatment. The owner was signed the consent (No. ES/003/2019) with principal investigator before the start of the study.

The space and feeding requirements were in line with regulatory requirements. Water was supplied via drinking nipples *ad libitum*.

Sample size estimation

OD negatively affects the productive parameters and sub-clinical infections have been described. The assumption was made that vaccination against OD should reduce the negative impact of the infection on the productive parameters, thus the average daily gain (ADG) was selected as the primary criteria.

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The sample size was calculated to detect a statistically significant difference in the ADG in the vaccinated piglets, with the previously described expected difference of 20 g between the vaccinated and non-vaccinated (control) piglets (SD = 40 g) (Creach et al. 2015), with a power of 80% and a 5% level of significance. A total of 63 piglets per group was required with the addition of a margin of 10%, thus, the sample size was targeted at 70 piglets per group.

Study design

A monocentric field efficacy study was performed using two groups in a parallel, randomised design, comparing the vaccinated group and negative control (non-vaccinated). The experimental unit was the piglet, but the treatment was allocated per litter to be in accordance with the field conditions. Moreover, the marketed preventive vaccination against OD is always administered to the entire litter in the field.

In total, 137 piglets from 13 randomly selected litters were randomly allocated to vaccinated (6 litters) and control (7 litters) groups at 3 days of age (DOA). The randomisation was stratified per batch of farrowings in a descending order of the day of farrowing and parity in a randomised block design. Litters were from different parity sows, farrowing at the same day (within 24 h), with an average parity of 4.2 (vaccine group) and 5.4 (control) and were balanced by removing the smallest piglets (minimum weight of 800 g) in order to have a max. 12–13 piglets per litter. All the piglets were individually weighed and examined by veterinarian to ensure their good health. Finally, 69 piglets were vaccinated intramuscularly at 4 DOA (ECOPORC SHIGA®; Ceva Sante Animale, Libourne, France) and 78 piglets served as the non-vaccinated control.

The litters were numbered with the sow ID used at the farm and each piglet was individually identified by a numbered ear tag in the left ear. Data from the piglets and sows were collected in paper form and later entered in an Excel datasheet.

After weaning (21 DOA), the piglets were transferred to the nursery, and the litters were placed in one compartment, according to the standard practice applied on the farm. Piglets were received commercial dry post-weaning diet, with 16.89% of crude protein, fibre content 4.64% and fat content 4.85%.

The body weight was measured for each piglet at 3 DOA, 21 DOA and 69 DOA and used to calculate the body weight gain and average daily gain (ADG). The mortality and individual antibiotic treatment were recorded for 7 weeks post-weaning, i.e., from 21 DOA to 69 DOA.

Any clinical signs of OD, such as nervous disorders like ataxia, paralysis of limbs, tonic-clonic convulsions, oedema of predilection sites such as the eyelids and subcutis of the face, were recorded by trained personnel on a daily basis throughout the whole experiment.

Detection and characterisation of STEC

The piglets from the non-vaccinated group were individually sampled at least once during the nursery period (50 and 68 DOA) in order to evaluate the effect of the *stx2e* gene presence/absence on the productive parameters, to confirm the negative effect of the toxin production and subclinical OD in positive animals during the nursery period. The non-vaccinated animals were selected due to the assumption that the negative effect of the Stx2e toxin would not be neutralised by the vaccination, contrary to the vaccinated animals, where such an effect is expected.

One gram of faeces was mixed with 10 ml of buffered peptone water (Oxoid, Hampshire, UK), homogenised and used for direct plating onto blood agar (Labmediaservis, Jaromer, Czech Republic). After incubation at 37 °C for 18–24 h, DNA from the resulting bacterial culture was prepared and used for PCR screening for the presence of the *stx2e* gene. In *stx2e* positive samples, the isolation of colonies carrying this gene was performed. A MALDI-TOF MS Biotyper v3.1 (Bruker Daltonics GmbH, Bremen, Germany) was used to identify the selected colonies. In the confirmed *E. coli* strains, the presence of adhesins F4, F5, F6, F18, F41 was tested by PCR and the serogroup was determined using the agglutination method (Salajka et al. 1992; Zhang et al. 2007).

Vero cell assay

The Vero cell assay was carried out as previously published (Gentry and Dalrymple 1980). Briefly,

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three colonies of *E. coli* strains were inoculated into 10 ml of a Brain Heart Infusion Broth (Oxoid, Basingstoke, UK) and incubated for 18 h at 37 °C with constant shaking. The cell-free STEC supernatants were used for the Vero cell assay *in vitro*. Therefore, the *E. coli* suspension was centrifuged at 12 000 *g*, for 30 min at 4 °C and filtrated using 0.22 µm (Gelman Sciences, Ann Arbor, USA) syringe filters. The filtrates were diluted in Dulbecco's Modified Eagle Medium (DMEM) (Biosera, Nuaille, France) and added in triplicate to the Vero cells for 72 hours. The Vero cell line was seeded on 96-well plates for 24 h before the treatment in a density of 10 000 cells/cm^2. The filtrate was diluted in a range of 1 : 100–1 : 64 000. Finally, a CyQUANT Cell Proliferation Assay (Thermo Fisher Scientific Inc., Waltham, USA) was used to quantify the cell proliferation. The effects on the Vero cells were correlated with the controls without the Shiga toxin treatment. The effective dilution of the Shiga toxin was designated as the Shiga toxin dilution that reduced 50% of the Vero cell proliferation. The experiment was performed in three independent repetitions and in triplicate.

Whole-genome sequencing (WGS)

The genomic DNA was extracted using a Blood and Tissue Kit (Qiagen, Hilden, Germany). The preparation of DNA libraries and sequencing on the Illumina platform (Nextseq 2 × 150 bp) were carried out by LGC Genomics GmbH (Berlin, Germany).

The obtained sequence data were assembled using SPAdes v3.14.1 (Nurk et al. 2013). Identification of the O- and H-antigen was performed using the SerotypeFinder tool from the Centre for Genomic Epidemiology (CGE) website (Joensen et al. 2015). Virulence genes were identified by VirulenceFinder (https://cge.cbs.dtu.dk/services/VirulenceFinder/). The presence of the potential adherence genes *eibG*, *lpfA*, *paa*, *bfp*, *sab*, *espP*, *orfA*, *orfB*, *efa1*, *fasA*, *saa*, *toxB*, *ehaA* was screened in BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Accession Nos. GU295813, AB161111, U82533, KJ641932, NZ_LHCM01000100, KY657280, EF601572, EF601573, AF453441, U50547, NZ_LHCZ01000131, JQ906697, NZ_NLZN01000004) (Baranzoni et al. 2016). The FimH type was identified using FimTyper v1.0 (Roer et al. 2017).

Statistical analyses

The primary criterion, ADG from 3 DOA to 69 DOA was analysed using an analysis of variance (ANOVA) model (MIXED procedure of the SAS v9.4 software program; SAS Institute, Cary, NC, USA). The model included the piglet weight at 3 DOA as the covariate, the group (vaccinated, non-vaccinated) as the fixed effect for the investigation and the sow within the group as a random factor. Additionally, the body weight evolution was analysed using a mixed-effects model (MIXED procedure) with the body weight at 3 DOA as the covariate and the level of the parameter as a dependent variable accounting for the fixed effects of the group (vaccinated, non-vaccinated), DOA (21 days, 69 days) and their different interactions. The DOA (21 days, 69 days) was used as a repeated measure, using an unstructured covariance matrix and the piglet (litter) as the subject. The random part of the model included the litter within the group. In both models, parity as a quantitative variable that was initially included was removed as not being significant.

Parity was entered in both models as a quantitative variable and was kept, if significant. The data from 3 DOA (baseline) were tested for differences between the two groups of animals using Student's *t*-test as well the difference in the body weight gain at 69 DOA between the non-vaccinated piglets that were positive or negative for STEC. The mortality during the nursery period was analysed by comparing the survival curves with the Mantel-Cox test considering all the animals still present at 21 DOA (i.e., 58 and 77 piglets in the vaccinated and non-vaccinated group, respectively) in GraphPad Software v8.4.3, 2020 (San Diego, CA, USA). A comparison of the percentage of the individual antibiotic therapy in the same period was performed between the treatment groups using Fisher's exact test. The differences were considered statistically significant when $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

RESULTS

Due to some early mortality possibly related to the OD ($n = 9$), the loss of ear tags ($n = 17$) and the premature removal due to the low milking ability of the sow ($n = 8$), a total of 113 piglets were kept for the final statistical analysis of the production parameters [see

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Table 1. Results (LSMEANS) of the average daily gain (ADG) and body weight (b.w.) by group (vaccinated and non-vaccinated)

Item	Group		SEM	*P*-value
	vaccinated	non-vaccinated		
Average daily gain from 3 DOA to 69 DOA (g/d)	339	312	12	0.040
Body weight (kg)[a]				
$BW_{21\ DOA}$	6.251	6.076	0.184	0.340
$BWG_{69\ DOA}$	24.212	22.487	0.775	0.028

[a]The body weight at 3 DOA is not included here because it was included as a covariate in the model

Flow Chart, Figure S1 in electronic supplementary material (ESM)] with 51 and 62 piglets in the vaccinated and non-vaccinated groups, respectively.

Production parameters

The piglets did not differ at inclusion into the study with an average weight of 1.85 ± 0.36 kg and 1.91 ± 0.32 kg in the vaccinated and non-vaccinated piglets (P = 0.350), respectively. The ANOVA model on the ADG from 3 DOA to 69 DOA showed a significant effect of the group (P = 0.040) with the least squares means ± standard error of the mean (LSMEANS ± SEM) for the ADG of 339 ± 9 g and 312 ± 8 g for the vaccinated and non-vaccinated piglets, respectively (Table 1), thus, an estimated difference of 27 g in favour of the vaccinated piglets.

This result is confirmed by the repeated measures model on the body weight evolution with a significant time and group effect. The overall body weight was significantly higher in the vaccinated piglets (group effect: P = 0.026). The DOA effect was also significant as expected (P < 0.001) and its interaction with the group was also significant (P = 0.040) with a higher body weight in the vaccinated piglets at 69 DOA (P = 0.028), while no difference was observed between the groups at 21 DOA (P = 0.340). Indeed, at the end of the study at 69 DOA, the final body weight was 1.72 ± 0.75 kg (LSMEANS ± SEM) higher in the vaccinated piglets than in the control piglets.

Productive parameters in the control pigs with subclinical infection

To further confirm the influence of the subclinical infection on the productivity, the non-vaccinated control group pigs were classified as positive or negative for STEC, and the body weight was compared between these pigs. The PCR screening of *stx2e* gene revealed 12 positive and 49 negative pigs in total, on days 50 DOA and 68 DOA. The weight gain between 21 and 69 DOA was 16.76 ± 3.65 kg in the negative pigs and 14.68 ± 3.73 kg in the pigs positive for the *stx2e* gene. The numerical difference in the weight gain between the groups was 2.07 kg in favour of the negative piglets, which was just short of showing significance at P = 0.084 (Figure 1).

Individual antibiotic therapy

An antibiotic treatment was required for one piglet from the vaccinated group (1/58, 1.7%). This piglet was treated with marbofloxacin to control symptoms not related to OD. Despite this, the piglet died the next day after the treatment at 42 DOA. Out of the non-vaccinated group, six piglets (6/77, 7.8%) were treated by marbofloxacin. Four of them exhibited clinical signs of OD (tonic-clonic convulsions, inability to move, lateral position, no fever) and two of them died despite the therapy. However, the difference was not statistically significant between both groups (P = 0.122).

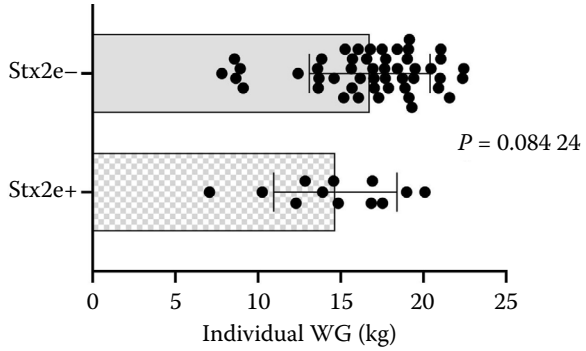

Figure 1. Individual weight gain differences between the infected (Stx2e+) and negative (Stx2e–) piglets
WG = weight gain

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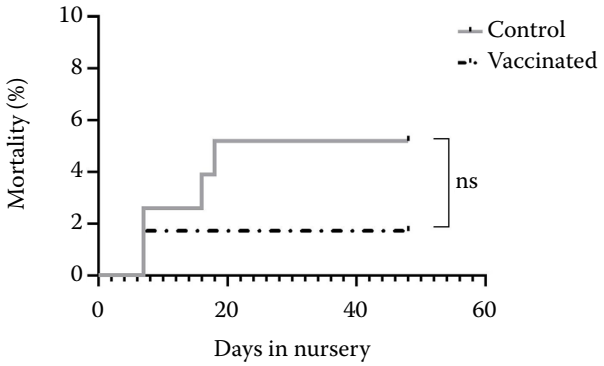

Figure 2. Development of mortality (%) in the 21–69 DOA period
ns = not significant

Mortality

The mortality during the nursery period was only numerically lower in the vaccinated pigs (1/58, 1.7%) compared to the controls (4/77, 5.2%) (P = 0.391, Figure 2). In two piglets from the control group, which died at 16 and 18 days in nursery (37 and 39 DOA), OD was determined as the presumptive cause of death by necropsy. The causes of deaths in all the remaining pigs were not associated with OD.

Characterisation of the isolated STEC

Out of 12 PCR positive samples, four *E. coli* strains harbouring the *stx2e* gene, were isolated. None of these strains carried genes for the adhesins F18 or F4, F5, F6 and F41. In two strains, the serogroup O9 was determined using the agglutination method, while in two strains, the serogroup was not specified.

The Vero cell assay results showed that three STEC strains produced the Shiga toxin at different levels. One strain had no cytotoxic effect on the Vero cells *in vitro* and, therefore, was excluded from further testing. One strain (No. 13856) produced

Table 2. Detailed characteristics of the detected *Escherichia coli* strains

13856	13857		lab. No.	
O9:H4	O100:H30		serotype	
fimH1339	fimH604		fim type	
100	8 000		effective dilution of Shiga toxin	
		gene presence	protein	function
■	■	*sta1*	heat-stabile enterotoxin ST-Ia	toxin
■		*stb*	heat-stabile enterotoxin II	
■	■	*stx2Ae*	Shiga toxin 2, subunit A, variant e	
■	■	*stx2Be*	Shiga toxin 2, subunit B, variant e	
■	■	*iha*	adherence protein	adherence
■		*papC*	outer membrane usher P fimbriae	
■		*paa*	porcine attaching and effacing associated	
■	■	*sepA*	Shigella extracellular protein A	invasin
	■	*cba*	colicin B	protectins
	■	*cea*	colicin E1	
■	■	*celb*	endonuclease colicin E2	
	■	*cma*	colicin M	
	■	*iss*	increased serum survival	
■	■	*traT*	outer membrane protein complement resistance	
■		*iutA*	ferric aerobactin receptor	iron acquisition system
■		*iucC*	aerobactin synthetase	
■		*etpD*	type II secretion protein	miscellaneous
■	■	*gad*	glutamate decarboxylase	
■	■	*terC*	tellurium ion resistance protein	

Grey colour = gene detected

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the Shiga toxin with little effect on the cell proliferation and an effective toxin dilution of 1 : 100 was determined. Strain No. 13857 was effective at a dilution of the Shiga toxin of 1 : 8 000.

Two strains with a cytotoxic effect on the Vero cells were selected for whole-genome sequencing (WGS) analysis. Using the VirulenceFinder tool, the presence of the *stx2e* gene was confirmed in both strains and additional virulence factors were detected (Table 2). No genes specific for F4 or F18 adhesins were found by the VirulenceFinder tool and their presence was not confirmed by BLAST (Accession No. Z26520). Using VirulenceFinder, the *iha* gene was confirmed in both strains. Screening of various gene encoding factors involved in adhesion and colonisation to the host revealed only the presence of the porcine attaching-effacing associated protein (paa) gene with 96.88% identity in 76% coverage (Accession No. U82533) in isolate 13856. The presence of *fimH* genes was confirmed and their type was determined (Table 2).

DISCUSSION

In this study, the effect of the vaccination against OD was assessed during the nursery period, in pigs 21–69 days of age. This period was selected since it represents the part of life with common OD manifestations (Imberechts et al. 1994; Fricke et al. 2015). A possible limitation of our study was the rather small size of the groups of animals which was influenced by ethical reasons, i.e., the history of high mortality during previous OD outbreaks on the farm.

We observed a positive vaccination effect on the productive parameters associated with the body weight, but only numerically, not a statistically significant difference in the decreased mortality and antibiotic treatment. A reduction in the overall morbidity and mortality due to the OD in this study was at a similar level to that reported earlier (Bastert et al. 2013; Johansen et al. 2013; Sidler et al. 2013). Similar data on the significant influence of the vaccination on production parameters, but a less evident effect on the mortality, were presented earlier (Scollo and Mazzoni 2017; Autret et al. 2018). The subclinical effect of lower Stx2e toxin levels might explain the difference in the weight gain parameters, despite the relatively small difference in the clinical cases of OD accompanied with mortality (Kausche et al. 1992; Scollo and Mazzoni 2017). The early vaccination of the piglets with the toxoid vaccine against OD, therefore, significantly improved the production parameters under the conditions of the presented study, whereas the positive effect of a decreasing mortality and the administration of antibiotics in pigs in the nursery period requires further investigation.

It has been also shown that the surviving pigs may have delayed growth and affected performance, depending on the quantity of absorbed toxin (MacLeod et al. 1991). Since, in clinically normal piglets with low levels of Stx2e in circulation, vascular lesions associated with decreased growth rate may develop, antibodies induced by the vaccination may neutralise the low levels of the Stx2e toxin in the circulation in the pigs without any clinical signs and, thus, improve their performance (Kausche et al. 1992; Scollo and Mazzoni 2017; Autret et al. 2018). The positive vaccination effect was previously confirmed on the farm with a history of OD used in the study, where neither clinical signs nor mortality differ ($P > 0.05$), but the daily feed intake and feed conversion rate were improved in the vaccinated pigs, supporting the negative effect of the low level of Stx2e on the pigs (Scollo and Mazzoni 2017).

To further investigate the influence of the subclinical infection on the productivity, non-vaccinated control pigs were classified as positive or negative for STEC, and the body weight was compared between these pigs almost showing a significant effect ($P = 0.084$) with a difference of 2.07 kg. All of this collectively explains the difference in the total weight gain during the trial, despite the relatively small difference in the number of clinical OD cases.

The additional handling of piglets from the non-vaccinated group due to the collection of samples to assess for the presence of STEC (two sampling points during the nursery period) should be taken into consideration as a limitation of study, because manipulation can cause additional stress with a potential negative impact on the performance.

OD diagnostics are based on detecting typical clinical signs and lesions, supported by isolation of the *E. coli* strains positive for the *stx2* gene and *fedF* gene (F18). The role of F18 adhesins in the strong attachment between the bacteria and the porcine brush border fragments was detected *in vitro* and they were described in porcine STEC (Imberechts et al. 1992; Weneburg and Thanassi 2018). In strains examined

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during this study, fimbriae F18 were not detected either by using PCR or by the VirulenceFinder tool. WGS revealed the presence of common pili type I (fimH type), usually expressed by most wild type *E. coli* strains (Hanh et al. 2000; Werneburg and Thanassi 2018). Also, the presence of another adherence associated *iha* gene was confirmed. The *iha* gene is associated with STEC strains of human origin, but was also found in almost 25% of the porcine STEC in the study by Baranzoni et al. (2016). Its role in the onset of an infection is still unclear. The sporadic occurrence of Shiga toxin-producing strains without F18 has been described in piglets without clinical symptoms, but as well in typical OD cases (Hornich et al. 1973; Osek 1999).

Our findings confirm that the Shiga toxin was the main virulence factor in the pathogenesis of OD, but also suggest the possible involvement of different types of adherences compared to typical F18 fimbriae. The potential of atypical strains to cause OD with a negative effect on the performance might be strain and farm specific as STEC strains without F18 have been previously isolated from piglets without clinical symptoms (Hornich et al. 1973; Osek 1999; Alexa et al. 2002). Nevertheless, a more detailed molecular characterisation, the presence of other adherence associated genes and the cytotoxic effect of isolated strains are generally missing in previous studies. Consequently, a case-based approach and careful diagnostics need to be applied in order to determine their role in the pathogenesis of OD.

The antibiotic treatment was rather ineffective once the clinical symptoms of OD had developed, further stressing the importance of vaccination. A reduction in the antibiotic use was reported in other studies assessing the effect of a vaccination based on toxoid vaccines on the antibiotic practice on the farms (Fricke et al. 2015). Compared to previous reports, the vaccination in this study only numerically reduced the need for individual antibiotic treatments and the difference was not significant. This could be explained by the rather small groups of animals and limited numbers of classical, clinical cases of OD, which needed antibiotic treatment. The early vaccination of the piglets with a toxoid vaccine against OD, therefore, significantly improved the production parameters only, with a numerical, positive effect on a decrease in the mortality and administration of antibiotics in pigs in the nursery period under the conditions of the presented study.

Conflict of interest

The authors DS, HK and JV declare that they are employees of the company Ceva Sante Animale France, which currently markets the vaccine ECOPORC SHIGA. The authors JV, NS, AK, IK, RK declare no conflict of interest. The funders had no role analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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