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ORIGINAL ARTICLE

Effect of lysozyme or antibiotics on faecal zoonotic pathogens in nursery pigs

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Abstract

Aims: The objective of this study was to determine the effect of lysozyme and antibiotics on zoonotic pathogen shedding in faeces from nursery pigs housed without and with an indirect disease challenge.

Methods and Results: Two replicates of approximately 650 pigs each were weaned and randomly assigned to one of 24 pens in either a nursery room that had been fully disinfected or a nursery room left unclean. Pigs were randomly assigned to control diet (*Control*), control diet + antibiotics (*Antibiotic*; chlortetracycline and tiamulin), or control diet + lysozyme (*Lysozyme*; 100 mg kg^{-1} diet). Rectal swab samples were collected on day 0 and 28 of treatment, and enriched and cultured for *Campylobacter* spp. and shiga-toxigenic *Escherichia coli* (STEC). Enrichments from rectal swab samples also were analysed for presence of enterohaemorrhagic *E. coli* (EHEC) virulence genes (*hly*A, *eae*, *stx*1 and *stx*2). Room hygiene had little effect on day 28 results. Percentage of samples culture positive for *Campylobacter* spp. was lowest for lysozyme diets ($P < 0{\cdot}01$), but similar for control and antibiotic diets (43·2, 83·7, and 84·8 respectively). Diet had little effect on the EHEC virulence genes *hly*A or *eae* ($P > 0{\cdot}1$), but there was a tendency for fewer samples positive for *stx*1/*stx*2 in antibiotic or lysozyme diet groups ($P < 0{\cdot}07$) compared to control diet (1·2, 2·1 and 5·8% respectively). *Salmonella* spp. and specific STEC types tested were rarely detected in the study.

Conclusions: In nursery swine, room hygiene had little effect on pathogen shedding. Dietary chlortetracycline and tiamulin did not reduce pathogen shedding but dietary lysozyme reduced faecal shedding of *Campylobacter*.

Significance and Impact of the Study: Lysozyme can effectively replace antibiotics in the diet of nursery swine and can be effective for pathogen control.

Introduction

Antibiotics have been fed to farm animals at sub-therapeutic levels for nearly 60 years to improve animal performance (Cromwell 2001). The young piglet benefits from dietary inclusion of antibiotics (Pluske *et al.* 2002; de Lange *et al.* 2010). However, public pressure has increased in recent years to remove antibiotics from food animal's diets and as a consequence there is an urgent need to identify alternatives.

Lysozyme is a 1,4-β-*N*-acetylmuramidase and this enzyme cleaves *N*-acetyl-glucosamine and *N*-acetylmuraminic acid linkages in bacterial cell wall peptidoglycan. The activity is antimicrobial and occurs widely in nature, and in mammals the enzyme is secreted in tears, saliva, and mucosa. In the gastrointestinal tract, the protein can modulate anti-inflammatory response (Goldman *et al.* 1986) and immune response (Kawano *et al.* 1981). As a naturally occurring antimicrobial, there is interest in determining the efficacy of the compound as a dietary supplement.

Early research utilizing transgenic vectors to deliver lysozyme indicated that piglets had improved intestinal morphology (Brundige *et al.* 2008), as well as metabolic

profile (Brundige *et al.* 2010). Recent research with egg-white lysozyme observed a performance benefit when fed to young piglets (May *et al.* 2012; Oliver and Wells 2013). However, these studies have been with small groups and determining the potential effect on pathogen shedding of naturally infected animals has been limited.

In pigs fed milk supplemented with lysozyme, changes in the intestinal microflora were observed (Maga *et al.* 2006, 2012), and in a small study with 10-day old piglets the *Campylobacter* prevalence was reduced (May *et al.* 2012). When pigs were challenged with enterotoxigenic *Escherichia coli* (ETEC), supplemented lysozyme reduced ETEC colonization and concentrations in the digesta (Nyachoti *et al.* 2012) and improved the recovery from clinical signs of infection (Cooper *et al.* 2013). Nonetheless, the potential benefit for dietary lysozyme to reduce pathogens in nursery piglets is unknown. This study was designed to evaluate lysozyme in nursery piglets in a typical production environment and evaluate its ability to control pathogens excreted in faeces.

Materials and methods

Animals, design and management

The experimental protocol was approved by the US Meat Animal Research Center Animal Care and Use Committee. Two replicates of approximately 650 pigs each (1287 pigs total) were weaned from the sow at 26 days of age. Pigs were blocked by litter and gender, and then randomly assigned to either a nursery room that had been fully cleaned and disinfected or a nursery room left uncleaned since the previous group of pigs (Bassaganya-Riera *et al.* 2001; Renaudeau 2009; Oliver *et al.* 2014). Within a room (24 pens per room), pigs were randomly assigned to a pen (experimental unit) with either control diets (*Control*; 2-phase nursery regime, Oliver *et al.* 2014), control diets + antibiotics (*Antibiotic*; chlortetracycline, 55 mg kg^{-1} diet and tiamulin hydrogen fumarate, 38 mg kg^{-1} diet (added as Denagard 10 premixed supplement per label (at 1·65 g kg^{-1} diet; Novartis Animal Health, Basel, Switzerland) or control diets + lysozyme (*Lysozyme*; 100 mg kg^{-1} in diet, sourced from egg whites; Entegard, Neova Technologies, Abbotsford, BC, Canada) and pigs in each pen were allowed to consume diets *ad libitum* for 4 weeks. The pigs were distributed across a total 96 pens in the study (averaged 13·4 piglets per pen; 32 pens per dietary treatment), with pen as the experimental unit for all statistical analyses. All diets met or exceeded NRC recommendations for required nutrients (NRC 1998). Animals were evaluated for performance (gain, intake, and gain to intake) and this data were reported previously (Oliver *et al.* 2014).

Sample collection

Piglets were sampled at day 0 (weaning), 14 and 28 of the study. A rectal swab was taken from each piglet and was placed in a 15-ml conical centrifuge tube containing 2 ml sterile Difco buffered peptone water (BPW; Becton Dickinson Co., Sparks, MD). After collection, tubes were immediately transported to the lab for processing. Tubes were vortexed vigorously and sample processing was done as described below for pathogen detection. A 500 μl aliquot was used for *stx* and shiga-toxigenic *E. coli* (STEC) detection, a 750 μl aliquot was used for *Salmonella* detection, and remainder in tube with swab was used for *Campylobacter* detection.

Microbiological analyses

Salmonella analyses

To determine *Salmonella*, a 750 μl volume was taken from swab sample tube and transferred to 15-ml conical tube containing 13 ml tetrathionate broth (Becton Dickinson) and capped (Wells *et al.* 2010, 2012). Tubes were incubated for 24 h at 37°C. Tubes were mixed well and a 50 microliter aliquot was transferred to 3 ml Rappaport–Vassiliadis Soya Peptone broth (RVS; Oxoid Ltd, Basingstoke, UK) in a deep well 48-well plate. The RVS deep well plates were covered and incubated at 42°C for 24 h. A 10 μl aliquot of the RVS enrichment was plated onto Difco Hektoen-Enteric agar (nHE; Becton Dickinson) supplemented with 20 μg per ml novobiocin (Sigma-Aldrich, St. Louis, MO). The nHE plates were incubated for 24 h at 37°C. Black colonies were picked and plated onto fresh nHE plate. Isolated colonies were grown in TSB and confirmed *Salmonella* by PCR for *inv*A gene (Ziemer and Steadham 2003).

Shiga-toxigenic Escherichia coli and virulence genes analyses

A 500 μl volume was taken from swab sample tube and transferred to deep well 96-well plate containing 500 μl of 50% glycerol for determination of *stx* and STEC. The samples in glycerol were held for up to 48 h at 2°C. A 500 μl volume was transferred to deep well 48-well plate containing 2·5 ml brain heart infusion broth (BHI; US Biological, Swampscott, MA, USA) for enrichment. The BHI enrichment plates were covered and incubated for 8 h at 37°C while shaken at 150 rev min^{-1} (Innova 44 Shaking Incubator; New Brunswick Scientific, Edison, NJ). A 10 μl sample of the BHI enrichment was added to 100 μl of BAX lysis buffer (Dupont Qualicon, Wilmington, DE), 25 μl screened for enterohaemorrhagic *E. coli* (EHEC) virulence factors *eae*, *hly*A, stx_1 and stx_2 using multiplex PCR in a 50 μl reaction as described previously

(Paton and Paton 1998), and another 25 μl screened for *E. coli* O-serogroups O45, O111, O121, O145 and O157 (Kalchayanand *et al.* 2013) genes, *E. coli* O-serogroup O26 gene (Fratamico *et al.* 2011) and *E. coli* O-serogroup O103 gene (Bai *et al.* 2012) using multiplex PCR in a 50 μl reaction as described previously (Kalchayanand *et al.* 2013). Potential STEC from enrichment samples positive for *stx* and the specific O-serogroups (O26, O45, O103, O111, O121, O145 and O157) were concentrated from 1 ml of the enriched sample using immunomagnetic separation (IMS; Rapid-Check STEC IMS kit with anti-*E. coli* O26, anti-*E. coli* O45, anti-*E. coli* O103, anti-*E. coli* O111, anti-*E. coli* O121, anti-*E. coli* O145 and anti-*E. coli* O157:H7 beads; SDIX, Newark, DE, USA). The IMS beads were suspended into 100 μl phosphate buffered saline (Sigma-Aldrich) and diluted 10-fold, and 50 μl of the diluted bead suspension was plated onto STEC differential agar (SDA; Kalchayanand *et al.* 2013). The SDA plates were incubated for 24 h at 37°C then allowed to rest at room temperature for 30 min prior to evaluation. Presumptive isolated STEC colonies were picked into deep well 96-well blocks with 1 ml TSB and grown 24 h at 37°C. Each putative STEC isolate was confirmed by PCR for specific O-serogroup and virulence factor genes as described above without BAX lysis buffer preparation. For each isolate, 1 μl of culture is heated to 100°C for 15 min in a sealed tube and PCR is performed directly on this preparation.

Campylobacter analyses

Campylobacter was determined by enrichment from remainder of sample (750 μl) with swab in sample tube. A 10 ml volume of Bolton selective enrichment broth with supplement (Oxoid Ltd) and lysed horse blood cells (Lampire Biological Labs, Pipersville, PA) was added to the sample tube (Wells *et al.* 2010). Tubes were gently mixed, capped tightly, and incubated 4 h at 37°C followed by 20 h at 42°C. A 20 μl aliquot was plated onto Campy-Cefex agar (Stern *et al.* 1992) and incubated using MicroAero Pack in AnaeroPack System (Mitsubishi Gas Chemical, New York, NY) for 48 h at 42°C. Presumptive colonies were verified *Campylobacter* positive by agglutination (*Campylobacter* Test Kit; Oxoid) and PCR for 23S rRNA gene PCR assays (Eyers *et al.* 1993; Fermér and Olsson Engvall 1999). Species assignments were made to the *Campylobacter* isolates using *lpx*A multiplex PCR assays (Klena *et al.* 2004).

Statistical analyses

This experiment was designed as a split-plot with pen being the experimental unit for dietary treatment (subplot) and pen within room being the experimental unit for the room hygiene treatment (whole plot). Pen was the experimental unit and prevalence for pathogens or pathogenic genes was calculated as a percentage of positive samples per pen. Data were analysed as a 2 × 3 factorial arrangement of treatments within the split-plot design using the GLIMMIX procedure (SAS Institute, Cary, NC). The effects of day, hygiene and dietary treatment, and the interactions, were included in the model as fixed effects. The effects of replication and replication*room were included in the model as random effects. The significance level for all tests was set at $P < 0{\cdot}05$ and tendencies at $P < 0{\cdot}10$.

Results

Swab samples from 1287 piglets in 96 pens were collected on 0 day as animals were sorted into pens going into the swine nursery and again on 28 day at the end of the nursery phase for this study. No significant interactions were observed for any of the statistical analyses. *Campylobacter* spp. prevalence in pens was 43·8% at the start of the study, and increased to 70·6% after 28 days in the nursery ($P < 0{\cdot}05$; Fig. 1). More than 90% of the *Campylobacter* spp. positive samples tested positive as *Campylobacter coli*. Samples were screened for *E. coli* O-serogroups O26, O45, O103, O111, O121, O145 and O157; STEC O103 (seven samples) was isolated at the start of the study and STEC O111 (one sample) was isolated after 28 days (data not shown). Non-shiga-toxigenic O26 was isolated from one sample at the start of the study and 16 samples at the end of the study, and non-shiga-toxigenic O103 was isolated from 12 samples at the start and one sample at the end of the study. At the start of the study, 27·5, 25·3 and 14·33% of samples were positive (Fig. 1) for STEC virulence genes *hly*A (haemolysin), *eae* (intimin) or stx_1/stx_2 (shiga-toxin 1/shiga-toxin 2), and the percentage of samples positive for each gene were different compared to day 28 (17·5, 78·7 and 3·0% respectively; $P < 0{\cdot}05$). Most of the *stx*-positive samples were positive for stx_1 (82·1% on day 0 and 100% on day 28; $P < 0{\cdot}05$). The stx_2 gene was rarely observed at the beginning of the study and no sample was positive on day 28. *Salmonella* spp. were only detected in a few samples and not affected by time of study (data not shown).

Prior to dietary treatment, thermophilic *Campylobacter* spp. and *Camp. coli* were lower in the antibiotic treatment pens compared to the lysozyme treatment pens ($P < 0{\cdot}05$; Fig. 2) and tended to be lower ($P < 0{\cdot}1$) compared to control treatment pens for *Camp. coli*. After the 28-day experiment, *Campylobacter* spp. were nearly 50% lower in the lysozyme treatment pens compared to the control and antibiotic treatment pens. Overall, there was a slight reduction from day 0 to day 28 in *Campylobacter*

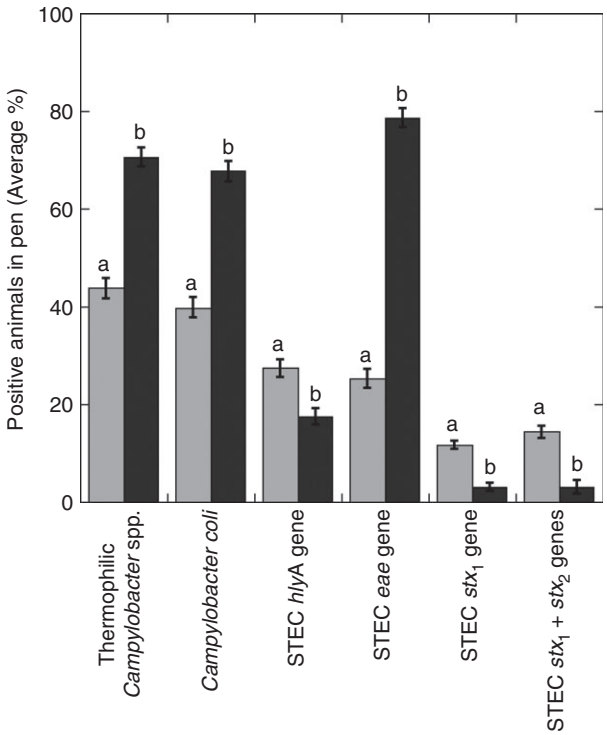

Figure 1 Average percentage of animal rectal swab samples in pen testing positive for thermophilic *Campylobacter* spp. (*Campylobacter jejuni, Campylobacter coli, Campylobacter lari* and *Campylobacter upsaliensis*) isolates, specifically for *Camp. coli* isolates, *hly*A (STEC haemolysin) gene, *eae* (STEC imtimin) gene, stx_1 (shiga toxin 1) gene or stx_1/stx_2 (shiga toxin 1 and shiga toxin 2) genes at weaning (day 0, light bars) or at end of the nursery phase (day 28, dark bars). Error bars denote the standard error. STEC, shiga-toxigenic *Escherichia coli*.

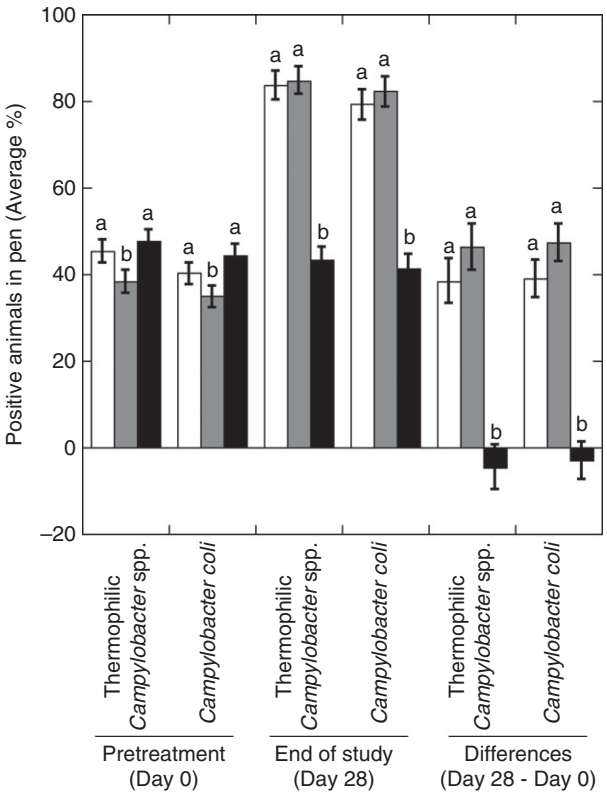

Figure 2 Average percentage of animal rectal swab samples in pen on treatment testing positive for thermophilic *Campylobacter* spp. (*Campylobacter jejuni, Campylobacter coli, Campylobacter lari* and *Campylobacter upsaliensis*) isolates or for *Camp. coli* isolates at weaning (day 0), at end of the nursery phase (day 28), and by difference from weaning (day 28-day 0). Control diet (white bars), antibiotic-supplemented diet (light grey bars), and lysozyme-supplemented diet (dark bars). Error bars denote the standard error, and treatment significant differences are signified by different letters above the error bars.

spp. for the piglets in the lysozyme-treated pens, whereas the control- and antibiotic-treated pens increased significantly over the course of the treatment period ($P < 0{\cdot}05$). Presence of *Campylobacter* spp. was not affected by room hygiene at beginning of the study and similar levels for *Campylobacter* spp. were observed in clean room pens and dirty room pens at the end of the 28-day study (Fig. 3).

The average pen prevalence specifically for STEC O-serogroups O26, O45, O103, O111, O121, O145 and O157 were each <1% and no effect of treatment was observed ($P > 0{\cdot}1$; data not shown). The average presence of the STEC virulence genes in pens for *hly*A (haemolysin) and *eae* (intimin) in faecal swabs were similar across treatments on 0 day and 28 day (Fig. 4). The average presence in pens of stx_1/stx_2 genes was also similar on 0 day across treatments (Fig. 5), but at end of the study on 28 day the average prevalence for these STEC virulence genes in pens differed between control and antibiotic treatment pens ($P < 0{\cdot}05$) and tended to be lower in the lysozyme-treated pens compared to the control pens ($P < 0{\cdot}1$). However, when the pen data were analysed as a difference between 0 and 28 days to account for slight differences in initial levels across treatments, no significant differences were observed for stx_1/stx_2 genes over the course of the study.

Piglets sorted into clean and dirty rooms had similar percentages of pen samples testing positive for haemolysin and intimin genes (Fig. 6). The percentage of pen samples testing positive for haemolysin or intimin did not differ statistically by day 28, nor did the pen values when calculated by difference from 0 to 28 days ($P > 0{\cdot}1$). Similarly, piglets sorted into clean and dirty rooms had similar percentages of pen samples testing positive for *stx*1 alone or both *stx*1/*stx*2 on 0 day ($P > 0{\cdot}1$; Fig. 7), and no difference in the percentage of pen samples positive was observed on 28 days or when calculated by difference from 0 to 28 days ($P > 0{\cdot}1$).

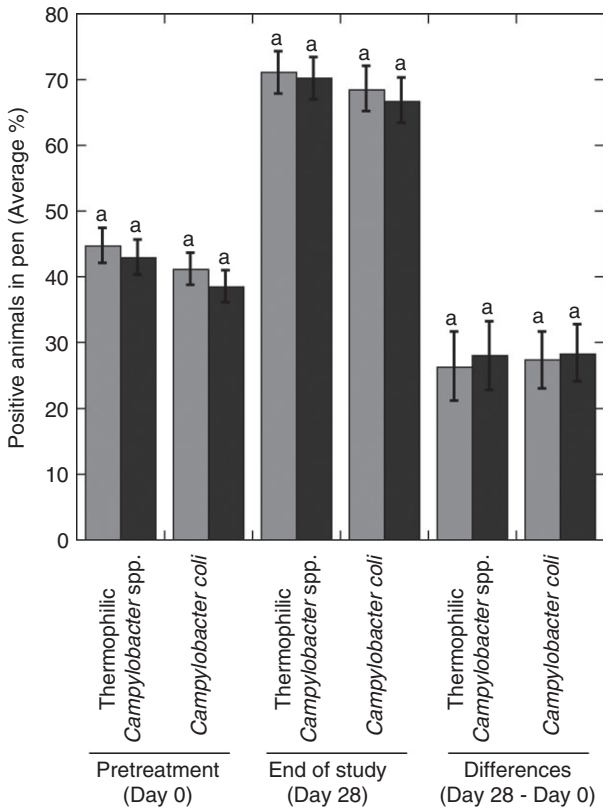

Figure 3 Average percentage of animal rectal swab samples in pen on treatment testing positive for thermophilic *Campylobacter* spp. (*Campylobacter jejuni, Campylobacter coli, Campylobacter lari*, and *Campylobacter upsaliensis*) isolates or for *Camp. coli* isolates in pens cleaned (Clean, light bars) or not cleaned (Dirty, dark bars) prior to being housed with piglets. Error bars denote the standard error, and room hygiene significant differences are signified by different letters above the error bars.

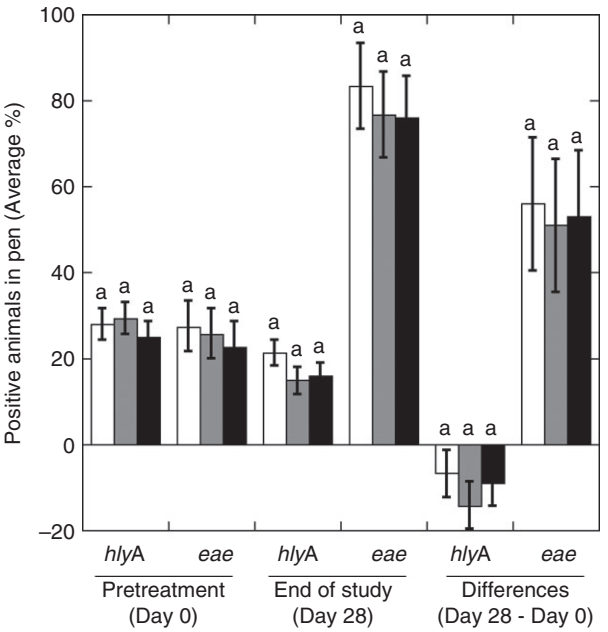

Figure 4 Average percentage of animal rectal swab samples in pen testing positive for *hly*A (STEC haemolysin) gene or *eae* (STEC imtimin) gene at weaning (day 0), at end of the nursery phase (day 28) and by difference from weaning (day 28-day 0). Control diet (white bars), antibiotic-supplemented diet (light grey bars) and lysozyme-supplemented diet (dark bars). Error bars denote the standard error, and treatment significant differences are signified by different letters above the error bars. STEC, shiga-toxigenic *Escherichia coli*.

Discussion

Inclusion of lysozyme in diets of young swine has been shown to improve performance (May *et al.* 2012; Oliver and Wells 2013; Oliver *et al.* 2014). The nursery piglets in the current study were fed a control diet or a diet supplemented with lysozyme or with the antibiotic combination chlortetracycline and tiamulin, and animals fed lysozyme or fed antibiotics performed better for weight gain and feed efficiency than the animals fed the control diet (Oliver *et al.* 2014). In addition, the animals were housed in either clean or dirty (left uncleaned from previous nursery batch) rooms to provide an indirect immune challenge and hygienic stress that reduced weight gain in control piglets but dietary treatments with lysozyme or antibiotics unaffected.

In this study with piglets fed control diets without treatment supplementation, *Campylobacter* spp. were detected in more than 40% of the faecal samples of piglets at weaning and nearly doubled to 80% following the 4-week nursery period. More than 95% of the *Campylobacter* spp. isolated was determined to be *Camp. coli*. We have previously observed high levels of *Campylobacter* in nursery piglets fed diets without supplemented antimicrobials (Wells *et al.* 2010). Quintana-Hayashi and Thakur (2012) observed nearly identical results to our current study in faecal samples from piglets at end of farrowing (i.e. weaning; 41·3%) and nursery (83·7%) phases for piglets in antibiotic-free production systems, and 99·8% of *Campylobacter* isolates were *Camp. coli*.

Campylobacter have been recovered throughout the gastrointestinal tract and from internal lymph node tissues (Nesbakken *et al.* 2003) and this pathogen appears to colonize animals at weaning (Harvey *et al.* 1999). However, the nursery phase appears to be an important phase for *Campylobacter* transmission as piglets from different litters are comingled. In older pigs sampled after the nursery phase, where *Campylobacter* prevalence was negligible, increases in *Campylobacter* prevalence were slow and the herd prevalence was <20% prevalence positive after 8-weeks of growing phase (Wells *et al.* 2012).

When piglets were monitored for faecal *Campylobacter* with repeated sampling over time, piglets that were recur-

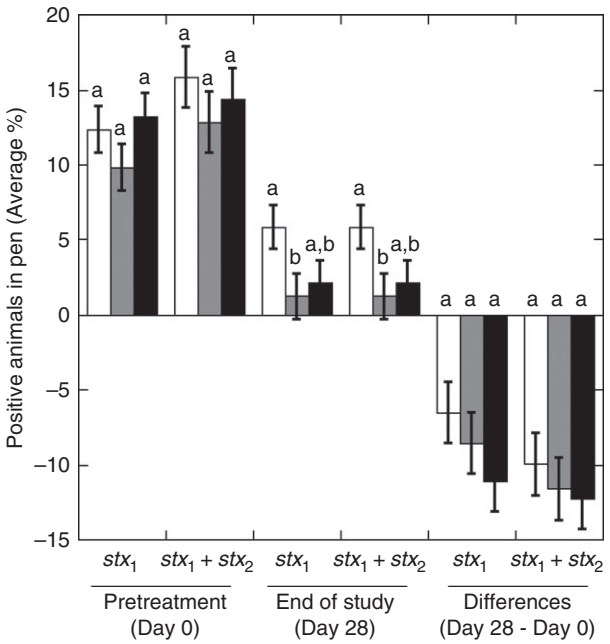

Figure 5 Average percentage of animal rectal swab samples in pen testing positive for stx_1 (shiga toxin 1) gene or stx_1/stx_2 (shiga toxin 1 and shiga toxin 2) genes at weaning (day 0), at end of the nursery phase (day 28), and by difference from weaning (day 28-day 0). Control diet (white bars), antibiotic-supplemented diet (light grey bars), and lysozyme-supplemented diet (dark bars). Error bars denote the standard error, and treatment significant differences are signified by different letters above the error bars.

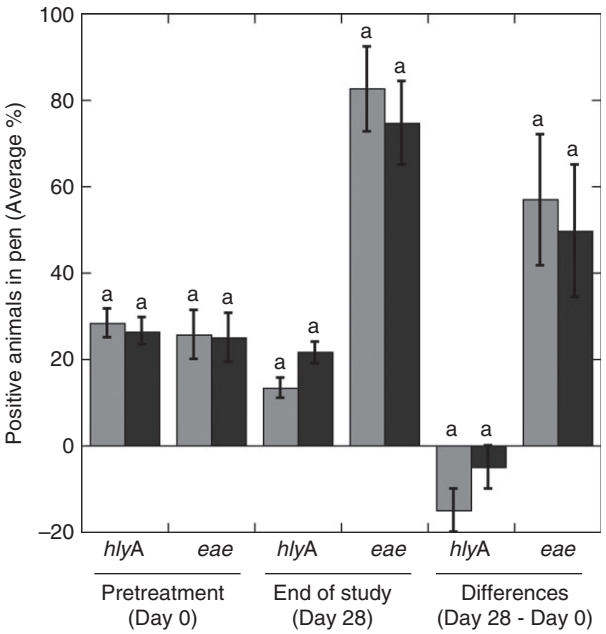

Figure 6 Average percentage of animal rectal swab samples in pen testing positive for *hly*A (STEC haemolysin) gene or *eae* (STEC imtimin) gene in pens cleaned (Clean, light bars) or not cleaned (Dirty, dark bars) prior to being housed with piglets. Error bars denote the standard error, and room hygiene significant differences are signified by different letters above the error bars. STEC, shiga-toxigenic *Escherichia coli.*

ring positive for *Campylobacter* were associated with lower gain (Wells *et al.* 2010). In modern conventional feeding of nursery swine, feeding of dietary antibiotics was associated with a slight (14%) reduction in *Campylobacter* (Quintana-Hayashi and Thakur 2012). Once colonized by *Campylobacter*, the prevalence for this pathogen remained high until harvest. Carbadox and copper sulphate feeding has been shown to reduce *Campylobacter* prevalence more than 75%, (Wells *et al.* 2010) but this dietary antimicrobial combination has not been utilized extensively in recent years. In this study, the addition of lysozyme to nursery swine diets reduced the prevalence of *Campylobacter* spp. detected in swine faecal samples collected at the end of the nursery phase nearly 50% compared to the piglet supplemented with antibiotics. The reduction in *Campylobacter* spp. may explain some of the gain benefits observed in lysozyme-fed piglets (May *et al.* 2012; Wells and Oliver 2013; Oliver *et al.* 2014).

As noted above, the antibiotic dietary treatment with chlortetracycline and tiamulin did not affect *Campylobacter* prevalence. Chlortetracycline is a tetracycline derivative with broad-spectrum antimicrobial activity commonly used in swine production but may have little effect against *Campylobacter* since most isolates harbour resistance (Quintana-Hayashi and Thakur 2012). Tiamulin is a pleuromutilin antibiotic fed to reduce swine dysentery caused by *Brachyspira* (*Treponema*) *hyodysenteriae* (O'Connor *et al.* 1979; van Duijkeren *et al.* 2014). Resistance against tiamulin is not widespread but *Campylobacter* appears to harbour resistance (Lykkegberg *et al.* 2007). It would appear from the current study that neither chlortetracycline nor tiamulin impact *Campylobacter* in nursery swine.

Shiga-toxigenic *E. coli*, including EHEC are known zoonotic pathogens, and can impact human health (Mathusa *et al.* 2010; Friedrich *et al.* 2002). In previous research with older growing pigs possible STEC O-serogroups O26, O103 and O145 were found in 35% of the samples, whereas STEC O-serogroups O111 and O121 were not observed (Wells *et al.* 2012). In nursery piglets in this current study, 0·5% of the piglets were STEC positive at weaning and <0·1% were STEC positive at the end of the nursery phase. Because the STEC prevalence was low, diet effects could not be demonstrated. In the nursery piglets, we only observed STEC O-serogroup O103 at weaning and O-serogroup STEC O111 at end of nursery phase. It would appear from this current research that STEC O-serogroups O26, O103, O111, O121, O145 and O157 types might not be significant inhabitants in the

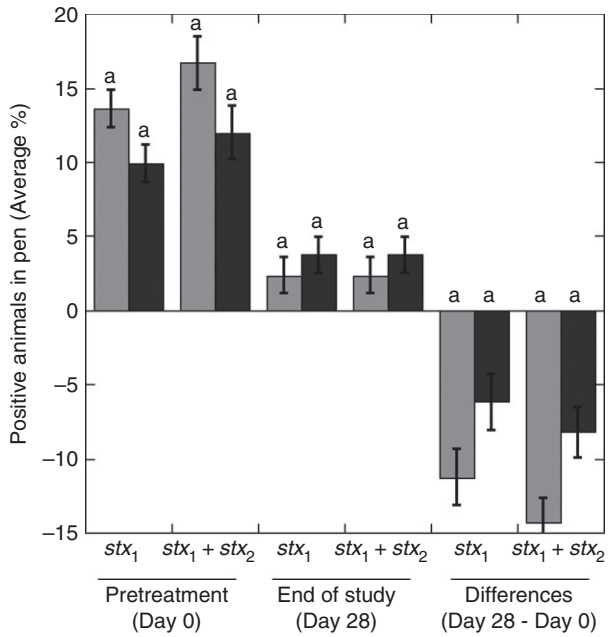

Figure 7 Average percentage of animal rectal swab samples in pen testing positive for stx_1 (shiga toxin 1) gene or stx_1/stx_2 (shiga toxin 1 and shiga toxin 2) genes in pens cleaned (Clean, light bars) or not cleaned (Dirty, dark bars) prior to being housed with piglets. Error bars denote the standard error, and room hygiene significant differences are signified by different letters above the error bars.

piglet during the nursery phase. However, based on the prevalence for *stx* genes detected in the faecal samples, other STEC serogroups may be present and abundant in the weaned piglet, but decrease after weaning. A recent report regarding swine in China did not observe these specific STEC serogroups, but did observe a significantly high prevalence for *stx* genes indicating that swine could be a significant reservoir for other STEC O-groups (Meng *et al.* 2014).

The *eae* gene encodes intimin, a virulence protein associated with pathogenic *E. coli*, and as such can be used as an indicator for EHEC and enteropathogenic *E. coli* (EPEC). The presence of this gene increased significantly in the faeces during the nursery phase. As EHEC bacterial grouping include STEC, and the presence of *stx* genes decreased over the nursery phase, much of the increase in *eae* is likely attributable to the EPEC pathogen groups. The role that EPEC might have in the young piglet is not well defined and this is the first research to indicate that, based on *eae* presence, EPEC might be present in many young piglets. Neither antibiotic nor lysozyme treatment affected this *eae* prevalence and thus unlikely to have affected EPEC.

Cleanliness of the room the piglets were weaned into had little effect on the specific pathogens monitored, even though gain was reduced in the control animals (Oliver *et al.* 2014). Although dietary treatments with lysozyme or antibiotics ameliorated the reduction in gain observed with control piglets in the dirty rooms (Oliver *et al.* 2014), we did not see an increase in pathogens in the dirty rooms for the control animals when we collected our bacterial samples after 4 weeks of housing. Rooms that were cleaned before animals were penned had numerical reductions in pathogen indicators such as *hly*A and *stx* genes, but neither reduction was statistically significant. However, we must note that room hygiene on day of animal placement may have had a greater impact on pathogen load in animals earlier in the nursery phase (e. g. weeks 1 and 2) when we did not sample.

Lysozyme previously has been shown to reduce ETEC in challenged piglets (Nyachoti *et al.* 2012). However, in a small study with young piglets, lysozyme did not reduce *E. coli* in the ileum (Maga *et al.* 2012), so lack of an effect on some of our pathogenic *E. coli* indicators was not unexpected. Maga *et al.* (2012) also noted an elimination of *Campylobacterales* in their small microbiome study with piglets fed lysozyme, and our study specifically shows that *Campylobacter* are significantly reduced with lysozyme. *Campylobacter* spp. are likely resistant to the enzymatic action of lysozyme (Hughey and Johnson 1987), so the potential mechanisms likely involve changes in the microbial ecology that affects *Campylobacter* (Maga *et al.* 2012).

Campylobacter annually account for 2·4 million illnesses, and *c.* 80% of these illnesses are foodborne transmissions (Mead *et al.* 1999). *Campylobacteraceae* were isolated from 15% of retail pork tested in a recent study from Ireland (Scanlon *et al.* 2013), and *Camp. coli* was species most often observed. *Campylobacter* were observed in 6·7% of plant samples and 1·3% of retail pork in the United States (Duffy *et al.* 2001) and *Camp. coli* in 1·3% of retail pork in New Zealand (Wong *et al.* 2007). Reduction in *Campylobacter* shedding early in the swine production cycle with lysozyme when animals appear most susceptible to colonization could reduce pathogens at harvest.

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Conflict of Interest

The authors declare that no conflict of interest exists.

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