American Association of Swine Veterinarians (AASV) - Project AASV Grant Application 2023

Title: Comparison of the pathology and clinical effects of an F18 enterotoxigenic *Escherichia coli* containing a *tia* adhesin gene against a contemporary F18 *Escherichia coli* strain

INVESTIGATORS

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1. Statement of the problem

Diarrhea due to Enterotoxigenic *Escherichia coli* (ETEC) remains one of the main enteric challenges to the United States (US) swine production (Francis 2002; Zhang et al. 2007), resulting in significant economic losses due to morbidity, mortality, decreased weight gain, and cost of treatment, vaccinations, and feed supplements (Fairbrother and Nadeau 2019). The Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) data shows that ETEC is the primary pathogen involved in post-weaning diarrhea (PWD) (Figure 1), and the number of confirmed cases has been on the rise since 2020 (Figure 2).

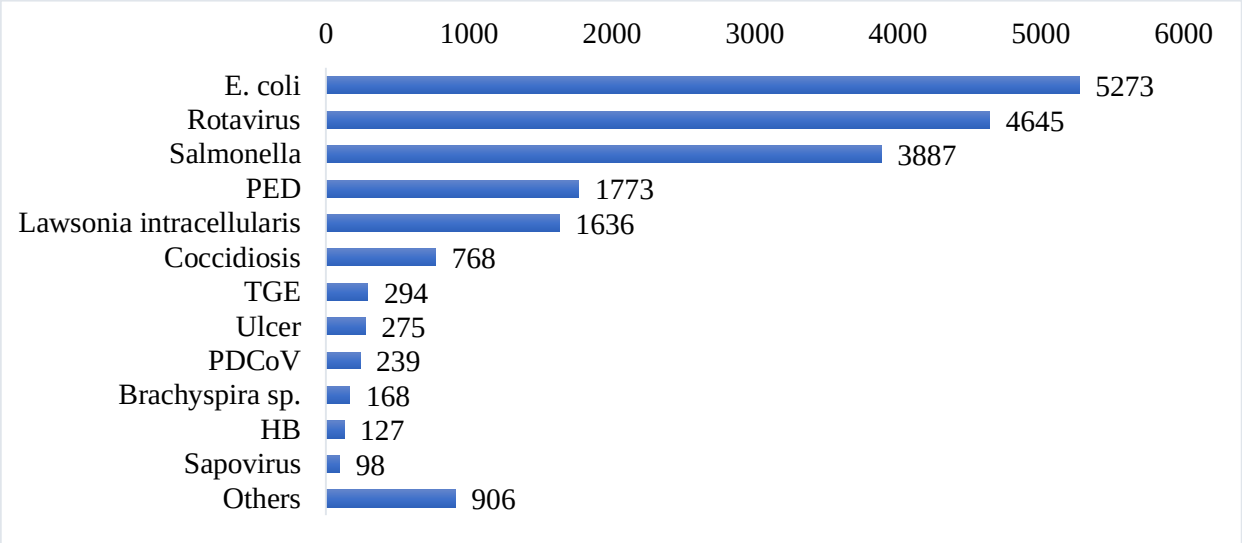

Figure 1. Cumulative cases of enteric diseases in growing pigs from ISU VDL from 2010 to 2022.

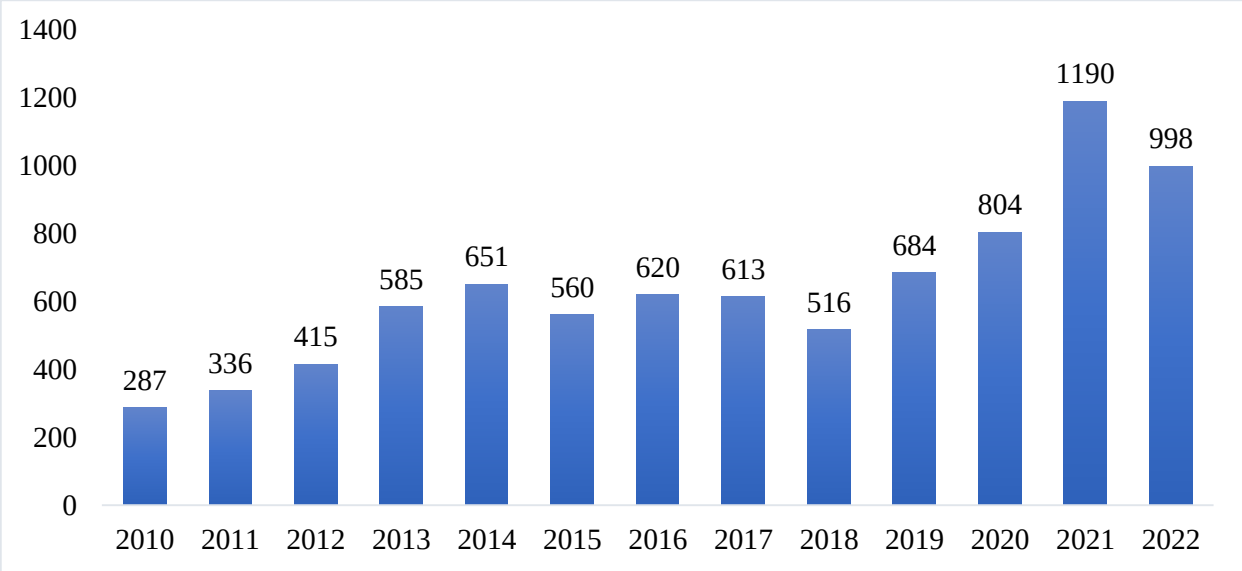

Figure 2. Number of cases per year of post-weaning colibacillosis diagnosed at the ISU VDL from 2010 to 2022.

Among the *E. coli* virulence factors, surface fimbriae are a key component that allows the bacteria to adhere to specific receptors on the intestinal mucosa (Fleckenstein 2013; Nagy and Fekete 2005). F4 (K88) isolates

are amongst the ETEC strains causing high mortality due to diarrhea during lactation and early after weaning. F18 strains can be responsible for either PWD or edema disease when Shiga toxin-producing *E. coli* (STEC) is present. STEC strains adhere to the intestinal mucosa by fimbrial adhesin F18ab and then produce Stx2e toxin, causing vascular damage (Fairbrother and Nadeau 2019; Gyles and Fairbrother 2010). Although much information is available about *E. coli* virulence factors, there is still much to learn about different pathogenic strains affecting pigs, especially how they evolve to survive in the environment and compete with other bacteria in the intestine (Gyles and Fairbrother 2010).

Two separate chromosomally encoded invasion loci (*tia* and *tib*) have been described in human-specific classical ETEC strains (Elsinghorst and Kopecko 1992; Elsinghorst and Weitz 1994; Fleckenstein et al. 1996; Mammarappallil and Elsinghorst 2000). These adhesins *tia* and *tib* likely increase the initial host-bacterial interaction, and it may play a role in ETEC pathogenicity (Mammarappallil and Elsinghorst 2000). The clinical relevance of *tia* gene in ETEC isolated on pigs is unknown; however, STEC strains containing the *tia* gene have been reported in a swine abattoir (Arancia et al. 2019). Recently, a PWD case investigation conducted at the ISU VDL confirmed the presence of adhesin gene *tia* in ETEC strains isolated from clinically affected pigs through whole-genome sequencing. Thus, this study aimed to understand the pathogenic role of F18 *tia*$^+$ and its potential impact on PWD diarrhea in pigs.

2. Objectives

This project aimed to:

1. To compare the clinical impact by measuring diarrhea score, rectal temperature, average daily gain as well as fecal shedding, bacterial attachment, and mortality on four-week-old pigs inoculated with ETECF18$^+$/*tia*$^+$ versus ETEC-F18$^+$/*tia*$^-$.
2. To assess the efficacy of a commercially available F18 *E. coli* vaccine in controlling postweaning diarrhea caused by an ETEC-F18$^+$/*tia*$^+$.

3. Material and Methods

Eligibility criteria

The pigs were selected following the criteria: (a) pigs from the same source and same week of birth (3-week-old); (b) males (n=36) and females (n=36) with similar weights.

Overview of study design

Seventy-two three-week-old pigs were purchased from a commercial farm and housed at the Iowa State University (ISU) Livestock Infectious Disease Isolation Facility (LIDIF). Pigs were individually weighted and randomly allocated into one of five treatments groups (Table 1) and housed in pens containing one barrow and one gilt each. After three days of acclimation (-2 days post-inoculation [dpi]), pigs in groups V/C-F18$^+$/*tia*$^+$ and V/C-F18$^+$/*tia*$^-$ were administered a commercial F18 *E. coli* avirulent live culture vaccine (Edema Vac^{tm}, Arko Laboratories) according to manufacturer's instructions. Pigs in the NC, NV/C-F18$^+$/*tia*$^+$, and NV/C- F18$^+$/*tia*$^-$ groups were administered 2 ml of phosphate buffer solution (PBS). Two days post-vaccination (0 dpi), pigs in the V/C-F18$^+$/*tia*$^+$ and NV/C- F18$^+$/*tia*$^+$ groups were inoculated with 10 ml (1.08 x 10^{10} CFU/ml) of a F18:LT:STa:STb:Stx2e:tia wild type isolate, while pigs in groups V/C-F18$^+$/*tia*$^-$ and NV/C- F18$^+$/*tia*$^-$ were inoculated xx ml (6.3 x 10^{10} CFU/ml) of a F18:LT:STa:STb:Stx2e wild type isolate were inoculated by oral gavage. The control group was sham-inoculated with 10 ml of PBS. All pigs were confirmed to be susceptible to F18 *E. coli* infection by FUT1 gene genotyping by PCR adapted from previously published methods (Jensen et al. 2012).

Table 1. Description of experimental treatments and number of pigs per treatment.

Treatment	Number of pigs
Negative control, non-vaccinated, non-challenged (NC)	8
Vaccinated, challenged with *E. coli* F18:LT:STa:STb:Stx2e (V/C-F18$^+$/*tia*$^-$)	16
Non-vaccinated, challenged with *E. coli* F18:LT:STa:STb:Stx2e (NV/C-F18$^+$/*tia*$^-$)	16
Vaccinated, challenged with *E. coli* F18:LT:STa:STb:Stx2e:*tia* (V/C-F18$^+$/*tia*$^+$)	16
Non-vaccinated, challenged with *E. coli* F18:LT:STa:STb:Stx2e:*tia* (NV/C-F18$^+$/*tia*$^+$)	16

Inoculum preparation

Two hemolytic *E. coli* wild type isolates, Ampicillin and Kanamycin resistant (Amp1 mg/ml and Kan 300 µg/ml), were selected based on their virulence factor profile. Isolates differ only in the presence of the *tia* gene, otherwise both isolates have a F18:LT:STa:STb:Stx2e genotype. Both isolates were selected from cases received at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL), where PWD was diagnosed from enteric disease outbreaks with high mortality. The inoculum for both *E. coli* strains was prepared according to methods previously described (Becker et al. 2020). Bacterial colony forming units (CFU) was calculated by 10-fold microdilution in Blood Agar of an overnight culture at 600 OD.

Clinical assessment and sampling

Pigs were weighed individually on dpi -5, -2, 0, and 7. Rectal swabs were collected on dpi 0, 1, 2, 3, 5, and 7. Rectal temperature and severity of diarrhea were evaluated daily. Pen fecal score was visually assessed using the following scale: 0 = solid, 1 = semi-solid, 2 = semiliquid, and 3 = liquid. A fecal score ≥ 2 was considered diarrhea. On dpi 7, all pigs were euthanized, and sections of duodenum, proximal, mid, and distal jejunum, and ileum were collected and fixed in 10% neutral buffered formalin and refrigerated for histopathological evaluation and bacteriology culture respectively.

Bacterial shedding assessment

The fecal swabs were plated onto TSA agar with 5% sheep blood with Ampicillin (1 mg/ml) and Kanamycin (300 µg/ml) and incubated overnight at 37°C. The hemolytic *E. coli* shedding was assessed based on a semiquantitative method measured using a 5-point scale ranging from 0 to 4 according to the number of streaked sections that had viable *E. coli*, where 0 corresponded to no growth, 1 corresponded to growth in the primary streak, 2 corresponded to growth extending into the secondary streak, 3 corresponded to growth into the tertiary streak, and 4 corresponded to growth into the quaternary section of the agar plate (Li et al. 2019). The presence of hemolytic *E. coli* on individual colonies isolates was confirmed using Matrix-Assisted Laser Desorption/Ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique (Singhal et al. 2015).

Bacterial attachment assessment

One section of the duodenum, proximal, mid, and distal jejunum, ileum, and colon were evaluated histologically. Sections were evaluated for the presence of bacterial attachment consistent with *E. coli* morphology.

Statistical analysis

Microsoft Excel® was used to combine all data collected. Fecal shedding, average daily gain, rectal temperatures, ETEC attachment on jejunum and ileum, and mortality were analyzed using a repeated measures model for longitudinal data through the R program (R. Core Team 2016). Differences were considered significant if p is ≤ 0.05 and a tendency if p is > 0.005 and ≤ 0.10.

4. Results

All treatments groups (V/C-F18$^+$/*tia*$^+$; NV/C-F18$^+$/*tia*$^+$; V/C-F18$^+$/*tia*$^-$; NV/C-F18$^+$/*tia*$^-$) were significantly different ($p \leq 0.05$) from the NC. There was a numerical difference in days of diarrhea per pig between NV/C-F18$^+$/*tia*$^+$ group compared with V/C-F18$^+$/*tia*$^-$; NV/C-F18$^+$/*tia*$^-$ and a significant difference compared with NC (Table 2). There was a numerical difference in weight (lbs) between NV/C-F18$^+$/*tia*$^+$ group compared to NC (table 3).

Table 2. Number of diarrheas per pig according to groups during the study period.

Treatment	Av. days of diarrhea per pig
NC	0.38 (03/08)
V/C-F18$^+$/*tia*$^-$	1.56 (25/16)
NV/C-F18$^+$/*tia*$^-$	1.56 (25/16)
V/C-F18$^+$/*tia*$^+$	1.69 (27/16)
NV/C-F18$^+$/*tia*$^+$	2.13 (34/16)

The fecal shedding score on groups V/C-F18$^+$/*tia*$^-$; NV/C-F18$^+$/*tia*$^-$; V/C-F18$^+$/*tia*$^+$; NV/C-F18$^+$/*tia*$^+$ was significantly higher ($p \leq 0.05$) compared to the NC group. There was a numerical difference in the average of fecal shedding score of the NV/C-F18$^+$/*tia*$^-$ group compared with V/C-F18$^+$/*tia*$^-$; V/C-F18$^+$/*tia*$^+$; NV/C-F18$^+$/*tia*$^+$ groups (Figure 1). The fecal shedding on dpi 0 was significantly higher ($p \leq 0.05$) on the V/C-F18$^+$/*tia*$^-$ group, compared with NV/C-F18$^+$/*tia*$^+$; V/C-F18$^+$/*tia*$^-$; NV/C-F18$^+$/*tia*$^-$ groups. The fecal shedding on dpi 7 was significant higher on the NV/C-F18$^+$/*tia*$^-$ group compared to NC, V/C-F18$^+$/*tia*$^-$, V/C-F18$^+$/*tia*$^+$, and NV/C-F18$^+$/*tia*$^+$ (Figure 3).

The fecal score was significantly different ($p \leq 0.05$) on treatments V/C-F18$^+$/*tia*$^-$; NV/C-F18$^+$/*tia*$^-$; V/C-F18$^+$/*tia*$^+$; NV/C-F18$^+$/*tia*$^+$) compared to the NC. By 7 dpi pigs on the NV/C-F18$^+$/*tia*$^+$ group had numerically higher average diarrhea score (fecal score ≥ 2) (Figure 4).

Table 3. Average body weight and overall body gain (lbs) on different treatment groups.

Treatment	Weight (lbs)		
	0 DPC	7 DPC	Gain
NC	17.9	24.1	6.1
V/C-F18^{+}/*tia*$^{-}$	16.5	20.6	4.1
NV/C-F18^{+}/*tia*$^{-}$	15.9	21.7	5.8
V/C-F18^{+}/*tia*$^{+}$	18.4	23.6	5.1
NV/C-F18^{+}/*tia*$^{+}$	17.4	21.9	4.5

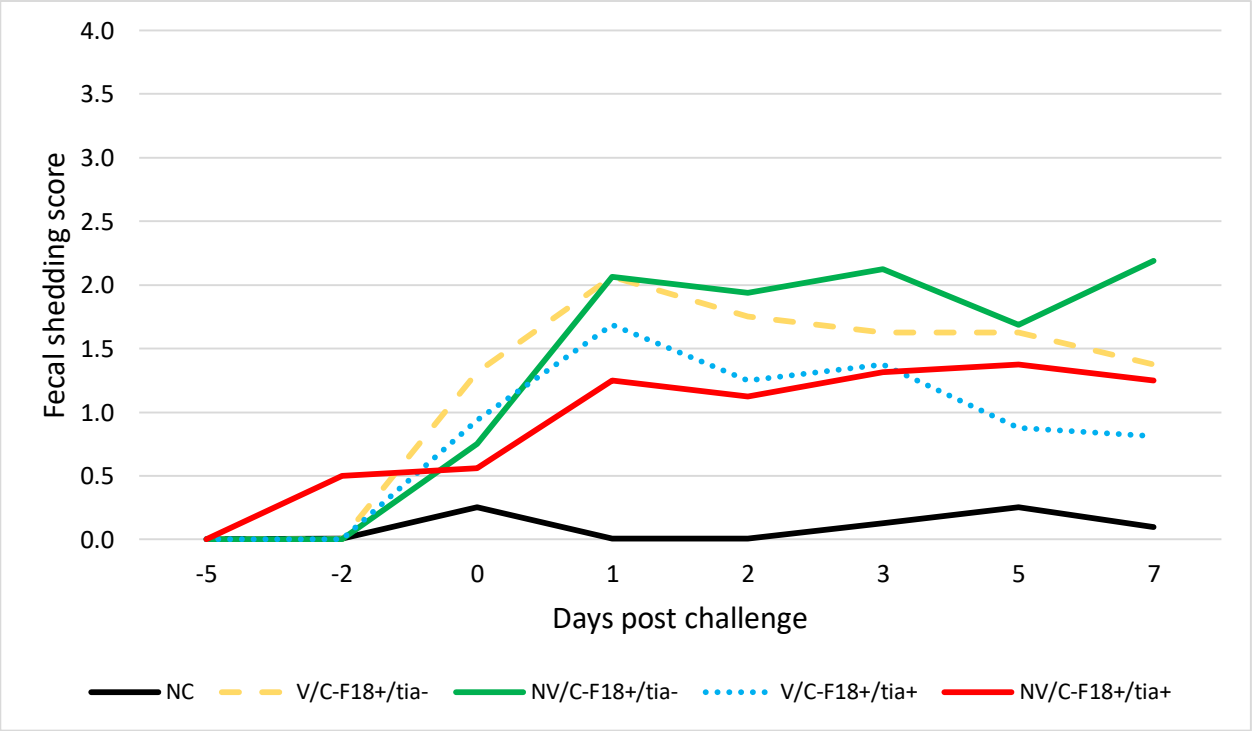

Figure 3. The fecal shedding of the groups in the study period.

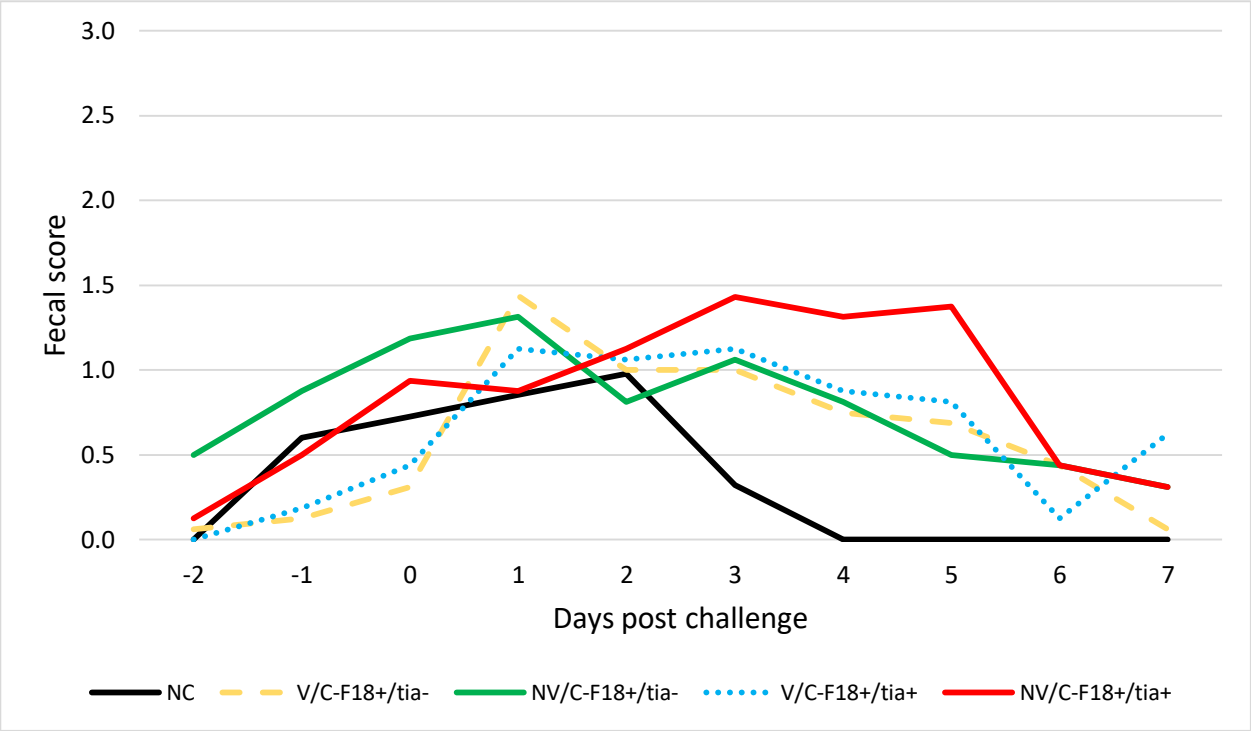

Figure 4. The fecal score of the groups in the study period.

5. Discuss the most significant findings and your recommendations.

Under the conditions of this study, both *tia*$^{+}$ and *tia*$^{-}$ *E. coli* isolates were associated with diarrhea in neonatal pigs. Since this trial was conducted with field isolates with different *tia* backgrounds, some limitations, such as the presence of other pathogenic genes other than *tia* can have implications on the clinical outcome. The vaccine was not associated as a competitive exclusion product. The vaccine used was not designed to be used as a competitive product, further studies to evaluate the challenge 14 days after vaccination will be valuable.

6. Describe how your findings will assist the practicing veterinarians

ETEC is an important challenge to the US swine production with significant economic losses. This study compared the clinical outcome of infection with two different *E. coli* field isolates with similar virulence factor profiles with the exception on the presence of *tia* gene. The role of this gen has not been evaluated in pig and its presence could be related with increased incidence on PWD in the field. The results of this study may help to better understand cases of enteric challenges in growing pigs without diagnostic confirmation and consider the novel E. *coli tia*$^{+}$ genotype as potential differential. Thus, veterinarians should consider the association of this gene with the enteric cases of diarrhea and trigger a deep investigation on the field cases. Vaccination was not effective in controlling clinical diarrhea or reducing fecal shedding when administered two days prior to challenge. However, this commercial product was not designed to be used as a competitive exclusion product. When faced with early *E. coli* challenge in the post-weaning period, alternative solutions must be pursued to prevent disease occurrence. Further time course

studies and potential field evaluation of competitive product on cases with this novel E. *coli tia*$^{+}$ genotype is warranted.

7. State what we can learn from this case, or the methods used to work up this case

An *E. coli* isolate containing the *tia* gene was capable to cause diarrhea in nursery pigs under experimental conditions. F18 *E. coli* can share genes through plasmid transfer, and therefore it is important to evaluate *E. coli* strains with unexpectedly higher morbidity and mortality in the field to understand potential implications on husbandry practices, prevention and treatment. Further investigation about the pathogenesis and the dynamic of isolates containing this gene is needed.

8. Itemize the take home message(s) for the audience

- The ISU VDL cases of *E. coli* have been higher than the historical average;
- An *E. coli* field isolate containing the *tia* gene was capable of causing diarrhea in challenged pigs and should be considered as a potential virulence factor contributing to disease in pigs post-weaning;
- There was a similar performance of vaccinated and non-vaccinated groups;
- Further investigation of this gene is needed to better understand its pathogenesis and impact to the swine industry.

9. References

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