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Clostridial disease in swine – a review

Rick Carter, Ph.D., Kemin Agrifoods Asia
Tom Marsteller, D.V.M., Kemin Agrifoods North America,
Mauro Di Benedetto, D.V.M., Kemin Agrifoods Europa

Abstract

This review outlines Clostridial disease in pigs including diagnosis and management. Whilst *Clostridium perfringens* type A and C and *C. difficile* infections have all been identified in pigs, *C. perfringens* type A is the most common condition in which suckling piglets exhibit clear clinical presentation with transmission being mainly from the lactating sow. Clostridial diarrhea is one of the most common causes of neonatal diarrhea associated with elevated pre-weaning mortality. However, the diagnosis of *C. perfringens* type A enteritis is often equivocal and may be confused with *E.coli*, coccidiosis or rotavirus. Whilst the major toxin produced by *C. perfringens* type A is α-toxin (cpa), most *C. perfringens* type A strains from disease cases produce ß-toxin (cpb). Procaine penicillin or oral dosing with *B. subtilis* PB6 (CLOSTAT™) can be diagnostic for *C. perfringens* infections in piglets, and CLOSTAT™ is effective in managing Clostridial disease in swine.

Key words: Clostridia, *Clostridium perfringens*, swine, pigs, CLOSTAT™

The organism & toxins

Clostridia are Gram positive, some oxygen tolerant, others obligately anaerobic, spore forming bacteria that are found in soil, water and in the intestinal contents of various mammals, birds and reptiles, and can cause disease in people and animals. Enteric *Clostridium perfringens* is classified according to the toxins produced by the different strains, disease being mediated by the production of these toxins, e.g. the convention has been that *C. perfringens* type A produces α-toxin (cpa) and *C. perfringens* type C produces α-toxin (cpa) and ß-toxin (cpb). *C. perfringens* types A and C, as well as *Clostridium difficile*, are the main Clostridial species associated with disease in swine. Whilst the netB toxin is essential for necrotic enteritis caused by *C. perfringens* type A in chickens (Keyburn *et. al.*, 2008), the toxin situation is less clear for swine. The major toxin produced by *C. perfringens* type A is α-toxin (cpa), but most *C. perfringens* type A strains from disease cases produce ß-toxin (cpb) (Songer & Uzal, 2005).

Rood *et. al.* (2018) updated and expanded the toxin typing scheme (table 1) to improve its epidemiological and diagnostic value, e.g.

- avian necrotic enteritis strains of *C. perfringens* type A producing the netB-toxin were re-classified as *C. perfringens* type G under the proposed scheme; and,
- strains of *C. perfringens* type A producing the cpe-toxin were re-classified as *C. perfringens* type F.

Table 1. *C. perfringens* toxin-based typing scheme with the names of toxin structural genes shown in brackets (Rood *et. al*., 2018)

Toxin type	α-toxin (plc or cpa)	ß-toxin (cpb)	Ɛ-toxin (etx)	ι-toxin (iap & ibp)	CPE (cpe)	NetB (netB)
A	+	-	-	-	-	-
B	+	+	+	-	-	-
C	+	+	-	-	±	-
D	+	-	+	-	±	-
E	+	-	-	+	±	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Disease presentation

The descriptions below are mostly quoted from Songer (2010).

C. perfringens type A:

- usually occurs in piglets in the first week of life
- non-hemorrhagic, mucoid (pasty, creamy) diarrhea within 2 days of birth and lasting for up to 5-7 days; feces may also become mucoid and sometimes pink
- farm managers may comment that the piglets “were born with it” (D.P. McKenzie, pers. comm.)
- high morbidity and variable mortality; mortality can be high in severe cases, including when necrotic enteritis is present (D.P. McKenzie, pers. comm.)
- piglets exhibit a rough hair coat and fecal staining of the perineal region
- poor litter homogeneity with affected pigs remaining light for their age through to finish
- at necropsy, the small intestine is flaccid, thin-walled, and gas-filled with watery contents
- mucosal inflammation is mild, occasionally with adherent necrotic material
- intestinal epithelial necrosis is mild in natural infections
- microscopic lesions in piglets may include superficial villous tip necrosis, but villi may appear normal
- note that the condition has also been characterized by mucosal necrosis, villus atrophy & serositis with lesions being most severe in the jejunum and ileum, and mucosal necrosis involving the epithelium and lamina propria, but *C. perfringens* type C affecting other intestinal layers (Songer & Uzal, 2005; see Appendix)
- absence of gross lesions or significant microscopic lesions suggest that *C. perfringens* type A enteritis is mainly a secretory diarrhea
- the large intestine may be distended with whitish, pasty contents but without lesions
- although no causal relationship is known, mesocolon edema syndrome in pigs less than 2 weeks old is strongly associated with the toxins produced by either *C. perfringens* type A (often with ß-toxin production) or *C. difficile*; the syndrome is characterized by localized edema and inflammation of the mesocolon around the spiral colon (Knudsen, 2018)
- disease associated with cpe-toxin producing strains of *C. perfringens* has been described in 3-month old pigs exhibiting mucoid diarrhea with minimal, if any, histological abnormalities (Songer & Uzal, 2005)

C. perfringens type C:
- hemorrhagic necrotic enteritis with high mortality in young piglets
- profound mucosal necrosis and emphysema in the small intestine

C. difficile:
- presents as diarrhea, colitis, pseudomembranous colitis, or fulminant colitis
- it may present as uncomplicated *C. difficile* enteritis or as a mixed infection
- affects piglets in their first week of life with a history of early onset scouring
- piglets without diarrhea, but sometimes with constipation or obstipation, may be toxin-positive
- intestinal contents may be intensely yellow in colour when *C. difficile* is the only isolated pathogen (as cited by Eastaugh, 2008)
- colonic serosal and mesenteric edema is common with the large intestine filled with pasty, yellowish feces
- gross lesions are minimal, but microscopic examination of the colon and cecum show suppurative foci with focal suppuration in the colonic lamina propria being the hallmark lesion, and
- mucosal erosion and 'volcano lesions' may be present (neutrophil and fibrin exudates into the lumen)

Transmission

Clostridial spores can persist in fecal matter and the piggery environment (Baker *et. al*., 2010). *C. perfringens* can also be isolated from feed (Songer & Uzal, 2005). Sows are the likely source of type A infections in piglets with antibodies against type A antigens commonly found in both finishing pigs and sows (Correa & Taylor, 1989).

Diagnosis

Songer (2010) stated that the diagnosis of *C. perfringens* type A enteritis is seldom unequivocal. Silva *et. al*. (2015) noted that the clinical signs of Clostridial diarrhea are similar to several other enteric diseases. Indeed, based on a case in the Netherlands in 1983, Nabuurs *et. al*. reported that 'white scours' were not always associated with rotavirus infections, pathogenic *E. coli*, coccidiosis, but with cpa-toxin producing *C. perfringens* type A. History, necropsy, and the specific age of affected pigs were listed as being important in diagnosing these clinically similar diseases (Larson & Schwartz, 1987). Collins *et. al*. (1994) reported a case of *C. perfringens* type A enteritis in piglets after vaccination for transmissible gastroenteritis (TGE), rotavirus, *C. perfringens* type C, and *E.coli* pilus types K88, K99, 987P and F41, could not prevent or reduce piglet morbidity on a continuous flow farm in the U.S.A.

Songer and Uzal (2005) state that compatible clinical presentation and isolation of large numbers of *C. perfringens* type A being mostly cpb2-positive from the affected jejunum and ileum, combine to be strongly suggestive of *C. perfringens* type A disease. Diagnosis is complicated because *C. perfringens* type A producing cpa-toxin is generally considered as commensal in the intestinal tract of healthy pigs (Songer & Uzal, 2005), but also causes enteric disease (Songer, 2010). Baker *et. al*. (2010) suggested that *C. perfringens* type A disease in piglets may be associated with an under-developed normal microbiota. *C. perfringens* type A cpa-toxin detection in gut contents, whilst supportive of diagnosis, does not alone, have diagnostic relevance

(Songer & Uzal, 2005). Songer and Uzal (2005) also note that differentiating virulent strains from the commensal population cannot be done reliably, but there are strain-to-strain differences in the virulence of type A strains (Baker *et. al*., 2010), and types A and C may both be present together. The virulence mechanisms of type A strains are not well understood, and the genetic diversity of Clostridia adds to the complication of identifying virulent strains (Baker *et. al*., 2010). However, Songer and Uzal (2005) state that any strain of *C. perfringens* type A could cause disease.

Songer (2010) notes that the pathogenesis is likely to be multi-factorial, but the jejunum or ileum is heavily colonized with *C. perfringens* with numbers reaching 10^8-10^9/gram of jejunal contents producing more than one toxin type including cpa and cpb toxins. Whilst there is no direct information on the role of specific toxins in pathogenesis, the strong association with cpb2 toxin suggest it as being a marker of virulence, i.e. more than 90% of *C. perfringens* type A strains isolated from neonatal enteritis were positive for cpb2 (Songer & Uzal, 2005). Resolution of day 1-7 diarrhea in piglets with procaine penicillin or oral dosing with *B. subtilis* PB6 (CLOSTAT™) has been proposed as diagnostic for *C. perfringens* infections (D. P. McKenzie 2019, pers. comm.). See Appendix for specific advice regarding on-farm diagnosis.

C. perfringens diarrhea in piglets was associated with injectable ceftiofur use (McKenzie & Carter, 2019) and *C. difficile* infections account for many cases of antibiotic-associated diarrhea in people (Songer *et. al*., 2000). *C. difficile* infections arise from spores germinating in the cecum and colon producing vegetative Clostridial cells when the normal microbiota is not well established or has been disrupted (Songer *et. al.,* 2000). Toxin types A and B are invariably detected in feces or colonic contents in *C. difficile* infections (Songer *et. al*., 2000).

Incidence

Baker *et. al*. (2010) noted that the diagnosis of neonatal scours due to *Clostridium* species had become increasingly common, and Chan *et. al.* (2012) stated that *C. perfringens* type A was considered by some to be one of the most common causes of neonatal diarrhea associated with increased pre-weaning mortality. Eastaugh (2008) cited reports on the incidence of Clostridial infections in pre-weaned pigs as follows:

- in 2001, a diagnostic laboratory in Illinois (U.S.A.) reported that 37% of diarrhea cases in pigs younger than 5 days were due to Clostridial infections and 21% due to Clostridia from 5 days to weaning;
- in 2004, Iowa State University (U.S.A.) reported that 61% of neonatal diarrhea cases presented in 2003 and 2004 were due to Clostridia infections with *C. perfringens* type A involved in the majority of cases;
- in 2007, Iowa State University (U.S.A.) noted that Clostridia were again involved in the vast majority of diagnostic cases studied in 2005 and 2006;
- a 2001-2003 investigation in the Czech Republic revealed that 39.2% of 153 farms with pre-weaning diarrhea were Clostridial cases;
- a 2005 study in Italy reported that 41% of cases of pre-weaning diarrhea were *C. difficile* infections

Rectal swabs were collected from 333 scouring neonatal piglets from 11 integrated pig production sites and from another 180 pigs from 16 regional sites in a survey of swine farms in the mid-west of the U.S.A (Baker *et. al*., 2010). With *C. perfringens* being ubiquitous and commonly found in the piggery environment and in

the intestinal tract of pigs, high Clostridia isolation rates were expected. All integrated sites were positive for *C. perfringens* and *C. difficile* with 89.8% of pigs being positive for *C. perfringens* and 57.7% of pigs being positive for *C. difficile*. All regional sites were positive for *C. perfringens* with 95.6% of pigs being positive, and 62.5% of regional sites were positive for *C. difficile* with 27.2% of pigs being positive. There were integrated and regional sites with both species present. Of the 502 isolates from regional sites, only 3 isolates of *C. perfringens* type C were found, and none found on integrated sites. Whilst all piglets were scouring at the time of sampling, the pathogenesis of *C. perfringens* type A disease is not well understood making diagnosis difficult, further complicated by not being able to distinguish commensal and pathogenic strains (Baker *et. al*., 2010).

Chan *et. al*. (2012) sampled feces from 48 farms in Ontario, Canada. Forty two of the 48 farms (i.e. 87.5%) were positive for *C. perfringens* as determined by cpa-toxin detection, and 25 farms (i.e. 52%) were positive for cpb2-toxin. From a total of 354 fecal samples, *C. perfringens* was isolated from 64% of samples, with:
- 89% isolation from gestating sows with log 4.3 cfu/gram of feces,
- 96% isolation from lactating sows with log 4.0 cfu/gram of feces,
- 98% isolation from suckling piglets with log 5.0 cfu/gram of feces,
- 34% from weanling pigs with log 1.3 cfu/gram of feces,
- 18% from grower-finisher pigs with log 0.6 cfu/gram of feces, and
- 75% from manure pit samples with log 2.7 cfu/gram of feces.

Treatment

Prophylactic and treatment approaches include antibiotics, probiotics and vaccines. Prophylaxis of *C. perfringens* type A enteritis in sows includes bacitracin which can also be used to treat piglets, as can amoxicillin (Songer, 2010). Lincomycin, sulfa-trimethoprim, penicillins, and tylosin have also been referred to, but Songer *et. al*. (2015) noted that studies have shown that most *C. perfringens* isolates are resistant to tylosin and oxytetracycline. Resistance to beta-lactam antibiotics has also been noted (Silva *et. al*., 2015). A vaccine containing *C. perfringens* recombinant toxoids α and ß was regarded as a candidate for a commercial vaccine against *C. perfringens* type A and C induced diarrhea in pigs (Salvarani *et. al*., 2013). Vaccines containing ß-toxins have been used to help control *C. perfringens* type C, and the use of autogenous toxoid preparations for cpe-toxin *C. perfringens* diarrhea have also been reported (Silva *et. al*., 2015). Whilst bacitracin may be effective in controlling *C. difficile*, antimicrobial therapy has produced inconsistent outcomes (Songer *et. al*., 2000) with multi-drug resistance recorded from *C. difficile* swine isolates in Spain (Palaez *et. al*., 2013). Anti-toxin immuno-prophylaxis may have a role in preventing *C. difficile* disease (Songer, 2010).

CLOSTAT™

(i) in vitro activity

Bacillus subtilis PB6 is the active probiotic bacteria in CLOSTAT™ and was shown to inhibit the *in vitro* growth of a 'culture collection' source of *C. perfringens* (Teo & Tan, 2005) and also inhibited the growth of field strains of *C. perfringens* obtained from swine farms in Australia (figure 1, table 2, figure 2; Kemin Industries, Singapore).

Figure 1. Antagonistic effect of *B. subtilis* PB6 against *C. perfringens (w*ell diffusion assay): A & B are Australian swine farm isolates & C is a *C. perfringens* 'culture collection'; (NC, negative control; PC, positive control (i.e. 10ppm amoxicillin); CFS, *B. subtilis* PB6 cell free supernatant; SPT, *B. subtilis* PB6 supernatant

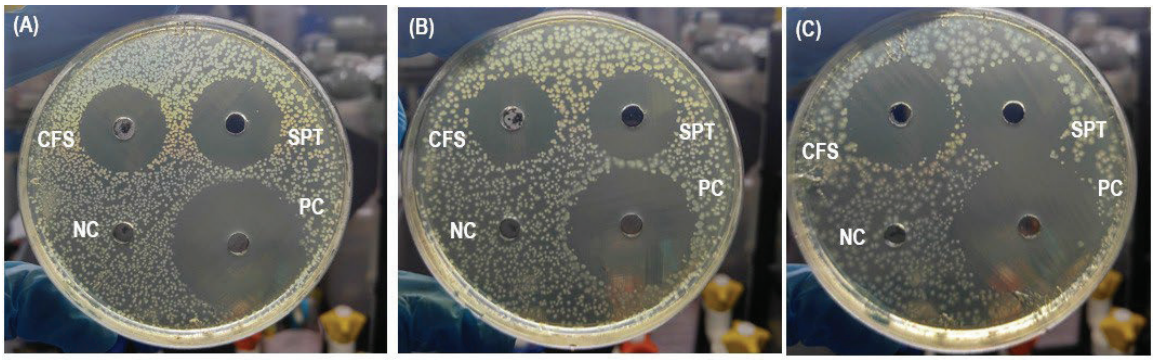
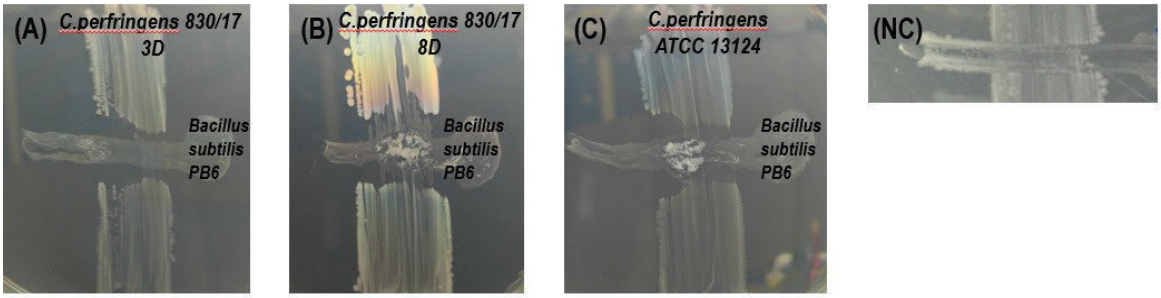


Table 2. Well diffusion zones of inhibition (mm) from Figure 1

Organism	SPT	CFS	PC	NC
C. perfringens farm isolate A	23.5	23.0	34.0	No effect
C. perfringens farm isolate B	24.5	23.5	36.0	No effect
C. perfringens ATCC 13124	25.0	26.0	36.0	No effect

Figure 2. Antagonistic effect of *B. subtilis* PB6 against *C. perfringens* (streak line assay): A & B are Australian swine farm isolates; C is a *C. perfringens* 'culture collection'; NC is negative control



This *in vitro* data is supported by trials conducted in swine as described below in which there were variations in the *B. subtilis* PB6 cfu counts administered to the pigs. The inclusion/dose rate of CLOSTAT™ is important in relation to efficacy and needs to be appropriate to the conditions on the farm.

(ii) Piglets & weanling pigs

A trial was conducted with the Murphy-Brown group in the U.S.A. (Hanson *et. al*., 2009) involving 360 sows and their litters. The sows were split into 2 groups of 180, a control group and a treatment group. The control group did not receive CLOSTAT™ whilst the treatment group received CLOSTAT™ as a daily top-dress on feed from 28 days prior to farrowing and during lactation until weaning of litters at 21 days. The litters from both sow groups were also split into control and treatment groups. The control litters did not receive CLOSTAT™ but all piglets in the treatment litters received an oral dose of CLOSTAT™ on the day of birth. The results for piglet weaning weight and the pre-weaning attrition rate are presented in Table 3.

Table 3. Average piglet weaning weight (21 days) & piglet attrition rate

Treatment	Weaning weight, kg	% attrition
1. control sow / control piglet	4.75[a]	25.4[a]
2. CLOSTAT™ sow / control piglet	4.83[a]	19.2[bc]
3. control sow / CLOSTAT™ piglet	5.04[b]	23.0[ab]
4. CLOSTAT™ sow / CLOSTAT™ piglet	5.04[b]	18.6[c]

[a,b,c], means with different letters within columns differ significantly, P<0.05

These results show that CLOSTAT™ treatment of piglets resulted in significantly increased weaning weight responses, and that treating the sows with CLOSTAT™ resulted in significantly decreased piglet attrition. The best numerical results were obtained when both sows and their piglets received CLOSTAT™.

Kirwan *et. al*. (2019) reported that piglets on a 550-sow unit in Italy with an average weaning age of 21 days experienced sudden and severe diarrhea immediately after birth in most litters, continuing for 5 days, and affecting all piglets in the affected litters. Antibiotics (i.e. enrofloxacin, florfenicol, amoxicillin) failed to resolve the diarrhea. Mortality was moderate, but loss of condition and lack of homogeneity were evident. Laboratory testing ruled out *E. coli*, but PRRS (porcine reproductive and respiratory syndrome) was confirmed. *Clostridium* was hypothesized as an opportunistic pathogen affecting immunosuppressed piglets and so CLOSTAT™ was included in the sow's liquid feed. Thirty days after the onset of the scouring outbreak which was 15 days after the commencement of supplementing with CLOSTAT™, diarrhea was reduced from most litters to only 5% of litters with no diarrhea in newborn piglets. Mortality also returned to normal and uniformity of pigs was restored.

A farm trial in Vietnam investigated the replacement of colistin with CLOSTAT™ from weaning at 4 weeks of age to 11 weeks of age (Nguyen & Carter, 2019). Landrace x Yorkshire x Duroc piglets were weaned and moved to nursery pens at 4 weeks of age with 120 pigs allocated to a control group (i.e. 3 pens of 40 pigs/pen with equal numbers of barrows and gilts/pen) and another 120 pigs to a treatment group (i.e. 3 pens of 40 pigs/pen with equal numbers of barrows and gilts/pen). The feed offered to the control pigs contained 180ppm of colistin, 300ppm of amoxicillin, and 150ppm of tiamulin from 5 days of age through to weaning at 4 weeks, and also from weaning to 9 weeks of age. From 9 to 11 weeks of age, the control feed contained 180ppm colistin and 40ppm florfenicol. The treatment feed was the same as the control feed, except it did not contain colistin at any stage, but contained CLOSTAT™ from weaning through to 11 weeks. Body weight, daily weight gain, feed conversion ratio, and pig deaths were recorded through to 11 weeks of age (table 4).

Table 4. Pig performance from weaning (4 weeks) to 11 weeks of age

Measure	**Control** (antibiotic regime)	**Treatment** (colistin replaced by CLOSTAT™)
4-week ave. weaning weight/pig, kg	6.2	6.2
11-week ave. weight/pig, kg	33.4[a]	35.0[b]
Ave. daily weight gain, g/pig	486	514
Feed Conversion Ratio	1.530	1.507
Number of deaths	1	5

[a,b], means with different letters in rows differ significantly, P<0.05

The 6 deaths in the trial were ascribed to complications from a live PRRS vaccine administered 3 days after weaning. The removal of colistin and its replacement with CLOSTAT™ in the treatment group did not result in decreased growth rate or feed conversion efficiency, but instead the 11 week body weight was significantly higher for the CLOSTAT™ treatment group with pigs being 1.6kg heavier on average which was associated with a 5.8% faster growth rate.

(iii) Sows & piglets

A study was conducted in North Carolina, U.S.A. to assess the effect of CLOSTAT™ on sow productivity and piglet performance in a herd with a history of piglet scouring (Kemin Technical Literature, 2010). CLOSTAT™ was included in both gestation and lactation sow feed for 12 weeks and compared with the previous 12 weeks as well as the same 12-week period from the previous year. The data collected included pre-weaning mortality, number of pigs weaned/litter, weaning weight, wean to first service interval, non-productive sow days (NPSD), and sow mortality. The results are shown in table 5.

Table 5. Sow & piglet performance associated with CLOSTAT™ inclusion in sow diets

Measurement	**Pre- CLOSTAT™ Jan. 18 – April 11**	**CLOSTAT™ period April 12 – July 4**	**Prior year**
Pre-weaning mortality, %	18.41	18.05	20.08
No. pigs weaned/litter	9.63	9.75	9.33
Ave. weaning weight, kg/pig	5.84	5.87	5.69
Wean – 1st service, days	6.36	6.51	7.04
NPSD, days	57.7	54.4	58.7
Sow mortality, %	9.52	9.05	9.31

CLOSTAT™ inclusion in this 'before-after' and 'current year-previous year' study was associated with:

- 0.36–2.03% reduction in pre-weaning mortality,
- 0.12-0.42 increase in pigs weaned/litter,
- 0.03-0.18kg increase in average weaning weight per pig,
- 3.3-4.3 reduction in non-productive sow days, and
- 0.47-0.26% reduction in sow mortality

(iv) Birth to finish

The anti-Clostridial property of CLOSTAT™ was a key element to a 'change management' program applied to 4 farms with highly competent managers in Australia (McKenzie & Carter, 2019). Other important elements to the program included varying stages of progression from continuous flow to all-in-all-out and batch farrowing, batch disinfection with biofilm control, and low protein starter diets with a balanced amino acid profile. The managers completed a questionnaire in relation to observed and recorded changes since implementing the 'change management' program with time on the program varying from 6 to 18 months at the time of completing the questionnaire. The questionnaire responses are shown in table 6.

Table 6: Responses to change management & CLOSTAT™ pre- & post-implementation

Measure	Farm 1	Farm 2	Farm 3	Farm 4
CLOSTAT™ use	dry & lac sow feed, weanling feed, sow udder spray[1] & piglet oral dose at processing[2]	dry & lac sow feed, weanling feed, sow udder spray[1] & piglet oral dose at processing[2]	dry & lac sow feed, weanling feed, sow udder spray[1] & piglet oral dose at birth & at processing[2]	dry & lac sow feed, weanling feed, sow udder spray[1] & piglet oral dose at processing[2]
Day 1-7 scour	90% to 5%	min. 90% reduction	noticeable improvement	80% reduction
Necrotic enteritis (day 8-10 weeks)	2-4 pigs/week to 1 per 2 months	99% reduction	no results provided	80% reduction
Injectable antibiotic use[3]	90% of pigs pre-weaning to zero	25% of pigs pre-weaning to zero	30% reduction	150/week pre-weaning to 0; 100/800 pigs post-weaning to 6-10 /800 pigs
Pre-weaning mortality	18% to 14%	11-15% to 10%	35 pigs/week to 20/week	halved
Ill-thrifty weanling pigs	4-5% to <1%	80% to 10%	40/140 pigs to 15/140	see post-weaning mortality
Post-weaning mortality	>10% to <3%	no results provided	12 pigs/week to 1/week	40 pigs/800 to 4-5/800
Live weight response	1 week earlier at same sale age & weight	1 week earlier at same sale age & weight	1kg/pig heavier at 22 day weaning & 2 weeks earlier at same sale weight	34kg to approx. 40kg at 12 weeks of age, & 5kg heavier at same sale age
In-feed antibiotic use	removed from weanling feed	1 product removed & 1 more to remove	1 product removed & another product reduced	85% reduction
Veterinary supply costs	not available	AU$1,000/month less	AU$800/month less	90% reduction
Piggery staff labor: -treating sick pigs; -time spraying udders	1.5 hours/day to zero; 30 min./day	1 hour/day to 15 min./week; 10 min./day	2 hours/day to zero; 10 min./day	2 hours/day treating sick pigs to 20 minutes/day to spray sow udders

[1], 50ml/udder of a CLOSTAT™-UHT milk preparation daily during lactation (see Appendix for details);
[2], 2-5ml/pig of CLOSTAT™-UHT milk preparation; [3], ceftiofur

The health and production changes were positive across all farms and were associated with cost and labor savings. Importantly, both injectable ceftiofur use and in-feed antibiotic inclusions were dramatically reduced in association with improved pig health and production. It was noteworthy that stopping pig deaths attributed to Clostridia lifted piggery staff morale and provided the catalyst for incorporating more elements of the program. See Appendix for specific advice regarding on-farm management of Clostridial disease.

Conclusions

C. perfringens is transmitted by breeding sows with disease mostly evident in suckling piglets as diarrhea in the first 5-7 days after birth, elevated pre-weaning mortality, and poor piglet and weanling homogeneity. Indeed, Clostridial diarrhea is one of the most common causes of neonatal diarrhea associated with elevated pre-weaning mortality. Diagnosis can be complicated and Clostridial diarrhea may be mis-diagnosed as *E.coli*, coccidiosis or rotavirus, but oral dosing with *B. subtilis* PB6 (CLOSTAT™) has been proposed as

diagnostic for *C. perfringens* infections in piglets. Effective management of Clostridial infections in swine can be achieved with CLOSTAT™.

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Appendix

A swine veterinarian's discovery of Clostridial disease

I didn't recognise Clostridial disease whilst working with large corporate operations. Clostridia were regarded as commensal. However, in my work with smaller farm operations during semi-retirement, I was confronted with severe suckling piglet diarrhoea. The scouring pigs did not fit the picture of *E. coli*, instead, typically presenting with diarrhea from birth with variable outcomes. Sequelae included inappetence, associated wasting, and death. Cases did not convincingly respond to treatment with sulpha or neomycin but were easily treated at their early stage with procaine penicillin. On autopsy, *E. coli* typically exhibits an 'angry', red,

small intestine, which is obvious on opening the abdomen, whereas the intestines from Clostridial diarrhea cases are usually of a dark disclosure with rapid autolysis. From my experience, *E. coli* does not exhibit necrotic enteritis. I observed very thin necrotic enteritic intestines, with most of the mucosa absent but with near-normal serosa and muscularis layers. All affected pigs had very empty stomachs. Multiplex ELISA (enzyme-linked immunosorbent assay) test results from fecal samples submitted to a local veterinary diagnostic laboratory revealed the only detected pathogen to be *Clostridium perfringens*. Similar scenarios were found at more sites, all being positive for *Clostridium perfringens* as determined by ELISA and with the occasional rotavirus, but no toxigenic *E. coli*. Based on the published anti-Clostridial effects of CLOSTAT™, I recommended 1kg of CLOSTAT™ Dry/tonne of lactating sow feed, i.e. $2x10^{11}$ *Bacillus subtilis* PB6 colony forming units (cfu)/tonne of feed, and 0.5kg of CLOSTAT™ Dry/tonne of gestating sow feed, i.e. $1x10^{11}$ *Bacillus subtilis* PB6 cfu/tonne of feed. The diarrhea condition almost fully resolved despite not having implemented significant improvements to hygiene.

Hygiene was progressively improved. This included cleaning with alkali to remove organic matter and biofilm, as well as disinfections with products that were effective in cold temperatures and in the presence of organic matter. Lactating and gestating sows continued to receive 1kg and 0.5kg of CLOSTAT™ Dry/tonne of feed respectively. Whilst this resulted in marked reductions in piglet scouring, improved sow lactation with fewer sows drying up (fully or partially), and consequent improved weaning weights, it seemed that the answer remained incomplete.

Prior to semi-retirement, I had great success from spraying lactating sows' udders with 50ml of *Lactobacillus* cultured in milk, the *Lactobacillus* strain known to be highly lethal to *E. coli* and Salmonella. As a result of this earlier experience, we cultured 2 teaspoons (10-12g) of CLOSTAT™ Dry (i.e. $2x10^{11}$ *Bacillus subtilis* PB6 cfu/kg of CLOSTAT™ product) per litre of UHT (ultra-high temperature processed) skim milk for 24 hours at 25-30^0C and sprayed lactating sows' udders with 50mls/udder/day. Most clients also orally dosed suckling piglets with 2-5ml of this preparation at processing or vaccination. Due to piggery staff shortages, udder spraying was sometimes not done on weekends resulting in piglet diarrhea observations on Monday, but this was resolved within 2 days by an oral dose of the CLOSTAT™-milk to all suckling piglets and the resumption of sow udder spraying.

This CLOSTAT™ programme was seen by most clients as one of the best actions that they had implemented in 20-30 years. It gave them confidence to progressively adopt more elements of my 'health by management' programme which is based largely on the work of Drs. Francois Madec (France) and Colin Cargill (Australia).

I no longer need to confirm the presence of Clostridia disease by laboratory testing. If I wish to confirm Clostridia disease, I use procaine penicillin on early scouring piglets because procaine penicillin is ineffective on all other enteric pathogens except Clostridia. The CLOSTAT™-milk is also a useful diagnostic tool due to its efficacy against Clostridia, as is $2x10^{11}$ *Bacillus subtilis* PB6 cfu/tonne of lactating sow feed (1kg/tonne of CLOSTAT™ Dry), with $4x10^{11}$ *Bacillus subtilis* PB6 cfu/tonne of lactating feed (1kg/tonne of CLOSTAT™ HC Dry) being even more diagnostic based on the following experience:

- according to history, pig health, and site hygiene, I predicted fecal *Clostridium perfringens* counts on a non-client's piggery would be very high, and indeed they were. The farm feed mill had a 2-tonne mixer

and arithmetic confusion regarding the inclusion level of CLOSTAT™ Dry resulted in 2kg of CLOSTAT™ Dry being included per tonne of lactating sow feed, 1kg/tonne in gestating sow and weanling diets, and 0.5kg/tonne in the post-weanling diet, i.e. double dosed. I visited the farm 10 weeks later and observed transformed pig health with no interventions other than CLOSTAT™. There was a marked reduction in pre-weaning mortality, an increase in weaning weight, a marked improvement in musculature of piglets and weaned pigs, and sow mortalities had largely ceased. The pigs reached their finishing weight about 2 weeks earlier than previously. CLOSTAT™ produced dramatic improvements which prompted the owner to update diet specifications for further productivity gains.

All my clients have now moved to $4x10^{11}$ *Bacillus subtilis* PB6 cfu/tonne of lactating sow feed and $1\text{-}2x10^{11}$ *Bacillus subtilis* PB6 cfu/tonne in other feeds. Most clients no longer spray lactating sows' udders with the CLOSTAT™-UHT milk preparation, but typically administer this preparation to piglets at processing and whenever suckling piglets are picked up. Historically, 21-day litter weights of 50-60kg were considered normal, but now with the combination of CLOSTAT™ and demonstrably improved management, a 21-day litter weight target of 70kg is relatively commonly achieved.

So, 4 features of the Clostridial syndrome had become evident:
- suckling piglet diarrhoea at all sites,
- necrotic enteritis at some sites,
- Clostridial sow deaths at some sites,
- CLOSTAT™ inclusion resulting in –
    - better growth rate in weanling pigs, grower pigs and finishing pigs,
    - good reduction in sow Clostridial deaths at sites where deaths were occurring, and,
    - particularly pronounced improvements with $4x10^{11}$ *Bacillus subtilis* PB6 cfu/tonne of lactating sow feed & $2x10^{11}$ *Bacillus subtilis* PB6 cfu/tonne of gestating sow feed.

Veterinarians are generally highly sceptical. Many do not accept that a commensal organism can be a pathogen - similar views were held when the commensal PCV2 virus was mooted as a pathogen!

Dr. Peter McKenzie, Seymour, Australia, April 2020