RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of a Chlorate Treatment on Post-Weaning Diarrhea Caused by *E. Coli* in Weaned Pigs - **NPB #02-061**

Investigator: Tom S. Edrington, Ph.D.

Institution: USDA-ARS-SPA, Food and Feed Safety Research Unit, Texas

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Abstract: A series of five experiments was conducted examining the effect of chlorate administration on post-weaning diarrhea caused by *E. coli* in weaned pigs. In the first two experiments, pigs were experimentally challenged with two strains of *E. coli* (containing the F18 and K88 fimbrial types) known to produce diarrhea in weaned pigs. Three chlorate levels were examined (0, 5, and 15 mM) and although no differences ($P > 0.10$) were observed in cecal or rectal populations of the challenge strains of *E. coli*, a slight reduction ($P < 0.05$) in the severity of scours was observed in the second experiment. We isolated a wild-type strain of *E. coli* that was later identified as having the F18 fimbriae. For the final three experiments, we purchased weaned pigs and allowed them to break naturally with post-weaning diarrhea at our laboratory. We examined the effect of chlorate (15, 100 and 200 mM) administered in successive doses via oral gavage at different time points in the post-weaning disease progression (upon detection of *E. coli* in rectal swabs; upon observing scours in any pig; and upon observing scours in 75% of the pigs). No effect ($P > 0.10$) was observed in populations of *E. coli* from the ileum, cecum, colon or rectum in any of the experiments. Nor were any differences ($P > 0.10$) noted in the severity or incidence of scours. Examination of the laboratory and wild-type strains in pure culture *in vitro* demonstrated that all of these strains were sensitive to chlorate. Chlorate has been shown to effectively reduce populations of *E. coli* in weaned pigs by other researchers. The reasons we did not see any benefit in these experiments may be related to dosing protocol, concentrations of chlorate examined or other unknown factors. Increasing the dosing frequency and/or the chlorate dose may still prove beneficial in alleviating the effects of post-weaning diarrhea caused by *E. coli*.

Introduction: Traditionally pigs were weaned at 10-12 weeks of age allowing for the dam's waning milk supply and the maturation of the young pig's digestive system. However, in the past 50 years weaning ages have decreased dramatically and pigs are now routinely weaned at 14-21 days of age. Following weaning, the piglets are susceptible to a variety of enteric diseases and conditions caused by pathogenic bacteria, most commonly collibacillosis or post-weaning diarrhea and edema disease.

Escherichia coli is a common member of the intestinal microflora and can act as a pathogen as well as being a commensal member. Post- weaning diarrhea is a communicable diarrhea characterized by the production of enterotoxins by specific strains of *E. coli*. It is estimated that over 50% of all losses in weaned pigs are due to *E. coli* infection.

While not all *E. coli* strains are pathogenic, pathogenic strains tend to share similar virulence factors. Fimbriae are projections from the cell that allow for attachment to the intestinal cell wall and certain fimbrial types have been implicated in development of diarrhea. The primary fimbrial types isolated from diarrhea cases have been F18 and F4 (a.k.a. K88). Along the epithelial wall there are specific receptors for fimbrial attachment, bringing the pathogenic bacterium proximate to the intestinal tissue. Not all pigs have receptors for pathogenic *E. coli*, therefore some pigs are genetically resistant to one or more of the *E. coli* strains capable of producing post-weaning diarrhea. However the presence of a receptor is a generally dominant trait. In addition to attaching to the intestinal wall these strains produce toxins that injure the intestinal epithelium. Nearly all *E. coli* involved in terminal cases of diarrhea produce a toxin.

As members of the family *Enterobacteriaceae*, *E. coli* and *Salmonella* possess a respiratory nitrate reductase enzyme. This enzyme is also capable of reducing chlorate to the lethal chlorite ion. Thus, bacteria such as *E. coli* and *Salmonella* that possess this enzyme are consequently killed by the chlorite, but bacteria not possessing the respiratory nitrate reductase enzyme (beneficial bacteria) are not affected. Therefore, we propose that administration of low levels of chlorate may selectively kill the *E. coli* responsible for post-weaning diarrhea in piglets.

Earlier related research: The addition of chlorate to ruminal and fecal fluid from cattle and fecal fluid from swine markedly reduced the populations of *Salmonella*, *E. coli* O157:H7 and generic *E. coli* (Anderson, et al., 2000). Further studies indicated that chlorate treatment in swine significantly reduced experimentally inoculated *Salmonella* and *E. coli* O157:H7 populations (Anderson, et al., 2001a, b). Chlorate administered in the drinking water of cattle and sheep significantly reduced *E. coli* populations (Callaway, et al., 2002). Additionally, the use of chlorate to reduce pathogens in food animals does not appear to have an impact on the ruminal or the cecal/colonic fermentation in ruminants or monogastrics. Selection of chlorate resistant mutants is unlikely because chlorate resistant mutants have been shown to be incapable of competing effectively against the intestinal microbial population (Callaway, et al., 2001).

Objectives: Evaluate the ability of chlorate administration in newly weaned pigs to prevent or reduce the incidence of post-weaning diarrhea caused by *E. coli*.

Procedures:

NPPC-EC-01-2002: Thirty weaned pigs (14-21 d of age) were purchased and brought to our laboratory. Immediately upon arrival pigs were weighed, dosed via oral gavage with two strains of *E. coli* (F18 and K88) and randomly assigned to pen (2 pigs/pen) and treatment [0 (Control), 5 (Low) or 15 (High) mM chlorate]. Feed and water were available for ad libitum consumption. Body weights were recorded again on days 4 and 7. The day following inoculation with *E. coli*, pigs were given their respective chlorate

treatment via oral gavage in 5 ml of de-ionized water. Scours and activity were scored daily according to the following scales:

Scours	Activity
0 = normal feces	0 = normal
1 = soft feces	1 = slightly lethargic, slow to move
2 = fluid feces	2 = lethargic, requires coaxing to move
3 = projectile feces	3 = down, won't move with coaxing
	4 = moribund

After seven days, pigs were euthanized and contents from the cecum and rectum collected for plating of challenge strains of *E. coli*.

NPPC-EC-02-2003: The experimental design was the same as above for the second study with the following exceptions:

- The study duration was 14 days.
- Pigs were challenged two days after acclimation to pen and diet.
- Chlorate was administered twice (via oral gavage): 2 and 6 days following inoculation with two strains of *E. coli*.
- Body weights were measured on day 1, 7 and 14.

In the previous two studies, a wild-type enterotoxigenic *E. coli* (F18) was isolated from the pigs approximately one week after arrival at our laboratory. Therefore, the remaining studies were conducted examining the effects of chlorate on a natural break of *E. coli* in weaned pigs. This natural strain of *E. coli* was found resistant to nalidixic acid, therefore all isolation was conducted on MacConkey's agar supplemented with 20 µg/ml naladixic acid. Intestinal contents collected in each study were serially diluted and plated on the agar described above for quantitative enumeration. In each study, *E. coli* isolates were sent to the Penn State Gastroenteric Disease Center for confirmatory typing.

NPPC-EC-03-2003: Twenty-eight weaned pigs (approximately 14 - 21 d of age) were purchased and brought to our laboratory. Upon arrival pigs were weighed, ear- tagged, a rectal swab taken, and pigs assigned to pen (2 pigs/pen; 7 pens/treatment) and treatment [Control (water only) or 15 mM chlorate)]. Rectal swabs were taken daily and plated for wild-type *E. coli* (F18) throughout the experimental period. Pigs were monitored daily for activity level and scours and when 75% of all pens had at least one pig with scours, treatments were initiated. Treatments were administered via oral gavage in 5 ml deionized water every other day for three days. Ten days following the last treatment dose, pigs were euthanized and contents collected from the cecum and rectum.

NPPC-EC-04-2003: Twenty-eight weaned pigs were purchased and processed as above for NPPC-EC-03-2003. Pigs were monitored daily for scours and when the first pigs was observed visually with scours, treatments administered. Pigs received one of two treatments [control (10 ml water only) or 100 mM chlorate in 10 ml water]

administered by oral gavage (in the afternoon) on three successive days. Sixteen hours following the last gavage treatment, the pigs in each pen were euthanized and tissue and luminal contents from the ileum, cecum, colon and rectum collected and plated as above. Body weights and scour scores were recorded throughout the experiment.

NPPC-EC-05-2003: Twenty-eight weaned pigs were purchased, brought to our laboratory, processed and penned as above. Pigs were monitored daily via rectal swabs for *E. coli* capable of growth on MacConkey's agar supplemented with naladixic acid. Immediately following detection of any pig shedding *E. coli*, all pigs were randomly assigned to receive one of two treatments: Control (water only) or 200 mM chlorate (in 10 ml water) administered via oral gavage in three successive doses 12 hours apart. The day following the last gavage treatment, pigs were euthanized and tissue and luminal contents from the ileum, cecum, colon and rectum collected and cultured as above. Body weights were recorded at the start and end of the experiment.

Statistical Analysis: Data for pig body weight and bacterial counts were subjected to analysis of variance appropriate for a completely randomized design using the general linear model of SAS (SAS Inst., Inc., Cary, NC) with pig as the experimental unit. Scour and activity scores were analyzed using the Proc Mixed procedure with pen as the experimental unit. Differences among means were considered significant at a 5% level of significance.

Results: The two strains of *E. coli* (K88 and F18) used in the first two experiments (Figure 1) and the wild-type strain of *E. coli* (F18) isolated in the last three experiments (Figure 2) were examined for sensitivity to chlorate in pure culture. As the graphs below show, all strains of *E. coli* were sensitive to chlorate.

Figure 1.

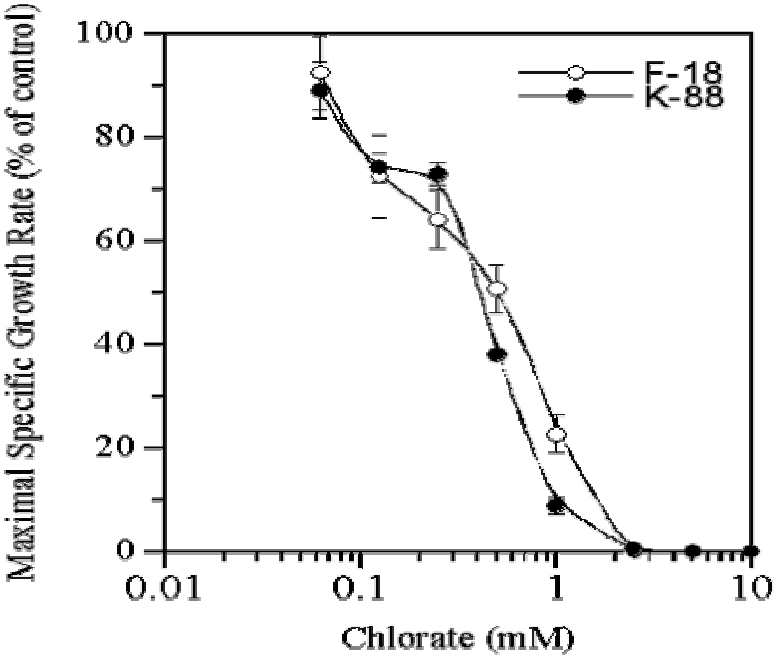
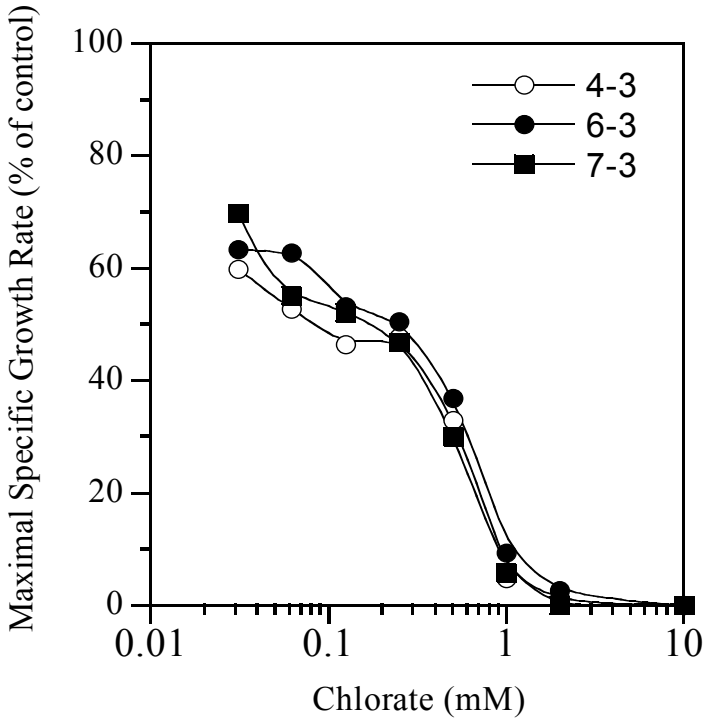


Figure 2.



NPPC EC-01-2002: Results from the first study are presented below in Table 1. Body weights were essentially the same among all treatments. Pigs in the Low chlorate treatment did not gain as well the first four days as the other two treatments, but when examined over the entire 7-day experimental period, weight change was not statistically different. Luminal concentrations of *E. coli* from the rectum were similar among treatments, however concentrations from the cecum were higher in the Low and High chlorate treatments for the K88 and F18 types, respectively. However, these concentrations were only a log higher, and probably not enough to affect any other changes. The average scour and activity scores for the 7-day period were not different among treatments.

Although some differences were noted in this study, the changes were rather insignificant, and do not suggest a benefit or harm from the chlorate treatment. We concluded that increasing the duration of the study may help to see any differences due to treatment on scours, performance, or mortality.

Table 1. Results of NPPC-EC-01-2002: Weight gain, bacterial concentrations and scour scores.

Item	Control	LOW	HIGH	SE[a]	P-value
Body wt. (kg)					
Initial	5.47	5.64	5.95	0.29	0.50
Change d 1 – 4	0.18[c]	0.04[b]	0.26[c]	0.07	0.02
Change d 4 – 7	0.42	0.31	0.37	0.07	0.58
Change d 1 – 7	0.25	0.35	0.11	0.10	0.29
E. coli CFU (log_{10}/g contents)					
F18 Cecum	5.68[c]	6.39[bc]	6.88[b]	0.31	0.03
F18 Rectum	5.58	6.46	6.42	0.43	0.28
K88 Cecum	5.66[c]	6.69[b]	6.49[bc]	0.29	0.05
K88 Rectum	5.67	6.38	5.92	0.48	0.58
Scours Score					
7 day average	0.93	0.85	0.85	0.15	0.89

[a]Standard error.
[bc]Values within a row with different superscripts differ ($P < 0.05$).

NPPC-EC-02-2003: No differences were seen in body weight change with pigs in all treatments gaining approximately 2.5 kg. Bacterial concentrations from cecal and rectal contents were not statistically different among treatments, although both F18 and K88 were one log lower in cecal contents from pigs on the high chlorate treatment. Average scour score for the 14 day period was significantly lower (about half) in the high treatment compared with control animals (Table 2). No differences were seen in activity score and no mortality occurred in the study.

It is important to note that in this study, rectal swabs taken prior to experimental challenge with *E. coli* K88 and F18 produced strains of *E. coli* capable of growth on MacConkey's plates supplemented with streptomycin and nalidixic acid. We assumed that using these antibiotics in the agar would prevent growth of anything other than the inoculated strains of *E. coli*. Therefore, the bacterial counts from the cecal and rectal contents may have contained the wild-type *E. coli* in addition to the inoculated strain. The wild-type *E. coli* was isolated and sent to Penn State University where it was identified as *E. coli* with the F18 fimbria.

Table 2. Results of NPPC-EC-02-2003: Weight gain, bacterial concentrations, and scour scores.

Item	Control	LOW	HIGH	SE[a]	P-value
Body wt. (kg)					
Initial	5.41	4.92	4.66	0.25	0.12
Change d 1 – 7	0.91	0.96	0.93	0.11	0.95
Change d 7 – 14	1.60	1.30	1.61	0.21	0.48
Change d 1 – 14	2.51	2.26	2.54	0.26	0.71
E. coli CFU (log_{10}/g contents)					
F18 Cecum	4.24	4.78	3.78	0.47	0.29
F18 Rectum	3.69	4.27	4.28	0.34	0.43
K88 Cecum	4.11	4.80	3.70	0.37	0.13
K88 Rectum	4.46	4.19	4.36	0.38	0.88
Scours Score					
7 day average	1.38[b]	1.14[b]	0.63[c]	0.15	0.01

[a]Standard error.
[ab]Values within a row with different superscripts differ (P < .05).

NPPC-EC-03-2003: No differences were observed in weekly body weights or in body weight change over the entire 21 day experimental period. We did observe a natural break of *E. coli* (F18) capable of producing scours in these weaned pigs, however, three days of successive treatment with 15 mM of chlorate did not significantly affect cecal or rectal concentrations of *E. coli* and had no effect on the incidence or severity of scours (Table 3).

Originally, we had hoped that chlorate would alleviate the negative effects of *E. coli* on performance and severity of scours. While we did see an effect of chlorate in the second experiment on scours, no other differences were observed. Examining cecal and rectal contents several days after chlorate treatment may have prevented us from seeing a chlorate effect on *E. coli* populations following treatment. Therefore, the next experiment was designed to more specifically examine the effect of chlorate on gut populations of *E. coli*.

Table 3. Results of NPPC-EC-03-2003: Weight gain, bacterial concentrations, and scour score.

Item	Control	15 mM Chlorate	SE[a]	P-value
Body wt. (kg)				
Initial	4.65	4.65	0.24	0.99
Change d 1 – 7	0.04	-0.07	0.08	0.30
Change d 7 – 14	0.59	0.90	0.16	0.19
Change d 14 – 21	1.44	1.22	0.27	0.58
Change d 1 – 21	2.09	2.05	0.35	0.95
E. coli CFU (log_{10}/g contents)				
Cecum	3.31	2.72	0.49	0.41
Rectum	2.77	2.61	0.49	0.82
Scours Score				
Average across days	0.01	0.14	0.04	0.03

[a]Standard error.

NPPC-EC-04-2003: No treatment differences were noted for weight gain over the 10 day experimental period. Concentrations of wild-type *E. coli* (F18) were not different among treatments in the contents collected from the ileum, cecum, colon, or rectum. Similary, no differences were observed in the incidence or severity of scours (Table 4).

In this experiment, chlorate treatment was initiated upon the first visual sign of scours. However, based on the high concentrations observed in all gut segments in this experiment, we felt that the population of *E. coli* was already too large and our chlorate treatment (although a higher dose than previously used) may have been administered too late to be effective against such a large and established population. Therefore, we designed the last experiment in an attempt to control the *E. coli* population before it had reached such a high population.

Table 4. Results of NPPC-EC-04-2003: Weight gain, bacterial concentrations and scour scores.

Item	Control	100 mM Chlorate	SE[a]	P-value
Body wt. (kg)				
Initial	6.52	6.49	0.21	0.93
Final (d 10)	6.81	6.58	0.24	0.49
Change d 1 – 10	0.29	0.09	0.13	0.29
E. coli CFU (log_{10}/g contents)				
Ileum	5.17	5.28	0.39	0.85
Colon	5.57	5.51	0.43	0.92
Cecum	5.28	5.36	0.50	0.91
Rectum	5.41	5.40	0.48	0.99
Scours Score				
Average across days	2.27	2.30	0.20	0.89

[a]Standard error.

NPPC-EC-05-2003: Immediately upon detection of a single positive rectal swab, three successive 200 mM chlorate doses were administered 12 hours apart. However, no significant differences were observed from any of the gut contents examined. Weight change was similar among treatments. Results are presented below in Table 5.

Table 5. Results of NPPC-EC-05-2003: Weight gain and bacterial concentrations.

Item	Control	200 mM Chlorate	SE[a]	P-value
Body wt. (kg)				
Change	0.21	0.21	0.10	0.98
E. coli CFU (log_{10}/g contents)				
Ileum	4.74	5.09	0.53	0.64
Colon	5.31	4.95	0.59	0.67
Cecum	4.98	4.68	0.62	0.73
Rectum	4.91	4.75	0.59	0.85

[a]Standard error.

Discussion: Results from the first two experiments, indicated that chlorate may be of some benefit to pigs with post-weaning diarrhea caused by experimentally infected *E. coli*. However, we later determined that these pigs also had a wild-type *E. coli* capable of causing scours. For the next three experiments, we had a model in which we used weaned pigs that broke naturally with post-weaning diarrhea caused by *E. coli* (F18). Unfortunately, the protocols we used for chlorate administration were not effective in reducing the gut concentrations of *E. coli* or in alleviating the incidence or severity of scours. These results are surprising because others have reported chlorate significantly reduced inoculated *E. coli* O157:H7 and wild-type *E. coli* in weaned pigs (Anderson et al., 2001b). We administered similar concentrations of chlorate in our later experiments, however the time frame between chlorate dosing was shorter (3 times, 8 hours apart) in the work by Anderson and co-workers (2001b). From our results it appears that the population growth of *E. coli* is so explosive that chlorate must be administered earlier, more frequently, and/or in larger doses to be effective. We chose to administer the chlorate via oral gavage vs treatment in the drinking water to ensure chlorate intake. A more constant exposure to chlorate as would be expected if chlorate was administered in the drinking water may be more beneficial in reducing *E. coli* populations in weaned pigs.

Lay Interpretation: In a series of five experiments, we evaluated the effect of chlorate on reducing gut populations of the *E. coli* capable of causing post-weaning diarrhea. In three of the experiments, we had a model using pigs that broke naturally with post-weaning diarrhea. We administered the chlorate by oral gavage and looked at several treatment times in an attempt to determine which time would be most effective in reducing gut populations of *E. coli*. Surprisingly, we did not see any effect of chlorate on gut populations or on the incidence of scours. Other researchers in our laboratory have seen a reduction of *E. coli* in chlorate-treated pigs using similar methodology. Increasing the dosing frequency of chlorate or including chlorate in the water, may be a more beneficial treatment method to reduce gut populations of *E. coli* in weaned pigs.

References:

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