The Cannulated Pig: A Model for Monitoring the Dynamics of Foodborne Pathogens In Vivo.

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Summary and Implications

We have developed a pig caecal cannulation model that allows us to evaluate the effects in vivo of feed withdrawal on (1) the caecal environment, including pH and volatile fatty acid (VFA) concentration, and (2) the growth of foodborne pathogens in the caecum. In vitro studies evaluated growth of *Yersinia enterocolitica* and *Salmonella typhimurium* at five concentrations of VFA at four pH levels. Minimal growth occurred in VFA and pH levels that simulated the caecum of a well-fed pig. Maximal occurs in the absence of VFA (0 mM/ml) at pH 7.0. When cultured in the caecal contents of a fasted pig, *Yersinia* and *Salmonella* replicate and survive. In contrast, caecal contents of a well-fed pig inhibit their growth *in vitro*. When instilled directly into the pig caecum, *Y. enterocolitica* and *S. typhimurium* were detected in fecal and cecal samples for up to 1 month. Infected pigs were subjected to four cycles of interrupted feeding. No predictable change occurs in the number of *Yersinia* or *Salmonella* in the caecum or in feces of pigs subjected to interrupted feedings compared with controls on a normal feeding regimen. In contrast, a fasting cycle predictably reduced VFA concentrations and increased the pH of the caecum. Thus, the pig caecal cannulation model is a practical way of monitoring the long-term dynamics of growth and survival of foodborne pathogens in the live animal.

Introduction

In ruminants, such as cattle and sheep, feed withdrawal prior to slaughter may reduce fecal contamination of carcasses. The effect of interrupted feeding in monogastric animals, such as the pig, is unknown. Volatile fatty acids (VFA), such as propionate, butyrate, and acetate, increase rumen pH and may inhibit the growth of potential human foodborne pathogens, such as *Salmonella* and *Yersinia*, in livestock. For hogs, fasting prior to slaughter saves on feed costs, reduces gut breakage, and reduces carcass fecal contamination. The impact of fasting on the hindgut (caecal) population and fecal shedding of potential human foodborne pathogens in pigs is unknown.

Salmonella and *Yersinia* are potential human foodborne agents of pork (5). The intent of this study to determine the effect in pigs of fasting prior to slaughter on fecal shedding of *Salmonella typhimurium* and *Yersinia enterocolitica.*

Materials and Methods

*Growth in varying(VFA and pH concentrations.*The growth rate (u per hr) was determined in a five VFA concentrations by four (pH) factorial design. VFA concentrations of 0 mM, 25 mM, 50 mM, 75 mM and 100 mM were tested at pH 5.5, 6.0, 6.5, and 7.0. Growth was determined by absorbance at 620 nm (7).

Surgery. The cannula was implanted into 40-lb pigs. To ensure adequate time for healing, caecal fluid was taken from pigs within 1 month following surgery.

Inoculum. Yersinia enterocolitica and *Salmonella* were used.

Instillation of bacterial pathogens. Pigs were held off of feed overnight prior to inoculation. *Yersinia* NADC 7959, a streptomycin-resistant mutant of ATCC 27729 (n=7 pigs, 10^8 CFU in 5 ml of PBS) and the nalidixic acid-resistant strain of *Salmonella* $_x$4232 (n=8 pigs, 10^8 CFU in 5

ml. of PBS) were instilled directly into the caecum.

Collection of fecal samples and caecal fluid. Fecal samples were collected with a 6-in cotton applicator stick and cultured for *Yersinia* and *Salmonella.* Caecal samples were collected into a 50-ml conical centrifuge tubes and cultured for *Salmonella* and *Yersinia.* pH and VFA levels were determined for clarified caecal fluids (7).

Results

Sensitivity of Salmonella and Y. enterocolitica to changes in the VFA and pH of the cecum of well-fed versus fasted pig. The growth of *S. typhimurium* in VFA concentrations (0, 25, 50, 75, and 100 mM) was tested at pH 6.0, 6.5, 7.0, and 7.5 and was measured spectrophotometrically (650 nm). Maximal growth was achieved in the absence of VFA (0 mm); minimal growth, at 100 mM VA (Figure 1). For *Y. enterocolitica* maximum growth occurred at 0 mM VFA, pH 5.6. VFA (>25 mM) inhibited growth at any pH (Figure 2).

Fasting decreases VFA and increases caecal pH (Figure 3). At day 0, the total VFA of a caecal sample of a well-fed market hog is >100 mM; pH 6.2 is minimal. On day 2, a decline in VFA and a rise in pH (6.5) occur in the caeca of this fasted hog (~18 hr after the last feed). On day 3, (~42 hr since last feed), caecal total VFA, propionate, butyrate and acetate are at a minimum. pH 7.9 is at maximum. By day 4, when the hog returns to a normal feeding cycle, VFA rises which decreases caecal pH (pH 6.3). By day 5, VFA and pH 6.1 reflect a normal feeding cycle. Thus, feed withdrawal decreases VFA and increases pH of the caecum.

Growth of S. typhimurium and Yersinia when cultured in vitro in caecal contents of well-fed versus fasted hog. Growth of *Salmonella* in caecal fluid of a market weight hog was evaluated. Exponential growth occurred when *Salmonella* (5×10^5 CFU/ml) was cultured in caecal fluid (10 ml) obtained from a fasted hog (~42 h since last feed). Caecal fluid from this same pig when normally fed inhibited *Salmonella* (Figure 4).

Yersinia survived in caecal fluid from a fasted hog (~42 hrs since last feed). Caecal fluid from this same hog when normally fed inhibited *Yersinia* (Figure 5).

Colonization and fecal shedding of these pathogens in well-fed versus fasted pigs. *Salmonella*(10^8 CFU/ml) was instilled into the caecum of pigs (n=8), and divided into two groups fed and fasted. Fecal shedding of *Salmonella* was unpredictable in both fasted and fed pigs.

Y. enterocolitica (10^8 CFU/pig) was given to pigs (n=7), which were divided into two groups: fasted and fed. *Yersinia* titers in the caecum and feces could not be predicted based on feeding regimens. In contrast, VFA and pH values for fed and fasted pigs reflect dietary fluctuations. VFA of <100mM and pH >6 were seen in fasted hogs. VFA >100mM and <pH 6.0 reflect the caecal environment of the well-fed pig.

Discussion and Conclusion

The pig caecal model is ideal for studying the dynamics of foodborne pathogen in vivo. It offers access to intestinal tract sampling and indicates that the caecal population may be more reliable gauge of foodborne pathogens kinetics following dietary changes than fecal sampling. The surgical technique used in this protocol was originally designed for studying restrained pigs (20~30 kg) over a limited time interval. In this current study, the cannulas were implanted when the pigs averaged 45~50 lb., and were still in place in 11 of 16 (69%) pigs which have achieved ~175 lb. Improvements in the canula design would prolong the life of the surgically implanted cannula. The caecal cannula model is economical in that a few pigs may be used repeatedly thus minimizing individual variation. If similar studies were to be conducted in pigs, multiple animals and multiple confinement rooms would be needed and the study would be fraught with individual pig variations. *Salmonella* and *Yersinia* instilled into the caeca colonized

and could be detected for up to ~3 weeks. When pigs were fasted, an increase in pH and a decline in VFA concentrations were evident. In contrast, no predictable alterations in the levels of *Salmonella* or *Yersinia* in either the caecal fluid or the feces could be seen when the feeding regimen was altered.

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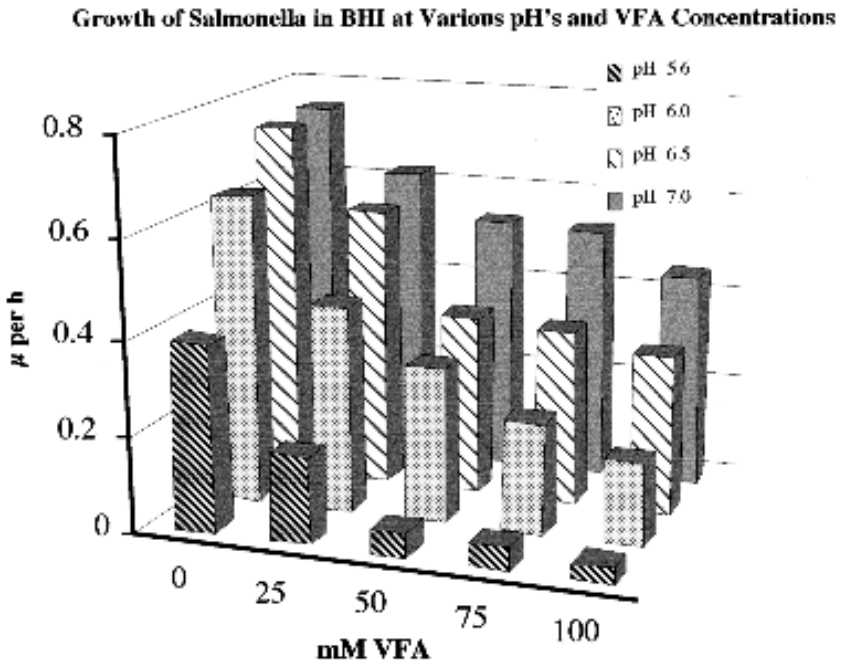
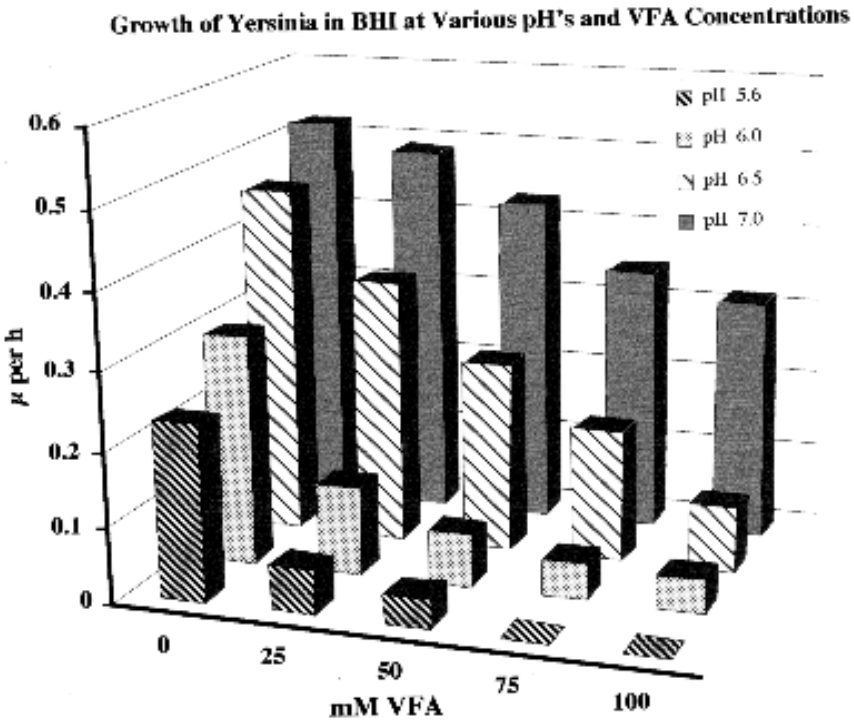


FIGURE 1



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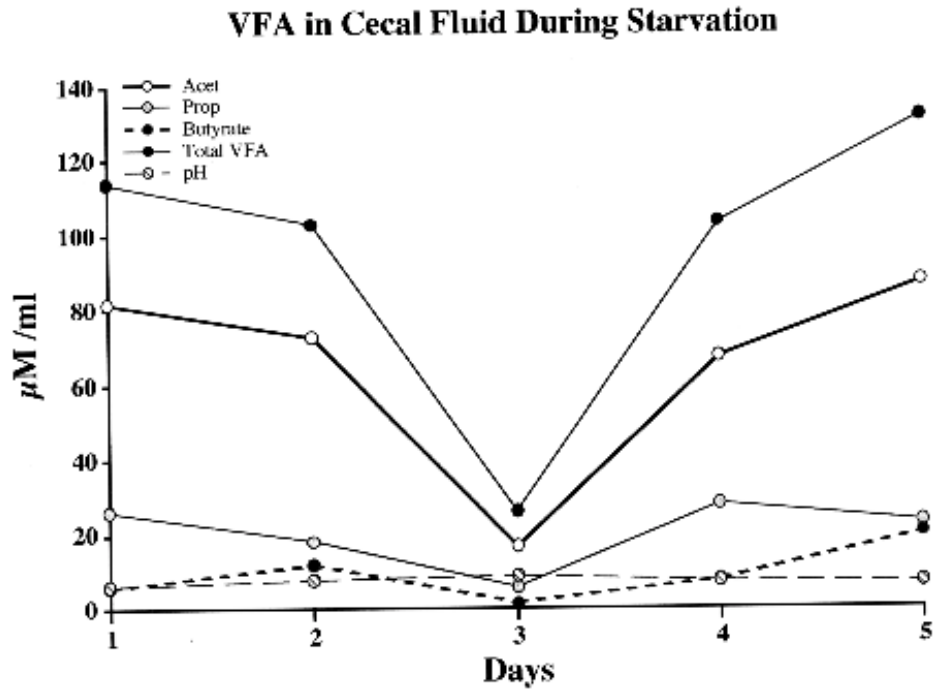

FIGURE 3

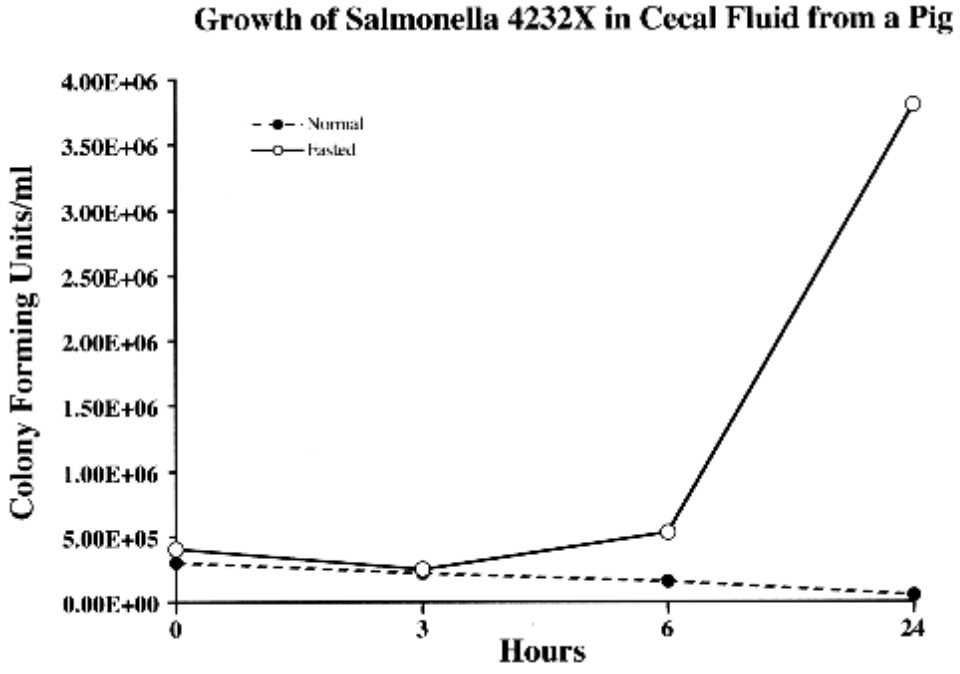

FIGURE 4

Growth of Yersinia in Fed and Fasted Cecal Fluid From Pig 322

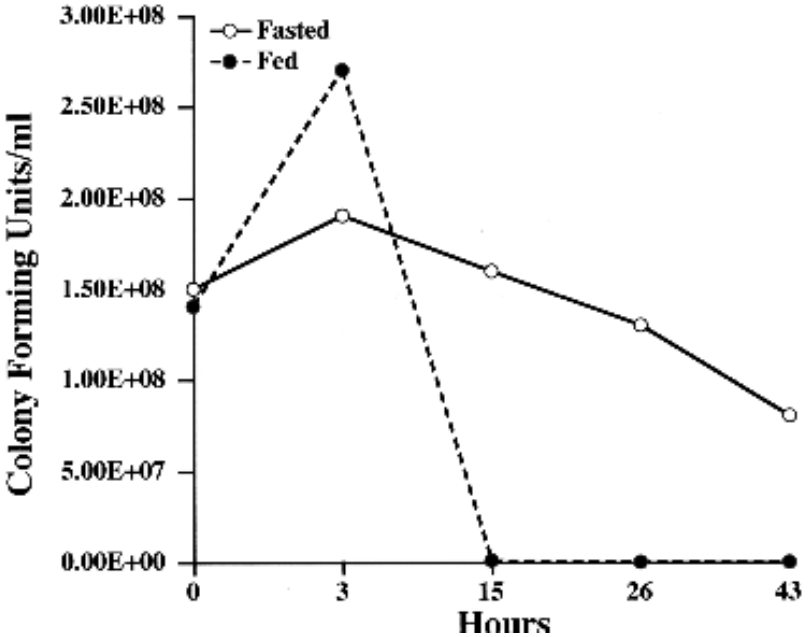

FIGURE 5