Development of an ex vivo model for investigating the bacterial association to the gut epithelium of pigs

Sugiharto, B. B. Jensen, and C. Lauridsen

Department of Animal Science, Faculty of Science and Technology, Aarhus University, AU-Foulum, Tjele, Denmark

ABSTRACT: To study enterotoxigenic Escherichia coli (ETEC) association to the gut of pigs, a simple and reproducible experimental model would be helpful. The aim of this experiment was to establish a model for studying the association of ETEC to the gut epithelium of pigs. Intestinal segments were prepared from 4 weaned pigs, which were tested susceptible to E. coli O149:F4 (homo- and heterozygotic; 2 pigs each) and O138:F18 (all homozygotic). Five segments were taken from 50% of the intestinal length measured from duodenum [mid small intestine (SI)], and 5 segments were taken from 90% distal to the duodenum (distal SI). The segments were immersed in Dulbecco’s Modified Eagle Medium (DMEM) and kept on ice. Polyethylene tubing was inserted into either end of the segment and tied. The tissue was washed with 50 mL of PBS. The other end of segment was tied, 10 mL of DMEM alone or DMEM containing either E. coli F4 or F18 was inoculated, and the segment was sealed with Teflon plug. The segment was immersed in DMEM in a 300-mL infusion bottle in a shaking water bath at 37°C. After 1 h the segment was removed, tissue was washed with 50 mL of PBS, weighed, and homogenized in PBS. Final dilution of 10⁻⁶ was prepared from the content and homogenate. The E. coli was enumerated on MacConkey agar. Data were analyzed according to a 2 × 3 × 2 parametric model including the effects of intestinal segment, E. coli strain, and site of SI with GLM procedure in SAS. A t-test was used to analyze the effect of genotype in F4-inoculated segment. The binding of E. coli on the tissue was 10 times higher (P < 0.001) for F4 than F18. The E. coli F18 was highest (P < 0.05) in mid SI whereas differences were not observed (P > 0.05) between sites of SI for F4. Fewer (P < 0.001) bacteria bound in the control and they associated more (P = 0.10) at distal than mid SI. The E. coli did not differ (P > 0.05) between genotypes in F4-inoculated segment. In conclusion, the ex vivo model may be feasible to investigate the ETEC association to the gut epithelium of pigs.

Key words: adherence, E. coli, intestinal segment


INTRODUCTION

Several unsuccessful attempts regarding experimental reproduction of Escherichia coli postweaning diarrhea (PWD) have been reported, and this has mainly been ascribed to the difficulty of introducing a diarrhea-like condition in the challenged pigs (Fairbrother et al., 2005). Therefore, to search for feed and food constituents with inhibitory effect on the adherence of enterotoxigenic E. coli (ETEC) to the gut, a reproducible, fast, and easy experimental model would be beneficial. To study the ETEC association, a number of in vitro cellular models have been used, but these are less suitable for ETEC pathogenesis study in pigs (Koh et al., 2008). In vivo loop models are available to study the invasion of bacteria to the porcine gut. However, this model is not always appropriate to investigate the inhibitory effect of feed constituents on the adherence of bacteria to the gut (Naughton et al., 2001). The latter authors have therefore developed a simple intestine organ culture model to investigate the binding of Salmonella and nonpathogenic E. coli to the gut of pigs. The objective of the present experiment was to further develop the porcine intestine organ culture model with special emphasis on the association of different ETEC strains (E. coli O149:F4 and O138:F18 that are most commonly isolated from pigs with PWD) to the different sites of porcine intestinal tissue.
MATERIALS AND METHODS

Intestinal segments from 50% [mid small intestine (SI)] and 90% (distal SI) of intestinal length measured from the duodenum were taken from 4 weaned pigs, which were tested (van Haeringen laboratorium B.V., Agro Business Park, Wageningen, The Netherlands) susceptible to both F4 (homo- and heterozygotic; 2 pigs each) and F18 (all homozygotic). Obtained intestinal segments were inoculated with either *E. coli* F4 or F18 or control (without bacteria).

The *E. coli* F4 and F18 were retrieved from bacterial culture stored at –80°C, streaked on MacConkey agar (MAC) and blood agar (BA; Oxoid), respectively, and grown at 37°C for 18 h. One colony of F4 was transferred into 10 mL Luria-Bertani (LB) broth and incubated at 37°C for 4 h. A 0.1 mL of incubated LB broth was suspended in 50 mL Dulbecco’s Modified Eagle Medium (DMEM), incubated overnight, and centrifuged at 2,000 × g for 10 min. The bacterial pellet was resuspended in 100 mL DMEM to get the inoculum of 5 × 10^8 cfu/mL. A loopful of F18 colony was taken from BA and suspended in 4 mL PBS. The suspension (0.1 mL) was poured onto iso-sensitest agar (Oxoid) and incubated at 37°C overnight. Incubated plate was harvested with 10 mL PBS, and this suspension was diluted 1:20 (vol/vol) with DMEM to get the inoculum of 5 × 10^6 cfu/mL.

Intestinal organ culture was prepared based on the model described by Naughton et al. (2001) with few modifications. Four pigs (8 wk of age; 25.73 ± 4.61 kg BW) fed standard Danish pig diet [based on barley (*Hordeum vulgare*), wheat, soybean (*Glycine max*) meal, animal fat, and fishmeal] were obtained from the herd at the Faculty of Science and Technology, Aarhus University, Foulum, Denmark. Immediately after slaughter, 5 intestinal segments (15 cm each) were taken aseptically from mid SI, and 5 segments were taken from distal SI, immersed in DMEM, and kept on ice. From each site of SI, 1 segment was used for control and the other 4 segments were inoculated with *E. coli*. Polyethylene tubing (Siltube; Eurpharm; 6 mm i.d.) was inserted into either end of the segment and tied with a suture to keep the tubing in place. Tissue was washed through with 50 mL of PBS (pH 7.2) using a FillMaster pump (Type 311; Delta Scientific Medical; flow rate of 7.7 cm/s). The other end of the segment was tied, 10 mL of DMEM alone (control) or DMEM containing either *E. coli* F4 or F18 was inoculated, and the segment was sealed with Teflon plug (5 mm i.d.). The gut segment was immersed in DMEM in a 300-mL infusion bottle in a shaking water bath at 37°C. The segment was removed after 1 h, the content of intestinal segment was obtained, and the tissue was washed with 50 mL of PBS, weighed, and homogenized using a Janke-Kunkel Ultra-Turrax T25 homogenizer (the Netherlands) for 20 s in PBS. Final dilution of 10^6 was prepared from the content and homogenate. The *E. coli* was enumerated on MAC after incubation of 16 h at 37°C.

Data were analyzed according to a 2 × 3 × 2 parametric model including the effects of intestinal segment (content or homogenate), *E. coli* strain (F4, F18, or control), site of SI (50% or 90%), respectively, and all interactions by using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). A t-test was used to analyze the effect of genotype in F4-inoculated segment.

RESULTS

The inoculum contained 8.64 ± 0.20 and 8.70 ± 0.26 log_{10} cfu/mL for F4 and F18, respectively, and no *E. coli* could be enumerated in the inoculum of the control. The number of *E. coli* in the content of intestinal segments was higher (*P < 0.05) than tissue homogenates when *E. coli* was inoculated (Figure 1). Compared to inoculum, there was an increase (*P = 0.07) of *E. coli* after 1 h incubation.
in the content of F4-inoculated segment, but change was not observed ($P > 0.05$) in F18. The binding of \textit{E. coli} to the tissue in F4-inoculated segment was 10 times higher ($P < 0.001$) than in F18-inoculated segment (Figure 1). There was an interaction ($P < 0.001$) between the strains of \textit{E. coli} and the sites of SI, as F18 was higher ($P < 0.05$) in mid SI than in distal SI whereas F4 did not differ ($P > 0.05$) between the sites of SI (Figure 2). With regard to the control treatment, fewer ($P < 0.001$) bacteria were enumerated in the content of intestinal segment and tissue homogenates when compared with the treatments (Figure 1), and they associated more ($P = 0.10$) at distal than mid SI (Figure 2). The number of \textit{E. coli} did not differ ($P > 0.05$) between genotypes in F4-inoculated segment; that is, tissue homogenate of homo- and heterozygous F4R$^+$ pigs contained $8.62 \pm 0.26$ and $8.45 \pm 0.12$ log$_{10}$ cfu/g (wet weight) of \textit{E. coli}, respectively.

**DISCUSSION**

The binding of ETEC on the intestinal tissue is determined by the presence and density of specific receptor on the enterocytes (Fairbrother et al., 2005). Thus, not all ETEC in the content of inoculated intestinal segments can bind to the intestinal tissue. The binding of F4 to the tissues was higher than determined for F18. The \textit{E. coli} F4 can colonize the gut very soon after inoculation whereas F18 need longer time to colonize (Verdonck et al., 2002). To cause diarrhea, ETEC must present in sufficient number ($10^9$ cfu/g tissue) following bacterial attachment to receptors in SI (Fairbrother et al., 2005). Therefore, when studying the adherence of pathogens using the ex vivo model, to incubate longer when inoculating with F18 compared with F4 is suggested. Concomitant with our control treatment, \textit{E. coli} is commonly present as commensal microflora in the distal ileum of pigs (Cassels and Wolf, 1995). With respect to pathogenic \textit{E. coli}, however, the site of association in the gut can differ (Naughton et al., 2001). In accordance to Fairbrother et al. (2005), our study showed that F4 associated in both mid and distal SI with a similar extent. In addition, differences between homo- and heterozygous F4R$^+$ pigs were not observed regarding the association of F4. Both genotypes have functional receptor for \textit{E. coli} F4 that can facilitate the binding of F4 to the intestinal tissue (Fairbrother et al., 2005). In this study we found that the binding of F18 was greatest in mid SI. However, Koh et al. (2008) reported that F18 strongly bound to cells derived from both jejunum and ileum of pigs. In conclusion, the model described herein might be feasible to investigate the ETEC association to the gut epithelium of pigs and therefore appropriate to study the inhibitory effect of feed constituents on the adherence of ETEC to the gut.

**LITERATURE CITED**


