

Title: Human Transmission of Enterotoxigenic *Escherichia coli* from Infected to Non-Infected pigs, NPB #01-011

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I. **Abstract:**

Post-weaning diarrhea in swine caused by enterotoxigenic *Escherichia coli* can be an important cause of mortality in weaned pigs and has become a frustration for many pork production units. On some farms post-weaning scours due to *E. coli* persists despite vaccination, aggressive use of antibiotics, and improved facility sanitation. One possibility is that *E. coli* is spread by caretakers tracking the pathogen from infected to susceptible groups of pigs. To determine the minimum standards for biosecurity measures to prevent such transmission 125, 19- to 21-day-old weaned pigs, negative to the challenge strain of enterotoxigenic *E. coli*, were randomly allocated to 5 rooms. Pigs in one room were inoculated with enterotoxigenic *E. coli* M1823B. Once a day for 10 consecutive days, a caretaker stood in the pen with these pigs for 10 minutes and then walked directly to another room of healthy pigs without using any biosecurity procedures and stood in the pen with these pigs for 10 minutes. The protocol was repeated to test two biosecurity procedures. The caretaker contacted sick pigs and then washed hands and changed outerwear before contacting a second group of healthy pigs. Next, the caretaker contacted sick pigs and then showered and changed outerwear before contacting a third group of healthy pigs. A separate caretaker cared for negative control pigs. Fecal samples were collected from pigs and cultured to detect the spread of *E. coli*.

The caretaker mechanically transmitted *E. coli* from sick pigs to healthy pigs when biosecurity procedures were not used. Handwashing and changing into clean outerwear did not prevent the mechanical transmission of *E. coli* by the caretaker. However, showering and changing into clean outerwear prevented the mechanical transmission of *E. coli* by the caretaker. The study also

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demonstrated that *E. coli* could spread among groups of pigs before diarrhea was observed in infected pigs. In conclusion, under the conditions of this study, showering and changing into clean outwear was the minimum biosecurity standard that could be implemented to prevent caretakers from mechanically transmitting *E. coli* among groups of pigs.

II. Introduction: Post-weaning diarrhea in swine caused by enterotoxigenic *Escherichia coli* has become a frustration for many pork production units. Vaccination of sows pre-farrowing, minimizing management stressors, pulse medication with antimicrobials in the water, and optimization of room cleaning and disinfection are the most common procedures used to control *E. coli* scours in pigs. However, on some farms post-weaning scours due to *E. coli* persists despite vaccination, aggressive use of antibiotics, and improved facility sanitation. Consequently, in these farms, other risk factors contributing to the spread of colibacillosis must be considered. One possibility is that *E. coli* is spread by caretakers tracking the bacteria from infected to susceptible groups of pigs. Determining effective biosecurity measures to prevent the transmission of *E. coli* by people will enable farm personnel, veterinarians, service personnel (electricians, plumbers etc), and visitors to move among farms and address herd needs, without compromising herd health.

IV. Objectives:

1. To determine if people can mechanically transmit enterotoxigenic *Escherichia coli* from infected to susceptible pigs
2. To determine if showering, and putting on clean clothes and boots, or just washing hands and changing into clean clothes and boots can prevent such transmission.

III. Procedures:

One hundred and twenty-five, 19- to 21-day-old weaned pigs, negative to the challenge strain of enterotoxigenic *E. coli* M1823B by fecal culture, were randomly allocated to one of 6 treatment groups:

- *E. coli*-Inoculated Pigs (n=20);
- Direct Pig Contact Sentinels (n=5);
- Direct Human Exposure Sentinels/ Positive Controls (n=25);
- Hand wash/Clean outwear Human Exposure Sentinels (n=25);
- Shower/Clean outwear Human Exposure Sentinels (n=25); or
- Negative Controls (n=25).

Facilities, Environment and Diet: On arrival each group of pigs was maintained in a separate room connected by a common hallway. Direct Pig Contact Sentinels were housed in the same room as Negative Controls for the first 17 hours of the study to avoid contact with challenge-inoculum. Rooms were 3.81 m x 5.18 m with sealed, epoxy-coated floors and two drains. Pigs were housed in 2.42 m x 1.82 m pens with elevated, plastic coated expanded metal flooring, a 1.21 m

long stainless steel nursery feeder, and two nipple waterers. Rooms were HEPA filtered and had negative pressure ventilation. Rooms were cleaned and disinfected prior to the start of the study. Swab samples from the pen floor, waterer, feeder, and room floor of each room were randomly collected and culturally examined. All environmental samples were determined to be sterile prior to pig entry. Pigs were fed a non-medicated diet formulated for nursery pigs ad libitum.

Study design: On arrival, 12 of 20 pigs allocated to the *E. coli*-Inoculated group were fed 2.23×10^{10} to 8.92×10^{10} *E. coli* strain M1823B (Kindly donated by Dr. Harley Moon) in trypticase soy broth diluted 1 vol: 1 vol in liquid strawberry gelatin (Jell-O gelatin dessert, Strawberry, Kraft Foods, Inc., Rye Brook, NY). The M1823B strain of *E. coli* is a K88+, F41+, LT+, and Stb+ ETEC O157:H43 porcine strain. Seventeen hours after arrival, five Direct Pig Contact Sentinels were placed in the same pen containing *E. coli*-Inoculated Pigs. Forty-eight hours after the initial challenge inoculation, eighteen *E. coli*-Inoculated pigs ingested fresh challenge inoculum. Each pig received 1.36×10^{10} to 2.04×10^{10} *E. coli* strain M1823B in trypticase soy broth diluted one vol: one vol in liquid strawberry gelatin.

Personnel procedures: A single caretaker cared for inoculated and sentinel pigs. The caretaker fed pigs, checked waterers, and stood in decks in direct contact with pigs for 10 minutes, in each room, once each day for 10 consecutive days. Pigs were cared for in a consistent order, testing increasing levels of biosecurity (Table 1). A separate caretaker cared for Negative Controls. Hallway traffic patterns were delineated such that caretakers did not cross paths. Each caretaker had a designated shower facility. Standard shower facilities with clean and contaminated sides were used.

Diagnostic evaluation: Caretakers recorded presence or absence of diarrhea in each pig daily. Rectal swabs for fecal culture were collected from all pigs at entry and on days two, four, seven, and 10 post-inoculation. Pigs other than *E.coli*-Inoculated pigs that exhibited diarrhea were removed from the room and were humanely euthanized. All *E.coli*-Inoculated pigs and pigs that did not exhibit diarrhea were humanely euthanized 11 days post-inoculation (at the end of the trial). Complete necropsies were performed on all pigs and gross lesions were recorded. Rectal swabs were collected for isolation of beta-hemolytic *E. coli* with characteristic antimicrobial sensitivity patterns of the challenge isolate. A maximum of six isolates per room were further characterized by detection of pilus and enterotoxin genes using multiplex PCR. Detection of *E. coli* strain M1823B in the feces and/or intestines of sentinel pigs was considered evidence of mechanical transmission of the organism.

Table 1: Pig care procedures.

Caretaker	Order of pig care	Biosecurity procedure to be implemented before entering treatment room	Groups
A	1	Shower in	Negative controls (n=25)
B	1	Shower in	<i>E. coli</i> -Inoculated pigs (n=20) Direct Pig Contact Sentinels (n=5)
B	2	None	Direct Human Exposure Sentinels (n=25)
B	3	None	<i>E. coli</i> -Inoculated pigs (n=20) Direct Pig Contact Sentinels (n=5)
B	4	Don room designated clothes and boots; Wash hands	Hand wash/Clean outerwear Human Exposure Sentinels (n=25)
B	5	None	<i>E. coli</i> -Inoculated pigs (n=20) Direct Pig Contact Sentinels (n=5)
B	6	Shower, don room designated clothes and boots	Shower/Clean outerwear Human Exposure Sentinels (n=25)

VI. Results:

Objective 1: To determine if people can mechanically transmit enterotoxigenic *Escherichia coli* from infected to susceptible pigs

Escherichia coli strain M1823B was isolated from 20 of 25 (80%) of Direct Human Exposure Sentinels based on antimicrobial sensitivity results. The identification of 6 of 6 (100%) isolates cultured from this group was confirmed by multiplex PCR. However, diarrhea was only observed in nine Direct Human Exposure Sentinels culture positive for *E. coli* M1823B. The results of this study support the assertion that people can act as mechanical vectors of *E. coli* when moving from groups of infected to susceptible pigs. Moreover, mechanical transmission occurred under conditions of routine pig contact where the caretaker simply stood in the pen with each group of pigs.

Objective 2: To determine if showering, and putting on clean clothes and boots, or just washing hands and changing into clean clothes and boots can prevent such transmission.

Escherichia coli strain M1823B was isolated from 23 of 25 (92%) of Hand wash/Clean outerwear Human Exposure Sentinels based on antimicrobial sensitivity results. The identification of 6 of 6 (100%) isolates cultured from this group was confirmed by multiplex PCR. Diarrhea was only observed in three Hand wash/Clean outerwear Human Exposure Sentinels culture positive for *E. coli* M1823B.

Diarrhea was observed in three Shower/Clean outerwear Human Exposure Sentinels; however, all pigs remained negative for *E. coli* strain M1823B by cultural examination.

Our results provided evidence that washing hands and donning clean outerwear was insufficient to prevent mechanical transmission of *E. coli* by the caretaker in our study. One reason for the failure of this intervention is that handwashing was not sufficient to remove all *E. coli* containing organic material from the caretaker. In contrast, showering and donning clean outerwear was sufficient to prevent such transmission. Showering provided a whole-body decontamination procedure that was more effective in removing organic material than handwashing.

The effectiveness of biosecurity interventions tested in this study may not reflect their efficacy under field conditions. Large populations of swine, susceptibility of these swine to infection, lack of compliance by personnel, large pathogen loads, and sub-optimal facility sanitation can all confound the efficacy of biosecurity procedures. Producers and veterinarians are encouraged to test biosecurity interventions on their farms to determine the most effective and appropriate biosecurity measures for their specific situation.

The presence of *E. coli* strain M1823B in pigs without diarrhea emphasizes the importance of strict personal hygiene to prevent mechanical transmission of pathogens. Many times, biosecurity procedures are implemented on farms in response to a disease outbreak. Biosecurity protocols must be implemented in a proactive manner because we can mechanically transmit organisms among groups of pigs before we observe clinical signs of infection.

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