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Evaluation of the German cockroach (*Blattella germanica*) as a vector for verotoxigenic *Escherichia coli* F18 in confined swine production

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Abstract

German cockroaches are common pests of confined swine production in North Carolina and other southeastern states. Vector competence of German cockroaches for one of the most important porcine bacterial pathogens, verotoxigenic *Escherichia coli* F18, was evaluated in laboratory bioassays using a culturing approach followed by multiplex PCR. In addition, the populations of fecal coliforms from the feces of piglets and cockroaches collected from a swine nursery were assessed. Viable and virulent cells of *E. coli* F18 were detected in cockroach feces for up to 8 days after the initial exposure. The population of fecal coliforms in cockroach feces was high (4.4×10^5 CFU g^{-1}) and comparable to that of piglet feces (1.9×10^6 CFU g^{-1}). This study demonstrates that cockroaches may serve as important mechanical vectors of pathogenic *E. coli*. Integrated management of cockroach populations should be incorporated into the disease prevention and control programs in the swine industry.

Keywords: *Escherichia coli* F18; German cockroach; Colibacillosis; Fecal coliforms

1. Introduction

Strains of *Escherichia coli* are the most frequently isolated swine pathogens in veterinary diagnostic laboratories (Fairbrother, 1999). Enterotoxigenic and verotoxigenic strains of *E. coli* are the primary cause of enteric colibacillosis in newborn and weaned pigs and edema disease in pigs after weaning (Bertschinger, 1999). *Escherichia coli* with the pilus F18, Shiga toxin (Stx II), and two heat stable toxins (STa, and STb) strikes around 10 days after weaning and causes edema disease and/or diarrhea which result in piglet mortality as high as 40% (Bosworth and Casey, 1997; Moon et al., 1999). Despite advances in research on virulent *E. coli*, the epidemiology and ecology of this pathogen are not well understood.

Increased numbers of hogs per farm and vertical integration of swine farms requires year-long housing in temperature-regulated buildings. This has created an optimal environment for pests that normally infest residential environments. Many farms in North Carolina have thus become heavily infested with large populations of the German cockroach, *Blattella germanica*

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(L.) (Waldvogel et al., 1999; Zurek et al., 2003; Gore et al., 2004). In severe infestations, many thousands of cockroaches can be seen in daytime on and around pig food, watering spouts and pig manure. At night, when cockroaches are more active, it is also common to see cockroaches on piglets and pigs. Vertical integration of the swine industry results in flow of animals, feed, and supplies from central processing facilities to contract farms. This arrangement potentially contributes to dissemination of cockroaches and other insect pests among farms, suppliers, and possibly even workers' homes (Waldvogel et al., 1999).

The objective of this study was to evaluate the vector competence of German cockroaches for one of the important porcine pathogens—verotoxigenic *E. coli* F18.

2. Materials and methods

2.1. Cockroaches

Several hundred cockroaches were collected with a modified vacuum cleaner from the nursery of a commercial swine farm located in Duplin County, NC and transported to the laboratory.

Forty adult cockroaches were randomly picked with soft forceps, divided into two groups of 20 (10 males, 10 females), and transferred to 5 l sterile plastic containers. One group of cockroaches was exposed to *E. coli* F18 (5.0×10^5 CFUs ml) in 10 ml of phosphate buffer solution (PBS, pH 7.2) (ICN Biomedicals, Aurora, OH) supplied in a sterile glass dish for 5 h. The second group of 20 cockroaches was the control and they received 10 ml of sterile PBS. After 5 h, the PBS was removed and replaced with sterile tap water supplied ad libitum in glass tubes with a cotton stopper. Sterilized (autoclaved) piglet feed ration (collected from the swine farm) was supplied ad libitum to both cockroach colonies.

2.2. Bioassays

Every 24 h (for 10 days), both groups of cockroaches were transferred to new sterilized containers with feed and water as described above. Fecal material was aseptically collected and 10 mg of cockroach feces was transferred to 1 ml of PBS and serially diluted. One hundred microliters of each dilution was spread on MacConkey agar plates (Becton Dickinson, Cockeysville, MA) and incubated at 37 °C for 24 h.

2.3. E. coli F18 detection

A maximum of five colonies from each dilution plate with morphology characteristic of *E. coli* were picked and streaked on blood agar plates (Remel, Lenexa, KS) and identified by API Rapid 20E (bioMerieux, Hazelwood, MO) following the manufacturer's instructions. Confirmed *E. coli* isolates were screened for four virulence factors (pilus F18, Stx II, STa, STb) by multiplex PCR (Bosworth and Casey, 1997). Briefly, a loopfull of each isolate was transferred to 200 μl of sterile distilled water in a 1.5 ml sterile microcentrifuge tube, cells were lysed by boiling for 10 min, incubated on ice for 5 min, and centrifuged for 2 min at 11,500 × *g*. Ten microliters of the supernatant was used per PCR reaction. Primers used in this study are described in Table 1. *Escherichia coli* 31385-97 (from the bacterial collection of the Rollins Animal Disease Diagnostic Laboratory, Raleigh, NC) was used as a positive control; *E. coli* ATCC 43894 was used as a non-pathogenic (negative) control. The master mix solution (50 μl) per reaction was composed of 5.0 μl of 10× buffer with $MgCl_2$ (15 mM), 1.0 μl of dNTPs mix (10 mM each), 2.2 μl of $MgCl_2$ (25 mM), 1.0 μl of *Taq* polymerase (all Promega, Madison, WI), 1.0 μl of each primer (50 μM), and 26.8 μl of deionized water. Amplification was done on a PTC 200 thermocycler (MJ Research, Waltham, MA). Cycling conditions were as follows: 90 °C for 5 min, 35 cycles of 90 °C for

Table 1
Primers used in multiplex PCR

Virulence factor	Primer sequence (5′–3′)	PCR product size (bp)
F18	TGG TAA CGT ATC AGC AAC TA ACT TAC AGT GCT ATT CGA CG	313
Sta	CAA CTG AAT CAC TTG ACT CTT TTA ATA ACA TCC AGC ACA GG	158
STb	TGC CTA TGC ATC TAC ACA AT CTC CAG CAG TAC CAT CTC TA	113
Stx II	AAT AGT ATA CGG ACA GCG AT TCT GAC ATT CTG GTT GAC TC	733

1 min, 55 °C for 1 min, 72 °C for 2.5 min, followed by 72 °C extension for 10 min and 4 °C hold. The PCR products were visualized by gel electrophoresis using 4.0% agarose (NuSieve 3:1, BMA, Rockland, ME).

2.4. Fecal coliforms

For assessment of the population size of fecal coliforms, 10 samples of fresh fecal material (approximately 10 g each) of piglets (7 days post-weaning) were collected in sterile plastic bags (Whirl-Pak, MB Co., New Haven, CT) and transported on ice to the laboratory. In addition, approximately 100 cockroaches (various stages) were collected from the piglet pens and transported in a sterile plastic container to the laboratory.

One gram of each fecal sample of piglets was transferred to sterile PBS and serially diluted in PBS to 10^{-6}. One hundred milligrams of fresh cockroach fecal material (up to 4 h old) was collected from a container with cockroaches, transferred to sterile PBS, and serially diluted in PBS to 10^{-5}. Each dilution was drop plated on mFC agar (Oxoid Limited, Basingstoke, Hampshire, England) plates and incubated at 44.5 °C for 20 h. Well-separated blue colonies were counted and the population size of fecal coliforms was expressed as the number of CFU per gram of feces.

3. Results and discussion

Although the population size of *E. coli* F18 in cockroach feces was not assessed, viable and virulent *E. coli* F18 cells (all four virulence genes present) were detected in cockroach feces for up to 8 days after their initial exposure (Fig. 1). The number of CFUs declined over time and no *E. coli* F18 cells could be detected 9 days after exposure. No *E. coli* F18 was detected in cockroach feces in the control group.

The mean population of fecal coliforms in cockroach feces was high ($4.4 \times 10^5\ g^{-1}$) and approximately similar to that found in piglet feces ($1.9 \pm 0.8 \times 10^6\ g^{-1}$).

Escherichia coli with the pilus F18 is different from those with more common pili (K88, 987P, F41, K99) because it only infects piglets at about 10 days after weaning (Bosworth and Casey, 1997; Franck et al., 1998). Apparently, the receptors necessary for *E. coli* attachment by the pilus F18 are not present in the intestinal wall of neonate pigs. *E. coli* F18 contains heat stable toxins (STa, STb) as well as Shiga toxin (Stx II). Consequently, infected piglets may suffer from diarrhea and/or edema disease (Moon et al., 1999).

To prevent and control dissemination of pathogens, the swine industry often adopts an all-in–all-out man-

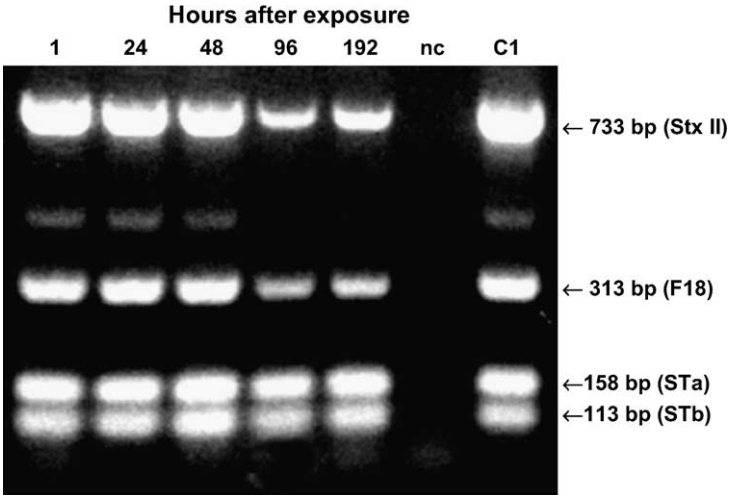

Fig. 1. Presence of virulence genes in *E. coli* isolates from the feces of German cockroaches collected periodically after an initial exposure—nc: negative control (*E. coli* ATCC 43894); C1: positive control (*E. coli* 31385-97).

agement system where sows are separated based on their reproductive stage and weaned piglets are kept in nurseries. Farrowing rooms and nurseries provide excellent conditions for insect pests. Relatively high and stable temperature and air humidity, ample food (swine feed) and water (drinking spouts and sprinklers), and voids in walls provide an ideal environment for large populations of German cockroaches. Visual 15 min counts, conducted during the day, when cockroaches normally hide out of sight, commonly exceed 25,000 cockroaches per farrowing room (Waldvogel et al., 1999; Zurek et al., 2003).

The potential of cockroaches to carry pathogenic bacteria has been investigated in several studies. For example, German cockroaches experimentally infected with *Salmonella typhimurium*, disseminated this pathogen for at least 4 days and contaminated non-infected cockroaches as well as chicken eggs (Kopanic et al., 1994). *Salmonella enteriditis* fed artificially to German cockroaches were recovered from the feces for 10 days and from the gut for up to 20 days after the initial exposure (Ash and Greenberg, 1980). Virulent mycobacteria (*Mycobacteria avium avium*), recovered from nymphs of Oriental cockroaches (*Blatta orientalis*) 10 days after the cockroaches were orally infected, caused avian tuberculosis in chickens (Fischer et al., 2003).

Our findings on the vector competence of cockroaches for *E. coli* F18 and the high number of fecal coliforms in cockroach feces may be important from an epidemiology perspective. Cockroaches have been observed at night on pig manure, feed, and around piglets and pigs (Waldvogel et al., 1999). Movement of cockroaches within infested farms is unrestricted, which leads to a high risk of localized pathogen transmission by this insect. During an outbreak of an infectious disease, every effort is taken to prevent spread of the pathogen within a farm and between farms. Areas of the farm with infected animals are usually isolated, washed, and disinfected. However, cleaning and sanitization temporarily drive cockroaches away from the infected rooms to adjacent areas of the farm. Considering that *E. coli* F18 cells remain viable and virulent after passage through the cockroach digestive tract and are disseminated for several days, there is a high likelihood of spread of this pathogen by cockroaches to different parts of the farm.

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