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The Common Feeder Cockroach *Blaptica dubia* Shows Increased Transmission Distance Based on Mode of Acquisition of Environmental Bacteria

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Running Title: *B. Dubia* Microbial Transmission Distance by Mode of Acquisition

Abstract

Although some researchers claim that cockroaches are masters of disease transmission, these claims have little to no scientific support. Most studies concerning cockroaches as a vector of disease only focus on the bacteria found on the body surface, not on whether cockroaches have actually transferred pathogenic bacteria via surface contact. We set out to determine if cockroaches would act as a mechanical vector for the transfer of the opportunistic pathogen, *E. coli*. Roaches were contaminated with Green fluorescent protein expressing *E. coli* (GFP-E. coli) broth by either walking the roach through a broth culture or by complete immersion in the culture. We then ran the roaches down a sterile agar track and measured the length of the glowing trail. Roaches were able to transmit *E.coli*, but only for a continuous distance of less than 50 cm, with the occasional sporadic colony growing after that. Roaches that were immersed in bacterial broth tracked the bacterium further than those that only walked through the solution. This suggests that while cockroaches are capable of acting as a mechanical vector, they are not capable of transporting transient flora over long distances. Future studies should explore this mechanism.

Introduction

The world we live in is full of pathogens. Our modern Western culture encourages sanitizing everything, yet we are not able to fully separate ourselves from all potential health threats. We still do not fully understand how and to what extent organisms that enter our homes bring microbial life with them. Humans are known to harbor the occasional pathogenic bacteria on our body surfaces (Chiller *et al.* 2001). *Vectors* are any organism that transmit bacteria, viruses or parasites from one organism to another. Vectors can transmit potential pathogens into our environments or even into our bodies in multiple ways. *Transmission* is the passing of a pathogen by direct contact to a new host (Tan *et al.* 1997). Transmission is *biological* if the pathogen reproduces or develops within a potential vector. Transmission is *mechanical* only if there is no reproduction or development of the pathogen in the vector (Mullen and Durden 2009).

Arthropods are regular, oftentimes, unknown visitors to our living spaces, and some are potential vectors of disease transmission. Cockroaches are common arthropods that have been associated with disease for generations, and are commonly found in and around dwelling places. (Moges *et al.* 2016). Because of their association with disease, people often assume that roaches will bring pathogens into these spaces biologically or mechanically (EL-Sherbini and Gneidy 2012).

The American cockroach *Periplaneta americana* and German cockroach *Blattella germanica,* common pest species of roaches, are both members of what the FDA deems the Dirty 22, which consists of the species most commonly associated with the spread of foodborne pathogens (Jones *et al.* 2013). Numerous studies have shown that wild-caught cockroaches do in fact carry various pathogenic bacteria such as *E. coli*, *Salmonella sp*, *P. aeruginosa*, etc., (Tatfeng *et al.* 2005; Fotetar *et al.* 2009; Hamu *et al.* 2014; Xue *et al.* 2009; EL-Sherbini and Gneidy 2012; Moges *et al.* 2016; Mpuchane *et al.* 2006).

Although many pathogens have been recovered from the bodies of natural populations of cockroaches, this does not necessarily mean that cockroaches serve as vectors for these pathogens. Isolation of pathogens from cockroaches may simply indicate the natural microbial fauna and flora of the domestic environment in which they were found (Mullen and Durden 2009). Cockroaches tend to live in dark, damp conditions, such as municipal sewer systems or septic tanks, and this can be a cause for concern because they also are commonly found in living spaces such as pantries and bathrooms. Yet, there have been few studies of the actual transmission of pathogenic bacteria by

cockroaches, or any arthropods. Transmission can be accomplished by behaviors that include walking or landing on a surface, feeding on a substrate, regurgitating on a surface, or defecating on a surface that will then be contacted by another organism such as a human (Foil and Gorham 2004). Few studies directly test the ability of cockroaches to transmit potential pathogens directly. While cockroaches may have been found to have pathogenic strains of bacteria on their surfaces, if they are not able to transmit a meaningful quantity of bacteria by walking across a surface, there would be no reason to see them as a significant vector of disease transmission.

In addition to a lack of direct implication of roaches as vectors, some suggest that chitin and chitosan found in roach bodies have some antimicrobial properties; this could possibly affect the potential transmission of bacteria (Basseri *et al.* 2019). Even knowing this, we hypothesized that the roaches would likely transmit some bacteria mechanically, based on research done on *Musca domestica* and their transmission of Rotavirus from their legs and wings (Tan *et al.* 1997).

In the process of determining if roaches could physically transmit bacteria, we also wanted to determine whether the mechanism of roach contamination would affect the distance that the bacteria would be tracked. Some species of roaches are capable of crawling through plumbing, having been immersed in potentially pathogen-filled fluids, while others may enter homes and merely walk across trash or contaminated surfaces. So, we asked if the mechanism of contamination would affect the ability and efficiency of physical transmission of bacteria.

The species we chose to use is *Blaptica dubia*, a common feeder roach that was readily available in the laboratory. In addition, we chose to use a non-pathogenic strain of *E. coli* (strain HB101 transformed with the pGLO plasmid) to reduce the danger of infection, while still using a microbe very similar to a common pathogen that might be encountered by both an escaping feeder roach or a home invader.

When determining the best course of action in testing our questions, we found there was no standard method of testing the physical transmission of pathogenic bacteria by arthropods. This study will be both a first test of direct physical transmission by a cockroach and also an introduction to a preliminary set of methods that can be altered and improved for future use. With this project, we can begin directly testing long-held assumptions about pathogens and arthropods that we encounter in our daily lives.

Materials and Methods

All animals that we used came from a well-maintained colony of *B. dubia* housed at Harding University. Roaches were not used more than once and were euthanized after exposure to bacterial contaminants.

In order to determine the length of track that we would need, roaches were run down a track made of a 1 m long piece of wood painted black with sides of aluminum flashing 8.89 cm tall. The roaches were placed in a dish of neon orange chalk in which they could walk around before moving down the long track. A dark hiding place was located at the end of the board to encourage the roach to move from the bright lights of the lab to the end of the track. A clear plastic sheet with 5 mm squares printed on it was laid over the board and the number of squares with chalk in them and the length of the trails was measured and recorded. The number of squares with chalk did not turn out to be as useful in analysis so the length of the trails in 5 mm square units was used as the dependent variable for future tests. This test demonstrated that a couple of roaches did track chalk beyond a 0.5 m distance and most chalk was deposited very close to the origin so a 1m track was used to both conserve agarose gel and provide an adequate distance to test for transmission.

Preparation of the Agar track and Bacteria

Aluminum flashing was wrapped in foil and autoclaved at 121 ℃ for 20 minutes. Next, 600 mL of LB agar with 10 % w/v arabinose sugar was poured into the base of the 1 m track made of the pre-sterilized aluminum flashing. This produced a single unbroken sheet of agarose. The arabinose added to the agar was necessary to activate the arabinose operon in the GFP-producing *E. coli.* Colonies that grew would glow under UV light. Plastic wrap was used to cover the opening at the top of the track. This allowed us to both see the roach running and allow light in to motivate roach movement, while limiting airborne bacterial contamination. A new batch of fresh liquid GFP *E. coli* was made up for every trial of the experiment. One hundred ml of *E. coli* was added to 250 ml of broth with 60 ml of arabinose and incubated overnight at 37 ℃.

Mechanical Transmission Assay

Equal numbers of both male and female roaches of at least 1.5 cm were chosen for each trial, and placed in

numbered centrifuge tubes. The roaches were then split into two groups with one group strictly walking through a small petri dish filled with 3 ml of bacterial culture broth and immediately across the agar track, and other roaches were shaken 10 times in a 50 ml conical tube containing 3 ml of bacteria broth solution. Methods were adapted from a study of bacterial sampling from roach and fly bodies in mechanical transmission of medical important parasites (EL-Sherbini & Gneidy 2012). We placed roaches at one end of the track and observed the roaches as they moved to the other end of the track. Normal room lights and a dark hiding spot provided by an egg crate at the end of the track was used to motivate animals to run the length of the track. We noted any stops and other activities of the roaches such as chewing the agar and walking in an irregular pattern. The roaches were euthanized after each trial and the tracks were incubated at room temperature for 48 hours. Next, we observed tracks under a black light and GFP *E. coli* colonies were counted using the plastic square method used in the chalk trials. We measured with a ruler and recorded any long continuous trails of *E.coli*. We also noted any other non-glowing bacterial and fungal colonies.

Data Analysis

We performed a power analysis to determine a proper sample size of 19-26, but were limited by time and resources to a sample size of 12 individual roaches, 8 in each group. We used length of trails made by roaches as the dependent variable in an ANCOVA following a test of normality that caused us to Log transform the data. Roach body length was used as the covariate and method of contamination was used as the independent variable. We used 12 total roaches, 6 shaken and 6 walk through. Roaches that did not leave trails or that did not finish running the track were removed from analysis. Descriptive statistics were graphed using Excel.

Due to our small sample size, we ran an estimate of power for the ANCOVA test of 0.4, using the equation found in McDonald (2015), we found that in order to achieve an 80% to 90% power increase we would have needed to test 19 to 26 individuals of each category of shaken and unshaken roaches. Due to time and money constraints, we were forced to stop our trials after 12 trials.

Results

We found that roaches that were shaken in tubes showed a trend of longer trails of glowing *E.coli* than roaches that walked through the bacteria (df=1; p=0.49). The power for our test was only 40%. The average length of the trail produced by the immersed group was 21.14 cm, while the average length of the walk through group was 4.25 cm (Figure 1). Occasionally isolated colonies were observed beyond the trail but were rare and most GFP *E. coli* were observed in continuous trails.

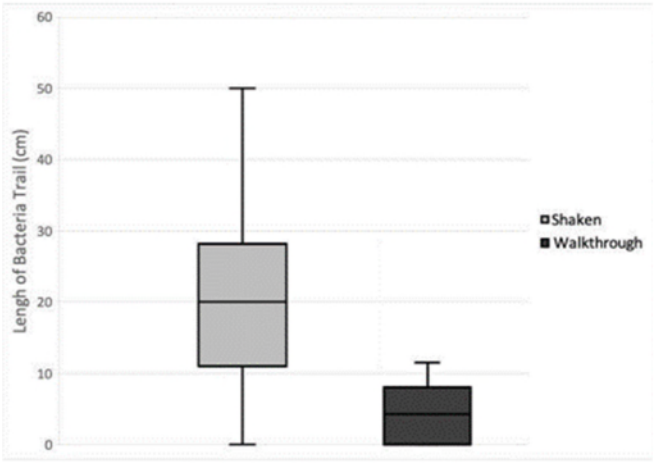

Figure 1: Transmission of GFP *E. coli* by *Blaptica dubia* by different means of exposure. Boxplot of the bacterial trail length in centimeters between *B. dubia* exposed to GFP *E.coli* by being shaken (full body exposure) and walking through a pathogenic broth

It is appropriate to note that other bacterial and fungal colonies were found to be growing on our tracks. We did not identify them at this time. It is assumed that the colony growth was transferred from the roach's body to the agar along with the *E. coli*. This was most likely due to the fact that we introduced our roaches to our E. *coli* broth, straight from their home environment in the lab. This would allow them to transmit some of their own native fauna and flora to our agar.

Discussion

Our tests indicated that roaches were capable of physically tracking *E. coli* by either walking through or by being immersed in it. The trail of bacteria in both situations was less than 0.5 m. The few isolated colonies observed, suggest the potential distance for

transmission is likely longer than our measured trails, but we did not have a large enough sample size to quantify this potential pattern. A power of 80-90% would have required a sample size of between 19-26 roaches per treatment. Despite our small sample size of 6 roaches per group Our observations suggest that roaches could be considered potential vectors, but they would not be efficient at transmission at distances greater than 0.5 m from the source of contamination. It was clear that roaches completely immersed in bacteria tracked microbes over a greater distance than those that merely walked through the petri dish of bacteria broth. Roaches tend to show positive *thigmotactic* behavior (Laurent *et al*. 2018) suggesting that they might be more likely to contact potential pathogens on multiple body surfaces in the tight places they prefer. Because of this, immersive environmental transmission might be the better approximate to a real-world transmission scenario.

Previous studies have repeatedly indicated that human habitations can have potentially-pathogenic bacteria in and around them (Tatfeng *et al.* 2005; Fotetar *et al.* 2009; Hamu *et al.* 2014; Xue *et al.* 2009; EL-Sherbini and Gneidy 2012; Moges *et al.* 2016; Mpuchane *et al.* 2006). This is the first study that has highlighted a roach directly transmitting an introduced bacterium from their body onto a surface. In this study, we did not directly test whether the transmission method was truly mechanical or biological, but due to the short period of time between infection and transmission, it can be assumed that this is an example of mechanical transmission with no pathogenic reproduction or development.

This study was also the first to use common feeder cockroaches as a model. All previous work has focused on well-known pest species, such as the American cockroach and the German cockroach that were caught in the wild, while ignoring the species that people intentionally bring into their homes. Despite many species of roach being considered major pests and health concerns, some people bring roaches into their homes to serve as food for exotic pets, or as the exotic pets themselves. There are a variety of common cockroach species that are found in the pet trade. Just because an arthropod is intentionally brought into a home, does not mean that it is not a health risk if it escapes and crawls through trash or other bacterially-infected substrates. In fact, we found that after our cockroaches were contaminated by the bacterial broth, they tracked the bacteria a relatively short distance, meaning the greatest threat is likely within centimeters of the source of contamination. Using these model organisms does limit our potential conclusions to the more common pest species; however, we have no reason to believe that roach anatomy and exoskeletal physiology differences between the species should prevent us from making tentative and testable predictions about species more relevant to public health. However, if this species can take the place of more troublesome species, then it may represent a good model for future transmission studies looking at potential contamination mechanisms. Further work is needed to support these data and to see if other species of pathogenic bacteria show different transmission patterns. Future tests should also look at whether the results seen in this species can be applied to the more pestiferous species.

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