PREVALENCE AND LOCATION

OF *CRONOBACTER* SPECIES AND ENTEROBACTERIACEA IN HOUSEHOLDS

by

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(Under the Direction of Mary Alice Smith)

ABSTRACT

Cronobacter (*E. sakazakii*) are a genus of bacteria known to cause rare and life-threatening illness in infants. Recent studies suggest *Cronobacter* species infection may be more common than previously thought. *Cronobacter* reservoirs are unknown, but isolates have been recovered from foods, natural environments, and human-made environments. The purpose of this study was to: 1) determine prevalence of *Cronobacter* in the home environment, 2) determine the locations where *Cronobacter* are likely to reside, and 3) to determine the prevalence and distribution of Enterobacteriaceae as an indicator of conditions favorable for *Cronobacter* growth. *Cronobacter* were found in all home types (78.5%); isolated from 24 out of 30 locations sampled, frequently found on floors and walkways; Enterobacteriaceae were found in all homes, all locations, and shared location frequency with *Cronobacter* isolation. *Cronobacter* are rare, but the presence of *Cronobacter* in the home still presents a risk and is a concern for susceptible populations.

INDEX WORDS: *Cronobacter*; *E. sakazakii*; *Cronobacter sakazakii*; powdered infant formula; infants; infection; environmental sampling; domestic environment; households; contamination; food safety

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by

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DEDICATION

I dedicate this to my parents for their love and support of me since I was small; and my siblings, Cassandra and Matthew, who were inadvertently dragged along throughout my life. You may not understand what I do, but I'm still glad you support me in doing it. I love you all and, as always, will "add oil".

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CHAPTER 1

INTRODUCTION

Food safety is a growing public health problem worldwide. Though preventable, foodborne illness occurs through the consumption of contaminated foodstuff. This contamination can occur during food production, food consumption, and any stages in between.

Cronobacter infection is rare and can occur in humans at any age, but typically occurs in the very young or elderly (Patrick et al., 2014). Although *Cronobacter* infection occurs more frequently in adults than children, the symptoms, severity of illness, and sequelae greatly differ.

These bacteria are ubiquitous and a natural reservoir has yet to be identified. It has been hypothesized that these are plant bacteria and have been isolated from a variety of environmental samples—plants, soil, insects, and rodents (C. Iversen & Forsythe, 2003; C. Iversen, Lehner, Mullane, Marugg, et al., 2007). The traits *Cronobacter* exhibit in nature allow for it to thrive and propagate within industrial and domestic environments. Contamination has been mostly associated with powdered infant formula (PIF) and weaning foods; but *Cronobacter* have been isolated from a variety of fresh produce and processed foods—meat, cheese, herbs, teas, and spices (Miriam Friedemann, 2007).

Contamination of food is a safety concern as foodborne illness may result from the consumption of contaminated food (World Health Organization, 2015). A majority of foodborne illnesses originate from the home (Azevedo, Albano, Silva, & Teixeira, 2014; Vaclavik & Christian, 2014). Domestic studies have found *Cronobacter* within the home in places like the refrigerator, sink, and vacuum dust (Kandhai, Reij, Gorris, Guillaume-Gentil, & van Schothorst, 2004; Agnes Kilonzo-Nthenge, Chen, & Godwin, 2008; A. Kilonzo-Nthenge, Rotich, Godwin, Nahashon, & Chen, 2012; Redmond & Griffith, 2009).

This study differs from past research. Home type has not been looked at specifically. Compared to prior domestic environmental studies, this study includes various representative sample locations from the whole home—bathroom, kitchen, thresholds, and floors. Seasonality and the effects of precipitation on *Cronobacter* presence have yet to be investigated.

The literature review (Chapter 2) provides a detailed look into the *Cronobacter* species—susceptible populations, associated illness, epidemiology, and pathogenicity. Additionally, the current understanding on *Cronobacter* sources and the implications of the presence of *Cronobacter* in the domestic environment are discussed.

In chapter 3, this study investigates the occurrence of *Cronobacter* in the domestic environment. Specific aims were 1) to determine if *Cronobacter* are prevalent in homes and 2) what locations in a domestic environment are possible sources of *Cronobacter*. The presence of *Cronobacter* in the home poses a risk for contamination of food products—from the food products themselves or cross contamination from other sources (Azevedo et al., 2014; Vaclavik & Christian, 2014). As a natural reservoir has yet to be identified, identifying sources where *Cronobacter* can survive will allow people to take action in reducing or eliminating the risk of contamination/exposure for susceptible populations. Enterobacteriaceae were also investigated as a general indicator of hygiene and as a potential indicator of habitable conditions for *Cronobacter*.

The appendices contain the flyer used for recruitment (Appendix A), a book chapter (Appendix B), and R coding and output (Appendix C). For Appendix B, we were invited to update a book chapter on intrauterine infections in 2015 for the "Comprehensive Toxicology" textbook published by Elsevier. This text discusses various factors that impact the infection during pregnancy; transmission of infection; adverse outcomes of infection; and a selection of bacterial, viral, parasitic, and fungal examples—including the Zika virus. Appendix C are the R source codes and outputs used in the results in chapter 3.

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CHAPTER 2

LITERATURE REVIEW

Foodborne disease is a serious threat to public health with more than 200 diseases caused by harmful bacteria, viruses, parasites, or chemicals found in food (World Health Organization, 2015). Although foodborne illness is preventable, there is an estimated 600 million people globally (approximately 1 in 10 people) that contract a foodborne illness after eating contaminated food resulting in 42,0000 annual deaths; 40% of these deaths are children under the age of five (WHO, 2015; World Health Organization, 2015).

Indicator organisms, typically bacteria, are used to provide evidence of poor hygiene, inadequate processing, or post-process contamination of foods. They are chosen due to their quick and simple detection. Enterobacteriaceae and coliform bacteria are two of the most common groups of indicator organism used by the food industry. Coliforms were historically the most common indicator group tested for by the food industry; but depending on the country and regulatory requirements, the food industry has started transitioning towards Enterobacteriaceae testing (Baylis, Uyttendaele, Joosten, & Davies, 2011).

ENTEROBACTERIACEAE

Commonly referred to as enterobacteria or "enteric bacteria"; the Enterobacteriaceae are a large family of Gram-negative bacteria that includes many of the more familiar pathogens such as *Salmonella, Escherichia coli*, *Yersinia pestis*, *Klebsiella*, and *Shigella*. Members of Enterobacteriaceae are rod shaped, non-spore-forming, and typically 1-5 μm in length (Baylis et al., 2011). They are facultative anaerobes and, with a few exceptions, most share the ability to reduce nitrate to nitrite. A uniquely common feature of this family is the lack of cytochrome C oxidase, with some

exceptions. Enterobacteriaceae ferment carbohydrates and can produce acid and gas from fermentation of D-glucose— an important diagnostic property for detection and enumeration.

The members of Enterobacteriaceae are widely distributed. Different levels of Enterobacteriaceae can be cultured from a variety of materials, depending on the origin of the raw material and on the control of hygiene. Their normal habitats include water, soil, the gut flora in the intestines of humans and other animals (Jung, Choi, & Lee, 2013; Kandhai, Reij, Gorris, Guillaume-Gentil, & van Schothorst, 2004; Khan, Jones, & Cerniglia, 1998; Mozrova, Brenova, Mrazek, Lukesova, & Marounek, 2014). The Enterobacteriaceae family have been used as indicator organisms by the food and water industry (Baylis et al., 2011).

CRONOBACTER SPECIES

Cronobacter species (*Cronobacter*) are motile, Gram-negative bacilli approximately 3 x 1 μm with peritrichous flagella, oxidase-negative, catalase-positive, facultative anaerobic, and uniquely able to hydrolyze α-glucosidase. Additionally, most *Cronobacter* strains (80%) produce a non-diffusible yellow, carotenoid-based pigment (a trait that contributes to UV tolerance) when grown on tryptic soy agar, blood agar, and brain heart infusion agar at 25°C for 48-72 hours; pigment production is temperature dependent (Kucerova, Joseph, & Forsythe, 2011).

Cronobacter are UV tolerant, able to survive long periods of desiccation, and are capable of growing between 8-58°C (C. Iversen & Forsythe, 2003; Kandhai et al., 2004; N. R. Mullane et al., 2007). These traits combined with the ability to form biofilms allow for *Cronobacter* to adhere to many surface types and persist for extended period of time in a variety of environments (Dancer, Mah, Rhee, Hwang, & Kang, 2009; Angelika Lehner et al., 2005). However, *Cronobacter* are unable to survive pasteurization (C. Iversen & Forsythe, 2003).

Susceptible Populations and Associated Illness. Most of the *Cronobacter* species (6 out of the 7) are infectious to humans. While infections are rare in humans, when infections occur, adults are primarily affected; however, invasive infections occur more in infants (Patrick et al., 2014). The

pathogen is commonly associated with severe and life threating illness of neonates and immunocompromised infants.

Newborns and infants, especially low birth weight infants (less than 2.5kg), are at a particular risk for *Cronobacter* infection. Approximately 50% of children infected are younger than one week old; susceptibility to infection decreases with increasing age (Arena N. Richardson, Pollak, Williams, Agyekum, & Smith, 2012). Contact with *Cronobacter* in the birth canal or post-birth environmental sources may result in infection; although, consumption of contaminated powdered infant formula (PIF) is more often implicated (Food and Drug Administration, 2012; Muytjens et al., 1983). The infection typically has a high case-fatality rate ranging from 40-80% (M. Friedemann, 2009). Symptoms of infection occur within a few days; they are often severe and may include poor feeding response, irritability, jaundice, grunting respirations, instability of body temperature, seizures, brain abscess, hydrocephalus and developmental delay (Food and Drug Administration, 2012). Infections leading to death originate from bloodstream and central nervous system infection—meningitis, bacteremia/sepsis, and necrotizing enterocolitis—those that survive often suffer from severe neurological sequelae (Food and Drug Administration, 2012; Lai, 2001). The susceptibility and severity of *Cronobacter* infection decreases between infants < 1 month and 1-11 months of age (FAO/WHO, 2008; Arena N. Richardson et al., 2012).

Adults are typically overlooked in regard to *Cronobacter* infection, but most infections occur in adults—particularly the elderly, immunocompromised (including pre-existing conditions), and those previously treated with antibiotics (Jason, 2015). In adults, *Cronobacter* infection causes symptoms typically insignificant to the patient's underlying medical problem and/or other infections (Jason, 2015). The *Cronobacter* incidence rates for adults ≥ 80 years of age and adults 70-79 years of age were higher than rates for any those < 1 year of age (Jason, 2015; Patrick et al., 2014). Symptoms of infection vary from wound infections, conjunctivitis, biliary sepsis, urosepsis, appendicitis, and pneumonia.

Cronobacter species and strains originate from different sources; it has been noted that only select few species and strains cause severe illness in infants or adults. Infants <1 year of age have the greatest incidence rates for invasive *Cronobacter* infection; only 3 species (*C. sakazakii*, *C. malonaticus*, and *C. turicensis*) have been isolated from infected neonates (Kucerova et al., 2010). PIF is the primary source of invasive infection in infants; sources of *Cronobacter* infection/colonization in adults have suggested to be infections either by nosocomial transmission and/or potentially contaminated nutritional (powderized) supplements (Jason, 2015; Kucerova et al., 2011).

Taxonomy. *Cronobacter* are a member of the Enterobacteriaceae family and are closely related to the *Enterobacter* and *Citrobacter* genera. The *Cronobacter* species have been subject to many taxonomic changes since its first recorded case. Revisions to the taxon of *Cronobacter* are still discussed with the advent of genomic sequencing and new analysis techniques.

Before the 1980's, *Cronobacter* were referred to as a single species of yellow pigmented *Enterobacter cloacae* due to the strains' ability to produce yellow-pigmented colonies on tryptic soy agar, blood agar, and brain heart infusion agar after 48-72 hours of incubation at 25°C (Baylis et al., 2011; Urmenyi & Franklin, 1961). In the 1980's, the pigmented coliform was named *Enterobacter sakazakii*— after the Japanese microbiologist Riichi Sakazaki in honor of his contributions to bacterial taxonomy— having been distinguished from *Enterobacter cloacae* based on DNA-DNA hybridization, pigment production, biotype assignment, and antimicrobial resistance; 15 *Enterobacter sakazakii* biogroups were described, with a single biotype commonly found (Baylis et al., 2011; Farmer, Asbury, Hickman, & Brenner, 1980).

In 2007, *Cronobacter* was proposed as a new genus, after using a polyphasic taxonomic approach—a combined fatty acid and DNA sequencing analysis, as to clarify the taxonomic relationship of the biogroups found among strains of *Enterobacter sakazakii* (C. Iversen, Lehner, Mullane, Bidlas, et al., 2007; Carol Iversen et al., 2008). The name "*Cronobacter*" originates from the Greek noun Cronos— who was one of the Titans of Greek mythology, who swallowed each of his

children as soon as they were born— and the New Latin noun bacter—a rod— resulting in a New Latin word for "rod that causes illness in neonates". The taxon was revised in 2008 naming 6 species and 3 subspecies in the newly formed genus (C. Iversen, Lehner, Mullane, Bidlas, et al., 2007; Carol Iversen et al., 2008).

There are currently 7 species in the genus— *C. sakazakii, C. malonaticus, C. turicensis, C. muytjensii, C. dublinensis, C. condimenti,* and *C. universalis* (*C. genomospecies 1*) (Joseph et al., 2012; Tall et al., 2014). An additional 3 non-pathogenic *Enterobacter* spp.—*E. pulveris*, *E. helveticus*, and *E. turicensis*— have been proposed to be included as members of *Cronobacter*, although these were excluded by Iversen et al. (2008) due to insufficient biological basis to support revision (Brady, Cleenwerck, Venter, Coutinho, & De Vos, 2013; Carol Iversen et al., 2008; Tall et al., 2014). *C. condimenti* is the only species not shown to be infectious to humans (Joseph et al., 2012).

The taxonomic changes are a complication in the study of *Cronobacter*. As the members of the *Cronobacter* genus were once known as a single species, it is uncertain which specific *Cronobacter* species were referred in publications pre-2008.

History and Epidemiology. Cases of *Cronobacter* infection have been documented, but not necessarily published, in infants and young children (primarily those under three years of age) since 1961 (FAO/WHO, 2008). The first reported cases originated in England by Uremenyi and Franklin in 1958. This case involved two infants initially recovering well from illness when their health declined rapidly to the point of death; causes were from neonatal meningitis by a "yellow-pigmented *Enterobacter cloacae*" (Urmenyi & Franklin, 1961). The surfaces in the hospital were investigated, but the sources of infection could not be determined (Urmenyi & Franklin, 1961). As the infants were separated, these two infants could not have contracted the infection from each other (Urmenyi & Franklin, 1961).

Sources of *Cronobacter* could not be determined at this time; but continued investigation of outbreaks with *Cronobacter* infection among neonates in neonatal intensive care units (NICU)

produced results indicating statistical and microbiological association between infection and contaminated PIF consumption (Biering et al., 1989; Simmons, Gelfand, Haas, Metts, & Ferguson, 1989; Van Acker et al., 2001). Two outbreaks from Belgium (1998) and the United States (2001) solidified this link; in both instances *Cronobacter* was isolated from unopened and opened cans of PIF from the lots used to feed neonates in the NICUs (CDC, 2002; Simmons et al., 1989; Van Acker et al., 2001).

The association with these infections and contaminated PIF prompted regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the World Health Organization/Food and Agriculture Organization (WHO/FAO) to establish guidelines for the safe preparation and handling of PIFs and a Call for Data was issued (FAO/WHO, 2004). The 2006 statistic for annual incidence among low-birth-weight infants (< 2,500g) and those < 1 year of age was 8.7 per 100,000 in the United States; no global active surveillance was available for tracking at this time (FAO/WHO, 2006). The WHO Expect Panel found 120 reported cases of infection were in children < 3 years of age tracked from 1961-2008 globally; actual cases were presumed to be under reported (Control & Prevention, 2009; FAO/WHO, 2008).

The year 2008 saw an outbreak in New Mexico involving two non-hospitalized unrelated infants born full term and with no complications (Control & Prevention, 2009). Although both infants consumed the same brand of PIF, clinical isolates of *Cronobacter* were different and were not linked to the manufacturer (Control & Prevention, 2009). *Cronobacter* was isolated from one of the infant's opened canisters of PIF and from vacuum dust from the same home (Control & Prevention, 2009). The PIF and clinical isolates were found to be the same (Control & Prevention, 2009). The vacuum dust was a different strain and was not implicated in the contamination of the PIF (Control & Prevention, 2009).

In 2011, four cases of *Cronobacter* infection from different states were reported—Florida, Illinois, Missouri, and Oklahoma (CDC, Media statement for immediate release, 2011). Laboratory

tests found *Cronobacter* in an opened container of PIF, an opened bottle of nursery water, and reconstituted PIF (CDC, Media statement for immediate release, 2011). No information supported contamination from production or shipping, suggesting local contamination (CDC, Media statement for immediate release, 2011).

Cronobacter have been associated primarily with infants and the contaminated formula they consume. This focus has led to great advances and awareness in food safety to the production and use of PIFs and weaning foods; but there is a large gap in the information about infection in adults. Adults are infected more frequently, particularly the elderly and immunocompromised (CDC, 2012; Patrick et al., 2014). Data on adult infections are scarce; but from a few studies it can be gleaned that there are some critical difference in how adults react to select species and particular strains compared to how neonates and young infants react (Jason, 2015; Patrick et al., 2014). The Centers for Disease Control and Prevention is typically informed of four to six cases in infants each year, but reporting is not required so the true number of cases is unknown.

Pathogenicity. *Cronobacter* species that have been associated with clinical infections are considered potentially pathogenic and vary in their virulence (Caubilla-Barron et al., 2007). The virulence of *Cronobacter* are dose-dependent due to characteristics of specific strains within a certain species (A. N. Richardson, Beuchat, Lambert, Williams, & Smith, 2010; Zhou et al., 2015). *Cronobacter* are intracellular pathogens, granting them the ability to evade immune responses from the host (A. N. Richardson, Pollak, Williams, & Smith, 2010). Some can invade human intestinal epithelial cells, replicate in macrophages, and invade the blood-brain barrier (Caubilla-Barron et al., 2007; Liu et al., 2012; Zhou et al., 2015).

It is presumed that *Cronobacter* gain entrance to the human body through the gastrointestinal track where they may cause necrotizing enterocolitis (Van Acker et al., 2001). Entrance into the systematic circulation through transcytosis of the intestinal epithelium was suggested in an in vitro model (Giri et al., 2012). Once in the in the blood, *Cronobacter* circulates and can invade tissues

throughout host and possibly cross the blood-brain barrier and enter the central nervous system (A. N. Richardson, Pollak, et al., 2010).

Treatment of *Cronobacter* infection involves antibiotics. An antibiotic or combination of antibiotics are administered to patients and successful treatment may take days (A. N. Richardson, Pollak, et al., 2010). The clearing of the infection varies due to the resistance and sensitivity of the particular species and/or strain causing the infection (A. N. Richardson, Pollak, et al., 2010).

***Cronobacter* reservoirs.** Ubiquitous in nature, isolates have been recovered from a variety of sources including the environment, clinical sources, and a wide range of foods (Miriam Friedemann, 2007; Gurtler, Kornacki, & Beuchat, 2005; Holy & Forsythe, 2014; A Lehner & Stephan, 2004). The characteristics and traits *Cronobacter* have allow for them to survive in a variety of environments. There are no known natural reservoir.

Cronobacter are presumably plant associated and have been associated with sources such as fresh produce, herbs, teas, and dried spices (C. Iversen, Lehner, Mullane, Marugg, et al., 2007; Schmid et al., 2009). Beyond these flora samples *Cronobacter* have been isolated from soil and animal sources—insects and rodents (C. Iversen & Forsythe, 2004; Khan et al., 1998; A. N. Richardson, Pollak, et al., 2010). Attempts to culture *Cronobacter* from surface water, rotting wood, grain, bird droppings, domestic animals, cattle, and cow's milk yielded no isolates (Muytjens & Kollee, 1990).

Although the initial sources are unknown, hospitals and clinical samples could be considered potential sources of nosocomial *Cronobacter* infections. *Cronobacter* isolates have been found in clinical samples of cerebrospinal fluid, blood, bone marrow, sputum, urine, inflamed appendix, neonatal enteral feeding tubes, and conjunctivae (Kucerova et al., 2011). Additionally, reports have been made that some humans may be asymptomatic carriers (intestines and throat) and could transfer *Cronobacter* to others by human-to-human transmission (Kucerova et al., 2011).

The presence of *Cronobacter* in human-made environments presumably originates from sources in nature. Though this may be the primary way of introducing the bacteria to the environment, traits of the bacteria allow for it to persist in environments by adhering to various surfaces such as silicon, latex, polycarbonate, and stainless steel (C. Iversen & Forsythe, 2003). *Cronobacter* have been reported to attach and form biofilm on glass and polyvinyl chloride allowing for persistence and potential cross contamination (Angelika Lehner et al., 2005). These common materials often are used during processing, machinery, or as structural elements in various environments such as food processing facilities, clinical settings, and domestic settings.

The relationship between *Cronobacter* infection and PIF can be linked to both food processing facilities (contaminated after pasteurization and drying processes and before packaging due to poor processing procedures or contaminated machinery) and clinical/domestic settings (contaminated ingredients after initial usage or contaminated preparation utensils) (N. Mullane et al., 2006; Muytjens, Roelofswillemse, & Jaspar, 1988). PIF is the vehicle most often implicated in *Cronobacter* infections especially in cases of infants (Miriam Friedemann, 2007; Angelika Lehner et al., 2005). These cases typically result from intrinsically or extrinsically contaminated reconstituted PFI and/or extrinsically contaminated equipment used to prepare the PIF (N. Mullane et al., 2006; Muytjens et al., 1988).

There are a couple of properties that PIF have that allow it to be susceptible for *Cronobacter* contamination and growth. PIF is not a sterile product, with no final "kill-step". A kill-step is the term typically used to describe a point in the food manufacturing process where potentially deadly pathogens are eradicated from the product— traditionally through cooking, pasteurization, pathogen-killing washes, irradiation, etc. Ingredients used during production may be heat treated and sterilized when combined; but there is no kill-step after the powdering and drying processes. Irradiation is a method used to sterilize PIF, but with some strains of *Cronobacter* having high tolerance to temperatures, the additional heat poses a threat to the degradation of the nutrients in PIF and not necessary the elimination of *Cronobacter* in the PIF (Giovannini et al., 2008). The

properties of reconstituted PIF provide favorable conditions for the growth of *Cronobacter*. Growth is encouraged contaminated reconstituted formula is left at room temperature for extended time periods (A. N. Richardson, Pollak, et al., 2010). Nutrients added to the formula, such as sialic acid—to promote infant development and growth— may be utilized by *Cronobacter* to grow. This is dangerous since there is a dose dependent link to infection (Arena N. Richardson et al., 2012).

Cronobacter have been isolated from the domestic environment (Azevedo, Albano, Silva, & Teixeira, 2014; Chap et al., 2009; Agnes Kilonzo-Nthenge, Chen, & Godwin, 2008; A. Kilonzo-Nthenge, Rotich, Godwin, Nahashon, & Chen, 2012). These isolates have come from various sources such as vacuum dust, cleaning sponges, dish cloths, refrigerators, dried spices, and other fresh or processed foods (Kandhai et al., 2004; Agnes Kilonzo-Nthenge et al., 2008; A. Kilonzo-Nthenge et al., 2012). Like in clinical settings, contamination has been linked to contaminated preparation utensils or ingredients (water) in cases of contaminated PIF (CDC, Media statement for immediate release, 2011).

Implications of *Cronobacter* in the domestic environment. Unlike food processing facilities and clinical settings, proper food safety cannot be enforced and hygiene varies from individual to individual (Azevedo et al., 2014; Vaclavik & Christian, 2014). The presence of *Cronobacter* in the domestic environment allows for potential contamination during food preparation.

The knowledge base of *Cronobacter* infection is heavily skewed towards neonates and infants, but infection occurs at higher rates in the elderly and immunocompromised (Jason, 2015; Patrick et al., 2014). The prevalence in these adult populations have increased, especially in individuals that may require the use of a protein or powdered nutritional supplement for their diet (FAO/WHO, 2008; Gosney, Martin, Wright, & Gallagher, 2006). This is a concern for public health as the population ages and the needs for synthetic, dehydrated nutritionals or adult supplements may be required to maintain good health in old age (FAO/WHO, 2008; Tall et al., 2014) .

CONCLUSION

Cronobacter infection, through rare, can affect the public. The progress in quality control and the practice of proper food safety has reduced the potential of *Cronobacter* exposure/contamination for PIF and other foodstuffs similar in production and reconstitution methods in the food processing industries. Still, a majority of foodborne illness originate from at home contamination due to improper food safety and personal hygiene.

The reservoirs for *Cronobacter* are still unknown; and sources are continually discovered. As infants are not the only vulnerable populations in a home. Determining sources in the home will allow for the better hygiene practices in the home, reduce risk of infection caused by *Cronobacter* contamination, and improve public health.

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CHAPTER 3

THE OCCURANCE OF *CRONOBACTER* SPECIES FROM URBAN, SUBURBAN, AND FARM DOMESTIC ENVIRONMENTS IN GEORGIA, UNITED STATES[1]

[1] Monica Y. Chan, Jordan Zambrana, Edith Marie McKenzie, Mary Alice Smith, 2016. To be submitted to the *Journal of Food Protection*.

ABSTRACT

Cronobacter species, has been implicated in cases of severe meningitis, sepsis, and necrotizing enterocolitis in premature and full-term infants. Infection has been linked to contamination of powdered infant formula with a particular species, *Cronobacter sakazakii*. The sources of contamination have been linked to production environments, product storage containers, and utensils used during food preparation suggesting contamination from the environment. A natural reservoir for its genus, *Cronobacter*, has not been identified, but isolates have been found in various foods, water, soil, and a myriad of environmental, industrial, and domestic environments. The presence of *Cronobacter* species in the home poses a risk for contamination of food products—from the food products themselves or cross contamination from other sources. Specific aims of this study were to determine if *Cronobacter* species are prevalent in homes and what locations in a domestic environment are possible sources. Enterobacteriaceae were similarly investigated as a general indicator of hygiene and as a potential indicator of habitable conditions for *Cronobacter* due to being related. The occurrence of *Cronobacter* species in different homes were estimated over the course of 18 months by taking 30 samples from locations in each of 65 homes. *Cronobacter* isolates were found in a majority of homes (78%). Home type (urban, suburban or rural), sample location, the season that samples were collected, average precipitation of the month the samples were collected, and the number of people that reside in the home significantly affected *Cronobacter* recovery. *Cronobacter* species can be found in homes, primarily on the floors and thresholds of the domestic environment.

BACKGROUND

The *Cronobacter* genus (*Cronobacter*), formerly known as *Enterobacter sakazakii (30, 31)*, are a diverse group of opportunistic Gram-negative bacilli of the Enterobacteriaceae family. The genus consists of seven species *(6, 31)*: *C. sakazakii*, *C. malonaticus*, *C. turicenisis*, *C. muytjensii*, *C. dublinensis*, *C. universalis*, and *C. condimenti (35)*. These hardy bacteria are motile *(11, 13)*, UV tolerant *(28)*, osmotolerant *(14, 17, 18)*, thermotolerant *(14, 49, 50)*, and have the ability to produce biofilms *(11, 29, 44)*. These traits which allow them to survive in various environments in nature *(22, 28, 39)*, food processing facilities *(20, 37, 46)*, hospitals *(7, 26, 48)*, and domestic settings *(37, 40, 41)*.

The species have been primarily associated with severe infection *(27)* in newborns and infants resulting in sepsis, meningitis, and necrotizing enterocolitis with estimated mortality rates of 40-80% *(21, 43)*; those that survive often develop chronic neurological issues resulting in permanent developmental challenges *(19, 43)*. Contact with *Cronobacter* in the birth canal or post-birth environmental sources *(48)* may result in infection; although, consumption of contaminated powdered infant formula (PIF) is more often implicated *(19, 48)*.

Outbreaks have been correlated with unopened and opened cans of PIF used to feed patients in neonatal intensive care units *(9, 15, 28)*. Cases of infection that were not linked to an intrinsic contamination sources were linked to extrinsic contamination on PIF preparation tools such as blenders and bottle-cleaning brushes *(10, 47)*. Infection is not limited to neonates and infants, as most infections occur in adults—particularly the elderly and immunocompromised *(33, 34, 51)*. The prevalence of infections increased for adults who have health conditions that requiring the use of supplements in their diet *(16, 23, 42)*. The epidemiology of these supplement-related infection cases suggested sources of contamination in the home environment *(25, 37, 40, 51)*.

Cronobacter are ubiquitous and a natural reservoir for the species is unknown. *Cronobacter* have been isolated from various foods *(3, 5, 55)* and in natural *(22, 28, 39)*, industrial *(20, 37, 46)*, clinical *(7, 26, 48)*, and domestic environments *(37, 38)*. Surveillance studies have implicated that the

presence of *Cronobacter* in dry ingredients *(32)* and dry environments *(12)* as the most likely source of contamination during production *(56)*.

Food production facilities are aware of and mandated for quality control, but food products can become contaminated at any point of production and distribution; in fact, a large proportion of foodborne disease incidents are caused by foods improperly prepared or mishandled at home, in food service establishments or markets *(57, 58)*. The presence of *Cronobacter* in the domestic environment allows for potential risk during food preparation. By determining how prevalent *Cronobacter* are in a home and sources where *Cronobacter* can survive, action can be taken to reduce or eliminate the risk of contamination. The purpose of this study is to investigate the occurrence of *Cronobacter* in the domestic environment, specifically: 1) to determine if *Cronobacter* are prevalent in homes and 2) what locations in a domestic environment are possible sources of *Cronobacter*.

MATERIALS AND METHODS

Recruitment and designation of households. The project was approved by the University of Georgia's Institutional Review Board. A total of 65 households participated in this study. Participants were recruited in the state of Georgia, United States via flyer postings in public areas, the University of Georgia Cooperative Extension Service, and word of mouth. Because having infants or infant formula in the home could be a confounder to the study, applicants were pre-screened by telephone to ensure that homes had no children under the age of five and/or had not had infant formula in the household within three years.

Study homes were separated into three home types depending on the location and population of the community: urban, suburban, and farm. Urban and non-urban households were separated into these categories based on the participant's home zip code, city associated with the zip code, and description of urban areas from the Federal Register on Qualifying Urban Areas for the 2010 Census *(8).* Non-urban households were subcategorized into suburban and farm homes. The farm category was based on the home with at least one person in the household who worked on the property to grow commercial crops and/or raise livestock.

Survey questionnaire collection and compensation. A survey was given to each participating household head to complete (FIG 3.1). The questionnaire inquired about composition of household and some socioeconomic information. All surveys and samples were coded with a unique identifier and all information was collected and stored anonymously. Compensation for time consisted of a $40 USD grocery gift card.

Sample collection. A total of 65 households participated in this study. A total of 30 sample locations, if available, were collected from four areas in the home: kitchen, bathroom, threshold (areas around defined doorways leading from the outside to inside the house), and floors (samples from floors and samples that represent high traffic areas). Samples were collected using 3M™ Sponge-Sticks with neutralizing buffer over a 1 m^2 area. Swabs with neutralizing buffer were also

used for the sampling of faucet and drains. Once collected, samples were stored on ice and transported to the lab to be processed within 24 hours.

Isolation of *Cronobacter* and Enterobacteriaceae. Processing methods were modified from Kilonzo-Nthenge et al. (2012) to isolate *Cronobacter (41)*. Samples were enriched with 90ml of BBL™ Buffered Peptone Water (Becton Dickinson) for 24 hours. Aliquots of enriched samples were taken for further enrichment of Enterobacteriaceae in E.E. Broth (Oxoid) or selective enrichment of *Cronobacter* with Cronobacter Screening Broth Base (Oxoid).

After incubation at 37°C overnight, samples enriched with E.E. Broth were isolated on to Violet Red Bile Glucose Agar (Becton Dickinson). Simultaneously, cultures of Cronobacter Screening Broth expressing a change of color indicating sucrose fermentation, were streak plated on to *Brilliance*™ Enterobacter sakazakii Agar (DFI) (Oxoid).

Violet Red Bile Glucose Agar plates that exhibited colonies characteristic to Enterobacteriaceae were isolated onto Difco™ Tryptic Soy Agar (Becton Dickinson) to be tested for the oxidase reaction. Blue-green pigmented colonies on DFI agar indicated an α-glucosidase hydrolysis reaction identifying presumptive *Cronobacter* species. Presumptive *Cronobacter* colonies were confirmed using RapID™ ONE System (Remel). Confirmed isolates were stored at -80°C on beads for future analysis.

Statistical analysis. All analyses were done in R 3.2.2. using logistic regression and Poisson regression *(52)*. Significance was set at $P \leq 0.05$.

RESULTS

Occurrence and Prevalence of *Cronobacter* species in households. *Cronobacter* isolates were recovered from 51 out of 65 households (78.5%): 19 out of 22 urban (86.4%), 18 out of 21 suburban (85.7%), and 14 out of 22 farm (63.6%) home types (FIG 3.2). All homes had Enterobacteriaceae present (100%) from some location in the household.

To determine whether home type (urban, suburban or farm homes) impacted whether *Cronobacter* are likely to be found in the domestic environment, we compared the prevalence of *Cronobacter* in these three home types. Thirty sample locations identified for sampling in each of the 65 participant households resulted in a total of 1,841 samples collected (TABLE 3.1). A total of 148 isolates out of 1,841 sample locations were identified as *Cronobacter* positive (8.04%); 1,410 out of 1,841 samples were identified as Enterobacteriaceae positive (76.6%). The average percentage of samples from which *Cronobacter* species were isolated from each home type were found to be significantly different— the farm homes were significantly less ($P \leq 0.05$) likely to have isolates than the urban and suburban homes (FIG 3.3).

Prevalence of *Cronobacter* isolates in samples collected from areas (categorized sample locations from a similar area) in the home were all found to be significantly different (FIG 3.4). Samples from the floor ($P \leq 0.05$) had the highest percentage of *Cronobacter* isolates followed by the threshold ($P \leq 0.05$), and kitchen ($P \leq 0.05$); the bathroom ($P > 0.05$) had the fewest *Cronobacter*.

The area in a home where *Cronobacter* was found most often was on the floor, as shown by the frequency from which *Cronobacter* was detected in vacuum cleaner dust (50%) and directly from floors (FIG 3.5; TABLE 3.1). A total of 24 out of 30 locations sampled in the homes were found to have *Cronobacter* in at least one of the 65 participant homes (FIG 3.2). *Cronobacter* was not isolated from 6 of the 30 sample types: from bathroom doorknob, bathroom sink faucet handle, bathroom sink faucet head, and toilet bowl, front entranceway doorknob, and tap water (TABLE 3.1).

External Effects and Resident Habits on *Cronobacter* Presence in a Home. Factors and habits, separate from the home type and structure, could affect the presence of *Cronobacter* in the home. We examined several of these factors including the season, average temperatures, and level of precipitation present when samples were collected. We also used a survey of each household to determine effects of number of people residing in the household, age, education level, etc. (FIG 3.2).

Seasons were determined by the astronomical seasons for 2014 and 2015: Spring = March 20 to June 20; Summer = June 21 to September 21; Autumn = September 22 to December 20; Winter = December 21 to March 19). Samples taken from a home during the autumn ($P \leq 0.05$) were found significantly more likely to have *Cronobacter* present in more locations than samples taken during the spring ($P \leq 0.05$) (FIG 3.6).

The average temperature and precipitation values were taken from the National Oceanic and Atmospheric Administration's National Climate Data Center within a set state by month. Higher precipitation values correlate with an increased number of *Cronobacter* samples ($P \leq 0.05$) (FIG 3.7). Average temperature did not significantly affect the number of samples recovered ($P > 0.05$).

Homes with more residents ($P \leq 0.05$) correlated with the increased number of *Cronobacter* isolates from location samples (FIG 3.8). Although the residents were instructed to abstain from any non-routine cleaning prior to the sampling visit, a question on the survey inquired about cleaning as well as several other household characteristics. Neither cleaning prior to the sampling visit ($P > 0.05$) nor the number of pets owned by the household ($P > 0.05$) affected *Cronobacter* isolate recovery from a home (data not shown).

DISCUSSION

Cronobacter was found in a majority of homes in our study regardless of whether it was urban, suburban, or farm. Farm homes had significantly fewer samples from which *Cronobacter* isolates were recovered than urban and suburban homes. *Cronobacter* have been isolated at a high frequency from external locations compared to indoor locations *(12)*. Assuming that movement in and out of urban, suburban and farm homes were relatively similar, we expected that farm homes would be more likely to have *Cronobacter* present due to occupation and proximity of the home to an agricultural setting. However, our data suggest that farm homes were less likely to have *Cronobacter* isolates. It was observed during sampling of farm homes that workwear was typically left outside of the home, for example boots and jackets were often found by the door or in the garage. This habit may reduce the amount of external particulates that are tracked into the house despite being exposed to environmental sources.

Specific areas within households were found to be significantly correlated in the recovery of *Cronobacter* isolates from sample locations. *Cronobacter* distribution have been linked to areas with frequent foot traffic such as walkways *(12)*. Household dust has been reported to contain *Cronobacter (37)*; for our study vacuum dust was a sample considered to be representative of all walkways in the home. Half of the homes sampled had *Cronobacter* present in their vacuum dust. Floors and thresholds are places in the home constantly exposed to various foot traffic, and frequently we isolated *Cronobacter* from these locations. The bathroom floor, one of the floor samples with fewer percentage of samples positive for *Cronobacter* (13.8%), had more isolates than the other samples from the bathroom. The reduction in retrieved isolates may be due to habits regarding cleaning of bathrooms, as the recovery of Enterobacteriaceae were also fewer in the bathroom than other places in the home and included the sink faucet head, the sample with the lowest percent Enterobacteriaceae recovery (36.5%).

Similar to the bathroom location, *Cronobacter* was isolated more frequently from the kitchen floor compared to other kitchen samples, and the kitchen floor had the most samples positive for *Cronobacter* (23.1%; TABLE 3.1). Prior studies have observed *Cronobacter* in domestic kitchens and refrigerators *(40, 41)*. The recovery of *Cronobacter* in this study are comparable to the results of Kilonzo-Nthenge et al. (2012) who examined the presence of *Cronobacter* in domestic kitchens— for example, they recovered 4% from sponges compared to the 6.8% recovery for sponges in this study *(41)*. Additional comparisons to the results of Kilonzo-Nthenge et al. (2012) to our recovery of *Cronobacter* in the same locations are similar— refrigerator handles (12%) and meat drawers (4%); compared to our refrigerator samples of door handle (1.5%), meat drawer (1.5%), vegetable drawer (3.1%), and primary shelf (1.5%) *(41)*. Variances in recovery may be due to location or in the refrigerator itself; use of the drawers and shelves in the refrigerator are not standard across homes, for instance many of the homes did not specify which drawer was for meats or vegetables. Isolate recovery from refrigerator vegetable drawers (3.1%) and the spice storage (3.1%) are the same; supporting literature suggests that *Cronobacter* may originate from plants and has been isolated from spices *(28, 32, 42)*.

Seasonal variation affected the number of *Cronobacter* isolates recovered from a home. *Cronobacter* seasonal variation has not been studied; but studies on seasonal variation for Gram-negative bacilli such as the closely related Enterobacter, may be comparable *(1, 53)*. Seasonal variation studies on Gram-negative bacilli have noted seasonal variation which have been linked to infection, such as increased urinary tract infections during particular seasons *(1, 53)*. Seasonality results may also be impacted with temperatures when people would be relatively more active outdoors. Literature suggests that the movement of air and particulates factor in the distribution of *Cronobacter*; increased foot traffic and environmental conditions coupled with the species' ability to withstand desiccation may explain the increased distribution in homes *(2, 12, 54)*.

Differences in the average temperature during sampling events were not significant in affecting the number of isolates recovered. Correlation was found between the amount of precipitation and the number of isolates recovered. Due to the anonymous nature of our data collection, exact temperatures and precipitation information was not recorded; instead, historical data regarding the average temperatures and precipitation levels were collected ex post facto. The data used were the averages in Georgia, United States taken from each month sampled and do not account for the nuanced changes of temperature or precipitation in a particular location. Temperature may not have affected presence due to samples being collected from the interior of the home where the environment is typically maintained separate from the environment. Habits of the residents during wet periods may affect *Cronobacter* distribution in a home in conjunction with the increased amount of environmental debris that may have adhered to clothing and shoes worn during and in rain.

Survey data was compiled to capture conditions of the entire household. The total number of people residing in the home increased the number of isolates recovered in the home. The more residents in a household, the more likely to have an increase in the foot traffic in the home. Having more individuals increase the potential incidents of contact with external sources of *Cronobacter.* Other factors such as having a gardening hobby, number of pets in the household, and cleaning prior to the study's sample collection did not significantly affect the prevalence of *Cronobacter* in homes.

Interestingly, the number of pets in the home did not significantly increase the likelihood of finding *Cronobacter* in the home. *Cronobacter* have been found in soil, insects and rodents; contact with these sources when a pet is outside could have increased the amount of positive isolates in the home *(36, 37, 39, 45)*. However, it was not the case in our study. It may be that the effects of the pets were masked by the already large number of floors and walkways that were positive for *Cronobacter*; the locations most likely to be effected by pets.

Those who participated in this study were instructed to avoid any intense cleaning outside of regular maintenance before we collected samples. The survey included a section regarding additional cleaning of places in the home to account for any radical reduction in bacterial retrieval. No significance was found, despite additional cleaning in 28 out of 65 homes (43.1%). This suggests that additional cleaning did not affect presence of *Cronobacter* in the home and/or indicate that the locations sampled were areas more difficult to clean or not typically cleaned thoroughly.

Homes with *Cronobacter* present were analyzed further to determine if there were any significant effects that would make a home more likely to produce isolates. A total of 1,442 samples were collected from 51 homes that had *Cronobacter* present; 148 samples (10.3%) were *Cronobacter* isolates and 1,125 samples (78.0%) were Enterobacteriaceae isolates. Similar to the overall data, locations and seasons were significant in determining the number of samples recovered in a home; the increasing number of residents in a home were positively correlated with the number of isolates recovered from a home. The only difference from the overall data was that there was no significant difference in isolate recovery from different home types ($P > 0.05$).

In this study, samples were examined for both *Cronobacter* and Enterobacteriaceae. Enterobacteriaceae was collected to determine general hygiene and used as an indicator for locations that may allow for the survival of *Cronobacter (4)*. Enterobacteriaceae were found in all homes from at least one location; home type was not significant in the number of locations from which Enterobacteriaceae was recovered in a home. The presence of Enterobacteriaceae were affected by the specific area sampled in the home and the seasons when the samples were collected. The average temperature, average precipitation, and total number of people in the home did not significantly affect the presence of Enterobacteriaceae. Locations where Enterobacteriaceae was recovered did differ from *Cronobacter*; for instance, the bathroom floor (96.9%) and the kitchen sink countertop (96.9%) were samples that frequently had Enterobacteriaceae present in a home. The comparison is interesting as it suggests that the kitchen sink countertop (typically used for food

preparation) is equally likely to have Enterobacteriaceae present as the bathroom floor (TABLE 3.1). Another interesting comparison is that vacuum dust (94.8%) samples did not have the most Enterobacteriaceae present like the *Cronobacter* vacuum dust (50%). Enterobacteriaceae survival in vacuum dust has been measured over a period of 60 days with a decline in bacterial counts with time; habits vary in the frequency of vacuuming, which may have attributed to this result *(24)*. Other factors such as number of residents in the home, having a gardening hobby, number of pets in the household, and cleaning prior to the study's sample collection did not significantly affect the prevalence of Enterobacteriaceae in homes.

Comparing *Cronobacter* and Enterobacteriaceae, there are similarities and differences. Both are affected by location and the seasons when the sample was taken. Enterobacteriaceae is not significantly affected by the number of residents in the home in contrast to *Cronobacter*. This suggests that *Cronobacter* distribution is dependent on humans and their movements/activity within the home.

In summary, *Cronobacter* was found in 78.5% of homes primarily on the floors and thresholds of the home. Farm homes were least likely to have *Cronobacter* in the home. The season when the samples were collected affected the amount of samples from a home with more found during the summer and autumn sampling and the less during the winter and spring. The number of people residing in the home is positively correlated to the number of *Cronobacter* isolates from a home.

While our study identified isolates for the genus *Cronobacter*, we did not identify the samples to the species level; thus, we could not determine if the species of *Cronobacter* most often associated with illness, *Cronobacter sakazakii*, is/are present in the home. Future sequencing of the collected *Cronobacter* isolates would yield insights into the exact species found in the homes. Determining the species will allow for a better risk estimate of exposure in the home.

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FIGURE LEGENDS

FIG 3.1. Questionnaire given to participants.

FIG 3.2. Prevalence of *Cronobacter* spp. in homes with *Cronobacter* spp. present in at least one location. No significant differences ($P < 0.05$) were detected between home types. Bars represent standard error. N= 65 homes (urban = 22, suburban = 21, farm = 22).

FIG 3.3. The average percentage of samples from which *Cronobacter* spp. were isolated from each urban, suburban, or farm home. Urban and non-urban homes groups were separated based on data provided by the United States' 2010 Census *(8)* (see methods section for exact details). The number of *Cronobacter* isolates recovered was determined to be significantly different in farm homes ($P \leq 0.05$). Bars represent standard error. ***** indicates significance. N = 65 homes (urban = 22, suburban = 21, and farm = 22); 1,841 samples (urban = 611, suburban = 603, and farm = 627) and; 148 *Cronobacter* isolates (urban = 63, suburban = 50, and farm = 35).

FIG 3.4. Prevalence of *Cronobacter* spp. from various areas in the home. Areas are grouped according to location and organized by proximity or "boundaries" within particular areas in a home. The Floors group is unique as it is not limited to a specific area in a home; samples in this group were taken from areas of high foot traffic in the home. A significance was detected in all areas within the home ($P \leq 0.05$). Floors ($P \leq 0.05$) and thresholds ($P \leq 0.05$)— samples located on or near the entrances and egresses of the home— were more likely to produce samples positive for *Cronobacter*. Bars represent standard error. A, B, C, and D indicate significance. N = 65 homes; 30 sample types from each home (bathroom = 7, kitchen = 13, threshold = 4, floors = 6); and 1,841 total samples collected from all homes (bathroom = 450, kitchen = 837, threshold = 245, floors = 309).

FIG 3.5. Prevalence of *Cronobacter* spp. isolated from each location within a home and organized in descending order of prevalence. Samples with higher prevalence primarily originate from

the floor. *Cronobacter* was not isolated from 6 locations—bathroom doorknob, sink faucet handle I, sink faucet head I, toilet bowl, front entranceway doorknob, and tap water—and were not included in this figure (TABLE 3.1).

FIG 3.6. Prevalence of *Cronobacter* from home types with *Cronobacter* present taken during different seasons. Seasons were determined by the astronomical seasons for 2014 and 2015 (Spring = March 20 to June 20; Summer = June 21 to September 21; Autumn = September 22 to December 20; Winter = December 21 to March 19). A significant difference was seen in the seasonal recovery of *Cronobacter* from samples ($P \leq 0.05$). Samples taken during the autumn were most likely to recover *Cronobacter* in various locations; samples taken during spring were from fewer locations. Bars represent standard error. A and B indicate significance. N = 51 homes (urban = 19, suburban = 18, farm = 14).

FIG 3.7. Comparison of the average measured precipitation (cm) of specific months in Georgia, USA and the number of *Cronobacter* samples recovered during the various precipitations recorded. Trend line show a positive correlation of increased precipitation and increased number of isolates. N = 65 homes (urban = 22, suburban = 21, farm = 22).

FIG 3.8. Comparison of the number of residents in a particular home type and number of *Cronobacter* positive sample locations in the household. Trend line for the all homes show a positive correlation between the number of *Cronobacter* recovered and number of people residing in the home. N = 54 homes (urban = 19, suburban = 18, farm = 17).

TABLE 3.1. Percentages of samples tested positive for the presence of *Cronobacter* and Enterobacteriaceae taken from different locations within study homes

Areas	Location Sample	N[a]	Number of *Cronobacter* Isolates	% of Samples positive for *Cronobacter*	Number Enterobacteriaceae Isolates	% of Samples positive for Enterobacteriaceae Isolates
Bathroom	Sink Countertop	65	2	3.10	52	80.0
	Tub/Shower Drain	63	1	1.60	46	73.0
	Sink Basin	65	1	1.50	52	80.0
	Bathroom Doorknob	64	0	0.00	24	37.5
	Sink Faucet Handle	65	0	0.00	36	55.4
	Sink Faucet Head	63	0	0.00	23	36.5
	Toilet Bowl	65	0	0.00	41	63.1
Kitchen	Sink Drain	65	10	15.4	55	84.6
	Sink Basin	65	7	10.8	60	92.3
	Sponge	59	4	6.80	54	91.5
	Sink Countertop	65	3	4.60	63	96.9
	Refrigerator Vegetable Drawer	65	2	3.10	57	87.7
	Spice Storage	65	2	3.10	50	76.9
	Pantry	64	1	1.60	52	81.3
	Sink Faucet Handle	64	1	1.60	57	89.1
	Refrigerator Door Handle	65	1	1.50	54	83.1
	Refrigerator Meat Drawer	65	1	1.50	52	80.0
	Refrigerator Primary Shelf	65	1	1.50	61	93.8
	Sink Faucet Head	65	1	1.50	39	60.0
	Tap Water	65	0	0.00	25	38.5
Threshold	Front Entranceway Floor	63	17	27.0	61	96.8
	Garage / Back Entranceway	57	8	14.0	48	84.2
	Back Entrance Doorknob	61	2	3.30	28	45.9
	Front Entranceway Doorknob	64	0	0.00	32	50.0
Floors	Vacuum Dust	58	29	50.0	55	94.8
	Garage Floor	45	17	37.8	42	93.3
	Kitchen Floor	65	15	23.1	62	95.4
	Main Walking Routes	55	12	21.8	52	94.5
	Bathroom Floor	65	9	13.8	63	96.9
	Mop Sample	21	1	4.80	14	66.7

[a] Total of 65 households were sampled. N= number of households where each sample was collected; for example, not all homes had a garage floor to be sampled.

FIG 3.1

Please read and answer all the questions below. Once completed, seal in provided envelope. No one will be able to associate the survey with the house.

1. **Gender of person answering questions:**
   Female Male

2. **Marital status:**
   _____ Married
   _____ Single
   _____ Divorced/ Separated
   _____ Widowed

3. **Race**:
   _____ African American
   _____ Asian/Pacific Islander
   _____ Hispanic
   _____ White
   _____ Other: _____

4. **Total Number of People in Household**:
   1 2-3 4-5 6+

5. **Age and number of all residents in each age group in household** *(Please indicate the number in household)*:
   _____ 0-4 years
   _____ 4-12 years
   _____ 13-17 years
   _____ 18-29 years
   _____ 30-44 years
   _____ 45-59 years
   _____ 60-69 years
   _____ 70+ years

6. **Education** *(Please indicate the number in household)*:
   _____ Infant/toddler, no school
   _____ Preschool-kindergarten
   _____ Elementary-high school
   _____ Less than High School
   _____ High school diploma or GED
   _____ Some college
   _____ College graduate
   _____ Graduate/postgraduate

7. **Employment status** *(Please indicate the number in household)*:
   _____ Full time
   _____ Part time
   _____ Unemployed
   _____ Retired
   _____ Student/homemaker

8. **Do you work in any of the following areas?**
   _____ Food Preparation
   _____ Farmer/Gardener
   _____ Child Care (Under the age of 5)
   _____ Health Care
   _____ None of the Above

9. **Household income:**
   _____ Less than $15,000
   _____ $15,000-$34,999
   _____ $35,000-$49,999
   _____ $50,000-$74,999
   _____ $75,000+

10. **Has an infant consuming powdered infant formula visited the house within the last 5 years?**
    Yes No

11. **Has powdered infant formula been prepared in the house within the last 5 years?**
    Yes No

12. **Have you used any disinfectants (bleach, Lysol, 409, etc…) in the past 4-5 days?**
    Yes No
    If "yes", what areas?
    _____ Kitchen
    _____ Bathroom
    _____ Floors

13. **Are there any pets in this Household?**
    Yes No
    If so, how many and what kind?
    _____ Dog(s)_____Cats(s) _____Other

14. **Do you use well water?**
    Yes No

FIG 3.2

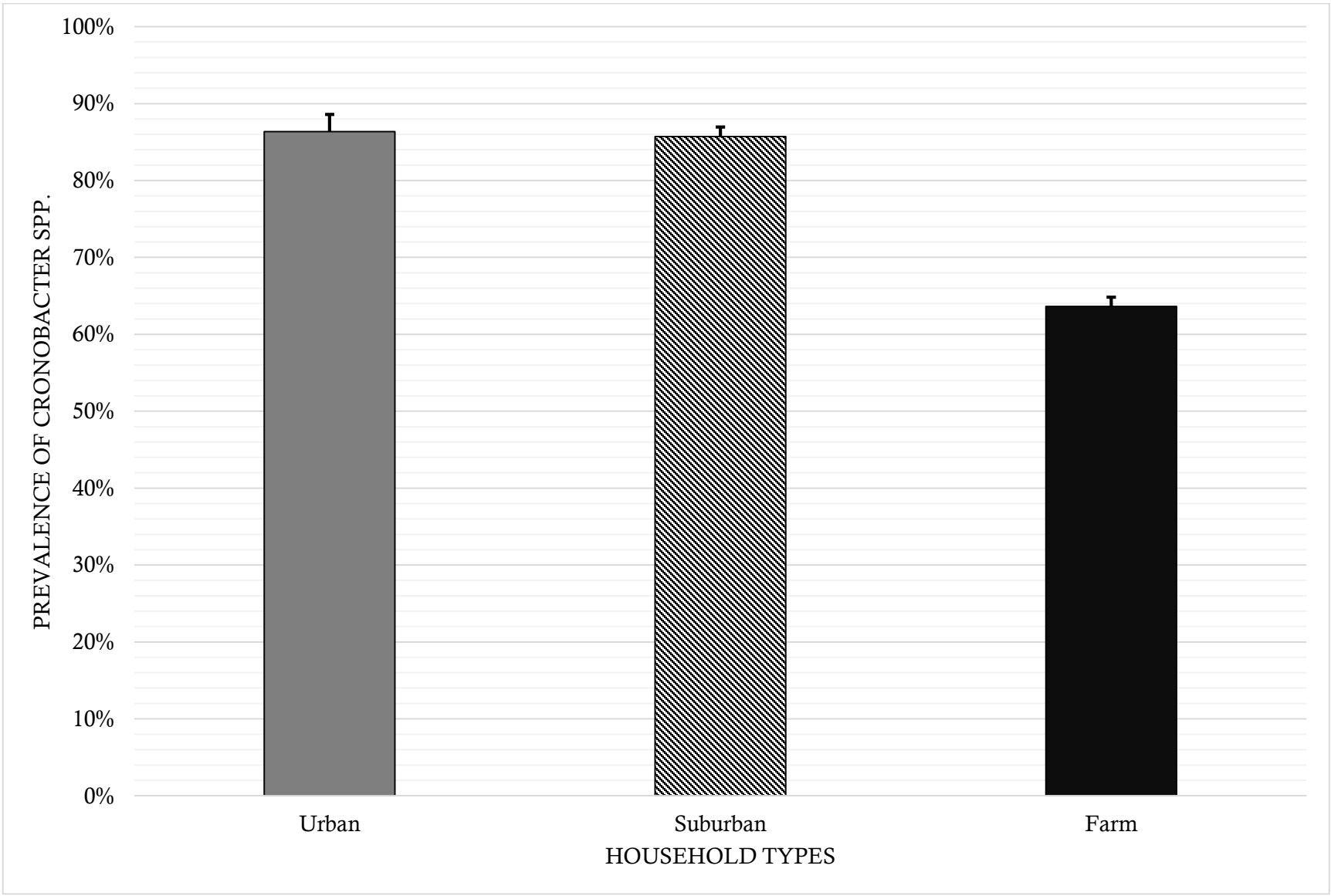

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
PREVALENCE OF CRONOBACTER SPP.
Urban
Suburban
Farm
HOUSEHOLD TYPES

FIG 3.3

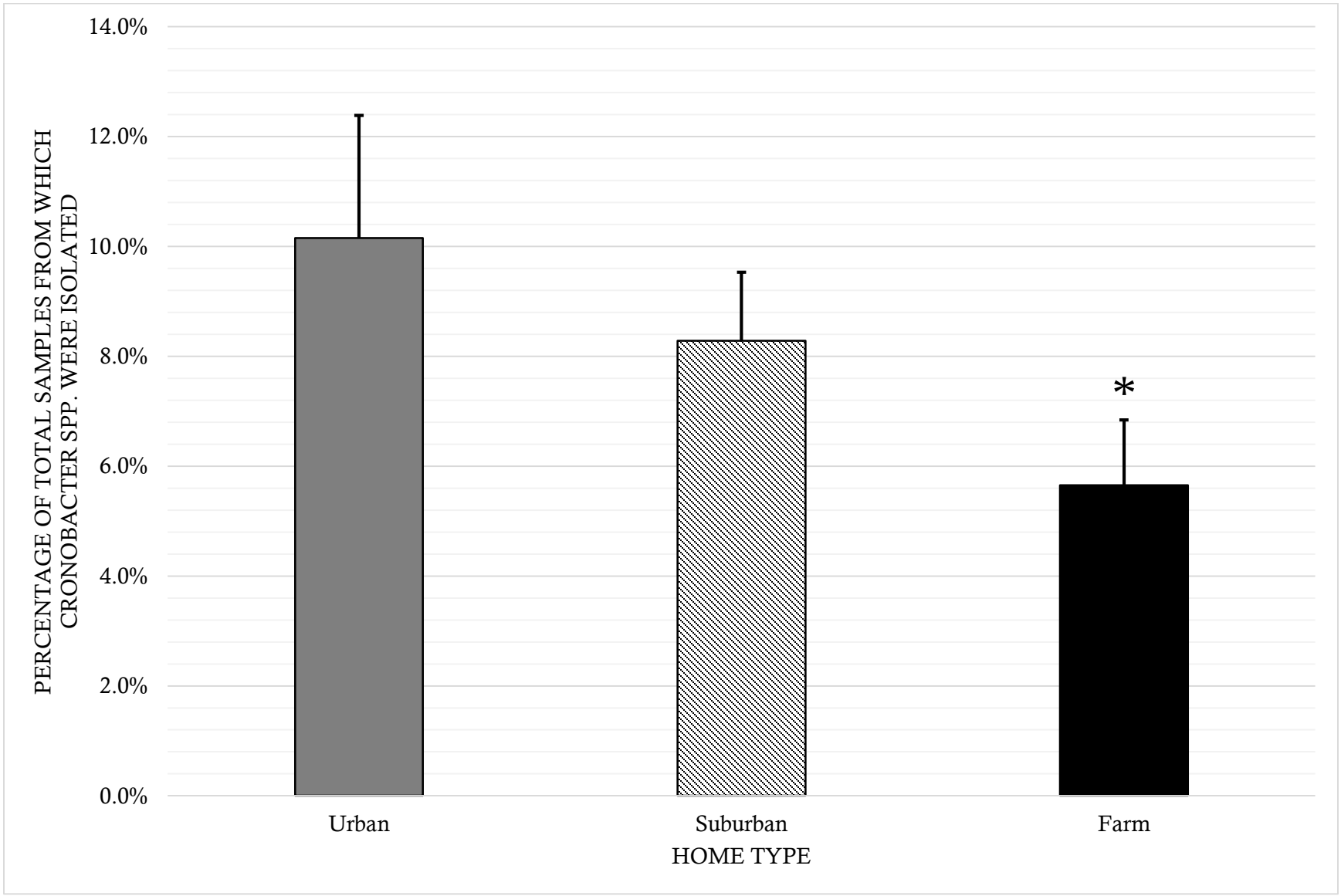

14.0%
12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
PERCENTAGE OF TOTAL SAMPLES FROM WHICH CRONOBACTER SPP. WERE ISOLATED
*
Urban
Suburban
Farm
HOME TYPE

FIG 3.4

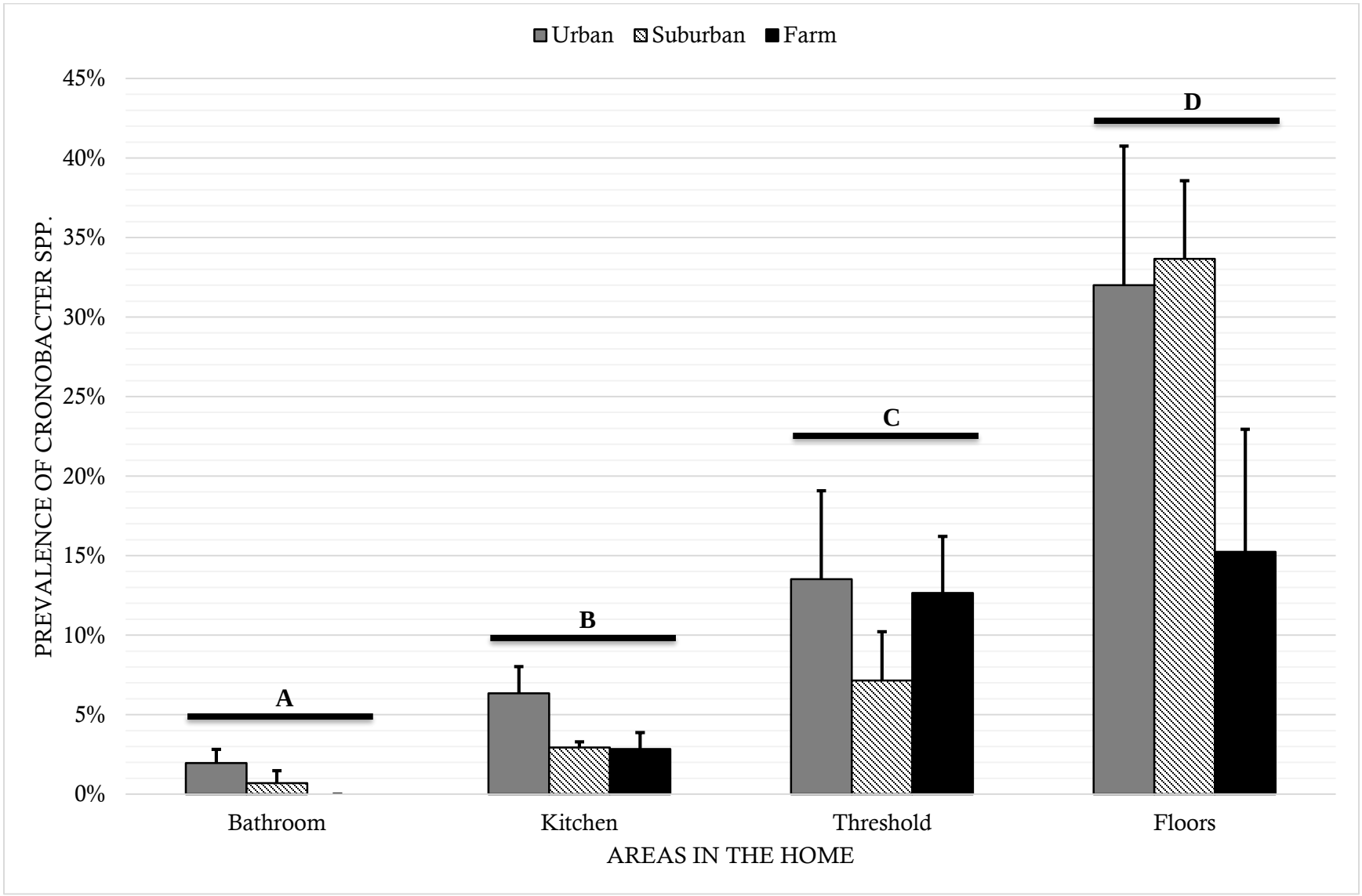

Urban
Suburban
Farm
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
PREVALENCE OF CRONOBACTER SPP.
A
B
C
D
Bathroom
Kitchen
Threshold
Floors
AREAS IN THE HOME

FIG 3.5

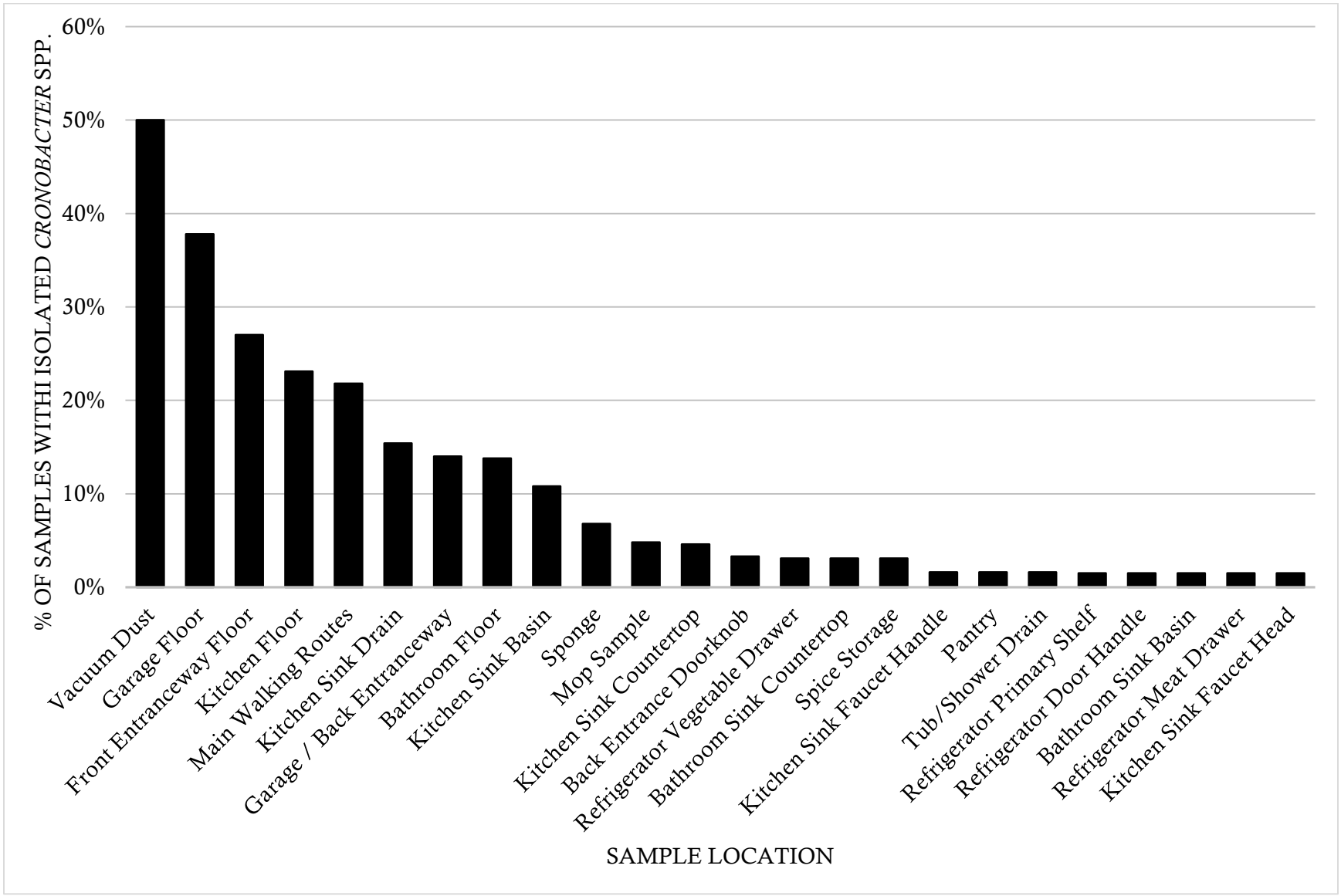

60%
50%
40%
30%
20%
10%
0%
% OF SAMPLES WITHI ISOLATED CRONOBACTER SPP.
Vacuum Dust
Garage Floor
Front Entranceway Floor
Kitchen Floor
Main Walking Routes
Kitchen Sink Drain
Garage / Back Entranceway
Bathroom Floor
Kitchen Sink Basin
Sponge
Mop Sample
Kitchen Sink Countertop
Back Entrance Doorknob
Refrigerator Vegetable Drawer
Bathroom Sink Countertop
Spice Storage
Kitchen Sink Faucet Handle
Pantry
Tub/Shower Drain
Refrigerator Primary Shelf
Refrigerator Door Handle
Bathroom Sink Basin
Refrigerator Meat Drawer
Kitchen Sink Faucet Head
SAMPLE LOCATION

FIG 3.6

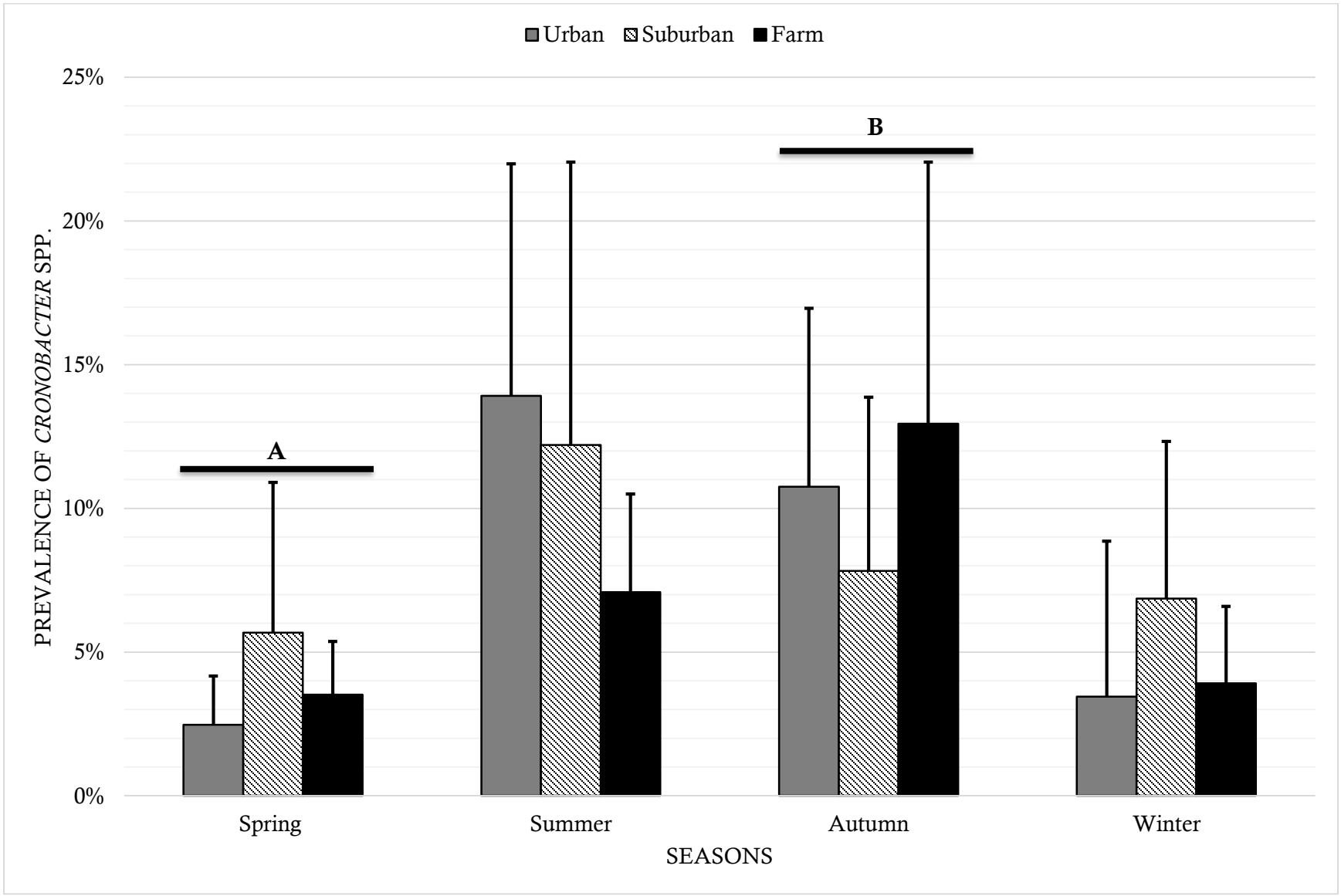

Urban
Suburban
Farm
25%
20%
15%
10%
5%
0%
PREVALENCE OF CRONOBACTER SPP.
A
B
Spring
Summer
Autumn
Winter
SEASONS

FIG 3.7

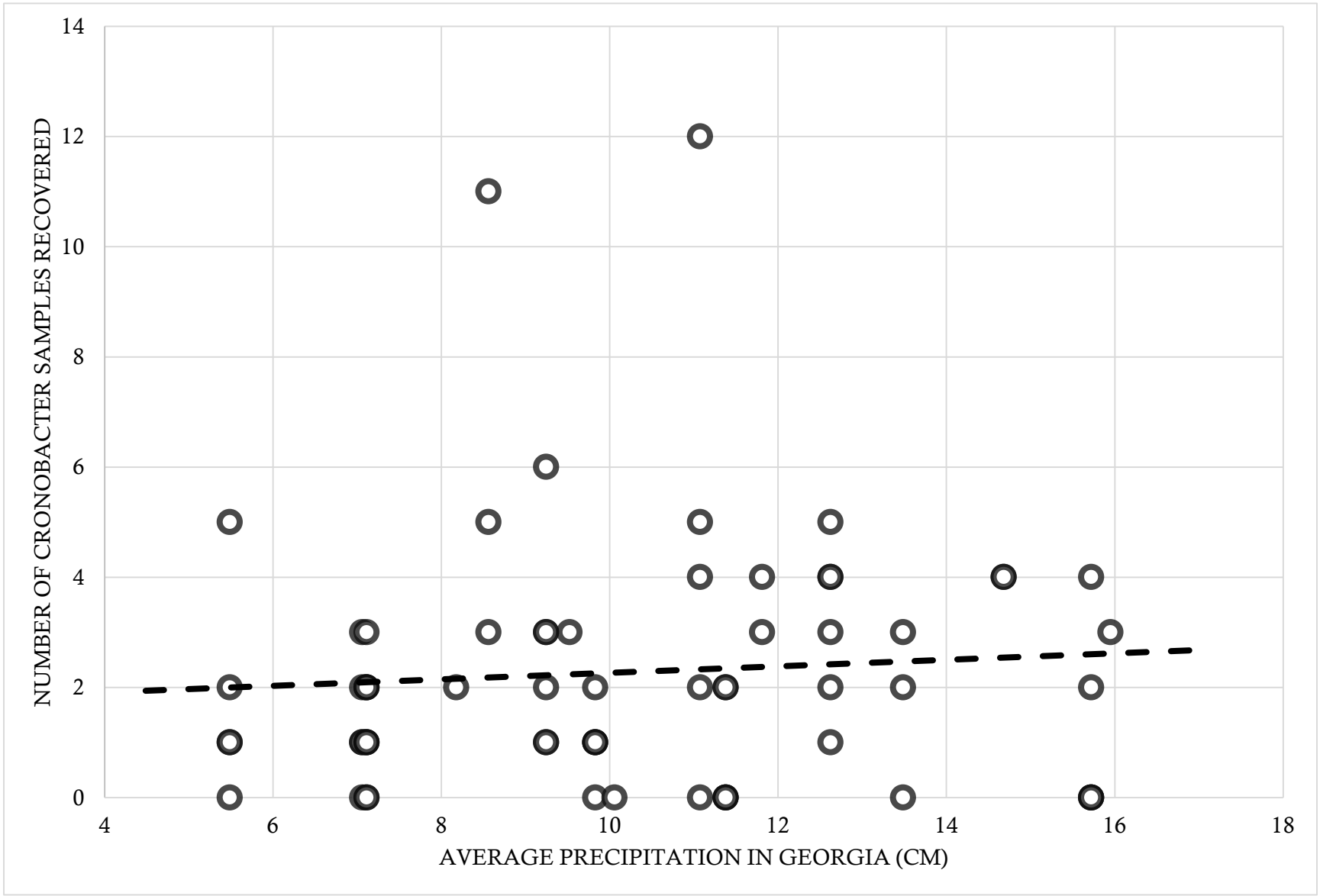
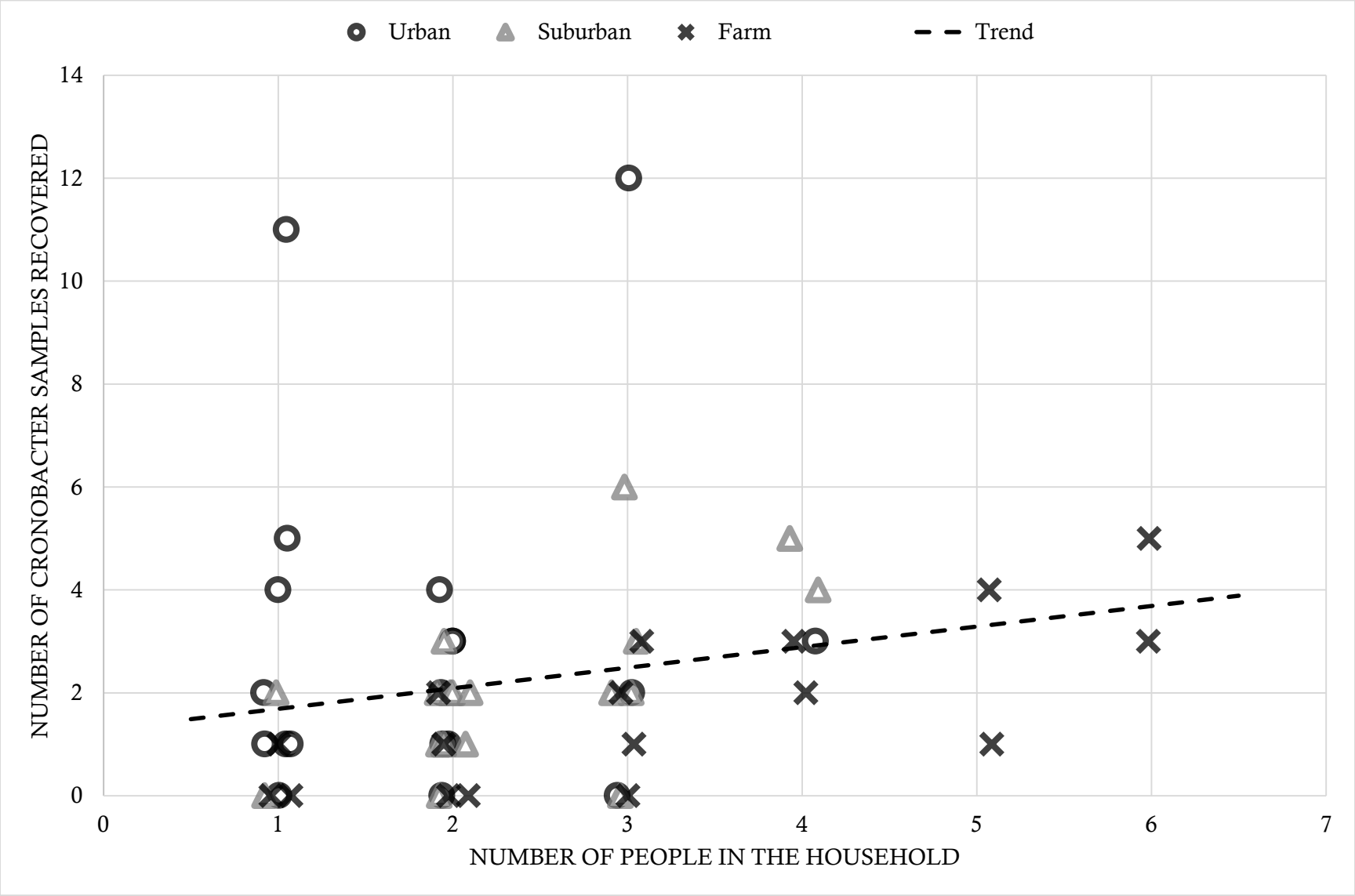

NUMBER OF CRONOBACTER SAMPLES RECOVERED
0
2
4
6
8
10
12
14
4
6
8
10
12
14
16
18
AVERAGE PRECIPITATION IN GEORGIA (CM)

FIG 3.8


Urban
Suburban
Farm
Trend
NUMBER OF CRONOBACTER SAMPLES RECOVERED
NUMBER OF PEOPLE IN THE HOUSEHOLD
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
7

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nov., respectively, and E. turicensis, E. helveticus and E. pulveris into Cronobacter as Cronobacter zurichensis nom. nov., Cronobacter helveticus comb. nov and Cronobacter pulveris comb. nov., respectively, and emended description of the genera Enterobacter and Cronobacter. *Systematic and Applied Microbiology*. 36:309-319.

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CHAPTER 4

CONCLUSION

Cronobacter infections are rare, but when they occur, can have life threatening consequences—especially for infants, elderly, and the immunocompromised. Infants who become infected with *Cronobacter* may develop bacteremia, meningitis, and necrotizing enterocolitis and have a 40-80% mortality rate (Friedemann, 2009; Zhou et al., 2015). Adults are infected or colonized more frequently than infants, symptoms of infection are typically less severe in adults (Jason, 2015; Patrick et al., 2014). The elderly and immunocompromised are the most vulnerable population of adults; when these populations are infected they are typically suffering from preexisting illnesses/conditions (Jason, 2015; Patrick et al., 2014).

It is well known that infections in neonates and young infants have been linked to *Cronobacter* contaminated powered infant formula (PIF), prepared PIF, and utensils used to prepare PIF (CDC, 2011). There are no known reservoir for *Cronobacter*; they have, however, been found from a variety of sources and environments (Iversen & Forsythe, 2003). In understanding the sources of *Cronobacter*, action can be taken to reduce or eliminate the risk for exposure or contamination.

Cronobacter have been found in the domestic environment from refrigerators and vacuum dust (Kandhai, Reij, Gorris, Guillaume-Gentil, & van Schothorst, 2004; Agnes Kilonzo-Nthenge, Chen, & Godwin, 2008; A. Kilonzo-Nthenge, Rotich, Godwin, Nahashon, & Chen, 2012). The objective of this research was to: 1) determine the prevalence of *Cronobacter* in homes, 2) to identify locations in the home where *Cronobacter* occurs, and 3) to use Enterobacteriaceae as an indicator of hygiene and environment suitable for *Cronobacter* growth.

Based on the results of this study, most houses (78.5%) had at least one location where *Cronobacter* was recoverable (FIG 3.2). Floors are the most common areas (FIG 3.4) where *Cronobacter* are found, particularly from the vacuum dust (50%) (FIG 3.5; TABLE 3.1). The

prevalence of *Cronobacter* changes during different seasons, with the recovery of *Cronobacter* from more locations in homes during the summer and autumn than winter and spring (FIG 3.6). Precipitation was found to positively correlate with the number of *Cronobacter* recovered during that particular month of precipitation (FIG 3.7). The number of people residing in a home is positively correlated with the number of recovered isolates from that home (FIG 3.8).

The literature suggests that distribution of *Cronobacter* are propagated by the movement of air, dust, and people. (Craven, McAuley, Duffy, & Fegan, 2010; A. Kilonzo-Nthenge et al., 2012). The results of this study support the literature's conclusions. The locations in homes from which *Cronobacter* was most frequently isolated were from the floors and from thresholds transitioning from outside to inside the house. Considering the positive correlation between the number of residents in the home and the increased number of locations from which Cronobacter was isolated, the data supports that movement of people contribute to the distribution of *Cronobacter* in a home. The finding that the season when the samples were collected in the households influences the number of locations with Cronobacter, also supports human traffic distribution in the home as summer and fall are seasons people are most active outside. The increase comings and goings of residents are likely to increase the distribution of *Cronobacter* in the domestic environment.

The data analyzed for Enterobacteriaceae were comparable to that of *Cronobacter*. Enterobacteriaceae were found in all homes with no significance detected between the type of homes. Location in the home were found to be significant in the bathroom, kitchen, and floors. Enterobacteriaceae were identified from all locations from at least one home. Locations with the least recovered isolates for Enterobacteriaceae were the same locations found at low levels to zero isolates of *Cronobacter*. Seasonality of Enterobacteriaceae were detected during the spring and autumn; this seasonality mirrors the *Cronobacter* results of this study. Average temperatures, average precipitation, and total number of residents were not found to be significant in regards to Enterobacteriaceae.

Although *Cronobacter* infection is rare, the presence of *Cronobacter* in the domestic environment presents a risk to susceptible populations residing in the home. Proper food safety practices should be used when preparing foodstuffs for susceptible populations.

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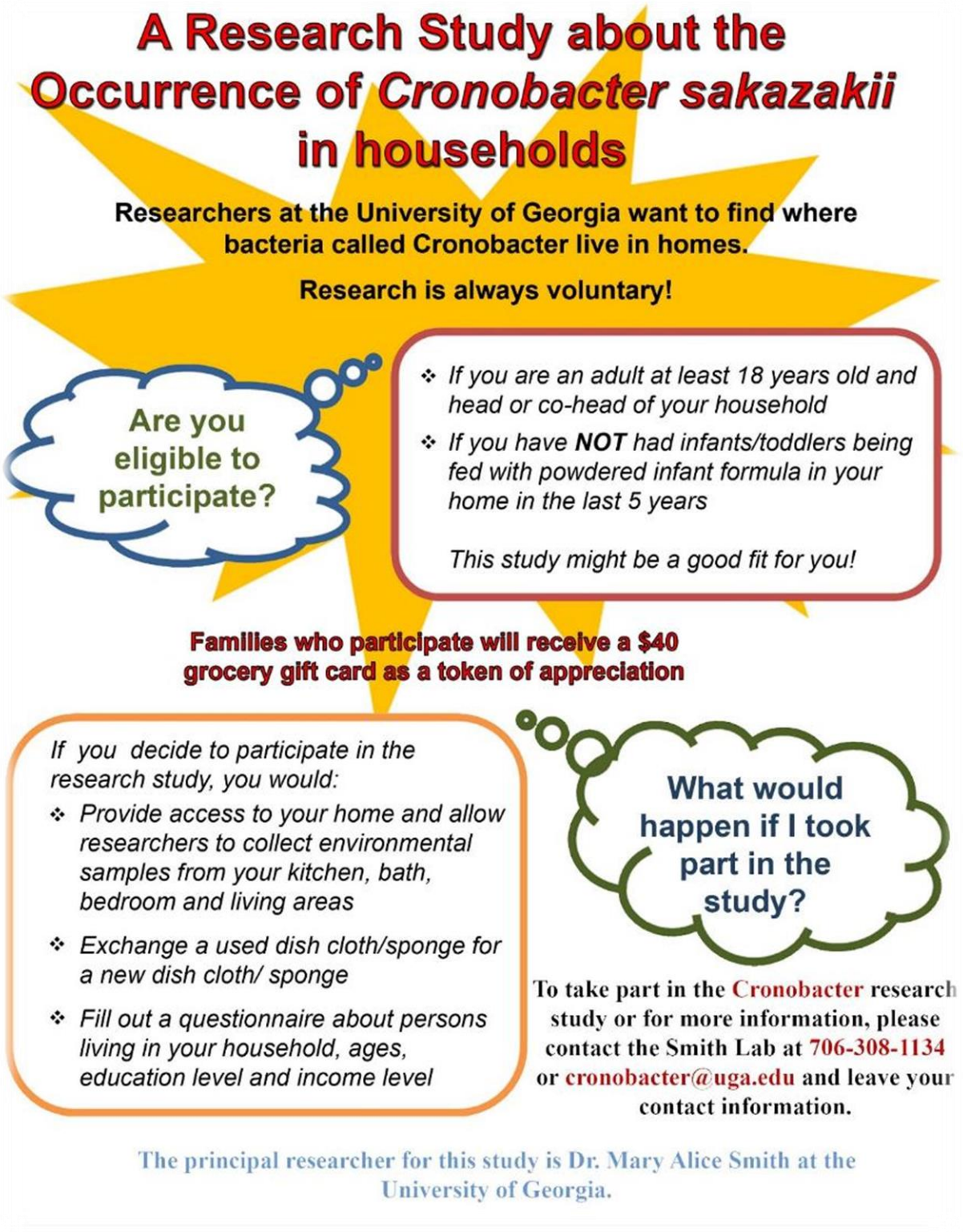
APPEDNDICES

APPEDNDIX A

RECRUITMENT FLYER FOR CHAPTER 3



APPENDIX B

INTRAUTERINE INFECTIONS[2]

[2] Monica Y. Chan and Mary Alice Smith, 2016. Submitted to *Comprehensive Toxicology*, 3rd ed. Elsevier.

ABSTRACT

Infections during pregnancy may affect a developing fetus. If left untreated these infections can lead to the death of the mother, fetus, or neonate and other adverse sequelae. There are many factors that impact infection during pregnancy such as the immune system changes during pregnancy, hormonal flux, stress, and the microbiome. We will review some of the outcomes of infection during pregnancy such as preterm birth, chorioamnionitis, meningitis, hydrocephaly, developmental delays, microcephaly, and sepsis. Transmission routes will be discussed regarding how a pregnant woman may pass their infection to their fetus. Followed by examples of infection during pregnancy: bacterial, viral, parasitic, and fungal infections. There are many known organisms that are capable of producing similar congenital defects during pregnancy; however, whether these infections share a common mechanism of action has yet to be determined. Additional research to better understand the mechanisms and risks associated with these organisms is needed to develop preventions, treatments, and/or therapies to lessen adverse outcomes due to intrauterine infection critical for the health of all potential mothers and their offspring.

I. INTRODUCTION

In the 1940's, serious birth defects in infants born to women infected with rubella during their first trimester provided the first recognition that adverse pregnancy outcomes could result from maternal infection. It has become well recognized that infections during pregnancy, in addition to affecting the mother, may affect or be passed to the developing fetus. Infections are caused by bacteria, virus, or parasites that have invaded body tissues or have released toxins disrupting normal functions and eliciting an inflammatory response to help combat the microorganism. Infections, or the inflammatory responses to an infection, can result in adverse effects including: preterm delivery, birth defects, developmental delays or stillbirths.

The main focus of this chapter is to explore the variety of pathogens that can cause adverse effects to the fetus or neonate. There are various factors that can impact infection and susceptibility during pregnancy, some of these will be examined in detail, focusing on new data and information. Databases searched for this information included PubMed, Google Scholar, and Web of Science focusing on publications from 2007 to 2016. Since the publication of the previous Comprehensive Toxicology 2nd Edition, new sections included in this chapter discuss: the transmission of intrauterine infections; the factors impacting infection; examples of classic and new pathogens capable of causing intrauterine infections; and an updated table of additional selected pathogens detailing the exposures, symptoms, and outcomes of infection.

I. TRANSMISSION

Bacteria, viruses, and other organisms are able to be passed from mother to child, otherwise known as transmission. The specific symptoms of transmitted infections depend on the individual pathogen and the stage of pregnancy at the time of infection. Transmission occurs either in the womb during pregnancy or after birth. Pathogens can enter the uterus and infect the fetus through several routes—hematogenous transmission, passage from abdominal cavity and through the fallopian tubes, ascending infection from maternal vagina, invasive medical procedures, or the birthing process (Richardson, Pollak, Williams, & Smith, 2010). The main routes of intrauterine infection transmission during pregnancy are vertical transmission and ascending infection from the mother's reproductive system.

A. Vertical Transmission

A vertically transmitted infection is caused by a pathogen transmitted directly from the mother to an embryo, fetus, or baby during pregnancy or childbirth; this type of transmission is also known as mother-to-child transmission. Vertical transmission during the perinatal period can be subcategorized as a perinatal infection.

When discussing vertical transmission, it generally refers to transplacental transmission. The mother must have the infection along with the presence of the pathogen in her blood (hematogenous transmission). These pathogens also must have the ability to overcome the placental barriers—syncytiotrophoblast interface, decidual-trophoblast interface, and physical obstacles (Robbins & Bakardjiev, 2012). Pathogens may overcome these barriers through: a damaged syncytiotrophoblast interface; invasion of the uterine-trophoblast interface directly or cell-to-cell (*Listeria monocytogenes*); or immune cells may allow for barrier crossing (transport of human immunodeficiency virus mediated by leukocytes) (Lagaye et al., 2001; Robbins & Bakardjiev, 2012). When the placenta is infected this may allow the pathogen to invade the fetus.

B. Ascending Infection from Maternal Reproductive System

Ascending infections occur when infectious pathogen residing in the external genitalia of the mother access the amniotic sac. Upon infection, the amniotic sac may become compromised and rupture. Once ruptured the pathogens have the potential to inoculate the amniotic fluid, spread as a biofilm over the exposed amnion, and invade choriodecidual tissue planes (M. J. Kim et al., 2009; Redline, 2012). The fetus can become infected by aspirating the microorganism to the lungs, ingesting the pathogens, or penetration to the ear canal. See section on chorioamnionitis for details post amniotic fluid infection.

II. FACTORS IMPACTING INFECTION

Susceptibility of infection in women is different due to natural variation; a number of other factors also influences this susceptibility. For intrauterine infections there are some common factors that should be considered to impact susceptibility to infection. Common factors of intrauterine infections that impact susceptibility are discussed below.

A. Immune System Changes during Pregnancy

Epidemiologic studies have shown that pregnant women have an increased incidence to a variety of infections like influenza, varicella, measles, severe acute respiratory syndrome, tuberculosis, listeriosis, pneumocystis, toxoplasmosis, and malaria (Jamieson, Theiler, & Rasmussen, 2006; Riley et al., 2011; Sappenfield, Jamieson, & Kourtis, 2013). The severity of infection has been suggested to vary at different stages of pregnancy, likely from the unique immunological alterations that occur during different stages of pregnancy (Faucette, Unger, Gonik, & Chen, 2015).

The maternal immune system changes to accommodate the genetic differences between the mother and the fetus to prevent allogenic rejection of the fetus (Jamieson et al., 2006). Hormone levels change during pregnancy and interplay between the sex hormones and immune system may alter the efficiency of the immune system. These changes are thought to contribute to an increase in

susceptibility towards infectious diseases which may alter the severity of illness and fetal mortality rates.

Pregnant women may be considered immunosuppressed, though not in the traditional sense of the word. A fetus derives half of its genetic material from its father; thus the fetus is essentially a foreign tissue in the maternal uterine environment. The fetus' susceptibility to rejection from its mother's immune system has been compared to an organ transplant (Jamieson et al., 2006). Evidence suggests that the maternal immune system tolerates fetal antigens by suppressing cell-mediated immunity while retaining normal humoral immunity and response (Jamieson et al., 2006). Though only occurring locally at the maternal-fetal interface, this suppression prevents the rejection of the fetal tissue; and has been proposed that it may affect maternal response to infection (Erlebacher, 2013; Jamieson et al., 2006). The exact immunological tolerance is not completely understood.

During pregnancy there is a systematic shift from T-helper 1 (Th1) to T-helper 2 (Th2) cell mediated responses in the mother. In a Th1 dominated response the Th1 type T lymphocytes and pro-inflammatory cytokines amplify cell mediated immunity allowing for recognition of the body's own cells that engulf the pathogen and express pathogen-related antigen on the their surfaces (Corr, Gahan, & Hill, 2007; Jamieson et al., 2006). When shifting to the Th2 cell mediated response, the Th2-stimulating cytokines dominate and suppress the Th1 T cell responses locally allowing for adequate humoral immune response while the cell mediated immunity is compromised (Thaxton & Sharma, 2010). Hormonal changes during pregnancy promote this shift from Th1 to Th2.

B. *Hormones*

Hormones can contribute to a modified immune response by altering the functions of immune cells and can have effects on the outcome of infection during pregnancy (Robinson & Klein, 2012). During pregnancy steroid sex hormone levels change drastically and are considerably higher than at any other time during a woman's life, particularly those pregnancy-associated hormones including: estradiol, estriol, progesterone, corticosteroids, and prolactin (Robinson & Klein, 2012).

The interplay between sex hormones and the immune system is complex and often contradictory—estradiol can enhance innate immunity in cell-mediated and humoral adaptive immune responses, yet progesterone can suppress the maternal immune response and alter helper T-cell responses (Kourtis, Read, & Jamieson, 2014; Robinson & Klein, 2012). Research is needed to fully understand the changes in hormones and the immune system during pregnancy and the interplay between the two systems.

C. *Stress*

Mechanisms linking maternal stress to infant development are complex. Stressors can enhance or suppress immune function which can change susceptibility to infections. Maternal stress may be mediated through biological and behavioral mechanisms like the neuroendocrine pathway, which can result in the activation of the maternal-placental-fetal endocrine systems, or an immune/inflammatory pathway, where maternal stress may modify the characteristics of systemic and local immunity by increasing susceptibility of the intrauterine and fetal environments to the immune inflammatory processes (Wadhwa et al., 2001). It is likely that the placenta plays a vital part in mechanisms linking maternal stress and infant development.

The duration of stress impacts the health of the individual. Suppressed immunity can occur by decreasing immune cell number and function or increasing active immunosuppressive mechanisms. Promotion of pro-inflammatory and type-2 cytokine driven responses changes leukocyte distribution within the body where the immune response occurs, timing of stress, and stress hormone exposure relative to initial immune response (Dhabhar, 2009).

Pregnancy is a stressful change in a woman's body. Additional stressors beyond that of a pregnancy can not only impact the health of the mother but the development of the fetus. Stress can either enhance or suppress the immune system and thereby change the mother's susceptibility and severity to certain immune responses.

D. *Microbiome*

Traditionally, intrauterine infections were thought to have originated from pathogens in the vaginal tract that ascend into the intrauterine environment. The placenta was considered sterile during gestation and the presence of any bacteria in clinical cultures was a diagnostic for intrauterine infection (Hillier et al., 1988). However, the presence of placental or membrane bacteria has been observed in the absence of histological infection and recognition that the presence of bacteria does not always have the expected corresponding clinical outcomes when observed (Buhimschi et al., 2009; Yiping W. Han, Shen, Chung, Buhimschi, & Buhimschi, 2009; Leviton et al., 2010; Mysorekar & Cao, 2014; Steel et al., 2005; Stout et al., 2013).

Of all the cells in the human body, only 10% are human somatic and germ cells; the remaining 90% are made of micro-flora (Savage, 1977). The microflora found in and on humans are composed of diverse niche communities of microbial species varying by site or organ. As a whole, all these communities make up the microbiome. With the initiatives set by the National Institutes of Health with the Human Microbiome Project in 2007, the microbiome has been found to be increasingly integral with the healthy functions of a human (Peterson et al., 2009; Turnbaugh et al., 2007).

Human-microflora interactions occur across body sites and have roles in metabolism, immunity, development, and behavior of the host (Cabreiro & Gems, 2013; O'Hara & Shanahan, 2006). The communities consist of a balance of commensal bacteria and beneficial bacteria that help humans with normal functions. When the balance is upset, it can result in disease and unfavorable alterations. Under certain conditions an outgrowth of potential pathogenic bacteria, a decrease in the number of beneficial bacteria, or an imbalance of commensal microflora may contribute to the pathogenesis of disorders which may affect the susceptibility rate for infections—including intrauterine infections (Hill & Artis, 2010; Honda & Littman, 2012; O'Hara & Shanahan, 2006; Round & Mazmanian, 2009). Infection with a pathogen leads to a change in the normal microbiome which can have an effect on the intrauterine system (Hacquard & Schadt, 2015). For

instance, a decrease in the diversity in the placental microbiome has been correlated with lower birth weight in human infants (Zheng et al., 2015).

Many of the diverse groups of oral cavity bacteria have been found in the intrauterine environment and associated with adverse pregnancy outcomes suggesting that the oral cavity may act as a reservoir for infection (Fardini, Chung, Dumm, Joshi, & Han, 2010; Y. W. Han, 2011; Mysorekar & Cao, 2014). These bacteria are capable of hematogenous transmission, similar to the transient bacteremia possible during periodontal infections (Fardini et al., 2010; Y. W. Han, 2011; Offenbacher et al., 1999). For example maternal oral infections, like acute gingival infection and chronic periodontal infections, are multifactorial disorders where microbial dental biofilms are considered the primary agent initiating inflammation (Chambrone, Guglielmetti, Pannuti, & Chambrone, 2011; Offenbacher et al., 2006; Xiong, Buekens, Fraser, Beck, & Offenbacher, 2006). Although inflammation is typically restricted to local periodontal tissues; the pathogens can stimulate bacteremia and translocate to distant tissues. (Champagne et al., 2000; Xiong et al., 2006).

Microorganisms have the ability to transfer from one location to another, as noted by studies on periodontal disease and its impact on pregnancy. As we continue to discover the complex roles and interactions between the human body and the microbiome that inhabits the body, it is likely that correlations between the health of the intrauterine microbiome and the susceptibility to intrauterine infection will be discovered.

III. ADVERSE PREGANACY OUTCOMES AFTER INFECTION

Once infected there are a multitude of adverse pregnancy outcomes— preterm births, chorioamnionitis, infections of the central nervous system, and sepsis— which will be detailed below. (Richardson et al., 2010).

A. Preterm Births

Premature births or preterm births is defined as births that occur prior to 37 completed weeks of gestation and is associated with 5-18% of pregnancies (Liu et al., 2012). Premature births are the leading cause of neonatal morbidity and mortality in the developed and developing world (R. L. Goldenberg, Hauth, & Andrews, 2000). Infants who are born prematurely have a higher risk of serious disability or mortality than their full-term counterparts; with those infants born before the 30th week of pregnancy being especially at risk of prenatal mortality and morbidity. Potential problems for premature babies are an increased risk of short-term complications due to immaturity of organ systems, neurodevelopmental disorders, intellectual disabilities, vision/hearing impairments, or even death (CDC, 2015e; Romero, Dey, & Fisher, 2014).

Intrauterine infection is a frequent and important mechanism that may contribute up to 40% of preterm births (Agrawal & Hirsch, 2012; Robert L. Goldenberg, Culhane, Iams, & Romero, 2008; Romero et al., 2007). However, this number may be higher because many infections are likely to be subclinical and the pathogenesis is not detected due to the lack of sensitivity of conventional culture techniques (Racicot et al., 2013). Outcomes of infection in a pregnant host are dependent upon varying factors including gestational stage of fetus, previous exposure and immunity of the mother, variable immunity among individuals, the placenta's ability to protect the fetus from infection, and the developing immune system in the fetus (Richardson et al., 2010).

There are different pathways by which pre-term birth from bacterial infection are known to occur. Two major events that are required for bacteria to induce preterm labor are: 1) bacterial colonization in gestational tissues or the fetus; and/or 2) bacterial counts reaching a threshold that elicits maternal immune inflammatory response that will induce preterm labor (Richardson et al., 2010; Romero, Espinoza, Chaiworapongsa, & Kalache, 2002). Preterm labor is more frequent when a fetal inflammatory response is triggered as diagnosed by an increase in IL-1 and IL-6 and a decrease in IL-10. (Romero et al., 2007).

The consequences of preterm birth resulting from intrauterine infection can be very serious. Fetal gestational age and weight have a direct correlation with the likelihood of neonatal survival

and risk of the development of morbidity in premature infants. Compared to term infants, preterm births have a higher incidence rate of temperature instability, respiratory distress, apnoea, hypocalcaemia, seizures, jaundice, kernicterus, feeding difficulties, periventricular leukomalacia, and hospitalizations (Saigal & Doyle, 2008). Effects of preterm birth can manifest later in childhood (Huddy, Johnson, & Hope, 2001). Studies on low birth weight have shown that most of these infants showed sequelae like cognitive defects, academic underachievement, grade failures and the need for increased remedial assistance during mid-childhood and adolescence continuing throughout life (Saigal & Doyle, 2008). There is still much about the pathogenesis and mechanisms of preterm birth that is not understood and animal models, such as sheep, have/are being utilized to gain knowledge that could lead to methods and treatments to improve neonatal outcomes (Robert L. Goldenberg et al., 2008; Racicot et al., 2013).

B. Chorioamnionitis

The condition in which the membranes that surround the fetus, the chorion and amnion, and the amniotic fluid are infected by bacteria is commonly referred to as "chorioamnionitis". Chorioamnionitis complications are associated with significant and/or long-term adverse outcomes for the mother (postpartum infections and sepsis) and/or the infant (stillbirth, premature birth, neonatal sepsis, chronic lung disease, and brain injury) (Tita & Andrews, 2010). It has been estimated that one of every three preterm neonates is born to a mother with intra-amniotic infection, identified with either cultivation or molecular microbiologic techniques (A. Berger et al., 2009). It is important to also note that microbial colonization of chorioamniotic membranes does not always result in a fetal or maternal inflammatory response (Romero et al., 2007). Chorioamnionitis is typically associated with bacteria that have low virulence and enter the uterus by ascending from the lower reproductive tract it is rare that transmission is spread hematogeneously (one notable exception: *Listeria monocytogenes*) (Richardson et al., 2010; Tita & Andrews, 2010). The presence of organisms in the amniotic cavity elicits an intense inflammatory response (Racicot et al., 2013). The

inflammatory response may intensify and progress into fetal inflammatory response syndrome which predisposes preterm neonates to short- and long-term consequences (Robert L. Goldenberg et al., 2008).

A maternal fever is an important criterion for the diagnosis of chorioamnionitis. The presence of maternal fever of greater than 38°C (100.4°F) and at least two of the following criteria: maternal leukocytosis (greater than 15000 cells/mm^3), maternal tachycardia (greater than 100 beats/minute), fetal tachycardia (greater than 160 beats/minute), uterine tenderness, and/or foul odor of the amniotic fluid. These thresholds are associated with higher rates of neonatal and maternal morbidity (Richard A. Polin & Committee on Fetus and Newborn, 2012).

Additional details of the mechanisms of chorioamnionitis can be found in Richardson, Pollak et al. (2010) and in Tita and Andrews (2010).

C. *Infection of Fetal Central Nervous System*

Adverse effects to the central nervous system (CNS) is a common result of intrauterine infections in neonates. Transversal of the blood brain barrier (BBB) must occur for a pathogen to access the central nervous system (R. A. Polin & Harris, 2001). The BBB secretes cerebrospinal fluid (CSF) while physically separating the brain from the intravascular department. The purpose of the BBB is to control the flow of molecules and ions that enter and leave the brain in order to maintain a neutral environment (K. S. Kim, 2003; Richardson et al., 2010). The extent of effect depends on the maturity of the developing brain, timing of infection, or amount of pathogens (I.-O. Kim, 2014).

a. Meningitis (Bacterial)

There are an estimated six cases of bacterial meningitis per 100,000 worldwide (NINDS/NIH, 2015b); however, there(NINDS/NIH, 2015b). There is little known about bacterial meningitis in the fetus. Generally pregnancy has not been associated with an increased risk of bacterial meningitis with the exception if the cause is due to *Streptococcus pneumoniae*, *Listeria monocytogenes* or *Cryptococcus neoformans* (Adriani, Brouwer, van der Ende, & van de Beek, 2012;

Baldwin & Roos, 2011). Clinical effects of meningitis are sepsis and increased intracranial pressure; chronic changes can occur such as hydrocephalus, encephalomalacia, or subdural effusion (I.-O. Kim, 2014). Neonatal meningitis is the most common and serious bacterial infection during the neonatal period; with the most common pathogens being enterobacteria, *E.coli*, and group B streptococcus (I.-O. Kim, 2014).

b. Hydrocephaly

Hydrocephaly is a condition where excess cerebrospinal fluid accumulates in the brain, it is also known as hydrocephalus or "water on the brain"(NINDS/NIH, 2015a). The excessive accumulations causes an abnormal expansion of the brain's ventricles producing pressure on the brain (NINDS/NIH, 2015a). Due to the malleability of an infant's skull, the added pressure on the brain may result in an enlarged head size for the infant as the skull widens to relieve pressure (Richardson et al., 2010). Causes of hydrocephalus are still not well understood; possible causes in the fetus are infections, genetic defects, developmental disorders, meningitis, premature birth complications, or traumatic head injury (NINDS/NIH, 2015a; Richardson et al., 2010).

c. Microcephaly

Microcephaly is a medical condition present at birth or developed within the first few years of life where the circumference of the head is smaller than normal because the brain has either not developed properly or growth has stopped (NINDS/NIH, 2015c). It is most often caused by genetic abnormalities during the early months of fetal development; but may be induced if the mother is infected or affected by pathogens, drugs or alcohol, or certain toxic chemicals during pregnancy (NINDS/NIH, 2015c). Recently, pregnant women infected with the Zika virus had a high incidence of babies born with microcephaly. See viral examples for a discussion on the Zika virus.

The result of having microcephaly varies. Babies born with microcephaly will have smaller than normal heads that fail to grow through infancy. Microcephaly can be an isolated condition or occur in combination with other major birth defects (CDC, 2016). Depending on the severity of

microcephaly, problems can range from mild to severe and are often lifelong such as: impaired cognitive development, developmental delay, facial distortions, dwarfism or short stature, hyperactivity, seizures, difficulties with coordination and balance, feeding problems, hearing loss, vision problems and other brain or neurological abnormalities (CDC, 2016; NINDS/NIH, 2015c). Severe microcephaly can also be life-threating (CDC, 2016). Yet some will develop normally and their head that will grow bigger especially if they are otherwise growing and developing normally (NINDS/NIH, 2015c).

d. Developmental Delays

Infections during pregnancy may result in developmental delays for the infant. Pathogens that are able to interact with the CNS have capabilities of causing morbidities in fetuses and premature infants. Some of these impairments may not manifest at birth or in the neonatal period, but later in life.

Many neonates are able to survive major insults, like infection, without any evidence of impairment due to the plasticity of the developing brain and improvements in medical care; yet, in others, these insults can cause varying degrees of long-term neurodevelopmental impairment such as cerebral palsy, with half of those continuing to develop cognitive and behavioral deficits (Mwaniki, Atieno, Lawn, & Newton, 2012; Stoll, Hansen, Fanaroff, & Lemons, 2004).

The exact mechanisms whereby pathogens cause developmental delays are not yet known. Molecular events such as the release of inflammatory cytokines by brain cells during infection may have a significant role in the brain injury (Dammann & Leviton, 1997). For instance, ascending intrauterine infections have been suggested to significantly increase the risk of fetal brain damage due to the initiation of fetal inflammatory response syndrome (R. Berger & Soder, 2015; Dammann & Leviton, 1997; de Vries, 2009). Yet, whether there are exposure-specific or pathogen-specific patterns by which cytokines act in response to inflammation during infection is unknown.

D. Sepsis

Sepsis is a systemic syndrome that occurs in response to infection of the blood and stems from an additional medical condition, like chorioamnionitis or a CNS infection (NIGMS/NIH, 2015). During sepsis, intravenous coagulation may occur, blocking blood flow to vital organs and creating hypoxic and ischemic conditions. Mortality in cases of sepsis is due to the failure of multiple organs—typically a single organ fails leading to the dysfunction of other organ systems

There is early and late onset of neonatal sepsis. Early onset is typically a consequence of fetal infection in utero or during parturition within the first week of life. Sepsis can begin in utero when the fetus inhales or swallows infected amniotic fluid; during parturition, sepsis is capable of developing within the hours or days after birth when colonized skin or mucosal surfaces are compromised (Richard A. Polin & Committee on Fetus and Newborn, 2012). Maternal treatment with intrapartum intravenous antimicrobial agents is the only intervention proven to reduce the incidence of early-onset neonatal sepsis (CDC, 2010b; Richard A. Polin & Committee on Fetus and Newborn, 2012). Late onset sepsis can result from colonization during birth via vertical transmission of a colonized mother's anorectal and vaginal areas; case reports have suggested possible horizontal transmission in the postnatal period through breast milk (Berardi et al., 2013; Richard A. Polin & Committee on Fetus and Newborn, 2012). Depending on the pathogen and the neonatologists' discretion, a full course of antimicrobials may be used to treat the neonate (Rubin et al., 2002). Vancomycin has been a frequent choice of empiric antimicrobial treatment of neonates with suspected late-onset sepsis (Rubin et al., 2002).

Chorioamnionitis is a major risk factor for neonatal sepsis and increased risk of early onset sepsis (Richard A. Polin & Committee on Fetus and Newborn, 2012; Wolfs et al., 2012). Additionally, pathogens that cause neonatal meningitis have the ability to induce neonatal sepsis. As mentioned previously, neonates that survive a CNS infection may experience developmental delays

and impairment. A study showed that the degree of impairment was more likely to be severe in septic preterm neonates than in nonseptic neonates (Mwaniki et al., 2012).

IV. Examples of Infection during Pregnancy

Infections acquired in utero or during birth are a significant cause of fetal and neonatal mortality which can contribute to early and later childhood morbidity. Organisms shown to cause these congenital conditions are included in the TORCH complex. The TORCH acronym represents ***T****oxoplasma gondii*, **O**ther infections, **R**ubella, ***C****ytomegalovirus*, and **H**erpes viruses as a concept emphasizing that these infections have been shown to cause still births, perinatal morbidity, and 2% to 3% of all congenital anomalies (Neu, Duchon, & Zachariah, 2015; Stegmann & Carey, 2002). These conditions are far from the only kinds that can cause congenital conditions. Specific examples of types of pathogens that affect pregnancy and the developing fetus are listed in Table 1. To explore some of the mechanism unique to pathogens (bacterial, viral, parasitic, and fungal) a few examples are elaborated in this section.

E. Bacterial Infection

a. *Listeria monocytogenes*

Listeriosis is predominantly a foodborne disease associated with the consumption of contaminated foods— primarily in dairy products. Populations most susceptible to *L. monocytogenes* infection are people with a compromised immune system, older people, pregnant women, and neonates. These populations account for at least 90% of reported infections (CDC, 2013a).

Pregnant women are about 10 times more susceptible to *L. monocytogenes* infection healthy non-pregnant adults; about one in seven (14%) cases of listeriosis occur during pregnancy (CDC, 2013a, 2013c). Infection during pregnancy can cause spontaneous preterm labor, fetal loss, and illness or death in newborn infants (CDC, 2013c; Richardson et al., 2010). Symptoms of infection may include gastrointestinal symptoms, influenza-like illness with fever, headache, myalgia and/or

backache; but 29% of the infection cases are asymptomatic (Jackson, Iwamoto, & Swerdlow, 2010; Mylonakis, Paliou, Hohmann, Calderwood, & Wing, 2002).

Animal studies on rhesus monkeys (M. A. Smith et al., 2003), guinea pigs (Williams, 2006), and gerbils (Roulo, Fishburn, Amosu, Etchison, & Smith, 2014) have shown a relationship between increasing bacterial load and adverse pregnancy outcomes. A fetus becomes infected when *L. monocytogenes* crosses the placenta, which has been suggested to be a reservoir for re-infection (Bakardjiev, Theriot, & Portnoy, 2006; De Luca et al., 2015). Neonatal infection occurs in 68% of the cases of maternal infection; 68% of the cases may make a full recovery, but in 12.7% of cases there may be long term sequelae (Mylonakis et al., 2002). Infection may lead to severe health conditions such as septicemia, pneumonia, or meningitis and in about 25% of cases, the occurrence of pre-term delivery with birth weights lower than normal or still birth may occur (Mylonakis et al., 2002). Septicemia is the main symptom neonates display with a 15-50% mortality rate. Other symptoms include respiratory problems, pneumonia, and the formation of minute abscesses in bodily organs (Farber & Peterkin, 1991). A neonate can develop late onset listeriosis through natural birth if the mother's vaginal tract has been colonized by *L. monocytogenes* (CDC, 2013c).

Bacteremia can be caused by ascending infection, transplacental passage of the organism or by inhalation of amniotic fluid (Becroft et al., 1971; De Luca et al., 2015). Second- and third-trimester infections can result in premature delivery followed by neonatal illness or pre-term delivery of a stillborn (Farber & Peterkin, 1991). For additional discussion of mechanisms, see Richardson, Pollak et al. (2010).

b. *Streptococcus*

Streptococcus are classified by their hemolytic properties with two species known to affect pregnancy—alpha-hemolytic streptococci (group A strep) and beta-hemolytic streptococci (group B strep). We will be detailing examples from group A strep and group B strep below because of their potential impact on pregnancy.

A Group A streptococci (GAS), *Streptococcus pneumoniae* or pneumococcus, is a significant human bacterium. It is part of the normal microbiome in the upper respired tract, but opportunistic, if conditions allow. It is a one of the most common causes of severe pneumonia and can cause pneumococcal meningitis, which is the most common form of meningitis and the most serious (CDC, 2015d; NINDS/NIH, 2015b). Coexisting disease in the mother increases the risk of contracting pneumonia in pregnancy.

A pregnancy complicated with pneumonia can have adverse fetal effects; 44% of women experienced preterm labor and of the preterm births 36% had respiratory failure (Munn, Groome, Atterbury, Baker, & Hoff, 1999). Pneumonia in pregnancy can result in inflammation and edema of alveoli, restricting the number available for oxygen transport. Fetal oxygen delivery decreases when the maternal oxygen saturation falls to <90% (Goodnight, 2005). Birth weights were significantly lower at deliver during pregnancies complicated by pneumonia (Goodnight, 2005).

Invasive Group B *Streptococci* (Group B Strep) or *Streptococcus agalactiae* is the leading infectious cause of maternal chorioamnionitis, puerperal endometritis and early-onset neonatal sepsis (CDC, 2010b; Phares et al., 2008). It causes invasive disease primarily in infants, pregnant/postpartum women, and older adults, with the highest incidence among young infants (Phares et al., 2008).

There are two main types of Group B Strep disease—early-onset disease and late-onset disease. Early-onset disease occurs during the first week of life; late-onset disease occurs from the first week through the first three months of life. During early-onset disease, Group B Strep commonly causes sepsis, pneumonia, and sometimes meningitis (CDC, 2014). For late-onset disease the same illnesses are found as in the early-onset, but meningitis is more common during this type of disease (CDC, 2014). The primary risk factor for neonatal infection transmission is colonization of the mother's genital tract through direct exposure during birth, or in fetuses through ascension into

the amniotic fluid then into the placenta. Group B Strep can also cause miscarriages, stillbirths, and preterm deliveries (CDC, 2014). The exact mechanisms are generally unknown (CDC, 2014).

c. Campylobacter

Campylobacter are found ubiquitously in the environment and considered normal intestinal flora in most mammals and birds. The consumption of raw or undercooked poultry or cross-contamination of other foods is the most common source of campylobacteriosis in industrialized nations, responsible for more than 90% of all sporadic cases in humans (Allos, 2001; Dasti, 2010). More than 90% of all human *Campylobacter* infections are caused by *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli*, with increasing rates of infection reported during the summer months.

Infants are one of the two most infected populations, the second group are patients between 15-39 years (Dasti, 2010). *C. jejuni*, *Campylobacter fetus* (*C. fetus*), and other strains have caused stillbirths/abortions in pregnant women (J. L. Smith, 2002). *C. jejuni* and *C. fetus* are two strains known to cause illness in pregnant/post-partum women and their fetuses/newborn infants. Though most cases have no complications, enteritis caused by *C. jejuni* infection can result in stillbirth or premature labor (Allos, 2001). Transmission of the pathogen to offspring can occur either transplacentally or through vaginal delivery (Richardson et al., 2010; J. L. Smith, 2002).

Additional details into the mechanisms of *Campylobacter* intrauterine infection and the impacts on the fetus—such as stillbirth— may be found in the 2nd edition of Comprehensive Toxicology, volume 8, Intrauterine Infections (Richardson et al., 2010).

F. Viral Infections

Rubella was the first virus recognized to result in birth defects. There are a number of viruses that can adversely affect the developing fetus (Table 1). Viruses act through different mechanisms of action, there are some common features of viral infections that adversely affect pregnancy. Transmission occurs vertically from the mother to the fetus. If the placenta is not infected, the virus does not appear to spread to the fetus. Various factors influence the likelihood of

the virus infecting the fetus such as infection state of the mother and gestational age of the fetus. Outcomes of viral infections are generally spontaneous abortion, stillbirth, prematurity, and organ pathogenesis. Below are examples of viruses, additional examples may be found in Table 1.

a. Cytomegalovirus

Cytomegalovirus (CMV) is a herpes virus that infects people of all ages. Once infected, the virus stays within a person's body for life. In the United States it is estimated that 50-80% of adults are infected with CMV by the age of 40 (CDC, 2010a). Most healthy children and adults infected with CMV exhibit no symptoms, those that do exhibit symptoms tend to be like other illnesses—fever, sore throat, fatigue, and swollen glands. CMV can cause serious disease in pregnant women and their babies, people who care for infants and children, and the immunocompromised. It is the most common virus infection in the developing fetus; approximately 1 in 150 children is born with congenital CMV infection.

Transmission of this virus is generally through direct contact with body fluids. CMV can be transmitted from a pregnant woman to her fetus during pregnancy by the virus crossing the placenta and infecting the fetus' blood.

Congenital CMV infection in developed countries occurs between 0.3-2.45 of all live births (Alford, Stagno, Pass, & Britt, 1990; Bonalumi, Trapanese, Santamaria, D'Emidio, & Mobili, 2011; Peckham, 1990). Most infants born with congenital CMV infection (90%) appear healthy at birth and 80% of the healthy appearing infants never develop symptoms or disabilities. Health problems or disabilities may appear two or more years after birth or never (CDC, 2010a). Fetal damage from CMV infection can include growth retardation and CNS abnormalities (Kimberlin et al., 2003).

Additional details regarding the mechanisms of CMV may be found in the 2nd edition of Comprehensive Toxicology, volume 8, Intrauterine Infections (Richardson et al., 2010).

b. Influenza

Influenza viruses are a group of RNA viruses and are the leading causes of serious wintertime respiratory morbidity worldwide. Studies about the effects of influenza-related illness during pregnancy have shown an impact on the health of pregnant women. Specific changes in a woman's immune system, heart, and lungs during pregnancy increase the susceptibility to severe illness, hospitalizations, and possibly death. There is an increased chance for serious problems for unborn babies, including premature labor and delivery (McNeil et al., 2011). There are three distinct genera of influenza viruses: A, B, and C. For this example, we will be looking at the outcomes specific to the subtype of influenza A— the 2009 H1N1 influenza A virus.

Women hospitalized with H1N1 infection were estimated to have a three times increased risk of delivering preterm (Yates et al., 2010). Emergency cesarean deliveries were frequently reported as urgent or emergent in one study regarding the 2009 H1N1 influenza (Haberg et al., 2013). The unusually high cesarean section delivery is a likely effect of deteriorating maternal condition that impacts the overall fetal status (Mendez-Figueroa, Raker, & Anderson, 2011). Infants of ill mothers can test positive for H1N1 influenza; but it is an unusual occurrence. This raises the possibility of vertical transmission, but additional study is needed about the impacts of maternal infection with influenza on neonatal outcomes. Long term follow-up studies on infants born to women with influenza infection should be performed to fully understand the impacts on overall outcomes when evaluating influenza in pregnancy. Vaccines are available and recommended to pregnant women; see Centers for Disease Control and Prevention (2015) for recommendations (CDC, 2015b).

c. Hepatitis

Hepatitis refers to the inflammation of the liver. Though inflammation can be caused by various means, most commonly it is due to a virus. There are five main types of hepatitis viruses— A, B, C, D, and E. Hepatitis A and E are generally caused by the intake of contaminated food or water; hepatitis B, C, and D typically occur as parenteral contact with infected bodily fluids.

Although, hepatitis E has a high vertical transmission rate (50%), there is still limited data on complications (Kumar, Beniwal, Kar, Sharma, & Murthy, 2004; Ornoy & Tenenbaum, 2006). For this example we will concentrate on Hepatitis B due to the effects it can cause on the pregnancy, to the fetus, and potential teratogenic effects of drugs used to prevent infection.

Maternal hepatitis B virus (HBV) infection has not been shown to increase major congenital defects in offspring. Reports of a higher incidence of low birth weight and prematurity have been noted during acute infection compared to the general population (Jonas, 2009). The higher incidence may be attributed to the changes in the maternal immune system contributing to a suppressed immune response against HBV.

Transmission of the HBV can occur prenatally or during birth. A high frequency of HBV perinatal transmission occurs in up to 90% of infants born to women positive for HBV infection. Specific risks for HBV transmission include cervical secretions and maternal blood. HBV is able to cross the placental barrier, but presumed to be the cause of a minority of infections for those mothers not immunized (Jonas, 2009). Increased likelihood of intrauterine transmission has been associated with the level of HBV DNA in the mother. Prevention of HBV transmission can be performed by identifying the hepatitis B surface antigen in pregnant women and providing hepatitis B immune globulin and hepatitis B vaccine to the infants within 12 hours of birth (CDC, 2015f).

d. Zika Virus

Zika virus is an arbovirus spread to people through mosquito bites. The virus was first identified in Africa in 1947 and is related to other flaviviruses including dengue and chikungunya (Triunfol, 2015). Transmission is typically through contaminated blood. However, competent Zika virus have been cited to have the capacity for sexual transmission it has been found in semen (2-10 weeks) after the onset of illness (Foy et al., 2011; Higgs, 2016; Oster, 2016). Symptoms appear 3-7 days after infection and are generally mild; severe disease is uncommon with no deaths reported (CDC, 2015g).

In October 2015, the Brazilian Ministry of Health received reports of an increase in the number of babies born with microcephaly, roughly 20 times higher than the typical cases seen in Brazil during a year (CDC, 2015g). Some samples from babies with microcephaly tested positive for Zika virus infection; several have tested negative using the same test (CDC, 2015g). Some mothers who birthed children with microcephaly may have had asymptomatic Zika virus (Dina Fine Maron, 2016). A case study of two pregnant women who presented with symptoms of Zika virus infection at 18 weeks' and 10 weeks' gestation were found to have the Zika virus genome in their amniotic fluid suggesting the virus can cross the placental barrier (Calvet et al., 2016). Other infections such as toxoplasmosis, rubella, cytomegalovirus, and herpes have been linked to microcephaly adding further information to the suspected link between Zika virus infection and microcephaly (CDC, 2016). Since the first reported case of Zika virus infection in May 2015, the virus has spread in many Brazilian states and other countries in Latin America.

There are no current reports in scientific literature of an association between Zika virus and microcephaly; yet, the Centers for Disease Prevention and Control (CDC) acknowledges the possible link between Zika virus infection in pregnant women and subsequent birth defects (CDC, 2015g; Triunfol, 2015). Brazil's Ministry of health made an announcement as of December 2015 for "…women in the northeast of the country not to get pregnant for the foreseeable future" (NPR, 2015). As of January 2016, the CDC has issued a travel notice Alert level 2 regarding the Zika virus in: South America, Central America, Mexico, the Caribbean, and Puerto Rico. The World Health Organization has declared a Public Health Emergency of International Concern on 1 February 2016 (WHO, 2016).

G. Parasitic Infections

Parasitic infections affect pregnant women worldwide, and directly or indirectly lead to a variety of adverse fetal effects. These effects include intrauterine retardation of growth, congenital malformations, and fetal loss. Depending on when the infection takes place and the stage of

pregnancy the fetus is in, severe fetal consequences may occur during development or be asymptomatic until later in the pregnancy.

a. Malaria

Malaria is a protozoan disease transmitted by female mosquitoes of the genus *Anopheles*. The parasites multiply in the host's liver and subsequently infect the red blood cells causing symptoms of fever, headache, and vomiting; if not treated the infection can become life threatening by disrupting the blood supply to vital organs. In 2015 there were an estimated 214 million malaria cases and 438,00 malaria deaths; young children, pregnant women, and those non-immune travelers are the most at risk when infected (WHO, 2014). In areas with high transmission rates, more than 70% of malaria deaths occur in the age group of children under the age of 5 (WHO, 2015b).

Pregnant women have increased susceptibility to *P. falciparum* malaria. Malaria during pregnancy increases the risk of maternal and fetal anemia, stillbirth, spontaneous abortion, low birth weight and neonatal death (WHO, 2015a). The effects of low birth weight has an odds ratio of two to seven times higher effects when it is the woman's first pregnancy compared to women who have had multiple pregnancies prior (Desai et al., 2007; White et al., 2014). In malaria-endemic countries, pregnant women are at increased risk of developing severe *P. falciparum* malaria with a very high mortality rate, approximately 50% (White et al., 2014). Maternal HIV infection also predisposes pregnant women to malaria, congenital malaria, and exacerbates reductions in birthweight. (Steketee, Nahlen, Parise, & Menendez, 2001).

The risk of infant death is high if maternal malaria occurs late in pregnancy (Bardaji et al., 2011; White et al., 2014). Congenital malaria occurs in roughly 5% of neonates but clears spontaneously in 62% of cases (Falade et al., 2007). The condition is typically defined as the presence of asexual forms of malaria parasites in the peripheral blood within the first 7 days of life; but frequently reported with the presence of the parasites in cord blood (Menendez & Mayor, 2007). *P. falciparum* contributes to 8-14% of low birth weight deliveries (White et al., 2014).

With increased malaria prevention (early diagnosis and treatment) and control measures, the malaria mortality rates have fallen globally among all age groups. There is, however, a concern of *P. falciparum* resistance in some countries to artemisinin, one of the core compounds in World Health Organization recommended treatments for uncomplicated malaria, (WHO, 2015b).

b. Toxoplasmosis

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*) that infects most species of warm blooded animals. It has a complex life cycle. Sexual reproduction can only occur in the digestive tract of Felidae hosts (cats). Asexual reproduction can occur in any warm blooded vertebrate. Humans can be infected by: eating undercooked meat of animals harboring tissue cysts; consuming contaminated food or drinking water; contact with contaminated environmental samples; blood transfusion or organ transplant; or by vertical transmission. In humans, those infected are generally asymptomatic; when symptoms occur, it is usually mild and flu-like lasting for weeks to months and until the cycle returns to a dormant state (CDC, 2013b). The parasites form tissue cysts and may remain throughout the life of the host (CDC, 2013b).

If a woman has contracted toxoplasmosis before becoming pregnant, the fetus will be protected due to the mother's developed immunity (Woudwyk et al., 2012). So maternal-fetal transmission only occurs when a pregnant woman is newly infected with *Toxoplasma*. Global estimates of maternal–fetal transmission are approximately 20–33% of newly infected pregnant women (Carlier, Truyens, Deloron, & Peyron, 2012). Although transmission rates during the first trimester of pregnancy are low (below 6%), when it does occur the damage to the fetus is the most severe at this time (Carlier et al., 2012). The second and third trimesters' transmission rates increase to 22-40% and 58-72 respectively; the highest proportions of transmission occur in the last few weeks before birth (Carlier et al., 2012).

Transmission may result in a miscarriage, stillbirth, or a child with congenital toxoplasmosis. Infants infected before birth may be asymptomatic at first but may develop later in life with potential

vision impairment/loss, mental disability, and seizures (CDC, 2013b). Treatment is available for pregnant women, newborns, and infants involving sulphonamides or spiramycin; but due to the parasites residing within cells in a less active phase it is difficult for medication to completely eliminate the cysts (CDC, 2013b; Richardson et al., 2010).

H. Fungal Infections

Fungi are ubiquitous; found outdoors, on many inside surfaces, and on human skin. Most fungi are harmless, but some types can be harmful to health. Anyone can be affected by fungal diseases (CDC, 2015c). Serious fungal infections are uncommon during pregnancy; in some cases infection may occur with higher frequency during pregnancy, potentially increasing maternal mortality and cause fetal loss or prematurity (Ostrosky-Zeichner & Rex). An extreme outcome of a fungal infection is the spread of the fungus through the blood to the spinal cord leading to fungal meningitis.

a. Candida

Candida is a genus of yeasts and is the most common cause of infections worldwide (Manolakaki et al., 2010). There are over 150 species. Many of the species are harmless commensals or endosymbionts under normal conditions; when conditions are sufficient they can invade and cause disease. *Candida albicans* (*C. albicans*) and *Candida parapsilosis* (*C. parapsilosis*) are the two most likely species to cause candidiasis and candidemia.

Up to 40% of pregnant women may have vaginal colonization with *Candida* spp. (DiGiulio, 2012; R. L. Goldenberg et al., 2000). *Candida* spp. have been isolated from amniotic fluid of spontaneous preterm birth mothers (Maneenil et al., 2015). Cases studied have shown intra-amniotic infection can cause fetal death or fetal candidiasis with impaired CNS outcomes in humans (Benjamin et al., 2006; Darmstadt, Dinulos, & Miller, 2000; Marelli, Mariani, Frigerio, Leone, & Ferrari, 1996; Siriratsivawong, Pavlis, Hymes, & Mintzer, 2014).

In neonatal candidiasis, *C. albicans* and *C. parapsilosis* are the most commonly isolated species, and cause the majority of fungal infections in very-low-birth weight infants. Though *C. albicans* is not specifically associated with the risk of preterm delivery, there has been some evidence that suggests the elimination of the yeast reduces the risk of a late miscarriage and preterm birth (Maneenil et al., 2015). *C. albicans* is usually vertically transmitted whereas *C. parapsilosis* is mostly transmitted by contact other than parent-child (Abi-Said et al., 1997; Chapman & Faix, 2000; Reef et al., 1998; Waggoner-Fountain et al., 1996). Pathogenesis likely involves ascension of the infection from maternal candida infection then leading to amniotic fluid infection and chorioamnionitis/funisitis (Siriratsivawong et al., 2014).

Approximately 9-10% of nosocomial blood infections in neonates are fungal infections; 85.6% of these infections are caused by *Candida* spp. (Blaszkowska & Goralska, 2014). The mortality rate is the second highest ranging from 40-70% (Blaszkowska & Goralska, 2014). At risk neonates can develop candidiasis, a dermatitis caused by *Candida* spp. The dermatitis usually resolves on its own in neonates born full-term; however, the condition may advance to candidemia in very low birth weight infants possibly leading to death. Some of the risk factors for dermatitis is vaginal birth, extreme prematurity, low birth weight, hyperglycemia, and steroid administration.

Congenital cutaneous candidiasis can present in a variety of symptoms—diffuse skin eruption in the absence of systemic illness to severe systemic disease leading to stillbirth or early neonatal death (Darmstadt et al., 2000). Cutaneous eruptions occur in 82% of cases and are typically detected within 24 hours of birth (Darmstadt et al., 2000). Diagnosis of congenital candidiasis may be delayed due to the similar appearance to more common rashes in newborns which increases risk for adverse sequelae (Siriratsivawong et al., 2014).

Adhesion is a major virulence factor for this fungus. *Candida* spp. has the capability of forming biofilm which allows it to adhere to skin, mucus layers, and surfaces of tubes/catheters that may be used on neonates during hospitalization. There is evidence that *Candida* spp. has the ability to form

biofilms on intrauterine devices (IUD). Due to IUD application directly affecting the vaginal ecosystem, there may be a need for the consideration of *Candida* spp. internal exposure for those women planning to get pregnant after removing the IUD (Caliskan, Ozcan, Cinar, Corakci, & Caliskan, 2011),

V. CONCLUSION

Since the 1940's outbreak of rubella, it has become well recognized that infections during pregnancy can affect the mother and fetus. For most women, pregnancy is a normal and healthy state. But, there are factors that may make pregnant women more susceptible to infection. Changes in the immune system and the altering effects of hormones affect the immune system's susceptibility and responses to infection. Stress is common during pregnancy, but high levels may increase the risk of certain problems with delivering a healthy neonate by influencing the already altered immune functions. The diversity in the human microbiome has been correlated with the health of humans; as we learn more about the interconnecting relations between the microbiome and our health promoting specific diversity may be able to protect the mother and fetus during pregnancy in the future.

Infection has long been established as the leading cause of preterm births which has its own risks like: the infection of the central nervous system or low birth weight, which can allow the neonate to be more susceptible to infections postpartum. Chorioamnionitis indicates the infection of the intrauterine environment and is a major risk factor for neonatal sepsis, which will cause those neonates to commonly present with multiple insults or lead to a still birth. Still births are an end to a pregnancy when the conditions in the mother are unable to keep the fetus alive.

Intrauterine infections are caused by a diverse group of organisms manifesting in a range of adverse outcomes in pregnancy. The TORCH complex is a reminder of the organisms that can produce congenital defects when infected during pregnancy. The mechanisms of some infections are

currently unknown and additional research is needed to understand how these pathogens cause adverse outcomes in pregnancy. Those that are understood may be preventable; others, like the Zika virus, are emerging and are only now being studied as outbreaks occur.

With the ever globalizing world, the risks of being exposed or contracting these infections rises as organisms can travel across continents in mere hours. Environmental factors such as changes in climate allow for those organisms limited to certain environments to move into more areas ranging into populated areas or reducing their environments into seclusion

The understanding of intrauterine infections is vital to ensure the health of all future mothers and their offspring. Many factors influence the susceptibility of infection. Contracting an intrauterine infection has health impacts on the mother and fetus. The fetus's development is a major concern. Being exposed to intrauterine infection, exhibiting a range of severity exhibited in the sequelae after birth or death may significantly impair normal development. There are many known organism that are capable of producing congenital defects during pregnancy. However, there are new emerging organisms that have yet to be studied. Additional research to understand the mechanisms and risks associated with these organisms is critical for the health of all potential mothers and their offspring.

Table 1: Effects of Selected Pathogens on Fetuses and Infants

Pathogen	Exposure	Symptoms	Outcome of Fetus/Infant	Prevention	Sources
Bacteria					
***Brucella* species**	Contact with infected animals; consumption of contaminated raw milk or milk products; inhalation of aerosols; horizontal transmission; breast milk *most commonly reported laboratory-acquired infection	Fever, chills, sweating, weakness, malaise, headache, and joint and muscle pain.	Spontaneous abortions	Avoid unpasteurized milk and unpasteurized milk products	(Food and Drug Administration, 2012; Kurdoglu et al., 2010)
Campylobacter jejuni	Raw milk, untreated water, raw and undercooked meat, poultry, or shellfish; vertical transmission	Diarrhea (sometimes bloody), stomach cramps, fever, muscle pain, headache, nausea, bacteremia, reactive arthritis	Intrauterine fetal infection, abortion, stillbirth, death in early life, enteritis, meningitis. Fetal harm increases when maternal bacteremia is present.	Careful food preparation.	(Blaser, 1997; Food and Drug Administration, 2012; J. L. Smith, 2002)
Clostridium perfringens	Meat and meat products, especially undercooked	Abdominal pain, diarrhea, occasional nausea and vomiting	Intravascular hemolysis, tissue necrosis, sepsis, fetal death	Careful food preparation.	(Blaser, 1997; Food and Drug Administration, 2012)
***Coxiella burnetii* (Q fever)**	Inhalation of aerosolized bacteria from bodily fluids of infected host animals (including humans); Unpasteurized milk and milk products or foods made with unpasteurized milk; tick bites;	fever; severe headaches; muscle aches; chills; heavy sweating; nausea, vomiting, or diarrhea; dry cough; and abdominal or chest pain; may be asymptomatic	Preterm birth, neonatal death	Avoid unpasteurized milk and unpasteurized milk products	(Food and Drug Administration, 2012; Langley et al., 2003)
Listeria monocytogenes	Unpasteurized milk and milk products or foods made with unpasteurized milk, cheeses (particularly soft cheeses), ice cream, raw vegetables, raw poultry and meats, fermented raw-meat sausages, hot dogs and deli meats, raw/smoked fish and other seafood	In pregnant women, flu-like symptoms, fever, headache, fatigue, Muscle aches, nausea, vomiting, diarrhea. Many times woman is asymptomatic.	Miscarriage, fetal death, stillbirths, bacteremia, meningitis.	Avoid unpasteurized milk and unpasteurized milk products; careful food preparation.	(Allerberger & Wagner, 2010; CDC, 2013c; Farber & Peterkin, 1991; Food and Drug Administration, 2012)

***Salmonella* Enteritidis**	Raw/undercooked eggs, raw meat, poultry, seafood, raw milk, dairy products, produce	Diarrhea, fever, vomiting, headache, nausea, stomach cramps	Sepsis, fetal death	Careful food preparation.	(Food and Drug Administration, 2012; Roll, Schmid, Menken, & Hanssler, 1996)
Viruses					
Coxsackie virus	Person-to-person through the fecal–oral route	Acute febrile illness, myocarditis, pericarditis, pleurodinia, exanthemas or enanthemas, hand, foot, and mouth disease, herpangina, acute hemorrhagic conjunctivitis, aseptic meningitis, encephalitis	Increased rate of insulin-dependent diabetes mellitus, severe respiratory failure, mental retardation, myocarditis, cardiac anomalies. Neonates may experience pneumonia, myocarditis, meningoencephalitis, and/or a severe maculopapular rash	Careful personal hygiene	(Bauer et al., 2002; Ornoy & Tenenbaum, 2006)
Cytomegalovirus (CMV)	Person-to-person through bodily fluids and vertical transmission to fetus	Mothers are generally asymptomatic	90–95% of affected infants are symptom-free at birth. Mental retardation, microcephaly, sensorineural hearing loss, and chorioretinitis are common symptoms. Fetal infection is most likely if maternal infection occurs in the first half of pregnancy	Careful personal hygiene, avoid organ and tissue transplantation from CMV-positive donors, antiviral drugs	(Gindes, Teperberg-Oikawa, Sherman, Pardo, & Rahav, 2008; Elie Shlyakhov, Shlomo Segev, & Ethan Rubinstein, 1998; Stegmann & Carey, 2002; Stern & Tucker, 1973)
Echovirus	Perinatal mother-to-fetus/infant	Rashes, diarrhea, respiratory infections, myositis, meningitis, encephalitis, pericarditis	Poor feeding, lethargy, jaundice, hepatic failure, seizures, apnea, and hemorrhaging	Avoid Caesarian sections; delay delivery to allow for transmission of antibodies from mother transplacentally	(Modlin, 1980; Modlin et al., 1981; Ornoy & Tenenbaum, 2006)
Hepatitis B	Horizontally by blood and sexual transmission. Vertically during pregnancy or labor	Loss of appetite, nausea, vomiting, fever, abdominal pain, jaundice	Low birth weight is common if infection occurs in the third trimester	Avoid contact with infected persons or blood	(Borgia, Carleo, Gaeta, & Gentile, 2012; CDC, 2015f) (Ornoy & Tenenbaum, 2006)
Hepatitis C	Typically, by blood. Rarely by sexual transmission. Low mother–fetus transmission	Information not available	There may be a greater risk of fetal complications; however, data are inconclusive	No vaccine. Avoid contact with those infected	(CDC, 2015f; Ornoy & Tenenbaum, 2006)
Hepatitis E	Fecal-oral route; Vertical transmission	Loss of appetite; nausea; abdominal pain; pain in joints; enlarged liver; jaundice; fever.	Preterm deliveries, perinatal mortality and morbidity	Careful food preparation; careful personal hygiene.	(Food and Drug Administration, 2012) (CDC, 2015f; Kumar et al., 2004)

		Pregnant women tend to get sicker and more likely to die; can cause hepatic damage and/or failure			
Herpes simplex virus (Herpes simplex)	Transmission from new HSV infections near delivery or reactivation of latent infection of prior maternal infection	Blistering and ulceration of external genitalia, fever or myalgia May also be asymptomatic	Abortion, stillbirths, congenital malformations, or severe neurodevelopmental disabilities	No vaccine Antiretroviral therapies (acyclovir and/or valaciclovir therapies)	(E. Shlyakhov, S. Segev, & E. Rubinstein, 1998; Stegmann & Carey, 2002)
Human immunodeficiency virus (HIV)	Vertical transmission from pregnant women to fetus not only during the last trimester but also during delivery or breastfeeding	Blood specimen positive 48 h after birth	Fetus/infant becomes infected with the virus	Antiretroviral therapies (especially zidovudine and combination therapies)	(Sauerbrei & Wutzler, 2007; E. Shlyakhov et al., 1998; Watts et al., 2003)
Influenza	Postnatal transmission, possibly vertical transmission	Mother with flu	Preterm birth, low birth weight	Vaccines are available	(CDC, 2015b; Mosby, Rasmussen, & Jamieson, 2011; Yates et al., 2010)
Measles (Morbilli, Rubeola, or Red Measles)	Person-to-person contact	Maternal rash, fever	Spontaneous abortion, premature labor, increased fetal/neonatal loss	Measles-mumps-rubella (MMR) vaccine, which has greatly reduced incidence in developed nations	(Chiba, Saito, Suzuki, Honda, & Yaegashi, 2003; Ornoy & Tenenbaum, 2006)
Rubella virus (German measles or three-day measles)	Vertical transmission from mother to placenta and fetus	Mother with rubella	Spontaneous abortion, stillbirth, damage to fetal organs, hearing loss, heart abnormalities, ocular defects, encephalopathy	Vaccination of children and female teenagers with MMR vaccine, which has greatly reduced incidence in developed nations	(E. Shlyakhov et al., 1998; Stegmann & Carey, 2002)
Varicella zoster virus (Chickenpox)	Contact with infected individuals	Chicken pox, shingles	Congenital limb hypoplasia, skin scarring, damage to the eyes and CNS	Chicken pox vaccine	(E. Shlyakhov et al., 1998)
Human parvovirus B19 (Fifth disease)	Vertical transmission to fetus	In children, slapped cheek rash	Hydrops fetalis, fetal death, acute arthritis	Avoid contact with infected individuals	(Stegmann & Carey, 2002) (E. Shlyakhov et al., 1998)
Zika virus	Likely by blood. Possibly by sexual transmission. Vertical transmission to fetus	Fever, rash, joint pain, conjunctivitis, muscle pain and headache.	Associated with microcephaly, Guillain-Barré Syndrome, and other neurological disorders	No vaccine available	(Foy et al., 2011; Higgs, 2016; Lavinia Schuler-Faccini et al., 2016; Triunfol, 2015; WHO, 2016)
Parasites					
Neospora caninum	Infected cats, dogs, cattle, and other animals	No occurrence has been found in humans. Zoonotic potential is unknown, but caution should still be taken	No occurrence has been found in humans. Zoonotic potential is unknown, but caution should still be taken	Use caution when disposing of pet waste (i.e., changing a litter box). Wash hands after dealing with animals.	(Dubey & Lindsay, 1996; Quinn, Ellis, & Smith, 2002)

***Plasmodium falciparum* (Malaria)**	Bites from female mosquitos carrying infected blood	Maternal anemia	Low birth weight	Use preventative measures against mosquito bites (insect repellant, bed nets, etc.). Chloroquine chemoprophylaxis and sulfadoxine-pyrimethamine intermittent treatment	(Bardaji et al., 2011; White et al., 2014; WHO, 2015b)
Toxoplasma gondii	Uncooked meat, cats/cat feces	Typically, asymptomatic. Fever, fatigue, muscle pain, sore throat, headache. Encephalitis may occur in immunocompromised patients.	Hydrocephalus, ventricle dilation, blindness, mental retardation, intracranial calcification, or chorioretinitis	Avoid consuming raw or undercooked meat, unpasteurized goat's milk, and untreated water; wash hands well after handling cats, litter boxes, and soil; possibly raw shellfish; pregnant women should not handle cat feces	(Food and Drug Administration, 2012; Stegmann & Carey, 2002)
Fungi					
***Candidia* infection (Thrush, Vaginal Yeast Infection, Invasive Candidasis)**	Unhygienic practices in medical setting; imbalance on persons	Maternal positive for *Candidia* infection	Chorioamnionitis, preterm birth, low birth weight, congenital cutaneous candidiasis	Careful personal hygiene; See healthcare provider; treatments available	(CDC, 2015a; Ito et al., 2013)

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APPENDIX C

R SOURCE CODE AND OUTPUT FOR CHAPTER 3

```
> #Data Retrieval
>   #Samples From ALL Homes
> AllSamples<-read.csv("Expanded Crono Data V2.csv")
>  #Samples From Crono Homes (65)
> CronoSamples<-read.csv("Expanded Crono Data V2 Crono ONLY.csv")
>   # Homes (65&54)
> Homes<-read.csv("Expanded Survey Data V2.csv")
>   ### Regression
>     ### Binomial
> # Fig 3.2
>   #Homes with Cono present in at least 1 location (NotSig)
> CronoPresent<-glm(CronoPresence~Htype, family = "binomial", data = Homes)
> anova(CronoPresent,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: CronoPresence

Terms added sequentially (first to last)


      Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                     64     67.731
Htype  2   4.1389        62     63.592   0.1263
> # Fig 3.3
>   # Samples (65)
> AllIso<-glm(CronoN~Htype+ZoneII+A.Seasons, family = "binomial", data = AllS
amples)
> anova(AllIso,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: CronoN

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                      1949    1047.67
Htype      2    8.913      1947    1038.76   0.01161 *
ZoneII     3  140.291      1944     898.47 < 2.2e-16 ***
A.Seasons  3   22.143      1941     876.32 6.092e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(AllIso)

Call:
glm(formula = CronoN ~ Htype + ZoneII + A.Seasons, family = "binomial",
    data = AllSamples)

Deviance Residuals:
```

```
    Min       1Q   Median       3Q      Max
-0.8750  -0.3661  -0.2393  -0.1573   2.9732

Coefficients:
                Estimate Std. Error z value Pr(>|z|)
(Intercept)      -4.7005     0.5533  -8.496  < 2e-16 ***
HtypeNon-Urban    0.3148     0.2409   1.307 0.191172
HtypeUrban        0.2926     0.2485   1.177 0.239052
ZoneIIFloor       3.4669     0.5186   6.685 2.31e-11 ***
ZoneIIKitchen     1.5605     0.5326   2.930 0.003388 **
ZoneIIThreshold   2.5923     0.5432   4.773 1.82e-06 ***
A.SeasonsSpring  -1.0152     0.3076  -3.300 0.000967 ***
A.SeasonsSummer   0.1561     0.2180   0.716 0.473967
A.SeasonsWinter  -0.7140     0.2950  -2.420 0.015512 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 1047.67  on 1949  degrees of freedom
Residual deviance:  876.32  on 1941  degrees of freedom
AIC: 894.32

Number of Fisher Scoring iterations: 7

> # Fig 3.4 & 3.5
>   # Samples (51)
> AllIso2<-glm(CronoN~Htype+ZoneII+A.Seasons+Garden+Cleaned+Pets, family = "b
inomial", data = AllSamples)
> anova(AllIso2,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: CronoN

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                      1619     865.60
Htype      2    7.494      1617     858.10  0.023586 *
ZoneII     3  115.253      1614     742.85 < 2.2e-16 ***
A.Seasons  3   13.716      1611     729.13  0.003318 **
Garden     1    7.375      1610     721.76  0.006612 **
Cleaned    1    2.725      1609     719.03  0.098777 .
Pets       1    1.162      1608     717.87  0.281101
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(AllIso2)

Call:
glm(formula = CronoN ~ Htype + ZoneII + A.Seasons + Garden +
    Cleaned + Pets, family = "binomial", data = AllSamples)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
```

```
-0.9982  -0.4087  -0.2538  -0.1625   3.0133

Coefficients:
                Estimate Std. Error z value Pr(>|z|)
(Intercept)      -5.1839     0.6478  -8.003 1.22e-15 ***
HtypeNon-Urban    0.2841     0.2794   1.017  0.30931
HtypeUrban        0.4972     0.2786   1.784  0.07437 .
ZoneIIFloor       3.2954     0.5229   6.302 2.93e-10 ***
ZoneIIKitchen     1.3278     0.5408   2.455  0.01408 *
ZoneIIThreshold   2.3899     0.5529   4.323 1.54e-05 ***
A.SeasonsSpring  -0.7974     0.3196  -2.495  0.01260 *
A.SeasonsSummer   0.5297     0.2914   1.818  0.06908 .
A.SeasonsWinter  -0.2003     0.3328  -0.602  0.54722
Garden            0.9185     0.3015   3.046  0.00232 **
Cleaned          -0.2668     0.2243  -1.190  0.23413
Pets             -0.2812     0.2603  -1.080  0.28007
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 865.60  on 1619  degrees of freedom
Residual deviance: 717.87  on 1608  degrees of freedom
  (330 observations deleted due to missingness)
AIC: 741.87

Number of Fisher Scoring iterations: 7

>   # CronoSamples (65)
> CronoIso<-glm(CronoN~Htype+ZoneII+A.Seasons, family = "binomial", data = Cr
onoSamples)
> anova(CronoIso,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: CronoN

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                      1529     972.60
Htype      2    2.201      1527     970.40  0.332758
ZoneII     3  143.579      1524     826.82 < 2.2e-16 ***
A.Seasons  3   15.497      1521     811.32  0.001438 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(CronoIso)

Call:
glm(formula = CronoN ~ Htype + ZoneII + A.Seasons, family = "binomial",
    data = CronoSamples)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.9325  -0.3915  -0.2663  -0.1682   2.9207
```

```
Coefficients:
                Estimate Std. Error z value Pr(>|z|)
(Intercept)     -4.31174    0.55139  -7.820 5.29e-15 ***
HtypeNon-Urban   0.11949    0.24762   0.483  0.62942
HtypeUrban       0.06065    0.24988   0.243  0.80823
ZoneIIFloor      3.52892    0.52010   6.785 1.16e-11 ***
ZoneIIKitchen    1.56775    0.53330   2.940  0.00329 **
ZoneIIThreshold  2.61644    0.54464   4.804 1.56e-06 ***
A.SeasonsSpring -0.89550    0.31872  -2.810  0.00496 **
A.SeasonsSummer  0.05570    0.22760   0.245  0.80666
A.SeasonsWinter -0.69744    0.30252  -2.305  0.02114 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 972.60  on 1529  degrees of freedom
Residual deviance: 811.32  on 1521  degrees of freedom
AIC: 829.32

Number of Fisher Scoring iterations: 7

>   # CronoSamples (51)
> CronoIso2<-glm(CronoN~Htype+ZoneII+A.Seasons+Garden+Cleaned+Pets, family =
"binomial", data = CronoSamples)
> anova(CronoIso2,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: CronoN

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                      1289     807.52
Htype      2    4.378      1287     803.15  0.112044
ZoneII     3  118.019      1284     685.13 < 2.2e-16 ***
A.Seasons  3   13.155      1281     671.97  0.004314 **
Garden     1    1.395      1280     670.58  0.237553
Cleaned    1    0.796      1279     669.78  0.372260
Pets       1    0.687      1278     669.09  0.407158
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(CronoIso2)

Call:
glm(formula = CronoN ~ Htype + ZoneII + A.Seasons + Garden +
    Cleaned + Pets, family = "binomial", data = CronoSamples)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.0504  -0.4078  -0.2729  -0.1838   2.8910

Coefficients:
```

```
                Estimate Std. Error z value Pr(>|z|)
(Intercept)      -4.5185     0.6585  -6.862 6.78e-12 ***
HtypeNon-Urban    0.1078     0.2901   0.371   0.7103
HtypeUrban        0.2557     0.2879   0.888   0.3744
ZoneIIFloor       3.3536     0.5246   6.393 1.63e-10 ***
ZoneIIKitchen     1.3338     0.5416   2.463   0.0138 *
ZoneIIThreshold   2.4116     0.5545   4.349 1.37e-05 ***
A.SeasonsSpring  -0.8727     0.3408  -2.561   0.0104 *
A.SeasonsSummer   0.1579     0.3175   0.497   0.6189
A.SeasonsWinter  -0.6802     0.3512  -1.937   0.0528 .
Garden            0.3666     0.3324   1.103   0.2701
Cleaned          -0.2673     0.2356  -1.134   0.2567
Pets              0.2263     0.2733   0.828   0.4077
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 807.52  on 1289  degrees of freedom
Residual deviance: 669.09  on 1278  degrees of freedom
  (240 observations deleted due to missingness)
AIC: 693.09

Number of Fisher Scoring iterations: 7

> ## Poisson
>   # Fig.3.7
>   # Survey (65)
> NOAA<-glm(N.Crono~RainCM*TempC, family = "poisson", data = Homes)
> anova(NOAA,test="LRT")
Analysis of Deviance Table

Model: poisson, link: log

Response: N.Crono

Terms added sequentially (first to last)


            Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                           64     137.08
RainCM       1   4.8781        63     132.20   0.0272 *
TempC        1   0.8328        62     131.37   0.3615
RainCM:TempC 1   0.2881        61     131.08   0.5915
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(NOAA)

Call:
glm(formula = N.Crono ~ RainCM * TempC, family = "poisson", data = Homes)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.3914  -1.0812  -0.0914   0.5829   4.9484

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
```

```
(Intercept)  -0.677140   1.429321  -0.474    0.636
RainCM        0.065402   0.071436   0.916    0.360
TempC         0.093129   0.130387   0.714    0.475
RainCM:TempC -0.003484   0.006496  -0.536    0.592

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 137.08  on 64  degrees of freedom
Residual deviance: 131.08  on 61  degrees of freedom
AIC: 280.62

Number of Fisher Scoring iterations: 5

>   # Fig. 3.8 (54)
> PPL<-glm(N.Crono~TotalPPL.+GardenHr+Total.Pets., family = "poisson", data =
Homes)
> anova(PPL,test="LRT")
Analysis of Deviance Table

Model: poisson, link: log

Response: N.Crono

Terms added sequentially (first to last)


           Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                         53     116.44
TotalPPL.   1   5.6206        52     110.83  0.01775 *
GardenHr    1   0.0761        51     110.75  0.78259
Total.Pets. 1   2.6364        50     108.11  0.10444
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(PPL)

Call:
glm(formula = N.Crono ~ TotalPPL. + GardenHr + Total.Pets., family = "poisson
",
    data = Homes)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.0859  -1.0191  -0.2788   0.2921   4.9386

Coefficients:
           Estimate Std. Error z value Pr(>|z|)
(Intercept)  0.54488    0.21372   2.549   0.0108 *
TotalPPL.    0.15887    0.07200   2.207   0.0273 *
GardenHr     0.01893    0.07266   0.260   0.7945
Total.Pets. -0.10425    0.06569  -1.587   0.1125
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 116.45  on 53  degrees of freedom
Residual deviance: 108.11  on 50  degrees of freedom
```

```
  (11 observations deleted due to missingness)
AIC: 233.77

Number of Fisher Scoring iterations: 5

> ### Entero
>     ## Binomial
> #Homes with Entero present in at least 1 location (NotSig)
> EnteroPresent<-glm(EnteroPresence~Htype, family = "binomial", data = Homes)
> anova(EnteroPresent,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: EnteroPresence

Terms added sequentially (first to last)


      Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                     64  0.000e+00
Htype  2        0        62  3.771e-10        1
> # Samples (65)
> AllIsoE<-glm(EnteroN~Htype+ZoneII+A.Seasons, family = "binomial", data = Al
lSamples)
> anova(AllIsoE,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: EnteroN

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                      1949     2301.1
Htype      2    4.563      1947     2296.5 0.1021416
ZoneII     3   67.436      1944     2229.1  1.51e-14 ***
A.Seasons  3   19.305      1941     2209.8 0.0002365 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(AllIsoE)

Call:
glm(formula = EnteroN ~ Htype + ZoneII + A.Seasons, family = "binomial",
    data = AllSamples)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.9891  -1.2488   0.6744   0.8283   1.2202

Coefficients:
                Estimate Std. Error z value Pr(>|z|)
(Intercept)       0.8222     0.1676   4.907 9.26e-07 ***
HtypeNon-Urban   -0.2664     0.1276  -2.088   0.0368 *
HtypeUrban       -0.1362     0.1421  -0.958   0.3379
```

```
ZoneIIFloor       0.6322     0.1509   4.189 2.80e-05 ***
ZoneIIKitchen     1.0072     0.1301   7.743 9.72e-15 ***
ZoneIIThreshold   0.2075     0.1627   1.275   0.2022
A.SeasonsSpring  -0.6558     0.1544  -4.247 2.17e-05 ***
A.SeasonsSummer  -0.1979     0.1482  -1.336   0.1816
A.SeasonsWinter  -0.2883     0.1647  -1.751   0.0800 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 2301.1  on 1949  degrees of freedom
Residual deviance: 2209.8  on 1941  degrees of freedom
AIC: 2227.8

Number of Fisher Scoring iterations: 4

> # EnteroSamples (65)
> CronoIsoE<-glm(EnteroN~Htype+ZoneII+A.Seasons, family = "binomial", data =
CronoSamples)
> anova(CronoIsoE,test="LRT")
Analysis of Deviance Table

Model: binomial, link: logit

Response: EnteroN

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                      1529     1768.4
Htype      2   12.766      1527     1755.7   0.00169 **
ZoneII     3   56.113      1524     1699.6 3.973e-12 ***
A.Seasons  3    6.080      1521     1693.5   0.10778
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(CronoIsoE)

Call:
glm(formula = EnteroN ~ Htype + ZoneII + A.Seasons, family = "binomial",
    data = CronoSamples)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.0955  -1.2465   0.6240   0.8244   1.1445

Coefficients:
                Estimate Std. Error z value Pr(>|z|)
(Intercept)       1.0415     0.1950   5.343 9.17e-08 ***
HtypeNon-Urban   -0.5401     0.1555  -3.474 0.000513 ***
HtypeUrban       -0.3868     0.1690  -2.289 0.022064 *
ZoneIIFloor       0.5490     0.1711   3.210 0.001329 **
ZoneIIKitchen     1.0359     0.1499   6.909 4.89e-12 ***
ZoneIIThreshold   0.1323     0.1845   0.717 0.473276
A.SeasonsSpring  -0.3405     0.1866  -1.825 0.068028 .
A.SeasonsSummer  -0.1458     0.1646  -0.886 0.375711
```

```
A.SeasonsWinter  -0.4236     0.1879  -2.255 0.024151 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 1768.4  on 1529  degrees of freedom
Residual deviance: 1693.5  on 1521  degrees of freedom
AIC: 1711.5

Number of Fisher Scoring iterations: 4

>     ## Poisson
> # Survey (65)
> NOAAE<-glm(N.Entero~RainCM*TempC, family = "poisson", data = Homes)
> anova(NOAAE,test="LRT")
Analysis of Deviance Table

Model: poisson, link: log

Response: N.Entero

Terms added sequentially (first to last)


             Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                         64     42.578
RainCM        1  0.28815        63     42.290   0.5914
TempC         1  1.35121        62     40.939   0.2451
RainCM:TempC  1  0.00002        61     40.939   0.9966
> # (54)
> PPLE<-glm(N.Entero~TotalPPL.+GardenHr+Total.Pets., family = "poisson", data
= Homes)
> anova(PPLE,test="LRT")
Analysis of Deviance Table

Model: poisson, link: log

Response: N.Entero

Terms added sequentially (first to last)


            Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                         53     33.035
TotalPPL.    1  0.26557        52     32.770   0.6063
GardenHr     1  0.57946        51     32.190   0.4465
Total.Pets.  1  0.27064        50     31.920   0.6029
```