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RISK FACTORS FOR SPORADIC NON-TYPHOIDAL SALMONELLA INFECTIONS IN MICHIGAN CHILDREN: A POPULATION-BASED CASE-CONTROL STUDY

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# RISK FACTORS FOR SPORADIC NON-TYPHOIDAL *SALMONELLA* INFECTIONS IN MICHIGAN CHILDREN: A POPULATION-BASED CASE-CONTROL STUDY

By

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## ABSTRACT

RISK FACTORS FOR SPORADIC NON-TYPHOIDAL *SALMONELLA* INFECTIONS IN MICHIGAN CHILDREN: A POPULATION-BASED CASE-CONTROL STUDY

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Epidemiologic investigations have consistently shown higher incidences of laboratory-confirmed *Salmonella* infections (salmonellosis) in children compared to adults. Our recent work investigating associations between demographic attributes and salmonellosis based on data from the Michigan Department of Community Health (MDCH) (1995-2001) revealed about an approximate 10-fold increase in risk for acquiring salmonellosis in children aged < 1 year and a 3-fold increase in risk for those aged 1-4 years when compared to adults aged 15-39 years. The majority (80 – 85%) of sporadic cases of salmonellosis in adult populations results from exposures to contaminated foods. However, few analytical studies have addressed the role of contaminated environmental exposures, which have been suspected in a large proportion of cases of *Salmonella* infections in children. We conducted a population-based case-control study of sporadic cases of non-typhoidal *Salmonella* infections in Michigan children aged ≤ 10 years to identify various food vehicles and environmental exposures associated with illness in this high-risk population. Laboratory-confirmed cases of *Salmonella* infections in children aged ≤ 10 years reported to MDCH, and healthy control children who did not experience symptoms of gastrointestinal illness during the past month, were recruited between December 15, 2006 and October 15, 2007. Controls were obtained using an on-line telephone directory. A pre-tested structured questionnaire, administered through trained

interviewers or self-administered by mail-in questionnaire, was used to gather data from parent(s) or caretakers. Information was collected on sociodemographic characteristics of children, child rearing (e.g., daycare, pre-school, elementary school attendance etc.), and various environmental exposures (e.g., contact with animals, contact with a person having symptoms of gastrointestinal illness). A total of 123 cases and 139 controls were enrolled during the study period. The final multivariate model, after adjusting for age group revealed that having salmonellosis was significantly associated with contact with cats (adjusted odds ratio (AOR) = 2.62, 95% CI: 1.17 – 5.87) and reptiles (AOR = 8.16, 95% CI: 1.55 – 42.88). Additionally, attending a daycare center (AOR = 4.86, 95% CI: 1.44 – 16.37) and contact with a person having symptoms of gastrointestinal infection during the 3 days prior to the onset of child's illness was significantly associated with *Salmonella* infections (AOR = 2.27, 95% CI: 1.02 – 5.44). Salmonellosis was not associated with exposures to other household- and food-related sources. Our study results suggest that environmental sources significantly contribute to the acquisition of *Salmonella* infections in children. This is in contrast to the adult population where a larger proportion of infections are acquired through food vehicles. Several public health recommendations have been made to educate parents and caretakers about the risk of *Salmonella* transmission to children from infected persons and animals, particularly reptiles. However, our study demonstrated that exposure to these factors continue to cause *Salmonella* infections in children. Additional efforts are needed to educate parents and caretaker about the risk of *Salmonella* transmission to children from cats and reptiles, along with the risk of 2% transmission following exposure to symptomatic individuals.

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| | |
|---|---|
| AOR | Adjusted Odds Ratio |
| AVMA | American Veterinary Medical Association |
| CDC | Centers for Disease Control and Prevention |
| CDR | Communicable Disease Rules |
| CFSAN | Center for Food Safety and Applied Nutrition |
| CI | Confidence Interval |
| CRIRB | Community Research Institutional Review Board |
| DHEC | Department of Health and Environment Control |
| ESR | Economic Research Services |
| FoodNet | Foodborne Disease Active Surveillance Network |
| FSIS | Food Safety Inspection Service |
| GI | Gastrointestinal |
| HACCP | Hazard Analysis Critical Control Point |
| IRB | Institutional Review Board |
| LHD | Local Health Department |
| LR | Logistic Regression |
| MDCH | Michigan Department of Community Health |
| MDSS | Michigan Disease Surveillance System |
| MDR | Multi Drug Resistant |
| MMWR | Morbidity Mortality Weekly Reports |
| NARMS | National Antimicrobial Resistance Monitoring System |
| OR | Odds Ratio |
| PAR | Population Attributable Risk |
| PHLIS | Public Health Information Laboratory System |
| PR-HACCP | Pathogen Reduction-Hazard Analysis Critical Control Point |
| RR | Rate Ratio |
| SES | Socioeconomic Status |
| US FDA | United States Food and Drug Administration |
| USDA | United States Department of Agriculture |

## BACKGROUND

### Foodborne infections:

Globally, foodborne illnesses are a major public health concern (1, 2). Foodborne infections are a common, unpleasant and sometimes life-threatening problem for millions of people worldwide. The Centers for Disease Control and Prevention (CDC) estimates 76 million people experience foodborne illnesses each year in the United States (US), accounting for 325,000 hospitalizations and more than 5,000 deaths (1, 3). A recent report noted that an estimated 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths annually are caused by foodborne pathogens where the etiologic agent is known. More than 250 foodborne infections have been described so far. However, in the US, the majority of bacterial foodborne infections are caused by *Salmonella*, *Campylobacter*, and *Escherichia coli* (4).

The symptoms of foodborne illnesses vary widely depending on the etiologic agent, dosage, and immunologic status of the host. However, diarrhea, vomiting, and abdominal discomfort are the most common symptoms (5). In the US, regulations for the control of foodborne and waterborne illnesses have been in place since the early 1900s. The CDC, in partnership with state and local counterparts, has been responsible for the investigation, control, and prevention of diseases spread by food and water since 1961. In 1996, the CDC established the Foodborne Disease Active Surveillance Network (FoodNet). FoodNet is the principal foodborne disease component of CDC's Emerging Infections Program (EIP). It is a collaborative project among CDC, state health departments in EIP sites, the Food Safety and Inspection Service (FSIS) of the United

States Department of Agriculture (USDA), and the Center for Food Safety and Applied Nutrition (CFSAN) of the US Food and Drug Administration (FDA) (6, 7). The objectives of FoodNet are to 1) determine the frequency and severity of foodborne diseases, 2) monitor trends in foodborne diseases over time and 3) study the association of common foodborne diseases with the consumption of specific foods. To address these objectives, FoodNet uses active surveillance and conducts epidemiologic studies. Between 1996 and 2006, the FoodNet surveillance population increased from 14.2 million persons (5% of the US population) in five states to 44.9 million persons (15% of the US population) in 10 states (8).

FoodNet specifically targets seven bacterial pathogens: *Campylobacter*, *E. coli* O157:H7, *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia* (7, 8). This report discusses the epidemiology of *Salmonella* infections.

## *SALMONELLA*

*Salmonella* are gram-negative, rod-shaped, nonlactose-fermenting, bacteria belonging to the family *Enterobacteriaceae* (9). *Salmonella* can survive and grow under a variety of environmental conditions outside of living hosts, ranging from dry surfaces to indigenous flora of living animals. *Salmonella* have been recovered from almost all vertebrate species. Inhibition of *Salmonella* growth occurs at pH <3.8 and temperature <7°C (10).

*Salmonella* serotypes are a common cause of zoonotic infections and are considered among the most ubiquitous pathogens, both in humans and animals (11). From the time of the first *Salmonella* isolation from a diarrheic pig in 1885 by Salmon and Smith and the first laboratory confirmed outbreak of salmonellosis in humans due to contaminated beef in 1888, *Salmonella* have been considered one of the most important foodborne pathogens worldwide (12). The large number of foodborne outbreaks associated with *Salmonella* infections is testimony to the importance of this bacterial genus (13). Additionally, the social and economic impact of *Salmonella* infections is considerable. They impose significant costs upon the public sector, on industry (especially the food industries), and upon infected persons and their families. In 1989, the costs of *Salmonella* infections were reported at $4 billion in the US and $486 million in Canada (14). The Economic Research Service (ERS), and the USDA have estimated that the annual economic costs due to *Salmonella* infections are $3 billion, and $2.9 billion of that cost is due to foodborne *Salmonella* infections. This estimate includes medical costs and the value of time lost from work due to acute illnesses, and the economic cost of premature deaths (15).

**Pathogenesis:**

*Salmonella* enters the human digestive system through *Salmonella*-contaminated food, water, or environmental sources (e.g., person-to-person transmission) and survives at low acidic conditions in the stomach by possessing an adaptive acid-tolerance response (particularly *Salmonella* serotype Typhimurium) (16). *Salmonella* passes into the small intestine via flagellal movement and swim chemotactically toward the mucosal surface. Their fimbriae adheres to intestinal epithelium using receptors present on the epithelium. After colonizing the lower intestine (ileum and cecum) (9), *Salmonellae* invades the mucosal cell, resulting in an acute inflammation. This inflammation leads to the activation of adenylate cyclase, increased fluid production, and release of fluid into the intestinal lumen, which results in diarrhea. *Salmonella* gastroenteritis has an 8-72 hours incubation period and may last from 2-7 days (17). The clinical presentation of salmonellosis varies by serotype, infectious dose, nature of the contaminated food, and host immune status. Certain serotypes are highly pathogenic for humans. However, the virulence of rare serotypes is not known.

**Transmission of *Salmonella* infections:**

*Salmonella* is typically transmitted through the fecal-oral route. Ingestion of contaminated food and water is the most important source of human infection. Although a large number of bacteria ($10^6$ cfu) are usually needed to cause an infection, the bacteria grow well in most types of food. In foods with a high fat content, such as chocolate and cheese, the infective dose is very low and only a few bacteria may be sufficient to cause infection (18). The following food items have been implicated in

outbreaks of human salmonellosis worldwide (5): meat products (raw meat, corned beef, salami, ham, cooked turkey meat, salami sticks); milk products (raw milk, infant dried milk, unpasteurized raw milk); soft cheese products (cheddar cheese, vacherin Mont d'Or cheese, mozzarella); eggs or products containing eggs (mayonnaise, custard in bakery goods, ice cream, confectionery products), fresh produce (mung bean sprouts, cantaloupe, fresh tomatoes, alfalfa sprouts, raw almonds); and other foods (potato salad, apple cider, roast cuttlefish, unpasteurized orange juice, peanut butter). Person-to-person transmission of *Salmonella* in households, daycares, nursing homes, and healthcare settings has been reported (19, 20). Having pets in the household, particularly reptiles, have also been associated with transmission of *Salmonella* infections in family members as these animals harbor *Salmonella* (21). Other factors such as lack of hygienic practices have also been shown to contaminate the environment with *Salmonella* (22) and result in indirect transmission of *Salmonella* infections. *Salmonella* can withstand the environment outside its host for a long period of time, therefore, inanimate objects that are contaminated can serve as a vehicle for the transmission of infection to a susceptible individual (22).

**Clinical manifestations:**

*Salmonella* causes illnesses ranging from mild to severe gastroenteritis, bacteremia, septicemia, localized infections, and a variety of long-term sequelae such as reactive arthritis and Reiter's syndrome (joint pain, irritation of eyes, and painful urination) (5). While human infection with a host-specific serotype (typhoidal serotypes) such as *S.* Typhi is associated with rather severe disease symptoms, the typical symptoms of

salmonellosis attributable to infection with non-typhoidal serotypes may include nausea, vomiting, fever, diarrhea, and abdominal cramps. Stools are typically loose, of moderate volume, and usually do not contain blood. Diarrhea is usually self-limited and subsides spontaneously in 3-7 days (18, 23). The mean duration of carriage of *Salmonella* in the stool is 4-6 weeks, but some carriers can be asymptomatic for months or even years. The susceptibility to infection varies, the critical infective dose is lower in young children, the elderly, and immunocompromised hosts (e.g., HIV infected individuals) (24). The likelihood of extraintestinal manifestations of *Salmonella* infections such as bacteremia, septic arthritis, cholecystitis, muscle abscesses, and vascular infection (25) are much higher for immunocompromised individuals (26, 27).

**Salmonellosis: Burden of the disease:**

Despite the implementation of several control and prevention measures, *Salmonella* infections remain a major public health problem worldwide (1). In England and Wales, incidence of *Salmonella* infections started to rise in the mid 1980s, primarily because of the epidemic of *S.* Enteritidis infections. Between 1987 and 1992, an overall 83% increase, from 40 cases per 100,000 population in 1987 to 73 cases per 100,000 population in 1992 was observed (28). However, in recent years, due to several control and prevention measures, a significant decrease in the overall incidence has been noticed. In 2004, a *Salmonella* incidence of 22 cases per 100,000 was reported (29). Figure 1 shows incidence of *Salmonella* infections per 100,000 population in England and Wales, between 1981 and 2004.

In Australia from 1996 to 2003, the average incidence of *Salmonella* was reported to be 28.99 cases per 100,000 population (30). Japan had a relatively low average incidence of 3.32 cases per 100,000 population between 1993 and 2004 compared to European countries (31). In Canada, mean annual incidence (1990-2004) was 19.4 cases per 100,000 population (32). The differences in *Salmonella* incidence could be partly explained by differences in the effectiveness of the existing public health measures that may limit extensive spread of contaminated poultry products and other foods potentially contaminated with the organism. Additionally, disease surveillance and the rate of case detection among these countries may also vary.

Figure 2 shows the annual incidence of *Salmonella* infections per 100,000 population between 1944 and 2002 in the US. A steady rise in the incidence of *Salmonella* infections has been observed between 1944 and 1980. However, a significant increase in incidence was observed after the 1980s. The incidence increased from 10 cases per 100,000 population in 1980 to 23 cases per 100,000 in 1994. As a result of prevention programs such as on-farm microbiologic testing for *Salmonella* and improved bio-security of food in the early 1990s, the incidence of *Salmonella* infections started to decline. The overall incidence of salmonellosis decreased from 16.6 cases per 100,000 population in 1996 to 14.2 cases per 100,000 population in 2001, although large outbreaks and sporadic cases continue to occur (4).

Although most culture-confirmed cases are reported to health authorities, the disease surveillance system underestimates the actual number of *Salmonella* infections as a result of surveillance artifacts. First, a person infected with *Salmonella* should develop

symptoms that are severe enough to seek medical care. Second, the physician must request and collect a specimen from the patient for bacterial culture. Third, the laboratory must test the specimen for *Salmonella* using a sensitive method and forward the isolate to a State Public Health Laboratory for confirmation. Fourth, the state laboratory must report results to the CDC. It has been estimated that the number of reported cases represent just 1% – 5% of the actual number of *Salmonella* infections that occur in the population (33). To better estimate the disease burden in the population associated with the foodborne infections, FoodNet conducts surveys of laboratories, physicians, and the general population at FoodNet sites. By estimating the proportion of the population seeking medical care for diarrheal symptoms, the proportion of physicians advising bacterial stool culture, and taking into account the influence of variations in laboratory testing for bacterial pathogens on the yield of number of culture-confirmed cases, FoodNet estimated in 1997 there were 1.4 million *Salmonella* infections, resulting in 113,000 physician office visits. Additionally, in recent years, salmonellosis has been attributed to 16,000 hospitalizations, and more than 500 deaths each year in the US (1).

Between 1996 and 2004, a decline in overall *Salmonella* incidence was observed, but when the data were stratified by the common serotypes, only one of the four most common (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, and *S.* Heidelberg) *Salmonella* serotypes, *S.* Typhimurium, declined significantly (34). In contrast, there were marked increase in the incidence of *S.* Javiana and monophasic serotype identified as *S.* I4,[5]12:i:- infections. No substantial declines in the incidence of the other common

*Salmonella* serotypes, *S.* Enteritidis, *S.* Newport, and *S.* Heidelberg, were observed (4). A contributing factor to the decline in *Salmonella* infections was a change in the industry and regulatory approaches to meat and poultry safety. In the mid 1990's, the UDSA-Food Safety Inspection Services (FSIS) implemented Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) systems regulations in meat and poultry slaughter and processing plants. The decline in the incidence of *S.* Typhimurium infections in humans may be related to changes in meat processing as evidenced by a decline in the prevalence of *Salmonella* isolated from FSIS-regulated meat and poultry products (35).

In 2005, a total of 6,655 laboratory-confirmed cases of salmonellosis, with an incidence of 14.55 cases per 100,000 population in FoodNet sites was reported, which accounted for 38% of all laboratory-confirmed cases of foodborne infections (36).

In 2006, a total of 6,655 laboratory-confirmed cases of *Salmonella* infections, with an incidence of 17.4 cases per 100,000 population, was reported by FoodNet surveillance sites. Of the 6,655 submitted samples, 5,957 (90%) of the *Salmonella* isolates were serotyped and seven serotypes accounted for 64% of the infections: Typhimurium, 1,157 (19%); Enteritidis, 1,109 (19%); Newport, 531 (9%); Javiana, 292 (5%); Montevideo, 250 (4%); Heidelberg, 239 (4%); and *S.* I 4,[5],12:i:-, 239 (4%) (8).

Healthy People 2010 objectives have been established for four foodborne pathogens under FoodNet surveillance (37). In recent years (2004-2006), the incidences of *Campylobacter*, Shiga-toxin producing *E. coli* (STEC O157), and *Listeria* were approaching their targets of 12.31, 1.00, and 0.25 cases per 100,000 population,

respectively. However, the incidence of *Salmonella* infections in 2006 remained much higher than the goal of 6.8 cases per 100,000 population by the year 2010 (8). Table 1 compares 2006 incidences of selected pathogens with the National Health Objectives 2010.

## OUTBREAKS OF FOODBORNE *SALMONELLA* INFECTIONS

As mentioned earlier, despite control and prevention efforts, foodborne outbreaks (defined by the CDC as two or more illnesses from a common source) caused by *Salmonella* have continued to occur. Salmonellosis outbreaks have been associated with family gatherings, restaurants, and community outbreaks either limited to a defined population or spread community-wide (35). Table 2 summarizes selected large foodborne outbreaks where *Salmonella* have been isolated as a cause of the outbreak.

It should be noted that the number of reported outbreaks represents a small proportion (6%-7%) of the total outbreaks that actually occurred in the population. Most outbreaks, particularly smaller ones, are never recognized, and those that are recognized frequently go unreported (38). The likelihood that an outbreak is brought to the attention of public health authorities depends on many factors, including general population and physician awareness, and motivation to report the incident as well as the resources and disease surveillance activities of local health and environmental agencies. Outbreaks that are most likely to be reported to health authorities include those that are large, multi-state, restaurant-associated, or that cause serious illness, hospitalization, or death (38).

During 1993-1997, *Salmonella* caused 357 (55%) of the 655 bacterial foodborne outbreaks with a known etiology. *Salmonella* serotype in the US. Enteritidis was the most frequently reported cause of foodborne disease outbreaks, accounting for 7% of all foodborne outbreaks (38).

In 2004, of the 6,498 *Salmonella* cases ascertained, 352 (5%) were identified as being outbreak-related. Of the outbreak-associated *Salmonella* cases, 78% were food-related, 20% were not food-related (e.g., person-to-person transmission) and for 2%, the mode of transmission was unknown. In the same year, *Salmonella* was responsible for 23% of nationally reported foodborne outbreaks in which an etiology was confirmed (4). In 2006, outbreak-associated cases accounted for 404 (6.1%) of 6,655 *Salmonella* cases ascertained at FoodNet sites, compared with 296 (4.6%) of 6,505 cases in 2005 (8).

## SELECTED OUTBREAKS OF SALMONELLOSIS

### Multi-state outbreak associated with contaminated milk, 1985: (35)

In 1985, an outbreak associated with *S.* Typhimurium causing 16,000 confirmed cases in 6 states was due to the consumption of milk from a dairy plant in Chicago, Illinois. Located in an industrial area of Chicago, the dairy plant was the sole supplier of milk to 217 supermarkets in Illinois, Indiana, Iowa, and Michigan. The dairy plant at one time processed about 1.5 million pounds of milk a day. This was the largest reported outbreak of foodborne salmonellosis in the US. Investigators from CDC and FDA could not identify the actual deficiency that led to the contamination of the pasteurized milk shipments but suspected the failing valves between the towers that contained the raw milk and those that hold the pasteurized milk.

### Multi-state Schwan's ice cream outbreak, 1994: (39)

In September and October of 1994, Minnesota's Health Department observed a sharp rise in the reported cases of salmonellosis. Investigators from the FDA and state officials determined that contaminated ice cream was the likely cause of a *S.* Enteritidis outbreak that may have sickened more than 3,000 people in as many as 41 states. Health officials suggested that *Salmonella* contamination occurred when raw, unpasteurized eggs were hauled in trucks that later carried pasteurized ice cream premix to the Schwan's plant. CDC received reports from 41 states of illness associated with Schwan's products: 740 cases from 30 states were confirmed by cultures, and 41 states reported an additional 3,423 suspected cases, however, no deaths were reported.

Analysis of egg samples from a tanker, ice cream from the plant, and stool specimens from infected consumers

revealed that all were contained with *Salmonella* of the same genetic type.

**Michigan bakery product outbreak, 2002: (40)**

In May 2002, *S.* Enteritidis associated outbreaks with the consumption of bakery products in Macomb County, Michigan resulted in 196 reported illnesses, among those 24 individuals were hospitalized. The state health authority, Michigan Department of Community Health (MDCH), concluded that black forest cakes and pastries were the vehicles for *Salmonella* transmission.

**Multi-state raw almond outbreak, 2004: (41)**

On May 12, 2004, the Oregon State Public Health Laboratory identified a cluster of five patients infected with *Salmonella* serotype Enteritidis. Further investigation led to the identification of at least 29 patients in 12 states and Canada as part of the *Salmonella* outbreak that began in September 2003. After an investigation by public health officials, the illnesses were linked to the consumption of raw almonds distributed by Paramount Farms and roughly 18 million pounds of raw almonds were recalled.

**South Carolina restaurant outbreak, 2005: (42)**

In May, 2005, the Department of Health and Environmental Control (DHEC) was informed about a possible outbreak of foodborne illness at Old South restaurant in Camden, South Carolina. The outbreak turned out to be one of the largest foodborne outbreaks in South Carolina history. Laboratory results from DHEC documented the

presence of *S.* Enteritidis in roasted turkey that had been consumed at an event catered by Old South. During the course of investigation, investigators determined that the convection oven used to cook the contaminated turkey had malfunctioned, thereby preventing the turkey from reaching a temperature sufficient to destroy *Salmonella*. A total of 304 laboratory-confirmed and suspected cases were identified and one person died as a result of these infections.

**Multi-state Orchid Island Juice outbreak, 2005: (43)**

In early July 2005, the FDA issued a nationwide warning to consumers against drinking unpasteurized orange juice products distributed under a variety of brand names by Orchid Island Juice Company of Fort Pierce, Florida. Fifteen cases had been directly linked to the product and at least 16 states had reported cases of *S.* Typhimurium infections that matched the outbreak strain. On July 15, 2005, with an increasing number of *Salmonella* illnesses traced to unpasteurized orange juice being reported to state health departments, the company agreed to issue a recall of all fresh and frozen juices.

**Multi-state tomato associated outbreak, 2005-2006: (44)**

During 2005-2006, four large multi-state outbreaks of *Salmonella* infections (with multiple serotypes) associated with eating raw tomatoes at restaurants were reported. These outbreaks resulted in 459 culture-confirmed cases of salmonellosis in 21 states. The investigation revealed that the tomatoes had been supplied to restaurants either whole or pre-cut from tomato fields in Florida, Ohio, and Virginia. Implicated

tomatoes were traced to a single packinghouse in Ohio supplied by three tomato growers from 25 fields in three counties. Tomato production had ended by the time the packinghouse was implicated.

**Multi-state peanut butter outbreak, 2007: (45)**

On February 14, 2007, the CDC and FDA announced that there had been 290 cases of *S.* Tennessee infections in 39 states that were linked to the consumption of Peter Pan and Great Value brand peanut butter that was manufactured in ConAgra's Georgia peanut butter plant. Peter Pan and Great Value brand peanut butter beginning with a particular product code was recalled in response to the outbreak investigation.

## SELECTED COMMON *SALMONELLA* SEROTYPES

Using the Kauffmann-White scheme based on antisera prepared to group and individual somatic and flagellar antigens (O and H antigens) from representative serotypes, over 2,500 different *Salmonella* serotypes have been identified in different parts of the world (46, 47). *Salmonella* can be classified into two main groups 1) *S. enterica* and 2) *S. bongori*. *Salmonella enterica* is further divided into six subspecies 1) *S. enterica* subsp. *arizonae*, 2) *S. enterica* subsp. *diarizonae,* 3) *S. enterica* subsp. *houtenae*, 4) *S. enterica* subsp. *indica*, 5) *S. enterica* subsp. *Salamae,* and 6) *S. enterica* subsp. *enterica.* The latest subspecies include all of the ~25,000 *Salmonella* serotypes. However, over 80% of all Salmonella isolated from humans and animals belong to about 20 serotypes (Table 3) (4).

Another way of classifying *Salmonella* serotypes is ecologically based on host adaptation (48, 49). *Salmonella* serotypes can be divided into two groups 1) host adapted and 2) ubiquitous (non-host adapted). Host-adapted serotypes typically cause systemic disease in a limited number of host species (Table 4) (49). For example, *S.* Typhi, *S.* Gallinarum, and *S.* Abortusovis are almost exclusively associated with systemic disease in humans, poultry, and sheep, respectively. However, some host adapted serotypes can also cause disease in more than one host species: Dublin and Choleraesuis, for example, are generally associated with disease in cattle and pigs, respectively but may also infrequently cause disease in other mammalian hosts including humans. Examples of non-host adapted serotypes include *S.* Typhimurium and *S.* Enteritidis, which are the most common serotypes that have been isolated from humans, animals, and environmental sources (49, 50).

Over periods of several years, incidences of certain *Salmonella* types have varied within large geographic regions. In 2001, approximately 60% of human cases reported to the CDC were caused by four serotypes, namely *S.* Typhimurium (22.1%), *S.* Enteritidis (17.7%), *S.* Newport (10.0%), and *S.* Heidelberg (5.9%). Of the 5,957 (90%) *Salmonella* isolates serotyped in 2006, the same four serotypes accounted for 51% of *Salmonella* infections: *S.* Typhimurium, 1,157 (19%); *S.* Enteritidis, 1,109 (19%); *S.* Newport, 531 (9%) and *S.* Heidelberg, 239 (4%).

Table 5 compares the percent change (2004 vs. 1996-1998) in reported incidence of the four common *Salmonella* serotypes (4). A significant decrease (41%) occurred in the

incidence of *S.* Typhimurium, while there was an increase in the other two common serotypes, *S.* Heidelberg, and *S.* Newport.

A brief description of the epidemiology of the four common *Salmonella* serotypes, *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport, is presented here.

***Salmonella enterica* serotype Typhimurium:**

*S.* Typhimurium is among the most prevalent human *Salmonella* serovars worldwide (4). *S.* Typhimurium accounted for 23% of all human *Salmonella* isolates reported in 2000. In regard to animals, *S.* Typhimurium is primarily a pathogen for cattle, but other species such as sheep, goats, pigs, and birds can be affected (51). Additionally, it has been isolated from various environmental sources.

Several risk factors have been identified for *S.* Typhimurium infections. Outbreaks have been associated with consumption of raw or undercooked ground beef (52), lamb kebabs (53), commercially processed salad (CDC, 2004b), unpasteurized milk (35), eating raw or undercooked eggs (54), cheese made of raw milk (55), chocolate (56), and salami sticks (57). In 2006, a multi-state outbreak of *S.* Typhimurium infections associated with tomatoes accounted for 58 (14%) of the outbreak-associated *Salmonella* cases identified by FoodNet (44).

Sporadic *S.* Typhimurium infections have been associated with the consumption of undercooked ground beef (58) and undercooked eggs or egg-product (59). Contact with farm animals and pets has also been associated with infection (21, 60).

Phage typing has enabled differentiation of *S.* Typhmurium into more than 200 Definitive phage Types (DTs). The recent public health concern related to *S.* Typhimurium was due to the emergence of multi-drug resistant (MDR) *S.* Typhimurium DT 104 (61). As a result of several control and prevention programs for salmonellosis, the incidence of *S.* Typhimurium infection decreased 24% from 1996 to 2001. However, an increasing proportion of isolates are resistant to multiple antimicrobial agents (62).

**Multi-drug resistant *Salmonella* Typhimurium DT104:**

Globally, the prevalence of *S.* Typhimurium resistant strains has increased several folds in the past few decades and caused a considerable number of outbreaks in North America since 1996. It has been suggested that drug-resistant *Salmonella* serotypes have emerged, primarily in response to antimicrobial use in food animals and the international trade of animals (51, 63). Among *Salmonella* serotypes, *S.* Typhimurium has shown the highest incidence of antibiotic resistance. The most frequent *S.* Typhimurium phage type associated with a multi-drug resistance pattern is DT104.

*S.* Typhimurium DT104 is commonly resistant to 5 antibiotics—ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (64). The first resistant strain of *S.* Tyiphimurium DT04 was detected in the UK in cattle during the late 1980s, and since then it has become common in other animal species such as poultry, pigs, and sheep (61).

In epidemiologic investigations, human infections with multiple antibiotic resistant DT104 isolates have been associated with the consumption of undercooked meat, poultry, beef, and pork. Table 6 shows the distribution of MDR and *S.* Tyiphimurium and definitive phage type 104 (DT04) in selected countries from 1992 through 2001. In most European countries and North America, MDR started to increase from the mid-1990s (61, 65).

Antimicrobial resistance to *Salmonella* serotypes, particularly DT104, appear to pose a significant health risk. In a recent review, it has been suggested that antimicrobial resistance in *Salmonella* results in about 300 excess hospitalizations and 10 deaths in the US each year (66). Additionally, a study conducted in a Danish population found that persons with resistant *Salmonella* infections had a higher mortality rate compared to those with antibiotic-susceptible *Salmonella* infections (67).

***Salmonella* enterica serotype Enteritidis:**

*S.* Enteritidis is the second most common *Salmonella* serotype in the US (50). An epidemic of *S.* Enteritidis began in the late 1970s in the northeast region of the country and some areas of Europe (68). In 1976, 1,207 *S.* Enteritidis isolates were detected nationwide with an incidence of 0.6 cases per 100,000 population. The incidence reached 2.4 cases per 100,000 by 1985. From 1980 to 1996, an increase of *S.* Enteritidis isolation from 5% to 25% of all *Salmonella* cultures was reported (38). Figure 3 shows the overall *S.* Enteritidis infection incidence in the US between 1970 and 2001. According to the CDC, 677 *S.* Enteritidis-related foodborne outbreaks were

reported between 1990 and 2001, resulting in 23,366 illnesses, 1988 hospitalizations, and 33 deaths (38, 69). During 1994, 1995, and 1996, *S.* Enteritidis surpassed *S.* Typhimurium to become the most common *Salmonella* serotype isolated in the US (70). The incidence of *S.* Enteritidis infection increased markedly from 1980 to 1995, but has decreased 22% from 1996 to 2001 (71).

Epidemiologic investigations of sporadic cases and outbreaks of *S.* Enteritidis infections have demonstrated that contaminated eggs and egg-products are major risk factors and that about 80-85% of all *S.* Enteritidis infections cases can be attributed to the consumption of contaminated egg products (1, 50). However, recent studies have identified that poultry has been associated with the transmission of *S.* Enteritidis infections. In a population-based case-control study, Kimura et al. reported that eating chicken outside the house doubled the risk of acquiring *S.* Enteritidis infection (OR = 2.6, 95% CI, 1.6-4.4) (72).

Moreover, in 2003, 12.8% of chickens sampled in slaughter plants in the FSIS-PR/HACCP Verification Testing Program were contaminated with *Salmonella.* USDA-FSIS reported an increase in the frequency of isolation of *Salmonella,* particularly *S.* Enteriditis, in chicken broiler during 2000-2005.

Furthermore, in 2005, an outbreak of *S.* Enteritidis associated with eating raw almonds was identified.

Unlike the increase in incidence of antibiotic-resistance seen in *S.* Typhimurium, *S.* Enteritidis has remained sensitive to most antibiotics (73).

### *Salmonella enterica* serotype Newport

*S.* Newport is the third most common *Salmonella* serotype in humans and has recently been named an emerging disease by the American Association of Veterinary Laboratory Diagnosticians. From 1973 through 1997, 149 *S.* Newport outbreaks caused 7,159 cases of illnesses. The median number of cases per outbreak was 17 (range: 2 to 700 cases) and 11.6% of case patients were hospitalized, while 0.1% died (62).

Unlike *S.* Typhimurium, the incidence of *S.* Newport increased in recent years—a 32% increase was observed from 1996 to 2001 (62).

Outbreaks and sporadic cases of *S.* Newport have been associated with consumption of hamburger (74), peanuts (75), cured ham (76), alfalfa sprouts(77), undercooked eggs (78), and pork sandwiches (79).

In addition to an overall increase in the incidence, the emergence of multi-drug resistant strains of *S.* Newport in recent years (80) is a significant health problem. Since 1996, the National Antimicrobial Resistance Monitoring System (NARMS) has identified an increasing number of *S.* Newport isolates that are resistant to at least nine of 17 antimicrobial agents tested: moxicillin/clavulanate, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (80). The increase of *S.* Newport multi-drug –resistant infections in humans has been

associated with exposure to animal products: ill cattle (81, 82) cheese made from unpasteurized milk (83), and raw or undercooked ground beef (80).

***Salmonella enterica* serotype Heidelberg:**

*S.* Heidelberg was the fourth most commonly reported *Salmonella* serotype in the US from 1993 through 1997. An average of 2,180 cases of *S.* Heidelberg infections were reported annually, accounting for about 6% of all culture-confirmed *Salmonella* infections in the US. However, culture-confirmed cases may represent only about ~2.6% of all illnesses of salmonellosis. Therefore, the actual burden associated with *S.* Heidelberg is estimated at 84,000 cases of illnesses annually with an incidence of 27.1 cases per 100,000 population (62).

Like other *Salmonella* serotypes, *S.* Heidelberg has largely been associated with food vehicles. However, reports of person-to-person or direct animal-to-person transmission have been reported (84). Investigations of outbreaks caused by *S.* Heidelberg have identified undercooked chicken, pork and cheddar cheese as food vehicles associated with *S.* Heidelberg (85, 86). Additionally, *S.* Heidelberg has been isolated from eggshells and shown to grow in eggs in in-vitro studies (87). In a population-based case-control study conducted at the FoodNet sites, Hennessy et al. (2004) reported eating eggs prepared outside the home at commercial food establishments as a significant risk factor (OR = 6.0; 95% CI, 1.2–29.6) for *S.* Heidelberg infections with a population-attributable fraction of 37% (88).

## *SALMONELLA* INFECTION IN CHILDREN

As stated earlier, the implementation of several control and prevention measures for salmonellosis across the nation in the mid 1990s resulted in a significant decrease in the overall incidence of *Salmonella* infections in recent years (1996-2003) (70). However, age-stratified analysis has shown a relatively stable infection rate in children at the national level (47). A large number of epidemiologic investigations, including reports from the CDC's FoodNet, have shown consistently higher incidences of laboratory-confirmed *Salmonella* infections in children compared to other age groups (3, 50, 89-91). Children aged < 5 years account for a large proportion of reported cases in the majority of the US disease surveillance systems (62). Between 1998 and 2003, the incidences of laboratory-confirmed cases of *Salmonella* infections reported at FoodNet sites were 122.7 cases per 100,000 population for children aged < 1 year and 50.6 cases per 100,000 population for children aged 1-4 years, compared to 10.8 cases per 100,000 population for those aged $\geq$ 5 years. Our recent work investigating associations between demographic attributes and the distribution of salmonellosis based on data from MDCH (1995-2001) revealed an approximately 10-fold increased risk for acquiring salmonellosis in children age < 1 year and a 3-fold increased risk for those aged 1-4 years when compared to adults aged 15-39 years (70, 92). Based on 13,877 salmonellosis case reports in Michigan, age-stratified analysis of recent MDCH data (1992-2006) showed an incidence of 70 cases per 100,000 population for children aged < 1 year, 22.2 cases per 100,000 population for children aged 1-4 years, and 10 cases per 100,000 population for children aged 5-9 years, compared to 4 cases per 100,000 population for those aged 10-34 years (Figure 4).

In addition to a several fold increased risk for acquiring salmonellosis, pediatric cases account for substantially more morbidity and mortality compared to adults (93, 94). The reasons for the observed higher incidence of salmonellosis in young children are not well understood. It is suggested that the higher incidence of salmonellosis in children could be due to their host immunoincompetent status, which makes them vulnerable to many infections including salmonellosis (94). Another reason cited in the literature is the increased case detection of *Salmonella* infections in children. In young children with gastrointesitinal symptoms, it is more likely that 1) the parents will seek medical attention and 2) the healthcare provider will submit a sample for culture (95).

As discussed earlier, the majority (80 - 85%) of sporadic cases of salmonellosis in the adult population results from consumption of contaminated food (96, 97). Another major risk factor in the adult population identified is traveling to areas (e.g., South America, Asia) where *Salmonella* is more prevalent. However, risk factors for salmonellosis in children have not been extensively evaluated in population-based epidemiologic studies. The majority of the studies in children have been conducted in response to salmonellosis outbreaks, particularly in daycare centers and nurseries. These investigations usually identify a point source as a cause for the outbreak. The risk factors identified in outbreaks may not be similar to those of sporadic cases of salmonellosis since exposures in sporadic cases vary widely.

Some analytical studies have shown a strong association between *Salmonella* infections and the consumption of contaminated food including raw or undercooked eggs in children aged < 5 years (59, 98-100), while a number of other investigations have failed

to implicate food as a source of infection for children < 5 years of age (93, 101-103). Additionally, in the majority of studies where investigators addressed the implication of specific food vehicles for salmonellosis, they did not explore the influence of mode of food preparation, food handling methods, and family kitchen hygiene practices as potential factors associated with *Salmonella* infections. There is an overall dearth of epidemiological data on the influence of these practices and the risk of sporadic *Salmonella* infections in households with children (96).

The risk factors for salmonellosis in children may be substantially different from adults because of markedly different behaviors and exposures. Table 7 shows the identified risk factors for *Salmonella* infections in children. It has been suggested that contaminated environmental sources contribute more than contaminated food vehicles in the acquisition of *Salmonella* infections in children. However, limited evidence exists to explain the influence of various environmental exposures in acquiring *Salmonella* infections in children (104). The majority of the literature showing an association between various environmental factors and *Salmonella* infections consists of case reports and case series. The few observational studies conducted, mainly outside the US, have shown mixed results (97, 102). Some investigations suggest that children have been infected from contaminated environmental sources(104). In contrast, other studies have not found associations between environmental sanitation or ownership of pets and salmonellosis in the pediatric population (97).

In a population-based case-control study, Delarocque-Astagneau et al. (1998) suggested that the predominant mode of *Salmonella* transmission differs in children by age (96).

They found that for children < 1 year of age, *Salmonella* infections are mainly related to exposure to an infected family member, whereas for children 1-5 years, infections are associated with the consumption of raw or undercooked egg products or chicken. However, the investigators did not collect the data on several established risk factors for *Salmonella* infections in children (e.g., exposure to reptiles, travel to endemic *Salmonella* zones). Hence confounding of the effect size by these known risk factors for salmonellosis in children cannot be determined (96).

In a few studies conducted in Guam, *Salmonella* have been isolated from kitchen counters (22), household dust and soil close to the house entrance (104). Moreover, in assessing an association between environmental contamination at shopping centers and *Salmonella* infections in children, a recent population-based study conducted using FoodNet sites identified that riding in a shopping cart next to meat and poultry was a risk factor for salmonellosis in children (89). However, this newly identified factor has yet to be validated.

State and local health authorities nationwide address *Salmonella* infections risk factors primarily for enteric outbreaks, which constitute about 6%-7% of all reported cases of salmonellosis (62). In the US, a few studies have been conducted to identify risk factors for sporadic infections of *Salmonella* infections. Furthermore, to date, no systematic population-based epidemiological study to identify risk factors for *Salmonella* infections in children has been conducted in Michigan.

**Objectives:**

***Primary objective:***

To assess the role of potential risk factors in the etiology of *Salmonella* infections in Michigan children, we conducted a population-based case-control study to determine 1) household-related factors (e.g., household density, family kitchen practices), 2) selected environmental exposures (e.g., contact with a person having symptoms of GI infection, contact with pets, and 3) consumption of various food vehicles (e.g., eating eggs, poultry and meat) in the etiology of sporadic non-typhoidal *Salmonella* infections in Michigan children aged ≤ 10 years.

**Secondary objectives:**

To assess select exposures (food vehicles and environmental exposures) by age group: 1) <1 year and 1-10 years.

To identify factors (food vehicles and environmental exposures) associated with *Salmonella* serotype Typhimurium infections.

## MATERIAL AND METHODS

***Study setting and population:***

The study was conducted in the state of Michigan. With an area of 96,810 square miles, Michigan is the 11th largest and eighth most populous state in the US. Michigan's major industries include car manufacturing, farming (corn, soybeans, and wheat), timber, and fishing. According to the 2000 US Census, Michigan has a total population of 9,956,111 and an average per capita income of $22,168. The target population for this study was all children aged ≤ 10 years with a permanent Michigan residential address. This age group constitutes about 15.6% (1,560,702) of the total population (105) .

***Source of Data:***

**Michigan Department of Community Health:**

Salmonellosis is a notifiable disease and under Michigan's Communicable Disease Rules, which require the reporting of the occurrence or suspected occurrence of all certain serious diseases and conditions (106). Therefore, physicians and laboratories across Michigan submit disease reports to their local health department (LHD) in either the jurisdiction where the individual with suspected or confirmed salmonellosis resides or where the reporting facility is located. The LHDs then submit these reports to the statewide communicable disease reporting system, Michigan Disease Surveillance System (MDSS), which is maintained by the MDCH. Figure 5 describes the surveillance of *Salmonella* infections in Michigan. MDSS is a centralized, statewide, web-based database of reportable diseases. In addition to reporting to the MDCH,

LHDs and clinical laboratories send clinical specimens to the Bureau of Laboratories, MDCH for testing. For all cases of salmonellosis, the *Salmonella* isolates are serotyped at MDCH Bureau of Laboratories and results are entered into MDSS.

**Study design:**

A case-control design was used to achieve study objectives. Case-control studies are used to identify factors that may contribute to a disease or condition by comparing a group of individuals who have a particular disease or condition to a group of individuals that do not. In this design, patients who have developed a disease are first identified, and their past exposure to suspected etiological factors are compared with that of controls or referents who do not have the disease. Case-control design allows for investigation of multiple exposures potentially associated with the given disease or condition. The use of a case-control approach facilitates rapid and cost-effective collection of data and allows scientific evaluation of the risk factors contributing to disease occurrence (107, 108). The other observational study design is a cohort or follow up study. In a cohort study, subjects who presently have a certain condition (exposed) and another group who are not affected by the condition (un-exposed group) are followed up longitudinally and compared for a defined period of time or till outcome of interest(s) (e.g., disease) occur (108). In our study, we wanted to study multiple potential risk factors associated with a disease (salmonellosis), cohort study design was not appropriate.

**Unmatched vs. matched case-control study:**

Matching refers to the selection of a reference (control) series- unexposed subjects in a case-control of cohort study- that' is identical, or nearly so, to the index series (cases)

with respect to the distribution of one or more potentially confounding factors (108). Matching control selection strategies are primarily done in case-control studies. When properly applied, matching may provide improved study efficiency and precision. Matching may be performed by subject to subject (individual matching) or for groups of subject (frequency matching). Individual matching usually involve one or more control subjects with matching-factor similar to those of the case subject (e.g., age, sex, race). Frequency matching involves selection of an entire stratum of control subjects with matching-factor similar to that of stratum of case subjects. Although matching does not offer advantages over unmatched control selection with regard to study validity under the case-control design, gains in study precision of results may be improved in matched design. Greater precision produces a smaller effect size (odds ratio) variance, and narrower confidence intervals. Disadvantages of matching may include loss of statistical efficiency and logistical issues with the enrollment of matched controls, particularly if the cases are matched with controls on multiple factors (difficult to find controls matched on several variables). Additionally, if the controls are matched on factors that are affected by exposure or disease (over matching), such as symptoms or sign of the exposure or the disease, such matching can distort the study data and yield biased estimates (107). In our study, we began enrolling participants using a neighborhood-matched design, but discontinued the matched design early in the course of data collection because of the lack of apparent differences between neighborhood-matched controls and non-neighborhood controls with regard to SES attributes and difficulty in finding the neighborhood matched controls (Table 19).

***Cases*:**

In this study, cases were defined as Michigan children aged ≤ 10 years with laboratory-confirmed *Salmonella* infections, except Typhi or Paratyphi, reported to MDCH between December 15, 2006 and October 15, 2007.

***Inclusion and exclusion criteria*:**

All individuals of age ≤10 years infected with any *Salmonella* serotypes other than Typhi or Paratyphi, isolated from any clinical specimen (e.g., stool, urine, blood, cerebrospinal fluid), were eligible for inclusion.

Cases were excluded from the study if 1) the case had a reported congenital malformation (e.g., birth defect), serious medical condition (e.g., leukemia, lymphoma), or a concomitant infection at the time of the *Salmonella* infection, 2) the case was reported as part of a salmonellosis outbreak investigated by public health officials, 3) more than one eligible cases were reported from the same household, the youngest case was selected for the study.

Additionally, case children were not included 1) if the child's family could not be contacted after two consecutive mailed invitation letters were returned to sender due to a wrong residential address in the MDSS and/or after about 20 phone call attempts, including evening and weekend calls, and 2) the contact established with the family but the caretaker (e.g., parent(s), grandparents) refused to participate in the study.

***Controls:***

Controls were children aged ≤ 10 years who were not diagnosed with any enteric infections (e.g., salmonellosis, campylobacteriosis) by a healthcare provider and did not experience any enteric disease symptoms (e.g., diarrhea, vomiting, nausea) during the 30 days prior to the interview day. After infection with *Salmonella,* stool shedding of the bacteria may last for up to 30 days, therefore, we choose the 30 days of symptoms-free period to exclude individuals with asymptomatic *Salmonella* carriage.

**Enrollment of cases and controls by age-groups:**

The incidence of salmonellosis and its risk factors vary by age within the ≤ 10 year old age group. To ensure enrollment of an adequate number of cases and controls in each age category, we used age-stratified sampling. We divided participants into three age categories: <1 year, 1-5 years, and 6-10 years, and enrolled cases and controls by these age categories.

Two methods were used to enroll controls:

**Method 1***:* Case parent(s) were asked to identify a child of similar age to their own within their county of residence.

**Method 2***:* To obtain potential controls, we used the on-line telephone directory available at www.whitepages.com. This website has a reverse address function that allowed us to find a list of household phone numbers within the same county. We obtained a list of potential control household phone numbers using case's addresses.

Each listed household was called, and after explaining the study's objective, we asked if they have a child of age ≤ 10 years. We interviewed consenting parent(s) or caretakers. If efforts to enroll a control from the compiled list were not successful, additional phone numbers were obtained using the same method working outward from the case's home address. This process was repeated to enroll the required number of controls.

***Control exclusion criteria:***

Controls were excluded if 1) the child had a congenital malformation (e.g., birth defect) or serious medical condition (e.g., leukemia, lymphoma) and/or 2) their caretaker (e.g., parent(s), grandparents) refused to participate in the study.

***Questionnaire development:***

We developed a structured questionnaire to collect data on sociodemographic characteristics (e.g., age and sex, household income, parental education), child feeding practices (e.g., breast feeding, formula milk, use of pacifier), child rearing (e.g., daycare, pre-school, or elementary school attendance), and various environmental exposures (e.g., contact with animals, contact with a person having GI symptoms). Additionally, we collected data on household kitchen practices. We included questions that had previously been used in similar research (e.g., food frequency questionnaire). The questionnaire was pilot-tested on volunteer parents, issues identified in this exercise were addressed, and appropriate changes were incorporated into the final version of the questionnaire (Appendix 4).

***Data collection methods:***

Several data collection methods are available for epidemiologic studies. The choice of data collection method is determined by several factors, including study population, response expectations, available resources, and the preferences of investigators (109). The methods of data collection we used, along with the rationale, are outlined below:

**Face-to-face (in-person) interview:**

To conduct face-to-face interviews, the fieldwork and its organization requires more resources compared to interviewing by telephone or using mail-in questionnaires and can be associated with interviewer bias. The advantages of face-to-face interview include a higher response rate, use of a longer survey instrument with complex skip patterns, more accurate recording of responses, low non-response on questions, and more appropriate for hard to reach populations (e.g., illiterate, institutionalized).

**Telephone interview:**

The telephone interview for data collection is very commonly used in epidemiologic research. The advantages include: less costly than face-to-face interview, higher response rates than mailed in questionnaires, relatively quicker access to participants, supervision of interviewers is feasible, and a better response rate with sensitive questions. The disadvantages include selection bias (e.g., persons without phones are not included) and a relatively high refusal rate.

**Mail-in-questionnaire:**

Mail-in-questionnaires are becoming more popular in public health research. Advantages include a lower cost compared to in-person interviews and anonymity (no threat of interviewer bias). Additionally, a self-administered questionnaire may produce more reliable and reduce non-response to sensitive questions. However, mail-in questionnaires are associated with a high rate of missing data, a low response rate, and are not suitable for population with low literacy (110).

**Electronic data collection:**

Computer-assisted data collection methods such as self-administered electronic questionnaires are being used in epidemiologic research. The instrument may either be an Internet questionnaire or an electronic questionnaire sent as an email attachment (111). This method facilitates the use of tailored questions and question branching and for different responses is easy to implement. It hastens data collection and also reduces the data entry error since the interviewer (or respondent) enters the data directly into a computer during the interview and can check for correctness. However, a potential problem of one type of electronic data collection is that not every household or individual has Internet access (110).

In our study because of the time and budget constraints we could not use face-to-face interview for data collection. Additionally, because of limited accessibility associated with electronic data collection methods, their use was not feasible. Therefore, parents

were given the option to fill out a self-administered mail-in questionnaire or participate in a 15-20 minute phone interview with a trained interviewer.

***Data collection process:***

An introductory cover letter describing the study's aim, methods, anticipated length of the interview, and potential risks and benefits associated with the study was mailed to the parents or guardians of each potential case. An informed consent form and self-addressed stamped envelope were also included (Appendix 2 & 3). If the informed consent form was not returned within one week after mailing the invitation letter, a follow-up phone call was made to inquire about the willingness to participate in the study. Cases with a missing residential address but a valid phone number were contacted over the phone. Either a signed consent form or oral agreement was secured from each case's legal guardian before the child was enrolled.

***Duration of exposure assessment:***

Based on the perceived period of (8 hours –72 hours) incubation before symptoms of *Salmonella* infections, food history and other exposures were collected for the three days preceding the illness onset date for cases. Similarly, for control exposure assessment, we gathered data for the three days prior to the interview day. This approach is similar to the data collection efforts of the CDC-FoodNet during their national investigation of the risk factors for foodborne diseases as well as case control studies conducted by other investigators (87, 112).

## ETHICAL CONSIDERATIONS

***Informed Consent:***

Parents or caretakers of children were adequately informed about the study's aims, methods, risks, and benefits. Informed consent was obtained from parent(s) or caretakers before enrolling the children into the study (Appendix 3).

***Data confidentiality:***

Data were de-identified by removing names and home addresses soon after the completion of the interviews. An identification code was assigned to each study subject before entering the information into a database. Data were stored in a password-protected computer. The parents were assured of the confidentiality of the information gathered.

***Approval from the Institutional Review Boards (IRB) for human research:***

The study protocol was reviewed and approved by the Community Research Institutional Review Board (CRIRB) at Michigan State University (MSU) and the Institutional Review Board (IRB) at the MDCH (Appendix 1).

## SAMPLE SIZE CALCULATIONS

A sample size calculation to achieve at least 80% study power, while restricting the probability of type I error to 5% (alpha), was based on the assumptions of prevalence of select exposures in the reference (control) population: family member had symptoms of GI prior to child's illness onset and exposures to pet, particularly reptiles. A case to control ratio of 1:1 was used.

In order to detect an unadjusted OR of 2.5 between cases and controls for select exposures having a prevalence ranging from 15% to 20% in the reference (control) population, we calculated the following sample sizes (58) (Table 21).

Since sample size of 124 x2=248 was large enough to detect the stated OR at desired precision at any presumed prevalence, we chose this sample size and recruited the required number of cases accordingly.

We assumed that 15% of reported cases would not have accurate contact information available (15% of 124=~17). Further, we anticipated a 'decline to participate'/ non-consenting rate of 19% based on published literature (19% of 124=~24).

Thus to enroll 124 cases, we needed to approach a minimum of 165, (124+17+24= 165) salmonellosis cases. Based on the past five-year MDCH data (2000-2004), an average 25 cases of salmonellosis of ages 10 or younger were reported to MDCH each month. The anticipated duration to enroll the required number of cases was about 8 months. Efforts to enroll controls were performed in parallel following each case's enrollment.

## DATA MANAGEMENT

Epi-info program was used to enter the data (version 6.04, Atlanta, GA; Centers for Disease Control and Prevention, 1995). All of the data were entered by one operator. About 10% of the entered questionnaires were randomly selected and checked for data entry errors. The error rate was < 3%. Data were cleaned to remove data entry and other logical error using Statistical Program for Social Sciences SPSS (version 10.0). All questions were coded into numerical terms. Continuous variables, if necessary, were categorized on biologically plausible or logical grounds.

A brief description of select variables follows:

**Variable description and transformation:**

***Dependant variable:***

Incident laboratory-confirmed *Salmonella* infections cases of human origin in children aged ≤ 10 and reported to MDCH via MDSS from December 15, 2006 through October 15, 2007 were included in this study.

***Independent variables:***

**Age**:

For cases, age of the child was extracted from the MDSS and was also verified by the parent(s) or caretakers. For control children, age reported by the caretaker was recorded. We categorized age into two groups for statistical analysis: <1 year, 1-10 years.

**Race:**

Race (of child) was reported by the parent(s) or caretaker of the case or control according to the race with which they most closely identify. Based on the distribution, we categorized race into Caucasian, African-American, Other (any race other than Caucasians and African-Americans e.g., Alaskan Indian, Middle Eastern, Asian and Pacific Islander etc) and Multiracial. Children born to parents of different races were classified as 'Multiracial.'

**Parental education:**

The highest level of schooling completed by the interviewing parents was recorded into one of the following categories: 1) no formal education to high school, 2) some college to a four year college degree, and 3) higher than college degree.

**Household income:**

Participants were asked to identify the income category in to which their household income would fall. The responses were categorized into the following income categories: 1) < $20,000, 2) $20,000 - $35,000, 3) $35,001 - $50,000, 4) $50,001 - $75,000, 5) $75,001 - $100,000, and 6) > $100,000. Annual household income included wages, salary, bonuses, or earnings from self-employment.

**Area of residence:**

Based on zip code level median household income reported by the US Bureau of Census 2000, we divided the area of residence into high income (income $ > 60,000), medium income (income between $38,000 and $ 60,000) and low-income ($< 38,000) neighborhoods.

**Breast-feeding:**

In this study, exclusive breast-feeding was defined as breast-feeding only with no formula or consumption of semi-solid or solid food in the three days before illness onset. A binary variable was created 'exclusively breast-fed vs. 'non-exclusively breast fed.'

**Formula milk used:**

Formula use was categorized into 'no formula use' and 'formula use' (if the caretaker used formula in addition to breast feeding, or formula alone) to feed the child during the 3 days prior to illness onset, the variable was categorized into 'formula use' and no 'formula use.'

**Pacifier use:**

Parents were asked if the child used a pacifier during the 3 days prior to illness onset, regardless of the duration of use. The responses were coded as 'used a pacifier' and 'did not use a pacifier'.

**Daycare / pre-school / school-related questions:**

Parents were asked if their children attended day care or school, and if they did, then greater detail such as number of hours per week child spent in daycare, total number of children enrolled in daycare, number of children sharing the same room in daycare, etc. were also asked.

**Food exposures:**

Several questions were used to collect information about the consumption of foods known to be associated with *Salmonella* infections. Egg consumption in the 3 days prior to the child's illness onset was categorized into three dummy variables: 1) ate fully cooked egg, 2) ate partially cooked egg, 3) ate fully cooked and partially cooked egg, and 4) did not eat egg. Similarly, parents were asked if the child had consumed poultry in the 3 days before illness onset, and the responses were grouped into: 1) ate at home; 2) ate outside home, 3) ate at home and outside, 4) did not eat, and 5) do not remember. In a separate question, similar inquiry was made about the consumption of meat other than poultry. In addition to capturing the data for 3 days prior to illness onset, we asked parent(s) about the average frequency of eating food at commercial food establishments and child's preferred food at these places.

**Kitchen practices:**

Family kitchen practices were assessed by asking multiple questions. The variable 'cleaning kitchen counter after preparing raw chicken and meat' was categorized into:

never, sometimes, and always. Questions regarding 'how do you clean the counters was categorized into 1) with soap and water, 2) with a disinfectant, and 3) both. We also asked whether the 'family kept eggs in a refrigerator', which was grouped into never, sometimes, and always.

**Contact with animals:**

Animals, particularly reptiles, are known to carry *Salmonella* bacteria (21). Parents were asked if the child had contact with any pet (household pet, someone else's pet, or an animal in a petting zoo) during the 3 days prior to illness onset. If the response was yes, then a second question asked about the type of animal (i.e., dog, cat, reptile, bird, hamster, gerbil, or ferret). The response was coded as a binary variable (e.g., contact with a reptile vs. no contact).

**Contact with a person with symptoms of GI upset:**

In two separate questions, parents were asked if the child had contact with a household member or house visitor with symptoms of stomach upset (e.g., diarrhea, vomiting) during the 3 days prior to the child's illness onset. Based on these responses, a dichotomous variable was created: 'contact with a person having symptoms of GI upset' and 'no contact'.

Parent(s) or caretakers who did not provide information in response to any of the above questions were coded as 'refused to answer'.

## STATISTICAL ANALYSES

***Descriptive statistics:***

Counts and percentages for each categorical variable were computed. Socioeconomic and demographic characteristics between cases and controls were compared using the chi-square test for two proportions (107).

***Inferential statistics:***

**Univariate analysis:**

Exposure variables were categorized into two or more levels, using the category with (or expected to have) the lowest risk of infection as the reference. Logistic Regression (LR) was used to examine associations between predictor variables (cases' socio-demographic characteristics and hypothesized risk factors) and *Salmonella* infections.

**Multivariate analysis:**

Since we did not match cases with controls on potential confounders, we used the unconditional multivariate statistical analysis to obtain the independent effect of exposures on the outcome variable while controlling for potential confounders. Adjusted odds ratios (AORs) with their respective 95% confidence intervals (95% CIs) were computed (107).

All variables with estimates of a p value $< 0.25$ on univariate analyses, along with those hypothesized a priori as putative risk factors for *Salmonella* infections, were considered for inclusion in the multivariate model. We checked the correlations between potential

correlated variables in our dataset. After identifying the variables, we examined the impact of co-linearity by separately entering the correlated variables (e.g., income, education, area of residence, and race) into the multivariate regression model. We developed several multivariate models and obtained the adjusted effect size for each collinear variable. Beside main effects, we also included several two way interaction terms in the model such as household income and race, household income and reptile ownerships, and age group and reptile ownership. We used the backward elimination method to obtain a parsimonious but yet plausible model. Exposure variables reported as risk factors for salmonellosis in previous studies such as consumption of eggs/egg-containing products, poultry,meat were kept in the final model regardless of their statistical association with the outcome variable. The final model was adjusted for age-group. The goodness-of-fit model was checked by using the Pearson chi-square test.

**Calculation of Population Attributable Risk (PAR%):**

Population attributable risk (PAR) for selected exposures was estimated using adjusted ORs from the final multivariate model (Table 12). We used Levin's formula (see below) for the calculation of PAR (113).

$$\frac{p(r-1)}{p(r-1)+1}$$

Where:

= Proportion of the population with exposure

= Adjusted Odd ratios

Levis formula has been shown by Leviton (114) to be algebraically identical to the formula:

$$\frac{\text{Incidence in total population} - \text{Incidence in non-exposed group}}{\text{Incidence in total population}}$$

Since, our study was a population-based study, had the control children developed *Salmonella* infections, they would have reported to MDCH. Therefore, our cases and controls arose from the same source population, which is a requirement for the calculation of PAR% from case-control design.

***Subgroup analyses:***

To better understand the relative contribution of certain exposures to *Salmonella* infections in children, we restricted our data to selected variables and performed the following subgroup analyses:

**Analysis 1:**

A few investigations have suggested that the magnitude of association between certain exposure variables and *Salmonella* infections varies by age within the pediatric population. To estimate the effect of selected risk factors (those with significant associations in multivariate analyses: contact with a person having GI upset and contact with reptiles and cats), we computed the AORs for the following age categories: < 1 year, and 1-10 years.

**Analysis 2:**

To identify associations between selected food related exposures and *Salmonella* infections in children aged <1 year

**Analysis 3:**

To identify associations between selected food-related exposures and *Salmonella* infections in children aged 1-10 years.

**Analysis 4:**

To study the risk factors for the most common *Salmonella* serotype Typhimurium, we restricted the data to cases (n=36) with *Salmonella* serotype Typhimurium only and compared it with controls (n=139) for selected exposures.

## RESULTS

**Case enrollment:**

During the 10 months of data collection (December 15, 2006 – October 15, 2007), a total of 862 cases of salmonellosis were reported to the MDSS, of which 228 (26.45%) occurred in children aged ≤ 10 years. Figure 6 describes the enrollment of cases. Of the 228 cases in children, 29 (12.72%) were infected with typhoidal *Salmonella* serotypes (*S.* Typhi and *S.* Paratyphi). Twenty-nine cases (12.72%) could not be included due to incomplete mailing addresses and/or missing home phone numbers. A letter of invitation was sent to 170 (74.56%) potential case households. The letter included a self-addressed stamp envelope and consent form. One case (0.59%) was ineligible on initial screening because of a coexisting chronic condition. Of 169 cases we attempted to enroll, 10 (5.91%) declined to participate: 4 (2.35%) on mail-in invitation letter and 6 (3.53%) over the phone. Of 159 (93.53%) eligible cases who agreed to participate either by returning the signed consent form via mail or on a follow up phone call, 36

(22.64%) could not be re-contacted again to complete the questionnaire despite repeated attempts. A total of 123 of 159 eligible cases were enrolled during the study period yielding a participation rate of 72.35%. Of the 123 interviewed cases, 102 (82.94%) were interviewed over the phone and 21 (17.06%) were interviewed through a mail-in questionnaire.

**Comparison of case participants and non-participants:**

Since public health officials routinely collect demographic information from all reported cases during the disease investigation process, we were able to compare the demographic characteristics (age, sex, race) of enrolled cases with non-enrolled children.

A total of 76 cases of salmonellosis in children aged ≤ 10 years reported during the study period were not enrolled in our study (Total cases in children aged ≤ 10 years – cases with Typhoidal serotypes – cases could not be contacted and refused to participate, 228-29-123=76). The enrolled cases (participants) did not differ significantly from non-enrolled cases (non-participants) with respect to demographic characteristics, including age group ($p = 0.42$), sex ($p = 0.55$), and race ($p = 0.78$). Table 8 shows a comparison of demographic characteristics between participants and non-participants in *Salmonella* case-control study, Michigan, 2007.

**Distribution of *Salmonella* serotypes among case children:**

*Salmonella* serotype information was available for 199 of 228 cases in children aged ≤ 10 years reported to MDCH during the study period. Among these cases, the four most common *Salmonella* serotypes included *S.* Typhimurium (20.10%), *S.* Enteritidis (8.54%), *S.* Newport (3.52%), and *S.* Heidelberg (3.52%) (Table 9).

**Comparison of *Salmonella* serotypes between cases aged ≤ 10 years and cases aged ≥ 11 years:**

The most common *Salmonella* serotype isolated among case children (participants and non-participants), *S.* Typhimurim, made up 20.10% of cases compared to 13.50% in cases aged ≥ 11 years during the study period. *S.* Enteritidis was the second most common serotype (8.54%) isolated in case children, while *S.* Enteritidis was the most common serotypes in cases aged ≥ 11 years accounting for 25.21% of the cases. Table 9 compares the distribution of *Salmonella* serotypes between children aged ≤ 10 years and reported cases ≥ 11 years during the study period.

**Control enrollment:**

Figure 7 describes the enrollment of controls. A total of 139 control children were obtained using one of the following two methods:

**Method 1:**

A total of 37 potential controls were identified by the interviewed case parents. Twenty-eight (75.68%) of the 37 households were contacted and interviewed, while 9 (24.32%) either could not be reached despite repeated phone calls or refused to participate in the study (Figure 7).

**Method 2:**

A total of 2,463 control addresses and phone numbers were obtained from the on-line white pages using the second method (Figure 7). Of these, 445 (18.07%) were

disconnected phone numbers and 53 (2.15%) were commercial phone numbers. After excluding these phone numbers, there were 1,965 potential control households used to identify appropriate controls. Of these potential controls, 1,134 (46.04%) could not be reached due to receiving an answering machine on repeated calling including evening calls, no answer, or a busy phone line. Additionally, 338 (13.72%) declined, hung up the phone, or said they were not interested in the study. Four hundred and ninety-three phone numbers were left of which 371 (15.06%) households had no children or no children aged $\leq$ 10 years of age. Of the 122 that scheduled an interview call, 11(0.45%) could not be contacted again after multiple calls. Therefore, a total of 111 parents (4.51%) of control children aged $\leq$ 10 years were interviewed using the second method.

**Descriptive statistics of cases and controls:**

Table 10 compares the socioeconomic and demographic characteristics of the enrolled 123 cases and 139 controls. The enrolled cases and controls did not differ by socioeconomic characteristics including parental education ($p = 0.94$) and annual household income ($p = 0.34$). Similarly, there were no significant age group ($p=0.54$) and gender differences ($p = 0.86$). However, significant differences in the distribution of racial composition ($p < 0.01$) were noted between enrolled cases and controls, which warranted control of this variable in the analysis stage. Forty-seven study subjects (22 cases and 25 controls) were aged < 1 year, 132 children (66 cases and 66 controls) were aged 1-5 years, and 83 subjects (35 cases and 48 controls) were aged 6-10 years. Overall, the rate of refusal to the question about parental education attainment was low (< 2%) among case and control households. However, the refusal rate for the question

regarding household income was lower (10.66%) among case households compared to refusal rate of 17.39% in control households.

One hundred and ninety-nine (75.95%) study subjects were Caucasian (107 cases and 92 controls), 36 (13.71%) were African-American (9 cases and 27 controls), and 24 (9.16%) belonged to other minority groups (6 cases and 18 controls).

**Inferential statistics**

***Univariate analyses:***

The results of the univariate analysis of putative risk factors for *Salmonella* infections in children aged ≤10 years are presented in Table 11 and briefly summarized here.

**Household related variables:**

Case and control subjects did not differ significantly with regard to household characteristics including number of people residing in the house, presence of other siblings aged ≤10 years, and type of family room flooring.

**Daycare and/or school-related exposures:**

Data showed that salmonellosis was significantly associated with attending a daycare facility (OR = 2.31, 95% CI: 1.01 - 5.40). However, no statistically significant association was found between attending a school and salmonellosis in the univariate analysis (OR = 0.84, 95% CI: 0.51 - 1.36).

**Food consumption and family kitchen practices:**

When studying associations between food consumption and *Salmonella* infections, univariate analysis did not show an association with the consumption of eggs or products containing eggs, eating poultry or meat, eating at commercial food establishments, and source of drinking water during the 3 days prior to the illness onset date (in cases) or interview date (in controls). Moreover, none of the family kitchen practices related variables was identified as a factor associated with the outcome variable.

**Person-to-person transmission:**

A total of 27 cases (21.95%) and 16 controls (11.5%) had contact with a sick person (having symptoms of GI upset) within 3 days prior to the onset of illness for cases and within 3 days before the interview day for controls. Our data suggest that contact with a person having symptoms of GI infection increases the odds for contracting salmonellosis (OR = 2.16, 95% CI: 1.10 - 4.24). Persons with symptoms of GI upset that had contact with the cases were either a family member, a visitor of the child's home, or someone the child visited.

**Contact with animals:**

Exposure to an animal during the 3 days prior to the child's illness onset was significantly associated with salmonellosis (OR = 2.69, 95% CI: 1.62 - 4.45). Analyses showed that having contact with reptiles (OR = 4.29, 95% CI: 1.53 - 12.02) was significantly associated with *Salmonella* infections. A total of 14 cases were

exposed to reptiles. The reptiles to which case children reported exposure included iguanas [1 (6.25%)], lizards [3 (18.75%)], snakes [6 (37.5%)], and turtles [7 (43.75%)]. In addition, 3 cases had contact with frogs and 1 case had contact with an alligator.

Contact with cats showed an association with salmonellosis (OR = 2.23, 95% CI: 1.21 - 4.10). Of the 138 cases interviewed, there were 35 cases that had contact with cats and 34 (89.4%) of these case children contacted cats aged > 1 year, while 4 (10.6%) contacted cats aged ≤ 1 year. A total of 53 cases had contact with dogs and, 33 (91.7 %) of these case children had contact with dogs aged >1 year, while 3 (8.3 %) had contact with dogs aged ≤ 1 year. Having contact with dogs, birds or hamsters did not show an association with salmonellosis.

**Other environmental exposures:**

Caretakers' handling packages of raw meat or chicken without gloves or plastic bags during grocery shopping with a child did not show an association with the outcome variable in our data. Additionally, there was no association found between the variable 'placing child on the floor without a blanket' and *Salmonella* infections.

***Multivariate analysis:***

Table 12 shows the results of a multivariate logistic regression model. The final multivariate model, after adjusting for age group revealed that having salmonellosis was significantly associated with contact with cats (adjusted odds ratio (AOR) = 2.62, 95% CI: 1.17 – 5.87) and reptiles (AOR = 8.16, 95% CI: 1.55 – 42.88). Additionally,

attending a daycare center (AOR = 4.86, 95% CI: 1.44 – 16.37) and contact with a person having symptoms of gastrointestinal infection during the 3 days prior to the onset of child's illness was significantly associated with *Salmonella* infections (AOR = 2.27, 95% CI: 1.02 – 5.44). None of the two-way interactions tested were statistically significant ($p > 0.05$). Therefore, the final multivariate model only included the main effects. The model was adjusted for age category and race. Pearson chi-square goodness-of-fit showed a good model fit ($p = 0.69$).

**Population Attributable Risk (PAR%) for selected variables:**

The PAR% of 19.98%, 19.65%, 20.45% and 12.75% was estimated for attending a daycare center, contact with cats, contact with reptiles and contact with a person having symptoms of GI infection respectively (Table 12).

***Findings from subgroup analyses:***

**Analysis 1:**

Contact with reptiles (AOR = 3.57, 95% CI: 1.04 - 12.25) and cats (AOR = 2.28, 95% CI: 1.01 - 4.28) were significantly associated with the *Salmonella* infections in children aged 1-10 years, while having contact with a sick person during the 3 days prior to illness onset did not show association in children aged 1-10 years.

None of the three variables were significantly associated with *Salmonella* infections in children aged < 1 year (Table 13).

**Analysis 2:**

Table 14 shows selected potential risk factors for *Salmonella* infections in children aged < 1 year. Salmonellosis was significantly associated with attending a daycare facility (OR = 2.31, 95% CI: 1.07 - 5.40) in children aged < 1 year. Additionally, a greater number (> 6 vs. ≤ 6) of children in the room of a daycare center increased the odds for *Salmonella* infections by 16-fold (OR = 15.99, 95% CI: 1.38 - 185.39). Other daycare-related variables including hours spent in a daycare per week, number of children in the daycare, number of children in diapers in the same room, having a child with symptoms of GI upset, and having a separate diaper changing area in the daycare, along with food-related exposures such as formula use and pacifier use did not show associations with the *Salmonella* infections.

**Analysis-3:**

Table 15 shows the results of a univariate analysis of selected risk factors associated with *Salmonella* infections in children aged 1-10 years. None of the school- or food-related risk factors were found significant with *Salmonella* infections.

**Analysis-4:**

Among case children, 36 (29.20%) were infected with *S.* Typhimurium, the most common *Salmonella* serotype in the US. To study the association between selected exposures variables and *S.* Typhimurium, we restricted our data to serotype *S.* Typhimirum cases and calculated the ORs with 95% CIs. Table 16 shows the demographic characteristics of cases and controls and Table 17 shows the results of

univariate analyses of selected risk factors for *S.* Typhimurium in children aged 1-10 years. Having contact with any animal (OR = 3.22, 95% CI: 1.47 - 7.08), reptiles (OR = 5.40, 95% CI: 1.37 - 21.29), and birds (OR = 12.45, 95% CI: 1.25 - 123.56) was significantly associated with *S.* Typhimurium.

# DISCUSSION

This population-based case-control study was designed to identify potential risk factors for *Salmonella* infections in Michigan children aged ≤ 10 years.

**Validity of findings:**

The validity of our study results, or degree to which the results are free from error for the study sample being studied has been evaluated by: 1) reviewing the study design, 2) selection of subjects, 3) comparability of cases with controls, 4) exposure assessment, and 5) statistical analysis based on selected variable specific to the case-control study design (interval validity) to identify if the results are threatened by any systematic (bias) or random error.

The case-control design was selected as the main approach because we wanted to study multiple potential risk factors associated with salmonellosis (prediction model). The case-control design was therefore the most appropriate design; investigation begins with diseased (cases) and non-diseased (controls) and retrospectively ascertains exposures between the two groups.

One of the central issues in case-control studies is the comparability of control subjects to case subjects. Since we used the MDCH database to identify cases, our cases were picked from across the state of Michigan in accordance with the times of their reporting. For the enrollment of controls, we adopted a method that would provide controls from the same base population. We have compared the recruited control subjects with cases and did not found that they were significantly different on selected socioeconomic characteristics.

In order to minimize the measurement error, we used validated questions used in other similar research studies, and our questions were pre-tested before their use on study subjects. Additionally, we used a detailed questionnaire to obtain data on plausible sources and a priori risk factors for salmonellosis reported in earlier studies, and control for confounders was possible in the multivariate analysis. Therefore, the results of our study may serve as fair and statistically unbiased estimate for the risk factors of salmonellosis in Michigan children.

**Criteria for causal relationship between exposure and outcome:**

Despite the inherent weakness of the case-control study design in establishing causal relationship between exposures and an outcome, a carefully designed case-control study can still be valuable in supporting the causal relationship between exposures and outcomes. We therefore evaluated our study findings using the Asutin Bradford Hills (1897-1991) Criteria of Causation - the conditions proposed to improve the likelihood of a causal relationship between risk factors of interest and disease being studied.

Although they are not rigid criteria the fulfillment of all may not be accomplished, they still give positive support to inferences about causality.

**Biological plausibility:**

It has been established that humans and animals harbor *Salmonella* serotypes and an infected person or animal can shed the bacteria in their feces. In a study conducted by Shutze et al in Arkansas n 1999, *Salmonella* has been isolated from household members, pets including cats and reptiles, and various places in the household such as

kitchen counters, bathrooms, and flooring. Through the fecal-oral route, direct or indirect transmission of an infection can occur.

**Magnitude of association:**

In this study, the magnitude of statistical association between the predictors and outcome variables is measured by the odds ratio. The stronger the association, the more likely it is that the relationship between the two variables is causal. In this study, the odds of exposure to the respective risk factors was at least 2 folds or higher among cases compared to controls, supporting a causal relationship between the specific exposure and salmonellosis.

**Consistency of findings:**

This criterion implies that if a relationship is causal, we would expect to find it consistently in different studies and in different populations. This is why numerous studies have to be done before meaningful statements can be made about the causal relationship. The associations between exposure variables and development of salmonellosis observed in our study are consistent with the results of similar studies using different settings, populations, and methods. A recent large population-based case-control study conducted by Jones and colleague at the FoodNet sites (89) showed that contact with reptiles and infected persons are significant risk factors for S*almonella* infection in children. Similarly, our results are consistent with the findings of studies carried out outside the US., in France and Island of Guam (96,104).

**Coherence:**

The association should be compatible with existing theory and knowledge. In other words, it is necessary to evaluate claims of causality within the context of the current state of knowledge within a given field. As mentioned earlier that both animals and humans harbor *Salmonella* and infected humans and animals can transfer infection through direct contact and indirectly by contaminated environment. Therefore, in our study the observed results are compatible with the existing theory of and knowledge of *Salmonella* infection transmission.

**Temporality:**

According to this criterion, exposure always precedes the outcome. Hill emphasizes the criterion of temporality as necessary, because in order for exposure to cause disease, exposure must precede disease in time. However, this criterion is usually restricted to prospective studies where we follow a cohort of exposed and unexposed people and look for the disease of interest within the cohort. However, in the majority of case-control studies temporal relationship between an exposure and an outcome cannot be ascertained. In our study, ascertainment of the exposures was made retrospectively for the 3 days prior to the child's illness onset. Based on the formulated questions to gather the exposure data in our study, it is likely that the exposure preceded the diseases particularly in cases of contact with animals and person with symptoms of GI infection.

**Dose-response relationship:**

An increasing amount of exposure increases the risk. If a dose-response relationship is present, it is strong evidence for a causal relationship. In our study, because of the limited sample size, we could not assess the dose- response relationship between exposure variables and *Salmonella* infections.

**Specificity:**

Specificity is established when a single putative cause produces a specific effect (outcome). This is considered as the weakest of all causal criteria. When specificity of an association is found, it provides additional support for a causal relationship. However, absence of specificity does not negate a causal relationship. Diseases are often caused by multiple factors, and it is rare to find a one-to-one cause-effect relationship between an exposure and a disease. Since humans can acquire salmonellosis from multiple sources, such as contaminated food, water, or environmental sources, this criterion is not met in our study.

Although the general findings of this study were not unexpected and in many ways in agreement with other studies conducted to identify risk factors for salmonellosis in children (89), the risk factors for *Salmonella* infections identified in this study differ from those identified in the adult population reported by previous studies. The differences may be partially attributable to markedly different dietary and other environmental exposures. Data from this study demonstrated that reported cases of laboratory-confirmed salmonellosis in children are associated with some potentially

modifiable risk factors and thus can be used as the base for strengthening the implementation of the disease prevention efforts.

**Person-to-person transmission:**

Data from this study suggest that children with *Salmonella* infections were more likely than controls to have been in contact with a person with symptoms of GI infection during the three days before to illness onset. Evidence of person-to-person transmission of *Salmonella* infections among family members of different age groups (115), nursing home residents (116, 117), children attending daycare (118, 119), children in schools (120) and hospital patients (121-124) has been well established in epidemiologic studies. A population-based case-control study conducted using FoodNet sites reported about a 13-fold increase for salmonellosis in children residing in households where a member had diarrhea in the 4 weeks before illness onset (125). Similarly, in France, Delarocque-Astagneau et al. (1998) reported an association between diarrheal symptoms in a household member and *Salmonella* infections in children aged < 5years (58, 96). Among household members, transmission may occur directly or indirectly (i.e., fomites). It has been suggested that an innoculum of about $10^7$-$10^8$ colony forming unit (cfu) of non-typhoidal *Salmonella* is usually required for person-to-person transmission (126). In our data, 27/123 (22%) cases reported contact with a person with GI upset symptoms during the 3 days prior to illness onset and 13 (48%) of these cases had family members that had been diagnosed with *Salmonella* infections. In a Dutch study in children, household members of cases revealed that about 42% of the families had at least one family member with a culture positive for

*Salmonella* (67). Similarly home investigations conducted in family members of children aged < 4 years diagnosed with *Salmonella* infections in Arkansas showed that about 14% of case children had a family member with a positive culture for *Salmonella* (101).

**Contact with animals:**

*Salmonella* is a well-recognized zoonosis (49). Animals are the predominant reservoirs for the bacteria, and the prevalence of *Salmonella* carriage varies by species. (11, 127). *Salmonella* serptypes have been isolated from most vertebrates including dogs and cats that have reported carriage rates of up to 36% and 18%, respectively (128). However, a much higher (up to 94%) *Salmonella* carriage rate has been observed in reptiles and amphibians (21). After infection, dogs and cats can remain asymptomatic and may tend to shed *Salmonella* for prolonged periods of time (129). In our study, children who had contact with cats during the three days before their illness onset were more likely to acquire *Salmonella* infections than their counterparts who did not have contact with cats. Most reports that have addressed the association between human salmonellosis and cats are case series where investigators reported the proportion of cats with positive *Salmonella* infections (130-133). The risk associated with *Salmonella* transmission from cats to humans has not been evaluated in analytical studies. Sources of *Salmonella* infections in cats vary and depend on whether the cats reside indoors or outdoors. For indoor cats, the most likely exposure is the consumption of food contaminated with *Salmonella* organisms, whereas outdoor cats may be exposed through scavenging and hunting prey, especially birds (128). *Salmonella* serotypes that

have been isolated from infected cats include *S.* Typhimuirum, *S.* Enteritidis, *S.* Anatum and *S.* Derby (128).

**Exposures to reptiles:**

Our study suggests that having contact with reptiles was significantly associated with *Salmonella* infections in children (AOR=8.16, 95% CI, 1.55-42.88). Additionally, reptile exposure had the highest PAR% (20.45) among the risk factors identified for salmonellosis. A large number of epidemiologic studies have repeatedly shown that exposure to turtles, lizards, and snakes have been associated with an increased risk of human salmonellosis, particularly for young children (134-138). In a recent case-control study conducted using FoodNet sites, Jones TF reported that reptile ownership is associated with a more than 5-fold increased risk of salmonellosis in children aged < 1 year (89). Similar or even higher magnitudes of association between children's contact with reptiles or amphibians and *Salmonella* infections have been observed in other epidemiological investigations (137).

The prevalence of *Salmonella* infections in exotic animals kept as pets is reportedly highest in reptiles and amphibians. An estimated 90% of all reptiles, in particular, turtles and iguanas, carry and shed *Salmonella* in their feces intermittently (139). Attempts to treat reptiles with antibiotics to eliminate *Salmonella* carriage have been unsuccessful and can increase the development of antibiotic resistance (140). *Salmonella* survives well in the environment (141) and can be isolated from surfaces contaminated by reptile feces for prolonged periods of time. Direct transmission of *Salmonella* occurs by handling of a reptile, and indirect transmission by contact with an

environment contaminated by reptile feces. However, it is more likely that they were infected indirectly through a *Salmonella*-contaminated environment.

Although our study sample consisted of children within a narrowly defined range (aged ≤ 10 years), transition of a child from an infant to toddler brings about significant changes in activities of children, that can profoundly affect their exposure to various known risk factors of *Salmonella*, particularly contact with household pets. We therefore performed an additional sub-group analysis of children < 1 year and those aged ≥ 1 year separately (Table 13). The analysis showed that exposures to cats and reptiles were significantly associated with salmonellosis in older children (≥ 1 year). This is a plausible finding because the mobility of older children (≥ 1 year) allows greater chances of direct contact with these pets and results in an increase acquisition of infection. The subgroup analysis in children aged < 1 years showed less significant associations between exposure to reptiles and contact with a person having symptoms of GI infection and salmonellosis. Although plausible, this finding may also be explained by the smaller sample size. In a recent case-control study, Jones and colleagues, identified both of these exposures as important risk factors for *Salmonella* infections in children aged <1 year. It is conceivable that this age group acquire *Salmonella* infections by indirect transmission from contaminated home environment or via parents or other family members.

*Salmonella* serotypes that are commonly isolated from exotic pets, particularly iguanas and turtles, include *S.* Java, *S.* Stanley, *S.* Poona, *S.* Litchfield, *S.* Manhattan, *S.* Miami, *S.* Jangwani, *S.* Tilene, *S.* Arizonae, and *S.* Rubislaw (142).

During the 1970s, small pet turtles were identified as a major source of *Salmonella* infections in the US. In 1975, the FDA banned the sale of small (i.e., < 4 in. long) turtles. This resulted in a substantial decrease in cases of salmonellosis (143). However, reptiles remain popular pets in the US. The increase in pet reptile popularity has been paralleled by an increase in the number of reptile-related *Salmonella* serotypes isolated from humans (137, 144). According to the American Veterinary Medical Association (AVMA), as many as 2.8 million reptiles were owned as pets in 2001 and 1.5- 2.5 million US households (1.6%) had a pet reptile (145). Applying these estimates to the Michigan population, 60,570 Michigan households would have reptiles as pets (142) .

Frogs and toads also carry *Salmonella* and have been linked to salmonellosis outbreaks in humans (21, 146). In one reported case-control study, ownership of amphibians was independently associated with *Salmonella* infections. It has been estimated that reptile and amphibian contact account for 74,000 (6%) of the approximately 1.2 million sporadic *Salmonella* infections each year in the US (21). In our study, however, associations between amphibian and salmonellosis were inconclusive because only three children reported contact with frogs.

Other animals, such as horses and cattle, have also been recognized as potential sources of *Salmonella* for exposed individuals (e.g., veterinary clinicians and students, and farmers) (147, 148). A recent study noted that inadvertent contamination of household carpets with *Salmonella* serotypes can occur when veterinarians have occupational exposure to cattle on farms (149). Additionally, pigs have been identified as a source

of *Salmonella* Choleraesuis infection associated with high mortality in humans (150). However, exposure to these animals did not show significant association with salmonellosis in our study.

**Daycare-related exposures:**

Children attending childcare centers experience a greater number of illnesses associated with infections, particularly enteric infections, compared to children cared for at home (151, 152). A study found that children who attended childcare required 40% to 80% more medical consultations for acute infections than their counterparts who remained at home (153). Wald and colleagues (1988) reported that children attending these centers had 51% more episodes of infection than their counterparts who were cared for at home (154). A large number of enteric infectious agents including *Salmonella* have been associated with attending daycare (152, 155, 156). Concurring with these studies, our data suggest that attending a daycare significantly increases the odds of salmonellosis (Table 11). Furthermore, our subgroup analysis in children aged <1 year (Table 14) showed that staying in a crowded room (> 6 children in the same room) at a daycare increases the odds of contracting *Salmonella* infections (OR=15.99, 95% CI, 1.38-185.39). The spread of infections in daycare centers is facilitated by crowding and microbial contamination of the childcare environment, as well as a greater susceptibility of young children to infections. It has been suggested that direct contact (person-to-person) is the major route of transmission in the majority of enteric infections in daycare settings. However, indirect contact through contaminated fomites, including toys and other shared items, can occur (157). Children, particularly young

infants, have habits that facilitate the dissemination of infection, such as putting their hands and objects in their mouth (158). A study of bacterial contamination in daycare centers found that the prevalence of infectious agents on the hands of daycare workers, daycare surfaces, and in air samples was inversely related to the age of the children attending the daycare. The likelihood of fecal contamination was greatest on the hands of young infants and their caretakers, and least on those of the older children (159, 160).

It has been suggested that the transmission rate of an enteric infectious agent within a childcare center is influenced by the characteristics of the daycare (number of children enrolled, room size), children attending (age, length of time enrolled, immunological status), and daycare workers (number of workers per child and workers' level of training) (155). Epidemiologic studies examining risk factors for diarrheal illness have found that daycare centers with non-toilet trained infants and those in which food-handling staff also changed diapers had higher diarrheal rates (155, 159).

Among identified risk factors for enteric infections in daycares, diaper changing is considered the procedure with the highest risk for transmission between children and workers (161). Although in a subgroup analysis, we evaluated the relationship between several daycare-related questions and *Salmonella* infections, only one factor, daycare crowding, achieved statistical significance with the outcome variable, likely because of our sample size (Table 14).

**Food-related risk factors:**

In the adult population, a wide variety of food-related potential sources of *Salmonella* infections have been identified, which vary by serotype. Consumption of contaminated poultry and meats, particularly ground beef, has been identified for *S.* Typhimurium (52) and *S.* Heidelberg, whereas eating eggs and egg products has been associated with *S.* Enteritidis (59, 99). In children, acquiring infections through environmental contamination is thought to be more common than via food vehicles (58, 96, 101).

In our study, food-related exposures such as consumption of chicken, meat, and eggs/egg-containing products within three days before the child's illness onset in cases and prior to the interview day in controls did not show a significant association with *Salmonella* infections. It is conceivable the actual magnitude of food exposure related risk for *Salmonella* infections was not evident in this study due to several factors. First, we obtained exposure information from interviewing surrogate sources—parents or caretakers. While certain exposures such as contact with animals and sick persons are more likely to be recalled, recall of consumption of specific foods is difficult and thus prone to measurement error. We suspect the use of surrogate sources in our study may have contributed to measurement error in the food-related exposures for older children. It is conceivable that some of the older children in our study population might have consumed food outside the home without informing their caretakers. This potential for inaccurate measurement could have resulted in the absence of an association between food exposures and *Salmonella* infections seen in our study.

Furthermore, the results of our subgroup analyses (2 & 3) examining the role of food-related sources and salmonellosis separately in children aged < 1 year and those aged 1-10 years did not show association with salmonellosis (Tables 13 & 14). However, our sample size calculations were based on the prevalence of mainly environmental exposures related to salmonellosis in the reference population. These null findings could have resulted because of the limited sample size our study had to detect food-related exposures between cases and controls. For example, breast feeding in children aged <1 year has been shown to prevent salmonellosis in epidemiologic studies. There is sufficient biologic evidence to support the protective effect of breast milk, that it can provide host defense for the breast-fed infant against the majority of infections including *Salmonella (162)*.

Another possible explanation for the protective effect of exclusive breast-feeding could be the characteristics of the environment of breast-fed infants. For example, non–breast-fed infants often drink powdered formula, which has been shown to be an important risk factor for infectious agents including *Salmonella*. Infant formula has been shown to support the growth of *Salmonella* in epidemiologic studies but few investigations have demonstrated that infant formula is associated with Salmonella infections (163). Contamination of formula with *Salmonella* usually occurs during preparation and handling, and growth of bacteria is highly likely if contaminated formula milk is kept at room temperature for several hours. In our study, however infant formula use did not show significant association with salmonellosis.

Pacifier contaminated with pathogens could also transmit infections to children. Some studies have demonstrated an association between pacifier use in young children and

infectious agents. In our study pacifier use did not show an association with salmonellosis.

When the data was restricted to serotype Typhimurium (subgroup analysis 4, Table 17), consumption of meat other than poultry both at home and restaurants during the three days before child's illness onset or interview day showed a significant association at the univariate level with *S.* Typhimurium infections (OR = 5.11, 95% CI: 1.07 – 24.30) in children aged 1-10. This finding corroborates with the results of other investigations that demonstrated an association between eating contaminated meat (164) and *S.* Typhimurium. A large number of reports of foodborne disease outbreaks have been traced back to the contamination of food during preparation and handling at restaurants. Epidemiologic studies of both sporadic and outbreak-associated enteric disease cases suggest that restaurants are an important source of foodborne disease in the US. During 1998-2004, the CDC reported there were 349 restaurant-associated outbreaks (165). In a recent case-control study, consumption of chicken prepared outside the home was associated with *Salmonella* serotype Enteritidis infection (72). Another case-control study identified eating eggs that were prepared outside of the home as a risk factor for *Salmonella* serotype Heidelberg infection (88).

In accordance with the findings of a case-control study conducted in the UK to identify risk factors of *Salmonella* infections in kitchens, none of the household-related variables in our study showed associations with the outcome variable (22, 141).

Drinking water is a major source of microbial pathogens in developing countries due to poor sanitation and hygiene practices (166).

In industrialized countries, drinking municipal tap water or water from private wells has not been identified as an independent risk factor for enteric infections. Accordingly, our study did not find an association between drinking tap or well water and salmonellosis. A few studies conducted in the US have shown association between drinking untreated water from a lake, river, or stream and enteric infections (167, 168). Few children drank water from these sources in our study.

**Other environmental exposures:**

A large number of studies have demonstrated that *Salmonella* can be efficiently transferred from contaminated environments to infect humans, particularly children (89, 97). A recent study reported that children aged < 1 year who ride in a shopping cart with meat or poultry placed next to them have a 4-fold increased risk for salmonellosis (89). It has been demonstrated that substantial levels of contamination with foodborne pathogens exist on the packaging of meats and poultry (169). In assessing the role of the contaminated environment in our study, we asked parents if the child accompanied a caretaker while grocery shopping and the whether the caretaker touched the packages of meat and/or poultry without gloves or plastic. However, this variable did not show an association with *Salmonella* infections in our study.

We also attempted to evaluate the role of in-house contamination and the risk of salmonellosis in children aged < 1 year. There was no significant association detected between the variable 'placing child on the floor without a blanket' and salmonellosis

(Table 14). Although some studies (104, 141) have reported the isolation of *Salmonella* from household dust, soil samples near the home, and samples from bathrooms, we did not evaluate contamination of the household environment through these means.

**Travel-associated salmonellosis:**

Individuals who travel to places where foodborne infections like salmonellosis are prevalent (e.g., South America, Asia) are at a greater risk of contracting enteric diseases. In contrast to the findings of other case-control studies conducted in adult populations that have identified travel-associated risk factors for salmonellosis and many other enteric pathogens, our study found no association between illness among children aged $\leq 10$ years and travel. Pathogens such as enterotoxigenic *Escherichia coli*, *Campylobacter jejuni,* and *Salmonella* serotypes account for the majority of diarrheal disease cases associated with travel. Nontyphoidal salmonellosis is mostly caused by the *Salmonella* serotypes Enteritidis and Typhimurium; however, other serotypes have been isolated from individuals with a history of recent travel.

**Public health recommendations:**

**Exposure to cats:**

Our study suggested a significant association between contact with cats and *Salmonella* infections. Additionally, exposure to cats had a PAR of 19.65% for salmonellosis. This finding should be viewed as a significant public health problem. Cats are among the most widely kept pets in the US—it is estimated that about 34% of households have at least one cat as a pet. The AVMA estimates that between 1996 and 2001, the US population of cats increased 16 %, reaching 78 million cats in 2001 (170).

Educating pet owners about the safe handling of their pets, disinfection of contaminated areas in the household, and restriction of contact with the family members who might be at greater risk for developing the disease, particularly young children. Additionally, cat owners should be informed of asymptomatic cat carriers of *Salmonella*. Older children (aged > 5 years) should be educated about hygiene practices such as hand washing after touching cats.

As stated earlier, risk of *Salmonella* transmission has not been examined in analytical studies, additional epidemiologic studies are needed to quantify the risk of *Salmonella* transmission from infected cats to humans.

**Exposure to reptiles:**

Numerous public health recommendations regarding ownership and care of reptiles and the potential risks of *Salmonella* exposure to children have been made (142). In 1999, the CDC recommended that children aged < 5 years and immunocompromised persons should avoid contact with reptiles and that reptiles should not be kept in homes where immunocompromised people or children < 5 years old reside (60, 135, 136). However reptile-associated salmonellosis continues to be a major public health problem in the US (60). Legislation requiring pet store owners to communicate the increased risks of salmonellosis to customers who wish to purchase reptiles exists in several States (142). Michigan requires consumer education regarding the risk of salmonellosis for the sale of turtles (135). In 1999, the National Association of State Public Health Veterinarians and the Council of State and Territorial Epidemiologists recommended that state and local authorities adopt regulations to prohibit the sale of reptiles without written point-

of-sale education to consumers about the risks for and prevention of reptile-associated salmonellosis (145). In 2003, the CDC gathered information from the health departments in all 50 states and New York City to determine whether such regulations existed. Among the 49 health departments responding, four states (Colorado, Illinois, Kansas, and Texas) required pet stores to provide information about salmonellosis to persons purchasing any reptile, and five states (California, Connecticut, Maryland, Michigan, and New York) required providing salmonellosis information to persons purchasing a turtle but not other reptiles. Tennessee prohibited the sale of all turtles, while NYC prohibited the sale of certain reptiles, including iguanas, small turtles, and boas, and required posting of information about reptile-associated salmonellosis where other kinds of reptiles were sold.

Pet-store owners, health-care providers, and veterinarians should educate owners and potential purchasers of reptiles and amphibians about salmonellosis prevention measures. A study reported that less than 50% of the families having iguanas as pets realize their pets may carry *Salmonella*, demonstrating an inadequate knowledge about potential *Salmonella* transmission (171).

It has been widely accepted that pets offer advantages in terms of providing companionship for lonely individuals, and helping children develop a sense of care and compassion. However, some pets, particularly exotic pets such as turtles and iguanas are known for their carriage status of enteric pathogens (e.g., E. Coli, Campylobacter and *Salmonella*), and thus can pose a risk to humans who contact them, especially children. The risk increases when such pets are mishandled, for example by placing turtles and iguanas in the bathtubs and failing to sanitize before human use (ref).

Mitigation of the risk for salmonellosis that is associated with exposure to pet animals has been the subject of intensive efforts by federal and state agencies along with the AVMA.

**Recommendations for the prevention of reptile-associated salmonellosis:**

Educating parents and caretakers regarding the risk of salmonellosis associated with exposure to animals can help reduce the disease burden.

**At the Federal or State level:**

- Periodic assessment of compliance with Federal laws regarding the sale of small sized turtles (<4 inches)
- Evaluation of the effectiveness of mandated point-of-sale education in reducing amphibian- and reptile-associated salmonellosis.
- Using mass media to educate parents regarding the risk of *Salmonella* transmission from reptiles
- Prohibition of day care centers and preschools to house reptiles or amphibians.
- Integration of human and veterinary surveillance systems and education of the veterinary community on its role in public health

**For veterinarians and healthcare providers:**

- Encourage pet retailers (pet store owners), veterinarians, and healthcare professionals to educate owners of reptiles or amphibians regarding the risk of *Salmonella* infections associated with reptiles.

- Provision of education by veterinarians to animal owners about the risk of *Salmonella* transmission whether or not pets are exhibiting symptoms of salmonellosis

**For pet owners:**

- Additional efforts to educate reptile and amphibian owners of the potential for *Salmonella* transmission from pets using mass media; also educate older children, aged > 5 years
- Encourage parents with young children not to keep reptiles and amphibians in the household
- Do not allow reptiles or amphibians to roam free in the living areas, particularly the kitchen
- Wear gloves when cleaning cages and treating animals, and wash hands thoroughly with soap and water each time a reptile or amphibian or its equipment is handled
- Do not clean reptile and amphibian cages and equipment in the kitchen or bathroom sinks or tubs
- Use designated tubs for cleaning equipment or bathing reptiles and disinfect with a bleach solution after use
- Immediately clean and disinfect areas contaminated with animal feces

**Attending a daycare:**

Illness in the daycare setting is a great concern of parents and a significant public health problem worldwide. Simple measures to control and prevent infections such as workers washing hands with soap and water after changing diapers, after assisting children with the toilet, and before handling food would help to substantially reduce the incidence of infections related to daycare. The effectiveness of hand washing has been illustrated by a study that showed a markedly reduced incidence of diarrhea among young children in child care centers after the introduction of an intensive hand washing program for the workers (172). Disposable gloves should be worn for changing the diapers and the changing station should be cleaned after each use. If possible, daycare workers who handle diapers should not prepare the food. Another key measure in controlling the spread of infections in daycares is to thoroughly clean the children's toys at the end of each day with hot water or disinfectant.

**Food-related exposures:**

Instituting safer food preparation practices in commercial kitchens could reduce much of the risk associated with eating at commercial restaurants. Commercial food establishments should take measures to ensure that meat, produce, and other foods are obtained from high-quality suppliers. Educating and training restaurant workers is important to ensure that safe food handling procedures are consistently followed. Public health authorities should also regularly perform inspections of food establishments and enforce regulatory policies. In addition, consumers should avoid consumption of high-risk foods such as undercooked eggs and meat in commercial food

establishments.

## STRENGTHS OF THE STUDY

**Incident enrollment of cases:**

We enrolled new cases of salmonellosis reported to MDCH. Parents of cases were contacted for an interview soon after they were reported in the disease surveillance system. Interviewing parents of case children soon after the onset of the disease and questioning control parents about the 3 previous days allowed for better recall of food history and related exposures.

**Laboratory-confirmed cases of salmonellosis:**

Bacterial cultures of samples obtained from patients with suspected *Salmonella* infections are cultured for *Salmonella* in local laboratories across Michigan and then sent to MDCH for confirmation and serotyping. We enrolled only laboratory-confirmed (ie., on stool, urine, cerebrospinal fluid, or blood culture) cases of salmonellosis reported to MDCH. Since microbiological laboratory culture is considered the gold standard for diagnosis of salmonellosis, the use of this highly sensitive and specific method for testing *Salmonella* infections minimized the chance of misclassification of the disease status.

**Generalizability of findings (external validity):**

The MDCH surveillance system collects information from the entire state of Michigan, therefore the participants in this case-control study are representative of all Michigan children. Although not all reported cases were enrolled in the study, enrollees (participants) and non-enrollees (non- participants) were drawn from the same

population base, and the two groups were similar with respect to demographic characteristics including sex, and race (Table 8). Additionally, cases and controls did not differ on socioeconomic and demographic characteristics including sex, parental education, annual household income and area of residence (Table 10). The enrollment of population-based community controls allows the generalization of our results to all Michigan children and possibly to children in similar neighboring states.

**Controlling for confounding variables:**

Our detailed questionnaire allowed us to collect information on a number of food- and environment-related variables to study their association with *Salmonella* infections. While building our statistical model, we were able to control for numerous potential confounders, including those reported in previous studies. However, chances of unmeasured (unknown) confounders could not be eliminated.

**Participation rate**

Our overall participation rate of 72% is higher than other population-based studies conducted to answer similar research questions. In a case-control study conducted at the FoodNet sites, Rowe SY reported a participation rate of 59% (162). Another recent population based case-control study carried out at the FoodNet sites (2002-2004), reported a response rate of 67% (89), while a study conducted in France by Delarocque-Astagneau and colleague (1995) reported a 60% response rate (58). The refusal rate among cases was much lower in our study compared to what has been reported in larger studies reported by Jones TF et al (2006) (11% vs. 19%) (89). The reason for higher

participation rate in our study was likely the result of our repeated attempts to contact parents of the study children (~ 20 phone calls), including evening and weekend calls.

**Post hoc power analysis:**

The post hoc power analysis for selected exposures showed that attending a daycare center, and having contact with cats or reptiles had a power of 80% at 5% probability of type-I error (Table 18). Therefore for the variables (risk factors) of interest, our study had sufficient power to detect significant association between the exposure variables in question and salmonellosis as the outcome, if such association really existed. Egg consumption, which is reported in the contemporary literature to be associated with *Salmonella* infections (88, 98), was not significantly associated with salmonellosis in our study. For factors that are highly prevalent in a population (and therefore in controls), detection of statistical significance requires large sample sizes. Our study was not powered to reveal statistically significant associations between salmonellosis and most of the prevalent risk factors such as consumption of eggs and poultry. Additionally, the high-risk foods are also commonly consumed by the general population. However, it is conceivable that cases may have consumed similar foods as controls but the contaminated one. This could only have been ascertained if we have had a microbiological testing of the food items listed by the cases and controls during the 3 days prior to the illness onset.

## STUDY LIMITATIONS

**Selection bias in enrolling cases:**

Our data were limited to laboratory-diagnosed cases, and are thus biased by factors that affect the probability of an illness being reported (173, 174). Cultures are not obtained in all cases of suspected foodborne diseases, including salmonellosis, for several reasons (1). First, the majority of foodborne illnesses are self-limiting and resolve spontaneously in about a week. Therefore, individuals with mild to moderate disease symptoms may not seek medical care and hence do not get reported. Second, physicians may not request stool or other specimen cultures for patients seeking care for gastrointestinal disease symptoms (1, 70, 95, 173, 174). Laboratory testing for salmonellosis is largely dependent upon a patient's presenting symptoms. In this study, we have only enrolled children $\leq$ 10 years, an age group likely to receive closer medical attention when they manifest gastrointestinal disease symptoms compared to adults.

**Recall bias:**

As an inherent weakness in case-control design, recall bias may be present in the measurement of some exposure variables (107). Parents or caretakers knew the disease status of their children prior to the interview and this may have influenced their responses (58, 162). Parents of case patients may recall exposures more accurately than parents of control subjects. In addition to questions related to specific exposures prior to illness onset, we asked questions about children's food preferences and the presence of common exposures (e.g., ownership of pets, family kitchen practices) in the household. This approach allowed us to study the association between these common

exposures and salmonellosis, in addition to measured exposures in the three-day period that may have caused the illness.

**Misclassification of outcome variable:**

We did not obtain specimens for culture from controls to exclude asymptomatic cases of salmonellosis. It is possible that some of our control children were asymptomatic *Salmonella* carriers and thus may have been misclassified. However, given the very low (1%) prevalence of chronic carriers of *Salmonella* in healthy populations, we expect very few to none misclassified controls (23, 175). Furthermore, there is no reason to assume that this misclassification may have been dependent on the presence or absence of any risk factor of interest for salmonellosis. Therefore, any misclassification that might have occurred because of inclusion of a few 'asymptomatic cases' as controls must necessarily be non-differential in nature. This may have yielded somewhat conservative (biased towards null) estimates of effect measure, but the validity of estimates of exposure, and their relationship with disease is not threatened.

**Interviewer bias:**

A total of 3 interviewers conducted telephone interviews from both cases and controls. Since we called parents of each of our study participants multiple times and at different times and day of the week, for logistical purpose we assigned separate interviewers for cases and controls. One person interviewed the cases and two persons interviewed the majority of controls. Because of the design of our study, we were not able to blind interviewers to the disease status of the study participants (107). This might have

introduced some bias in the interviewing process. However, prior to conducting the study, all interviewers received standard training for conducting phone interviews. They were informed about ways to prevent the introduction of bias during the interview process when assessing exposures. Interviewers were provided written instructions on how to administer the questionnaire to all participants following a similar approach/protocol. They read from a common script irrespective of the disease status of the interviewee. All pre-testing, which also served as practical training of the interviewers, was supervised by at least one of the investigators at all times.

**Selection bias in control enrollment:**

For control selections, we used the on-line white pages, which only provide landline phone numbers and do not include cell phone or Internet phones. It is possible that our enrolled controls (households that have landline phones) might have been different from household that did not have a landline phone in regard to certain socioeconomic attributes. However, the National Health Interview Survey (2005) estimated that about 2% of households do not have any telephone service (wireless or landline) and only about 7.8% of adults lived in households with only a cell phone. Moreover, the majority of cell phone only households belonged to younger and single individuals (37). Since our study enrolled households with children, it is less likely that we have missed a large proportion of households without a landline phone while enrolling control children. Therefore, it is likely that our case households are similar to the control households with regard to landline phone status.

**Two methods of enrolling controls:**

We used two methods to enroll control subjects. In method 1, only 28 appropriate subjects suggested by cases caretakers based on their familiarity with them (friends or relatives), were included among the control sample of this study. This approach could yield a control group similar to the case group with regard to certain socioeconomic and life style characteristics (unplanned matching) (108). In method 2, we enrolled controls using an online telephone directory, which provide a community or population control group. Comparing controls enrolled using the two methods, based on household income and parental education attainment revealed that the two groups were significantly different with regard to selected socioeconomic attributes (Table 20) perhaps due to the difference in sample size of the compared groups (28 vs. 111). However, overall comparison of cases and controls (Table 10) did not reveal significant difference with regard to socioeconomic difference, except the racial distribution between the two.

**Subgroup analyses:**

Some of the subgroup analyses were based on a small numbers of observations, resulting in a lack of adequate statistical power. Therefore, findings based on subgroup analyses should be interpreted with caution. This however does not jeopardize the main findings of the study. Additionally, results of subgroup analyses (II, III, IV) were based on univariate analysis and did not control for potential confounders.

**Age and area of residence as risk factors:**

We used age-stratified sampling to enroll a sufficient number of cases and controls in each age stratum. Moreover, as mentioned earlier, we started enrolling our controls using a neighborhood matched design. However, early in the course of data collection based on apparent lack of difference between the neighborhood matched and non-matched controls with regard to certain socioeconomic attributes (Table 19), we dropped the neighborhood matching design. Therefore, we did not analyze age and area of residence as potential risk factors for salmonellosis in our study. However, the final multivariate model was adjusted for age group.

Our cases and controls differed with regard to race. Although the reason for this difference is not very well understood, food consumption, handling, and preparation, along with lifestyle factors, have been reported to vary among different racial and ethnic groups (176) . Additional studies are needed to clearly delineate the risk of *Salmonella* infections associated with these populations.

## CONCLUSIONS

This is the first population-based case-control study designed at identifying risk factors for *Salmonella* infections in children in Michigan. Our study revealed that contact with cats and reptiles within three days before the onset of child's illness is a risk factor for infection with *Salmonella* serotypes. Additionally, attending a daycare center and contact with a person with symptoms of GI upset is also associated with significant risk for *Salmonella* infections in children. In agreement with other studies aimed at examining factors associated with *Salmonella* infections in children, our data suggest that the contribution of environmental sources plays an important role in the acquisition of *Salmonella* infections in children, compared to the adult population where a larger proportion of infections are acquired through food vehicles. Several recommendations have been made to educate parents and caretakers about the risk of *Salmonella* transmission to children from infected persons and household pets, particularly reptiles. However, our study showed that exposure to these factors continued to cause *Salmonella* infections in children. Additional efforts are needed to educate parents and caretakers about the risk of *Salmonella* transmission to children from cats and reptiles, along with individuals having GI symptoms.

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## CONFLICT OF INTEREST

No conflict of interest to declare

Table 1. Comparison of the 2006 incidences of infections with major enteric pathogens and the US National Health Objective 2010 [Annual disease summary, CDC, 2004 (modified)].

| Pathogen | 2006 incidence* | 2010 objective** |
|---|---|---|
| Shiga toxin-producing *Escherichia coli O157* | 0.80 | 1.00 |
| *Campylobacter* | 12.80 | 12.31 |
| *Listeria* | 0.27 | 0.25 |
| *Salmonella* | 14.61 | 6.80 |

*Reported cases per 100,000 population
**2010 Healthy People Objective

Table 2. Selected large foodborne outbreaks where *Salmonella* serotypes were identified as etiologic agents (1974-2007). [Source: compiled from various MMWR and FoodNet reports]

| Year | Number of reported cases | Source/vehicle | Location |
|---|---|---|---|
| 2007 | 60 | Veggie Booty | Nationwide |
| 2006-2007 | 425 | Peanut butter | Nationwide |
| 2006 | 183 | Restaurant tomatoes | Nationwide |
| 2006 | 84 | Deli | IN |
| 2006 | 29 | Frozen chicken dinners | MN |
| 2005 | 31 | Orange juice | Nationwide |
| 2005 | 300 | Under-cooked turkey | SC |
| 2004 | 300 | Roma tomatoes | PA, OH, MD, VA, WV |
| 2004 | 29 | Raw almonds | Canada and US |
| 2003 | 99 | Hospital cafeteria | MO |
| 2002 | 47 | Unpasteurized milk | OH |
| 2002 | 141 | Roma tomatoes | FL |
| 2002 | 27 | Cantaloupe | Western US |
| 2001 | 1000 | Bakery products | MI |
| 2001 | 20 | Cantaloupe | CA |
| 2001 | 225 | Deli sandwiches | VA |
| 1998 | 209 | Toasted oats cereal | Nationwide |
| 1998 | 58 | Chile relleno | AZ |
| 1998 | 50 | Mexican cake | MD |
| 1998 | 71 | Ziti | NV |
| 1997 | 79 | Cheese / raw milk | CA |
| 1996 | 44 | Chile relleno | GA |
| 1996 | 52 | Roast beef | SD |
| 1996 | 66 | Chicken | MA |
| 1995 | 62 | Orange juice (unpasteurized) | FL |
| 1995 | 241 | Alfalfa sprouts | 6 States & Finland |
| 1995 | 133 | Alfalfa sprouts | OR, BC |

| (Table 2 continued) | | | |
|---|---|---|---|
| 1994 | 158 | Raw ground beef | WI |
| 1994 | 224,000 | Ice cream | 41 states |
| 1993 | 19 | Egg rolls | TX |
| 1993 | 23 | Hollandaise and béarnaise sauce | CA |
| 1993 | 22 | Mayonnaise | CA |
| 1990 | 690 | Bread pudding | IL |
| 1989 | 164 | Mozzarella and shredded cheese from a single plant | MN, WI. NY |
| 1985 | 16,000 | Milk | IL, MI, IN, IA |
| 1974 | 3,400 | Potato salad | Not known |

Table 3. The 20 most frequently reported *Salmonella* serotypes from human sources reported to CDC in 2004 [Annual disease summary, CDC, 2004 (modified)].

| Order | Twenty most common *Salmonella* serotypes | No. | % |
|---|---|---|---|
| 1 | Typhimurium | 6842 | 19.2 |
| 2 | Enteritidis | 5012 | 14.1 |
| 3 | Newport | 3325 | 9.3 |
| 4 | Javiana | 1772 | 5.0 |
| 5 | Heidelberg | 1757 | 4.9 |
| 6 | Montevideo | 870 | 2.4 |
| 7 | I 4,[5],12:i:- | 739 | 2.1 |
| 8 | Muenchen | 739 | 2.1 |
| 9 | Saintpaul | 692 | 1.9 |
| 10 | Braenderup | 684 | 1.9 |
| 11 | Infantis | 588 | 1.6 |
| 12 | Mississippi | 558 | 1.6 |
| 13 | Oranienburg | 495 | 1.4 |
| 14 | Thompson | 493 | 1.4 |
| 15 | Berta | 409 | 1.1 |
| 16 | Agona | 406 | 11 |
| 17 | Paratyphi B var. L(+) tartrate+ | 354 | 1.0 |
| 18 | Typhi | 306 | 0.9 |
| 19 | Hadar | 277 | 0.8 |
| 20 | Anatum | 250 | 0.7 |
| | **Total** | **26568** | **74.5** |

Table 4. Examples of *Salmonella* serotypes by host adaptation. [Source: Uzzau S, 2000]

| Serotype | Natural host | Other host(s) |
|---|---|---|
| *S.* Typhi | Human | - |
| *S.* Paratyphi A | Human | - |
| *S.* Paratyphi C | Human | - |
| *S.* Sendai | Human | - |
| *S.* Abortusovis | Ovine | - |
| *S.* Gallinarum | Poultry | - |
| *S.* Typhisuis | Swine | - |
| *S.* Abortusequi | Equine | - |
| *S.* Choleraesuis | Swine | Human |
| *S.* Dublin | Bovine | Human, Ovine |

Table 5. Percent change (2004 vs. 1996-1998) in the incidence of reported cases of four of the most common *Salmonella* serotypes under surveillance at FoodNet sites. [Annual disease summary, CDC FoodNet, 2005 (modified)]

| Pathogen | Percent change in incidence |
|---|---|
| *Salmonella* Typhimurium | - 41 |
| *Salmonella* Enteritidis | 0 |
| *Salmonella* Heidelberg | 3 |
| *Salmonella* Newport | 40 |

Table 6. Distribution of multi-drug resistant (MDR) *Salmonella* Typhimurium and definitive phage type 104 strains in selected countries, 1992–2001. [WHO, 2003]

| | % MDR, % DT104 | | | | |
|---|---|---|---|---|---|
| **Country** | **1992–1993** | **1994–1995** | **1996–1997** | **1998–1999** | **2000–2001** |
| **Ireland** | 40.3, 38.9 | 66.8, 61.2 | 76.1, 70.0 | 70.7, 65.4 | 63.3, 45.2 |
| **Scotland** | NA, NA | NA, NA | NA, NA | 75.0, 63.1 | 79.7, 56.3 |
| **Denmark** | NA, NA | 1.5, 1.5 | 5.0, 3.0 | 21.9, 15.8 | 23.1, 12.7 |
| **Austria** | NA, 17.0 | NA, 14.6 | 13.7, 32.7 | 13.1, 28.9 | 35.8, 29.6 |
| **Germany** | 14.3, 3.1 | 30.2, 9.2 | 44.3, 32.1 | 49.0, 32.1 | 57.1, 44.0 |
| **Netherlands** | 10.3, 6.7 | 8.9, 15.3 | 26.3, 23.5 | 29.5, 29.6 | 33.9, 37.2 |
| **Canada** | NA, 17.7 | NA, 27.3 | 16.2, 46.1 | 44.1, 43.8 | 47.8, 35.5 |
| **USA** | NA, NA | 19.8, NA | 45.7, 29.1 | 40.5, 34.0 | 39.0, NA |
| **Japan** | NA, 2.1 | NA, 3.1 | NA, 8.8 | NA, 13.7 | NA, 9.8 |
| **Australia** | 3.8, 0.1 | 2.7, NA | 1.5, NA | 1.8, 0.2 | 3.2, 0.7 |
| **New Zealand** | NA, 0.8 | NA, 0.5 | NA, 0.3 | NA, 0.4 | NA, 0.1 |

NA: data not available.

Table 7. Identified risk factors for *Salmonella* infections in children.

| Risk factors | Location(s) | Investigator/Year* (Reference) |
|---|---|---|
| **Food-related exposures** | | |
| Raw or undercooked egg | France<br>Netherlands | Delarocque-Astagneau E/1998 (96)<br>Doorduyn Y/2005 (177) |
| Consumption of ground beef | France | Delarocque-Astagneau E/2000 (58) |
| Infant formula | FoodNet sites, US<br>Gangwon, Korea<br>Island of Guam | JonesTF/2006 (89{Rowe, 2004 #3)<br>Park J/2002 (178)<br>Haddock RL/1991 (163) |
| **Environmental exposures** | | |
| Person-to-person transmission | France<br>France<br>FoodNet sites, US<br>Wisconsin, US | Delarocque-Astagneau E/1998 (96)<br>Delarocque-Astagneau E/2000 (58)<br>Rowe SY/2004 (162)<br>Wilson R/1981 (67) |
| Daycare attendance | FoodNet sites, US | JonesTF/2006 (89) |
| Contact with reptiles | FoodNet sites, US | JonesTF/2006 (89) |
| Contaminated home environment | Island of Guam | Haddock RL/1994 (104) |
| Riding a shopping cart during grocery shopping | FoodNet sites, US | JonesTF/2006 (89) |
| Playing in a sandbox | Netherlands | Doorduyn Y/2005 (177) |
| Travel outside US | FoodNet sites, US | JonesTF/2006 (89) |

*Year of reporting

Table 8. Comparison of demographic characteristics between enrolled case children (participants) and non-enrolled case children (non-participants) in *Salmonella* case-control study, Michigan, 2007

| | Participant children n=123 | | Non-Participant children n=76 | | P-value* |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| **Age (year)** | | | | | 0.42 |
| <1 | 22 | (17.89) | 17 | (22.37) | |
| 1-5 | 66 | (53.66) | 37 | (48.68) | |
| 6-10 | 35 | (28.46) | 22 | (28.93) | |
| **Sex** | | | | | 0.55 |
| Male | 61 | (49.60) | 41 | (53.95) | |
| Female | 54 | (43.90) | 28 | (36.84) | |
| Unknown* | 8 | (6.50) | 7 | (9.21) | |
| **Race** | | | | | 0.78 |
| Caucasians | 55 | (7.32) | 29 | (38.16) | |
| African- | 9 | (2.44) | 6 | (7.89) | |
| Americans | 3 | (44.71) | 2 | (2.63) | |
| Asian | 4 | (3.25) | 6 | (7.89) | |
| Other | 52 | 42.28) | 33 | (43.42) | |
| Unknown** | | | | | |

* Computed using the Chi-square test for two proportions

**Information was missing in the MDSS.

Public health officials at Local Health Departments collect data on demographic characteristics (e.g., age, sex, and race) from reported cases during the disease investigation process and report to MDCH. The information for this table was obtained from the Michigan Disease Surveillance System (MDSS). Cases infected with *S.* Typhi and *S.* Paratyphi were excluded.

Table 9. Distribution of *Salmonella* serotypes in children aged ≤ 10 years and ≥ 11years reported to MDCH during the study period (Dec 15, 2006 - October 15, 2007).

| ***Salmonella* serotype** | **Age groups** | | | | |
|---|---|---|---|---|---|
| | **≤ 10 years**[a] | | **≥ 11 years** | | |
| | n=199 | | n=602 | | |
| | No. | % | No. | % | P-value[d] |
| Typhimurium | **40** | **20.10**[b] | 88 | 13.50 | 0.06 |
| Enteritidis | 17 | 8.54 | **164** | **25.21**[c] | <0.1 |
| Newport | 7 | 3.52 | 34 | 5.20 | 0.23 |
| Heidelberg | 7 | 3.52 | 34 | 5.20 | 0.23 |
| Oranienburg | 5 | 2.51 | 8 | 1.20 | - |
| Braenderup | 4 | 2.01 | 7 | 1.00 | - |
| Stanley | 3 | 1.51 | 7 | 1.00 | - |
| Saintpaul | 2 | 1.01 | 8 | 1.20 | - |
| Pomona | 2 | 1.01 | 2 | 0.30 | - |
| Infantis | 2 | 1.01 | 7 | 1.00 | - |
| Hartford | 2 | 1.01 | 6 | 0.90 | - |
| Cotham | 2 | 1.01 | 0 | - | - |
| Thompson | 5 | 2.51 | 15 | 2.30 | - |
| Soerenga | 1 | 0.50 | 0 | - | - |
| Rough O's:[e,h:1,5] | 1 | 0.50 | 4 | 0.60 | - |
| Norwich | 1 | 0.50 | 2 | 0.30 | - |
| Muenchen | 3 | 1.51 | 9 | 1.38 | - |
| Montevideo | 5 | 2.51 | 3 | 0.46 | - |
| Meleagridis | 1 | 0.50 | 2 | 0.30 | - |
| Mbandaka | 3 | 1.51 | 3 | 0.46 | - |
| Kentucky | 1 | 0.50 | 1 | 0.15 | - |
| Hadar | 3 | 1.51 | 7 | 1.00 | - |
| Bovismorbificans | 1 | 0.50 | 1 | 0.15 | - |
| Bareilly | 1 | 0.50 | 0 | - | - |
| Adelaide | 1 | 0.50 | 0 | - | - |
| Sp., 4,5,12:b:- | 4 | 2.01 | 8 | 1.20 | - |
| Sp., 4,5,12:i:- | 15 | 7.54 | 36 | 5.50 | - |
| Abony | 0 | 0.00 | 1 | 0.15 | - |
| Agona | 2 | 1.01 | 6 | 0.90 | - |
| Anatum | 6 | 3.02 | 13 | 2.00 | - |
| Anecho | 0 | | 1 | 0.15 | - |

| (Table 9 continued) | | | | | |
|---|---|---|---|---|---|
| Baildon | 0 | - | 1 | 0.15 | - |
| Berta | 2 | 1.01 | 4 | 0.60 | - |
| Chester | 4 | 2.01 | 11 | 0.15 | - |
| Corvallis | 0 | - | 1 | 0.15 | - |
| Derby | 1 | 0.50 | 4 | 0.60 | - |
| Dublin | 1 | 0.50 | 2 | 0.30 | - |
| Gnesta | 0 | - | 1 | 0.15 | - |
| Group B,4,12:i:- | 2 | 1.01 | 5 | 0.77 | - |
| Group B,4,5,12:-:1,2 | 0 | - | 2 | 0.30 | - |
| Group B,4,5,12:nonmotile | 0 | - | 1 | 0.15 | - |
| Group C1 | 3 | 1.51 | 7 | 1.00 | - |
| Havana | 0 | - | 1 | 0.15 | - |
| Javiana | 4 | 2.01 | 9 | 1.40 | - |
| Kiambu | 0 | - | 1 | 0.15 | - |
| Kottbus | 2 | 1.01 | 1 | 0.15 | - |
| Litchfield | 2 | 1.01 | 5 | 0.77 | - |
| Manhattan | 0 | - | 1 | 0.15 | - |
| Miami | 0 | - | 1 | 0.15 | - |
| Muenster | 0 | - | 2 | 0.30 | - |
| Ohio | 0 | - | 1 | 0.15 | - |
| Oslo | 0 | - | 1 | 0.15 | - |
| Reading | 0 | - | 2 | 0.30 | - |
| Sanjuan | 0 | - | 1 | 0.15 | - |
| Schwarzengrund | 0 | - | 2 | 0.30 | - |
| Subgroup 1 | 0 | - | 2 | 0.30 | - |
| Subgroup IIIA | 0 | - | 1 | 0.15 | - |
| Subgroup IIIB | 0 | - | 2 | 0.30 | - |
| Subgroup IV | 0 | - | 1 | 0.15 | - |
| Telelkebir | 0 | - | 1 | 0.15 | - |
| Tennessee | 5 | 2.51 | 16 | 2.50 | - |
| Virchow | 0 | - | 2 | 0.30 | - |
| Weltevreden | 2 | 1.01 | 5 | 0.77 | - |
| Not named | 3 | 1.51 | 0 | - | - |
| Serotype was not listed* | 21 | 10.55 | 74 | 11.35 | - |

*Information was missing in the Michigan Disease Surveillance System

[a] All cases of salmonellosis in children aged $\leq$ 10 years (participants and non-participants)

[b] *S.* Typhimurium was the most common *Salmonella* serotypes reported in children aged $\leq$ 10 years

[c] *S.* Enteritidis was the most common *Salmonella* serotype reported in aged $\geq$ 11 years.

[d] P-value calculated for selected

Cases infected with *S.* Typhi and *S.* Paratyphi were excluded

Table 10. Socioeconomic characteristics of children aged ≤ 10 years enrolled in a population-based case-control study to identify risk factors for *Salmonella* infections, Michigan, 2007.

| Socioeconomic characteristics | Cases n=123 | | Controls n=139 | | p-value* |
|---|---|---|---|---|---|
| | No. | (%) | No. | (%) | |
| **Age (year)** | | | | | 0.54 |
| < 1 | 22 | (17.89) | 25 | (17.99) | |
| 1-5 | 66 | (53.66) | 66 | (47.48) | |
| 6-10 | 35 | (28.46) | 48 | (34.53) | |
| **Sex** | | | | | 0.86 |
| Female | 66 | (53.66) | 76 | (54.68) | |
| Male | 57 | (46.34) | 63 | (45.32) | |
| **Race** | | | | | < 0.01* |
| Caucasians | 107 | (87.70) | 92 | (67.15) | |
| African-Americans | 9 | (7.38) | 27 | (19.71) | |
| Other minorities** | 6 | (4.92) | 18 | (13.14) | |
| **Parental education** | | | | | 0.94 |
| Elementary to High school | 30 | (24.39) | 33 | (23.91) | |
| Some college to college degree | 72 | (58.54) | 85 | (61.59) | |
| Post-graduate degree | 19 | (15.45) | 18 | (13.04) | |
| Refused to answer*** | 2 | (1.63) | 2 | (1.45) | |
| **Annual income household** | | | | | 0.34 |
| <$ 35,000 | 26 | (21.31) | 25 | (18.12) | |
| $35,001- $50,000 | 17 | (13.93) | 17 | (12.32) | |
| $50,001- $75,000 | 28 | (22.95) | 39 | (28.26) | |
| >$75,000 | 38 | (31.15) | 33 | (23.91) | |
| Refused to answer | 13 | (10.66) | 24 | (17.39) | |
| **Area of residence** | | | | | 0.31 |
| High income: $>60000 | 31 | (25.20) | 35 | (25.18) | |
| Medium income: $38000 - $60000 | 56 | (45.53) | 74 | (53.24) | |
| Low income: $<38000 [δ] | 36 | (29.27) | 30 | (21.58) | |

*Significant at $P < 0.05$; computed using Chi-square test for two proportion **Asian, Middle Eastern, Alaskan Indian and other racial minority groups

***Participants refused to provide the answer/response

[δ] Categorized based on zip code level median household income obtained from the US Bureau of Census, 2000.

Table 11. Univariate analyses of putative risk factors for *Salmonella* infections in children aged ≤10 years, assessed in a population-based case- control study, Michigan.

| Variables | Cases n=123 | | Controls n=139 | | Unadjusted OR* (95% CI**) |
|---|---|---|---|---|---|
| | No. | (%) | No. | (%) | |
| **Household related variables** | | | | | |
| **Number of people in household** | | | | | |
| ≤ 4 | 82 | (66.67) | 77 | (55.40) | Reference |
| > 4 | 41 | (33.33) | 62 | (44.60) | 1.61(0.97-2.66) |
| **Number of children aged ≤10 years** | | | | | |
| 1 | 40 | (35.52) | 58 | (41.73) | Reference |
| 2-3 | 73 | (59.35) | 72 | (51.80) | 1.47(0.87-2.46) |
| ≥ 3 | 10 | (8.13) | 9 | (6.47) | 1.61(0.60-4.32) |
| **Number of bedrooms** | | | | | |
| > 3 | 36 | (29.27) | 67 | (48.20) | Reference |
| 2-3 | 70 | (56.91) | 46 | (33.09) | 2.83 (1.63-4.90) |
| ≤ 2 | 17 | (13.82) | 26 | (18.71) | 1.21 (0.58-2.53) |
| **Family room flooring** | | | | | |
| Wood | 17 | (13.82) | 12 | (8.63) | Reference |
| Carpet | 88 | (71.54) | 117 | (84.17) | 0.53 (0.24-1.16) |
| Other and combination | 18 | (14.63) | 10 | (7.19) | 1.27 (0.43-3.70) |
| **Attend a daycare** | | | | | |
| No | 106 | (86.18) | 130 | (93.53) | Reference |
| Yes | 17 | (13.82) | 9 | (6.47) | 2.31 (1.01-5.40) |
| **Child attends school other than daycare** | | | | | |
| No | 69 | (56.10) | 72 | (51.81) | Reference |
| Yes | 54 | (43.92) | 67 | (48.20) | 0.84(0.51-1.36) |
| **Food related exposures** | | | | | |
| **Ate eggs/egg containing product** | | | | | |
| No | 44 | (40.74) | 60 | (44.44) | Reference |
| Yes | 64 | (59.26) | 75 | (55.56) | 1.16(0.69-1.94) |
| **Ate poultry** | | | | | |
| No | 13 | (11.30) | 17 | (12.50) | Reference |
| Yes | 78 | (67.83) | 95 | (69.85) | 1.07(0.49-2.34) |
| **Ate poultry at:** | | | | | |
| Home | 15 | (41.67) | 78 | (56.52) | Reference |
| Outside home at a restaurant | 3 | (8.33) | 3 | (2.17) | 2.08 (0.57-7.51) |
| Both home and outside home | 4 | (11.11) | 10 | (7.25) | 5.20 (0.95-28.26) |
| **Ate meat** | | | | | |
| No | 36 | (30.51) | 51 | (37.78) | Reference |
| Yes | 58 | (49.15) | 60 | (44.44) | 1.36 (0.78-2.39) |
| **Ate meat at:** | | | | | |
| Home | 9 | (25.00) | 46 | (33.33) | Reference |
| Outside home at a restaurant | 2 | (5.56) | 6 | (4.35) | 1.70 (0.29-9.80) |
| Both home and outside home | 4 | (11.11) | 4 | (2.90) | 5.11 (1.07-24.30) |

| Table 11 continued (univariate analysis) | Cases | | Controls | | Un-adjusted OR* (95% CI**) |
|---|---|---|---|---|---|
| | n=123 | (%) | n=139 | (%) | |
| **Drinking water source** | | | | | |
| Bottled | 25 | (20.33) | 38 | 27.34 | Reference |
| Municipal tap | 73 | (59.35) | 81 | (58.27) | 1.04(0.63-1.71) |
| Private well water | 25 | (20.33) | 20 | (14.39) | 1.51(0.79-2.89) |
| **Family Kitchen practices** | | | | | |
| **Keeps eggs refrigerated** | | | | | |
| Always | 118 | (95.93) | 136 | (97.84) | Reference |
| **Never or sometimes** | **5** | **(4.07)** | **3** | **(2.16)** | **1.92 (0.44-8.20)** |
| **Clean kitchen counters with** | | | | | |
| **Soap and disinfectant** | **45** | **(36.89)** | **70** | **(50.36)** | **Reference** |
| **Soap and water only** | **26** | **(21.31)** | **25** | **(17.99)** | **1.61(0.83-3.14)** |
| **Disinfectant only** | **51** | **(41.80)** | **44** | **(31.65)** | **1.80 (.84-3.12)** |
| **How often clean kitchen counter** | | | | | |
| Daily | 109 | (90.08) | 130 | (93.53) | Reference |
| More than once a week/once a Week/less than once a week | 12 | (9.92) | 9 | (6.47) | 1.59 (0.64-3.91) |
| **Other environmental exposure** | | | | | |
| **Handled packages of raw meat/eggs while shopping with child** | **64** | **(52.03)** | **87** | **(62.59)** | **Reference** |
| Did not go to shopping with child | 15 | (12.20) | 16 | (11.51) | 1.27(0.58-2.76) |
| Handled packages with plastic/gloves | 44 | (35.77) | 36 | (25.90) | 1.66(0.96-2.86) |
| Handled packages without plastic/gloves | | | | | |
| **Contact with a person having GI upset** | 96 | (78.05) | 123 | (88.49) | Reference |
| No | 27 | (21.95) | 16 | (11.51) | **2.16(1.10-4.24)** |
| Yes | | | | | |
| **Contact with animal** (vs. no contact) | | | | | |
| Any animal contact | 81 | (65.85) | 58 | (41.73) | **2.69 (1.62-4.45)** |
| Dogs | 53 | (43.09) | 47 | (33.81) | 1.48 (0.89-2.44) |
| Cats | 35 | (28.46) | 21 | (15.11) | **2.23 (1.21-4.10)** |
| Reptiles | 17 | (13.82) | 5 | (3.60) | **4.29 (1.53-12.02)** |
| Birds | 4 | (3.25) | 1 | (0.72) | 4.63 (0.51-42.07) |
| Hamster | 1 | (0.81) | 2 | (1.44) | 0.56 (0.05-6.26) |
| **Travel history** | | | | | |
| No | 90 | (73.17) | 106 | (76.26) | Reference |
| Yes | 31 | (25.20) | 33 | (23.74) | 1.10 (0.62-1.94) |

*Odds ratio, ** Confidence interval. All exposure data were gathered for during the 3 days of child's illness onset for cases and 3 days before the interview for controls

Table 12. Multivariate analysis of putative risk factors for *Salmonella* infections in children aged ≤ 10 years, assessed in a population-based case-control study, Michigan, 2007.

| | Adjusted OR[a] (95% CI[b]) | PAR%[c] |
|---|---|---|
| Ate eggs / egg-containing product | | |
| No | Reference | |
| Yes | 1.52 (0.73-3.15) | 22.41 (-17.43-83.61) |
| Ate poultry | | |
| No | Reference | |
| Yes | 1.57 (0.60-4.09) | 32.36 (-50.55-72.17) |
| Ate meat | | |
| No | Reference | |
| Yes | 1.14 (0.57-2.30) | 7.03 (-30.24-41.24) |
| Attended a daycare | | |
| No | Reference | |
| Yes | 4.86 (1.44-16.37) | 19.98 (2.72-49.84) |
| Attended a school | | |
| No | Reference | |
| Yes | 0.89 (0.35-2.26) | -5.60 (-45.34-37.77) |
| Contact with cats | | |
| No | Reference | |
| Yes | 2.62 (1.17-5.87) | 19.65 (2.43-42.36) |
| Contact with reptiles | | |
| No | Reference | |
| Yes | 8.16 (1.55-42.88) | 20.45 (1.93-60.06) |
| Contact with a person having symptoms of gastrointestinal infection | | |
| No | Reference | |
| Yes | 2.27 (1.02-5.44) | 12.75 (0.22-33.77) |

[a] Odds ratio, [b] Confidence interval, [C] Population attributable risk estimate ranges based on the adjusted OR and the 95% CI. All exposure data were assessed for the periods: 3 days prior to child's illness onset for cases and 3 days before the interview for controls. Model adjusted for age category and race.

**Subgroup analysis-1**

Table 13. Multivariate analysis of selected risk factors for *Salmonella* infections by age groups in children population aged ≤10 years, assessed in a population-based case-control study, Michigan, 2007.

| Exposures | Age groups | |
|---|---|---|
| | **< 1 year** AOR* (95% CI**) | **1-10 years** AOR (95% CI) |
| **Contact with a person having symptoms of GI upset** [δ] | | |
| No | Reference | Reference |
| Yes | 2.20 (0.22-21.60) | 2.06 (0.92-4.59) |
| **Contact with reptiles** | | |
| No | Reference | Reference |
| Yes | 4.33 (0.23-80.26) | **3.57 (1.04-12.25)** |
| **Contact with cats** | | |
| No | Reference | Reference |
| Yes | 0.55 (0.05-6.01) | **2.08 (1.01-4.28)** |

*Adjusted odds ratio, **Confidence interval
Model adjusted for race; All exposures data were gathered for during the 3 days prior to child's illness onset for cases and 3 days before the interview for controls

**Subgroup analyses-2**

Table 14. Assessment of selected putative risk factors for *Salmonella* infections in children aged < 1 year, assessed in a population-based case- control study, Michigan, 2007.

| Exposures (aged < 1 year) | Cases n=22 | | Controls n=25 | | Un-adjusted OR* (95% CI**) |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| **Attend a daycare** | | | | | |
| No | 17 | (77.27) | 16 | (84.00) | Reference |
| Yes | 6 | (27.27) | 4 | (16.00) | **2.31 (1.07-5.40)** |
| **Hours spent in daycare per week** | | | | | |
| ≤ 15 | 5 | (4.07) | 3 | (2.16) | Reference |
| > 15 | 12 | (9.76) | 6 | (4.32) | 1.20 (0.21-6.80) |
| **Number of children attending daycare** | 6 | (4.88) | 3 | (2.16) | Reference |
| ≤ 15 | 9 | (7.32) | 5 | (3.60) | 0.90 (0.15-5.25) |
| > 15 | | | | | |
| **Number of children in the same room as the enrolled child** | | | | | |
| ≤ 6 | 1 | (0.81) | 4 | (2.88) | Reference |
| > 6 | 16 | (13.01) | 4 | (2.88) | **15.99 (1.38-185.39)** |
| **Number of children in diapers I n same room** | | | | | |
| ≤ 1 | 9 | (7.32) | 1 | (0.72) | Reference |
| > 1 | 7 | (5.69) | 2 | (5.04) | 0.11 (0.01-1.12) |
| **Placing child on floor/carpet without blanket** | | | | | |
| Once a day | 3 | (2.44) | 1 | (0.72) | Reference |
| More than once a day/other | 10 | (8.13) | 14 | (10.07) | 0.23 (0.02-2.63) |
| **Exclusively breast fed** | | | | | |
| Yes | 7 | (5.69) | 7 | (5.04) | Reference |
| No | 19 | (15.45) | 17 | (12.23) | 1.11 (0.32-3.84) |
| **Formula fed** | | | | | |
| No | 5 | (4.07) | 2 | (1.44) | Reference |
| Yes | 20 | (16.26) | 22 | (15.83) | 0.36 (0.06-2.08) |
| **Pacifier used** | | | | | |
| **No** | **9** | **(7.32)** | **9** | **(6.47)** | **Reference** |
| **Yes** | **15** | **(12.20)** | **15** | **(10.79)** | **0.86 (0.32-2.25)** |
| **Child ate food containing eggs** | | | | | |
| **No** | **17** | **(13.93)** | **21** | **(15.11)** | **Reference** |
| **Yes** | **6** | **(4.92)** | **3** | **(2.16)** | **2.47 (0.53-11.36)** |

*Odds ratio, **Confidence interval All exposure data were gathered for during the 3 days of child's illness onset for cases and 3 days before the interview for controls.

**Subgroup analyses-3**

Table 15. Univariate analyses of selected risk factors for *Salmonella* infections in children aged 1-10 years, assessed in a population-based case- control study, Michigan, 2007.

| Exposures (aged ≥ 1 year) | Cases n=101 | | Controls n=114 | | Un-adjusted OR* (95% CI**) |
|---|---|---|---|---|---|
| | No. | (%) | No. | (%) | |
| **Consumed unpasteurized milk or cheese** | | | | | |
| No | 95 | (77.24) | 110 | (79.14) | Reference |
| Yes | 4 | (3.25) | 5 | (3.60) | 0.92 (0.24-3.55) |
| **Ate food containing eggs** | | | | | |
| Ate at home | 72 | (58.54) | 65 | (46.76) | Reference |
| Ate outside of home | 6 | (4.88) | 25 | (17.99) | 0.21 (0.08-0.56) |
| Ate both at home and outside | 3 | (2.44) | 1 | (0.72) | 2.70 (0.27-26.68) |
| **Ate poultry** | | | | | |
| Ate at home | 62 | (50.41) | 79 | (56.83) | Reference |
| Ate outside | 8 | (6.50) | 10 | (7.19) | 1.01 (0.38-2.73) |
| Ate both at home and outside | 8 | (6.50) | 3 | (2.16) | 3.39 (0.86-13.3) |
| **Ate meat other than poultry** | | | | | |
| Ate at home | 41 | (33.33) | 46 | (33.09) | Reference |
| Ate outside | 6 | (4.88) | 6 | (4.32) | 1.12 (0.33-3.75) |
| Ate both at home and outside | 5 | (4.07) | 4 | (2.88) | 1.40 (0.35-5.57) |
| **Frequency of eating at commercial food establishments** | | | | | |
| Daily to more than once a week | 6 | (4.88) | 9 | (6.47) | Reference |
| Once a week | 45 | (36.59) | 51 | (36.69) | 1.20 (0.33-4.36) |
| Never to once a month | 48 | (39.02) | 55 | (39.57) | 1.58 (0.66-3.79) |
| **Preferred food at fast food establishment** (vs. never to once a month) | | | | | |
| Hamburger | 11 | (12.79) | 22 | (19.13) | 0.65 (0.27-1.54) |
| Chicken | 36 | (41.86) | 39 | (33.91) | 1.20 (0.62-2.31) |
| Other/combination | 9 | (10.47) | 15 | (13.04) | 0.78 (0.30-2.02) |
| **Child attends school other than daycare** | | | | | |
| No | 69 | (56.10) | 72 | (51.81) | Reference |
| Yes | 54 | (43.92) | 67 | (48.20) | 0.84 (0.51-1.36) |
| **School food usually prepared by:** | | | | | |
| Family member | 8 | (8.08) | 12 | (10.53) | Reference |
| School café/cook | 14 | (14.14) | 21 | (18.42) | 1.00 (0.32-3.06) |
| Other/combination/ do not eat at school | 8 | (8.08) | 8 | (7.02) | 1.50 (0.39-5.65) |

*Odds ratio, **Confidence interval

All exposure data were gathered for during the 3 days of child's illness onset for cases and 3 days before the interview for controls.

**Subgroup analysis-4**

Table 16. Demographic characteristics of cases of *Salmonella* serotype *S.* Typhimuriun and controls in children aged ≤ 10 years, assessed in a population-based case- control study, Michigan, 2007.

| Demographic characteristics | Cases n=36 | | Controls n=139 | | |
|---|---|---|---|---|---|
| | No. | (%) | No. | (%) | p-value |
| **Age (year)** | | | | | 0.69 |
| < 1 | 6 | (16.67) | 25 | (18.12) | |
| 1-5 | 20 | (55.56) | 66 | (47.83) | |
| 6-10 | 10 | (27.78) | 47 | (34.06) | |
| **Sex** | | | | | 0.08 |
| Female | 14 | (38.89) | 76 | (55.07) | |
| Male | 22 | (61.11) | 62 | (44.93) | |
| **Race** | | | | | 0.02* |
| Caucasians | 31 | (86.11) | 91 | (66.91) | |
| Minorities ** | 5 | (13.89) | 45 | (33.09) | |
| **Parental education** | | | | | 0.15 |
| Elementary to High school | 6 | (16.67) | 33 | (24.09) | |
| Some college to college degree | 29 | (80.56) | 85 | (62.04) | |
| Post-graduate degree | 1 | (2.78) | 17 | (12.41) | |
| Refused to answer [χ] | 0 | - | 2 | (1.46) | |
| **Annual income household** | | | | | 0.13 |
| ≤ $ 35,000 | 9 | (25.00) | 25 | (18.25) | |
| $35,001- $50,000 | 7 | (19.44) | 17 | (12.41) | |
| $50,001- $75,000 | 8 | (22.22) | 39 | (28.47) | |
| >$75,000 | 11 | (30.56) | 32 | (23.36) | |
| Refused to answer [χ] | 1 | (2.78) | 24 | (17.52) | |
| **Area of residence** [δ] | | | | | 0.04* |
| High income: $>60000 | 9 | (25.00) | 35 | (25.36) | |
| Medium income: $38000 - $60000 | 10 | (27.78) | 73 | (52.90) | |
| Low income: $<38000 | 17 | (47.22) | 30 | (21.74) | |

*Significant at $p < 0.05$ (p-value obtained using a chi-square test for two proportions)
**African-Americans, Asian, Middle Eastern, Alaskan Indian and other racial groups
[χ] Parents refused to provide the answer/response
[δ] Categorized based on zip code level median household income obtained from the US Bureau of Census, 2000.

Table 17. Univariate analyses of putative risk factors for *Salmonella* serotype Typhimurium infections in children aged ≤10 years, assessed in a population-based case- control study, Michigan, 2007.

| Subgroup analysis – 4 (continued) | Cases n=36 | | Controls n=138 | | Un-adjusted OR* (95% CI**) |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| **Ate eggs/egg containing product** | | | | | |
| No | 4 | (11.11) | 21 | (15.22) | Reference |
| Yes | 2 | (5.56) | 3 | (2.17) | 3.50 (0.43-28.13) |
| **Ate poultry** | | | | | |
| Ate at home | 15 | (41.67) | 78 | (56.52) | Reference |
| Ate outside home | 3 | (8.33) | 3 | (2.17) | 2.08 (0.57-7.51) |
| Ate both home and outside home | 4 | (11.11) | 10 | (7.25) | 5.20 (0.95-28.26) |
| **Ate meat** | | | | | |
| Ate at home | 9 | (25.00) | 46 | (33.33) | Reference |
| Ate outside home | 2 | (5.56) | 6 | (4.35) | 1.70 (0.29-9.80) |
| Ate both home and outside home | 4 | (11.11) | 4 | (2.90) | **5.11 (1.07-24.30)** |
| **Frequency of eating at commercial food establishments** | | | | | |
| Never | 3 | (8.33) | 17 | (12.32) | Reference |
| Once a month | 12 | (33.33) | 37 | (26.81) | 1.83 (0.45-7.37) |
| Once a week | 13 | (36.11) | 51 | (36.96) | 1.44 (0.36-5.68) |
| Daily to more than once a week | 2 | (5.56) | 9 | (6.52) | 1.25 (0.17-8.96) |
| **Contact with animal** | | | | | |
| Any | 25 | (69.44) | 57 | (41.30) | 3.22 (1.47-7.08) |
| Dogs | 17 | (47.22) | 46 | (33.33) | 1.78 (0.85-3.76) |
| Cat | 9 | (25.00) | 21 | (15.22) | 1.85 (0.76-4.50) |
| Reptiles | 5 | (13.89) | 4 | (2.90) | **5.40 (1.37-21.29)** |
| Birds | 3 | (8.33) | 1 | (0.72) | **12.45 (1.25-123.56)** |
| **Contact with a person with GI symptoms** | | | | | |
| No | 30 | (83.33) | 122 | (88.41) | Reference |
| Yes | 6 | (16.67) | 16 | (11.59) | 1.52 (0.55-4.22) |
| **Travel outside the states** | | | | | |
| No | 26 | (72.22) | 106 | (76.81) | Reference |
| Yes | 10 | (27.78) | 32 | (23.19) | 1.27 (0.55-2.92) |

*Odds ratio, **Confidence interval. All exposures data were gathered for during the 3 days of child's illness onset for cases and 3 days before the interview for controls

Table 18. Post hoc power analysis of selected potential risk factors for *Salmonella* infections in Michigan children assessed in a population-based case-control study, 2007

| Variables | Observed exposure in controls (%) | Actual sample size | Estimated AOR[a] | Power |
|---|---|---|---|---|
| Ate eggs / egg-containing product | 55.55 | 108 | 1.52 | 29% |
| Ate poultry** | 83.92 | 91 | 1.57 | 13% |
| Ate meat** | 54.05 | 94 | 1.14 | 5% |
| Attended a daycare | 6.47 | 123 | 4.86 | 97% |
| Attended a school | 48.20 | 123 | 0.89 | 5% |
| Contact with cats | 15.10 | 123 | 2.62 | 86% |
| Contact with reptiles | 3.59 | 123 | 8.16 | 99% |
| Contact with a person having symptoms of gastrointestinal infection | 11.51 | 123 | 2.27 | 62% |

[a] Adjusted odds ratio; Not assessed in children aged < 1 year

Table 19. Comparison of controls: neighborhood matched vs. non-neighborhood matched

| | Neighborhood Controls (n=36) | Unmatched Controls (n=36) | P-value* |
|---|---|---|---|
| **Annual household income** | | | 0.82 |
| Some high school | 4 (11.11) | 3 (8.33) | |
| High school or GED | 5 (13.89) | 7 (19.44) | |
| Some college | 12 (33.33) | 8 (22.22) | |
| Four year college degree | 9 (25.00) | 11 (30.56) | |
| Graduate degree | 4 (11.11) | 2 (5.56) | |
| Post Graduate degree | 1 (2.78) | 3 (8.33) | |
| Refused to answer | 1 (2.78) | 2 (5.56) | |
| **Parental education** | | | 0.63 |
| ≤ $20,000 | 3 (8.33) | 6 (16.67) | |
| $20,000-$35,000 | 2 (5.56) | 3 (8.33) | |
| $35,001-$50,000 | 7 (19.44) | 1 (1.78) | |
| $50,001-$75,000 | 7 (19.44) | 10 (27.78) | |
| $75,001-$100,000 | 4 (11.11) | 4 (11.11) | |
| >$100,000 | 5 (13.89) | 5 (13.89) | |
| Refused to answer | 8 (22.22) | 7 (19.44) | |

*Computed using Chi-square test for two proportions

e parents (method-1)

and from the landline telephone directory (method-2)

| Variables | Controls | | P-value* |
|---|---|---|---|
| | **Method-1 (n=28)** | **Method-2 (n=111)** | |
| **Race**** | | | <0.01 |
| Caucasians | 10 (35.71) | 83 (74.77) | |
| African-Americans | 16 (57.14) | 11 (9.91) | |
| Other minorities | 2 (7.14) | 2 (15.32) | |

| | | | |
|---|---|---|---|
| **Parental education** | | | 0.04 |
| High school | 4 (14.29) | 29 (26.13) | |
| Some college | 15 (53.57) | 29 (26.13) | |
| Four year college degree | 6 (21.43) | 35 (31.53) | |
| Post Graduate degree | 3 (10.71) | 18 (16.22) | |
| **Annual household income** | | | 0.02 |
| ≤ $35,000 | 7 (25.00) | 18 (16.22) | |
| $35,001-$50,000 | 7 (25.00) | 10 (9.01) | |
| $50,001-$75,000 | 11 (39.29) | 28 (25.23) | |
| >$75,001 | 2 (7.14) | 31 (27.93) | |

*Computed using chi-square test for two proportions, **Asian, Middle Eastern, and Alaskan Indian

Table 21. Sample size calculations for Michigan *Salmonella* case-control study

| **Power** | **Exposure in control group** | **Odds Ratio** | **Number of cases required** |
|---|---|---|---|
| **80%** | **15%** | **2.5** | **124** |
| 80% | 18% | 2.5 | 111 |
| 80% | 20% | 2.5 | 105 |

Figure 1. Incidence of non-typhoidal *Salmonella* infections per 100,000 population, England and Wales, 1981-2004.

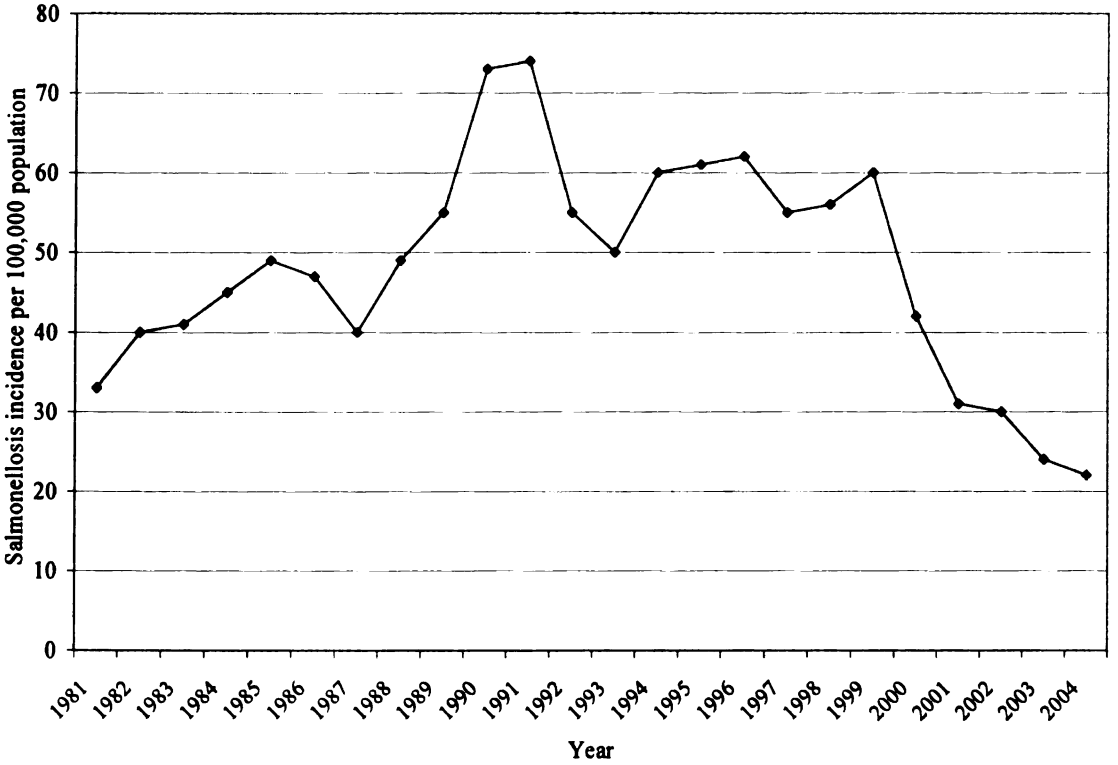


Figure 2. Incidence of non-typhoidal *Salmonella* infections per 100,000 population, US, 1944-2002.

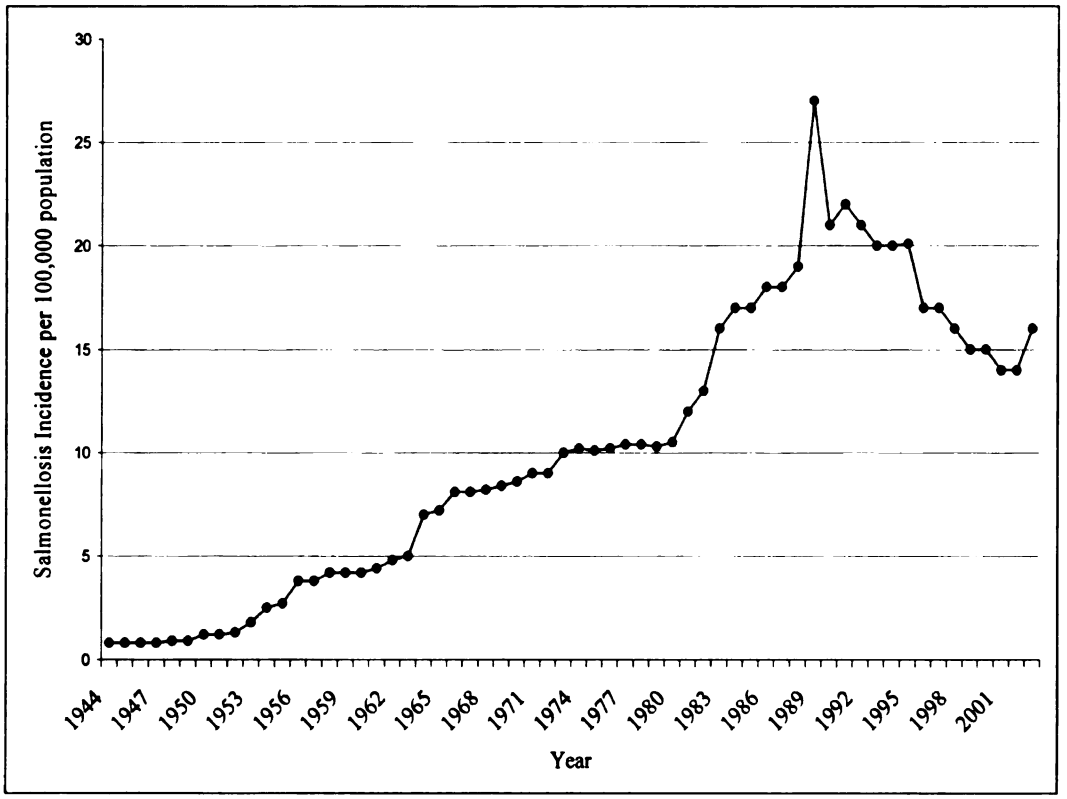


Figure 3. *Salmonella* Enteritidis infections incidence in the United States, 1970-2001

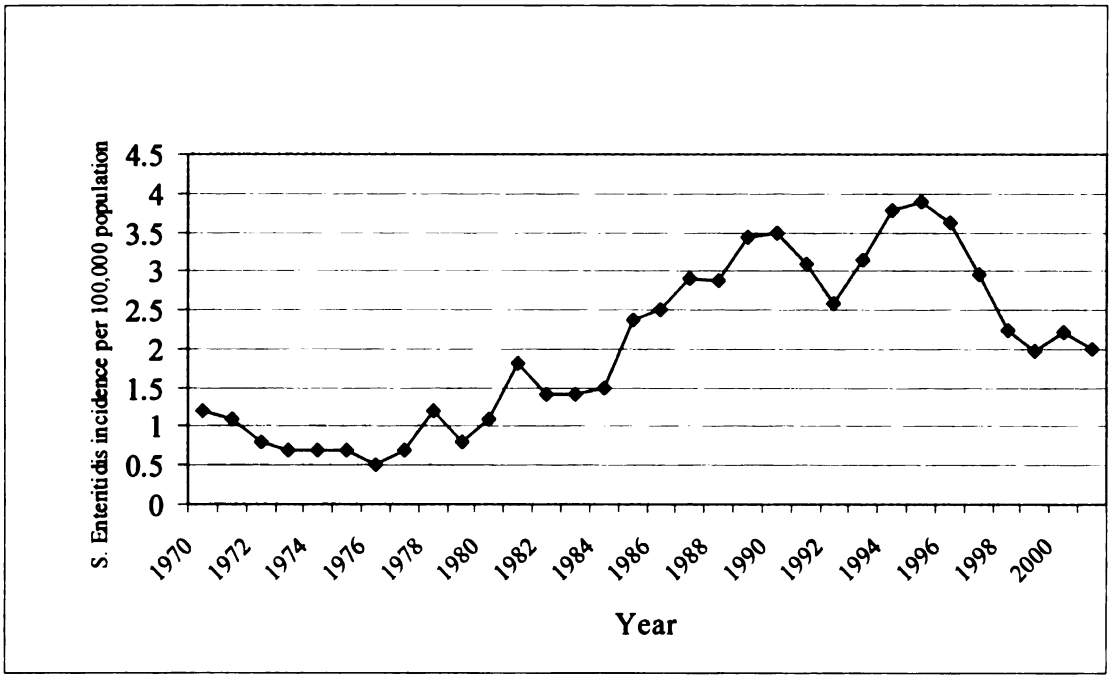


Figure 4. Age-stratified salmonellosis incidence, Michigan, 1992-2006.

(n = 13,877)

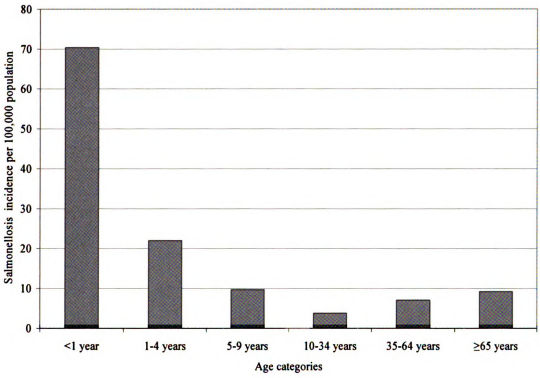


Figure 5. Surveillance of *Salmonella* infections in Michigan

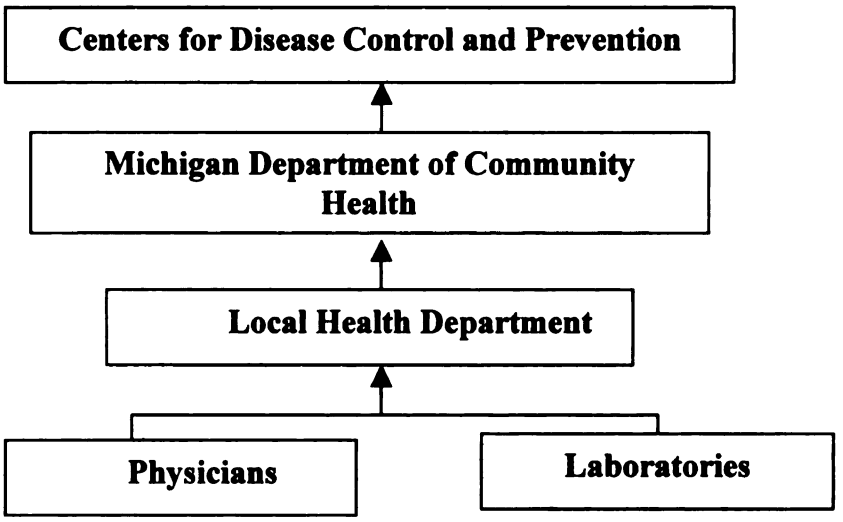


Figure 6. Michigan *Salmonella* case-control study, 2007: Enrollment of cases (12/15/06 - 10/15/2007)

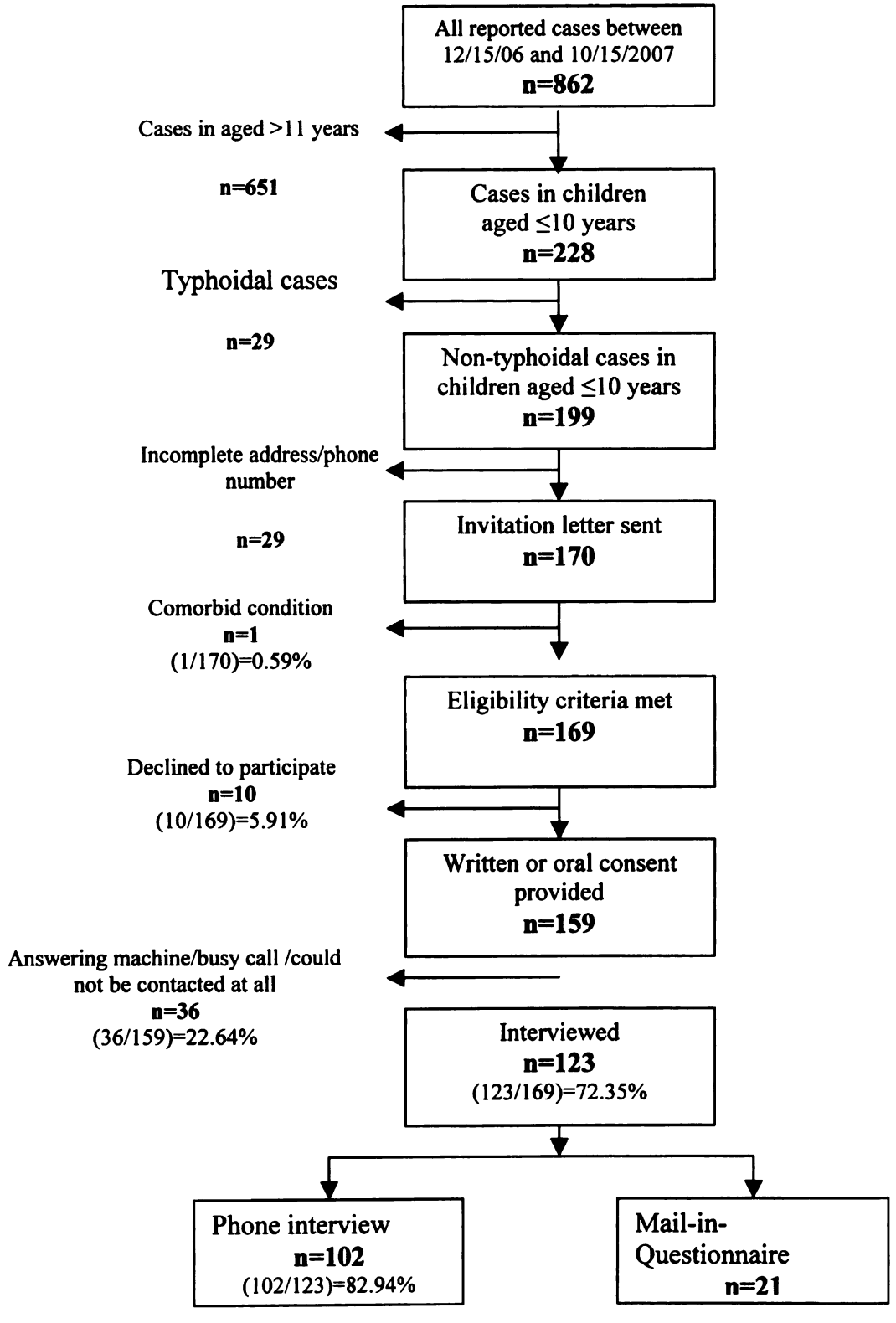
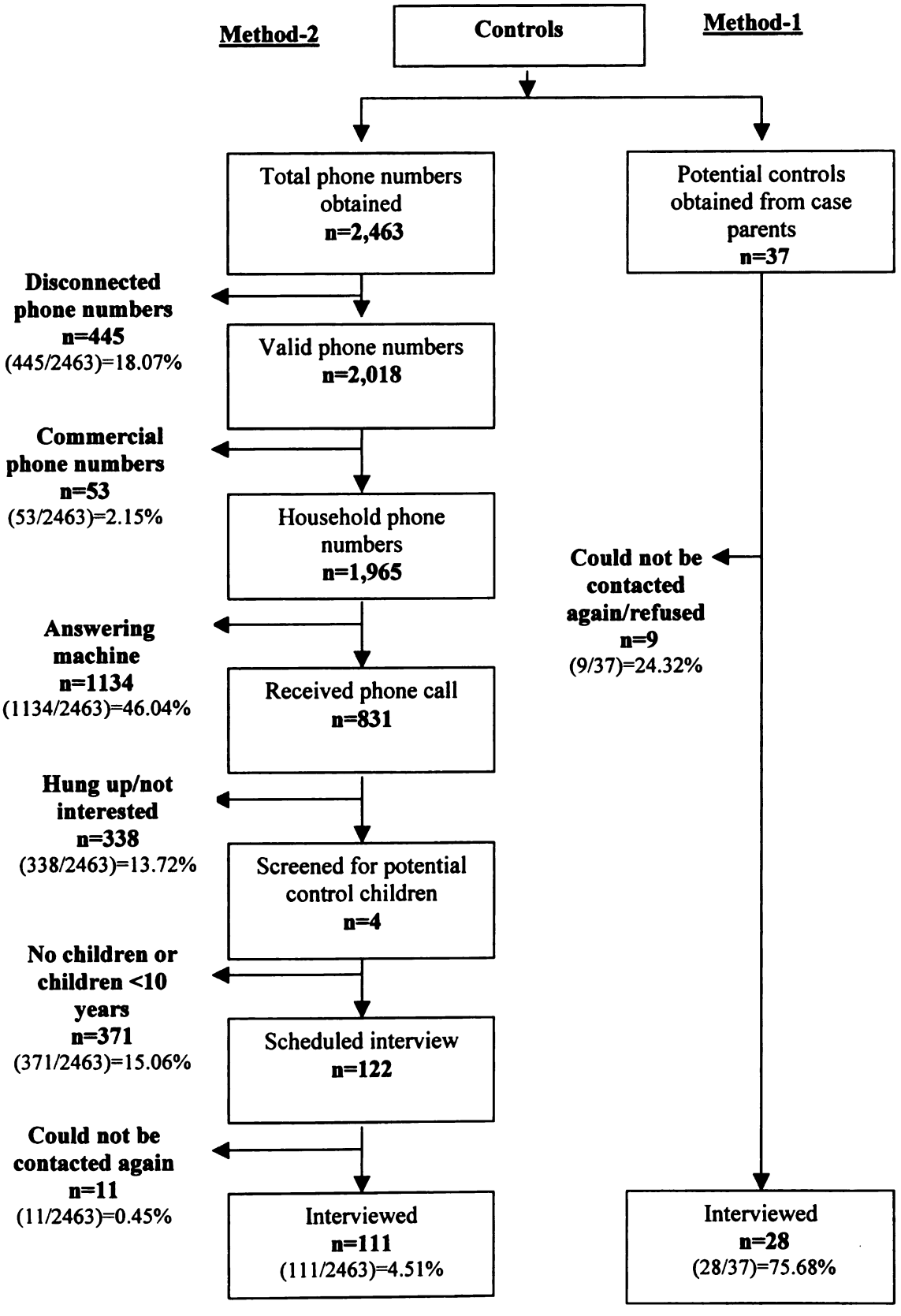


Figure 7. Michigan *Salmonella* case-control study, 2007: Enrollment of controls (12/15/06 - 10/15/2007)



# APPENDIX

**Contents:**

## CASE-CONTROL STUDY INVITATION LETTER

MICHIGAN STATE
UNIVERSITY

Date: ____/____/_____

Name: _____

Address: _____

Dear _____

I am writing to you because your child is a possible candidate for a statewide *Salmonella* Study, which is being conducted by researchers from Michigan State University (MSU) and Michigan Department of Community Health (MDCH). The main objective of this study is to identify the risk factors and conditions for *Salmonella* infections in Michigan children less than ten years of age.

We need to contact parents of children who have experienced recent *Salmonella* infection. Hospitals and Physicians are required by law to report diagnosed cases of *Salmonella* infection to Michigan Department of Community Health (MDCH). The MDCH records are being used to identify contact information of reported cases. In this research, each child's parent will be interviewed regarding food intake history, food handling and cooking practices, and household sanitation. The phone interview will take about 15-20 minutes. Alternatively, you may choose to fill out the same questionnaire that can be mailed back in a provided self-addressed, stamped envelope.

This survey is a very common way to study the causes of *Salmonella* infection. It is an important study since it will contribute to the understanding of the risk factors related to *Salmonella* infection in young children. Michigan State University and MDCH Internal Review Boards have reviewed and approved this research project according to the most recent patient rights and privacy rules.

Enclosed with this letter, you will find an information sheet that describes the study as well as a consent form to participate in this study. If you need more information on this study, you may contact professor A. Mahdi Saeed, Ph.D. at 517-432-9517.

***Although your participation is voluntary, it is very important for the success of this study. We will appreciate the return of the consent form even you elect not to participate. This confirms that you were successfully contacted. Please respond promptly.***

Sincerely,

**Dr. Mahdi Saeed**
Professor of Public Health,
College of Human Medicine,
Michigan State University

**Dr. Melinda Wilkins**
Director, Division of Communicable Diseases,
Bureau of Epidemiology,
Michigan Department of Community Health

**Please return via the included self-addressed, stamped envelope to:**

Dr. Melinda Wilkins/Michigan *Salmonella* study

Room #508

Capital View Building,

Michigan Department of Community Health

201 Townsend Street, Lansing, MI 48913

## CASE-CONTROL STUDY INFORMATION AND CONSENT FORM

Michigan State University and the Michigan Department of Community Health (MDCH) are conducting research to identify factors that increase the risk of illness from Salmonella in children.

**Importance of the study:**

*Salmonella* is a bacterium, which can cause illness of the digestive system. Among adults it is commonly caused by eating contaminated food. Our study attempts to determine the role of kitchen and household practices that may contaminate food and objects, and cause illness in younger children.

**Description of the study:**

You are being contacted either because your child's illness was reported to the health department (*Salmonella* illness is legally reportable to the health department) or just at random as part of a comparison group who were not ill. If you agree to participate you will be asked questions about your child's food intake and things like your household food handling, cooking, and cleaning/sanitation practices. The interview will take about 15-20 minutes and you do not have to answer any questions you don't want to answer.

**Risk/Benefits:**

There is no direct benefit to you for participation, but we hope it will help us learn more about this illness. The only potential risk is to your confidentiality.

**Confidentiality:**

The information from your questionnaire will be put into an electronic file that does not identify you or your child. Once that is done we will destroy study records that could identify you or your child. The confidentiality of your information will be protected to the maximum legal extent.

**Contact details:**

If you have any questions regarding the study, you may contact Dr. Mahdi Saeed at 517-432-9517.

For information about your/your child's rights as a research subject you may contact:

Peter Vasilenko, Ph.D.

Director of Human Research Protections

Michigan State University

202 Olds Hall Lansing,

East Lansing, MI 48823-1047

Phone: (517) 355-2180

Fax: (517) 432-4503

E-mail: irb@msu.edu

## CASE-CONTROL CONSENT FORM

I agree to allow a researcher to contact me to complete the study questionnaire.

**I agree** ☐ **I don't agree** ☐

If you agree:

**Please indicate if you prefer to complete the interview:**

Over telephone ☐

Myself (you will be mailed the questionnaire) ☐

If you chose telephone interview, please write:

Home telephone number: _____

Best Time of Day to Call: _____

Best Time of Week to Call: _____

Best Days of Week to Call: _____

Dates to Avoid or on which you are Unavailable over the next 60 days: _____

***Thank you in advance for your contribution in this project. We will be happy to send you a summary of the study findings upon your request.***

Sincerely,

**Please return via the enclosed self-addressed, stamped envelope to:**

Michigan *Salmonella* Study

Michigan Department of Community Health, Division of Communicable Disease

Division, Bureau of Epidemiology, Capitol View Building,

201 Townsend Street, Lansing, Michigan

**Interview starts (time)---------- Interview ends (time)----------**

## Michigan *Salmonella* Case-Control Study

**STUDY QUESTIONNAIRE**
[Telephone interview Form]

**Study ID:**_____

**Interviewer:**_____

**Date of interview:**_______________

**Study Introduction:**

Hello, my name is _____ and I work for Michigan State University (MSU).

Are you the parent or guardian of *(_____)*?

*Insert child's name*

MSU is conducting a study, in collaboration with the Michigan Department of Community Health (MDCH), to identify factors and conditions that make some children more likely than others to get *salmonellosis*, a foodborne illness. Children are also at a higher risk of getting salmonellosis compared to adults. Therefore, we are trying to study the causes of this higher infection rate. *Salmonella* infection is a reportable disease by law in Michigan. Your contact information was obtained with the permission of our collaborator, Michigan Department of Community Health.

We are very hopeful that you will be willing to participate in this project to enable us to generate very much needed information on the conditions associated with the disease in Michigan children.

Your participation is voluntary. However, we are asking for your help because the knowledge gained through this study may contribute to the control and prevention efforts of *Salmonella* infections in Michigan's children.

The type of effort needed from you, as a participant, is to complete a short questionnaire. You can answer the questions over the phone or by filling out the questionnaire mailed to you. There are no known physical and/or psychological side effects associated with these questions. The questionnaire will only take about 10 minutes for older children, and 15-20 minutes for younger ones.

All information gathered from you will remain confidential. Data will be reported in a summary form and no individually identifiable responses will be presented or published. You may decide to withdraw from the study even after the interview, and you can decline to answer any question that makes you uncomfortable. Do you have any questions?

**Are you willing to take part in this research?** _____Yes "Thank you in advance for your contribution to this project. "

_____No "Thank you for your time"

| | | | | |
|---|---|---|---|---|
| **Singed consent:** | **Yes** | ☐ | **No** | ☐ |
| **Verbal consent** | **Yes** | ☐ | **No** | ☐ |

(Please read the consent form over telephone)

**How would you like to fill out the questionnaire ____ phone or by _____mail?**

_____By mail **(Confirm the address):**_____

_____**By phone interview**

**Is now a good time to talk to you?** _____Yes "Thanks, we will now begin the questionnaire"
_____No "When can I call you back?

Day and date: _____ Time: _____

**Eligibility Criteria: To determine the eligibility of (_____) for this study, could you please tell us if (_____) has any serious medical conditions (e.g cancer: leukemia, lymphoma) or birth defects?**

_____**Yes** [We apologize, we cannot enroll (_____) as a participant in this particular study because having a serious medical condition will complicate the understanding of *Salmonella* infection risk factors.

_____**No** [please proceed with the interview]

**Below is the information that will be obtained from the MDSS* database**

## DEMOGRAPHIC INFORMATION

**1) Illness Onset date _____/_____/_____ (dates three days prior to illness onset ___/____ to ____/____)**

*For example illness onset 7/17/2006 so three days prior would be 7/14 – 7/16, use the above dates throughout questionnaire*

*For controls for this case, use the three-day period prior to interview date throughout the questionnaire*

**2) Child's name** _____

**3) Age of the child at time of illness onset** (in completed months): [1] _____
Days/Months/Years *(number of days if the child is less than a month)*

**4) Child's gender:** _____ **Male** ____ **Female**

*Michigan Disease Surveillance System maintained by the Michigan Department of Community Health

**5) Zip code of child's permanent residence** _____

**6) County of child's permanent residence** _____

**7) Child's race**

**_____Caucasian\White**
**_____African American\Black**
**_____Asian**
**_____Pacific Islander**
**_____Unknown**

**8) Child's ethnicity**

**_____Hispanic/Latino**
**_____Non-Hispanic/Latino**
**_____Unknown**
**_____Other (specify): _____**

**9) Case is reported as part of an outbreak**
*(Only for cases)*

**_____Yes _____No _____Unknown**

Information for this part of a questionnaire will be obtained from a parent/guardian (preferably the mother) of the child. Respondent must be a parent or guardian, or permission must be granted from a parent or guardian for someone else (nanny, grandparent, step parent) to provide this information.

**10) What is your relationship to (_____):**
*child's name*

**_____Mother**
**_____Father**
**_____Other (with parent or guardian's permission)**

## HOUSEHOLD INFORMATION

**First, I will ask some questions about your household during the 3 days prior to (_____) illness, from (_____to_____)**

**[For cases: All questions should refer to the 3 days preceding the illness onset date]**
**[For controls: All questions should refer to the 3 days time preceding the interview date]**

**11) How many people live in your home?** _____

**12) How many children in your home are less than 10 years of age? _____**

***13) How many children in your home are in diapers?*** **_____**

**14) How many bedrooms are in your house? _____**

**15) What kind of flooring do you have in the family room?**

**_____Carpet**
**_____ Wood**
**_____Tile/linoleum**
**_____Does not have a family room**
**_____Other (specify):_____ indicate rugs here**

**16) What kind of flooring is in (_____) bed room?**
*child's name*

**_____Carpet**
**_____ Wood**
**_____Tile/linoleum**
**_____Other (specify):_____indicate rugs here**

**CHILD CARE**

| |
|---|
| Now, I will ask you about (_____) childcare during the 3 days prior to (his/her) illness, from (_____to_____). (insert same date's as above) |

**17) Does (_____) attend a day care outside of your home?** ____Yes
_____No *(If No, go to Q#18)*

**17a) How many hours per week does (*he/she*) usually spend in day care?**
_____ hours/wk

**17b) How many total children attend (_____) daycare?** _____ children
*Child's name*

**17c) About how many children share the same room as (_____)?**

_____ children *Child's name*

**17d) About how many children in your child's room are in diapers? _____children**

**17e) About how many day care workers attend to this room?** _____ workers

**17f) Is there a separate room for changing diapers in the day care?**

**17g) Is there a sink with soap and water next to the diaper-changing area in the day care?**
__Yes __No _Don't know

**17h) In the day care, approximately how far in feet is the diaper-changing area from the area where food, milk, and other beverages are handled?** _____ft

**17i) Are you aware of any child at the daycare who experienced vomiting, diarrhea, or abdominal cramps during the 3 days prior to (_____) illness?**
____Yes _____No _____Don't know *(If No or Don't know, go to Q #17k)*

**17j) How many children had nausea, vomiting, diarrhea or abdominal cramps during the 3 days prior to (_____) illness?** _____children
*Child's name*

**17k) Who usually prepares the food *(child's name)* eats while at the daycare?**
*(Mark all that apply)*
**_____Mother**
**_____Father**
**_____Other family member**
**_____Daycare personnel**
**_____Other (specify):_____**

**18) Does (_____) attend a preschool, kindergarten, or elementary school?**
_____Yes _____No
*(If No, go to Q #19)*

*Can be in addition to daycare – such as before or after school care programs.*

**18a) Who prepares the food that (_____) eats while at school?**
***(Check all that apply)***
**_____Mother**
**_____Father**
**_____Other family member**
**_____Cafeteria/cook**
**_____Other (specify): _____**

**19) Did you take (_____) with you while grocery shopping during the 3 days prior to (his/her) illness?**

_____Yes _____No ____Don't know *(If No or don't know, go to Q #20)*

**19a) Did you use gloves or plastic bags when handling packages of raw chicken, meat, and egg products while grocery shopping that time?**

____Yes ____No ____ Don't know __ Did not handle meat or egg products

**This part of the questionnaire asks you about (child's name) food history and activities (skip if over age 1 year)**

**20) Did you put (_____) on the floor or carpet without a blanket during the 3 days prior to (_____) illness?**

_____Yes _____No *(If No, go to Q #21)*

**20a) About how often was (_____) placed on (or played on) the floor or carpet without a blanket in the 3 days prior to his/her illness?**

**_____Never**
**_____Once a day**
**_____More than once a day**
**_____Other (specify):_____**

**21) Was (_____) breast-fed during the 3 days prior to (his/her) illness?**
_____Yes _____No _____Don't know

**22.) Did you use formula to feed (_____) during the 3 days prior to** (his/her) **illness?**
____Yes _____No ___Don't know *(If No or Don't know, go to Q# 23)*

**22a) What type (e.g., milk, soy, rice-based) and brand of formula did you feed (*child's name*) during the 3 days prior to (*his/her)* illness?**

_____

Please record exact brand and type if known.
If not known, use list below to prompt recall.
*(Check all that apply)*

**_____Isomil**
**____ Enfamil**
**_____Bright Beginnings**
**_____Nestle**
**_____Similac**
**_____Store brand (e.g. Meijers, Krogers etc)**
**____ Other (specify): _____**

**23) Did (_____) use a pacifier during the 3 days prior to (*his/her*) illness?**
____Yes _____No ___Don't know

**24) Did (_____) *eat egg during the 3* days prior to (*his/her*) illness?**
____Yes (*If yes, how it was prepared?* ____ *fully cooked* ____ *partially cooked*)

_____ No _____Don't know

**25) Did (_____) eat any food that contained eggs during the 3 days prior to (*his/her*) illness?**

____Yes _____No ___Don't know

**Food History**
*(Skip if less than 1 year of age and go to*

**26) Did (_____) eat or drink any unpasteurized milk, or cheeses such as queso fresco made with unpasteurized milk during the three days before your illness?**

____Yes _____ Probably yes ____ Probably not _____No _____Don't know

**26a) Did (_____) *eat egg during the 3* days prior to (*his/her*) illness?**
*child's name*
_____Yes *(If yes, how it was prepared? ____fully cooked ____half cooked ?)*

___No ___ Don't know

**26b) Did *(_____)* eat any food that contained eggs (such as: cookie dough, salad dressings, mayonnaise, ice cream, custard, cake mix) during the 3 days prior to *(his/her)* illness?**

Yes _____ if yes, prepared at home: Yes _____ No _____

No_____ Don't know _____

**26c) Did *(_____)* eat any food that contained poultry (such as chicken, or turkey) during the 3 days prior to *(his/her)* illness?**

Yes _____ if yes, prepared at home: Yes _____ No _____

No_____ Don't know _____

**26d) Did *(_____)* eat any food that contained meat other than poultry (such as hamburger) during the 3 days prior to *(his/her)* illness?**

Yes _____ if yes, prepared at home: Yes ____ No ____

No_____ Don't know _____

**26e) In the three days before (_____) illness, did he/she eat at any of the following types of commercial food establishment?** *(mark all that apply)*

____Restaurant

____Fast-food establishment

____Cafeteria

____Deli

____Read-to-eat food served in a supermarket or department store

____Street-vended food

____Concession stand at sporting event

____Snack bar

____Gas station

If don't remember then ask Q26 f and g

____Other (specify)

**26f) How often does (_____) eat at fast food restaurants?**
*child's name*

_____Daily

_____More than once a week

_____Once a week

_____Once a month

_____Never

*Other(Specify):*_____

**26g) What is (_____) preferred food at fast food places?**
*child's name*

_____Hamburger

_____Chicken

_____Other (specify

Question about source(s) of drinking water

**27) Now I am going to ask about the types of water sources (*child's name*) drank during the 3 days prior to (*his/her*) illness? Did *(child's name)* drink water from:**

| | | | |
|---|---|---|---|
| Municipal tap water | _____Yes | _____No | _____Don't know |
| Private well water | _____Yes | _____No | _____Don't know |
| Untreated surface water (river, pond, lake) | _____Yes | _____No | _____Don't know |
| Bottled water | _____Yes | _____No | _____Don't know |

Other:_____

## INTRAFAMILIAL TRANSMISSION OF *SALMONELLA*

**This part of the questionnaire asks you about your family's possible exposure to *Salmonella* during the 3 days prior to illness**

**28) Was anyone in your household ill with symptoms of stomach upset, which may include nausea, vomiting, diarrhea, and abdominal cramps during the 3 days prior to (_____) illness?**
____Yes _____No ____Don't know *(If No or Don't know, go to Q# 29)*

**28a) Did (he or she) seek medical care for these symptoms?**
____Yes _____No ____Don't know *(If No or Don't know, go to Q29)*

**28b) What was the diagnosis?** _____diagnosis or ____Don't know

**29) During the 3 days prior to (_____) illness, did *(he/she)* visit any friends or relatives who had symptoms of stomach upset, which may include nausea, vomiting, diarrhea, and abdominal cramps?**
____Yes _____No ____Don't know

**30) During the 3 days prior to (_____) illness, did anyone who had symptoms of stomach-upset visit your home?**

____Yes _____No ____Don't know

## FAMILY KITCHEN PRACTICES

**This part of the questionnaire asks you about your family's kitchen practices**

**31) Do you keep your eggs in a refrigerator?**
_____Never _____Sometimes _____Always

**32) Do you wash your kitchen counters, sinks, and cutting boards after preparing raw chicken?**
_____Never _____Sometimes _____Always *(If never go to Q#35)*

**33) How do you clean your kitchen counters?**

_____with soap and water _____with a disinfectant

**34) How often do you clean your kitchen counters?**

_____Less than once a week
_____Once a week
_____More than once a week
_____Daily

**ANIMAL EXPOSURE**

**This section of the questionnaire asks you about pets**

**35) During the 3 days prior to (_____) illness, did (he/she) have contact with any type of pet, your pet or someone else's pet or animals in a petting zoo setting?**
____Yes _____No ____Don't know *(If No or Don't know, go to Q# 36)*

**35a) What kind of pet(s) did (_____) have contact with during the 3 days prior to (his/her) illness?**
(Check all that apply, get as much detail as possible)

_____Dogs *(if yes, how many?)* ____# *Dog(s) age(s)* ____*weeks, months, adult*
_____Cat *(if yes, how many?)* ____# *Cat(s) age(s)* ____*weeks, months, adult*
_____Reptiles *(if yes, how many)* ____# *describe* _____ *(iguana, cornsnake etc)*
_____Birds *(if yes, how many)* ___# *describe* _____
*(chicken, duckling, parakeet etc.)*
_____Hamster
_____Gerbil
_____Ferret
_____Other (specify):
_____

**35b) Were any of these animals noticeably ill with diarrhea?**
____Yes _____No ____Don't know

**TRAVEL HISTORY**

**This section of the questionnaire asks you about your child's travel history**

**36) Did (_____) travel anywhere during the 3 days prior to (his/her) illness?**

____Yes _____No ____Don't Know *(If No or Don't know, go to Q #37)*

**36a) Did (_____) meet any person with symptoms of stomach upset during your visit?**

____Yes _____No ____Don't know

**SOCIOECONOMIC HISTORY**

**Just a couple more questions about your income and education, you don't need to answer if you are uncomfortable**

**37) What is the highest level of education you have completed?**

_____Some High School
_____High School or GED
_____Some college or technical training
_____4 year college degree
_____Graduate degree
_____Post graduate degree

**37a) What is your total annual household income?**

_____ less than $20,000
_____$20,000 - $35,000
_____$35,001 - $50,000
_____$50,001 - $75,000
_____$75,001 - $100,000
_____more than $100,000
_____Refused to answer

"That's it! Thank you so much for your time, we really appreciate that you have shared this important information with us as we try to research this important childhood disease If you have any questions related to the study you may contact Dr. Mahdi Saeed, the principal investigator of this research, at 517-432-9517."

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