Epidemiology and Molecular Identification of Salmonella Infections in Children

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Objective: To explore the role of foods and the environment in the development of infections with Salmonella in infants and children.

Design: Case-controlled survey and the use of pulsed-field gel electrophoresis to establish DNA fingerprint patterns.

Setting: Ambulatory and hospitalized patients at a children’s hospital.

Patients or Other Participants: A consecutive sample of children younger than 4 years old who were infected with Salmonella and 3 age-matched controls per patient were to be surveyed. Of the 103 eligible cases of salmonellosis, 90 cases and 264 controls were included in the study.

Data Analysis: Univariate analysis was done using the Mantel-Haenszel χ² test or the Fisher exact test. The Bonferroni correction was used for multiple comparisons. DNA fingerprints were inspected for identical banding.

Results: Results demonstrated similar diets between cases and controls with the exception of more potato or macaroni salad or coleslaw consumption in the control group (P < .001). DNA fingerprints of Salmonella newport and Salmonella typhimurium demonstrated that all cases were due to unique isolates except in 5 instances involving 12 patients. Seven of these patients could be connected geographically.

Conclusions: Most of the cases of salmonellosis in children younger than 4 years are of a sporadic nature and the major source of infection remains unidentified. For patients infected with identical isolates of Salmonella, a common food source could not be incriminated with the methods used. Environmental contamination or other sources of Salmonella are suggested by these epidemiological data.


ACH YEAR in the United States more than 40,000 cases of human salmonellosis are reported to the Centers for Disease Control and Prevention with almost one third of these cases involving children younger than 5 years.1 Previous data from Arkansas have demonstrated that the case rates for these infants and children exceed those of the rest of the nation and that most of these cases are sporadic in nature without an identifiable source of infection.2 Physicians still tend to emphasize contaminated food or food prepared by infected individuals as the major source of infection for these young patients based on what is understood with adults and salmonellosis. Recent data, however, have demonstrated that conclusions drawn from the investigations of food poisoning outbreaks must be of questionable value in explaining the epidemiology of salmonellosis for infants and young children.3 Investigators have suggested that since the Salmonella bacterium is so common in our environment, virtually all infants and young children are exposed to contamination on a daily basis. It is postulated that this daily contamination from the environment in conjunction with factors that affect host resistance may be the most important determinants of infection. The purpose of this study, therefore, was to explore the role of foods and the environment in the development of infections with Salmonella in infants and children.
METHODS

Investigators were informed when a child younger than 4 years had Salmonella isolated from any specimen submitted to the clinical microbiology laboratory from the outpatient clinics or emergency department or the inpatient service at Arkansas Children’s Hospital, Little Rock, from July 1993 through June 1995. The decision to submit specimens for bacterial cultures was at the discretion of the treating physician. Contact by telephone or in person with a parent or guardian of the child was made within 48 hours after notification of the positive culture findings. After informed consent was obtained, demographic data were collected and a questionnaire was administered concerning parental education and occupation, government assistance (eg, Women-Infants-Children Program participation), medical history of the patient having had salmonellosis, involvement of others in the family with a similar illness, preparation and consumption of foods considered to be high risk, pet ownership and type of pet (reptile vs mammal), smoking in the household, day care attendance, and family income. When applicable, questions concerning formula preparation and/or consumption and breast-feeding were also interviewed on the same day the case was identified as a control group after informed consent was obtained. The first 3 patients with a matching age who had scheduled appointments to the clinic on the day the case was interviewed were chosen as controls. If they refused, the next available patient meeting entry criteria was interviewed. All interviews of cases and controls were conducted by a single, trained interviewer (E.L.F.).

Salmonella isolates were serogrouped in the clinical microbiology laboratory according to the Kaufman-White scheme and sent to the Arkansas Department of Health for serotyping. The 2 most common serotypes underwent further investigation through the use of DNA restriction fragment analysis using pulsed-field gel electrophoresis (PFGE) to identify identical isolates among like serotypes. DNA fragment patterns were visually assessed and each distinct pattern was assigned a pattern number. Since the isolates being compared were of the same serotype, they were considered to be genetically different if there was greater than 1 band variation in PFGE patterns. Epidemiological information obtained from the family of patients infected with identical Salmonella serotypes was reviewed extensively looking for a connection that might indicate a common source of infection.

STATISTICAL ANALYSIS

Data were collected using a questionnaire constructed to work efficiently with Epis-Info (Version 5.0B). All data were double-entered and verified. Univariate analyses were conducted using the Mantel-Haenszel χ² test or the Fisher exact test, whichever was appropriate based on expected cell size. The Bonferroni correction was used for the evaluation of multiple comparisons. In 8, 3 were already included in the study, and 2 refused to participate. Seventy percent of the cases occurred between April and September. The case and control groups did not differ in relation to age, race, sex, or history of chronic diseases (Table 1). During the week prior to

DNA PREPARATION, RESTRICTION ENDONUCLEASE DIGESTION, AND PFGE

DNA Preparation

Salmonella organisms were grown overnight at 37°C in nutrient broth and were sedimented by centrifugation and washed in 100-mmol/L Tris hydrochloride (pH 7.5), 100-mmol/L EDTA, and 150-mmol/L sodium chloride. The pellet was mixed with molten 2% low-melting point agarose gel (In Cert Agarose, FMC BioProducts, Rockland, Me) and the suspension was added to plug molds (Bio-Rad, Hercules, Calif). The agarose gel blocks were incubated in solution containing 6-mmol/L Tris hydrochloride (pH 7.5), 1-mol/L sodium chloride, 100-mmol/L EDTA, 0.5% polyoxyethylene 20 cetyl ether (Brij 58, Sigma, St Louis, Mo), 0.2% sodium deoxycholate, 0.5% Sarkosyl, and 1-mg/mL lysosome for 15 hours at 37°C, followed by deproteinization in 0.4-mol/L EDTA (pH 9.3), 1% Sarkosyl, and 1-mg/mL protease K at 50°C for 24 hours. The cell debris and protease K were removed by multiple washes in CHEF-TE (clamped homogeneous electric field) with 0.1-mol/L Tris and 0.1-mol/L EDTA buffer—100-mmol/L Tris hydrochloride (pH 7.5)—and 100-mmol/L EDTA. DNA plugs were stored at 4°C in CHEF-TE buffer until digestion with a restriction enzyme.

Restriction Enzyme Digestion

Genomic DNA was restricted with 1 Xba I (5'-TCTAGA-3'), and Spe I (5'-ACTAGT-3'). Isolates with similar fingerprint patterns with Xba I and Spe I underwent subsequent restriction with Nhe I (5'-GCTAGC-3'), Avr II (5'-CCTAGG-3'), Asel (5'-ATTAAT-3'), or NotI (5'-GGGGCGC-3') (New England Bio Labs, Beverly, Mass, and Fromega Co, Madison, Wis). The agarose gel blocks were washed for 3 hours at 4°C in TM buffer—100-mmol/L Tris hydrochloride (pH 8) and 5-mmol/L magnesium chloride—followed by equilibration in the appropriate restriction enzyme buffer for 1 hour at 4°C. Fresh buffer containing 5 to 25 units of the chosen enzyme was added to the plugs and they were incubated for 16 hours at 37°C. The enzyme reaction was terminated by adding EDTA (pH 8) to a final 1-mol/L concentration.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis of the digested samples was performed by contour-clamped homogeneous electric field on a CHEF-DR II system (Bio-Rad Laboratories, Richmond, Calif) in 1% agarose gels in 0.3 X TBE buffer—0.5-mol/L Tris, 0.1-mol/L boric acid, 0.2-mmol/L EDTA (pH 8) for 14 or 24 hours at 200 V and 14°C. The ramped-pulse times varied according to the enzyme, ranging from 1 to 60 seconds. The DNA size standards used were a bacteriophage lambda ladder consisting of concatemers starting at 48.5 kilobase pairs (Bio-Rad Laboratories, Richmond, Calif) as well as a midrange II marker with the smallest band at 24 kilobase pairs (New England Bio Labs). The gels were stained with ethidium bromide and photographed on a UV transilluminator.

RESULTS

During the study period, 103 cases of human salmonellosis in eligible patients were identified. Thirteen cases were excluded because contact could not be established
the development of the patients’ illness there was no difference in the type of medications or the number of illnesses in other family members for cases and controls regardless of age. Specifically, 17% of cases and 14% of controls described another member of the family with diarrhea. For infants younger than 3 months, 29% of cases and 22% of controls (P > .2) had at least 1 other family member with diarrhea. There was also no difference between the groups in regards to pet ownership, smoking in the household, day care attendance, parental education, employment in the poultry industry, and household income of less than $10,000. The control patients, however, were more likely to be supported by the Women-Infants-Children Program (73% vs 60%; P < .02) and Medicaid (63% vs 43%; P < .01) than the cases.

Review of the food data demonstrated that the only difference in food consumption noted was that the controls were more likely to have eaten potato or macaroni salad or coleslaw (Table 2). There were also no differences noted between cases and controls for infants still consuming formula (Table 3). Among those patients who did not boil their water prior to mixing the infant’s formula, the source of the water was similar for cases and controls (mean age, 13 months). Specifically, no notable environmental or food exposures were identified when the data were evaluated based on the organism isolated.

Pulsed-field gel electrophoresis was performed on 29 S. newport and 22 S. typhimurium isolates (Figure 1). Restriction endonuclease analysis patterns of S. newport and S. typhimurium generated by PFGE after XbaI and SpeI digestion were 12 and 9, respectively, for the S. newport isolates and 20 and 18, respectively, for the S. typhimurium isolates. Combining XbaI and SpeI patterns for S. newport demonstrated 23 distinct combinations of patterns. Nineteen of these combinations represented single patients, while 4 of the identical patterns involved 10 patients. Restriction endonuclease patterns generated with NheI, AvrI, NotI, or Asel were not helpful in further dif-
ferentiating the isolates. Combining the results of both enzymes for \textit{S. typhimurium} demonstrated 21 distinct combinations, each represented by a single patient except for 1 common fingerprint pattern that was noted among 2 patients. Restriction endonuclease patterns generated with \textit{NheI} and \textit{NotI} were not helpful in the further differentiation of \textit{S. typhimurium} isolates.

The demographic data of patients infected with isolates that had similar DNA fingerprint patterns were further reviewed (Figure 2). The average age of the children involved in these clusters was 15.5 months, which was not different from the other 78 cases (13.8 months). All patients except for 2 (1 with \textit{S. newport}, 1 with \textit{S. typhimurium} identified) used city water. None of the children involved had similar diet histories. Of the 4 common clusters involving 10 patients with \textit{S. newport}, 2 clusters involved 5 patients who lived in or adjacent to the same city as another patient with the same isolate although as much as 3 months may have separated the illnesses. In the 1 pair of identical DNA fingerprint patterns involving \textit{S. typhimurium}, the 2 patients were infected 7 months apart and lived in separate cities and counties. The second child, however, had spent the day in the home town of the first child within 72 hours of the beginning of their illnesses. No other common source could be identified.

\textbf{COMMENT}

Human epidemics of salmonellosis are commonly attributed to contaminated foods, but the role of foods in the development of sporadic disease in infants and children is not understood. Data generated in this study in infants and young children would suggest that the dietary habits prior to the development of infection between cases and controls were remarkably similar. Recent data have suggested that contaminated eggs remain important vehicles for the transmission of \textit{Salmonella} in adults even in the absence of recognized outbreaks. Such data for young children are not supported by the current study because of the lack of egg consumption by our patients, the lack of infections due to \textit{Salmonella enteritidis}, and the fact that Arkansas has not had a problem with egg contamination.

Risk factors for infants who do not consume table foods have identified the use of iron-fortified formulas and the lack of breast-feeding as major risk factors for the development of disease. Although questions about the iron content of formulas were not added until the second year of the survey, no differences were noted between groups in the use of such formulas. The numbers of infants breast-feeding or using any breast milk in their diet in this study were too few to demonstrate the protective effect that has been previously described in our

\begin{table}[h]
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\begin{tabular}{|l|l|l|}
\hline
\textbf{Cases} & \textbf{Controls} \\
(\textit{n} = 46) & (\textit{n} = 128) \\
\hline
Breast milk & 3 (7) & 9 (7) \\
Supplement breast-feeding with formula & 2 (4) & 4 (3) \\
Pump and save breast milk & 2 (4) & 2 (2) \\
Table food & 10 (22) & 38 (30) \\
Vegetable jar food & 18 (39) & 49 (38) \\
Egg yolk jar food & 0 (0) & 4 (3) \\
Fruit jar food & 20 (43) & 60 (47) \\
Rice cereal & 24 (52) & 68 (53) \\
Ready-to-feed formula & 13 (28) & 22 (17) \\
Iron-fortified formula$\dagger$ & 15 (79) & 48 (94) \\
Powdered milk & 10 (22) & 22 (17) \\
Used concentrated formula & 26 (57) & 89 (70) \\
Boil water before mixing formula & 13 (28) & 59 (46) \\
Plastic bottle with refillable bags & 11 (24) & 30 (23) \\
Glass or plastic bottles that require washing & 44 (96) & 112 (88) \\
\hline
\end{tabular}
\caption{Eating Habits of Infants Still Consuming Formula$^*$}
\end{table}

$^*$\textit{P} values were adjusted for multiple comparisons. The critical value of statistical significance was 0.05/15 = .0033. Values are expressed as number (percentage).

$\dagger$Data based on 19 cases and 51 controls.
OTHER RECOGNIZED risk factors for the development of salmonellosis such as chronic diseases or lower socioeconomic class were not found to predominate in our cases as compared with our controls. The socioeconomic status of our patients and controls was similar. The fact that more control children were supported by Medicaid and the Women-Infants-Children Program was probably because of the fact that the controls were patients who received their routine medical care in our general pediatric clinic where an emphasis on enrolling eligible patients into these programs exists. When evaluating our patients and controls by absolute income there was no difference noted between the 2 groups. Specifically, there was no difference between the 2 groups regarding yearly incomes of less than $10,000. An attempt to identify environmental or food risk factors based on the organisms isolated also was unsuccessful. *Salmonella newport* and *S typhimurium* have been demonstrated in previous investigations to be the 2 most common isolates in Arkansas. The discovery that *S typhimurium* was more commonly found to infect the older child is of interest, but at present, its meaning is unclear.

Analysis of chromosomal DNA restriction patterns by PFGE was chosen for our study since it is widely available and has been proven to demonstrate genetic variations among like serotypes of *Salmonella*. In the current study, enough diversity was observed among 44 (86%) of the fingerprint patterns that it appears the use of *XbaI* and *SpeI* are useful epidemiological tools for *S newport* and *S typhimurium*. With the use of these restriction enzymes, the 2 most common serotypes were found to have 44 different fingerprint combinations. This would indicate that most of the isolates were not clonally related, which is consistent with the conclusion that most of the cases are sporadic in nature. Further evaluation of isolates with identical patterns using other enzymes was not found to aid in differentiating the serotypes in the 5 clusters of patients with similar patterns. Because of the diversity of the DNA fingerprint patterns obtained with the use of these restriction enzymes, the appearance of identical patterns suggests either the presence of a common contaminating source of infection or that there is limited sequence divergence in isolates of these particular serotypes.

Our epidemiological data, however, support the former. Seven of the children had a history of living in or traveling to the same town as others with identical isolates. Because these infants and children had different diet histories, the source of their infection was more likely from their immediate environment and not directly from contaminated foods. Past studies have demonstrated that cultures taken from the households of infected infants and children have demonstrated that the dirt from around the home, the local playground, and samples from vacuum cleaners or broom straws from the home have been found to harbor *Salmonella*. Since infants and children are very inquisitive, are orally fixated, and are constantly exploring their environment, this type of contamination would be of great importance but would not explain how patients could be infected with identical organisms separated by long distances and time.

An infected person contaminating the child’s environment, however, could explain the issues with time and distance. The median duration of excretion of *Salmonella* from all infected individuals is approximately 5 weeks and although 90% of adults stop excreting organisms after 9 weeks and only 1% are excreting at 1 year, 60% of children younger than 5 years are excreting after 20 weeks and approximately 5% still excrete the organism at 1 year after the development of their illness. In previous bacteriologic investigations of household contacts of infants and young children with salmonellosis, it has been noted that approximately one third of household contacts are shedding *Salmonella* with approximately 50% being asymptomatic. Infants and young children are more likely to have extended or more intimate periods of handling by their caretakers than older children, thus increasing their risk of exposure to infection if the caretaker is excreting *Salmonella*. Our data, however, demonstrated that an equal number of patient and control families had other individuals in the home with diarrhea. This was also true even among the patients younger than 3 months, an age that has been associated with intrafamilial spread. Unfortunately, we had no method for detecting asymptomatic infections among family members; the importance of such an individual cannot be overlooked.

In conclusion, this study demonstrates the complexities of identifying the source of human salmonellosis in infants and children. For patients infected with identical isolates of *Salmonella*, a common contaminating food source was not identified with the methods used but environmental contamination or other sources of *Salmonella* might account for these infections. For patients with unique isolates, a major source of infection remains unidentified. Further evaluations of these patients to include bacterial evaluations of their homes, contacts, and foods would be required to adequately support these findings. Based on these data, however, when clinicians encounter patients with human salmonellosis who are younger than 4 years, time spent questioning the family about the environment surrounding the patient rather than a dietary history may deliver more useful information.
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REFERENCES


Announcement

Free Patient Record Forms Available

Patient record forms are available free of charge to ARCHIVES readers by calling or writing FORMEDIC, 12D Worlds Fair Dr, Somerset, NJ 08873-9863, telephone (908) 469-7031.