Infant Diet and Salmonellosis

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ABSTRACT

Background: The Island of Guam has experienced a high incidence of infant salmonellosis for a number of years (age-specific incidence rate of approximately 3700 cases per 100 000 infants in 1984). Interviews of case parents suggested that the use of high-iron infant formula was associated with this problem.

Methods: A case-control study was conducted to test this hypothesis. Information on feeding practices and a variety of medical and socioeconomic factors was collected for 78 laboratory-confirmed *Salmonella*-case infants and 167 control infants.

Results: Case infants were less likely to have been breast-fed (odds ratio [OR] = 9.15; 95% confidence interval [CI] = 2.71–30.9) and more likely to have been fed infant formula with an iron content of 10 mg/L or greater (OR=2.96; 95% CI = 1.24–7.08) than were control infants.

Conclusions: Although the precise means by which infants are most commonly exposed to *Salmonella* bacteria remain obscure, breast-feeding apparently protects against the development of physician-diagnosed clinical salmonellosis, while the feeding of high-iron infant formula has the opposite effect. (*Am J Public Health.* 1991;81:997–1000)

Introduction

The incidence of nontyphoid salmonellosis on the island of Guam increased from 78.6 cases per 100 000 population in 1975 to a peak of 218 cases per 100 000 in 1984. The incidence has declined to 110 per 100 000 in 1989, still more than 5 times the US rate for the same year.[1] Infants typically account for about 50% of *Salmonella* cases on Guam. Another unusual characteristic of the epidemic is the presence of *S. waycross,* a serotype that is rare elsewhere in the world but accounts for about 30% of all human *Salmonella* isolates on Guam. In 1984, only 3 of 35 862 cases of salmonellosis reported in the United States were due to *S. waycross;*[2] all three cases were residents of Guam who were referred to the state of Hawaii for medical treatment (A. P. Liang, personal communication, 1987).

Several investigations have failed to link infant cases of this epidemic to the consumption of foods with an animal origin (including milk), to secondary spread from infected family members, or to the ownership of pets or domestic animals.[3–5] Infant salmonellosis cases occur with approximately equal frequency in both civilian and military communities as well as in families of both higher and lower economic status.[3] Investigations of infant day care and formula preparation practices have not provided any clues to the origin of infant salmonellosis cases.[4]

During the course of an earlier unpublished study of this problem, we were impressed by an apparent association between infant salmonellosis cases and a history of being fed iron-supplemented brands of infant formula. Samples of these products, obtained both from case households and unopened from grocery shelves, were invariably sterile, however.

In 1984 an article in the veterinary literature reported that weanling pigs administered therapeutic amounts of iron orally developed more severe *Escherichia coli* diarrhea than pigs to which the same amounts of iron were administered by intramuscular injection; the authors theorized that increased amounts of available iron in the intestinal tract promoted the multiplication of otherwise harmless numbers of potentially pathogenic bacteria, thus rendering them better able to cause overt disease.[9]

Since iron-enriched formula is commonly fed to infants, we initiated a study to determine if the iron content of formula could be contributing to the problem of infant salmonellosis on Guam.

Methods

A case-control study was carried out using as cases, infants born on Guam who developed laboratory-confirmed salmonellosis before their first birthday. Cases were identified prospectively as laboratory reports were received from the Guam public health reference laboratory. (All medical laboratories on Guam, including

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This paper was submitted to the journal May 17, 1990, and accepted with revisions February 19, 1991.

TABLE 1—Crude odds ratios (OR) for Iron Content of Formula Fed at Onset of Illness in Non-Breast-Fed Cases of Infant Salmonellosis and Non-Breast-Fed Age-Matched Controls

Formula	Cases	Controls	OR[a]	95% CI
Low-iron	13 (17.3%)	37 (30.0%)	1.00	
High-iron	62 (82.7%)	88 (70.0%)	2.96	1.24–7.08

[a]OR and 95% CI estimated retaining age-matching.

military facilities, routinely submit isolates of *Salmonella, Shigella,* and *Vibrio* to this laboratory for confirmation and serotyping.)

Controls were identified by selecting the next two births listed in the Guam birth registry following the case-birth registration, thereby matching for age and season of birth. If an infant selected as a control was deceased, or a salmonellosis case, or had previously been selected as a control, the next registered birth was selected. If a selected infant had been treated by a physician for diarrhea or if efforts to locate parents were not successful, the next birth certificate was selected, and the process was repeated until two controls had been enrolled in the study.

Parents of case infants were asked what milk their infant was being fed when the diarrhea developed, and parents of control infants were asked what milk their infant was being fed at the same age. Formula brands were assigned to one of two categories: low iron (less than 10 mg/L) or high iron (10 mg/L or more). Upon completion of each interview, data on the age, ethnicity, education, and marital status of the parents, infant birthweight and APGAR score, complications of pregnancy or delivery, month of pregnancy during which prenatal care began, number of prenatal physician visits, and number of previous births to the mother were excerpted from birth certificates.

Names of all study infants, without identification as to whether they were cases or controls, were submitted to the Special Supplemental Food Program for Women, Infants and Children (WIC) staff to determine if they had been enrolled in that program.

Statistical Methods

Cross-tabulations and simple statistical tests, including χ^2 tests, were performed using the computer package PC-SAS.[10] Odds ratios (OR) and their 95% confidence limits (CI) were estimated by conditional logistic regression analysis, retaining the matching of controls to cases as suggested by Breslow and Day,[11] using the computer package EGRET.[12]

Results

Of 96 cases of salmonellosis reported in children less than 1 year of age during this study, 18 (18.8%) could not be traced. In selecting controls, birth certificates of 389 infants were examined. Of these, 131 (34%) were excluded from the study for various reasons, 112 being untraceable. Of the remaining 258 controls, 167 had no history of treatment by a physician for diarrhea and were enrolled in the study.

Cases were less likely to be receiving breast milk at the time they developed salmonellosis than their age-matched controls (3.9% vs 25.2%), suggesting that failure to breast-feed infants on Guam is associated with an approximately ninefold increase in the risk of symptomatic salmonellosis infection (OR = 9.15, CI = 2.71–30.9). This association remained after taking into account potentially confounding variables.

To compare infants with regard to the iron content of infant formula received, those infants receiving breast milk were excluded, leaving a total of 75 cases and 125 controls. Cases were more likely than controls to be receiving infant formula with a high iron content (OR = 2.96, CI = 1.24–7.08; Table 1). Ninety-six percent of cases enrolled in the WIC program received a high-iron formula compared with 75% of cases not enrolled. Among controls, 80% of infants in the WIC program received a high-iron formula compared with 67% of those not in the program. No association between education of parents or marital status of mother and type of infant formula used was observed.

Each of the variables included in Table 2 was then considered individually as a potential confounder of the association between type of infant formula and risk for salmonellosis using the technique of conditional logistic regression. Controlling WIC enrollment led to the greatest reduction in the estimate of the odds ratio (OR = 2.54, CI = 1.02–6.28), while controlling marital status led to the greatest increase (OR = 3.85, CI = 1.51–9.84). A conditional logistic regression model including the variables listed in Table 2 was then fitted. Problems of collinearity prevented the simultaneous inclusion in the model of terms for father's ethnic group and age. All other variables were included. When father's ethnic group was omitted from the model, the estimated OR of the association between high-iron infant formulas and salmonellosis was 4.74 (CI = 1.28–17.57). Excluding father's age from the model produced an OR estimate of 4.04 (CI = 1.09–14.94).

Discussion

It has been suspected for some time that infants born on Guam may be infected with *Salmonella* bacteria by some means other than the more commonly recognized modes of transmission. The discovery that both indoor and outdoor environments on Guam were heavily contaminated with *Salmonella* bacteria[13,14] and that the incidence of infant salmonellosis in particular was linked to seasonal changes in environmental conditions[15] seemed to support the hypothesis that infant salmonellosis might frequently be a consequence of contamination of the infant's environment rather than the result of consuming contaminated food products or contact with infected family members.[16] It was also suspected that the immunologic naivete of newborn infants was a key factor in facilitating infection by the relatively small number of *Salmonella* bacteria that were likely to be encountered in an apparently clean home environment and that were harmless to other family members. The results of this study suggest that absence of breast-feeding and high levels of iron in some brands of infant formula may also be factors that contribute to an increased risk for infants' developing clinical salmonellosis on Guam.

Reported complications associated with pregnancy or delivery was the only other variable to show a substantially elevated OR in our univariate analysis (Table 2). This trend remained in both multivariate models (OR = 2.79, CI = 0.76–10.26, father's ethnic group excluded, and OR = 3.68, CI = 1.08–12.55, father's age excluded). Complications reported in more than one case included wrapping of umbilical cord around neck of infant (14), meconium staining of infant (8), Caesarean delivery (6), and premature rupture of membranes (2). These infants may have been at increased risk of being identified as salmonellosis cases because of decreased resistance to infection or because they received more intensive medical attention following delivery. While we did not measure either disease-specific or general infant immunity levels, our analysis did use the number of antenatal physician visits as a proxy measure of the tendency of parents to utilize medical services.

Other possible biases in this study include loss of 18 (18.9%) cases and 112 (28.8%) controls. Factors that contributed to the high rate of unlocateable cases and controls included the mobility of younger families, the concurrent use of as many as three different house-numbering schemes in some areas, and the frequent absence of addresses or telephone numbers in patient records. The fact that case address information obtained from health care providers was more current than control address information listed on birth certificates may have affected the success rates for locating individuals from these two groups.

Of initially selected controls, 91 were excluded from the analysis because they had a history of physician treatment for diarrhea and might therefore have been unidentified cases of salmonellosis. Trial inclusion of these children in the analysis led to some reduction in the final estimate of the OR of the association between type of infant formula and risk of salmonellosis, as might be expected if misclassification of disease status had occurred (OR = 2.34, CI = 1.22–9.16), but would not materially alter our interpretation of the results.

A biased estimate of the OR might also have arisen through failure to control other confounding variables. While socioeconomic status has not been controlled directly in the analysis, maternal and paternal education, both indicators of socioeconomic status, have been controlled. Enrollment in the WIC program, which is restricted to low-income families, has also been controlled. Cost differential between low- and high-iron formulas is not a factor, as both formulations cost the same.

Weaning practices could also be a confounding factor, but since 60% of children in the study were age 3 months or less, it seems unlikely that this could have played a substantial role in the observed associations. Formula preparation practice was not examined because an earlier investigation suggested that this was not a risk factor for infant salmonellosis on Guam.[4]

TABLE 2—Crude odds ratio (OR) for Various Physical, Demographic, and Socioeconomic Variables: Infant Salmonellosis Cases vs Age-Matched Controls

	Cases		Controls			
Variable	No.	Percentage	No.	Percentage	OR	95% CI
Sex						
Males	48	62	97	58	1.00	
Females	30	38	70	42	0.89	0.51–1.57
Birth order						
First	30	38	60	36	1.00	
Later	48	62	106	64	0.90	0.53–1.56
Birth weight (g)						
2500 or more	72	92	157	94	1.00	
<2500	6	8	10	6	1.25	0.43–3.60
Mother's status						
Married	61	78	114	68	1.00	
Unmarried	17	22	53	32	0.62	0.33–1.15
Ethnic group of mother						
Pacific Islander	44	56	90	54	1.00	
Asian	18	23	48	29	0.86	0.44–1.67
Other	16	21	29	17	1.22	0.52–2.84
Ethnic group of father						
Pacific Islander	36	52	56	43	1.00	
Asian	17	25	37	29	0.79	0.38–1.65
Other	16	23	36	28	0.67	0.27–1.62
Complications of pregnancy or delivery						
No	44	56	116	69	1.00	
Yes	34	44	51	31	2.10	1.07–4.15
Hospital of birth						
Civilian	59	76	122	73	1.00	
Military	19	24	45	27	0.87	0.30–2.50
Mother's age (years)						
<20	8	10	23	12	1.00	
20–29	49	63	108	64	1.24	0.52–2.95
≥30	21	27	36	24	1.68	0.65–4.37
Father's age (years)						
<20	4	6	5	4	1.00	
20–29	33	49	69	53	0.63	0.16–2.41
≥30	31	46	55	43	0.73	0.18–2.87
Mother's education						
Secondary	47	60	112	67	1.00	
College	31	40	54	33	1.43	0.81–2.52
Father's education						
Secondary	38	60	76	60	1.00	
College	25	40	50	40	1.07	0.57–2.03
Number of prenatal visits						
1–8	30	38	63	38	1.00	
9–11	24	31	56	34	0.95	0.48–1.89
≥12	24	31	46	28	1.12	0.51–2.44
APGAR score at 1 min						
1–8	53	68	113	68	1.00	
9–10	25	32	52	32	1.03	0.56–1.91
APGAR score at 5 min						
1–8	11	14	21	13	1.00	
9–10	67	86	144	87	1.00	0.41–2.45
WIC client						
No	51	65	130	78	1.00	
Yes	27	35	37	22	1.74	0.94–3.21

Note: OR and 95% CI estimated retaining age-matching.

It has previously been reported that breast-feeding and available dietary or serum iron can influence the course of infections.[17–22] Since mother's milk contains only about 1.5 mg of iron per liter and normal full-term infants exclusively breast-fed for 4 to 6 months by mothers in normal health are unlikely to develop iron deficiency,[23] it may be that maintaining a relatively low concentration of iron in the neonatal gastrointestinal tract is one of nature's strategies for protecting infants from bacterial infections during the critical first few months of life. Commercial iron-supplemented infant formula, on the other hand, contains from 12 to 19 mg of iron per liter and would provide the intestinal flora of infants fed these products with from 8 to 12 times more iron than is available to breast-fed infants.

Although one of the objectives of the Guam WIC program is to encourage breast-feeding, this study found that breast-feeding was less common among WIC program participants than among nonparticipants (19% vs 28% among controls). The enticement of offering free infant formula to new mothers may be more effective in determining actual nursing practice than present educational efforts designed to foster knowledge and attitudes favorable to breast-feeding. Current regulations of the WIC program stipulate that infant formula containing less than 10 mg of iron per liter may be provided only when a physician determines that the recipient infant has an existing medical condition that contraindicates the use of iron-supplemented formula. This study suggests that such a requirement may contribute to the problem of infant salmonellosis on Guam.

For the 10-year period 1981 to 1990, the average annual age-specific incidence rate for infant salmonellosis on Guam peaked at age 2 months (approximately 4580 cases per 100 000) but fell by more than 50% by age 6 months (approximately 2140 cases per 100 000). This result suggests that it would be advisable to breast-feed Guam newborns for at least 6 months and to delay feeding iron-supplemented infant formula for the same period when breast-feeding is not possible.

While we have only studied the relationship between salmonellosis and infant diet, it is possible that other bacterial diseases are similarly affected. It has already been demonstrated using an animal model that oral iron supplementation can cause an exacerbation of diarrhea due to *E. coli* infection,[9] and both pathogenic strains of *E. coli* and *Campylobacter* (a bacteria similar epidemiologically to *Salmonella*) are known to be important causes of childhood diarrhea in many countries.[24–26]

Although a relatively high nonresponse rate among both cases and controls and the other potential sources of bias outlined in this report suggest that a cautious interpretation of our conclusions is warranted, we believe that the data presented here, together with a biologically plausible mechanism previously reported, warrant further investigation. Certainly any inexpensive and readily implemented strategies that have the potential to significantly affect the important worldwide problem of infant diarrheal disease merit serious consideration. □

Acknowledgment

This work was supported by grant WP 82005 from the Diarrhoeal Diseases Control Program of the World Health Organization, Geneva.

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