# Prevention of Invasive *Cronobacter* Infections in Young Infants Fed Powdered Infant Formulas

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*Cronobacter*, *sakazakii*, infant formula, neonatal infection

AAP—American Academy of Pediatrics
BM—breast milk
CDC—Centers for Disease Control and Prevention
*Cronobacter*—*Cronobacter* multispecies complex, formerly *Enterobacter sakazakii*
EBF—exclusively breastfed
FDA—US Food and Drug Administration
HMF—human milk fortifier
PFGE—pulse field electrophoresis
PIF—powdered infant formula
RTF—ready-to-feed formula
WHO—World Health Organization
WIC—Supplemental Nutrition Program for Women, Infants, and Children

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*The author has acted as an expert witness in legal cases related to* Cronobacter *infection; therefore, if this study leads to preventive action, it is, in a practical sense, contrary to her personal financial interests.*

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Invasive *Cronobacter* infection is a rare but devastating disease known to affect hospitalized premature or immunocompromised infants fed powdered infant formulas (PIFs). PIF labels imply that powdered formulas are safe for healthy, term infants if the label instructions are followed.

*Cronobacter* can also infect healthy, term infants in the first months of life, even if PIF label instructions are followed. Invasive Cronobacter infection is extremely rare in exclusively breastfed infants or those fed commercially sterile, ready-to-feed formulas.

## abstract

Invasive *Cronobacter* infection is rare, devastating, and epidemiologically/microbiologically linked to powdered infant formulas (PIFs). In 2002–2004, the US Food and Drug Administration advised health care professionals to minimize PIF and powdered human milk fortifier (HMF)'s preparation, feeding, and storage times and avoid feeding them to hospitalized premature or immunocompromised neonates. Labels for PIF used at home imply PIF is safe for healthy, term infants if label instructions are followed.

1) Medical, public health, Centers for Disease Control and Prevention, US Food and Drug Administration, and World Health Organization records, publications, and personal communications were used to compare 68 (1958–2003) and 30 (2004–2010) cases of invasive *Cronobacter* disease in children without underlying disorders. 2) The costs of PIFs and ready-to-feed formulas (RTFs) were compared.

Ninety-nine percent (95/96) of all infected infants were <2 months old. In 2004–2010, 59% (17/29) were term, versus 24% (15/63) in 1958–2003; 52% (15/29) became symptomatic at home, versus 21% (13/61). Of all infected infants, 26% (22/83) had received breast milk (BM), 23% (19/82) RTF, and 90% (76/84) PIF or HMF. Eight percent received BM and not PIF/HMF; 5%, RTF without PIF/HMF. For at least 10 PIF-fed infants, label instructions were reportedly followed. Twenty-four ounces of milk-based RTF cost $0.84 more than milk-based PIF; 24 ounces of soy-based RTF cost $0.24 less than soy-based PIF.

*Cronobacter* can infect healthy, term (not just hospitalized preterm) young infants. Invasive *Cronobacter* infection is extremely unusual in infants not fed PIF/HMF. RTFs are commercially sterile, require minimal preparation, and are competitively priced. The exclusive use of BM and/or RTF for infants <2 months old should be encouraged. *Pediatrics* 2012;130:1–9

*Cronobacter* multispecies complex, formerly classified as *Enterobacter sakazakii* (*Cronobacter*), are pathogenic, Gram-negative, non–spore-forming, coliform enteric bacteria.[1,2] Invasive *Cronobacter* infection was first reported in 1961 and is now recognized as a rare, often devastating, infection predominantly affecting infants.[3–6] *Cronobacter* infection appears to have a low infectious dose and short incubation period[6–9] and (R. Mittal, PhD, personal communication, 2011). Liquefying meningitis is a frequent complication, and severe neurologic impairment or death is common.[6] In the United States, only 1 state, Minnesota, requires *Cronobacter* reporting. These infections are likely underrecorded, as evidenced by recent events. In late 2011, single reports of *Cronobacter* illness in infants in Missouri and Illinois caused the Centers for Disease Control and Prevention (CDC) to ask public health officials around the country to look for other cases of *Cronobacter* infection among infants. This generated reports of 2 additional cases, 1 in Oklahoma and 1 in Florida, bringing the 2011 US case total to 13.[10,11]

Ten NICU *Cronobacter* outbreaks have been reported.[6]* In 8, nutritional sources were evaluated; all affected infants had received some specific powdered infant formula (PIF). In 3 outbreaks, epidemiological and microbiologic studies were done. There was no evidence of infant-to-infant or environmental transmission and the implicated PIF yielded *Cronobacter*.[12–14] These findings, nonoutbreak cases, and a 2002 US Food and Drug Administration (FDA) study isolating *Cronobacter* from 23% of sampled PIFs[15] prompted the World Health Organization (WHO) to state[16]: "Contaminated powdered infant formula has been convincingly shown, both epidemiologically and microbiologically, to be the vehicle and source of infection in infants."

A 2002 FDA Letter to Health Care Professionals[17] and subsequent cautionary material from formula manufacturers and the International Formula Council (see, for example, references 18 and 19)† warned that premature infants and infants with underlying medical conditions could become infected with *Cronobacter*, recommended PIF be avoided in NICUs unless there was no alternative, and suggested the chance of infection could be decreased by (1) reconstituting only a small amount of formula at a time, (2) minimizing "holding time" between preparation and feeding, (3) refrigerating and using formula within 24 hours after preparation, and (4) not exceeding 4 hours "hang time" for continuous enteral feeding. Parents did not receive similar information but formula companies gradually changed PIF instructions and labels for at-home use to indicate that PIF should not be fed to premature or immunocompromised infants and, for infants' safety, caretakers should (1) feed PIF immediately or refrigerate and use it within 24 hours and (2) use warmed formula within 1 hour or discard it. (see, for example, references 20–22) Since 2004–2005, PIF labels have stated that PIF is not sterile but, in a 2005–2006 US national survey, when mothers of 2-month-old infants were asked if various formulas were "likely to contain germs," only 29.5% responded affirmatively for PIF, whereas 31.1% did so for commercially sterile, ready-to-feed formula (RTF), and 35.0%, for commercially sterile concentrates.[23]

In an August 28th, 2003 letter to the FDA, the American Academy of Pediatrics (AAP) wrote, "While sampling large batches of product can be problematic, and product sterility cannot be absolutely assured, all powdered formula should be E. sakazakii free. The AAP also recommends that the standards regarding powdered formula be the same for premature as well as term infants. The AAP sees no reason that they should be different, as the absolute risk, even to term infants, is not zero."

This study analyzes all obtainable 1958–2010 reports of invasive pediatric *Cronobacter* infection occurring worldwide in children without underlying disorders, to examine if the frequency, place of occurrence, or characteristics changed after warnings were disseminated to health care professionals. In addition, the costs of PIF, RTF, and concentrates were compared to determine if the latter 2 might be economically viable home-use alternatives to PIF for young infants who are not exclusively breastfed (EBF).

Reviewed material included (1) CDC and FDA files obtained through Freedom of Information Act requests, (2) published cases and literature reviews,[4–6,24] (3) all cases reported by WHO as of July 15 to 18, 2008,[25] (4) personal communications with publication authors, and (5) nonconfidential information from parents, medical records, and legal documents. Children were not included in these analyses if their infections were noninvasive or they had underlying birth defects, medical conditions, or signs of immunodeficiency. Other exclusion criteria are provided in Supplemental Information 1. Of note, all children meeting these criteria were ≤87 days of age at symptom onset.

Definitions for terms used herein include the following: healthy, no recorded evidence of a preexisting immunodeficiency, underlying disorder, or birth

*United Kingdom (1961), Netherlands (1983), Greece (1987), Iceland (1989), United States (1989 and 2002), Belgium (2001), Israel (2001), France (2004), and New Zealand (2004).

†The International Formula Council is an international association of manufacturers and marketers of formulated nutrition products (eg, infant formulas and adult nutritionals) whose members are predominantly based in North America. It was formed in 1998 through the consolidation of the Infant Formula Council (founded in 1970) and the Enteral Nutrition Council (founded in 1983).

defect; neonatal, in the first month of life; premature, gestational age <37 weeks at birth; and low birth weight, <2500 g. Nutritional intake was based on the best obtainable information. The estimated general population rate of newly diagnosed primary immunodeficiency, underlying disorders, and birth defects in newborns (ie, <5%) was based on data from a large, local US population[26] and Birth Defects OMNI-Net.[27] US rates of prematurity (13% in 2005), low birth weight births (8%), and breastfeeding of 1-month-olds (46% EBF and an additional 23% fed breast milk [BM] in combination with other foods) were based on CDC data.[28,29] The proportion of US newborns remaining in the hospital because of clinical problems/complicating diagnoses (29% in 2000) was based on US Agency for Healthcare Research and Quality data.[30] Comparisons excluded unknowns and were made by using 2-tailed Fisher exact tests and the Freeman-Halton extension of the Fisher exact tests for 2×3 and 2×4 tables.

Cost data for PIF, RTF, and concentrate formulations of 3 milk-based and 3 soy-based products marketed for US neonates were obtained in September 2011 from 5 Web sites with free-shipping options: www.amazon.com, www.babiesrus.com, www.cvs.com, www.diapers.com, and www.walmart.com.

The proportion of invasively infected infants with a preexisting disorder/immunodeficiency did not change significantly between 1958–2003 and 2004–2010 (9/77, 12% vs 6/36, 17%) and was higher than the general population rate (<5%). The worldwide average annual number of reported invasive *Cronobacter* infections in infants without preexisting conditions, that is, those examined further herein, was 1.5 in 1958–2003 (68 cases in 46 years) and 4.3 in 2004–2010 (30 cases in 7 years).

The proportion of infected infants who were neonates (83%) was stable (Table 1). Only 1 infant was >2 months old at symptom onset. During both time periods, the proportions of *Cronobacter*-infected infants who were premature and/or of low birth weight were higher than in the general population (prematurity, 13%; low birth weight, 8%); however, the proportions of cases involving term and normal birth weight infants were significantly higher in 2004–2010, compared with 1958–2003. Similarly, the proportion of invasive *Cronobacter* infections occurring in a hospital exceeded the proportion of US infants requiring prolonged postnatal hospitalization (29%), but the majority of 2004–2010 infections occurred at home, even though 2 infants who became symptomatic at home on the day of postnatal discharge were placed into the "hospital" category for this analysis. Consistent with these findings, the proportion of reported invasive *Cronobacter* infections involving necrotizing enterocolitis was lower in 2004–2010 than in 1958–2003. In both time periods, most reported *Cronobacter*-infected infants had meningitis.

Nutritional information (Table 2) was wholly absent for 19% of cases in 1958–2003 and no case in 2004–2010. Ninety percent of invasively infected infants had received a powdered product, that is, PIF or human milk fortifier (HMF). This proportion did not differ significantly between time periods, but in 2004–2008 proportionately more infants received multiple types of nutrition. Nineteen infants received RTF; where timing was specified, RTF was initiated before postnatal discharge; at least 9 infants were not receiving it on the day they became symptomatic. The proportions EBF (1/53 in 1958–2003 and 2/29 in 2004–2010) were much lower than the rate for all US neonates

**TABLE 1** Characteristics of All Reported Infants Without Underlying Disorders, Invasively Infected With *Cronobacter*, by Time Period

| Characteristic[a] | 1958–2003 | 2004–2010 | Total | *P*[b] |
|---|---|---|---|---|
| <1 mo old at onset of symptoms | 53/66 (80%) | 27/30 (90%) | 80/96 (83%) | NS |
| Premature | 48/63 (76%) | 12/29 (41%) | 60/92 (65%) | |
| Term | 15/63 (24%) | 17/29 (59%) | 32/92 (35%) | .002 |
| BW <2500 g | 44/55 (80%) | 10/24 (42%) | 54/79 (68%) | |
| BW ≥2500 g | 11/55 (20%) | 14/24 (58%) | 25/79 (32%) | .001 |
| Premature, BW <2500 g | 42/54 (78%) | 8/24 (33%) | 50/78 (64%) | |
| Term, BW ≥2500 g | 6/54 (11%) | 14/24 (58%) | 20/78 (26%) | <.0001 |
| Other[c] | 6/54 (11%) | 2/24 (8%) | 8/78 (10%) | |
| Place of symptom onset | | | | |
| Hospital | 48/61 (79%) | 14/29 (48%)[d] | 62/90 (69%) | |
| Home | 13/61 (21%) | 15/29 (52%) | 28/90 (31%) | 0.007 |
| Diagnoses[e] | | | | |
| Meningitis | 38/68 (56%) | 22/30 (73%) | 60/98 (61%) | NS |
| Bacteremia | 21/68 (31%) | 14/30 (47%) | 35/98 (36%) | NS |
| NEC | 22/68 (32%) | 1/30 (3%) | 23/98 (23%) | 0.001 |
| UTI | 1/68 (2%) | 0/30 (0%) | 1/98 (1%) | NS |

See Methods section and Supplemental Information 1 for details concerning data sources and selection criteria. BW, birth weight; NEC, necrotizing enterocolitis; NS, not significant; UTI, urinary tract infection.

[a] An infant was considered term if the records indicated that was the case and/or the gestational age was specified as being at least 37 weeks. An infant was considered premature if the records indicated that was the case and/or the gestational age was <37 weeks. Table excludes patients for whom the specified data are unknown; there were a total of 68 infants in 1958–2003 and 30 in 2004–2010.

[b] Fisher exact tests and Freeman-Halton extension of the Fisher exact probability test for a 2 × 3 table. Not significant if $P \geq .05$. Totals percents may not equal 100 because of rounding.

[c] Term, BW <2500 g or premature, BW ≥2500 g. When "other" category is excluded, *P* remains <.0001.

[d] This category includes 1 infant who became ill 12 hours after leaving the hospital and another who was noted to be ill on the day of hospital discharge and was reportedly symptomatic while in the hospital.

[e] Some patients had >1 diagnosis. Specifically, 18 patients with meningitis also had proven bacteremia and 2 also had NEC. One patient with bacteremia also had NEC and one also had a UTI. *P* values are for proportion with each individual diagnosis.

**TABLE 2** Number and Proportion of Reported Infants Without Underlying Disorders, Invasively Infected With *Cronobacter*, by Time Period and Nutrition Source

| Nutrition Source[a,b] | 1958–2003 | 2004–2010 | Total | *P* Value[c] |
|---|---|---|---|---|
| Noted[d] | 55/68 (81%) | 30/30 (100%) | 84/98 (86%) | .020 |
| Not indicated | 13/68 (19%) | 0/30 (0%) | 13/98 (13%) | |
| PIF, no BM[b] | 43/53 (81%) | 17/30 (57%) | 60/83 (72%) | .022 |
| BM & PIF | 4/53 (7%) | 6/30 (20%) | 10/83 (12%) | NS |
| BM & HMF | 3/53 (6%) | 2/30 (7%) | 5/83 (6%) | NS |
| BM, no PIF/HMF[b] | 3/53 (6%) | 4/29[d] (14%) | 7/82 (8%) | NS |
| Any PIF or HMF[e] | 51/54[d] (94%) | 25/30[d] (83%) | 76/84 (90%) | NS |
| Any BM[e] | 10/54[d] (18%) | 12/29[d] (41%) | 22/83 (26%) | .036 |
| Any RTF[e] | 6/53[d] (9%) | 13/29[d] (45%) | 19/82 (23%) | .003 |
| Any concentrate[e] | 1/53[d] (2%) | 2/29[d] (7%) | 3/82 (4%) | NS |

See Methods section and Supplemental Information 1 for details on data sources and selection criteria. Pertinent details on individual cases are provided in the Supplemental Information, but not all previously published details concerning outbreak-associated cases are provided therein. NS, not significant.

[a] Documented nutrition at any time before onset of symptoms, based on the best available information, including from medical records, CDC files, parent report, publications, and communications with publication authors. Total percents may not equal 100 because of rounding. Denominators include only those for whom data were known.

[b] The "PIF, no BM" category includes 9 infants who were also fed RTF, 7 of whom were not receiving RTF at the time of symptom onset and 2 of whom also received concentrate. One of these 2 was receiving only concentrate on the day of symptom onset. The "BM & PIF" category includes 4 infants who also received RTF, one of whom additionally received concentrate. The "BM, no PIF/HMF" category includes 4 infants who were also fed RTF, one of whom may also have been fed his twin's PIF (see Supplemental Information 2 for details).

[c] Fisher's exact tests. Not considered significant if $P \geq .05$.

[d] This category includes 1 infant who received formula that was likely but not definitely PIF and definitely did not receive BM (J. Burdette, MD, personal communication, 2011). This infant is included in the denominator for "any BM" and not in any numerators. The category also includes an infant who definitely received a recalled, contaminated lot of PIF but I could not determine if he received BM or other formulas as well (Belgium 2002). This infant is included in the numerator and denominator for "any PIF." A third infant in this category is a term newborn recorded on a CDC line list as not having received PIF but without information concerning what, if any, enteral feeding she did receive (AZ 2009). This infant is included in the denominator of "Any PIF or HMF" and is not included in "Any BM," "Any RTF," and "Any concentrate."

[e] Categories are not mutually exclusive; therefore, total percent is >100. Numbers are for those who had the specified nutrition noted.

(46%), but the proportions who had been fed BM and other nutrition were not (9/54 = 17% and 10/29 = 34%, vs 23%).[29] The EBF-infected infants lived in Brazil (2003), India (2006), and Slovania (2006). One US neonate diagnosed on the day of his postnatal discharge (2007) and 3 hospitalized infants (United States 1998–2001, United States 2003, Spain 2007) were fed only BM and RTF. Supplemental Information 2 provides the available case-specific clinical, epidemiological, and microbiologic testing details, broken down by nutrition received.

BM was cultured and negative in 5 cases, breast pumps in 2, and pump tubing in 1. Water samples were tested and negative in 10 PIF-related incidents involving 29 patients. One or more PIF product samples of some sort were *Cronobacter* tested in 29 incidents involving 62 patients and positive in 12 of the incidents (41%), involving 44 of the patients (71%). Investigators considered a *Cronobacter* isolate indistinguishable from the patient(s)' isolate(s) in 9 (75% of positive) incidents involving 35 patients. Environmental testing was never described in detail but was noted to have been done in 17 incidents involving 28 patients, with something positive in 6 (35%) incidents involving 16 (57%) patients. These involved formula preparation areas (sink, splash area, counter, water storage area, dish drawer); 2 were considered indistinguishable from patient isolates. FDA records for 1 case indicate that a bottle nipple was positive for *Cronobacter*; in another, a pacifier. (See Supplemental Information 3 for summaries of available microbiologic information, including the techniques used by investigators to compare isolates.)

Records for 4 hospital and 11 at-home US cases unrelated to outbreaks contained comments concerning the caretakers' PIF or HMF feeding and storage techniques (15/35, 43%). For 1 hospitalized infant, it was noted that BM/HMF feedings were given over 30 minutes; for another, that 6 hours-worth of PIF was mixed at a time, refrigerated for <24 hours, and warmed immediately before feeding. For the remaining 2, BM and HMF were mixed immediately before feeding, hang time was <4 hours, and BM was either stored frozen or refrigerated for <6 hours. Records of 8 infants who became symptomatic at home specified that PIF was mixed immediately before each feeding and never stored; another infant's parent made 2 bottles at a time, fed 1 immediately, and stored the other in the refrigerator just until the next feeding; another parent usually mixed formula for each feeding, occasionally made 1 or 2 extra bottles, stored these in the refrigerator, and used them within the day. In addition, 7 records specifically noted that unfinished remainders of feedings were always discarded; 5, that hands and/or preparation areas were washed before PIF preparation; and 6, that bottles, caps, and nipples were sterilized. Of note, these data were not collected systematically by case investigators and absence of information from a record does not indicate that a guideline was not followed. To summarize, for at least 2 infected, hospitalized infants, FDA guidelines reportedly were followed; for at least 10 infants infected at home, label instructions reportedly were followed.

Table 3 provides September 2011 online-shopping costs and relative costs for 6 formulas commonly used from birth to 6 or 12 months of age. These products are all available in PIF, RTF, and concentrate formulations. Prices varied relatively widely within and among brands, products, formulations, and stores. Approximate daily (4 ounces of formula every 4 hours) costs of feeding a neonate the least expensive

**TABLE 3** Per Ounce Prices and Price Differences, by Brands and Forms of Infant Formulas

| Type of Infant Formula | Price Range[a] | Mean (Median) Cost Differences Compared With Powdered | |
|---|---|---|---|
| | | % | Absolute[a] |
| Within-brand differences[b] | | | |
| Milk-based | | | |
| Powdered | 0.121–0.192 | NA | NA |
| RTF | 0.156–0.417 | 26–60 (31–42) | 0.040–0.103 (0.047–0.071) |
| Concentrate | 0.137–0.193 | 11–15 (13–15) | 0.017–0.024 (0.020–0.024) |
| Soy-based | | | |
| Powdered | 0.140–0.198 | NA | NA |
| RTF | 0.130–0.451 | 6–82 (6–55) | 0.011–0.134 (0.010–0.087) |
| Concentrate | 0.140–0.399 | 30–36 (15–35) | 0.050–0.062 (0.026–0.055) |
| Prices for all brands[c] | Mean (Median) | | |
| Milk-based formulas | | | |
| Powdered | 0.160 (0.162) | NA | NA |
| RTF | 0.237 (0.206) | 48 (27) | 0.077 (0.044) |
| Concentrate | 0.180 (0.184) | 12 (14) | 0.020 (0.022) |
| Soy-based formulas | | | |
| Powdered | 0.170 (0.171) | NA | NA |
| RTF | 0.232 (0.203) | 36 (19) | 0.062 (0.032) |
| Concentrate | 0.224 (0.212) | 32 (24) | 0.054 (0.041) |
| Both milk- & soy-based combined | | | |
| Powdered | 0.165 (0.169) | NA | NA |
| RTF | 0.235 (0.203) | 42 (20) | 0.070 (0.034) |
| Concentrate | 0.202 (0.192) | 22 (14) | 0.037 (0.023) |
| Least-expensive available products[d] | Actual cost/ounce | Actual Cost Differences Compared With Powdered | |
| Milk-based formula | | | |
| Powdered | 0.121 | NA | NA |
| RTF | 0.156 | 29 | 0.035 |
| Concentrate | 0.137 | 13 | 0.016 |
| Soy-based formula | | | |
| Powdered | 0.140 | NA | NA |
| RTF | 0.130 | −7 | −0.010 |
| Concentrate | 0.140 | 0 | 0 |

Costs were determined for 6 formulas available for neonates and young infants (and for use by a premature or immunocompromised infant as/if recommended by that infant's pediatrician): Enfamil (milk-based) (5 stores for PIF and RTF, 2 stores for concentrate); ProSobee LIPIL (soy-based) (5 stores for PIF, 3 stores for RTF, and 2 stores for concentrate); Good Start with iron, Gentle or Gentle plus (milk-based) (5 stores for PIF, 4 stores for RTF, and 3 stores for concentrate); Good Start soy, Supreme or Supreme Plus (4 stores for PIF, 3 stores for RTF, and 2 stores for concentrate); Similac Advance (milk-based) (5 stores for PIF, RTF, and concentrate); and Isomil (soy-based) (5 stores for PIF, 4 for RTF and concentrate). Prices were obtained in September 2011, for the least expensive packaging options, from the following Internet sites: Amazon.com, Babies-R-Us, CVS, Diapers.com, and Walmart. Not all sites carried all brands of each product, but all sites carried at least 1 brand each of a powdered, RTF, and concentrate product. Price ranges are for any of the assessed brands at any of the assessed Internet sites. NA, non applicable.

[a] In dollars per fluid ounce of prepared formula.

[b] Brand-specific ranges for differences in mean and median costs of each product type (RTF and Concentrate), compared with PIF, by using prices from all stores carrying the specific product type. Median values are in parentheses.

[c] All brands of specified product type are included in analyses. Medians are provided in parentheses.

[d] Lowest priced product of any brand, at any store. Numbers reflect actual costs and cost differences for those products.

formula of each type were compared. Milk-based RTF cost 84 cents more a day than milk-based PIF and milk-based concentrate cost 38 cents more than milk-based PIF. Soy-based concentrate cost no more than soy-based PIF and soy-based RTF, 24 cents less a day than soy-based PIF.

The major findings in this study are that the majority of reported invasive pediatric *Cronobacter* infections now occur in nonhospitalized and term infants, 99% were <3 months old, and 90% had received PIF. These findings raise a number of issues, including study limitations, potential sources of *Cronobacter* infection other than PIF and related to PIF, and implications in terms of parent education and infant feeding, taking into consideration that approximately half of US parents (those living at or below 185% of the federal poverty level) receive nutrition assistance through the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC).

This study has at least 5 limitations. First, these data span a wide time period, during which NICU care, infant-feeding practices, and formula processing have changed in ways that cannot be fully addressed in these analyses. Second, I could examine only available records from known cases of invasive *Cronobacter* infections. Europe has a surveillance system for product contamination (European Rapid Alert System for Food and Feed), but few countries have active surveillance for clinical *Cronobacter* infections. Current automated bacterial identification systems can accurately identify *Cronobacter* but several cases' medical records suggest that not all health care providers recognize that *Cronobacter* is an unusual pathogen. Cases reported after a public health alert[10,11] support that health authorities are not proactively informed of all *Cronobacter* infections. Third, reporting may be biased in regard to case characteristics and information collected. For example, most neonatologists are likely aware of *Cronobacter* infection in premature infants. This might lead to better reporting from NICUs and a relative underestimation of infections in healthy, nonhospitalized infants. Also, infections in breastfed infants are disproportionately represented in published case

reports, even though these provide no or minimal epidemiological or environmental microbiologic data, whereas infections in PIF-fed infants dominate CDC records, review articles, and footnotes in published microbiologic studies. Fourth, information concerning feeding preparation and storage techniques was not provided in response to standardized questionnaires and therefore is incomplete and varies between records. Fifth, I could not document data validity. Much information was obtained by public health investigators at the time of the illness, but some preparation and storage information was obtained in subsequent years. Parental recall may have been inaccurate or influenced by grief, stress, and/or a sense of guilt.

For 3 cases involving PIF-fed infants at home, *Cronobacter* was isolated from kitchen surfaces; for another, from a pacifier; and, for a fifth, from a bottle nipple. Epidemiological investigations could not determine whether these were contaminated by PIF or reflected an extrinsic source of PIF contamination or infection. *Cronobacter* has been found in a number of food substances, some used in PIF and some commonly present in household kitchens.[31,32] In a recent study, it was recovered from environmental sampling in 21 of 78 kitchens of recruited, predominantly low-income, middle Tennessee households.[33] These findings, the seven reported cases of invasive infection in non-PIF-fed infants, and occasional *Cronobacter* infection or colonization of immunocompromised, hospitalized adults,[34] indicate that *Cronobacter* infections are sometimes related to non-PIF sources. However, epidemiological and microbiologic data strongly implicate PIF as a source of pediatric *Cronobacter* infections. Furthermore, *Cronobacter* has been isolated repeatedly from PIF, including as recently as 2010.[9,15,31,35–39] *Cronobacter* (and *Enterobacteriaceae*) are established and ubiquitous in PIF dry processing environments; eradication is not considered possible.[16,40] PIF, RTF, and concentrate manufacturing begin with nonsterile nutritional components being put into solution, homogenized, and then pasteurized, resulting in commercial sterility. PIF is then dried in a nonsterile environment and nonsterile components often are added after pasteurization.[40] Drying- and dry-processing areas can be kept free of *Salmonella* through environmental, component, and end-product surveillance and microbiologic testing; however, 6 PIF-associated salmonellosis outbreaks have been reported since 1995, in Canada, France, Korea, Spain, the United Kingdom, and the United States. The most recent, in 2005, involved 141 French infants.[41]

One of the statistical assumptions in the FDA's *Cronobacter* end-product testing protocol is that *Cronobacter* contamination in PIF is not clustered or clumped[42]; however, *Cronobacter* has been described as tending to form clumps that are "sort of stuck together."[43] A recent study provided evidence of this. A 22 000 kg, released-to-market lot (ie, batch) of PIF was recalled because postmarket testing by authorities found 1 package to be positive for *Cronobacter*.[39] Examination of the retrieved material showed that contamination varied among production-time-specific samples. Most samples were below detectable limits but 3- to 560-cell clusters occurred sporadically in 8 of 2290 1-g samples. The 2 largest clusters, of 123 and 560 cells, originated from just 2 product bags. Of note, the investigated lot contained $>1$ contaminated product bag, but that does not preclude the possibility of more confined, even single-bag, contamination occurring in other lots of PIF.

*Cronobacter* has never been isolated from BM, unopened bottled water, treated US municipal drinking water, unopened RTF, or unopened concentrates. Only 7 reported, invasively infected infants were not fed PIF. PIF labels imply the product is safe if label feeding and storage instructions are followed. AAP and WHO PIF guidelines recommend cleaning hands and preparation areas, cleaning and sterilizing equipment, discarding unfed warmed, prepared formula after 2 hours, and storing prepared formula in a refrigerator and for no more than 24 hours.[44,45] Cases of invasive *Cronobacter* infections have occurred when these preparation and feeding guidelines, as well as label directions, reportedly were followed or exceeded (in that formula was always prepared as individual servings immediately before feeding and never stored).

WHO guidelines also recommend that water be boiled and cooled for up to 30 minutes before being added to PIF to achieve a reconstitution temperature of 70°C, because WHO consultants determined this inactivated all tested *Cronobacter* strains.[45] Not all organizations agree with this recommendation.[45] In 2002, the FDA and the US Department of Agriculture reversed their own recommendations that health professionals use boiled water to reconstitute PIF, citing potential loss of heat-sensitive nutrients, changes in some formulas' physical characteristics, inadequate destruction of *Cronobacter*, and injury to personnel preparing formula.[17,45] The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition also disagreed with the WHO recommendation, because of possible adverse effects on nutrients.[45] AAP's current instructions do not recommend boiling water unless the safety of the water source is uncertain.[44] Two case records reviewed herein indicated that the *Cronobacter*-infected infants had received boiled water, but there was no indication it was done as recommended by WHO. Of note, in a recent report of two 2010 noninvasive

*Cronobacter* infections in Mexico, associated with a US-manufactured PIF, the authors determined that the health care providers had attempted to follow WHO guidelines. However, retrospective investigation suggested that the boiled water was likely 45°C, not 70°C, at the time of PIF reconstitution.[9]

The AAP recommends exclusive breastfeeding for the first 6 months of infancy.[46] The data herein suggest that invasive *Cronobacter* infection rarely occurs in EBF infants. However, the proportion of *Cronobacter*-infected infants who were partially breastfed was similar to the rate for all US 1-month-olds. In a 2007 survey of breastfeeding-related maternity practices at US hospitals and birth centers, 70% of facilities reported providing breastfeeding mothers with discharge packs containing formula samples.[47] It might be helpful to discontinue these samples or limit them to RTF, which is commercially sterile, requires minimal, albeit careful, handling, and is comparably priced to PIF if parents are willing and able to comparison shop.

Comparison shopping is not a primary option for families on WIC. WIC has instituted policies to encourage breastfeeding, with some apparent success; in 1 non-nationally representative, US survey, 47% of WIC neonates were EBF in the previous week, compared with 26% of non-WIC neonates.[23] Infant formula is purchased by WIC at a discount, through a state-by-state exclusive contract bidding process, and provided to nonbreastfeeding or BM-supplementing mothers. RTF is available through WIC, but PIF is the predominant type of formula currently used by the program. The options for parents on WIC could be improved if WIC could provide RTF for infants in the first 2 months of life.

Premature and immunocompromised PIF-fed neonates continue to be disproportionately represented in reports of invasive *Cronobacter* infection, relative to their proportion in the general population. However, the majority of cases now involve nonhospitalized and term, PIF-fed infants. Parents, like health care professionals, need education concerning the proper handling and storage of infant nutrition, as well as accurate information concerning the relative number of enteric infections, including *Cronobacter*, in EBF, RTF-fed, and PIF-fed infants, so they can make informed decisions about their infants' nutrition.

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Fifteen children with evidence of an underlying birth defect, medical disorder, or immunodeficiency were included in the calculations related to the proportion of invasively infected infants who had birth defects, underlying disorders, or evidence of immunodeficiency. They were not included in the calculations related to Tables 1 and 2.

The following reports and cases were excluded from this study:

- Thirty-one infants who were asymptomatic, colonized, or had noninvasive disease.
- Four patients who were asymptomatic, colonized, or had noninvasive disease and also had evidence of an underlying birth defect, medical disorder, or immunodeficiency.
- Two cases involving multiple organisms, 1 patient whose culture was judged to be a postmortem contaminant, a Greek outbreak with inadequate data ($n = 2$ infants), a Brazilian outbreak linked to a contaminated intravenous solution ($n = 5$), a case series of apparently noninvasive infections in Utah during 2004–2005 ($n = 5$–10), and 87 laboratory surveillance reports submitted in response to WHO's 2008 Call for Data and for which age ranges were the only information provided (59 British and Welsh records from 1992–2007 and 28 Philippine records from 1998) [FAO/WHO 2008=reference 25].
- Information could not be obtained to review whether the following possible cases in Friedemann[24] met the inclusion criteria herein: 6 possible cases associated with 12 Polish NICU isolates from 1997 to 2007, 10 possible cases listed in that article's Table 5, and an unspecified number of Spanish cases related to 5 positive blood isolates from a university general hospital in 1991–2006, [Friedemann, 2009=reference 24].
- A 2009 Minnesota CDC case involving a bacteremic female <1 week old, who had not received PIF, and for whom I could obtain no other information.
- The author of a 2000 North Carolina case confirmed that the infant was fed only formula but could not confirm that it was powdered [Burdette 2000=reference 48 and personal communication, JH Burdette, 10/6/11]. This infant is included in the denominator for calculations related to receipt of breast milk.
- A 2002 Belgium case [Nestlé 2002=reference 37] involved an infant who received powdered infant formula (PIF); there was no information on whether he received any breast milk. When the PIF manufacturer was informed of this infant's infection, additional, postmarketing culturing of the PIF lot/batch used by this infant was done. The additional testing was positive for *Cronobacter*, although pre-release testing of the lot had been negative. The lot was recalled. This infant is included in the numerator and denominator for "any PIF" calculations but not included in the breast milk-related calculations.
- For a 2009 AZ case recorded on a CDC line list, it was noted that the infant had not received PIF but not what, if any, enteral feeding was received. (It was noted that the infection happened at a birthing center and the mother's stool was negative.) This infant was included in the denominator of "any PIF or HMF" but not included in "any breast milk", "any RTF", or "any concentrate" calculations.
- This category includes three infants fed solely breast milk: A Brazilian term neonate who became symptomatic with meningitis while at home [Barreira 2003=reference 49], a preterm, 10-day-old, Slovenian infant with hyaline membrane disease who developed symptoms of sepsis while being fed expressed

‡As described in medical records, legal records, CDC files, and the references cited. Does not include infants determined to have been exclusively PIF-fed.

breast milk (2004 case)[Pavcnik-Arnol 2007=reference 50], and a two-month-old, breastfed Indian infant admitted for bronchiolitis and transferred to a pediatric intensive care unit (ICU) with signs of sepsis on day three of hospitalization (2006 case) [Ray 2007=reference 51 and personal communication, P. Ray, March 19, 2012].

- The Brazilian case involved a 14-day-old, nonhospitalized infant who became acutely symptomatic over a matter of hours, with hypoactivity, pallor, vomiting and "food refusal". The perinatal history was normal; delivery was at term. The infant's birth weight was 2650 grams and Apgar scores were 9/10. No epidemiologic or environmental evaluation appears to have been done. The author postulated vertical transmission but appears to have based this solely on the age of the infant. No microbiologic or epidemiologic data were provided to support this theory [Barreira 2003=reference 49]. Of note, there has never been a documented case of vertical transmission of *Cronobacter* and a number of cases have occurred in infants born via Caesarean sections.
- In the Slovenian case, breast milk and a breast pump were tested and negative for *Cronobacter*. The author of the Slovenian report indicated that she did not have information on the hand washing, nipple and bottles cleaning, or the expressed breast milk handling techniques used in the ICU. She thought this was the only *Cronobacter* case in the ICU in the previous 12 years (case occurred between September 2004 and November 2005)[Pavcnik-Arnol 2007=reference 50 and personal communication, M. Pavcnik-Arnol, March 14, 2012].
- The author of the Indian case report referred to it as nosocomial. Since the infant had been hospitalized for 3 day when she became symptomatic, the infection met that definition. The case occurred in 2006 but was recognized retrospectively from Bacteriology Laboratory records. Her medical records mentioned she had been breast fed prior to admission and during admission; there was no mention of tube feeding. No attempt was made to trace the source and breast milk was not cultured [P. Ray 2007=reference 51 and personal communication, March 19, 2012].

- This category also includes four infants who received liquid or ready-to-feed formula (RTF) as well as breast milk [2/03 KY, Stoll 2004=reference 52 (case occurred in 1998–2001), Aguirre-Conde 2007=reference 50, 8/07 AZ #1]. These cases are described further in the following paragraphs.
  - A pair of twins in a US neonatal ICU (NICU) became infected in 2003. Both twins received breast milk and liquid formula. An order was written for one twin to receive a specially formulated PIF. Investigation suggested that the other twin might have also received the PIF (in error). The open PIF can was taken by the manufacturer's representative and the company subsequently reported it as negative for *Cronobacter*; it could not be retrieved for further testing at CDC. The sterile water used in mixing the PIF was culture-negative, as were rectal swabs of five other infants in the nursery. There is no indication in records that the mother's breast milk was cultured. Based on pulse field electrophoresis (PFGE), investigators considered the twins' isolates indistinguishable from one another and similar to the isolate of a Colorado infant who received a PIF produced at the same factory. One of these two twins is listed in this category [2/03 KY]; the other is listed in the breast milk and PIF category.
  - One case was identified through the 1998–2001 US Neonatal Research Network data set; the infant was in a NICU and received breast milk and RTF. No investigation was done and further information is unavailable [Stoll 2004=reference 52] (case occurred in 1998–2001).
  - In one case in Spain, breast milk, RTF, environmental, and the mother's vaginal cultures were negative and the authors found no breaches in food preparation or storage techniques [Aguirre-Conde 2007=reference 53].
  - One US infant had RTF and breast milk in a newborn nursery and had only breast milk at home. He may have been symptomatic prior to his discharge but became clearly symptomatic within a day of release from the hospital. The mother's breast milk, breast pump tubing, and environmental samples were negative for *Cronobacter* [8/07 AZ #1].

- One infant received RTF concurrently with PIF [7/06 LA ]. I could not determine the temporal relationship for one infant [2010 TX]. Two infants were receiving concentrates at the time they became symptomatic [6/03 MI, 5/04 IL].
- Seven infants in this category received RTF in the newborn nursery but were not receiving it on the day they became symptomatic [2/00 KY, 12/02 WI, 6/03 MI, 5/04 IL, 12/04 CO, 5/05 MN, 9/06 SC]. These include the following four patients.
  - An infant who was born at a gestational age of 31 weeks and a birth weight of 837 grams was discharged in good condition at 51 days of life. She was fed RTF in the nursery and sent home with a bottle of RTF and a can of PIF. She finished the RTF four days after discharge and began the PIF. Twenty-four hours later she became symptomatic. [2/00 KY].
  - A 2002 US case, fed RTF in the nursery, following discharge on day 2 of life, was switched to PIF on day 3 of life, and became ill between 2 and 3 days later [12/02 WI].
  - A 2003 U.S. case received RTF for the first four days of life and then was switched to PIF, on which he had intermittent vomiting. He was changed to concentrate after four days on PIF and, after four days on concentrate, became symptomatic. Both an open can and reconstituted PIF were negative at the FDA but positive for multiple *Cronobacter* isolates, as well as other organisms, when tested at the MI State laboratory [6/03 MI].
  - A 2004 US case received RTF in the hospital for 2 days and developed symptoms at seven days of age, while on PIF at home [12/04 CO].
  - A 2006 US case received RTF until her discharge at 2 days of age, after which she was fed only from a sample can of PIF provided at hospital discharge. She became ill on day 5 of life. A small amount of formula left in the can and samples from the same lot tested negative for *Cronobacter* [9/06 SC].
- Three infants in this category received PIF and breast milk in a hospital [Biering 1989=reference 54, 12/03 KY, Jarvis 2005=reference 55]; one of these infants also received liquid formula [12/03 KY]. Five infants in this category received PIF at home [1/01 OH, 11/04 NC, 7/05 MI, 9/07 VA, 10/07 IA]. Two of these also received RTF [7/05 MI, 9/07 VA] and one also received RTF and concentrate [11/04 NC]. One infant's location at the onset of illness could not be identified (case occurred in 2008) [Japan Food Safety 2008=reference 56].
- Two infants in this category were involved in an Iceland NICU outbreak in which a *Cronobacter* isolate from an unopened can of PIF had the same plasmid profile and antibiogram as the patient isolates (cases occurred in 1986–1987) [Biering 1989=reference 54]. The investigators were unable to obtain samples from the mothers.
- One infant in this category was in a New Zealand NICU outbreak in which infants received two PIFs and breast milk. I could not locate information on whether the breast milk fed to this infant was tested but both PIFs were *Cronobacter* positive. An isolate from a can of one PIF was considered indistinguishable from the case patient's (and four other patients') isolates, based on PFGE. Other environmental samples were negative (case occurred in 2004)[Jarvis 2005=reference 55].
- Individual cases in this category include:
  - One infant's mother began breast feeding after delivery. When the infant appeared to be hungry after feedings, the hospital staff suggested the mother supplement breast feeding with PIF, which was done. The mother was given a supply of PIF on discharge. She had previously taken a course on food safety and, at home, sterilized the infant's bottles, washed her hands and cleaned the preparation area before preparing individual feedings, cleaned the kitchen counter several times a day, and cleaned the drains and faucets twice a week. She mixed the formula in the same area every time, never stored mixed formula, and threw out mixed, unfinished formula. The infant did not use a pacifier, receive vitamins, or have any intake other than PIF and breast milk. The mother cleaned her breasts before breastfeeding and noted they were never dry or sore and had no signs of infection. There is no record of either the breast milk or the PIF being tested for *Cronobacter* [1/01 OH].
  - In a 2003 U.S. case, the infant received PIF, breast milk, and

liquid formula; his twin may have received this case's PIF in error. The investigation for these siblings was described above, in the section on infants fed breast milk and no PIF [12/03 KY].

- One U.S. infant was breastfed for the first two weeks of life but received only PIF, without breast milk, for the eleven days prior to onset of symptoms. The infant received three types of PIF, as well as RTF and concentrate. In this case's investigation, PIF from two open cans were negative and a sink culture was positive and was considered by investigators to be indistinguishable from the patient's isolate, based on PFGE. [11/04 NC].
- One infant received RTF in the hospital after delivery. The mother was given RTF and PIF samples at infant's discharge, which she used to supplement breastfeeding. Supplements were stopped on day five and the infant became symptomatic on day eight of life. A *Cronobacter* isolate from a sample of an open can of the infant's PIF was considered indistinguishable from the infant's isolate, based on PFGE; a closed can was not available. The splash area around the sink was also positive but the isolate was reportedly not similar to the patient isolate. Breast milk was apparently not cultured [7/05 MI].
- One infant had received RTF and six feedings of PIF before becoming symptomatic. Two kitchen counter swabs were positive but the isolates were reportedly not similar to the patient's isolate: one from where formula was prepared and one from where the bottled water used in making formula was stored. Other environmental samples were negative, including the cupboard area where PIF was stored and the refrigerator shelf where RTF were kept. Samples from the bottled water and open and closed PIF cans were negative when tested at the CDC and the FDA. A sample of the same lot, tested at the FDA, was negative. I could find no evidence that breast milk was cultured [9/07 VA].
- One 2007 U.S. case had no breast milk after a week of age and became ill at one month of age. Lot numbers and PIF samples were not available for evaluation [10/07 IA].
- An infant in a Japanese NICU received PIF in the first few days of life, received only breast milk thereafter, and became ill on day 22 of life. There is no information concerning whether any epidemiologic or microbiologic testing was done (2008 case) [Japan Food Safety=reference 56].

- All five infants in this category received HMF in a hospital [2/02 MN, 3/02 TN, 12/03 TX]; two also received RTF [5/07 IA, 11/07 IL].
- In two cases, breast milk and environmental samples were negative for *Cronobacter* and an adequate amount of HMF was not available for testing [5/07 IA, 11/07 IL].
- Cases with more details include:
  - One infant who received HMF, RTF, and breast milk in the hospital. The breast milk, a small amount of the same lot of HMF, water samples, and environmental samples were all negative for *Cronobacter*. CDC records indicate that, based on PFGE, the infant's isolate appeared to be similar to those of two unassociated US cases. The infant's twin, who received breast milk and the same lot of RTF but not HMF did not become infected [5/07 IA].
  - One infant who had culture-negative breast milk and HMF samples. Investigators apparently considered his clinical isolate to be similar to those of two unrelated cases, based on CDC's PFGE [11/07 IL].

- Microbiologic and/or environmental testing was often not done. When done, information concerning the testing was often absent, incomplete, or unclear.
- Product testing, when done, was often not of material from unopened containers, often used a sample size and/or culture techniques not consistent with FDA/CDC protocols, sometimes was not done at a laboratory experienced in isolating this organism from non-clinical, dry-stressed samples, and always assumed a homogeneous distribution of contamination, which may not be consistent with the nature

§Cases as described in CDC files, FDA files, or the references cited.

of *Cronobacter* PIF contamination [Jongenburger=reference 39].

- Testing often did not include all products and materials fed to the infant and, when there was material remaining in the can used by the infant, the amount was often insufficient for adequate analysis.
- Very commonly, PIF testing was done on the lot/batch thought to be associated with the material fed to the infected infant, not the actual formula being consumed at the time of symptom onset. When the lot was tested, the production time, in relation to the can in question, was not noted. It does not appear that attempts were made to test product that approximated the production time of the PIF fed to the infant.
- In a few instances, testing appears to have been done on material from a batch that was not in any way related to the PIF consumed by the infant.
- In regard to cases involving HMF, it should be noted that HMF is distributed in small-volume packets. Therefore, there was little, if any, related material available for testing.

- In 12 incidents reviewed herein, involving 44 patients with invasive disease, *Cronobacter* was isolated from some associated PIF product (s) [Muytjens 1983=reference 57, Biering 1989=reference 54, Simmons 1989=reference 12, Van Acker 2001=reference 13, Bar-Oz 2001=reference 58, Nestlé 2002=reference 37, Himelright 2002=reference 59, 6/03 MI, Jarvis 2005=reference 55, 7/05 MI, Coignard 2006=reference 60, Caubilla-Barron 2007=reference 61].
  - In six of these incidents, involving 28 patients with invasive disease, *Cronobacter* was isolated from previously unopened PIF product(s) [Biering 1989=reference 54, Van Acker 2001=reference 13, Nestlé 2002=reference 37, Himelright 2002=59, Coignard 2006=reference 60, Caubilla-Barron 2007=reference 61].
  - In three of these incidents, involving five patients with invasive disease, the patients were also receiving breast milk [Biering 1989=reference 54, Jarvis 2005=reference 55, 7/05 MI].
  - In nine of these incidents, involving 35 patients with invasive disease, the investigators considered a PIF isolate to be indistinguishable from patient(s)' isolate(s) [Biering 1989=reference 54, using plasmid profiles and antibiograms; Simmons 1989=reference 12, using plasmid analysis and multilocus enzyme profiles; van Acker 2001=reference 13, using arbitrarily primed pcr; Bar Oz 2001=reference 58, using SpeI endonuclease restriction and PFGE; and Himelright 2002=reference 59, Jarvis 2005=reference 55, 7/05 MI, Coignard 2006=reference 60, and Caubilla-Barron 2007=reference 61, using PFGE].
  - In one Belgium outbreak a *Cronobacter* isolate from an open can of PIF was considered indistinguishable from patients' isolates, based on arbitrarily primed pcr. In that outbreak, when newborn intensive care unit (NICU) use of the implicated PIF was stopped, no new infections occurred. Subsequently, another infant was fed the formula and developed necrotizing enterocolitis. At that time, *Cronobacter* was isolated from a closed can; it was considered indistinguishable from that infant's isolate. Use was then terminated and there were no further infections [Van Acker 2001=reference 13].
  - In an Israeli outbreak involving two infected and three colonized infants, *Cronobacter* was isolated from prepared formula and a blender used to prepare formula. These isolates were considered indistinguishable from one another and from the five patients' isolates, based on PFGE [Bar-Oz 2001=reference 58].
  - In 17 incidents included herein, involving 18 patients with invasive disease, some form of PIF was tested by some laboratory, in some fashion, and all the results were negative [12/02 WI, 12/03 KY #1, 5/04 IL, 11/04 NC, 5/05 MN, 12/05 TN, 7/06 LA, 9/06 SC, 5/07 IA, 9/07 VA, 11/07 IL, 4/08 IA, 7/08 MD, 2008 NM#1, 5/10 TX, 2010 IN, and Switzerland 2005, twins A & B from Essers 2006=reference 62, Mange 2006=reference 63, and personal communications, A. Lehner, March 20, 2012]. These cases include one for which not all brands of PIF products consumed were tested [11/04 NC], one for which the product was tested at the infant's hospital, not CDC or FDA [12/02 WI], two that were tested by the manufacturer [12/03 KY#1, 5/04 IL], two for which the lot tested was noted to have possibly not been the implicated lot [5/04 IL, 7/08 MD], and four for which the amount tested was noted to have been

small [Muytjens 1983†=reference 57, 9/06 SC, 5/07 IA, 10/07 IL].

*In the five cases in which breast milk was noted to have been tested,* all samples were negative, including a breast pump in two cases and tubing in one case [Pavcnik-Arnol 2007=reference 50, Aguirre-Conde 2007=reference 53, 5/07 IA, 8/07 AZ #1, 11/07 IL].

*In one case, RTF was noted to have been tested* and was negative [Aguirre-Conde 2007=53]. In another case, FDA notes indicate that one of two unopened cans of RTF was negative for Cronobacter but positive for *pseudomonas aeruginosa*, suggesting the possibility of intrinsic contamination [5/10 TX].

*In ten incidents involving 29 patients with invasive disease, the water used to mix the PIF or HMF was tested* and found to be negative for *Cronobacter* [Muytjens 1983=reference 57, Van Ackers 2001=reference 13, Bar-Oz 2001=reference 58, Himelright 2002=reference 59, 12/03 KY, 7/05 MI, 5/07 IA, 9/07 VA, 4/08 IA, 7/08 MD].

- In 11 incidents involving 12 infants with invasive disease, environmental testing was not fully described but was noted to have been negative [Biering 1989=reference 54, Himelright 2002=reference 59, 2004 NZ, 12/05 TN, 5/07 IA, 8/07 AZ #1, Aguirre-Conde 2007=reference 53, 4/08 IA, 2008 NM#1, 5/10 TX, and Switzerland 2005, twins A & B from Essers 2006=reference 62, Mange 2006=reference 63, and personal communications, A. Lehner, March 20, 2012].
- In six incidents involving 16 patients, environmental testing was positive [Muytjens 1983=reference 57, Simmons 1989=12, Bar-Oz 2001=58, 11/04 NC, 7/05 MI, 9/07 VA]. Four of these involved areas (sink, splash area, counter, water storage area, dish drawer) [Muytjens 1983=57, 11/04 NC, 7/05 MI, 9/07 VA] and/or objects (serving spoon, blender) [Simmons 1989=reference 12, Bar-Oz 2001=reference 58] involved in formula preparation. In two instances, positive samples were considered indistinguishable from the patient(s) isolates [Bar-Oz 2001=reference 58, 11/04 NC].
- WHO's Annex 1 line list [FAO/WHO] indicates that, for one infant, PIF from open and sealed cans were negative but may not have been from the same lot as the one used by the infant. FDA records that appear to be related to this case indicate that nine environmental samples, two water samples, and a sample from an open can of PIF were tested at CDC and found to be negative but a nipple from a clean bottle set in a cabinet was positive for *Cronobacter*. FDA appears to have tested one unopened can from the home and two ten-can samples from two PIF lots; all were negative. The records I reviewed did not indicate the relationship between this material and the PIF consumed by the infant [7/08 MD].
- In another case, FDA notes indicate that environmental samples were negative but a pacifier was positive [2010 IN].

Microbiologic information of note, concerning cases not included in the analyses herein:

- *Cronobacter* contamination of eleven cleared and distributed lots of PIF were reported to the European Rapid Alert System for Food and Feed (FASFF) between 2002 and 2007 [Friedemann 2009=reference 24]. *Cronobacter* infections were reportedly associated with three of these contaminated products, one of which is included in the analyses herein [Nestlé 2002=reference 37]. Ten potential cases associated with the other two lots were not considered for inclusion herein because I was unable to obtain information to confirm or characterize them (nine listed as being in the Netherlands in 2004 and one in Switzerland in 2007) [reference 24].
- In at least three incidents (involving four cases) not meeting the criteria for inclusion in these analyses, a PIF product was positive and the isolate was considered to be indistinguishable from a patient isolate, based on PFGE [10/07 MI, 2008 NM#2, Parra Flores 2011=reference 9, and personal communications, J. Parra Flores, March 13, 2012].
  - The third of these instances involved two hospitalized Mexican infants with non-invasive disease (bloody diarrhea), fed solely U.S.-produced PIF. In the investigation of these two cases, *Cronobacter* was isolated from closed cans of the PIF, as well as prepared PIF. The PIF isolates were considered indistinguishable from the fecal matter isolates, in terms of biotypes, adhesion and invasiveness factors, and PFGE profiles [Parra Flores 2011=reference 9 and personal communications, J. Parra Flores, March 13, 2012].

†At the March 18th, 2003 FDA Food Advisory Committee Meeting on *Enterobacter sakazakii* (ie., *Cronobacter*) contamination of PIFs, Dr Klontz of the FDA stated that he telephoned Dr Muytjens, who indicated he cultured only 4 to 10 g of the implicated PIF products. Dr Muytjens also noted that no cases had occurred in 8 years, after the NICU switched from PIF to RTF.