

## 2. EPIDEMIOLOGY AND PUBLIC HEALTH ASPECTS

### 2.1 ORGANISMS OF CONCERN

#### 2.1.1 *Enterobacter sakazakii*

*Enterobacter sakazakii* is a gram-negative, non-spore-forming bacterium belonging to the Enterobacteriaceae family. On occasion, it has been associated with sporadic cases or small outbreaks of sepsis, meningitis, cerebritis and necrotizing enterocolitis. While *E. sakazakii* has caused disease in all age groups, the focus of this meeting was on cases that are reported in infants under 28 days old. Although incomplete, the published data on these infants indicate that approximately half of them had a birth weight of less than 2 000 g, and two-thirds were premature, being born at less than 37 weeks gestation. It is also likely that immunocompromised or medically debilitated infants are more susceptible to infections with *E. sakazakii*. The pattern of disease in term infants is less clear, with some having a major congenital abnormality (e.g. neural tube defects and Trisomy 21 [Down syndrome]), while others have no reported evidence of a compromised host defence, yet have been afflicted with *E. sakazakii* sepsis or meningitis (Lai, 2001). *E. sakazakii* bacteraemia has also been identified among older infants and infants at home (CDC, unpublished data). In addition, asymptomatic infants have been identified with *E. sakazakii* in their stools or urine (Biering et al., 1989; CDC, 2002; Block et al., 2002) and stool carriage has been demonstrated for up to 18 weeks (Block et al., 2002).

Mortality rates from *E. sakazakii* infection have been reported to be as high as 50 percent or more, but this figure has declined to under 20 percent in recent years. Significant morbidity in the form of neurological deficits can result from infection, especially among those with bacterial meningitis and cerebritis. While the disease is usually responsive to antibiotic therapy, a number of authors have reported increasing antibiotic resistance to drugs commonly used for initial treatment of suspected *Enterobacter* infection. Reports have also been made of  $\beta$ -lactamases and cephalosporinases from *E. sakazakii* (Pitout et al., 1997). Long-term neurologic sequelae are well recognized (Lai, 2001; Clark et al., 1990).

While the reservoir for *E. sakazakii* is unknown in many cases, a growing number of reports have established powdered infant formula as the source and vehicle of infection (Biering et al., 1989; Simmons et al., 1989; Van Acker et al., 2001; CDC, 2002). In several investigations of outbreaks of *E. sakazakii* infection that occurred among neonates in neonatal intensive care units, investigators were able to show both statistical and microbiological association between infection and powdered infant formula consumption (Simmons et al., 1989; Van Acker et al., 2001; CDC, 2002). These investigations included cohort studies which implicated infant formula consumed by the infected infants. In addition, there was no evidence of infant-to-infant or environmental transmission; all cases had consumed the implicated formula (Simmons et al., 1989; Van Acker et al., 2001; CDC, 2002). The formula consumed by infected infants in each of these outbreaks yielded *E. sakazakii*; in two outbreaks, formula from previously unopened cans from the same

manufacturing batch also yielded *E. sakazakii*. A combination of typing methods (plasmid analysis, antibiograms, chromosomal restriction fragment analysis, ribotyping, multilocus enzyme electrophoresis) were used to evaluate the isolates from each outbreak as to their relatedness. Though the typing methods differed, the isolates among cases and those obtained from the implicated formula shared the same typing pattern in each of these investigations.

In addition, the stomach of newborns, especially of premature babies, is less acidic than that of adults: a possible important factor contributing to the survival of an infection with *E. sakazakii* in infants. The frequency of intrinsic *E. sakazakii* contamination in powdered infant formula is of concern, even though intrinsic concentration levels of *E. sakazakii* appear to be typically very low.

In a study of the prevalence of *E. sakazakii* contamination in 141 powdered infant formulas, 20 were found culture-positive, yet all met the microbiological specifications for coliform counts in powdered infant formula (<3 cfu/g) of the current Codex code (Van Acker et al., 2001; Muytjens, Roelofs-Willemse, and Jasper, 1988). Such formula has been linked to outbreaks (Van Acker et al., 2001). Furthermore, outbreaks have occurred in which the investigators have failed to identify lapses in formula preparation procedures (Van Acker et al., 2001; CDC, 2002). Thus, it seems that neither high levels of contamination nor lapses in preparation hygiene are necessary to cause infection from *E. sakazakii* in powdered infant formula. While it can be assumed that lapses in preparation hygiene or extended holding at non-refrigerated temperatures could lead to increases in the levels of contamination at the time of consumption, it is not possible to assess the contribution that these factors have on the cases of infection that have been associated with powdered infant formula that contained low levels of *E. sakazakii*. Thus it must be currently assumed that low levels of *E. sakazakii* in infant formula (<3 cfu/100 g) can lead to infections.

Formula preparation equipment contaminated by *E. sakazakii* has been demonstrated to have caused two outbreaks (Noriega et al., 1990; Block et al., 2002), but the original source of *E. sakazakii* was not determined in either case. Environmental swabbing of formula preparation areas in the course of outbreak investigations has not demonstrated *E. sakazakii* in the general environment. *E. sakazakii* has been identified in the environments of milk powder production facilities and other food production facilities, as well as in households (Kandhai, Reij, and Gorris, 2004). Not all infants with *E. sakazakii* infection have been exposed to powdered infant formula, and *E. sakazakii* infections can also occur in adults (Lai, 2001). Thus, although an environmental source of *E. sakazakii* infection other than infant formula has not been strictly identified, other sources undoubtedly exist. The relative contribution of powdered infant formula sources and other sources to the burden of *E. sakazakii* disease is unknown.

There is very little known about virulence factors and pathogenicity of *E. sakazakii*. The work done by Pagotto et al. (2003) was the first describing putative virulence factors for *E. sakazakii*. Enterotoxin-like compounds were produced by some strains. Using tissue cultures, some strains produced a cytotoxic effect. Two strains (out of 18 isolates) were capable of causing death in suckling mice by the peroral route. Therefore, there appear to be differences in virulence among *E. sakazakii* strains, and some strains may be non-pathogenic. Brain abscesses due to *E. sakazakii* and the related bacterium (*Citrobacter koseri*) are morphologically similar and may be due to similar virulence mechanisms (Kline, 1988).

## 2.1.2 Other relevant organisms of concern

Although liquid, ready-to-feed infant formula is commercially sterile, powdered infant formula is not. Enterobacteriaceae were present in 52 percent of 141 different formulas from 35 countries in one 1988 study (Muytjens, Roelofs-Willemse, and Jasper, 1988). Enterobacteriaceae are also common aetiologies for systemic infection in neonates and, to a lesser extent, older infants. *E. sakazakii* may be a sentinel organism, receiving attention due to its relative rarity. Other Enterobacteriaceae from powdered infant formula may also be responsible for systemic infections in infants, but there is little reported information to determine their role. One outbreak of *Citrobacter freundii* infections in a neonatal intensive care unit did identify formula as the vehicle of infection, though it was unclear if the formula was intrinsically (the source) or extrinsically contaminated.

*Salmonella* contamination of infant formula has been responsible for multiple outbreaks (Picket and Agate, 1967; Rowe et al., 1987; CDC, 1993; Usera et al., 1996; Threlfall et al., 1998; Olsen et al., 2001; Bornemann et al., 2002). Similar to *E. sakazakii*, low-level intrinsic contamination of powdered infant formula with *Salmonella* was epidemiologically and microbiologically associated with infections in infants in these outbreaks. Rates of salmonellosis are also highest in infants compared to any other age group (Olsen et al., 2001). The factors that give newborns a relatively high risk of infection include the relative gastric achlorhydria, the buffering capacity of the milk, the use of high iron infant formula and the need for frequent diaper changing (Miller and Pegues, 2000). Unlike *E. sakazakii* and other Enterobacteriaceae, however, *Salmonella* is rarely found in surveys of powdered infant formula. In the study surveying 141 different formulas by Muytjens, Roelofs-Willemse, and Jasper (1988), no samples yielded *Salmonella*.

Powdered infant formula has never been convincingly identified as a vehicle or source of infection for sporadic cases as opposed to outbreaks of infection with *Salmonella*, but this may well be due to the greater difficulty of identifying vehicles for sporadic infection. It would be illogical to conclude that sporadic infection due to powdered infant formula never occurred, but its frequency is unknown. Ongoing, large, sporadic case-control studies in the United States will be valuable in determining any potential association between significant numbers of sporadic salmonellosis cases and powdered infant formula.

## 2.2 SCOPE/CASE DESCRIPTION

The meeting considered illnesses in infants (i.e. children <1 year) linked to microorganisms (or their toxins) associated with powdered infant formula either epidemiologically or microbiologically.

### 2.2.1 Identification of products considered

The products under consideration were those in powdered form, specially manufactured and presented to be used by infants, either as a breastmilk substitute after preparation with water, or to modify prepared breastmilk substitutes or fortify human milk. Included products are, therefore, infant formula (as defined in Codex Stan 72-1981<sup>1</sup>), follow-up formula (as defined in Codex Stan

<sup>1</sup> Available at: [ftp://ftp.fao.org/codex/standard/en/CXS\\_072e.pdf](ftp://ftp.fao.org/codex/standard/en/CXS_072e.pdf).

156-1987<sup>2</sup>), formula for special medical purposes intended for infants (as defined in Appendix V of Codex Alinorm 04/27/26<sup>3</sup>), formulas for special medical purposes for the partial feeding of infants (covered by Codex Stan 180-1991<sup>4</sup>) and human milk fortifiers.

Breastmilk substitutes are needed when infants do not have access to breastmilk for various reasons. Commercial infant formulas are usually used as breastmilk substitutes for normal healthy infants under 6 months, and are formulated industrially in accordance with the appropriate Codex standards. Formulas for special medical purposes intended for infants are breastmilk substitutes for sick infants (patients). Although other milks may be used after 6 months, follow-up formulas can substitute for breastmilk in the older infant who also eats complementary food. These three types of formula only need the addition of water to be ready for consumption and, therefore, are often also available in liquid, ready-to-feed form. However, formulas for special medical purposes for the partial feeding of infants require the addition of measured amounts of other foods, quite often also in powdered form, to satisfy the special nutritional needs of the individual infant patient.

Human milk fortifiers are powdered supplements which can be added to expressed human milk when the nutritional requirements of low-birth-weight infants (<2 500 g) and especially very low-birth-weight infants (<1 500 kg) are not satisfied by human milk alone. This can include thickening agents, such as starches or simple cereals which are specially manufactured for the purpose of increasing the consistency of the liquid food, and can be added to formula intended for infants with gastro-oesophageal reflux.

Throughout the following report, the term “powdered infant formula” includes all of the products mentioned above, but excludes cereals.

### 2.2.2 Case definition

The meeting considered illnesses in infants (i.e. children <1 year) due to microorganisms (or their toxins) associated with powdered infant formula consumption either epidemiologically or microbiologically. Figure 1 provides a graphic account of the issues included and excluded in the scope of the work. The area of intersection of all three circles, i.e. area 7, represents the scope of the meeting.

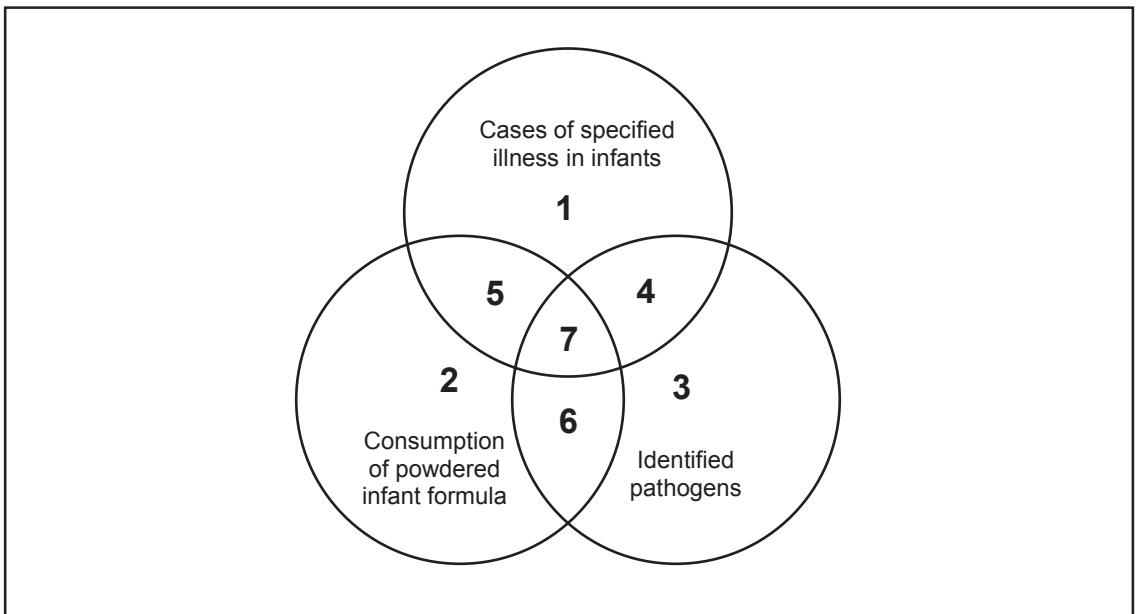
It should be noted that certain illnesses in infants caused by powdered infant formula may have been excluded, including those for which no microorganism has been reliably identified (area 5 in Figure 1) and those for which the microorganism has not been reliably associated with powdered infant formula (area 4 in Figure 1). In addition, bacteria detected in infant formula were not considered by the meeting if there was no evidence that these bacteria are associated with illness in infants (area 6 in Figure 1).

<sup>2</sup> Available at: [ftp://ftp.fao.org/codex/standard/en/CXS\\_156e.pdf](ftp://ftp.fao.org/codex/standard/en/CXS_156e.pdf).

<sup>3</sup> Available at: [ftp://ftp.fao.org/codex/alnorm04/al04\\_26e.pdf](ftp://ftp.fao.org/codex/alnorm04/al04_26e.pdf).

<sup>4</sup> Available at: [ftp://ftp.fao.org/codex/standard/en/CXS\\_180e.pdf](ftp://ftp.fao.org/codex/standard/en/CXS_180e.pdf).

Young children (i.e. >1 year) consume powdered formula and may experience illness associated with the microbiological contamination of powdered formula. However, these illnesses were not considered by the meeting, as it was believed that the spectrum of illness could be adequately represented by infants under 1 year (and subpopulations of infants <1 year) and these same groups were considered to be the populations most at risk.



**Figure 1.** Graphic representation of the issues considered in defining the scope of the meeting.

1. Cases of illness in infants
2. Consumption of powdered infant formula
3. Identified pathogens
4. Illness in infants caused by a specific microorganism (powdered infant formula not related to illness)
5. Illness in infants associated with consumption of powdered infant formula (microorganism unknown)
6. Specific microorganisms in powdered infant formula but not causing illness
7. Specific microorganisms in powdered infant formula that resulted in cases of illness in infants = scope

