Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on the microbiological risks associated with the consumption of certain foods for children aged 0 to 3

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Abstract

Children under 3 years old are particularly susceptible to foodborne diseases, due in part to the immaturity of their immune systems. The rate of foodborne diseases among this age group is much higher than it is for the general population. Considering the strict sanitary controls in place during paediatric stage, methods of prevention based on risk communication and health education might be the most effective for the infants and young children population group.

Therefore, in order to determine some basic principles for implementing management activities and risk communication, the Scientific Committee has drafted a report, examining certain foodborne pathogens which present a particularly high risk for infants and young children, in each case analysing the factors which affect their survival and growth, as well as the most effective prevention methods, and highlighting what people can do themselves.

A distinction is made between the main microbiological risks for each age group: infants (either breastfed or fed with formulae), children on soft foods and children on solid foods.

In the case of breastfed children, infections that should absolutely be avoided are reviewed (brucellosis, HIV, HTLV), as well as ones that should generally be avoided. Meanwhile, infants fed with formulae are especially at risk of infection from *Salmonella* and *Cronobacter*, and there is clear evidence for a causal relationship between its presence in prepared infant formulae and them developing an illness. The key food hygiene precautions to take when preparing and using feeding bottles are presented.

In terms of children with a soft or solid food diet, there is an emphasis on the need to include instructions for handling food hygienically at home in risk communication campaigns. Lastly, a list of foods which present a risk to this population group is included.

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Key words

Infants, young children, microbiological risks, *Salmonella*, *Cronobacter*, virus, foodborne pathogens, infant formulae, preparation of infant feeding bottles, infant food, hygienic food handling.

1. Glossary

The terms and definitions used in this document are those listed in the applicable legislation:

- Royal Decree 867/2008, of 23 May, approving the specific Technical and Health Regulations for infant and follow-on formulae, implementing Directive 2006/141/EC (BOE, 2008):
  - Infants: children under the age of 12 months.
  - Young children: children between the age of 1 and 3 years.
  - Infant formulae (IF): foodstuffs intended for particular nutritional use by infants during the first months of their life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary feeding.
  - Follow-on formulae: foodstuffs intended for particular nutritional use by infants when appropriate complementary feeding is introduced and constituting the principal liquid element in a progressively diversified diet of such infants. These products, as indicated elsewhere in the Royal Decree, are suitable only for particular nutritional use by infants over the age of 6 months. They should only form part of a diversified diet, and must not be used as a substitute for breast milk during the first 6 months of life. The decision to begin complementary feeding, including any exception to 6 months of age, should only be made on the advice of independent persons having qualifications in medicine, nutrition or pharmacy, or other professionals responsible for maternal and child care, based on the individual infant's specific growth and development needs. This information must be included on the labelling of these foodstuffs.
- Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (EU,2005):
  - Foodstuffs intended for infants: foodstuffs particularly intended for infants, as defined in Directive 2006/141/CE.
  - Foods for special medical purposes: dietary foods intended for special medical purposes, as defined in Directive 1999/21/EC.
- Royal Decree 490/1998, of 27 March, approving the specific Technical and Health Regulations for cereal-based foods and baby foods for infants and young children, implementing Directive 96/5/CE (BOE, 1998).
  - Cereal-based foods, which are divided into the following four categories:
    a) Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids.
    b) Cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid.
    c) Pastas which are to be used after cooking in boiling water or other appropriate liquids.
    d) Rusks and biscuits which are to be used directly or, after pulverisation, with the addition of water, milk, or other suitable liquids.
  - Baby foods other than cereal-based foods.

2. Introduction

Children under the age of 3 years are particularly susceptible to food-borne diseases due to a number of physiological factors, including the immaturity of their immune system, the reduced level of production of gastric secretion (Rabet et al., 2008) or the existence of risk factors associated to their specific behaviour (crawling, putting hands and objects in the mouth, etc.) which increase the probability of exposure to enteric pathogens (Sockett and Rodgers, 2001). The incidence of food-borne diseases in this age group is much higher than for the general population, even considering that they are diagnosed more often (Scallan et al., 2013).

The neonate and the infant have immunological differences to the adult immune system. Neonates and healthy children are immunocompetent but, due to the unspoilt condition of their immune system, they react differently to antigens and are temporarily less competent when facing certain pathogens. They are therefore more susceptible to infection. Immunological competence develops gradually and is an adaptation process through exposure to antigens and pathogens.

In normal conditions, previous exposure does not exist and the immune system must adapt to life outside the womb. This inexperience and the prevalence of suppressor factors during pregnancy increase the susceptibility of the neonate and small child to infection.

One important factor for the effectiveness of the immune system is the gestational age. The neonate born full-term (>37 weeks) is more competent than the premature baby (<37 weeks). At birth, the child has received maternal IgG through the placenta and has levels and a specificity pattern comparable to that of the mother. In premature babies these values decrease in proportion with the gestational age. The figures for IgG fall during the first weeks of life after birth, they are metabolised and gradually replaced by produced IgG. Other immunoglobulins do not cross the placenta, although the foetus is able to synthesise IgA and IgM in response to intrauterine infection (Gronlund et al., 2000).

After birth, breast milk is responsible not only for feeding, but also for supplying protective elements, the majority of which are cells and soluble factors that are absent in the neonate (Martín et al., 2003). The intestinal colonisation of the neonate starts immediately after birth and is essential for the maturation of the lymphoid tissue associated to the intestine. During breastfeeding this is the only moment at which the human being not only receives all the necessary nutrients in one single food, but also this food meets the requirements suited to the functional immaturity of the digestive, kidney and immune systems of the baby (Bahl et al., 2005).

In the healthy child, the introduction of artificial formulae for feeding and of supplementary foodstuffs at later stages is one of the first immunological and nutritional challenges faced by the infant and small child.

From the age of 6 months, the energy and nutrient requirements begin to exceed the levels provided by breast milk. From this moment, it is necessary to cover the energy and nutrient needs.

In many countries, the period of supplementary feeding, from 6 to 23 months, is when the number of infectious diseases peaks, and delayed growth and micronutrient deficiencies are seen (Dewey and Adu-Afanwuah, 2008).

3. Main food pathogens affecting infants and young children

3.1 Bacterial pathogens

Almost any food-borne pathogenic microorganism can affect young children. A difference must be drawn between infants (breast milk, infant and follow-on formulae), those children who eat pureed food and those who have a solid food diet.

With regard to powdered formulae, the FAO/WHO Committee of Experts (Food and Agriculture Organisation of the United Nations/World Health Organization) identifies three categories of microorganisms based on the strength of the evidence of a causal relationship between their presence in infant formulae and the development of disease in these infants (FAO/WHO, 2004a, 2006):

1. Microorganisms with clear evidence of causality. This category includes *Cronobacter* spp. and *Salmonella* spp.
2. Microorganisms with plausible causality, that is, they produce disease in infants and have been found in formulae, but it has not yet been demonstrated (epidemiologically or microbiologically) that the contaminated formulae are the source of infection. This includes species from the family *Enterobacteriaceae*, such as *Citrobacter* spp.
3. Microorganisms for which causality is less plausible, such as those that cause disease in infants but have not been identified in the formulae or which have been isolated from the formulae but have not been implicated as agents of disease in infants. This group includes *Escherichia coli*, *Serratia* spp., *Acinetobacter* spp., *Bacillus cereus*, *Clostridium difficile*, *Clostridium perfringens, Clostridium botulinum, Listeria monocytogenes* and *Staphylococcus aureus.*

In addition to the microorganisms listed in these reports, it is also important to consider the specific data available in this respect in Spain. The Microbiological Information System (SIM) provides the results of food-borne diseases by age group. Thus, it can be seen that in children under the age of one, in addition to the above-mentioned microorganisms, *Campylobacter jejuni, Campylobacter coli* and *Yersinia enterocolitica* are also of note, together with *Cryptosporidium* and *Giardia lamblia* protozoa (SIM, 2015). Lastly, this list could include other microorganisms not mentioned above but which are recognised as a major cause of paediatric disease in developed countries, especially *Shigella* spp., *Aeromonas caviae* and *Aeromonas hydrophila* (Wilcox et al., 1992) (Vzvan Pelt et al., 2003) (Koehler et al., 2006).

In the case of children fed on solid food (in the epidemiological studies it is usual to separate the age group of children from 1 to 4 years), among the microorganisms reflected in the SIM and in studies conducted in developed countries *Campylobacter* spp., *Salmonella* spp., *Yersinia enterocolitica, Shigella* spp., some pathogenic types of *E. coli* and *Listeria monocytogenes* are listed as the most frequent (Van Pelt et al., 2003) (Koehler et al., 2006).

3.1.1 *Cronobacter* spp.

Cronobacter spp. is a genus of Gram-negative bacteria belonging to the family *Enterobacteriaceae*, whose members are considered to be opportunistic pathogens. This genus (previously called *Enterobacter sakazakii* and reclassified in 2007) comprises, at present, seven species: *C. sakazakii, C. turicensis, C. malonaticus, C. muytjensii, C. dublinensis, C. universalis* and *C. condimenti.* Based on the currently available data, none of these species can be dismissed as without risk for neonates and infants (FAO/WHO, 2008a).

Cronobacter causes a serious disease in newborns, leading to meningitis, septicaemia and necrotising enterocolitis, and is often linked to infant formulae. Symptoms start with fever, loss of appetite, crying and lethargy. In the adult population, symptoms may include fever, diarrhoea and urinary tract infection. Although it can affect all age groups, the vast majority of cases are observed in babies under the age of 28 days (EFSA, 2004). Premature infants, with a low birth rate or with immunosuppression, are part of the high risk group. Mortality rates of 40 to 80 % have been described and survivors often suffer from severe neurological sequels (Bowen and Braden, 2006) (Friedemann, 2009) (Holy and Forsythe, 2014). The disease usually responds to treatment with antibiotics, although several authors have reported a growing resistance (FAO/WHO, 2004a). Up until 2008, approximately 120 cases had been reported around the world in children up to the age of 3 years (FAO/WHO, 2008a), although it is suspected that there may be many more (Iversen and Forsythe, 2003). Of these, 6 were diagnosed in children aged between 6 and 11 months old and 2 in children between 1 and 3 years old (FAO/WHO, 2008a) and, at least 27 of these had a fatal outcome. From the United States Centers for Disease Control and Prevention (CDC) database, it can be estimated that there are six new cases of infection by *Cronobacter* every year all over the world (Healy et al., 2010).

The main transmission route is thought to be oral. The infective dose has not been established, but it is estimated to be low (10-100 microorganisms). Nevertheless, its widespread distribution suggests that the consumption of low numbers of microorganisms in infant formulae and follow-on formulae by healthy babies and children does not entail any disease (EFSA, 2004). The possibility of vertical transmission has been dismissed, as the microorganism has not been isolated from the intestinal tract or from the vagina of mothers of infected children (Iversen and Forsythe, 2003). Nor are there any records of transmission between infants or through the environment (FAO/WHO, 2004a). To date, the specific molecular mechanisms of the pathogenicity of *Cronobacter* have not been identified, although some strains produce some compounds similar to enterotoxins (Pagotto et al., 2003). In addition, there appear to be differences between the virulence of clinical, environmental and food isolates (FAO/WHO, 2008a). The fact that the stomach of a newborn and, in particular, of a premature baby, is less acid that an adult one may contribute to the survival of *Cronobacter* in infants.

Its minimum growth temperature is between 5.5 and 8 ºC and the maximum between 41 and 45 ºC, although some strains are able to multiply at 47 ºC. Thus, the optimum temperature is estimated at between 37 and 43 ºC, depending on the culture medium (Iversen et al., 2004). It is able to grow at pH values of between 3.9 and 9.0 (Breeuwer et al., 2003) (Dancer et al., 2009) and

at water activity values of up to 0.94 (Dancer et al., 2009). In addition, many strains produce an exopolysaccharide, allowing them to create biofilms and to resist adverse conditions on different surfaces (Iversen et al., 2004).

There is no agreement as to whether it is more tolerant to heat treatment than other non-spore-forming bacteria. It has been suggested that *Cronobacter* is one of the most heat-resistant members of the *Enterobacteriaceae* family (Nazarowec-White and Farber, 1997b) (Dancer et al., 2009), although it has been demonstrated that its resistance to heat varies significantly among the different strains, with D values of between 0.1 and 15 min at 58 ºC and z values of between 3.1 and 10.9 ºC in infant formulae and different laboratory mediums (Nazarowec-White and Farber, 1997b) (Asakura et al., 2007) (Al-Holy et al., 2009) (Arroyo et al., 2009) (Dancer et al., 2009) (Osaili et al., 2009) (Huertas et al., 2015). This heat-resistance is affected by different factors, including the culture medium, the stage of growth or the prior application of thermal shock (Gadjosova et al., 2011).

It is considered to be an ubiquitous microorganism and has been isolated in a large variety of sources, including very different foods, of both plant and animal origin, mixed dehydrated raw materials, waste water and other domestic environments, hospitals and food production lines (Norberg et al., 2012). Its prevalence is greater in dried food. Its main competitive advantage compared to other microorganisms causing food-borne disease with which it shares approximately similar temperature, pH and water activity growth limits, is its high resistance to desiccation, making it particularly relevant in dry environments, such as those of infant formulae and processing plants. In fact, *Cronobacter* has been confirmed to be more resistant to osmotic stress and desiccation than other enterobacteria (Breeuwer et al., 2003).

Infant formulae and follow-on formulae have been epidemiologically associated with outbreaks of disease caused by *Cronobacter*. The manufacturing processes for both types of food are essentially identical, the only difference being that the follow-on formulae contain a higher number of ingredients. Different studies reveal its presence at variable frequencies, from 0 to 14 % in infant formulae and other food for newborns (Muytjens et al., 1988) (Nazarowec-White and Farber, 1997a) (Iversen and Forsythe, 2004) (Kandhai et al., 2010) (Sonbol et al., 2013) (Li et al., 2014) (Pan et al., 2014). In any case, *Cronobacter* is generally present in infant formulae at very low levels (between 0.36 and 66.0 CFU/100 g) (Muytjens et al., 1988) (Forsythe, 2005). Nevertheless, according to the FAO and the WHO, the low levels of *Cronobacer* present in infant formulae, even when less than 3 CFU/100 g, can result in infection (FAO/WHO, 2004a). Even so, the European Food Safety Authority (EFSA) highlighted that, already in 2004, the large-scale production of infant formulae and follow-on formulae, both of which are distributed all over the world, combined with the relatively low number of cases in neonates and children, are signs that the products are usually safe. Although infant formulae are the main source of this microorganism, they are not the only one, as cases have been diagnosed in children not fed with infant formulae (Stoll et al., 2004). Moreover, cases have been reported in adults.

The contamination of infant formulae with *Cronobacter* may occur during the production process (Asakura et al., 2007). There is wide consensus that *Cronobacter*, in spite of its high level of resistance to heat, is unable to survive the pasteurisation treatments normally applied

during the processing of the infant formulae. This therefore implies that contamination most probably takes place after the heat treatment (Iversen and Forsythe, 2004), either as a result of the addition of heat-sensitive raw materials and nutrients (vitamins, minerals, etc.) after pasteurisation, or due to the contamination of the equipment and processing lines and packaging, or due to food handlers, carriers of the microorganism. Another source of contamination is found in contaminated utensils (such as blenders or spoons) which may be used in the home to prepare the infant formulae (Noriega et al., 1990).

Cronobacter is not able to multiply in dried infant formulae, but has been shown to be able to survive during the industrial dehydration process (Arku et al., 2008). Other studies have revealed its capacity to survive in dry atmospheres for long periods of time (Mullane et al., 2008) (Terragno et al., 2009). It has also been demonstrated to be able to survive in infant formulae with a water activity as low as 0.2 for periods of up to two and a half years (Caubilla-Barron and Forsythe, 2007).

Reconstituted infant formulae are, on the other hand, an ideal medium for its proliferation. Some studies have found that *Cronobacter* can survive the thermal stress applied when the infant formula is reconstituted using warm water (Asakura et al., 2007) (Osaili et al., 2009). The FAO and the WHO recommend the reconstitution of infant formulae using water at a temperature of more than 70 ºC to reduce the potential risk of *Cronobacter* (FAO/WHO, 2006). This would result in a reduction of its population by 4-6 log cycles, depending on the product type (Osaili et al., 2009). In addition, it has been found that its exposure to gentle heating or to acid conditions may lead to greater viability during the subsequent rehydration of the infant formulae at higher temperatures and even in the acid conditions of the stomach (Yang et al., 2015). Once the infant formulae has been reconstituted, *Cronobacter* is able to multiply depending on the preparation and storage conditions, and consequently it should be stored at temperatures of less than 5 ºC (FAO/WHO, 2006).

Regulation (EC) No 2073/2005, of 15 November 2005, on microbiological criteria for foodstuffs, and its subsequent amendments, establishes a maximum microbiological limit of absence of Cronobacter in each of 30 samples (n=30) of 10 g for dried infant formulae and dried dietary foods intended for special medical purposes for infants under the age of 6 months, throughout the product shelf-life (EU, 2005). For follow-on formulae a hygiene criteria of the processes for enterobacteria is established, with absence in each of five samples of 10 g taken at the end of the manufacturing process. In the event of a positive result, an analysis for *Cronobacter* must be conducted (Annex I).

3.1.2 *Salmonella* spp.

Salmonella, as is *Cronobacter*, is a well-known cause of disease, including diarrhoea, septicaemia or meningitis, in infants (FAO/WHO, 2004a). Just as in the cases of *Cronobacter*, the rates of salmonellosis in infants are higher than in other groups (Olsen et al., 2001), indicating the greater susceptibility of this population group. A recent study which analysed the incidence of the five principal food-borne pathogens in the United States, determined that *Salmonella* is the main cause of bacterial disease in children under the age of 5 years (Scallan et al., 2013).

Systemic infection is a complication which appears in approximately 5 % of the cases, and is

most frequent in immunosuppressed patients, especially those with cellular immune alterations (Chen et al., 2013). Secondary bacteraemia is associated with extra intestinal manifestations such as meningitis, encephalopathy, endocarditis, pneumonia, abscesses, osteomyelitis, cellulitis or arthritis. In children aged between 0 and 6 years with bacteraemia, the risk of meningitis is 24 % (Sánchez-Vargas et al., 2011). In addition, a recent study links a history of gastroenteritis due to *Salmonella* in children to a higher risk of irritable bowel syndrome when adult (Cremon et al., 2014).

The foods most frequently involved in the transmission of *Salmonella* include unpasteurised milk and milk derivatives, raw or undercooked meat and poultry, raw or undercooked eggs, raw sprouts (alfalfa, soy, radish), raw vegetables and any dish prepared using the above, including salads, desserts, sauces, etc. (Wattiau et al., 2011).

Salmonella has been isolated occasionally from infant formulae (FAO/WHO, 2004a). Between 1995 and 2006 at least six outbreaks of salmonellosis were linked to powdered milk products in different countries (FAO/WHO, 2006). The contamination of infant formulae with low levels of *Salmonella w*as sufficient to cause infection in infants. In these cases, errors in the production process, for example the presence of water in normally dry areas, which permitted the multiplication of *Salmonella*, or the presence of *Salmonella* in zones difficult to access or clean, were identified as the source of the contamination (FAO/WHO, 2006).

Nevertheless, and unlike other outbreaks caused by *Salmonella,* it has not been possible to convincingly demonstrate from an epidemiological or microbiological aspect that infant formulae are the source or the vehicle of the infection in sporadic cases (FAO/WHO, 2004a). In addition, in studies conducted on infant formulae, *Salmonella* has rarely been detected. In a study by Muytjens et al. (1988), *Salmonella* was not found in any of the 141 samples analysed.

As occurs with *Cronobacter, Salmonella* is not able to multiply in dried infant formulae, although it can survive for long periods of time in the dried formula. Once reconstituted, the infant formula is the perfect medium for the growth of *Salmonella*. Storage of the reconstituted infant formulae at temperatures of less than 5 ºC would avoid the multiplication of *Salmonella* (FAO/WHO, 2006).

Salmonella is excreted in faeces after infection for a period of 5 weeks, or longer in children under the age of 5 years (Sánchez-Vargas et al., 2011). As is the case with all enteric pathogens, good hygiene and good practices when handling food are the keys to prevent transmission.

Regulation (EC) No 2073/2005 establishes a food safety microbiological criteria of absence of *Salmonella* in each of 30 samples (n=30) of 25 g for dried infant formula and for dried dietary foods intended for special medical purposes for infants under the age of 6 months, and for dried follow-on formulae, throughout the self-life of these products (EU, 2005) (Annex I).

3.1.3 *Listeria monocytogenes*

This is a Gram-positive, non-sporulating bacterium, which causes a very serious food-borne disease (FAO/WHO, 2004b).

In the European Union (EU), listeriosis is an uncommon but very serious food-borne disease, in comparison with other food-borne processes, such as salmonellosis. In addition, it has high rates of morbidity, hospitalisation and mortality in vulnerable population groups (EFSA, 2013).

The mortality rate may reach 20-30 %. *Listeria monocytogenes* is an opportunistic pathogen that almost always affects individuals with severe underlying pathologies accompanied by immunosuppression. In neonates, the most serious and precocious form is infantiseptic granulomatosis, with disseminated abscesses and granuloma particularly in the liver, spleen, lung and brain. There is a later and more frequent neonatal form which appears between the first and eighth week of the child's life, where the contagion probably took place in the delivery channel. The most frequent symptoms are meningitis, with lethargy, refusal to feed and irritability.

Listeria monocytogenes is widely distributed in the environment and may contaminate food at various stages in the food chain. The usual heat treatment used in the preparation of food (>65 ºC) is sufficient to eliminate it, but, due to its ubiquity, it is able to re-contaminate food after the processing; this, linked to its capacity to develop at refrigeration temperatures, means that some ready-to-eat products, especially those with a medium or long shelf-life (semi-preserved fish, heat-treated meat products, soft cheeses), are particularly dangerous (FAO/WHO, 2004b) (EFSA, 2013). Regulation (EC) No 2073/2005 establishes a food safety microbiological criteria for this microorganism for ready-to-eat foods intended for infants in which the absence is required in 25 g of the product throughout its shelf-life (EU, 2005) (Annex I). For ready-to-eat foods not intended for infants, the maximum limit is 100 CFU/g during the shelf-life of the food, considering that the risk of developing listeriosis due to the intake of contaminated food with counts below this limit for the general population is very low (FAO/WHO, 2004b) (EU, 2005).

3.1.4 *Campylobacter* spp.

Campylobacter spp. is the principal bacterial zoonotic agent in the world (EFSA, 2005) (Epps et al., 2013). Species from the thermophilic group (*C. jejuni* and *C. coli*) are widespread throughout the environment and their main reservoir is the digestive tract of birds and some mammals, such as the pig, and to a lesser extent, cattle and other ruminants (EFSA, 2005). Its capacity for survival in the environment is limited, as it is unable to grow at temperatures below 30 ºC and requires low oxygen concentrations (5 % optimum); it is also sensitive to drying, sodium chloride at concentrations of more than 2 %, a pH of less than 4.9 and numerous disinfectants, and to heat treatments such as pasteurisation or complete cooking (Park, 2002). The majority of *Campylobacter* infections are sporadic and outbreaks are infrequent. Poultry meat is the principal source of transmission of the disease, either due to the consumption of undercooked contaminated meat, or due to cross contamination (Acheson and Allos, 2001) (FAO/WHO, 2009). In developed countries there is a distribution of cases with a higher rate in children under the age of 1 year (Acheson and Allos, 2001). In the case of Spain, the figures from the SIM also show a high number of cases in children aged between 1 and 4 years.

Campylobacteriosis is a gastrointestinal disease similar to that produced by other enteric pathogens and in most cases it is self-limiting. There may occasionally be gastrointestinal or systemic complications, which, although infrequent, are more likely in young children (EFSA, 2005). The mortality from the infection is low; nevertheless, the possibility of developing severe

sequels, such as reactive arthritis or Guillain-Barré syndrome, and the terrible consequences that these sequels may have in the infant population, require maximum precaution to prevent its transmission (EFSA, 2005).

In the case of neonates, the majority experience a moderate disease, although the infection evolves more often than in adults towards neonatal sepsis or the appearance of meningoencephalitis. The latter complication, more frequent in the case of *C. fetus*, can be fatal or may leave severe neurological sequels (Smith, 2002). In neonates, the mortality rate due to *Campylobacter* reaches 2.5 % (McDonald and Gruslin, 2001).

The most efficient preventive measures at home are good personal hygienic practices (hand-washing) and prevention of cross contamination when handling raw food, paying special attention to poultry.

3.1.5 *Escherichia coli*

All the pathogenic groups of *Escherichia coli* are agents of gastrointestinal disease in children. It is known that the infective dose in children under the age of 5 years is far less than for adults. In addition, it has been demonstrated that the period of excretion through faeces after the symptoms have disappeared is longer than in adults, implying a higher risk of acquisition in schools and kindergartens (Woteki and Kineman, 2008). Most of the pathogenic groups of *Escherichia coli* are uncommon in developed countries, as its principal reservoir is humans and transmission is related to the presence of faecal contamination in water and the lack of hygienic measures during the preparation of food (Nataro and Kaper, 1998) (Kaper et al., 2004). Nevertheless, the Shiga toxin-producing strains (STEC/EHEC) and the atypical strains of enteropathogenic *Escherichia coli* (aEPEC) are associated with production animals, mainly dairy cattle, and are often transmitted through food of animal origin (Nataro and Kaper, 1998) (Trabulsi et al., 2002).

5-10 % of the patients infected by EHEC develop haemolytic uremic syndrome (HUS), with a mortality rate of 3-5 %. HUS is the primary cause of acute kidney failure in small children and is associated with severe neurological complications (convulsions, coma, etc.) in 25 % of the cases, and with chronic kidney failure in approximately 50 % of the survivors (Lim et al., 2010).

The principal outbreaks of infection due to EHEC are associated with the consumption of undercooked beef, especially hamburgers, undercooked meat derivatives such as salami or sausages, unpasteurised milk, raw sprouts, fresh spinach, tomatoes, lettuce and unpasteurised apple juice (Ferens and Hovde, 2011). In this respect, the consumption of raw milk is a risky practice, as there are confirmed cases of HUS for this reason in children (Allerberger et al., 2003). A study conducted in France on paediatric cases of HUS over 1 year revealed that *Escherichia coli* O157 was the principal agent of this disease and that the greatest incidence occurred in children under the age of 1 year, followed by the age group of up to 5 years (all together, children under 5 years made up 81 % of the cases). Some of the cases of disease are also linked to the consumption of dairy products made with raw milk (Decludt et al., 2000). Children under the age of 5 years are also more susceptible to the development of disease as a consequence of secondary transmission at home when there are individuals with gastroenteritis (Parry and Salmon, 1998).

Consequently, by way of prevention the consumption of raw food of animal origin should be avoided and good personal hygienic practices emphasised.

3.1.6 *Clostridium botulinum*

Clostridium botulinum is an anaerobic Gram-positive bacillus able to form spores. Seven subtypes are distinguished according to the neurotoxin produced, four of these cause the disease in humans, and only two, A and B, are responsible for the majority of cases of infant botulism. Infant botulism affects children aged between 1 week and less than 1 year, although 95 % of the cases occur in children under the age of 6 months (AECOSAN, 2011).

Infant botulism occurs when the child swallows spores of *Clostridium botulinum*, after which they germinate and multiply in the digestive tract, releasing the botulinum toxin which passes to the blood stream. The botulinum toxin combines with the cholinergic receptors of the nerve terminals at the neuromuscular joints, preventing their normal functioning. The minimum infective dose of *Clostridium botulinum* is considered to be between 10 and 100 spores (AECOSAN, 2011). Symptoms include a combination of hypotonia, weakness and flaccid paralysis of the skeletal muscle system, although a wide range of clinical presentations may be observed. Children are particularly sensitive to colonisation by *Clostridium botulinum* due to the immaturity of their gut microbiota (Rosow and Strober, 2015).

In the majority of cases of infant botulism, the source of the spores of *Clostridium botulinum* has not been identified (AECOSAN, 2011). In many patients for whom the origin of the infection has not been found, it is assumed to be the result of inhaling spores attached to microscopic dust particles present in the air. Children living in rural zones are at greater risk of developing infant botulism than those in urban areas, presumably due to their greater exposure to dust particles (Rosow and Strober, 2015).

In one case of infant botulism in the United Kingdom, an infant formula was suspected, among other foods, to be linked, although no conclusive results were obtained (Anon, 2001). In fact, there has been no confirmation of a case of infant botulism from an infant formula or follow-on formula (EFSA, 2004). Nevertheless, *Clostridium botulinum* has occasionally been found in honey, a standard ingredient in follow-on foods (Rosow and Strober, 2015). Current epidemiological data give reason to believe that the risk of developing the disease is low in children under the age of 12 months, if the intake of honey and/or infusions of plant species is avoided (AECOSAN, 2011).

3.1.7 *Clostridium difficile*

Clostridium difficile is the main cause of nosocomial diarrhoea in adults in developed countries, although its prevalence in children under 2 years is not clear, as it frequently colonises their intestinal tract, usually without clinical manifestations, making its diagnosis more difficult (Santiago et al., 2015). The colonisation of the intestinal tract by *Clostridium difficile* occurs shortly after birth and increases progressively during the first year, exceeding 70 % of children under the age of 2 years (Enoch et al., 2011).

In the cases of infected children in which symptoms appear, these take the form of slight and

self-limiting diarrhoea, although in risk groups it may lead to pseudomembranous colitis (Enoch et al., 2011). The frequency of *Clostridium difficile* in children hospitalised with diarrhoea is 3 cases per 1 000 hospital admissions (Enoch et al., 2011) (Santiago et al., 2015). It is of particular note in children under the age of 2 years, with another underlying disease or who have been administered antibiotics.

Among the sources of transmission, maternal, cross contamination between patients and through the atmosphere (Enoch et al., 2011), are especially listed, but it has not been linked to the intake of contaminated infant formulae.

3.1.8 *Clostridium perfringens*

Clostridium perfringens is an aerotolerant, anaerobic, Gram-positive, spore-forming species that produces several toxins. The majority of food-borne diseases are caused by the toxigenic type A, although fewer than 5 % of the strains of *Clostridium perfringens* are bearers of the gene responsible for the production of the enterotoxin A (cpe). The toxigenic type C causes an uncommon but severe necrotising enterocolitis.

The disease due to *Clostridium perfringens* appears as a self-limiting gastroenteritis with watery diarrhoea, general malaise and stomach cramps, with a low mortality rate. Children and elderly people are more susceptible than the general population (McClane et al., 2013).

The spores survive refrigeration and freezing with a reduction of less than 1 log after 6 months. Heating to 70 °C for 2 minutes during cooking destroys the vegetative cells but not the spores.

The enterotoxin is produced during sporulation. As it is a thermolabile protein, which is inactivated at 60 ºC for 5 minutes, intoxication rarely occurs due to the intake of preformed toxin. Typically, the disease appears when a food contaminated with spores and undercooked cools slowly. The spores germinate and the vegetative cells reproduce until reaching the infective dose. When the food is swallowed, sporulation takes place in the intestine with the release of the enterotoxin (McClane et al., 2013).

Clostridium perfringens is ubiquitous and spreads extensively in soil, dust and plants. It also colonises the intestine of human beings and animals. The main sources of infection are undercooked and incorrectly handled meat and poultry, which are involved in up to 90 % of the outbreaks (Grass et al., 2013). Prevention includes thorough cooking and suitable storage of the dishes until consumption (McClane et al., 2013).

The bacteria have not been linked to the appearance of outbreaks due to the consumption of infant formulae. Some studies have detected the presence of high concentrations of *C. perfringens* in the intestine of premature newborns who developed necrotising enterocolitis (De la Cochetiere et al., 2004). Nevertheless, it is not clear whether its presence is linked to the appearance of the disease or is a consequence of the accompanying dysbiosis (Cilieborg et al., 2012).

3.1.9 *Yersinia* spp.

The genus *Yersinia* is currently formed of 18 species of Gram-negative bacteria belonging to the *Enterobacteriaceae* family. This genus includes two enteropathogenic species, namely *Y.*

pseudotuberculosis and some types of *Y. enterocolitica*; although the latter is more frequently associated with human infections, the incidence of *Y. pseudotuberculosis* has increased in some parts of Northern Europe (EFSA, 2007). *Y. enterocolitica* is a psychrotropic bacterium, able to multiply at refrigeration temperatures and is divided into six biotypes (1A, 1B, 2, 3, 4 and 5) characterised by its biochemical properties. All the pathogenic strains are bearers of a virulent plasmid, called pYV and, depending on the function of other factors of virulence, three pathogenic types are distinguished: with high pathogenicity, moderate pathogenicity and no pathogens (EFSA, 2007) (Robins-Browne, 2007). In Europe, strains of moderate pathogenicity are most commonly isolated, corresponding to the serotypes O:3 (biotype 4) and O:9 (biotype 2), with a high incidence rate in children under 5 years (Rosner et al., 2012). The infection appears as gastroenteritis with self-limiting diarrhoea, but may lead to severe complications, including recurrent acute infection, pseudoappendicitis and septicaemia, or long-term sequels, such as reactive arthritis (EFSA, 2007) (Bottone, 2015). Pigs are thought to be the main reservoir of pathogenic strains and the consumption of pork and its derivatives that have not been heat treated correctly is a frequent cause of the appearance of sporadic cases (Rosner et al., 2012). It has also been linked to pasteurised milk and dairy products, which have been contaminated after the heat treatment, possibly due to the use of contaminated water (EFSA, 2007). In an extensive study carried out in Germany by Rosner et al. (2012), it was observed that the consumption of undercooked minced pork was the principal risk factor in the appearance of cases of yersiniosis, and home preparation contributed to increasing this risk. Given these aspects, the main preventive measure is to educate consumers about the risks associated with the consumption and handling of pork-derived products.

3.1.10 *Citrobacter* spp.

The species of the genus *Citrobacter* may cause infections in neonates, children and immunosuppressed adults (Doran, 1999). They are Gram-negative bacteria belonging to the family of enterobacteria, distributed widely, and are found in the human and animal gastrointestinal tract, and in the environment (soil, water and food).

Citrobacter freundii has caused several nosocomial outbreaks and in one of them, in a neonatal intensive care unit, the vehicle of transmission was an infant formula, although the source of contamination was never clearly defined (Thurm and Gericke, 1994). In general, the infection is considered to be transmitted vertically from the mother to the neonate, but there are huge gaps in the knowledge about these transmission pathways. The disease may have very severe consequences, frequently developing sepsis and meningitis, with mortality rates reaching 30 % (Doran, 1999).

3.1.11 *Shigella* spp.

Bacteria of the genus *Shigella* are enterobacteria which cause a serious gastrointestinal disease, shigellosis, characterised by bloody diarrhoea that may lead to dysentery. Although the disease may occur in any individual, it occurs most frequently in children under the age of 4 years (Lampel and Maurelli, 2007). The principal reservoirs are humans, and consequently transmission is through the faecal-oral route and person-to-person, and almost any food may be contaminated

(Warren et al., 2006). Given these aspects and the low infective dose, prevention is based on the observance of good hygienic practices.

3.1.12 *Bacillus cereus*

Bacillus cereus is a Gram-positive, spore-forming microorganism, widely distributed in the environment. It is a frequent contaminant of milk, entering through environmental contamination and the milking equipment. The production process for powdered milk and similar products is not enough to inactivate all the spores; in addition many strains are psychrotrophic, able to multiply in refrigeration. This means that this type of products have a high percentage level of contamination, although the counts are generally low (Becker et al., 1994). In addition, it is also present in cereals used in infant food (Becker et al., 1994) (Granum and Lund, 1997). The intake of a low number of cells or spores is not considered harmful, but the germination of spores or the multiplication of vegetative cells in the prepared food may result in a dangerous concentration of the microorganism (higher than 10^5 CFU/g) (Kramer and Gilbert, 1989). Consequently, although no outbreaks of disease have been reported as associated with powdered milk or other infant food products, it is possible that the incorrect handling of these products (mainly the abuse of the temperature of reconstituted food) allows for the development of this microorganism (Becker et al., 1994) (EFSA, 2004). In the EU, there is a hygiene criteria for the processes for dried infant formulae and dried dietary foods intended for special medical purposes for infants under the age of 6 months with a sampling plan in three classes (n=5, c=1, m=50 CFU/g and M=500 CFU/g), which is considered satisfactory if, at the end of the manufacturing process, the five samples (n=5) have values less than or equal to 50 CFU/g (m), acceptable if one of these five samples (c=1) has counts between 50 (m) and 500 CFU/g (M) and unacceptable if one of these five samples has values higher than 500 CFU/g or more than one of these five samples have values of between 50 and 500 CFU/g (EU, 2005) (Annex I). By way of prevention, the rapid consumption of cooked food or its immediate refrigeration is recommended, minimising the time it remains in the temperature interval of 10-50 ºC (Kramer and Gilbert, 1989).

3.1.13 *Aeromonas* spp.

The genus *Aeromonas* includes species of Gram-negative bacteria, of widespread distribution, although it is generally related to the aquatic environment. It appears as a contaminant in drinking water and in numerous foods of diverse origin, including milk and dairy products (Isonhood and Drake, 2002) (Janda and Abbott, 2010). Some species are considered pathogenic for humans, producing sporadic cases of gastrointestinal disease which may occasionally result in extra intestinal complications (Wilcox et al., 1992) (Janda and Abbott, 2010). Aeromonas caviae is the most significant species as an agent of disease in children, especially those under the age of 3 years, with a seasonal peak in the hottest months (Wilcox et al., 1992). Given the gaps in the knowledge about the transmission pathways of this microorganism, it is difficult to establish preventive measures, but it is important to pay special attention to the use of drinking water, especially in the hot months, when preparing any food to be consumed by children.

3.1.14 *Staphylococcus aureus*

Staphylococcus aureus is a bacterium that produces minor food poisoning and is often linked to milk and dairy products (Le Loir et al., 2003). There are records of some significant outbreaks linked to powdered milk, although they did not affect young children, and were attributed to shortcomings in hygiene and temperature abuse in the liquid milk, before drying (Anderson and Stone, 1955) (Asao et al., 2003).

In addition, attention is being given to the infections caused by methicillin resistant *Staphylococcus aureus* (MRSA), due to its resistance to β-lactam antibiotics and the severity of the diseases that it can cause, especially in hospitalised or immunosuppressed patients (Doyle et al., 2012). In recent years, outbreaks of food poisoning caused by MRSA have been reported. Although this does not imply greater virulence of the disease, it causes concern regarding the dissemination of these microorganisms, which are able to produce serious and difficult-to-treat infections (Jones et al., 2002). Strains of MRSA have also been observed in production animals which may be transmitted to derived food products, but it does not appear that the risk of human disease is increased for this reason (EFSA, 2009).

The preventive measures are well-known and focus on the training of the handlers in hygienic practices and on the correct use of the heat treatments and maintenance of the refrigeration conditions (Doyle et al., 2012).

3.1.15 Other microorganisms

Other species from the *Enterobacteriaceae* family, such as *Citrobacter diversus, Citrobacter koseri, Klebsiella oxytoca, Klebsiella pneumoniae, Pantoea agglomerans, Escherichia vulneris, Hafnia alvei, Serratia marcenscens* and *Enterobacter cloacae*, have also been found in infant formulae, but it has not been clearly demonstrated that, from an epidemiological or microbiological aspect, the infant formulae are the vehicle and the source of infection (FAO/WHO, 2004a). These species, nevertheless, are assuming more and more importance as neonatal pathogens and should also be considered opportunistic pathogens (EFSA, 2004).

Infant formulae have also been linked to the conveyance of a strain of *Bacillus licheniformis*, which produces a toxin with similar characteristics to that of the emetic toxin of *Bacillus cereus* (EFSA, 2004). It has not been possible to establish a clear relation with infant formulae in these cases either.

3.2 Main viral food pathogens

Enteric viruses are faecal-oral transmission viruses and may be present in food and water that have been contaminated by faecal matter. These include various agents which cause gastroenteritis, such as Norovirus (NoV), Sapovirus (SaV), Astrovirus (AstV), Rotavirus (RV) and Adenovirus (AdV), and the enteric hepatitis agents (A and E), causing acute hepatitis (Koopmans and Duizer, 2004) (Newell et al., 2010) (BIOHAZ, 2011). Less frequently, enterovirus infections may be transmitted through water and food, and may cause different clinical manifestations, ranging from diarrhoea to meningitis or rashes (Muehlenbachs et al., 2015). On the whole, all these

pathogens occur more frequently in the child population, with the exception of the hepatitis A virus (HAV), which, in children under the age of 6 years, usually causes asymptomatic infections. In addition to the above viruses, others such as the bird flu virus, the tick-borne encephalitis virus, the MERS virus or the Nipah virus have also been included in some reports as viruses potentially food-borne to the general population (FAO/WHO, 2008b). Although the impact of these viruses on health may be significant, they are not discussed in this report given the low frequency in which cases may occur. We will focus on the gastroenteritis and hepatitis viruses.

The raw foods at greatest risk of being contaminated at source by a viral pathogen are bivalve molluscs, fresh vegetables eaten in salad and berries which have been irrigated with water contaminated with faecal matter. All these foods are usually eaten raw or undercooked, thereby maximising the risk of infection. Sometimes they are sold in frozen form, but this does not reduce the risk of infection. Recently, given that certain strains of the hepatitis E virus may infect animals, including the pig or the wild boar, cases have been reported of adults infected from eating raw or undercooked pork or game (Van der Poel, 2014). Lastly, any other food may also be contaminated due to poor hygienic practices by handlers, both symptomatic and asymptomatic, carriers of the virus. Given that the infective doses of the viral pathogens are very low, although a virus can never multiply in the food, minimum contamination levels may be sufficient to cause infection.

Therefore, in order to maximise the precautions to prevent this type of infection in young infants, we should avoid feeding them with these foods unless they have been thoroughly cooked, and it must be remembered that freezing does not reduce the risk of viral infection. In addition, at home, good personal hygienic practices (hand washing) and avoiding cross contamination when handling raw food are essential.

3.2.1 Rotavirus

Group A rotaviruses are the primary cause of hospitalisation due to gastroenteritis in young infants and cause more than 450 000 deaths in children under the age of 5 years in developing countries (Ruggeri and Fiore, 2012). In these countries, it has been estimated that almost all children are infected by rotavirus before the age of 2 years. Since 2006, many countries have recommended the vaccination against rotavirus for young infants with either of the two approved vaccines (RotaTeq® by Merck and Rotarix® by GlaxoSmithKline), but in the majority of countries it is an optional and costly vaccination. Since 2009, the WHO has recommended the inclusion of this vaccination in the immunisation programmes of all countries. In spite of the huge diversity of circulating strains, it would appear that both the infection and the vaccination protect against future gastroenteritis due to the majority of the viral strains circulating in the population (Jiang et al., 2010) (Wang et al., 2010), although it cannot be dismissed that asymptomatic infections may be caused in adults.

In spite of their impact, transmission pathways other than person-to-person have not been characterised in detail, but rotavirus is known to be highly stable in the environment, in water, on food and on surfaces. In developing countries, rotavirus may be carried in water with faecal contamination and the use of formula milk reconstituted with low quality water appears to be a

source of likely infection. In addition, given that healthy adults are able to act as a reservoir, it is also important to ensure good personal hygienic practices during the preparation of food for young infants. Although many of the strains currently in circulation have a zoonotic origin, it is thought that the transmission of rotavirus from animals to humans does not occur frequently.

In countries with warm climates, infections from rotavirus in children are noticeably seasonal, with the highest incident rates in the colder months. Although the majority of cases are sporadic infections, epidemic outbreaks in schools, kindergartens, hospitals and geriatric nursing homes have also been reported. The source of contamination in some of these outbreaks was contaminated drinking water with a high viral load due to accidental contamination (Gallay et al., 2010) (Räsänen et al., 2010). Food contaminated after cooking has also been reported as the cause of an outbreak in a sanatorium for mothers and children in Germany (Mayr et al., 2009).

3.2.2 Norovirus (NoV)

After rotavirus, the NoV are considered to be the second most frequent cause of acute nonbacterial gastroenteritis in children. Due to the use of the vaccination against rotavirus in many countries, it is possible that they may soon be in first place. In industrialised countries it is estimated that they cause 12 % of the hospitalisations due to gastroenteritis in children under the age of 5 years. In developing countries it is estimated that they cause 218 000 infant deaths (Patel et al., 2008). The NoV affect all age groups and have a high incident rate all over the world, both in the form of epidemic outbreaks and as sporadic cases, that are often undervalued due to a lack of data. Most infections are not severe and are cured after approximately 24-60 hours. Some studies identify them as the cause of more than 50 % of all food borne infections (Scallan et al., 2011) (Robilotti et al., 2015.). With respect to the outbreaks, different studies indicate that the percentage of outbreaks originating from food ranges between 12 and 54 % (FAO/WHO, 2008b) (Sabria et al., 2014), but there is little available data about the percentage if only infant infections are considered. The greatest food outbreak in Europe during 2012 was an outbreak of NoV in Germany associated with the consumption of frozen strawberries, affecting almost 11 000 individuals, the majority of which were school or pre-school aged.

In comparison with children between 2 and 4 years, in children under the age of 2 years, the gastroenteritis has been observed to last longer (7 days versus 3.5 days) and with a greater level of severity (Murata et al., 2007). In neonates, infections by NoV have been described which have complicated to necrotising enterocolitis, causing death in some cases (Turcios-Ruiz et al., 2008) (Stuart et al., 2010). In one of these outbreaks, one of the carers reported having symptoms of gastroenteritis in the days before the outbreak (Turcios-Ruiz et al., 2008).

3.2.3 Other viruses causing gastroenteritis

After rotavirus and norovirus, there is a very long list of other viruses which cause acute gastroenteritis in children, of which the best known are the enteric Adenoviruses (types 40 and 41), the Astroviruses and the Sapoviruses (Koopmans and Duizer, 2004) (Carter, 2005). In addition, in recent years other etiological agents of infant gastroenteritis have been discovered, such as

the Aichiviruses (Kitajima and Gerba, 2015) or the Picobirnaviruses (Ganesh et al., 2012). The majority usually only cause gastroenteritis in children, and although adults may also suffer from the infection, it is often asymptomatic. There are few systematic studies that provide data on the prevalence of each of these agents as a cause of gastroenteritis in children; especially due to that fact that many of them are only identified by research laboratories and that the diagnosis is not the same in all countries (Guarino et al., 2008). As for rotavirus and NoV, there is usually a greater incidence in the colder months and they are faecal-oral transmitted viruses. The role of food in the transmission of the infection has not been well-documented. As the majority may cause asymptomatic infections, one possible transmission pathway is through the handling of foods during preparation when personal hygiene standards are inadequate. Although the study was not exclusively limited to virus, the following were included among the principal risk factors in children with gastroenteritis in industrialised countries: recent trips abroad, contact with symptomatic individuals (in particular in kindergartens), hospitalisation, contact with dogs with diarrhoea, consumption of products containing powdered milk, low level of education of parents and previous diagnosis of different types of atopic diseases (Ethelberg et al., 2006). In the case of viral gastroenteritis, contact with symptomatic individuals was the most significant risk factor.

3.2.4 Viruses causing acute hepatitis

The acute hepatitis that may have food origin are those caused by hepatitis A (HAV) and hepatitis E virus (HEV).

In developing countries, HAV is endemic and most children are asymptomatically affected and immunised for life (Pintó et al., 2010). According to the WHO, only 10 % of children under the age of 6 years suffer from jaundice. In industrialised countries with higher standards of hygiene, the virus is no longer in circulation and the vaccination against HAV is frequently included in the routine vaccination schedule. The Vaccine Advisory Committee of the Spanish Association of Paediatrics recommends the vaccination against HAV in certain risk groups, and since 2015, the vaccination has been universally used in Catalonia and in the cities of Ceuta and Melilla. Preventive measures against HAV include the vaccination of children, and avoiding feeding them with raw foods of risk. As the virus is no longer in circulation in most industrialised countries, it is unlikely that national food would be contaminated, but the risk is significant when referring to food imported from endemic regions (Collier et al., 2014) (Guzmán-Herrador et al., 2014) (Terio et al., 2015). In addition, it is important to be alerted about the increase of symptomatic cases in children under the age of 5 years, observed in Spain and associated with a certain genotype of the virus (D'Andrea et al., 2015).

The information regarding whether or not HEV infections in children are also asymptomatic is not conclusive. While the WHO web page indicates that infection in children is usually asymptomatic (WHO, 2015), other sources consider that HEV infections appear with symptoms and may be particularly serious in children under the age of 2 years (Sayed et al., 2015). HEV infections in endemic regions are mainly transmitted through the faecal-oral route, often in the

form of waterborne outbreaks, but in non-endemic developed countries such as Europe, the number of native infections is increasing. It is thought that this is in part due to the transmission of certain genotypes which also infect pigs and other animals (Hoofnagle et al., 2012). Consequently, the HEV is today considered an emerging zoonotic food-borne pathogen. Although the consumption of undercooked pork and pork derivatives is described as a risk factor for hepatitis E, the percentage of infections with this origin is unknown (BIOHAZ, 2011). Lastly, although in China a vaccination against HEV has been approved since 2012, it is not yet marketed in any other country. The best strategies for prevention continue to be the consumption of thoroughly cooked food, handled in accordance with good hygiene standards. In addition to pork derivatives, and as with other enteric viruses, the HEV is also observed in food in contact with faecal-contaminated water such as shellfish, berries and salads. Poor management of pig manure and slurry or its use as fertiliser may also increase the risk of crop contamination (BIOHAZ, 2011).

3.3 Main parasites

The main transmission pathways of pathogen parasites are water and person-to-person contamination via the faecal-oral route. According to the data from the SIM, parasitic protozoa belonging to the genera *Cryptosporidium* and *Giardia* are found among food-borne disease agents affecting the 1-4 year age group. In Spain, it has also been observed that young children may be frequent asymptomatic carriers of these microorganisms (Mateo et al., 2014).

The symptoms produced by other food-borne parasites are not significantly different in children to those of adults, and the prognosis of the disease seems to be linked more to the immune status of the patient than to their age. So, for example, congenital toxoplasmosis is a transplacental infection of the foetus and preventive measures are mainly directed at pregnant mothers. The adoption of good hygienic practices when preparing food and thorough cooking of the food are the most efficient preventive measures for avoiding these risks.

3.3.1 Cryptosporidium

Cryptosporidium parvum and other species of this genus are the agents of cryptosporidiosis, a gastrointestinal disease characterised by diarrhoea and abdominal cramps that may last several weeks. The life cycle of the protozoa is completed in only one host and ends with the elimination of mature oocytes (that is, fully infectious) in the faeces. These oocytes may contaminate water and remain viable for long periods of time (up to 6 months) or be transmitted by direct contact or through the incorrect handling of foods (Nichols, 2000) (Dawson, 2005).

The majority of the cases of cryptosporidiosis in Spain occur in the hottest months, possibly associated to the recreational use of water, and the most affected age group are children between 0 and 4 years (Semenza and Nichols, 2007). Some outbreaks have been reported affecting pre-school age children, as occurred in Guadarrama in 1998, associated with the consumption of incorrectly treated water (Rodríguez et al., 2000).

The cysts are quite resistant to disinfectant, especially chlorine-based disinfectants. They are also partially resistant to freezing up to -20 ºC. The most effective treatment for its elimination is

drying and heat treatment (thorough cooking of food, pasteurisation, boiling of water), in addition to good hygienic practices at home (Doyle, 2003) (Dawson, 2005).

3.3.2 *Giardia*

Giardia intestinalis (also known as *G. lamblia* or *G. duodenalis*) is the most frequent intestinal parasite in developed countries, with around 1 000 cases reported every year in Spain, according to the figures from the SIM. Giardiasis is produced as a consequence of the intake of cysts, transmitted via the faecal-oral path, or through contaminated water, incorrectly handled food or direct contact with carriers (Doyle, 2003) (Dawson, 2005). The disease appears with gastrointestinal symptoms (nausea, diarrhoea) accompanied by stomach cramps, a bloated feeling and flatulence. The diarrhoea may last several days or weeks and is accompanied by a loss in weight.

In Spain, children under the age of 5 years are the most affected age group. In addition, certain recorded outbreaks of giardiasis took place in schools and educational centres, mainly due to direct person-to-person transmission, although there have also been outbreaks associated with the consumption of water (Carmena, 2012).

The cysts of *Giardia* are quite resistant to chlorine treatments, although less so than those of *Cryptosporidium*, and consequently the treatments described above are also applicable for *Giardia* (Doyle, 2003) (Dawson, 2005).

4. Microbiological risks of breastfeeding

Breast milk is not a sterile product and often contains bacteria from the mother's skin. Breastfeeding in a healthy child brings with it the healthy bacterial colonisation in the child. Breastfeeding is the optimum method for feeding an infant. The WHO recommends breastfeeding exclusively until 6 months of age, and accompanied by appropriate supplementary food until the age of 2 years or more.

In practice, there are very few infections which contraindicate breastfeeding (Díaz-Gómez, 2005). The presence of clinical infection in a lactating mother usually implies that the child is exposed to this pathogen at the same time. Stopping breastfeeding does not, therefore, have a preventive effect and should not usually be recommended. In general terms, if the common bacterial, fungal and viral diseases do not compromise the health of the mother they do not pose a contraindication for the infant.

4.1 Principal pathogens that can be transmitted through breast milk

Some of the principal types of infectious disease that may discourage breastfeeding are listed below. They can be classified into a few contraindications and certain situations that should be assessed individually. The recommendations included in this section must not, in any event, replace the instructions of a paediatrician.

4.1.1 Bacterial diseases

4.1.1.1 Brucellosis

This can be transmitted through human milk. If the mother is diagnosed after breastfeeding has started, it is highly likely that the child is infected and both need treatment. There is no agreement as to whether it is necessary to stop breastfeeding until the treatment has finished (Díaz-Gómez, 2005).

4.1.1.2 Lyme's disease

Although the spirochete *Borrelia burgdorferi* has been isolated in breast milk, the transmission of the disease has not been confirmed. If it is diagnosed after birth, treatment should be started immediately, and for the child also if he or she has any symptoms. Once treatment of the mother has been started, breastfeeding may continue.

4.1.1.3 Severe bacterial infections

When the mother is suffering from sepsis or another serious infection, the germs may pass to the milk, but the child will also receive antibodies from the mother to protect against the microorganism causing the infection, and the antibiotics given to the mother. It is reasonable to establish that the general health of the mother should take priority over the maintenance of breastfeeding either temporarily or permanently. If the disease significantly affects the general condition of the mother, breastfeeding may be stopped during the initial stages of the treatment, and continued later, as recommended by the doctor (Díaz-Gómez, 2005).

4.1.2 Viral diseases

When the mother suffers from a viral disease, the virus is sometimes transmitted to the breast milk, posing a risk for the baby. Apart from transmission through the milk, the baby may also be infected when feeding due to direct contact with lesions on the breast and nipple. Nevertheless, only in cases of potentially serious disease for the baby should stopping the breastfeeding be considered.

Table 1 summarises the principal viral pathogens with a potential risk of transmission through these paths and for which information is available (Lanari et al., 2012) (Civardi et al., 2013). The decision must always be weighed against the numerous and important benefits of breastfeeding for the child, considering the risk of transmission and the severity of the potential disease. In developing countries, the benefits of breastfeeding always compensate any risk with the exception of mothers infected by the human immunodeficiency virus (HIV) but only in certain circumstances. In these countries, it is estimated that breastfeeding reduces the risk of mortality due to other causes by 20 % (Edmond et al., 2006). In industrialised countries, the mortality rate due to malnutrition and other infections is much lower, and breastfeeding is contraindicated in certain cases: infection with the human immunodeficiency virus (HIV) and the human T-cell lymphotropic virus (HTLV) (AAP, 2012). If it is deemed necessary to stop breastfeeding temporarily, the mother should frequently express the milk manually or with a pump, to prevent the milk supply from drying out and to permit the return to breastfeeding without problem.

In certain cases it is possible to resort to breast milk banks to feed the baby. Women who are HIV, hepatitis B or C, HTLV or syphilis positive, or who are at greater risk of the Creutzfeldt-Jakob disease should not donate breast milk (NICE, 2010).

The risk of transmission of HIV through breast milk ranges between 15 and 44 % and treatment with antiviral drugs has recently been observed to reduce the risk of transmission (WHO, 2007). In industrialised countries, breastfeeding is contraindicated for mothers who are HIV seropositive, but in developing counties this is not always the case. Since 2010, the WHO has recommended that women infected by HIV who are able to receive treatment with antiviral drugs, continue to breastfeed until the infant reaches the age of 12 months (WHO, 2010).

The risk of transmission of the type 1 human T-cell lymphotropic virus (HTLV-I) through breast milk is also high, around 20 % when breastfeeding lasts 6 months or more (Moriuchi et al., 2013). This virus is an oncogenic retrovirus which infects the CD4 T lymphocytes and which may induce lymphomas and myelopathies (Moriuchi et al., 2013). 10-20 million people are estimated to be infected, concentrated in Japan, Central and West Africa, the Caribbean and Central and South America, and the risk of suffering from a T-cell lymphoma from the adult among infected individuals is calculated to be 5 %. The vertical transmission of HTLV mainly occurs through breast milk, but as with HIV, breastfeeding should only be stopped for this reason in industrialised countries and in situations in which the correct nutrition of the baby through artificial milk can be guaranteed.

Although the transmission of cytomegalovirus (CMV) through breast milk has also been proven, with figures ranging between 4 and 38 % (Hamprecht et al., 2001) (Hayashi et al., 2011) (Lanzieri et al., 2013), the majority of infections in children are asymptomatic. The probability of infection and disease from CMV in children born full-term is very low, probably due to the transfer of antibodies against the virus during pregnancy. The only situations in which breastfeeding in seropositive mothers would be contraindicated is in premature babies or babies born with a weight of less than 1.5 kg (Lanzieri et al., 2013), in which, CMV infection may cause hepatopathy, thrombocytopenia, neutropaenia, petechiae, respiratory distress syndrome and the "sepsis-like" syndrome.

For other infections from the same family, as for example the herpes simplex virus or the varicella zoster virus, breastfeeding is only advised against in cases in which the mother's infection is very recent, for example, in the case of herpetic lesions on the mother's breasts or cracked nipples. If the mother is infected by varicella 5 days before the birth or 2 weeks afterwards, the baby should be kept away from the mother to reduce the risk of infection, but the breast milk can be expressed and fed to the baby. For infections of the mother with the rubella or measles viruses, the situation is similar, although there is far less available data. In general, breastfeeding is only to be advised against during a primary infection of the mother, when the concentrations of the virus in the milk may be at their highest. Nor is there any great risk of vaccinating lactating mothers against infections such as varicella, rubella, mumps or measles, all of which are live vaccines. Although there are few recent studies in this area, in some cases it has been demonstrated that the live virus may be transmitted through the milk to the child, but no serious infections have been reported in infants (Alain et al., 2012).

Although the risk of transmitting the hepatitis B or C virus through milk is not zero, there have been no reported cases of the transmission of these infections through breast milk. The Centers for Disease Control and Prevention (CDC) and the American Academy of Paediatrics (AAP) consider that infections from this virus do not contraindicate breastfeeding, although it has also been suggested that breastfeeding should be stopped temporarily if the infected mother has bleeding cracks on her nipples. Children born from mothers infected with the hepatitis B virus should receive passive and active immunity against the virus in the first 12 hours of life. The second dose should be administered at the age of 1-2 months, and the third dose at 6 months, but it is not necessary to delay breastfeeding until the baby is fully immunised.

In pregnant women infected by HEV, the infection may be transmitted to the foetus and cause a high rate of mortality (Krain et al., 2014), but there have been no reported cases of transmission through breast milk and therefore breastfeeding is not contraindicated. In addition, the antibodies present in breast milk may help to protect the child against infection. The situation may be similar for the HAV, and under no circumstances is breastfeeding advised against.

Lastly, for mothers infected with the West Nile virus (WNV), existing data are very scarce. Transmission through milk cannot be dismissed, but in the only cases in which it has been confirmed, no symptomatic disease was observed in the infant (Hinckley et al., 2007).

Table 1. Pathogens which can potentially be transmitted to the baby through the breast milk and which are better documented

Pathogen	Presence in breast milk	Risk of transmission	Breastfeeding indication
Bacterial pathogens			
Brucella spp. (brucellosis)	Yes	Possible	Not recommended if the mother is not receiving treatment
Borrelia burgdorferi (Lyme's disease)	Yes	Very low	Not recommended in industrialised countries if the mother is not receiving treatment
Viral pathogens			
HIV	Yes	Significant	Not recommended in industrialised countries
HTLV (I & II)	Yes	Significant	Not recommended in industrialised countries
Cytomegalovirus (CMV)	Yes	Significant	Recommended except in premature infants or infants with an immunodeficiency, and in mothers with a current primary infection
Varicella Zoster Virus (VZV)	Yes	Possible	Recommended unless there are lesions on the nipples
Herpes Simplex Virus (HSV)	n.a.	n.a.	Recommended unless there are herpetic lesions on the nipples
Hepatitis B Virus (HBV)	Yes	Low	Recommended except in mothers with current primary infection
Hepatitis C Virus (HCV)	Yes	Low	Recommended except in cases of lesions on nipples and mothers with current primary infection
Hepatitis A Virus (HAV)	n.a.	Very low	Recommended
West Nile Virus (WNV)	Yes	Very low	Recommended

n.a.: information not available. **Adapted from**: (Civardi et al., 2013).

4.1.3 Other pathogens not transmitted through breast milk which may be transmitted to the baby during breastfeeding. Recommendations on the convenience of breastfeeding

Sometimes, due to the close contact between the mother and the baby, certain infections may be transmitted from the mother to the baby via paths other than through breast milk, for example through respiratory or faecal-oral routes. While precautions and hygiene measures should be maximised to prevent contamination, the majority of common acute infections do not contraindicate breastfeeding. In case that the mother expresses the milk and either she herself, or another person, are required to handle it, it is important to observe good hygienic practices for handling food, especially in those cases in which there are symptoms of infection. Table 2

summarises the information available regarding the recommendations on the convenience of breastfeeding when the mother has an infection.

4.1.3.1 Mammary abscesses and mastitis

Mastitis or a mammary abscess should not be an absolute indication to stop breastfeeding. In a breast with abscesses and with pain and pus, it is possible to continue feeding temporarily from the other breast, and emptying the affected breast.

4.1.3.2 Untreated active tuberculosis

The tuberculosis bacillus has not been isolated in breast milk. Transmission occurs through the respiratory route. If the tuberculosis is diagnosed during pregnancy, treatment should be started immediately, to prevent the risk of contamination. If it is diagnosed at the end of the pregnancy or after birth, there is some controversy as to whether or not the child should be separated from the mother. The WHO recommends not separating them and giving the child isoniazid for 6 months if the mother has been receiving treatment for less than 2 months at the time of birth; while the American Association of Paediatrics and other authors recommend the separation of the mother and child until after the first 2 weeks of treatment when the mother is no longer infectious (Díaz-Gómez, 2005).

4.1.3.3 Food-borne diseases

There is no evidence that these diseases are transmitted through breast milk unless they cause bacteraemia. In addition, the infant has already been exposed to infection through contact with the mother during the prodromic stage. When the mother is in the symptomatic phase, she has formed antibodies that may be transmitted through the breast milk, protecting the child against infection and reducing the severity of the symptoms. In these cases, breastfeeding may be continued and treatment given to the mother, if required. During the infectious period, the mother should maximise personal hygiene measures.

The only contraindication would be if the intoxication is systemic (enteric fever due to *Salmonella*, for example); in this case breastfeeding should be avoided.

4.1.3.4 Respiratory infections

When these diseases are acquired, the body immediately produces antibodies that pass directly to the breast milk. Transmission occurs airborne, not through the breast milk, and therefore preventive measures should be adopted (face masks, hand washing, etc.) without it being necessary to stop breastfeeding.

4.1.3.5 Skin diseases or sexually transmitted diseases

Almost none of the sexually transmitted diseases are transmitted through breast milk. The presence of syphilis or herpes skin lesions on the breast or nipple contraindicates breastfeeding, as it may contain treponemes or viruses (Díaz-Gómez, 2005).

There is no indication to stop breastfeeding, even temporarily, in the case of urinary infection or other diseases, as long as the general condition of the mother so permits and always observing the doctor's recommendations (Lamounier et al., 2004).

4.1.3.6 Malaria

Breastfeeding should be continued if the mother's clinical condition so permits. Precaution is necessary with the antimalarial drugs which are compatible with breastfeeding except in cases of glucose 6-phosphate dehydrogenase deficiency, G6PD, with the withdrawal of quinine.

4.1.3.7 Chagas disease

The transmission of *Trypanosoma* through breast milk is exceptional. Heat treatment inactivates the parasite and therefore after expressing the milk, this procedure may be used in the acute phase of the disease followed by its subsequent administration.

4.1.3.8 Scabies

Skin infection from the parasite *Sarcoptes scabei* or scabies mite. Highly contagious by direct maintained contact. When either the mother or the child is diagnosed with scabies, it is most likely that both present it, and therefore there is no point in separating them. If scabies is diagnosed right at the time of birth, the newborn child should be isolated from the mother for the first day while the treatment dose takes effect, maintaining breastfeeding by expressing the milk manually or with a pump, and feeding it to the newborn baby. All individuals who are infected or cohabiting should be treated to prevent re-infection.

4.1.3.9 Candidiasis

Vaginal candidiasis may result in a colonisation in the child. It should be treated with anti-fungal drugs but, given appropriate hygiene measures, breastfeeding should not be stopped.

Table 2. Other infections that may potentially be transmitted to the baby during breastfeeding and recommendations on the convenience of breastfeeding

Bacteria	**Recommendations**
Mastitis and abscesses	Continue breastfeeding, maximising hygiene measures. It may be necessary to reject milk from the infected breast
Tuberculosis	Postpone breastfeeding until the mother has received at least 2 weeks of treatment
Urinary infection	Continue breastfeeding
Postcesarean infection of the abdominal wall	Continue breastfeeding
Bacterial diarrhoea	Continue breastfeeding, maximising hygiene measures
Other bacterial infections without general compromise	Continue breastfeeding, maximising hygiene measures
Parasites	
Malaria	Continue breastfeeding
Chagas Disease	Continue breastfeeding
Other parasites, scabies	Avoid contact between breast and mouth until cured. Maximise hygiene measures
Fungi	Continue breastfeeding, maximising hygiene measures

4.1.4 Summary: contraindications of breastfeeding

These can be classified into a few contraindications and certain situations that should be assessed individually.

4.1.4.1 Contraindications

HIV, human T-cell leukaemia virus, cytomegalovirus in pre-term neonates.

4.1.4.2 Individual assessment

Active tuberculosis, herpes simplex, hepatitis C, brucellosis, Lyme's disease, severe bacterial infection, syphilis, varicella, measles, rubella, parotiditis, malaria, Chagas disease, scabies.

4.1.4.3 False contraindications

Common infections, hepatitis A, hepatitis B, mastitis or mammary abscess, vaginal candidiasis.

4.1.4.4 Immunizations

Breastfeeding is not a contraindication for the administration of vaccines, but their desirability is disputable except in certain cases (tetanus). It appears to be unnecessary or inappropriate except in cases of prolonged lactation and a very high risk for the mother of contracting the disease.

4.2 Hygiene recommendations for the handling and conservation of breast milk

Breast milk can be expressed manually or with a pump for use at a later stage, ensuring its suitable conservation at room temperature, in a refrigerator or freezer, depending on the length of time before it will be used to feed the baby. A daily shower is usually enough to ensure the hygiene of the breasts, but it is important to wash hands thoroughly before expressing the milk. The milk should be expressed in a hygienic and quiet location; bathrooms are not safe places. If using a breast pump, all the pieces of the pump and the containers for collecting and storing the milk must be clean and disinfected prior to use, in accordance with the manufacturer's instructions. The breast pump should be for personal use only. According to the Breastfeeding Committee of the Spanish Association of Paediatrics, expressed breast milk can be stored at a temperature of 4 ºC for 8 days, at 19-22 ºC for 10 hours, at 25 ºC for between 4-6 hours, and at 30-38 ºC for a maximum of 4 hours (AEP, 2012). Correctly washed glass containers or commercially-sold bags intended for this purpose should be used for the storage of the milk. Ideally, the expressed milk should be cooled quickly (in a recipient of cold water) and frozen as soon as possible. If the milk is expressed at home and is not going to be used the same day, it should be frozen. If it is expressed away from home, it can be stored in a portable cool box with ice blocks and frozen on arrival home. If the milk is contaminated intrinsically or extrinsically by any pathogenic microorganism, freezing will reduce the contamination, but will not eliminate it completely.

The milk can be thawed by submerging the container in a bowl of hot water, or at room temperature. The use of microwaves is to be avoided as the temperature distribution is not uniform. If the breast milk is to be taken to a kindergarten or infant school, it should be expressed or thawed on the same day or the day before and kept refrigerated during transportation and for the necessary time prior to administration.

5. Risk practices in the preparation and handling of powdered infant formulae and hygiene measures to minimise these risks

5.1 Microbiological risk factors in infant formulae

Prevention and the efficient fight against food-borne disease are based on identifying the hazards associated with the production, treatment and preparation of the food, assessing its risks and establishing the operations in which certain methods of control are efficient. In this respect, knowledge of the factors contributing to the outbreak of food-borne disease is essential, together with knowledge of the results of research into the ecology, multiplication and inactivation of the pathogens transmitted by food (Bryan, 1992).

In general, the risk of food-borne disease (FBD) is linked to four main factors (Bryan, 1992):

1. Properties of the food, with regard to its epidemiological history and its characteristics and possibilities for allowing the survival and development of pathogens.
2. Preparation, in relation to the methods normally used in its manufacture and subsequent handling to ensure the inactivation of pathogens or to prevent their proliferation.

3. Volume of food prepared, with respect to the time and conditions of maintenance that may favour the bacterial development during the interval between preparation and consumption.
4. Consumer susceptibility, with regard to the destination population which may include high risk populations.

The current manufacturing processes for infant formulae and follow-on formulae do not guarantee that these are sterile. The FAO, the WHO and the EFSA find that the principal risk vector associated with the use of infant formulae neonatal feeding is *Cronobacter*, although the presence of other microorganisms including *Salmonella* has also been observed (EFSA, 2004) (FAO/WHO, 2006). The presence of other microorganisms in infant formulae such as *Citrobacter freundii*, has been reported, although it has not been possible to demonstrate that these infant formulae are the vehicle and the source of the infection (FAO/WHO, 2004a). There are other microorganisms that cause disease in infants, but these are not found in infant formulae. This is the case of *Clostridium botulinum* or *Clostridium difficile* (FAO/WHO, 2004a).

5.2 Sources of contamination of the infant formulae

The FAO/WHO expert work groups (2004a, 2006) set up to assess the risk of pathogenic microorganisms in powdered infant formulae, identified different types of microorganisms associated with the contamination of these products: *Cronobacter* spp., *Salmonella enteritidis, Enterobacter agglomerans, Hafnia alvei, Klebsiella pneumoniae, Citrobacter koseri, Citrobacter freundii, Klebsiella oxytoca, Enterobacter cloacae, Escherichia coli, Serratia* spp., *Acinetobacter* spp., *Bacillus cereus, Clostridium difficile, Clostridium perfringens, Clostridium botulinum, Listeria monocytogenes* and *Staphylococcus* spp. However, they concluded that the pathogens of most concern were *Cronobacter* and *Salmonella*. Therefore, this study refers preferably to these two pathogens, on the understanding that the majority of the considerations are applicable to other pathogenic agents that are potentially present in infant formulae.

The microbial contamination of infant formulae may occur along two ways: intrinsic, during the elaboration process and/or extrinsic, during the reconstitution and handling of the powdered formula.

The International Food Safety Authorities Network (INFOSAN, 2005) indicates that in 50-80 % of the cases, the powdered infant formula is both the source and the vehicle of the infection produced by *Cronobacter*, and in 20-50 % it is the vehicle, but the source of origin is the lack of hygiene during reconstitution and handling.

Contrary to the case of *Cronobacter*, it is unlikely that the cases of salmonellosis in infants are due to an intrinsic contamination of the infant formulae (FAO/WHO, 2004a).

5.2.1 Intrinsic contamination

Although sterile liquid infant formulae are available, dried infant formulae are the most frequently used products as a source of food for this population group. The manufacturing processes currently applied cannot guarantee a sterile powdered formula without altering the nutritional

properties, and consequently, microbiological safety depends on the strict observance of good hygienic practices throughout the manufacturing process.

To guarantee the safety of the product throughout its useful life, the companies which manufacture dried infant formulae and dried dietary foods intended for special medical purposes for infants under the age of 6 months, must comply with the food safety criteria relating to *Cronobacter* and *Salmonella* established in Regulation (EC) No 2073/2005, on microbiological criteria for foodstuffs and its subsequent amendments. This establishes a maximum microbiological limit of absence of *Cronobacter* in each of 30 samples of 10 g and absence of *Salmonella* in each of 30 samples of 25 g (Annex I).

This regulation also establishes microbiological criteria of hygiene for the process relative to *Enterobacteriaceae* (absence in each of 10 samples of 10 g) and presumed *Bacillus cereus* (n=5, c=1, m=50 CFU/g and M=500 CFU/g) required of the industries at the end of the manufacturing process (Annex I), together with their obligation to take samples from the equipment and work zones to guarantee compliance with these microbiological criteria.

A recent study (Parra et al., 2015) reports viable mesophilic counts considered unacceptable (>10 000 to <50 000 CFU/g) in 8 % of the infant formulae samples from different countries (Chile, Mexico and Holland). In addition, the enterobacteria counts obtained in seven samples of powdered milk for premature infants with <100 CFU/g (two samples), of 100 to 500 CFU/g (four samples) and 1 000 CFU/g (one sample) are of note. Furthermore, *Cronobacter sakazakii* was identified in two batches of infant milk produced in Chile.

The presence of enterobacteria in infant formulae indicates inadequate conditions of hygiene in the manufacturing environment and has been shown to be linked to the presence of pathogens associated with infection in infants (Reich et al., 2010). Therefore, their absence may be considered as a safety factor, especially in the case of products intended for feeding premature infants, underweight newborns and immunocompromised infants.

A number of reviews published in recent years (FAO/WHO, 2004a) (Gurtler et al., 2005) (Strydom et al., 2012) (Holy and Forsythe, 2014) (Huertas et al., 2015) include bibliographic references that reveal the presence of *Cronobacter* in commercial infant formulae, with a prevalence rate ranging between 0 and 18 %. In 2006-2009, *Cronobacter* was detected in percentages of 0.4 % (Slovakia) and 5 % (Austria) in infant formulae available for sale in countries of the European Union (Helwigh et al., 2012).

The detection of the pathogen in unopened packages of infant formulae is evidence of the intrinsic path of contamination during the product manufacturing process (Holy and Forsythe, 2014).

Although the levels of contamination observed in the majority of cases are less than 1 CFU/g, the positivity of *Cronobacter* in infant formulae is considered a significant risk of infection considering, on the one hand, the low infective dose associated with this pathogen (10-100 organisms) and the consumption of variable quantities of milk several times a day and, on the other hand, the ease of proliferation in the rehydrated product.

The FAO/WHO (2004a) estimated that a significant reduction in the frequency of the

contamination of Infant formulae might reduce the relative risk of *Cronobacter* by between four or five times.

Contrary to the case of *Cronobacter, Salmonella* has rarely been observed in Infant formulae (Muytjens et al., 1988). However, infant formulae have also been involved in outbreaks of salmonellosis in children. For example, the outbreak of gastroenteritis due to *Salmonella Poona* declared in Spain in 2010-2011, for which a statistically significant association was found to the consumption of two brands of powdered milk manufactured by the same company. However, the results of tests carried out in the production environment were negative for *Salmonella* (ISCIII, 2011).

In addition, it has been suggested that the intrinsic contamination of the product was the cause of other outbreaks of salmonellosis in infants, where the low level of *Salmonella* detected in the involved milk was common in all the cases, making it more difficult to detect in routine controls (Cahill et al., 2008).

Infant formulae are manufactured from various ingredients: including milk, soya protein, carbohydrates, fats, minerals and vitamins, using three different types of process: wet mixing process, dry mixing process and combined process.

Depending on the process applied, different risk factors can be identified which potentially result in the contamination of the product obtained. These factors include the following:

5.2.1.1 Heat treatment

In the wet mixing process all the ingredients are handled in a liquid phase, subjected to pasteurisation by heat treatment and then dried to obtain the powdered product.

Some studies have revealed that the pasteurisation process applied during the manufacture of infant formulae is adequate for inactivating *Cronobacter sakazakii* and other enterobacteria present (Fu et al., 2011), although it has been shown that the resistance to heat of this pathogen may vary with the strain, the pH, the water activity and the thermal stress (Arroyo et al., 2009). Furthermore, the combinations of time and temperature used to achieve the pasteurisation should consider properties of the product such as the fat content, dry material or total solids, due to the possible effects that they may have on the heat resistance of the pathogenic microorganisms in question.

In one of the most recent studies (Huertas et al., 2015) treatments of 58 ºC/2.99 minutes or 62 ºC/0.17 minutes were established to obtain a reduction of 5 log cycles of a strain of *Cronobacter sakazakii* particularly tolerant to stress.

In addition the heat treatment applied in the infant formulae manufacturing process is considered adequate for the inactivation of other vegetative pathogenic microorganisms such as *Salmonella, Listeria monocytogenes* or *Staphylococcus aureus*, while spore-forming pathogens such as *Bacillus cereus* and *Clostridium botulinum* are partially inactivated, depending on the manufacturing conditions (FAO/WHO, 2004a).

Considering that the pasteurisation processes used in the wet mixing manufacturing process are sufficient to eliminate the pathogens, contamination of the product with *Cronobacter* or

Salmonella in this type of process is due to a new contamination after the heat treatment (FAO/WHO, 2004a).

5.2.1.2 Ingredients added during the manufacturing process

In the dry mixing process, the ingredients prepared separately are dry mixed until the product formulation is obtained, and in the combined process, some of the ingredients are added to the liquid mixture and undergo pasteurisation heat treatment, whereas other ingredients including the vitamins, minerals or carbohydrates are added after the heat treatment, during the mixing phase.

In neither case, can the manufacturer guarantee microbial inactivation in the added ingredients, which become a potential source of contamination of the product. *Cronobacter* has been isolated from different ingredients including powdered skimmed milk, lactose, lecithin, but starch has the highest rate of prevalence (FAO/WHO, 2004a).

In these cases, the safety of the final product depends on strict compliance by suppliers with the food safety and hygiene regulations necessary to ensure that the ingredients comply with the same microbiological requirements as those of already finished powdered formulae.

5.2.1.3 Preparation environment during drying or packaging

After the heat treatment, the principal contamination risk refers to the preparation environment during the dry phases of the process (drying and packaging). During the drying process, the liquid mixture is dried almost instantly in hot air, and the resultant powder is cooled, sieved and transported to storage silos or directly to the packaging lines. This process constitutes a risk factor for contamination, especially in the case of the more ubiquitous pathogens such as *Cronobacter*.

Although the usual reservoir of *Cronobacter* remains unknown, the fact that it has been isolated in a huge variety of environments (food, powdered milk production facilities and other food production plants and domestic environments) is a reflection of its ubiquitous nature.

Various studies reveal the risk of recontamination with *Cronobacter* in the preparation environment. In one study carried out by Reich et al. (2010) a total of 867 samples, obtained from 12 different locations at an infant formulae processing plant, were analysed and *Cronobacter* was detected in 33 samples of powder taken from facilities involved in the drying and packaging processes; on the contrary, the 175 samples taken from surfaces produced negative results. Nevertheless, *Enterobacteriaceae* with <100 CFU/g was found in all the samples analysed except for seven locations that gave counts of >500 CFU/g, five of these coinciding with the positive results for *Cronobacter*.

Other studies advise of the importance of the correct installation and maintenance of the air filters (Mullane et al., 2008), or the cleaning and disinfection of the external parts of the equipment and the environment around the processing lines (Craven et al., 2010), in order to reduce the spread of *Cronobacter* and other biological hazards in the infant formulae production environment.

The osmotolerance capacity of *Cronobacter* may be linked to its persistence in the environment and with the post-preparation risk of contamination of infant formulae (FAO/WHO, 2004a).

Another remarkable aspect is the fact that *Cronobacter* and other enterobacteria are microorganisms able to survive attached to the interior surfaces of the equipment in direct contact with the product, thereby increasing the risk of recontamination after the application of the inactivation heat treatment.

In addition, *C. sakazakii* has also been isolated from the intestinal tract of the fly *Stomoxys calcitrans*, which is found all around the world (Hamilton et al., 2003) (Mramba et al., 2006) and of the Mexican fruit fly *Anastrepha ludens* (Kuzina et al., 2001). These findings make these insects a secondary source of contamination and their presence in the manufacturing environment is an additional risk factor.

The prevention of intrinsic contamination focuses on the following areas:

1. Pasteurisation heat treatment to reduce the vegetative forms of pathogens to a level at which they do not pose a threat to health.
2. Microbiological quality control of the raw material added to the product after pasteurisation, with a careful selection of the ingredients, inspections to assess the supplier processes, control and monitoring of the procedures and regular checks of the ingredients obtained.
3. Environmental monitoring programmes to reduce the levels of enterobacteria in the general and processing environments (equipment and processing lines).
4. Labelling of infant formulae to include the information that the product is not a sterile product.

5.2.2 Extrinsic contamination

Extrinsic contamination is produced as a result of poor hygienic practices during the handling, preparation or administration of the infant formulae, both in the domestic environment and in hospitals.

In this case, the factors of risk can be divided into three groups: 1) factors associated with the contamination of the product, 2) factors associated with the survival of pathogens in the powdered product, and 3) factors associated with the proliferation of pathogens in the rehydrated powdered product.

5.2.2.1 Risk factors associated with the contamination of the milk

In the domestic environment, the principal risk factors favouring the contamination of the product once the container has been opened include the materials used in the preparation and administration of the milk, the water used for rehydration and the people involved in the preparation.

Feeding bottles and materials used in the preparation

Cronobacter has been isolated from utensils, blenders and spoons used for the preparation of infant formulae (FAO/WHO, 2004a), and from the environment in which the feeding bottle is prepared in the home (Kandhai et al., 2004).

As indicated above, certain pathogenic bacteria are able to easily form biofilms making them more resistant to inactivation by physical agents (temperature and drying) and chemical agents (detergents and disinfectants) (Beuchat et al., 2009). The capacity of *Cronobacter* to attach itself to inert materials regularly used in the preparation of infant food, including silicone, latex, polycarbonate, stainless steel, glass or polyvinyl chloride has been proven in experiments (Iversen et al., 2004) (Hurrell et al., 2009).

In addition, the formation of biofilms is enhanced by the availability of nutrients in the environment. Temperature may also play a significant role as it is stimulated at 25 ºC and inhibited at 12 ºC, even in the presence of nutrients (Kim et al., 2007) (Beuchat et al., 2009). Other members of the *Enterobacteriaceae* family including *Salmonella* and *Escherichia coli* are also associated with the formation of biofilms on inert surfaces (Zogaj et al., 2003).

Consequently, the pathogenic bacteria remain on the surface of the materials used in the preparation of the milk or even in the feeding bottle itself, making these a source of cross contamination although no traces of milk are observed.

In a microbiological assessment study of the preparation of infant formulae in hospital environments in France (Tudela et al., 2008) species of *Bacillus* were observed in 54 % of formulae for premature infants and in 19 % of other types of formulae, together with the presence of coagulase-negative staphyloccocci in two samples and *Clostridium* bifermentans in another one. In addition, microorganisms were found on 4.3 % of the surfaces analysed, including *Enterobacter cloacae, Pseudomonas fluorescens, Bulhkholderia cepacia* and *Staphylococcus aureus.*

These results demonstrate the huge importance of a thorough cleaning of materials in contact with the product, as a measure for preventing the development of biofilms and avoiding their persistence in the milk preparation environment.

In addition, it has been shown that raw animal and plant foods are reservoirs of pathogens including *Salmonella* spp., *Campylobacter jejuni, Clostridium perfringens, Yersinia enterocolitica, Listeria monocytogenes* and *Staphylococcus aureus. Cronobacter* has also been isolated from different foods of animal and plant origin including meat, cheese, herbs, spices and ready-to-eat salads (Holy and Forsythe, 2014). Therefore, food must be considered a potential source of cross contamination in the domestic environment in which powdered formula is prepared, either via the hands of the individuals preparing it or via surfaces or kitchen cloths.

The risk assessment drafted by the FAO/WHO (2004a) estimated a relative reduction of the risk of infection by *Cronobacter* of 1.2 times, which can be attributed to the improvement in hygiene in the powdered formula preparation environment.

The WHO (2007) recommends the sterilisation of feeding bottles and of all the materials used in the preparation process after use, using a commercial steriliser (observing the manufacturer's instructions), pressure cooker or boiling water. Prior to the sterilisation process, all the material should be washed in warm soapy water, using a specific special brush to remove traces of milk from feeding bottles and teats. Moreover, clean kitchen cloths should be used and preparation surfaces must be kept clean.

Reconstitution water

The preparation of the infant formulae for consumption requires the reconstitution of the formula by adding water and then stirring until obtaining a uniform mixture.

The WHO (2008) in "Guidelines for Drinking-water Quality" establishes that safe drinking-water does not represent any significant risk to health over a lifetime of consumption, including the different sensitivities that may occur in individuals at different life stages. Nevertheless, one consequence of the different sensitivities of individuals to the pathogenic agents is that exposure to drinking water of a particular quality may have different effects on health in different populations. Therefore, in the case of infants and young children, and given their sensitivity to microorganisms whose presence in water is not normally of concern, additional precautions are required.

The principal microbial risks associated with drinking-water are referred to faecal pathogens (bacteria, viruses, protozoa and helminths). The destruction of these agents with disinfection is of unquestionable importance for the supply of safe drinking-water. Nevertheless, standard chemical disinfection using products such as chlorine does not guarantee the safety of the water, in view of its limited efficiency against pathogenic protozoa and certain viruses. The normal conditions of chlorination reduce the risk of *Escherichia coli*, Rotavirus, hepatitis A and poliovirus type 1 infections by 99.9 %. However, the dose needs to be 150 times higher to inactivate the cysts of *Giardia* and $7x10^6$ times higher to inactivate the oocysts of *Cryptosporidium* (WHO, 1996).

Therefore, the verification of the microbiological quality of the water measured as the absence of faecal pollution indicators *(Escherichia coli)* does not necessarily imply that these pathogens are not present.

A study carried out in Spain reported the detection of parasites in drinking water in the region of Galicia, *Cryptosporidium* spp. in 40.1 % and Giardia duodenalis in 33.8 % of the samples analysed by PCR (Castro-Hermida et al., 2015). Moreover, both parasites were detected in the mains water supply in the city of Sao Paulo in Brazil, in concentrations ranging from 0.1 to 97 cysts/l of *Giardia* in 49.5 %, and between 0.1 and 6 oocysts/l of *Cryptosporidium* in 9.2 % of the water samples analysed (Sato et al., 2013).

The increase in the number of cases of cryptosporidiosis reported in 2012 in the United Kingdom, Holland and Germany is also of note. Although the causes have not been established, the possibility of contaminated bottled water has been suggested as one of the factors involved (ECDC, 2012).

There is no evidence that *Cronobacter* is transmitted through drinking water, although it may be present in poor quality water. In a recently published review (Holy and Forsythe, 2014) the importance of water as a vehicle of transmission for *Cronobacter* was stressed, together with the lack of attention given to the potential reservoir for this pathogen. Nevertheless, the organism is sensitive to disinfectants and its presence can be avoided with adequate disinfection.

From the toxicological point of view, bottled water is suitable for use in the preparation of feeding bottles, but this is not synonymous to ensuring that these are sterile and therefore free of microorganisms.

Some investigations carried out to compare the microbiological quality of distribution drinking water and of bottled mineral water indicate that the use of bottled water does not offer a greater guarantee that the reconstituted milk will be harmless, unless the water is sterilised (Zamberlan da Silva et al., 2008). Varga (2011) investigated the microbiological quality of 246 samples of non-carbonated bottled mineral water from Austria, Croatia, France, Germany and Italy and found coliforms in 9.3 %, *Escherichia coli* in 2.8 %, *Enterococcus* spp. in 0.8 % and *Pesudomonas aeruginosa* in 2.4 %; on the contrary, all the samples produced negative results for sulphite-reducing anaerobes. Also of note were the counts above 100 CFU/ml of microorganisms at 22 and at 37 ºC in 24 and 20 % of the samples, respectively.

According to the CDC (1995), boiling water for the preparation of powdered milk for 1 minute guarantees the inactivation of protozoa, bacteria and virus. Similarly, the WHO recommends boiling water for 1 minute (counted from when it starts to boil at the surface), adding 1 minute per 1 000 metres above sea level.

In light of the above, it is a good idea to include the practice of boiling both tap water and bottled water used for the reconstitution of infant formulae. This is an additional measure for guaranteeing the destruction of the non-pathogenic microbiota that may be present in the water, normally not of concern, but which may pose a problem for the infant given his or her reduced immune capacity.

Personnel

Food handlers may be carriers of *Cronobacter, Salmonella* and other pathogens. Therefore, if food is handled without considering good hygienic practices, it may be contaminated.

In a study carried out in Holland between 2001 and 2005 to determine the possible reservoirs of *Cronobacter*, the pathogen was isolated in 1 out of 98 samples of faeces and 1 out of 116 samples of skin from the handlers. These results indicate the possibility that personnel may be a strong source of contamination of the powdered milk during its handling (Kandhai et al., 2010).

In addition, infected individuals or asymptomatic carriers of *Staphylococcus aureus, Salmonella* or *Shigella*, infected by norovirus or by other viruses such as the hepatitis A virus even during the incubation period, are a risk factor, especially when handling food that has not been subjected to heat treatment prior to consumption and is intended for a risk population, such as that of infants.

5.2.2.2 Risk factors associated with the survival of pathogenic agents in infant formulae

Both *Cronobacter* and *Salmonella* are able to survive in dry food for long periods of time by remaining in a latent state and recovering the capacity to grow when environmental conditions are more favourable.

Cronobacter has been shown to have a greater tolerance to drying in comparison to other enterobacteria such as *Escherichia coli* and *Salmonella* (Breeuwer et al., 2003). This tolerance to drying is well documented in the scientific literature, there is evidence that it can survive in infant formulae with water activity between 0.25 and 0.5 (Strydom et al., 2012) (Huertas et al., 2015) and

it has been demonstrated that it may remain in the infant formulae for up to 2.5 years (Barron et al., 2007).

Moreover, *Cronobacter* has been shown to have a significant resistance to acid pH (FAO/WHO, 2004a, 2006).

Once the resistance to drying of these pathogens has been recognised together with their viability in the powdered product for long periods of time, the interest in its control focuses on the possibility of inactivation during the milk reconstitution process and in preventing its multiplication in the reconstituted product.

In accordance with the risk assessment drafted by the FAO/WHO (2004a), the two factors that result in the greatest reduction of the risk associated with *Salmonella* and *Cronobacter* are the length of consumption time and the inclusion of a bactericide treatment at the time of rehydration.

The principal factors of risk linked to the survival capacity of pathogens in powdered formulae and in the rehydrated product are described below.

Conditions for the conservation of the product in powder

Beuchat et al. (2009) observed that the survival capacity of *Cronobacter* in infant powdered milk formulae was higher, the lower the availability of free water in the medium and the lower the conservation temperature (30, 21 and 4 ºC). Conversely, no differences were observed in the survival capacity with respect to the composition of the infant formulae.

Although the reduction of *Cronobacter* during the useful life of the Infant formulae has been proven experimentally, this is very slow (0.001 $\log_{10}$ CFU per day) and does not imply a significant reduction of the relative risk (FAO/WHO, 2006).

In addition, it does not seem likely that the water content of the infant formulae after opening and during storage would increase sufficiently to enhance the growth of the pathogens that would have contaminated the product (FAO/WHO, 2004a). Nevertheless, the powdered product should be kept closed, in a dry atmosphere, with a relative humidity of less than 70 %, and at a temperature of less than 20 ºC for a maximum of 1 month (Vargas-Leguás et al., 2009).

Temperature of water reconstitution

The temperature of the water used for the rehydration of the infant formulae may have a significant role in the control of the risk associated with *Cronobacter*, and there are many studies that link this factor to the risk of survival of the pathogen and its subsequent proliferation in reconstituted milk.

Although the species of the genus *Cronobacter* have been considered to be more thermotolerant than other enterobacteria (Nazarowec-White and Farber, 1997b), the majority of the studies of heat resistance generally display relatively low D values (at 58 ºC): 0.27-0.5 minutes (Breeuwer et al., 2003); 2.6 minutes (Iversen et al., 2004); 4.2 minutes (Nazarowec-White and Farber, 1997b) and 9.9 minutes in the case of a particularly heat resistant strain (Edelson-Mammel and Buchanan, 2004). These differences may be due to the influence of the strain. It has been shown in infant formulae reconstituted at 56-70 ºC, that the heat resistance of 12 different strains of *Cronobacter*

may vary up to 20 times (Edelson-Mammel and Buchanan, 2004), reaching the conclusion that there are two different types of heat resistant phenotypes (FAO/WHO, 2004a). At 70 ºC the D value estimated for *Cronobacter* in infant formulae is 0.07 minutes (Edelson-Mammel and Buchanan, 2004).

The scientific literature contains various studies aimed at establishing the factors that may affect the heat resistance of *Cronobacter* in the rehydration process. In general, the results obtained show that 1) prolonged storage of the infant formulae increased the susceptibility of the pathogen to heat during the rehydration process with hot water (Osaili et al., 2008); 2) the thermal shock at temperatures below 47 ºC for 15 minutes improved the thermal tolerance (Chang et al., 2009); and 3) the stationary-phase cells maintained between 20 and 37 ºC (D60=0.9 min) were more resistant than those maintained at 10 ºC (D60=0.2 min) and, moreover, the heat resistance increased at neutral pH and a low free water content (Shaker et al., 2007) (Arroyo et al., 2009).

Other studies have focussed on studying the survival of *Cronobacter* in reconstituted milk according to the temperature of the water added. Osaili et al. (2008, 2009) obtained a reduction of 5.3 $\log_{10}$ with water at 70 ºC and of 6 $\log_{10}$ when hot water at 80-100 ºC was added. Similar results are offered by Edelson-Mammel and Buchanan (2004), who reported reductions of 1 and ≥4 $\log_{10}$ with water at 60 and 70 ºC, respectively. Other recent studies reveal that although the reconstitution of milk with water at 70 ºC implies a significant reduction of the population of *Cronobacter*, the surviving microorganisms can multiply up to $1.57x10^3$ CFU/ml after 24 hours at room temperature (Huertas et al., 2015).

It is also possible that hot water (70 ºC) may activate spores of pathogenic bacteria present in the formula, which may multiply if the prepared feeds are kept at temperatures above the refrigeration temperature for prolonged periods of time (WHO, 2007).

The risk assessment carried out by the FAO/WHO (2004a) estimates a relative risk reduction for *Cronobacter* of 10 000 times when a treatment able to reduce its initial population by 4 log cycles is applied. On the contrary, in terms of risk, rehydration with water at 40-50 ºC is the worst case-scenario compared to water temperatures of less than 40 ºC (FAO/WHO, 2006).

Even considering the existence of strains of *Cronobacter* with a greater thermotolerance than the majority of enterobacteria (Edelson-Mammel and Buchanan, 2004), the FAO/WHO (2004a, 2006) concluded that the inactivation of *Cronobacter* may be achieved in a short time with temperatures above 70 ºC, and recommends rehydration with water at 70 ºC to reduce the risk of infection in infants (WHO, 2007).

5.2.2.3 Risk factors associated with the proliferation of pathogens

As explained above, *Cronobacter* survives in the powdered product for long periods of time and once the product has been reconstituted, even at 70 ºC, it is able to easily multiply depending on the ambient temperature (Huertas et al., 2015).

Iversen and Forsythe (2003) indicated that *Cronobacter* can grow at temperatures of between 6-47 ºC, with an optimum growth temperature of 39 ºC; however, some strains are inhibited at temperatures of over 44 ºC (Nazarowec-White and Farber, 1997a) (Iversen et al., 2004) while other

strains are able to grow at 5 ºC (Nazarowec-White and Farber, 1997b). At 4 ºC both *Cronobacter* and other pathogenic enterobacteria remain inactive.

The capacity for growth is also higher at a pH of 7.2, whereas it is slightly inhibited at a pH of 11.0 (Fu et al., 2011). The resistance of *Cronobacter* to osmotic stress is reflected in its capacity to grow at water activity of 0.94.

The principal risk factors linked to the proliferation of pathogens in rehydrated milk are listed below.

Cooling of the reconstituted product

The study by Huertas et al. (2015) shows certain risk factors linked to the proliferation of *Cronobacter* in reconstituted infant formulae up until the moment of consumption. They assessed the effect of cooling the product rehydrated with water at 70 ºC until reaching the optimum temperature for feeding the infant of 37 ºC. Given an initial concentration of 1.4 $\log_{10}$ CFU/ml, they confirmed that after 24 hours at ambient temperature, the milk cooled slowly using tap water had lower counts (2.82 $\log_{10}$ CFU/ml) than the milk cooled quickly using refrigerated water (5.39 $\log_{10}$ CFU/ml). These results indicate that the cooling of milk reconstituted with hot water at 70 ºC may have a significant impact on the survival and subsequent growth of *Cronobacter* and that in order to reduce the risk of infection, the practice of cooling the milk with tap water until reaching the optimum temperature for feeding of approximately 37 ºC is to be recommended.

Waiting time and conservation of the reconstituted product between preparation and feeding to the infant

Beuchat et al. (2009) studied the conditions of growth of *Cronobacter* in reconstituted infant formulae and did not detect growth when the milk was kept at 4 ºC although the pathogen remained viable for 72 hours after rehydration. On the contrary, depending on the initial concentration of the inoculum, values of 1 $\log_{10}$ CFU/ml were obtained or exceeded when the milk was kept at 12, 21 and 30 ºC for 48, 12 and 8 hours, respectively, regardless of the infant formula.

Iversen et al. (2004) studied the growth rate of *Cronobacter* in reconstituted infant formulae and confirmed that the generation times at 6, 21 and 37 ºC were 13.7 hours, 1.7 hours and 19-21 minutes, respectively; these results demonstrate the capacity of *Cronobacter* to grow quickly if the product is kept at ambient temperature.

Rosset et al. (2009) identified various risk factors for the growth of *Cronobacter* in feeding bottles prepared in a hospital environment, including the initial temperature of the milk, the storage temperature and time of the bottle prior to administration and the reheating temperature. They concluded that the risk of infection was the consequence of the combination of more than one risk factor.

The risk assessment conducted by the FAO/WHO (2004a) estimates a reduction of the relative risk for *Cronobacter* by 30 if the milk is administered within 2 hours of preparation. Conversely, the risk increases by 30 after 6 hours, by 1 000 after 8 hours and by 30 000 if the time is extended to 10 hours.

The growth of other pathogens, including *Bacillus cereus*, has also been studied in samples of reconstituted powdered milk experimentally inoculated. It has been observed that milk refrigerated at 7 ºC for 24 hours maintains the same concentration levels of *Bacillus cereus* as at the start, whereas after 6 hours at 31 ºC or 12 hours at 25 ºC levels were obtained corresponding to a risk of food poisoning (Rodríguez Máuriz et al., 1996).

Given the available information, the majority of Public Health institutions establish control measures based on the maintenance of the rehydrated infant formulae in refrigerated environments at temperatures of less than 5 ºC for a maximum of 24 hours, and the disposal of any product that has been left at ambient temperature or higher for more than 2 hours.

Reheating of rehydrated product

In accordance with the risk assessment drafted by the FAO/WHO (2006), reheating of the infant formulae rehydrated at 37 ºC is a factor which increases the risk of *Cronobacter* regardless of the rehydration temperature applied, unless a temperature of 70 ºC is applied.

5.3 Hygiene measures to minimise the microbiological risks associated with the preparation of infant formulae

In 2007, the WHO and the FAO drafted a set of guidelines for the safe preparation of feeding bottles. These guidelines were the basis of the Code of hygienic practice for powdered formulae for infants and young children (CAC/RCP 66-2008) published with the objective of providing practical guidance and recommendations on the hygienic manufacture of infant formulae and on the subsequent hygienic preparation, handling and use of reconstituted formulae.

In addition, a number of organisations and public health authorities have published recommendation for the safe handling of infant formulae (EFSA, 2004) (FSA, 2006, 2010, 2013) (BDA, 2007) (FSAI, 2012) (NHS, 2012) (Health Canada, 2012) (ACSA, 2013) (ANSES, 2013) (BSNA, 2013) (FDA, 2014).

The hygiene standards recommended for reducing the risk of infection from *Cronobacter* and *Salmonella*, associated with the handling, preparation and administration of infant formulae are listed below, understanding that, in general, these are also applicable to the control of other microbiological risks associated with these products:

5.3.1 Measures on the use of sterile or decontaminated liquid formulae

Whenever possible and feasible, commercially sterile liquid formula, or formula that has undergone an effective point-of-use decontamination procedure, should be used, especially when intended for high-risk infants.

5.3.2 Measures for the conservation of the powdered product

- Keep the powdered product well closed in dry atmosphere for a maximum of 1 month.
- Dispose of any product stored in inadequate conditions.

5.3.3 Measures prior to the preparation of the feeding bottle

- Wash hands prior to starting to handle the powdered product.
- Individuals suffering from diarrhoea or gastroenteritis must not prepare the feeding bottle.
- Use clean cloths and clean work surfaces before use in the preparation of the feeding bottle. These practices prevent cross contamination with raw food or other contaminated materials.
- All materials used in contact with the powdered or rehydrated formula, including feeding bottles, teats, spoons and brushes must be washed with warm soapy water. Special brushes should be used to remove traces of milk, as higher-risk pathogens are capable of producing biofilms on the walls of glass, plastic and teats.
- Disinfection of utensils and bottles prior to use. This practice guarantees the elimination of pathogens from the surfaces in contact with the milk and prevents cross contamination, provided they are protected from environmental contamination and are handled in hygienic conditions.

5.3.4 Measures for the reconstitution of the powdered formula

- Cover infant formula container immediately after use.
- During the preparation process, the milk container lid and the spoons must only be placed on clean surfaces.
- Only use safe drinking for the reconstitution of the milk. This should be boiled for 1 minute. If bottled water is used, the same procedure should be observed. Both mains water and bottled water that meet legal criteria are suitable for the preparation of infant formulae (AECOSAN, 2014b).
- The powdered formula should be reconstituted with drinking water at a temperature of 70 ºC. It should be used within 30 minutes of boiling. Hot water reduces the number of potentially present pathogenic vegetative cells.
- Cool the well-closed bottle by holding under a running tap, making sure that the water does not enter the teat.
- Microwaves should not be used, as the temperature distribution is uneven.
- When it is not possible or suitable to use hot water, rehydrate the powder formula using water at 20 ºC, shake well and give feed immediately, disposing of any excess 2 hours after its preparation.

5.3.5 Feeding and storage measures for the reconstituted product

- Reduce the time between the preparation of the dehydrated milk formula and its consumption. The feed should be prepared and consumed immediately to prevent possible growth of the microorganism.
- The reconstituted formula must not be kept at room temperature for more than 2 hours, even if water at a minimum temperature of 70 ºC has been used to prepare it.
- If immediate consumption is not possible, keep the recently reconstituted formula in containers and in volumes that permit rapid cooling and storage at temperatures of less than 5 ºC for a maximum of 24 hours.

- After refrigeration, the feed may be heated for a maximum of 15 minutes in a container with hot water, disposing of any excess milk that has been reheated and has not been consumed within 2 hours.
- Do not use bottle heaters for prolonged periods of time, as this will keep the milk in favourable conditions for the growth of pathogenic microorganisms.
- Away from home, the milk should not be reconstituted until it is time for the feed, unless it is possible to guarantee storage at temperatures of less than 5 ºC for a maximum of 2 hours.

6. Microbiological risks associated with the intake of ground and solid foods

In the European Union, cereal-based foods and pureed infant food are subject to strict control. These are safe foods from a microbiological aspect, provided the Good Practices and quality assurance systems are observed during production in order to guarantee compliance with the microbiological requirements of the law.

Home-made pureed food may pose a much greater risk due to factors including the combination of ingredients (meat, vegetables, fruit), the pureeing process, and the intrinsic characteristics (water activity, pH, nutrient content) which make these foods an excellent culture medium for microorganisms, together with the high levels of handling involved in the preparation and storage.

A number of documents are available regarding the principal hygienic measures for preventing microbiological risks during the preparation, storage and consumption of this type of food.

To conclude, a summary is given of the principal hygienic measures (Scott, 2000) (Sockett et al., 2001) (Moore, 2008) (WHO, 2009) (Smith, 2011) (AAP, 2014) (AECOSAN, 2014a) (FDA, 2015):

6.1 For commercial products

- Make sure that the jar is hermetically sealed and that the seal is intact. Where applicable, check that the safety button on the lid of the jar is facing downwards. Do not use the product if this button is not released when the jar is opened. Reject any jars that are cracked, dented, damaged or have rusty lids.
- The manufacturer's instructions regarding the storage and administration of the product must be strictly observed.
- Do not use after the best-before date given on the label.
- If the child is fed directly from the jar, any left-over food must not be stored in the refrigerator. It is best to serve the usual portion on a plate and store the remaining food left in the jar in the refrigerator. Any left-over food on the plate must be discarded.
- Once the jar is open and the food has been heated, do not leave it at room temperature for more than 2 hours.

6.2 For home-made pureed food and solid food

General standards of hygiene during preparation and storage

- Use drinking water to prepare food and to clean utensils and wash hands.
- Wash hands often with warm water and soap, for at least 20 seconds, and always before and

after handling food, after contact with any dirty material (waste, animals), and especially after using the bathroom and after any contact with faeces-contaminated material (nappies, baby changer, underwear…).

- Individuals suffering from diarrhoea or gastroenteritis must not prepare this food.
- Use warm water and soap to wash liquidisers, grinders or blenders or any other utensil in contact with food. Rinse thoroughly with warm water after washing.
- Avoid cross contamination, using different utensils for raw and cooked food. Cooked food must be stored in the refrigerator in a separate compartment, away from the raw food.
- Wash fresh fruit and vegetables thoroughly under a running tap even if they are going to be peeled. This process should not be carried out prior to storage but immediately before eating.
- Wash and dry fruit before preparing fresh fruit juices.
- Store raw meat, poultry, fish and dairy products in the coldest part of the fridge immediately after purchase.
- Cook food, especially meat and fish, making sure the correct temperature is reached in all parts of the food in order to destroy any microorganisms: a temperature of 71 ºC should be reached for at least 1 minute (until the meat changes colour in the centre of the product).
- Make sure that the refrigerator maintains the correct temperature (less than 5 ºC).
- When reheating food that has been cold-stored, procedures must be used to guarantee that temperatures of more than 65-70 ºC are reached in less than 1 hour. In addition, this should be carried out as close as possible to the moment of eating.
- Any left-over reheated food must be thrown away.

In the case of pureed food, these hygiene measures must be strictly observed. Some specific measures include:

- When preparing pureed food it is best to use fresh food wherever possible. Frozen food may be used if it has been correctly defrosted.
- Although tinned food may be used, food that has been canned at home or food with a damaged container should be avoided: for example if the tin is rusted, dented, swollen, porous, etc.
- Once the food has been prepared and pureed it can be kept in the refrigerator in a clean and hermetically sealed container for 24-48 hours.
- During the cooling period (from cooking to refrigeration or freezing) it should be kept in the recipient in which it was cooked, covered and untouched. Pureeing should be done immediately before putting the food in the refrigerator or freezer.
- Freeze pureed food in sealed containers, each one containing an individual portion. As a general rule, it can be kept for a maximum of 3 months (1-2 months for purees containing meat or fish; 3-6 months for vegetables), after which time it must be discarded.
- Do not leave food at room temperature for more than 2 hours. If travelling, carry the food in a cold box with thermal insulation.
- Do not store any left-over food in the refrigerator.

6.3 Foodstuff that may pose a risk in young children's food

Children under 3 years are advised not to eat:

- Raw milk and raw milk derivatives.
- Soft cheeses prepared with raw milk.
- Raw fruit and vegetables that have not been washed first.
- Raw sprouts (alfalfa, soybean...).
- Unpasteurised juices, unless they have been prepared immediately before consumption and made from fruit which has been washed.
- Eggs which are not fully "set". Food containing raw egg, including home-made sauces and mayonnaises, mousse, meringues and home-made pastries, tiramisu, home-made ice-creams and eggnog.
- Raw meat, rare or medium-rare meat. Smoked or marinated meat that is not going to be cooked later, including Frankfurt-type sausages. These foodstuffs must always be cooked correctly.
- Raw bivalve molluscs. Some authors advise against any type of raw, smoked, refrigerated or marinated fish which is not going to be cooked afterwards.
- Foods produced by individuals for their own consumption (for example products from their own kitchen garden, eggs from domestic hens…).
- Children under the age of 1 year should not eat honey.

References

Acheson, D. and Allos, B.M. (2001). Campylobacter jejuni Infections: Update on Emerging Issues and Trends. *Clinical Infectious Diseases*, 32 (8), pp: 1201-1206.

ACSA (2013). Agencia Catalana de Seguridad Alimentaria. Fichas de seguridad alimentaria. La preparación de biberones. Available at: www.gencat.cat [accessed: 2-11-15].

AECOSAN (2011). Agencia Española de Consumo, Seguridad Alimentaria y Nutrición. Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) sobre el botulismo infantil. *Revista del Comité Científico de la AESAN*, 14, pp: 9-26.

AECOSAN (2014a). Agencia Española de Consumo, Seguridad Alimentaria y Nutrición. Informe del Comité Científico de la Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN) en relación con los riesgos microbiológicos asociados al consumo de determinados alimentos por mujeres embarazadas. *Revista del Comité Científico de la AECOSAN*, 19, pp: 11-49.

AECOSAN (2014b). Agencia Española de Consumo, Seguridad Alimentaria y Nutrición. Informe del Comité Científico de la Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN) sobre los criterios necesarios para poder efectuar en las aguas minerales naturales la mención "indicada para la preparación de alimentos infantiles". *Revista del Comité Científico de la AECOSAN*, 20, pp: 11-43.

AEP (2012). Asociación Española de Pediatría. Protocolo para la alimentación con leche materna en las escuelas infantiles. Available at: http://www.aeped.es/comite-lactancia-materna/documentos/protocolo-alimentacion-con-leche-materna-en-las-escuelas-infanti [accessed: 2-11-15].

Al-Holy, M.A., Lin, M., Abu-Ghoush, M.M., Al-Qadiri, H.M. and Rasco, B.A. (2009). Thermal resistance survival and inactivation of *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered and reconstituted infant formula. *Journal of Food Safety*, 29, pp: 287-301.

Alain, S., Dommergues, M.A., Jacquard, A.C., Caulin, E. and Launay, O. (2012). State of the art: Could nursing mothers be vaccinated with attenuated live virus vaccine? *Vaccine*, 30, pp: 4921-4926.

Allerberger, F., Friedrich, A.W., Grif, K., Dierich, M.P., Dornbusch, H.-R., Mache, C.J. and Zimmerhackl, L.B. (2003). Hemolytic-uremic syndrome associated with enterohemorrhagic *Escherichia coli* O26:H infection and consumption of unpasteurized cow's milk. *International Journal of Infectious Diseases*, 7 (1), pp: 42-45.

AAP (2012). American Academy of Pediatrics. Breastfeeding and the Use of Human Milk. *Pediatrics*, 129 (3), pp: e827-e841.

AAP (2014). American Academy of Pediatrics, and the Center for Foodborne Illness. Young Children and Foodborne Illness.

Anderson, P.H.R. and Stone, D.M. (1955). Staphylococcal Food Poisoning Associated with Spray-Dried Milk. *Epidemiology & Infection*, 53 (4), pp: 387-397.

Anon (2001). Infant botulism: update. *CDR Weekly*, 11 (33), pp: 4.

ANSES (2013). Agence nationale de sécurité sanitairede l'alimentation, de l'environnement et du travail. Infant feeding bottles: how should they be prepared and stored? Using dried infant formula. Available at: www.anses.fr/en/content/infant-feeding-bottles-how-should-they-be-prepared-and-stored [accessed: 2-11-15].

Arku, B., Mullane, N., Fox, M., Fanning, S. and Jordan, K. (2008). *Enterobacter sakazakii* survives spray drying. *International Journal of Dairy Technology*, 61, pp: 102-108.

Arroyo, C., Condón, S. and Pagán, R. (2009). Thermobacteriological characterization of *Enterobacter sakazakii*. *International Journal of Food Microbiology*, 136, pp: 110-118.

Asakura, H., Morita-Ishihara, T., Yamamoto, S. and Igimi, S. (2007). Genetic characterization of thermal tolerance in *Enterobacter sakazakii*. *Microbiology and Immunology*, 51, pp: 671-677.

Asao, T., Kumeda, Y., Kawai, T., Shibata, T., Oda, H., Haruki, K. and Kozaki, S. (2003). An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiology & Infection*, 130 (1), pp: 33-40.

Bahl, R., Frost, C., Kirkwood, B.R., Edmond, K., Martines, J., Bhandari, N. and Arthur, P. (2005). Infant feeding patterns and risks of death and hospitalization in the first half of infancy: multicentre cohort study. *Bulletin of the World Health Organization*, 83, pp: 418-426.

Barron, J.C. and Forsythe, S.J. (2007). Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated powered infant formula. *Journal of Food Protection*, 70, pp: 2111-2117.

BDA (2007). British Dietetic Association. Guidelines for making up special feeds for infants and children in hospital. Available at: www.food.gov.uk [accessed: 2-11-15].

Becker, H., Schaller, G., von Wiese, W. and Terplan, G. (1994). Bacillus cereus in infant foods and dried milk products. *International Journal of Food Microbiology*, 23 (1), pp: 1-15.

Beuchat, L.R., Kim, H., Gurtler, J.B., Lin, L., Ryu J. and Richards, G.M. (2009). *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation. *International Journal of Food Microbiology*, 136, pp: 204-213.

BIOHAZ (2011). Panel on Biological Hazards. European Food Safety Authority. Scientific Opinion on an update on the present knowledge on the occurrence and control of foodborne viruses. *The EFSA Journal*, 9.

BOE (1998). Real Decreto 490/1998, de 27 de marzo, por el que se aprueba la Reglamentación técnico-sanitaria especifica de los alimentos elaborados a base de cereales y alimentos infantiles para lactantes y niños de corta edad. BOE Nº 83 de 7 de abril de 1998, pp: 11638-11643.

BOE (2008). Real Decreto 867/2008, de 23 de mayo, por el que se aprueba la Reglamentación técnico-sanitaria específica de los preparados para lactantes y de los preparados de continuación. BOE Nº 31 de 30 de mayo de 2008, pp: 25121-25137.

Bottone, E.J. (2015). Yersinia enterocolitica: Revisitation of an Enduring Human Pathogen. *Clinical Microbiology Newsletter,* 37 (1), pp: 1-8.

Bowen, A.B. and Braden, C.R. (2006). Invasive *Enterobacter sakazakii* disease in infants. *Emerging Infectious Diseases*, 12, pp: 1185-1189.

Breeuwer, P., Lardeau, A., Peterz, M. and Joosten, H.M. 2003. Desiccation and heat tolerance of *Enterobacter sakazakii*. *Journal of Applied Microbiology*, 95, pp: 967-973.

Bryan, F.L. (1992). Hazard analysis critical control point evaluations: a guide to identifying hazards and assessing risks associated with food preparation and storage Available at: http://apps.who.int/iris/handle/10665/40138?locale=en&null [accessed: 2-11-15]. World Health Organization.

BSNA (2013). British Specialist Nutrition Association. Chief Medical Officer Re-States Advice on the Safe Preparation of Infant Formula. Available at: www.bsna.co.uk/news/91293/Chief_Medical_Officer_ReStates_Advice_on_the_Safe_Preparation_of_Infant_Formula_ [accessed: 2-11-15].

CAC (2008). *Codex Alimentarius*. Código de prácticas de higiene para los preparados en polvo para lactantes y niños pequeños (CAC/RCP 66-2008). Available at: www.codexalimentarius.org [accessed: 2-11-15].

Cahill, S.M., Wachsmuth, I.K., Costarrica, M.L. and Ben Embarek, P.K. (2008). Powdered Infant Formula as a Source of *Salmonella* Infection in Infants. *Clinical Infectious Diseases*, 46, pp: 268-273.

Carmena, D. (2012). Current situation of *Giardia* infection in Spain: Implications for public health. *World Journal of Clinical Infectious Diseases*, 2 (1), pp: 1.

Carter, M.J. (2005). Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology*, 98, pp: 1354-1380.

Castro-Hermida, J., González-Warleta, M. and Mezo, M. (2015). *Cryptosporidium* spp. and *Giardia* duodenalis as pathogenic contaminants of wáter in Galicia, Spain: The need for safe drinking water. *International Journal of Hygiene and Environmental Health*, 218, pp: 132-138.

Caubilla-Barron, J. and Forsythe, S.J. (2007). Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated powdered infant formula. *Journal of Food Protection*, 70, pp: 2111-2117.

CDC (1995). Centers for Disease Control and Prevention. Assessing the public health threat associated with waterborne cryptosporidiosis: Report of a Workshop. *Morbidity and Mortality Weekly Report,* 44 (RR-6), pp: 1-18.

Chang, C.H., Chiang, M.L. and Chou, C.C. (2009). The effect of temperature and length of heat shock treatment on the thermal tolerance and cell leackage o *Cronobacter sakazakii* BCRC 13988. *International Journal of Food Microbiology,* 134, pp: 184-189.

Chen, H.M., Wang, Y., Su, L.H. and Chiu, C.H. (2013). Nontyphoid *Salmonella* infection: Microbiology, clinical features, and antimicrobial therapy. *Pediatrics and Neonatology*, 54, pp: 147-152.

Cilieborg, M.S., Boye, M. and Sangild, P.T. (2012). Bacterial colonization and gut development in preterm neonates. *Early Human Development*, 88 (1), pp: S41-49.

Civardi, E., Garofoli, F., Tzialla, C., Paolillo, P., Bollani, L. and Stronati, M. (2013). Microorganisms in human milk: lights and shadows. *Journal of Maternal-Fetal & Neonatal Medicine*, 26 (2), pp: 30-34.

Collier, M.G., Khudyakov, Y.E., Selvage, D., Adams-Cameron, M., Epson, E., Cronquist, A., Jervis, R.H., Lamba, K., Kimura, A.C., Sowadsky, R., Hassan, R., Park, S.Y., Garza, E., Elliott, A.J., Rotstein, D.S., Beal, J., Kuntz, T., Lance, S.E., Dreisch, R., Wise, M.E., Nelson, N.P., Suryaprasad, A., Drobeniuc, J., Holmberg, S.D. and Xu, F. (2014). Outbreak of hepatitis A in the USA associated with frozen pomegranate arils imported from Turkey: an epidemiological case study. *The Lancet Infectious Diseases*, 14, pp: 976-981.

Craven, H.M., McAuley, C.M., Duffy, L.L. and Fegan, N. (2010). Distribution, prevalence and persistence of *Cronobacter (enterobacter sakazakii)* in the nonprocessing and processing environments of five milk powder factories. *Journal of Applied Microbiology*, 109, pp: 1044-1052.

Cremon, C., Stanghellini, V., Pallotti, F., Fogacci, E., Bellacosa, L., Morselli-Labate, A.M., Paccapelo, A., Di Nardo, G., Cogliandro, R.F., De Giorgio, R., Corinaldesi, R. and Barbara, G. (2014). Salmonellosis Gastroenteritis During Childhood Is a Risk Factor for Irritable Bowel Syndrome in Adulthood. *Gastroenterology*, 147, pp: 69-77.

Dancer, G.I., Mah, J.H., Rhee, M.S., Hwang, I.G. and Kang, D.H. (2009). Resistance of *Enterobacter sakazakii* (*Cronobacter* spp.) to environmental stresses. *Journal of Applied Microbiology*, 107, pp: 1606-1614.

D'Andrea, L., Pérez-Rodríguez, F.J., de Castellarnau, M., Manzanares, S., Lite, J., Guix, S., Bosch, A. and Pinto, R.M. (2015). Hepatitis A virus genotype distribution during a decade of universal vaccination of preadolescents. *International Journal of Molecular Sciences*, 16, pp: 6842-6854.

Dawson, D. (2005). Foodborne protozoan parasites. *International Journal of Food Microbiology*, 103 (2), pp: 207-227.

De la Cochetiere, M.F., Piloquet, H., des Robert, C., Darmaun, D., Galmiche, J.P. and Roze, J.C. (2004). Early intestinal bacterial colonization and necrotizing enterocolitis in premature infants: the putative role of *Clostridium*. *Pediatric Research*, 56 (3), pp: 366-370.

Decludt, B., Bouvet, P., Mariani-Kurkdjian, P., Grimont, F., Grimont, P.A., Hubert, B. and Loirat, C. (2000). Haemolytic uraemic syndrome and Shiga toxin-producing *Escherichia coli* infection in children in France. *Epidemiology and Infection*, 124 (2), pp: 215-220.

Dewey, K.G. and Adu-Afarwuah, S. (2008). Systematic review of the efficacy and effectiveness of Complementary feeding interventions in developing countries. *Maternal and Child Nutrition*, 4 (s1), pp: 24-85.

Díaz-Gómez, N.M. (2005). ¿En qué situaciones está contraindicada la lactancia materna? *Acta Pediatra Española*, 63, pp: 321-327.

Doran, T.I. (1999). The Role of Citrobacter in Clinical Disease of Children: Review. *Clinical Infectious Diseases*, 28 (2), pp: 384-394.

Doyle, M.P. (2003). Foodborne parasites. Wisconsin: Food Research Institute. Available at: https://fri.wisc.edu/files/Briefs_File/parasites.pdf [accessed: 4-11-15].

Doyle, M.E., Hartmann, F.A. and Lee Wong, A.C. (2012). Methicillin-resistant staphylococci: implications for our food supply? *Animal Health Research Reviews*, 13 (2), pp: 157-180.

ECDC (2012). European Centre for Disease Prevention and Control. Increased *Cryptosporidium* infections in the Netherlands, United Kingdom and Germany in 2012. Stockholm. Available at: www.ecdc.europa.eu [accessed: 4-11-15].

Edelson-Mammel, S.G. and Buchanan, R.L. (2004). Thermal inactivation of *Enterobacter sakazakii* in rehydrated infant formula. *Journal of Food Protection*, 67, pp: 60-63.

Edmond, K.M., Zandoh, C., Quigley, M.A., Amenga-Etego, S., Owusu-Agyei, S. and Kirkwood, B.R. (2006). Delayed Breastfeeding Initiation Increases Risk of Neonatal Mortality. *Pediatrics*, 117, pp: e380-e386.

EFSA (2004). European Food Safety Authority. Opinion of the Scientific Panel on biological hazards (BIOHAZ) related to the microbiological risks in infant formulae and follow-on formulae. *The EFSA Journal*, 113, pp: 1-35.

EFSA (2005). European Food Safety Authority. Opinion of the Scientific Panel on biological hazards (BIOHAZ) related to *Campylobacter* in animals and foodstuffs. *The EFSA Journal*, 173, pp: 1-10.

EFSA (2007). European Food Safety Authority. Monitoring and identification of human enteropathogenic *Yersinia* spp. *The EFSA Journal*, 595, pp: 1-30.

EFSA (2009). European Food Safety Authority. Scientific Opinion of the BIOHAZ Panel: Assessment of the Public Health significance of meticillin resistant *Staphylococcus aureus* (MRSA) in animals and foods. *The EFSA Journal*, 993, pp: 1-73.

EFSA (2013). European Food Safety Authority. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011 Part A: *Listeria monocytogenes* prevalence estimates. *The EFSA Journal*, 11 (6), pp: 3241.

Enoch, D.A., Butler, M.J., Pai, S., Aliyu, S.H. and Karas, J.A. (2011). *Clostridium* difficile in children: Colonisation and disease. *Journal of Infection*, 63 (2), pp: 105-113.

Epps, S.V.R., Harvey, R.B., Hume, M.E., Phillips, T.D., Anderson, R.C. and Nisbet, D.J. (2013). Foodborne *Campylobacter*: Infections, Metabolism, Pathogenesis and Reservoirs. *International Journal of Environmental Research and Public Health*, 10 (12), pp: 6292-6304.

Ethelberg, S., Olesen, B., Neimann, J., Schiellerup, P., Helms, M., Jensen, C., Böttiger, B., Olsen, K.E.P., Scheutz, F., Gerner-Smidt, P. and Mølbak, K. (2006). Risk Factors for Diarrhea Among Children in an Industrialized Country. *Epidemiology*, 17, pp: 24-30.

EU (2005). Regulation (EC) No 2073/2005 of the Commission, of 15 november 2005, on microbiological criteria for foodstuffs. OJ L 338 of december 22th, 2005, pp: 1-26.

FAO/WHO (2004a). Food and Agriculture Organization/World Health Organization. *Enterobacter sakazakii* and other micro-organisms in powdered infant formula. Rome: FAO.

FAO/WHO (2004b). Food and Agriculture Organization/World Health Organization. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Roma: FAO.

FAO/WHO (2006). Food and Agriculture Organization/World Health Organization. *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting report. Roma: FAO.

FAO/WHO (2007). Food and Agriculture Organization/World Health Organization. Guidelines for the safe preparation, storage and handling of powdered infant formula. Available at: http://www.who.int/foodsafety/publications/micro/pif2007/en/ [accessed: 4-11-15].

FAO/WHO (2008a). Food and Agriculture Organization/World Health Organization. *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formulae: Meeting Report. Rome: FAO.

FAO/WHO (2008b). Food and Agriculture Organization/World Health Organization. Virus in food: Scientific advice to support risk management activities.

FAO/WHO (2009). Food and Agriculture Organization/World Health Organization. *Salmonella* and *campylobacter* in chicken meat. ROMA: FAO.

FDA (2014). Food and Drug Administration. Consumer Health Information. FDA Takes Final Step on Infant Formula Protections. Available at: www.fda.gov/ForConsumers/ConsumerUpdates [accessed: 4-11-15].

FDA (2015). Food and Drug Administration. Consumer Health Information. Infants & Toddlers. Available at: http://www.fda.gov/Food/FoodborneIllnessContaminants/PeopleAtRisk/ucm047530.htm [accessed: 4-11-15].

Ferens, W.A. and Hovde, C.J. (2011). *Escherichia coli* O157:H7: Animal reservoir and sources of human infection. *Foodborne Pathogens and Disease*, 8, pp: 465-487.

Forsythe, S. (2005). Enterobacter sakazakii and other bacteria in powdered infant milk formula. *Maternal and Child Nutrition*, 1, pp: 44-50.

Friedemann, M. (2009). Epidemiology of invasive neonatal *Cronobacter (Enterobacter sakazakii)* infections. *European Journal of Clinical Microbiology*, 28, pp: 1297-1304.

FSA (2006). Food Standards Agency. Guidance for health professionals on safe preparation, storage and handling of powdered infant formula. Available at: www.food.gov.uk/multimedia/pdfs/formulaguidance.pdf [accessed: 4-11-15].

FSA (2010). Food Standards Agency. FSA reminds parents of advice on making up infant formula. Available at: http://www.food.gov.uk [accessed: 4-11-15].

FSA (2013). Food Standards Agency. Safe preparation of powdered infant formula. Available at: http://www.food.gov.uk [accessed: 4-11-15].

FSAI (2012). Food Safety Authority of Ireland. Guidance Note No. 22.Information Relevant to the Development of Guidance Material for the Safe Feeding of Reconstituted Powdered Infant Formula (Revision 2). Available at: www.fsai.ie [accessed: 4-11-15].

Fu, S., Gao, J., Liu, Y. and Chen, H. (2011). Isolation of Cronobacter spp. Isolates from Infant Formulas and Their Survival in the Production Process of Infant Formula. *Czech Journal of Food Sciences*, 29 (4), pp: 391-399.

Gajdosova, J., Benedikovicova, K., Kamodyova, N., Tothova, L., Kaclikova, E., Stuchlik, S., Turna, J. and Drahovska, H. (2011). Analysis of the DNA region mediating increased thermotolerance at 58ºC in *Cronobacter* spp. and other enterobacterial strains. *Antonie Van Leeuwenhoek*, 100, pp: 279-289.

Gallay, A., De Valk, H., Cournot, M., Ladeuil, B., Hemery, C., Castor, C., Bon, F., Mégraud, F., Le Cann, P. and Desenclos, J.C. (2010). A large multi-pathogen waterborne community outbreak linked to faecal contamination of a groundwater system, France, 2000. *Clinical Microbiology and Infection*, 12, pp: 561-570.

Ganesh, B., Banyai, K., Martella, V., Jakab, F., Masachessi, G. and Kobayashi, N. (2012). Picobirnavirus infections: viral persistence and zoonotic potential. *Reviews in Medical Virology*, 22, pp: 245-256.

Granum, P.E. and Lund, T. (1997). *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiology Letters*, 157 (2), pp: 223-228.

Grass, J.E., Gould, L.H. and Mahon, B.E. (2013). Epidemiology of foodborne disease outbreaks caused by *Clostridium perfringens*, United States, 1998-2010. *Foodborne Pathogens and Disease*, 10 (2), pp: 131-136.

Gronlund, M., Arvilommi, H., Kero, P., Lehtonen, O. and Isolauri, E. (2000). Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0-6 months. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, 83 (3), pp: F186-F192.

Guarino, A., Albano, F., Ashkenazi, S., Gendrel, D., Hoekstra, J.H., Shamir, R. and Szajewska, H. (2008). European Society for Paediatric Gastroenterology, Hepatology, and Nutrition/European Society for Paediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: executive summary. *Journal of Pediatric Gastroenterology and Nutrition*, 46, pp: 619-621.

Gurtler, J.B., Kornacki, J.L. and Beuchat, L.R. (2005). *Enterobacter sakazakii*: a coliform of increased concern to infant health. *International Journal of Food Microbiology*, 104, pp: 1-34.

Guzmán-Herrador, B., Jensvoll, L., Einoder-Moreno, M., Lange, H., Myking, S., Nygard, K., Stene-Johansen, K. and Vold, L. (2014). Ongoing hepatitis A outbreak in Europe 2013 to 2014: imported berry mix cake suspected to be the source of infection in Norway. *Euro Surveill*, 19.

Hamilton, J.V., Lehane, M.J. and Braig, H.R. (2003). Isolation of *Enterobacter sakazakii* from Midgut of Stomoxys calcitrans. *Emerging Infectious Diseases*, 9 (10), pp: 1355-1356.

Hamprecht, K., Maschmann, J., Vochem, M., Dietz, K., Speer, CP. and Jahn, G. (2001). Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. *Lancet*, 357, pp: 513-518.

Hayashi, S., Kimura, H., Oshiro, M., Kato, Y., Yasuda, A., Suzuki, C., Watanabe, Y., Morishima, T. and Hayakawa, M. (2011). Transmission of cytomegalovirus via breast milk in extremely premature infants. *Journal of Perinatology*, 31, pp: 440-445.

Health Canada (2012). Preparing and handling powdered infant formula. Available at: www.healthycanadians.gc.ca/eating-nutrition/safety-salubrite/formula-nourrisson-eng.php [accessed: 4-11-15].

Healy, B., Cooney, S., O'Brien, S., Iversen, C., Whyte, P., Nally, J., Callanan, J.J. and Fanning, S. (2010). *Cronobacter (Enterobacter sakazakii)*: an oportunistic foodborne pathogen. *Foodborne Pathogen and Disease*, 7, pp: 339-350.

Helwigh, B., Korsgaard, H., Grønlund, A.J., Sørensen, A.H., Jensen, A.N., Boel, J. and Borck Høg, B. (2009). External Scientific Report Microbiological contaminants in food in the European Union in 2004-2009. Technical University of Denmark. Supporting Publications 2012: EN-249.

Helwigh, B., Korsgaard, H., Anne, J., Grønlund, A.J., Sørensen, A.H., Jensen, A.N., Boel, J. and Høg, B.B. (2012). Microbiological contaminants in food in the European Union in 2004-2009. Technical University of Denmark. Supporting Publications 2012: EN-249.

Hinckley, A.F., O'Leary, D.R. and Hayes, E.B. (2007). Transmission of West Nile Virus Through Human Breast Milk Seems to Be Rare. *Pediatrics*, 119, pp: e666-e671.

Holy, O. and Forsythe, S. (2014). *Cronobacter* spp. Emerging cause of healthcare-associated infection. *Journal of Hospital Infection*, 86, pp: 169-177.

Hoofnagle, J.H., Nelson, K.E. and Purcell, R.H. (2012). Hepatitis E. *The New England Journal of Medicine*, 367, pp: 1237-1244.

Huertas, J.P., Álvarez-Ordóñez, A., Morrissey, R., Ros-Chumillas, M., Esteban, M.D., Maté, J., Palop, A. and Hill, C. (2015). Heat resistance of *Cronobacter sakazakii* DPC 6529 and its behavior in reconstituted powdered infant formula. *Food Research International*, 69, pp: 401-409.

Hurrell, E., Kucerova, E., Loughlin, M., Caubilla-Barron, J. and Forsythe, S.J. (2009). Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii, Salmonella* serovars and other *Enterobacteriaceae. International Journal of Food Microbiology*, 136, pp: 227-231.

INFOSAN (2005). Red Internacional de autoridades de inocuidad de los alimentos. *Enterobacter sakazakii* en las fórmulas infantiles en polvo. Nota informativa no.1/2005-*Enterobacter sakazakii*. Available at: www.who.int/foodsafety/fs_management/No_01_Esakazakii_Jan05_sp.pdf [accessed: 4-11-15].

ISCIII (2011). Instituto de Salud Carlos III. Brote supracomunitario de gastroenteritis por *Salmonella* Poona en 2010-2011 Centro Nacional de Epidemiología. *Boletín Epidemiológico Semanal*, 19 (13).

Isonhood, J.H. and Drake, M. (2002). Aeromonas species in foods. *Journal of Food Protection*, 65 (3), pp: 575-582.

Iversen, C. and Forsythe, S. (2003). Risk profile of *Enterobacter sakazakii*, and emergent pathogen associated with infant milk formula. *Trends in Food Science and Technology*, 14, pp: 443-454.

Iversen, C. and Forsythe, S. (2004). Isolation of *Enterobacter sakazakii* and other *Enterobacteriaceae* from powdered infant formula milk and related products. *Food Microbiology*, 21, pp: 771-777.

Iversen, C., Lane, M. and Forsythe, S.J. (2004). The growth profile, thermotolerance and biofilm formation of *Enterobacter sakazakii* grown in infant formula milk. *Letters in Applied Microbiology*, 38, pp: 378-382.

Janda, J.M. and Abbott, S.L. (2010). The Genus Aeromonas: Taxonomy, Pathogenicity, and Infection. *Clinical Microbiology Reviews*, 23 (1), pp: 35-73.

Jiang, V., Jiang, B., Tate, J., Parashar, U.D. and Patel, M.M. (2010). Performance of rotavirus vaccines in developed and developing countries. *Hum Vaccin*, 6, pp: 532-542.

Jones, T.F., Kellum, M.E., Porter, S.S., Bell, M. and Schaffner, W. (2002). An Outbreak of Community-Acquired Foodborne Illness Caused by Methicillin-Resistant Staphylococcus aureus. *Emerging Infectious Diseases*, 8 (1), pp: 82-84.

Kandhai, M.C., Heuvelink, A.E., Reij, M.W., Beumer, R.R., Dijk, R., van Tilburg, J.J.H.C., van Schothorst, M. and Gorris, L.G.M. (2010). A study into the occurrence of *Cronobacter* spp. in The Netherlands between 2001 and 2005. *Food Control*, 21, pp: 1127-1136.

Kandhai, M.C., Reij, M.W., Gorris, L.G., Guillaume-Gentil, O. and Van Schothorst, M. (2004). Occurrence of *Enterobacter sakazakii* in food production environments and households. *Lancet*, 363, pp: 39-40.

Kaper, J.B., Nataro, J.P. and Mobley, H.L.T. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology*, 2 (2), pp: 123-140.

Kim, S.H. and Park, J.H. (2007). Thermal resistance and inactivation of *Enterobacter sakazakii* isolates during rehydration of powdered infant formula. *Journal of Microbiology and Biotechnology*, 17, pp: 364-368.

Kitajima, M. and Gerba, C.P. (2015). Aichi virus 1: environmental occurrence and behavior. *Pathogens*, 4, pp: 256-268.

Koehler, K.M., Lasky, T., Fein, S.B., DeLong, S.M., Hawkins, M.A., Rabatsky-Ehr, T. and Vugia, D.J. (2006). Population-Based Incidence of Infection with Selected Bacterial Enteric Pathogens in Children Younger Than Five Years of Age, 1996-1998. *The Pediatric Infectious Disease Journal*, 25 (2), pp: 129-134.

Koopmans, M. and Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90, pp: 23-41.

Krain, L.J., Atwell, J.E., Nelson, K.E. and Labrique, A.B. (2014). Fetal and Neonatal Health Consequences of Vertically Transmitted Hepatitis E Virus Infection. *The American Journal of Tropical Medicine and Hygiene*, 90, pp: 365-370.

Kramer, J.M. and Gilbert, R.J. (1989). Bacillus cereus and other Bacillus species, pp: 21-70. In book: *Foodborne bacterial pathogens*. Doyle, M.P. Marcel Dekker, New York. pp: 21-70.

Kuzina, L.V., Peloquin, J.J., Vacek, D.C. and Miller, T.A. (2001). Isolation and identification of bacteria associated with adult laboratory Mexican fruit flies, *Anastrepha ludens* (Diptera: Tephritidae). *Current Microbiology*, 42, pp: 290-410.

Lamounier, J.A., Moulin, Z.S. and Xavier, C.C. (2004). Recommendations for breastfeeding during maternal infections. *Jornal de Pediatria*, 80 (5), pp: S181-S188.

Lampel, K.A. and Maurelli, A.T. (2007). *Shigella* species. In book: *Food Microbiology: Fundamentals and Frontiers*. Doyle, M.P. y Beuchat, L.R., Washington, D.C., ASM Press. pp: 323-341.

Lanari, M., Sogno Valin, P., Natale, F., Capretti, M.G. and Serra, L. (2012). Human milk, a concrete risk for infection? *Journal of Maternal-Fetal & Neonatal Medicine*, 25 (4), pp: 75-77.

Lanzieri, T.M., Dollard, S.C., Josephson, C.D., Schmid, D.S. and Bialek, S.R. (2013). Breast Milk–Acquired Cytomegalovirus Infection and Disease in VLBW and Premature Infants. *Pediatrics*, 131, pp: e1937-e1945.

Le Loir, Y., Baron, F. and Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research*, 2 (1), pp: 63-76.

Li, Y., Chen, Q., Zhao, J., Jiang, H., Lu, F., Bie, X. and Lu, Z. (2014). Isolation, identification and antimicrobial resistance of *Cronobacter* spp. Isolated from various foods in China. *Food Control*, 37, pp: 109-114.

Lim, J.Y., Yoon, J.W. and Hovde, C.J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*, 20, pp: 5-14.

Martín, R., Langa, S., Reviriego, C., Jimínez, E., Marín, M.L., Xaus, J., Fernández, L. and Rodríguez, J.M. (2003). Human milk is a source of lactic acid bacteria for the infant gut. *Journal of Pediatrics*, 143 (6), pp: 754-758.

Mateo, M., Mateo, M., Montoya, A., Bailo, B., Saugar, J.M., Aguilera, M. and Carmena, D. (2014). Detection and Molecular Characterization of *Giardia* duodenalis in Children Attending Day Care Centers in Majadahonda, Madrid, Central Spain. *Medicine*, 93 (15), pp: e75.

Mayr, C., Strohe, G. and Contzen, M. (2009). Detection of rotavirus in food associated with a gastroenteritis outbreak in a mother and child sanatorium. International *Journal of Food Microbiology*, 135, pp: 179-182.

McClane, B.A., Robertdon, S.I. and Li. J. (2013). *Clostridium perfringens*. In book: *Food Microbiology: Fundamentals and Frontiers*. 4th edition. Doyle, M.P. y Buchanan, R.L. American Society for Microbiology. pp: 465-490.

McDonald, S.D. and Gruslin, A. (2001). A review of *Campylobacter* infection during pregnancy: A focus on C. jejuni. *Primary Care Update for Ob/Gyns*, 8, pp: 253-257.

Moore, D.L. (2008). Foodborne infections. *Paediatric Child Health*, 13 (9), pp: 779-782.

Moriuchi, H., Masuzaki, H., Doi, H. and Katamine, S. (2013). Mother-to-child Transmission of Human T-cell Lymphotropic Virus Type 1. *The Pediatric Infectious Disease Journal*, 32, pp: 175-177.

Mramba, F., Broce, A. and Zurek, L. (2006). Isolation of *Enterobacter sakazakii* from stable flies, Stomoxys calcitrans L. (Diptera: Muscidae). *Journal of Food Protection*, 69 (3), pp: 671-673.

Muehlenbachs, A., Bhatnagar, J. and Zaki, S.R. (2015). Tissue tropism, pathology and pathogenesis of enterovirus infection. *Journal of Pathology*, 235, pp: 217-228.

Mullane, N.R., Healy, B., Meade, J., Whyte, P., Wall, P.G. and Fanning, S. (2008). Dissemination of *Cronobacter* spp. *(Enterobacter sakazakii)* in a powdered milk protein manufacturing facility. *Applied and Environmental Microbiology*, 74, pp: 5913-5917.

Murata, T., Katsushima, N., Mizuta, K., Muraki, Y., Hongo, S. and Matsuzaki, Y. (2007). Prolonged norovirus shedding in infants <or=6 months of age with gastroenteritis. *Pediatric Infectious Disease Journal*, 26, pp: 46-49.

Muytjens, H.L., Roelofs-Willemse, H. and Jaspar, G.H.J. (1988). Quality of powered substitutes for breast milk with regard to members of the *Enterobactericeae*. *Journal of Clinical Microbiology*, 26, pp: 743-746.

Nataro, J.P. and Kaper, J.B. (1998). Diarrheagenic *Escherichia coli. Clinical Microbiology Reviews*, 11 (1), pp: 142-201.

Nazarowec-White, M. and Farber, J.M. (1997a). Incidence, survival and growth of *Enterobacter sakazakii* in infant formula. *Journal of Food Protection*, 60, pp: 226-230.

Nazarowec-White, M. and Farber, J.M. (1997b). Thermal resistance of *Enterobacter sakazakii* in reconstituted dried-infant formula. *Letters in Applied Microbiology*, 24, pp: 9-13.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., der Giessen, Jv. and Kruse, H. (2010). Food-borne diseases-The challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, 139, pp: S3-S15.

NHS (2012). National Health Service. Guide to Bottle feeding. How to prepare infant formula and sterilise feeding equipment to minimise the risks to your baby. 2012. Available at: www.nhs.uk/start4life/documents/pdfs/start4life_guide_to_bottle_feeding.pdf [accessed: 4-11-15].

NICE (2010). National Institute for Health and Care Excellence. Donor milk banks: the operation of donor milk bank services. *Clinical guideline* 93.

Nichols, G.L. (2000). Food-borne protozoa. *British Medical Bulletin*, 56 (1), pp: 209-235.

Norberg, S., Stanton, C., Ross, R.P., Hill, C., Fitzgerald, G.F. and Cotter, P.D. (2012). *Cronobacter* spp. in powdered infant formula. *Journal of Food Protection*, 75, pp: 607-620.

Noriega, F.R., Kotloff, K.L. and Schwalbe, R.S. (1990). Nosocomial bacterimia caused by *Enterobacter sakzakii* and Leuconostoc mesenteroides resulting from extrinsic contamination of infant formula. *The Pediatric Infectious Disease Journal*, 9, pp: 447-449.

Olsen, S.J., Bishop, R., Brenner, F.W., Roels, T.H., Bean, N., Tauxe, R.V. and Slutsker, L. (2001). The changing epidemiology of *Salmonella*: Trends in serotypes isolated from humans in the United States, 1987-1997. *Journal of Infectious Diseases*, 183, pp: 753-761.

Osaili, T.M., Al-Nabulsi, A.A., Shaker, R.R., Ayyash, M.M., Olaimat, A.N., Abu Al-Hasan, A.S., Qadora, K.M. and Holley, R.A. (2008). Effects of extended dry storage of powdered infant milk formula on susceptibility of *Enterobacter sakazakii* to hot water and ionizing irradiation. *Journal of Food Protection*, 71, pp: 934-939.

Osaili, T.M., Shaker, R.R., Al-Haddaq, M.S., Al-Nabulsi, A.A., and Holley, R.A. (2009). Heat resistance of *Cronobacter* species *(Enterobacter sakazakii)* in milk and special feeding formula. *Journal of Applied Microbiology*, 107 (3), pp: 928-935.

Pagotto, F.J., Nazarowec-White, M., Bidawid, S. and Farber, J.M. (2003). *Enterobacter sakazakii*: infectivity and enterotoxin production *in vitro* and *in vivo*. *Journal of Food Protection*, 66, pp: 370-375.

Pan, Z., Cui, J., Lyu, G., Du, X., Qin, L., Guo, Y., Xu, B., Li, W., Cui, Z. and Zhao, C. (2014). Isolation and molecular typing of *Cronobacter* spp. in commercial powdered infant formula and follow-up formula. *Foodborne Pathogens and Disease*, 11, pp: 456-461.

Park, S.F. (2002). The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *International Journal of Food Microbiology*, 74 (3), pp: 177-188.

Parra, J.F., Oliveras, L.V., Rodriguez, A.F., Riffo, F.S., Jackson, E. and Forsythe, S. (2015). Risk of *Cronobacter sakazakii* in powdered milk for infant nutrition. *Revista Chilena de Nutrición*, 42, pp: 83-89.

Parry, S.M. and Salmon, R.L. (1998). Sporadic STEC O157 infection: secondary household transmission in Wales. *Emerging Infectious Diseases*, 4 (4), pp: 657-661.

Patel, M.M., Widdowson, M.A., Glass, R.I., Akazawa, K., Vinje, J. and Parashar, U.D. (2008). Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerging Infectious Diseases*, 14, pp: 1224-1231.

Pintó, R., Costafreda, M.I., Pérez-Rodriguez, F., D'Andrea, L. and Bosch, A. (2010). Hepatitis A Virus: State of the Art. *Food and Environmental Virology*, 2, pp: 127-135.

Rabet, L.M., Vos, A.P. Boehm, G. and Garssen, J. (2008). Breast-Feeding and Its Role in Early Development of the Immune System in Infants: Consequences for Health Later in Life. *Journal of Nutrition*, 138, pp: 1782S-1790S.

Räsänen, S., Lappalainen, S., Kaikkonen, S., Hämäläinen, M., Salminen, M. and Vesikari, T. (2010). Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak. *Epidemiology & Infection*, 138, pp: 1227-1234.

Reich, F., König, R., vonWiese, W. and Klein, G. (2010). Prevalence of *Cronobacter* spp. In a powdered infant formula processing environment. *International Journal of Food Microbiology*, 140, pp: 214-217.

Robilotti, E., Deresinski, S. and Pinsky, B.A. (2015). Norovirus. *Clinical Microbiology Reviews*, 28, pp: 134-164.

Robins-Browne, R.M. (2007). *Yersinia* enterocolitica. In book: *Food Microbiology: Fundamentals and Frontiers.* Doyle, M.P. y Beuchat, L.R., 3rd Edition. Washington, D.C.: ASM Press. pp: 293-322.

Rodríguez Máuriz, C., Valdés Amey, E., Lara Ortiz, C. and Vilalta Remón, A. (1996). Multiplicación del *Bacillus Cereus* inoculado en leche en polvo reconstituida. *Revista Cubana de Alimentación y Nutrición*, 10 (2), pp: 87-89.

Rodríguez Salinas, E.P., Peña, A., José, A., Allue Tango, M., Pérez, L., Angeles, M. and José, M. (2000). Brote de Criptosporidiosis en Guadarrama. (Comunidad Autónoma de Madrid). *Revista Española de Salud Pública*, 74 (5-6), pp: 527-536.

Rosner, B.M., Stark, K., Höhle, M. and Werber, D. (2012). Risk factors for sporadic *Yersinia* enterocolitica infections, Germany 2009-2010. *Epidemiology & Infection*, 140 (10), pp: 1738-1747.

Rosow, L.K. and Strober, J.B. (2015). Infant botulism: review and clinical update. *Pediatrics Neurolgy*, 52, pp: 487-492.

Rosset, P., Noel, V. and Morelli, E. (2007). Time-temperature profiles of infant milk formula in hospitals and analysis of *Enterobacter sakazakii* growth. *Food Control*, 18, pp: 1412-1418.

Ruggeri, F.M. and Fiore, L. (2012). Vaccine preventable viral diseases and risks associated with waterborne transmission. *Annali dell'Istituto Superiore di Sanità*, 48, pp: 460-472.

Sabria, A., Pinto, R.M., Bosch, A., Bartolome, R., Cornejo, T., Torner, N., Martinez, A., de Simon, M., Dominguez, A. and Guix, S. (2014). Molecular and clinical epidemiology of norovirus outbreaks in Spain during the emergence of GII.4 2012 variant. *Journal of Clinical Virology,* 60, pp: 96-104.

Sánchez-Vargas, F.M., Abu-El-Haija, M.A. and Gómez-Duarte, O. (2011). *Salmonella* infections: An update on epidemiology, management, and prevention. *Travel Medicine and Infectious Disease*, 9, pp: 263-277.

Santiago, B., Guerra, L., García-Morín, M., González, E., Gonzálvez, A., Izquierdo, G., Martos, A., Santos, M., Navarro, M., Hernández-Sampelayo, M.T. and Saavedra-Lozano, J. (2015). *Clostridium difficile* isolation in childre hospitalized with diarrhoea. *Anales de Pediatría*, 82, pp: 417-425.

Sato, M.I.Z., Galvani, A.T., Padula, J.A., Nardocci, A.C., de Souza Lauretto, M., Razolini, M.T. and Hachich, E.M. (2013). Assessing the infection risk of Giardia and *Cryptosporidium* in public drinking water delivered by surface water system in Sao Paulo State, Brazil. *Science of the Total Environment*, 442, pp: 389-396.

Sayed, I.M., Vercouter, A-S., Abdelwahab, S.F., Vercauteren, K. and Meuleman, P. (2015). Is HEV an emerging problem in industrialized countries? *Hepatology*, 62, pp: 1883-1892.

Scallan, E., Griffin, P.M., Angulo, F.J., Tauxe, R.V. and Hoekstra, R.M. (2011). Foodborne illness acquired in the United States-unspecified agents. *Emerging Infectious Diseases Journal*, 17, pp: 16-22.

Scallan, E., Mahon, B.E., Hoekstra, R.M. and Griffin, P.M. (2013). Estimates of Illnesses, Hospitalizations and Deaths Caused by Major Bacterial Enteric Pathogens in Young Children in the United States. *Pediatric Infectious Disease Journal*, 32 (3), pp: 217-221.

Scott, E. (2000). Relationship between cross-contamination and the transmission of foodborne pathogens in the home. *Pediatric Infectious Disease Journal*, 19 (10), pp: S111-113.

Semenza, J.C. and Nichols, G. (2007). *Cryptosporidiosis* surveillance and water-borne outbreaks in Europe. Euro Surveillance: Bulletin Européen Sur Les Maladies Transmissibles. *European Communicable Disease Bulletin*, 12, pp: 120-123.

Shaker, R., Osaili, T., Al-Omary, W., Jaradt, Z. and Al-Zuby, M. (2007). Isolation of *Enterobacter sakazakii* and other *Enterobacter* sp. from food and food production environments. *Food Control*, 18, pp: 1241-1245.

SIM (2015). Sistema de Información Microbiológica. Instituto de Salud Carlos III. Available at: http://www.isciii.es/ISCIII/es/contenidos/fd-servicios-cientifico-tecnicos/fd-vigilancias-alertas/sistema-informacion-microbiologica.shtml [accessed: 4-11-15].

Smith, A. (2011). Alimentos caseros para bebés: prepárelos de manera segura. Foodsafety.gov. Available at: http://espanol.foodsafety.gov/blog/182t/alimentos-caseros-para-beb%C3%A9s.html [accessed: 4-11-15].

Smith, J.L. (2002). *Campylobacter jejuni* infection during pregnancy: long-term consequences of associated bacteremia, Guillain-Barré syndrome, and reactive arthritis. *Journal of Food Protection*, 65, pp: 696-708.

Sockett, P.N. and Rodgers, F.G. (2001). Enteric and foodborne disease in children: A review of the influence of food-and environment-related risk factors. *Paediatrics & Child Health*, 6 (4), pp: 203-209.

Sonbol, H., Jospeh, S., McAuley, C.M., Craven, H.M. and Forshythe, S.J. (2013). Multilocus sequence typing of *Cronobacter* spp. from powdered infant formula and milk powder production factories. *International Dairy Journal*, 30, pp: 1-7.

Stoll, B.J., Hansen, N., Fanaroff, A.A. and Lemons, J.A. (2004). *Enterobacter sakazakii* is a rare case of neonatal septicemia or meningitis in VLBW infants. *Journal of Pediatrics*, 144, pp: 821-823.

Strydom, A., Cawthorn, D.M., Cameron, M. and Witthuhn, R.C. (2012). Species of *Cronobacter*-A review of recent advances in the genus and their significance in infant formula milk. *International Dairy Journal*, 27, pp: 3-12.

Stuart, R.L., Tan, K., Mahar, J.E., Kirkwood, C.D., Andrew Ramsden, C., Andrianopoulos, N., Jolley, D., Bawden, K., Doherty, R., Kotsanas, D., Bradford, J, and Buttery, JP. (2010). An outbreak of necrotizing enterocolitis associated with norovirus genotype GII.3. *Pediatric Infectious Disease Journal*, 29, pp: 644-647.

Terio, V., Bottaro, M., Di Pinto, A., Catella, C., Chironna, M., Bozzo, G., Kingsley, D.H., Bonerba, E., Morea, A. and Martella, V. (2015). Outbreak of Hepatitis A in Italy Associated with Frozen Redcurrants Imported from Poland: A Case Study. *Food and Environmental Virology*, 7, pp: 1-4.

Terragno, R., Salve, A., Pichel, M., Epszteyn, S., Brengi, S. and Binsztein, N. (2009). Characterization and subtyping of *Cronobacter* spp. from imported powdered infant formulae in Argentina. *International Journal of Food Microbiology*, 136, pp: 193-197.

Thurm, V. and Gericke, B. (1994). Identification of infant food as a vehicle in a nosocomial outbreak of Citrobacter freundii: epidemiological subtyping by allozyme, whole-cell protein and antibiotic resistance. *Journal of Applied Bacteriology*, 76 (6), pp: 553-558.

Trabulsi, L.R., Keller, R. and Gomes, T.A.T. (2002). Typical and Atypical Enteropathogenic *Escherichia coli*. *Emerging Infectious Diseases*, 8 (5), pp: 508-513.

Tudela, E., Croizé, J.A., Lagier, A. and Mallaret M.-R. (2008). Surveillance microbiologique des échantillons de laits infantiles et des surfaces dans une biberonnerie hospitalière. *Pathologie Biologie*, 56, pp: 272-278.

Turcios-Ruiz, R.M., Axelrod, P., St John, K., Bullitt, E., Donahue, J., Robinson, N. and Friss, H.E. (2008). Outbreak of necrotizing enterocolitis caused by norovirus in a neonatal intensive care unit. *Journal of Pediatrics*, 153, pp: 339-344.

Van der Poel, W.H.M. (2014). Food and environmental routes of Hepatitis E virus transmission. *Current Opinion in Virology*, 4, pp: 91-96.

Van Pelt, W., de Wit, M.S., Wannet, W.J.B., Ligtvoet, E.J.J., Widdowson, M.A. and van Duynhoven, Y.T.H.P. (2003). Laboratory surveillance of bacterial gastroenteric pathogens in The Netherlands, 1991-2001. *Epidemiology and Infection*, 130 (3), pp: 431-441.

Varga, L. (2011). Baceriological quality of bottled natural mineral waters commercialized in Hungary. *Food Control*, 22, pp: 591-595.

Vargas-Leguás, V., Rodríguez Garrido, R., Lorite Cuenca, R., Pérez-Portabella, S.Y. and Campins Martí, M. (2009). Guía para la elaboración de fórmulas infantiles en polvo en el medio hospitalario. Sistema de análisis de peligros y puntos de control críticos. *Anales de Pediatría*, 70 (6), pp: 586-593.

Wang, F.T., Mast, T.C., Glass, R.J., Loughlin, J. and Seeger, J.D. (2010). Effectiveness of the Pentavalent Rotavirus Vaccine in Preventing Gastroenteritis in the United States. *Pediatrics*, 125, pp: e208-e213.

Warren, B.R., Parish, M.E. and Schneider, K.R. (2006). *Shigella* as a Foodborne Pathogen and Current Methods for Detection in Food. *Critical Reviews in Food Science and Nutrition*, 46 (7), pp: 551-567.

Wattiau, P., Boland, C. and Bertrand, S. (2011). Methodologies for *Salmonella* enterica subespecie. enterica subtyping: Gold standards and alternatives. *Applied and Environmental Microbiology*, 77, pp: 7877-7885.

WHO (1996). World Health Organization. Protozoa. Guidelines for drinking-water quality. Vol 2. Health criteria and other supporting information. 2nd ed. Geneva. pp: 52-67.

WHO (2007). World Health Organization. HIV Transmission Through Breastfeeding. A review of available evidence. Updated 2007.

WHO (2008). World Health Organization. Guidelines for drinking-water quality - Volume 1: Recommendations, 3rd edition. Available at: http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/ [accessed: 4-11-15].

WHO (2009). World Health Organization. Children's Health and the Environment. Available at: http://www.who.int/ceh/capacity/food.pdf [accessed: 4-11-15].

WHO (2010). World Health Organization. Guidelines on HIV and infant feeding 2010. Principles and recommendations for infant feeding in the context of HIV and a summary of evidence.

WHO (2015). World Health Organization. Hepatitis E. Fact sheet Nº 280. Available at: http://www.who.int/mediacentre/factsheets/fs280/en/ [accessed: 4-11-15].

Wilcox, M.H., Cook, A.M., Eley, A. and Spencer, R.C. (1992). *Aeromonas* spp as a potential cause of diarrhoea in children. *Journal of Clinical Pathology*, 45 (11), pp: 959-963.

Woteki, C.E. and Kineman, B.D. (2008). Food Safety. In book: *Nutrituion in Pediatrics: Basic science, clinical applications*. Duggan, C., Warkins, J. and Walker, W.A. BC Dekker.

Yan, L., Feng, Q., Mei-ling, W. and Wei, W. (2012). Screening for Enterobacteriaceae Bacteria in Infant Formula Powder. *Journal of Northeast Agricultural University*, 19 (1), pp: 68-72.

Yang, H.Y., Kim, S.K., Choi, S.Y., You, D.H., Lee, S.C., Bang, W.S. and Yuk, H.G. (2015). Effect of acid, desiccation and heat stresses on the viability of *Cronobacter sakazakii* during rehydration of powdered infant formula and in simulated gastric fluid. *Food Control*, 50, pp: 336-341.

Zamberlan da Silva, M.E., Getirana Santana, R., Guilhermetti, M., Camargo Filho, I., Harua Endo, E., Ueda-Nakamura, T., Vatura Nakamura, C. and Prado Dias Filho, B. (2008). Comparison of the bacteriological quality of tap wáter and bottled mineral water. International *Journal of Hygiene and Environmental Health*, 211, pp: 504-505.

Zogaj, X., Bokranz, W., Nimtz, M. and Römling, U. (2003). Production of cellulose and curli fimbriae by members of the Family Enterobacteriaceae isolated from the human gastrointestinal tract. *Infection and Immunity*, 71, pp: 4151-4158.

Annex I. Microbiological safety and hygiene criteria affecting foods and formulae intended for infants and young children

1. Safety Criteria

Food category	Microorganism/ its toxins, metabolites	Plan for sampling	Limits	Analytical method of reference	Phase at which criteria are applied
Ready-to-eat food intended for infants[4]	*Listeria monocytogenes*	n=10 c=0	Absence in 25 g	EN/ISO 11290-1	Products marketed during their useful life
1.22. Dried infant formulae and dried dietary food intended for special medical purposes for infants under the age of 6 months	*Salmonella*	n=30 c=0	Absence in 25 g	EN/ISO 6579	Products marketed during their useful life
1.23. Dried follow-on formulae	*Salmonella*	n=30 c=0	Absence in 25 g	EN/ISO 6579	Products marketed during their useful life
1.24. Dried infant formulae and dried dietary food intended for special medical purposes for infants under the age of 6 months[14]	*Cronobacter* spp. *Enterobacter sakazakii*	n=30 c=0	Absence in 10 g	ISO/TS 22964	Products marketed during their useful life

[4]Under normal circumstances, regular tests regarding this criteria are not required for the following ready-to-eat food products: • foods which have undergone heat treatment or other effective process for eliminating *L. monocytogenes*, when recontamination is not possible after this treatment (for example, heat-treated products in their final packaging), • fresh fruit and vegetables, whole and untransformed, excluding sprouted seeds, bread, biscuits and similar products, • bottled or packaged water, soft drinks, beer, cider, wine, spirits or similar, • sugar, honey and sweets, including cocoa-based products and chocolate, • live bivalve molluscs.
[14]An analysis for the detection of *Enterobacteriaceae* and *E. sakazakii* will be run in parallel unless a correlation has previously been established for these microorganisms on the scale of specific plants. If *Enterobacteriaceae* are detected in any of the samples taken from said plant, then an analysis will be made for *E. sakazakii*. The manufacturer will be obliged to prove, to the satisfaction of the competent authority, whether said correlation exists between the *Enterobacteriaceae* and *E. sakazakii*.

2. Hygiene criteria

Food category	Microorganism/ its toxins, metabolites	Plan for sampling	Limits	Analytical method of reference	Phase at which criteria are applied	Action in the event of unsatisfactory results
2.2.9. Dried infant formulae and dried dietary food intended for special medical purposes for infants under the age of 6 months	*Enterobacteriaceae*	n=10 c=0	Absence in 10 g	ISO 21528-1	End of manufacturing process	Improvements in production hygiene to minimise contamination[9]
2.2.10. Dried follow-on formulae	*Enterobacteriaceae*	n=5 c=0	Absence in 10 g	ISO 21528-1	End of manufacturing process	Improvements in production hygiene to minimise contamination
2.2.11. Dried infant formulae and dried dietary food intended for special medical purposes for infants under the age of 6 months	Presumed *Bacillus cereus*	n=5 c=1	m=50 CFU/g M=500 CFU/g	EN/ISO 7932[10]	End of manufacturing process	Improvements in production hygiene. Prevention of recontamination. Selection of raw materials

[9]An analysis for the detection of *Enterobacteriaceae* and *E. sakazakii* will be run in parallel unless a correlation has previously been established for these microorganisms on the scale of specific plants. If *Enterobacteriaceae* are detected in any of the samples taken from said plant, then an analysis will be made for *E. sakazakii*. The manufacturer will be obliged to prove, to the satisfaction of the competent authority, whether said correlation exists between the *Enterobacteriaceae* and *E. sakazakii*.

[10]1 ml of inoculum is sown on a Petri dish (140 mm diameter) or three Petri dishes (90 mm diameter).