Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on infant botulism

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Abstract

Infant botulism is a disease that occurs in infants between 1 and 52 weeks years of age when spores of *Clostridium botulinum* are ingested. These spores germinate and produce neurotoxins in the large intestine lumen. These toxins act at the neuromuscular junction blocking release of acetylcholine neurotransmitter. Clinical severity varies from a mild hipotony to systemic flaccid paralysis. It has even been considered as the cause of infant sudden death. *C. botulinum* spores are commonly found in soil samples and aquatic sediments throughout the world. Honey is the most well-documented food source of *C. botulinum* spores for infants although corn syrup, infant formulas, dry cereals, plants infusions and dust have been also associated with infant botulism. However, for the majority of cases the source of the causative spores remains unclear.

Researchers have also stressed the importance of the child's natural environment that may facilitate the exposure to *C. botulinum* spores. Contact with dust or soil in houses from rural areas, where farming activities are carried out, has also been cited as a risk factor. As well as urban areas with big areas under construction involving earthmoving or restoration works in buildings.

It is considered that the minimum infective dose of spores of *C. botulinum* may be as low as 10-100 spores. Current epidemiological data indicate that the risk of disease is low in infants less than 1 year old by avoiding the consumption of honey and/or plants infusions.

Key words

Infant botulism, botulinum neurotoxin, honey, plants infusions, dust.

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Introduction

In view of the severity of infant botulism and in spite of the low incidence, the existence of cases in Spain has led the Executive Director of the Spanish Agency for Food Safety and Nutrition (AESAN) to ask the Scientific Committee to draft a report assessing the levels of knowledge concer-ning infant botulism and its relation to food consumption.

The term botulism refers to a serious neurological disease affecting humans and animals, and is characterised by flaccid paralysis and produced by neurotoxins (Hatheway, 1990) (Midura, 1996). At present, four types of botulism are recognised: classic botulinum intoxication, wound botulism, infant botulism and inhalation botulism.

Classic botulinum intoxication is caused by the intake of foods containing neurotoxins synthesised by *C. botulinum* as it multiplies in foods. In 1821, Justinus Kerner studied a series of food intoxications which were attributed to eating incorrectly prepared sausages, calling the disease botulism, from the Latin *botulus* (sausage). Kerner extracted a toxic compound from the sausages which he identified as the cause of the disease. In 1897, the Belgium microbiologist, Emile-Pierre Van Ermengen isolated a sporulating, Gram-positive and anaerobic bacteria in a ham responsible for a food intoxication, calling it *Bacillus botulinus* (*C. botulinum* at present*)*.

In 1951, a description was given of wound botulism which breaks out when wounds are infected with the spores of *C. botulinum*, that germinate, multiply and produce neurotoxins. Cases have been observed in injecting drug users (Kuusi et al., 1999) (Barry et al., 2009).

Infant botulism was not recognised until 1976 (Midura and Arnon, 1976) (Pickett et al., 1976). It occurs in children between 1 and 52 weeks of age, after the ingestion of spores of *C. botulinum*. The spores, in the large intestine, germinate, multiply and produce neurotoxins that act on the neuromuscular junction preventing the release of the acetylcholine. The severity of the resultant disease varies from a slight hypotonia of cranial pairs followed by descending, symmetric, acute, flaccid paralysis without fever to systemic flaccid paralysis, and has even been considered as the cause of sudden death in infants . The varied symptomatology and unspecified appearance means that it is a difficult disease to diagnose and can be confused with other processes, principally with the *Guillain-Barré* syndrome and *Myasthenia gravis* (Arnon, 2004). Infant botulism has been detected in many countries, although levels of reporting are variable. The absence of cases is usually attributed more to difficulties in diagnosis and the reporting system than to the non-existence of the disease. A similar form of botulism to that of infant botulism has also been described in adults, appearing linked to gastrointestinal infections, prolonged treatment with antibiotics and intestinal surgery (Chia et al., 1986) (Freedman et al., 1986) (Griffin et al., 1997).

Botulism due to the inhalation of neurotoxins is associated to bioterrorism. One gramme of purified toxin is thought to be able to cause the death by inhalation of more than one million individuals, although distribution might pose certain technical difficulties (Arnon et al., 2001).

Hazard identification

In 1976, infant botulism was first recognised as a clinical form of botulism different to classic botulinum intoxication (Midura and Arnon, 1976) (Pickett et al., 1976). However, records exist of one documented but incorrectly diagnosed case in California in 1931 (Arnon et al., 1979).

Unlike classic botulinum intoxication that breaks out due to the intake of neurotoxins present in food, infant botulism may be considered an opportunistic event in that the spores of *C. botulinum* penetrate the large intestine where the immaturity of the intestinal flora allow the spores to germinate, multiply and produce neurotoxins. Furthermore, strains of *Clostridium butyricum* and of *Clostridium baratii* have also been responsible in some cases of type E and F infant botulism (McCroskey et al., 1991) (Gimenez et al., 1992) (Fenicia et al., 2002) (Arnon, 2004) (Barash et al., 2005) (Brook, 2007) (Abe et al., 2008).

Since 1976 more than 2,900 cases have been reported on a global level and today it is considered to be the most frequent form of botulism in many countries (Koepke et al., 2008) (Barash et al., 2010) (Lúquez et al., 2010).

Hazard characterisation

1. Characteristics of the agent and its pathogeny

C. botulinum is a Gram-positive, strictly anaerobic and sporulating bacillus. The spores of *C. botulinum* are widespread in soils and in aquatic sediments, and may contaminate different types of food (Tanzi and Gabay, 2002) (EFSA, 2005) (Lindström et al., 2010).

C. botulinum, depending on the toxins elaborated, is classified into seven types (A, B, C, D, E, F and G). Types A, B, E, F and G are associated to botulism in humans, while types C and D affect animals. However, sporadic cases have been reported in humans of botulism caused by types C and D (Fenicia and Anniballi, 2009). In general, each strain of *C. botulinum* produces a unique toxin although there are strains able to synthesize more than one (Arnon, 1984), in which case they are classified according to the main toxin elaborated (Ab, Af, Ba and Bf). Strains of *C. butyricum* and *C. baratii* have also been found that, while maintaining all the typical characteristics of these two species, produce E and F type toxins respectively (Brook, 2007).

The strains of *C. botulinum* are also classified into four groups (I-IV) according to the different biochemical properties and particularly according to their proteolytic capacity. The strains of *C. botulinum* that cause botulism in humans belong to groups I (proteolytic strains that produce type A, B and F toxins) and II (non-proteolytic strains that produce type B, F and E toxins). The spores of the strains from group I are more resistant to heat than those in group II. The toxins, on the contrary, are all thermolabile (Lindström et al., 2010).

With respect to infant botulism, the types of *C. botulinum* most frequently isolated are types A and B, whereas types E and F have been described to a lesser degree (Lúquez et al., 2010). Furthermore, in the bibliography a case produced by *C. botulinum* type C is quoted (Koepke et al., 2008). Strains that produce the Ab, Ba and Bf toxins have also been identified (Fathalla et al., 2008) (Fenicia and Anniballi, 2009). At the same time, strains of *C. butyricum* that produce the E neurotoxin and strains of *C. baratii* that produce the F neurotoxin have been associated with cases of infant botulism (McCroskey et al., 1991) (Barash et al., 2005) (Abe et al., 2008). The majority of strains of *C. botulinum* responsible for infant botulism are proteolytic (Brook, 2007).

The botulinum toxin is considered to be one of the most lethal toxic compounds for humans. Some authors establish the lethal oral dose for humans at 1 µg/kg (Sobel, 2005).

Botulinum toxins synthesised inside the cell have a molecular weight of approximately 150 kDa and very little toxicity. They are released into the environment as the cell lyses although this may also occur at the end of the exponential phase of growth. The botulinum toxin is not released in free form but linked to other known proteins such as progenitors of the toxin (hemagglutinins and non-hemagglutinant proteins), whose function is to protect the toxin from degradation and facilitate its attachment to intestinal cell receptors. Once released, the botulinum toxin breaks down into a heavy chain (100 kDa) and a light chain (50 kDa) as a result of the action of proteases produced by the microorganism or present in the tissues (Smith, 2009). The two subunits remain attached by a disulphide bond and the breakdown occurs approximately one third from the N-terminal end. The light chain is an endopeptidase that contains Zn^{2+} and hydrolyses one or more of the three intracellular proteins (synaptobrevin, syntaxin and SNAP-25 complex) required for the vesicular fusion and the release of the acetylcholine neurotransmitter (Pellizzari et al., 1999) (Brook, 2007) provoking, finally, the flaccid paralysis characteristic of this disease.

In infant botulism, the most frequent symptoms appear after a period of incubation of approximately three days. These include initial constipation, loss of appetite and a progressive decrease in movement, loss of facial expression, weak cry, deglutition dysfunction with loss of the gag reflex, ocular paralysis, weakness of trunk and general symmetric and descending hypotonia. The evolution of the disease is variable and ranges from rapid recovery when the child is hospitalised before the appearance of respiratory systems (Midura, 1996), to death. As mentioned in the introduction, *C. botulinum* has also been associated with cases of sudden death in children. Böhnel et al. (2001) carried out a study over five years in which they analysed 75 cases which included 57 that were classified as sudden death. Of the samples investigated, the toxin or microorganism was detected in 15, therefore the authors concluded that in these cases the death could be attributed to *C. botulinum,* recommending that health authorities investigate its presence in the cases of sudden death in children under 1 year old.

Infant botulism is confirmed with the detection of the neurotoxins in faeces or by the isolation in faeces of the microorganisms producing the toxin (Fenicia and Anniballi, 2009). Observable levels of neurotoxin are rarely found in blood samples. The presence of *C. botulinum* spores is investigated in suspect foods and if discovered, these are checked to see whether they are the same type as those isolated in the patient, subsequently comparing the strains using genetic techniques (Brett et al., 2005) (Barash et al., 2010).

The conventional treatment for adults with equine botulinum antitoxin is not recommended for children, as its efficiency has never been evaluated in controlled trials and the appearance of anaphylactic reactions is common (Fox et al., 2005) (Cárdenas et al., 2007). In 2003, the FDA (Food and Drug Administration) approved the use of human specific immunoglobulin (BabyBIG^{R}) for the treatment of infants suspected of having the disease. It is made from human antibodies obtained from the plasma of immunised adults with the capacity to neutralise the botulinum toxin circulating in the blood. Since 2005, BabyBIG^{R} has been available for the treatment of infants outside the United States. Before this drug became available, infant botulism was accompanied by a high morbid-mortality rate (Cárdenas et al., 2007) (López Laso et al., 2008).

2. Dose-response ratio

In infant botulism, the minimum infective dose of *C. botulinum* spores, the intake of which could lead to the symptoms, is not precisely known, although it is considered to be between 10 and 100 spores (Bianco et al., 2008). In an intragastric dose-response study carried out by Sugiyama and Mills (1978) in infant mice, the infective dose 50 (ID_{50}) was found to be 700 spores of *C. botulinum* and 1,500 spores in rats (Moberg and Sugiyama, 1980).

3. Susceptible population

Age is the only predisposition factor recognised for infant botulism. The majority of the cases recorded occur in children aged between 1 and 52 weeks old, and the average age is 13 weeks (Brook, 2007) (Koepke et al., 2008). In very exceptional cases, it may also affect older children with intestinal disturbances, or after a prolonged treatment of antibiotics (Chia et al., 1986) (Freedman et al., 1986) (Griffin et al., 1997).

Healthy adults and older children normally ingest *C. botulinum* spores in food without developing illness. The reason is related to the host intestinal microflora, which is quantitatively and qualitatively simpler in young infants, and does not efficiently prevent colonisation of the intestinte by spores of neurotoxigenic clostridia (Midura, 1996) (Arnon, 2004).

4. Risk factors

Some authors have suggested that the difference between breast and formula-fed infants, the length of time the child is breast-fed, the age at which new foods are introduced, etc., could have an influence on the appearance of infant botulism (Nevas et al., 2005).

The type of feeding was one of the main factors analysed, due to the differences in the immunological composition of breast milk compared to formula milk, with respect to its influence on the intestinal microflora that have to compete with the spores of *C. botulinum* in intestinal colonisation. The age at which the symptoms appear is lower in those children fed with formula milk (Arnon, 2004), where the severity of the process is usually greater, which could be connected to a reduced presence of immunoglobulin A, lactoferrin, lysozyme or with the type of intestinal flora. With respect to breast-fed infants, the critical period is considered to be that which coincides with the introduction of new foods, that would be accompanied by a significant change in the type of intestinal flora. Nonetheless, several authors have concluded that it is not possible to establish a direct relation between the type of food and the appearance of the disease (Fenicia and Anniballi, 2009).

Another factor which could favour the appearance of infant botulism is the decline in intestinal motility, measured as the frequency of defecation. Less than one bowel movement per day is considered to be a risk factor both for breast-fed and formula-fed infants. The existence of Meckel diverticula may be a predisposing factor in the case of infant botulism produced by *C. butyricum*. Nonetheless, this relation has not been demonstrated in the case of *C. botulinum* (Arnon, 2004).

Attempts have been made to find links between a variety of foods and infant botulism (honey, corn syrup, sugar, formula milk powder, cereals, plant infusions, etc.), and of all these, honey appears the most often as responsible in those cases where it has been possible to identify the source (Arnon et

al., 1979) (Midura, 1996) (Satorres et al., 1999) (SCVMPH, 2002) (Van der Vorst et al., 2006) (Koepke et al., 2008) (Bianco et al., 2009). However, it should be noted that in the majority of cases investigated, the origin of the spores causing the disease was not clarified.

Many researchers have highlighted the importance of the environmental conditions surrounding the child which facilitate his/her exposure to the spores from *C. botulinum,* quoting contact with dust or earth in homes in rural farming areas, or urban areas with many areas under construction (involving earth movements) as risk factors (Midura, 1996) (Fox et al., 2005).

5. Current world situation

To date, cases of infant botulism have been documented in 26 countries from all the continents except for Africa. The countries with the highest number of cases are the United States, Argentina, Australia, Italy, Canada and Japan (Fenicia and Anniballi, 2009). The lack of cases reported in Africa could be due more to difficulties in diagnosis and the registration system than to the non-existence of the disease (Koepke et al., 2008).

It should be remembered that almost all the information available about infant botulism comes solely from the study of those patients hospitalized, which could bias the results obtained (Arnon, 2004). In fact, the current perceived rate is more a reflection of the capacity of health personal to detect the disease than of its true frequency.

In the Americas, in the United States and in Argentina, infant botulism is currently the most frequently occurring form of botulism (Arnon, 2004) (Lúquez et al., 2007), with the United States reporting the highest number of cases at global level (Koepke et al., 2008).

In Europe, cases of infant botulism have been detected in a more evenly distributed manner through the different countries. This may be due to a similar capacity for diagnosing the disease in all the countries, or to a more homogenous distribution of the spores, or even to both. In the European Union, it is obligatory to report botulism. Nonetheless, reporting the disease does not require separation by age or appearance, therefore specific data available about infant botulism mainly refer to documented and published cases. According to Koepke et al. (2008), until 2006 a total of 65 cases of infant botulism had been reported in Europe and the highest number of notifications was in Italy.

In Spain, according to the study carried out by Koepke et al. (2008), from 1985 to 2002 there are references to nine cases of infant botulism. Of these nine cases, the toxin type was established in four cases, being A in two cases and B in the other two cases. In the bibliography consulted, references have been found to a few more cases (Lizarraga et al., 1996) (Cárdenas et al., 1997) (López Laso et al., 2008).

The variability between countries and even within the same country when reporting cases of infant botulism could reflect the heterogeneous environmental distribution of the spores. In Argentina, in some studies, a direct relation has been established between the incidence of infant botulism and the presence of *C. botulinum* spores in certain regions (Lúquez et al., 2005, 2007).

In addition to the varying capacities for detecting the disease and the documentation of cases, variability in the incident rate between countries may be attributed to cultural practices relating to the feeding of infants with honey, infusions or other infant foods, together with a different exposure to the dust

or soil and also to variations in the susceptibility of patients due to causes not yet known (Koepke et al., 2008).

Evaluation of exposure

1. Presence of *Clostridium botulinum* in foods and other sources

The spores of *C. botulinum* are widespread in soils and in aquatic sediments, and may contaminate different types of food (Tanzi and Gabay, 2002) (EFSA, 2005) (Lindström et al., 2010).

Different studies have evaluated the presence of spores of *C. botulinum* in soils and in aquatic sediments in the United States, Canada, Central and South America, Europe, Africa, Indonesia, Australia and New Zealand, and have revealed that the incidence of infant botulism and the type of toxin responsible are directly related to the degree of contamination of the soils and the type of spores found in these soils (Fenicia and Anniballi, 2009).

In the United States, in 1982 the FDA carried out an extensive study in which they examined ten categories of infant foods to establish the possible presence of *C. botulinum* spores. A large number of samples were analysed including dried cereals (90), commercial baby formula (100), non fat dry milk (100), whole milk (90), canned fruit (100), fruit juice (100), honey (100), corn syrup (40), sugar (90) and cooked carrots (100). The results obtained only proved the presence of spores from *C. botulinum* type A in two of the 100 samples of honey analysed and the presence of spores from *C. botulinum* type B in eight of the 40 samples of corn syrup. As there was no prior data indicating the presence of spores of *C. botulinum* in corn syrup, a wider sample at national level was thought necessary. Samples were taken from different supermarkets and commercial establishments to ensure a large number of suppliers. Of the 961 canned samples analysed, five contained spores from *C. botulinum* type B in approximate levels of 1.25 spores/25 g (Kauter et al., 1982). On the basis of the results obtained, the FDA recommended that honey and corn syrup should not be ingested by children under the age of one. It should be highlighted that the United States is the country which reports the highest number of cases of infant botulism. In 2009, according to the data provided by the CDC (Centers for Disease Control and Prevention), 121 cases of botulism were recorded, of which 84 (69%) were infant botulism (CDC, 2009).

A fairly similar study was carried out in Canada in 1988 to establish the presence of spores of *C. botulinum* in infant foods. 150 samples of honey, 43 samples of corn syrup and 40 samples of dried cereals were analysed. The results only revealed the presence of spores from *C. botulinum* in one sample of rice and one sample of honey suspected of being involved in a case of infant botulism (Hauschild et al., 1988).

Honey

As indicated earlier, of the possible foods responsible for infant botulism, honey is the food to have been most frequently linked to cases of the disease, not only in the United States but also in Europe (Aureli et al., 2002) (Tanzi and Gabay, 2002).

Honey consumption among children under the age of one is relatively low, and even lower since the health recommendations in some countries which advise against its use (Arnon et al., 1979)

(SCVMPH, 2002). A number of manufacturers in the United Sates and the United Kingdom already label honey recommending that is not given to infants less than 12 months of age (Arnon, 2004) (Koepke et al., 2008). Following the use of such labelling, the number of cases of infant botulism in those countries appears to have fallen (Fenicia and Anniballi, 2009).

The presence of *C. botulinum* spores in honey may come from the pollen, from the digestive tract of the bees, dust, air, earth, water and nectar. Other sources of contamination are the equipment, tools, cross contamination, etc., during the subsequent process (SCVMPH, 2002). The number of spores in the honey samples associated to cases of the disease ranged from 5 to 80 spores/g.

In Europe, one of the first cases of infant botulism attributed to the consumption of honey was described by Fenicia et al. (1993). In this case, the same strain of *C. botulinum* type B was isolated in both the faeces of the nine-week infant and in the samples of honey taken from the jar from which the infant had been fed. The information collected highlighted that the honey had been fed to the infant to calm it and stop it from crying. In this respect, a survey in Italy of 270 women, revealed that 25% gave honey to infants without being aware of the risks of this practice (Aureli et al., 2002).

Castell and Nieto (1999) describe a similar case in Spain associated to the consumption of honey in a 2-month old child. From the epidemiological background, it was found that the child had been fed with breast milk and formula milk, and the mother stated that on occasions the dummy had been dipped in honey. The botulinum type B toxin was detected in the faeces and blood serum analyses. The results of the analysis of the formula milk consumed by the child were negative, but spores of *C. botulinum* type B were found in the samples of honey. In this case it was possible to demonstrate the presence in honey of *C. botulinum* spores that produce the same toxin type as those found in the child's faeces. The authors believe that undiagnosed cases are likely to exist in Spain in view of the variable clinical spectrum of the disease and, consequently, the difficulty in diagnosis.

In a later study, Nevas et al. (2002), in Finland, analysed 190 samples of honey to detect the presence of spores of *C. botulinum* using the Polymerase Chain Reaction (PCR). The results revealed the presence of *C. botulinum* spores in 8 (7%) of the 114 samples of Finnish honey analysed and in 12 (16%) of the 76 samples of honey imported from 16 countries. The number of spores in the samples ranged from 18 to 140 spores/kg. The gene which codified for the neurotoxin A was observed in 14 samples and that of the neurotoxin B in 2 samples. In another study, the same authors analysed 294 samples of honey produced in Denmark, Norway and Sweden using a multiplex PCR technique. The percentage of positive samples varied according to the origin. In honeys coming from Denmark, the isolations were greater with 26% of positive samples, followed by those from Norway with 10% and Sweden with 2%. The *C. botulinum* type B spores were the most frequently identified spores. The higher number of positive samples in honeys from Denmark was related in this study to the high number of pig farms in the country. A prior study carried out by Dahlenborg et al. (2001), revealed a prevalence of *C. botulinum* spores of 62% in faecal samples from pigs slaughtered in the abattoir. Denmark is known for the high levels of pork production with more than 13 million animals in an area of only 43,094 km^2. The regular use of purines as fertilisers may contribute to a wider distribution of spores in the soil and water, so that bees easily come into contact with the spores. According to the authors this would explain the higher number of positive samples in honeys from Denmark. The presence of

C. botulinum spores in honey is a reflection of the environmental contamination. An analysis of soils carried out in Denmark and Sweden revealed the presence of *C. botulinum* spores in 30% of the soil samples analysed (Huss, 1980).

Nevas et al. (2006) carried out a study to identify the possible presence of *C. botulinum* spores in hives and honey processing facilities. They analysed 1,168 samples from 100 hives and equipment during 2001-2003. Data concerning the processing methods and environmental conditions were recorded. The results revealed the presence of *C. botulinum* spores throughout the production chain, and *C. botulinum* type B was the most frequently identified. The approximate number of spores in the positive samples ranged between 60 and 1,200 spores/kg, with an average of 180 spores.

Other studies, in which samples of honey purchased in retail outlets were analysed, detected *C. botulinum* spores in honeys from the United States (10%), Japan (7.5%), Italy (6.5%) and Turkey (12.5%) (SCVMPH, 2002) (Küplülü et al., 2006). It should be noted that the application of a heat treatment able to destroy the *C. botulinum* spores found in the honey is not feasible as it would modify the organoleptic characteristics of the end product (Nevas et al., 2006).

The sources of exposure to honey of children aged under one are varied and are highly linked to the customs in each country and even in each region. The most usual paths were the addition of honey to infant foods, spreading honey on the mother's nipple before breast feeding, on the baby's lips or on the baby's dummy to reduce anxiety or crying (SCVMPH, 2002). As mentioned above, several institutions follow the FDA and the WHO (World Health Organisation) in recommending that honey is not consumed by children under the age of one.

Powdered milk formulae

In addition to honey, other foodstuffs analysed as a potential source of exposure to *C. botulinum* spores are formula milk powder. In principle, the presence of *C. botulinum* spores in recently obtained milk cannot be excluded, although the levels are probably low. Furthermore, spores may enter the process at a later stage and survive the heat treatments applied (Lindström et al., 2010).

Although previous studies of samples of formula milk powder have not revealed the presence of *C. botulinum* spores (Kauter et al., 1982) (Hauschild et al., 1988), the reporting in 2001, in the United Kingdom of a case of infant botulism in a 5-month old child associated with this type of product triggered alarms and the need to determine the presence of spores. In the United Kingdom, given the low frequency of the appearance of infant botulism, two independent studies were carried out to clarify the source of the *C. botulinum* spores. The results published by Johnson et al. (2005) revealed that the case reported had come from a strain of *C. botulinum* type B isolated in the infant faeces and in the samples of the formula consumed by the child. Samples were also analysed from five formulas from the same batch that had not yet been opened. Microbiological cultivation techniques used to characterise the strains included phenotypical characterisation, neurotoxin typification and Pulsed Field Gel Electrophoresis (PFGE). The PFGE band patterns of the strain isolated in the faeces and in the formula that had been ingested by the child were similar, whereas the pattern obtained from a strain, isolated in the sample taken from the unopened packet of milk in which the presence of spores had been confirmed, was different. The authors concluded that it was not possible to establish with absolute

certainty whether the origin of the spores in the formula milk consumed by the child was in the formula itself, or whether the product had been contaminated after opening. Studies of samples taken from the child's environment were not carried out, although the home was close to a building site, so that the movement of earth and resultant dust could have released the spores. Furthermore, the researchers indicated that if the contamination of the batch had been high, as the batch consisted of 122,388 units manufactured in October 1998 and consumed by approximately 30,000 infants over a three-year period, more cases would have been expected of infant botulism, but this was not the case.

The other laboratory study related to this case was carried out by Brett et al. (2005). These researchers confirmed the presence of *C. botulinum* type B neurotoxin in the faeces of the infant. Furthermore, two different profiles were identified in nine isolations in faeces of *C. botulinum* type B, using the Amplified Fragment Length Polymorphism (AFLP) analysis. In the analysis of samples taken from the patient's home, *C. botulinum* type A was isolated in an open packet of dried rice pudding with fruit and *C. botulinum* type B in an open packet of formula milk powder that had been consumed by the child. From the ten isolations obtained of *C. botulinum* type B, four different profiles were obtained using the AFLP technique, of which two were similar to those obtained in the faeces isolation. In the analysis of 14 samples taken from unopened packets of the same batch as that of the suspect formula, *C. botulinum* was only identified in the samples from one packet and this had a different profile. Analyses of ten unopened packets of dried rice pudding with fruit from the same batch were negative. The authors concluded that multiple strains of *C. botulinum* might be found in the intestinal tract of the patients.

Given this background, Barash et al. (2010) carried out an interesting study over two years to determine the possible presence of *C. botulinum* and of other *Clostridium* in the formula powder consumed by children hospitalized with infant botulism. Furthermore, they analysed samples taken at commercial establishments. Of the 39 samples analysed, *Clostridium* spores were isolated in 12 (31%), but none of the samples were found to contain spores from *C. botulinum, C. baratii* or *C. butyricum*. The species identified include *C. perfringens*, *C. septicum*, *C. novyi/haemolyticum* and *C. esporogenes*. The most likely number of spores in positive samples ranged between 1.1 and 23 spores/100 g. *C. esporogenes* was the species to be most frequently isolated. The majority of the samples analysed contained, moreover, spores belonging to different species of the *Bacillus* genus. Although spores from *C. botulinum* were not isolated, the presence of other spores of *Clostridium* and *Bacillus* with similar habitats revealed the possibility that this type of formula could also contain spores from *C. botulinum*.

Dried infant formulae are not sterile products and in fact on some occasions they have been linked to foodborne diseases in which the microorganisms identified are *Salmonella* spp. and *Cronobacter sakazakii* (Baker, 2002). In the United States, the FDA considers that additional controls to those established for *Salmonella* spp. and *Cronobacter sakazakii* (FDA, 2006) are not necessary in dried infant formulae. This opinion derives from the decision adopted by the FAO/WHO (2006), which concludes that these two microorganisms are the ones most frequently linked to foodborne disease in infants associated to the consumption of this type of formula.

In Europe, Commission Regulation (EC) No 1441/2007 of 5 December 2007 (EU, 2007) establishes food safety criteria in dried infant formulae. The established criteria are the absence of *Salmonella* in

25 g and the absence of *Cronobacter sakazakii* in 10 g of sample. The same regulation introduces in the process hygiene criteria, the control at the end of the manufacturing process of spores of presumptive *Bacillus cereus* with an m value of 50 cfu/g and an M value of 500 cfu/g. When the results are unsatisfactory, the regulation establishes improvements in the production hygiene, prevention of recontamination and improvements in the selection of the raw materials. Although current regulations do not include any control of *C. botulinum* spores in dried infant formulae, it might be advisable for industries to adopt preventive measures that guarantee the absence of spores of this bacterium in the final product.

Plant infusions

The presence of *C. botulinum* spores in plant species used in infusions may come from the air, water or soil in which they are grown, or from cross contamination during the subsequent process and/or final preparation for consumption. The heat treatment applied to the infusion is not sufficient to guarantee its destruction.

In several countries in Central and South America, just as in countries in the Mediterranean area such as Spain or Italy, it is a relatively normal practice to prepare infusions in order to treat intestinal colic in children, mainly in infants, or for use simply as a drink (Satorres et al., 1999) (Fenicia and Anniballi, 2009).

Satorres et al. (1999) published a study in which they analysed 100 samples from different plant species in order to detect the presence of *C. botulinum* spores. Of the 100 samples, only four (4%) contained *C. botulinum* type A spores. The isolations occurred in Penny Royal (*Lippia turbinata)*, Khaki weed (*Alternanthera pungens)*, Anis (*Pimpinella anisum)* and Senna (*Senna acutifolia)*, although the number of samples of each botanical species was low and the work did not specify whether the spores were found in one, several or all of the samples taken from the same plant.

Bianco et al. (2008) analysed 200 samples of chamomile (*Matricaria chamomilla*) in order to detect the presence of *C. botulinum* spores. 7.5% of the samples contained spores. The results revealed a higher number of spores in the samples obtained by the weight than in those obtained in packets. The number of spores in the positive samples ranged between 0.3-0.4 spores/g of chamomile. In the study, types A, B and F were identified in 53.3%, 6.7% and 13.3% of the positive samples respectively, whereas in 6.7% a mixture of types A and F were isolated. In the remaining 20% typification was not possible. The authors concluded that although the number of spores in the samples was lower than the number considered necessary to cause the disease, infusions should not be consumed by children under one year old, as these infusions are usually prepared with several grammes of the plant, and may also be administered several times a day and on successive days. As the presence of spores in the plants appears to be a direct consequence of their existence in the soil, in an earlier work the authors analysed 2009 samples of soil from five regions of Argentina and found *C. botulinum* spores in 23.5% of the samples (Lúquez et al., 2005).

The presence of *C. botulinum* spores was also analysed in lime (*Tilia* spp.), a plant also used in the preparation of infusions for administration to infants. 100 samples taken from the direct harvest of flowers on the tree and another 100 samples from commercial preparations packed in bags for infu-

sions were analysed. The results revealed that 3% of the samples of harvested flowers contained an average value of 30 spores/100 g. *C. botulinum* type A was identified in two samples and *C. botulinum* type B in one. In contrast, *C. botulinum* spores were not isolated in any of the commercial preparations (Bianco et al., 2009).

In Spain, López Laso et al. (2008) analysed a case of infant botulism that appeared in 2007 in Cordoba in a 40-day old baby fed with a formula milk who had also consumed a chamomile and fennel (*Foeniculum vulgare*) infusion. The fact that two months later a second case was detected in Huelva in another infant who had consumed the same type of infusion led to suspicions that this could be the origin of the *C. botulinum* spores. Nonetheless, *C. botulinum* spores were not isolated in any of the samples taken from the formula milk or from the infusion consumed by the infant. The type B neurotoxin was detected in the faecal analysis together with *C. botulinum* type B spores.

As the processing methods cannot guarantee the absence of *C. botulinum* spores in some plant infusions, their consumption should be avoided in children under 12 months old. Inclusion of this information on the product labels could help to reduce the incidence of the disease.

Presence of *Clostridium botulinum* in dust

The *C. botulinum* spores present in dust may also contaminate food given to the infants. Furthermore, the spores may be directly inhaled by the child, passing to the intestinal tract, or may reach the child orally when he/she is crawling and comes into contact with contaminated objects or places his/her dusty hands in his/her mouth.

Nevas et al. (2005) demonstrated in an 11-week old child who had died suddenly, from what was later confirmed as infant botulism, that the origin of the *C. botulinum* spores was house dust. Tests of the formula consumed by the child produced negative results, and honey was rejected as a source of exposure to the spores. Therefore the researchers took samples of soil from the house plants and of dust from the domestic vacuum cleaner. Using a PCR technique, the presence of *C. botulinum* type B spores was confirmed in the dust taken from the vacuum cleaner. These spores were similar to those isolated in the intestinal content of the child using Pulsed Field Gel Electrophoresis (PFGE) and Random Amplification of Polymorphic DNA (RAPD) techniques. The production of the B neurotoxin was confirmed in both strains using the mouse bioassay. The remaining samples taken from the house were found to be negative.

The movement of earth, for example on construction sites, roads, etc., contributes to the dispersion of the spores which may easily enter nearby houses. Farming practices involving work on the soil, or living in windy areas, are considered as risk factors as they increase exposure to spores. In other countries, the majority of cases have appeared in cities and are most likely to be related to the dust generated during property renovation work (Fenicia and Anniballi, 2009).

Characterisation of the risk

Infant botulism appears in children aged between 1 and 52 weeks old, who consume *C. botulinum* spores. Of all the possible foods that have been analysed in attempts to link them to infant botulism (honey, corn syrup, formula milk powder, cereals and plant infusions), honey is the most frequently

occurring food to be identified as responsible in those cases in which the source is identified. Therefore, the risk of contracting the disease can be classified in general terms as low if the consumption of honey and of plant infusions is avoided in children under the age of 12 months.

To further reduce the risk, the exposure of infants to dust in their homes (whether in rural farm areas or in urban areas with many construction sites involving the movement of earth, or during property renovation work) should be minimised.

Conclusions of the Scientific Committee

1. Age is the only predisposition factor recognised for infant botulism. The majority of the cases recorded occur in children aged between 1 and 52 weeks old, and the average age is 13 weeks. In infant botulism, the minimum infective dose of *C. botulinum* spores which could lead to a break-out of the symptoms is considered to be between 10 and 100 spores.
2. The majority of research carried out indicates that it is not possible to establish a direct relation between the type of food consumed by the child (breast milk compared to formula milk, the length of time the child is breast-fed, age at which new foods are introduced, etc.) and the appearance of the disease.
3. It should be remembered that almost all the information available about infant botulism comes solely from the study of those patients hospitalized, which could bias the results obtained. In fact, the current perceived rate is more a reflection of the capacity of health personal to detect the disease than of its true frequency.
4. Contamination of soils and aquatic sediments with *C. botulinum* spores determines their potential presence in food not subjected to heat treatments that guarantee their destruction. Of all the possible foods analysed in an attempt to link them to infant botulism, honey is the most frequently occurring food that appears as responsible in those cases in which a source is identified. To a lesser degree, some plant infusions have also been involved. However, on the majority of occasions it has not been possible to clarify the origin of the spores responsible for the disease. Furthermore, *C. botulinum* spores present in dust may be directly inhaled by the child, passing to the intestinal tract, or may reach the child orally when he/she is crawling and comes into contact with contaminated objects.
5. As the processing methods cannot guarantee the absence of *C. botulinum* spores in honey or in some plant infusions, this Scientific Committee recommends that their consumption be avoided in children under 12 months old. Inclusion of this information on the product labels could help to reduce the incidence of the disease. Furthermore, it is important to avoid the practice of spreading honey on the mother's nipple prior to feeding, on the baby's lips or on the baby's dummy in an attempt to reduce anxiety or crying.
6. When cases of infant botulism are investigated, the analysis of samples should contain (in addition to samples taken from suspect food), samples of dust from domestic vacuum cleaners, earth from flower pots or dust in the child's environment, especially in homes in rural farming areas, or in urban areas with many construction sites involving the movement of earth, or during property renovation work.
7. It is important to reinforce health education regarding this disease, particularly through health personnel working in primary health care who should convey the above recommendations to parents and carers of nursing children.

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