



# Infant Botulism: In Search of *Clostridium botulinum* Spores

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## Abstract

Infant botulism is now the most common form of human botulism in Canada and the United States. Infant botulism is a severe neuroparalytic disease caused by ingestion of the spore-forming neurotoxic clostridia, including *Clostridium botulinum* that colonize the large intestine and subsequently produce botulinum neurotoxin in situ. It has been over a century since the first surveys documenting the ubiquitous prevalence of *C. botulinum* in soils around the world. Since then, honey has been identified as the only well-known risk factor for infant botulism despite a multitude of international environmental surveys isolating *C. botulinum* spores from ground soil, aquatic sediments, and commonly available infant foods. Associations of infant botulism cases with confirmed sources of *C. botulinum* exposure have primarily implicated outdoor soil and indoor dust, as well as commonly ingested foods including honey, dry cereals, and even powdered infant formula. Yet the origin of infection remains unknown for most infant botulism cases. This review summarizes the various surveys from around the world for *C. botulinum* in environmental soils and sediments, honey, and other infant foods, as well as laboratory-confirmed associations with documented infant botulism cases. Additional factors are also discussed, including the composition of infant gut microbiota and the practice of breastfeeding. We make several recommendations to better identify sources of exposure to *C. botulinum* spores that could lead to effective preventive measures and help reduce the incidence of this rare but life-threatening disease.

## Introduction

*Clostridium botulinum* is a Gram-positive, spore-forming, obligate anaerobic bacterium that causes the neuroparalytic disease of botulism by producing botulinum neurotoxin (BoNT). BoNTs cause muscle paralysis by cleaving pre-synaptic vesicle proteins responsible for releasing acetylcholine from cholinergic nerve terminals at neuromuscular junctions. Some rare pathogenic strains of closely related species including *C. baratii*, *C. butyricum*, *C. argentinense*, and *C. sporogenes*, can also produce BoNTs [1]. *C. botulinum* is categorized into four different physiologically and genetically distinct groups (I-IV) that express at least eight different serotypes of BoNT (A-H), including the putative novel toxin BoNT type X. Groups I and II *C. botulinum* are most

associated with human botulism and express BoNT types A, B, F, and B, E, F, respectively [2]. The proteolytic group I *C. botulinum* can form spores that are highly heat resistant and have a higher optimal growth temperature (35 °C) than the non-proteolytic group II *C. botulinum*, which form heat-sensitive spores and can grow at a lower minimum temperature (4 °C). Group III *C. botulinum* expresses BoNT types C and D that are associated with animal botulism [3], while group IV *C. botulinum* expresses BoNT type G that has been implicated in human disease but is exceptionally rare [4].

Botulism is categorized by the different routes of exposure to BoNTs. Foodborne botulism is caused by ingestion of foods contaminated with *C. botulinum* that have been permitted to grow and produce BoNT in the food. Thus, foodborne botulism is an intoxication caused by ingestion of preformed BoNT. Adult colonization botulism and wound botulism are caused by *C. botulinum* colonization of the gastrointestinal tract and infected wounds, respectively, with production of BoNTs in situ. Adults with healthy gastrointestinal tracts are resistant to *C. botulinum* colonization and may unknowingly ingest spores on a regular basis. Infant botulism is now the most common form of botulism in Canada and the United States [5, 6], and is caused by

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colonization of the gastrointestinal tract in infants predominantly less than one year of age. Infants are particularly susceptible to colonization by *C. botulinum* at an age between three to five months old [7] that coincides with perturbations in gut microbiota after weaning from breast milk to solid foods [8], although cases have been described at younger than one week old [9, 10]. Infant botulism is most associated with group I *C. botulinum* types A and B, yet cases have also been caused by group II *C. botulinum* type E [11], as well as *C. butyricum* type E [12–16], and *C. baratii* type F [10, 17–19]. In a single instance, group III *C. botulinum* type C causing infant botulism has been described [20].

In contrast to a foodborne botulism outbreak, in which multiple patients can be traced to a single-shared food source, infant botulism is caused by the ingestion of *C. botulinum* spores commonly found in the environment and occurs sporadically as individual cases. Over a century has passed since the pioneering surveys by Meyer and colleagues in 1922 that found viable *C. botulinum* spores in the ground soils, aquatic sediments, and food products in the United States, Canada, Europe, and China [21–25]. Since then, environmental surveys from around the world have isolated *C. botulinum* spores from soils and sediments, as well as foods commonly fed to infants such as honey, dry cereals, fruits, and vegetables, and even powdered infant formula [26, 27]. Honey is the most well-known risk factor for infant botulism [28]. Household dust and outdoor soils that were phylogenetically identical to patient isolates have also been confirmed as sources of infection [29]. The largest case–control study of infant botulism in the United States to date, conducted from 1976 to 1983, identified the following risk factors for infant botulism associated with illness hospitalization: breastfeeding, the occurrence of less than one bowel movement per day, ingestion of honey or corn syrup, dust exposure, and residence in a windy area [30]. Yet source attribution for infant botulism remains a significant challenge. In Canada, from 1979 to 2019, only 6% of cases were attributed to a laboratory-confirmed source, all of which were from honey [5].

This article summarizes the findings of published *C. botulinum* surveys including infant foods and environmental sources from around the world. The eligibility criteria included primary research articles from all peer-reviewed journals from all countries with no limit to the year of publication. Information sources included PubMed® (National Institutes of Health, USA) and Google Scholar. By creating a complete and unified summary of available information, we identify several gaps in knowledge where future investigations could reveal common food or environmental reservoirs where infant exposure to *C. botulinum* is most likely to occur, thereby leading to simple and effective preventive measures to reduce the incidence of this life-threatening childhood disease.

## Discussion

### Honey Surveys

Honey was recognized as a potential source of *C. botulinum* spores in 1976 when infant botulism was first described by Midura and Arnon [31]. By 1979, several case associations had been made linking *C. botulinum* isolates from patients' clinical specimens to honey isolates, and several different surveys had also recovered *C. botulinum* from retail honey samples [32–36]. In 1981, the American Academy of Pediatrics issued a policy statement advising against feeding honey to infants [37], which has since resulted in a significant decline in infant botulism cases associated with honey ingestion [38]. In total, at least 70 published surveys, independent of associations with infant botulism cases, have been conducted globally from 1978 to 2023 on honey sampled directly from apiaries or retail sources and are listed in Supplementary Table 1. Overall, *C. botulinum* was found in 173/4,310 (4%) of honey samples from around the world. Most of the isolates recovered were type A (67/173, 39%) and type B (63/173, 36%). This agrees with the observation that *C. botulinum* type A and B spores are most associated with mainland soils and are accumulated by bees during nectar collection [39]. In addition, BoNT type was unknown in 33/173 (19%) specimens. Type E and F were recovered in 5/173 (3%) specimens each, and a type AB (dual-toxin-producing strain) was recovered in 1/173 (<1%) specimen. Honey poses no risk for foodborne botulism due to its low water activity (0.5–0.65) that inhibits the growth of vegetative *C. botulinum* cells (>0.94 required) [40], and as such no instance of preformed BoNT has ever been reported in honey.

Honey produced from different countries had widely differing prevalences of *C. botulinum* contamination. Denmark was found to produce honey at the highest prevalence with 29/112 (26%) of samples testing positive from a single survey [41]. In the United States, *C. botulinum* was found in 42/587 (7%) of honey samples from nine different surveys. In Germany, only 1/354 (<1%) of honey samples tested positive from three different surveys and honey from the UK and Canada contained 0/122 and 0/112 positive samples, respectively. In 1998, a case of infant botulism from Norway was linked to honey containing *C. botulinum* type A from Argentina [42]. Honey produced in Argentina has a *C. botulinum* prevalence of 12/318 (4%) and high proportion of type A (9/12, 75%) when six independent surveys are combined. Several studies tested small batches of retail honey produced from around the world. For example, in 2002 a survey tested retail honey sold in Finland that was produced in 16 different countries spanning

three continents [43]. This investigation found that honey tested in small sample sizes from some countries had a high prevalence of contamination, such as Argentina (3/4, 75%), Australia (2/7, 29%), Spain (2/8, 25%), Italy (1/2, 50%), Cuba (1/5, 20%), and France (1/5, 20%). However, a much lower prevalence was found after combining multiple surveys, or incorporating surveys performed at higher scales. For example, when combining all three surveys on honey produced in Italy, the incidence was 3/139 (2%) overall, and combining the two surveys on honey produced in France the incidence was 1/95 (1%) overall. This demonstrates the importance of incorporating a higher sample size for individual surveys or combining multiple surveys together when inferring the prevalence of *C. botulinum* spores in a given food or environmental sample.

### Other Infant Food Surveys

In addition to honey as a source of spores, other infant foods such as corn syrup, dry cereals, and powdered infant formulas, were surveyed for the presence of *C. botulinum*. In total, at least 18 different surveys from 1922 to 2020 were conducted on retail infant food items and are summarized in Supplementary Table 2. In 1982, a survey of retail corn syrups sold in Washington, DC, isolated *C. botulinum* from 8/40 (20%) of samples tested, and a follow-up survey found 5/961 (<1%) of samples tested positive [44]. Corn syrup manufacturers revised their production methods in the 1980s and a subsequent large-scale survey of retail corn syrups in 1991 failed to isolate *C. botulinum* from 738 samples tested, which led the authors to conclude that corn syrup is unlikely to be a source of infection for infant botulism [45, 46]. In 1992, a survey in Japan recovered *C. botulinum* from raw, but not refined, sources of sugar sampled directly from producers, including sugar for apiculture (2/56, 4%), raw sugar (2/22, 9%), molasses (2/5, 40%), brown sugar (2/41, 5%), and corn syrup (1/16, 6%) [47]. A series of interesting surveys in 2008 and 2009 from Argentina found *C. botulinum* in chamomile (15/200, 8%) and linden flower (3/100, 3%) tea leaves when sampled directly from producers, yet all 100 samples of linden flower tested from retail sources were negative [48, 49]. Retail samples of dry cereals were found to be negative when tested in surveys from 1979 (0/30), from 1982 (0/90), and also from 1983 (0/12). However, in 1988, a survey from Canada isolated *C. botulinum* in one sample of retail dry rice cereal (1/40, 3%) [50]. The contamination was only found in 1/3 replicates and a follow-up investigation of three cereals of the same lot was all negative.

In 2005, a case of infant botulism in the UK was linked to an opened can of powdered infant formula [26]. An opened container of dried rice pudding also contained *C. botulinum*, but of a different BoNT type to the patient. A follow-up survey of retail powdered infant formulas isolated *C. botulinum*

from 1/15 (7%) of samples from the same batch, but the isolate was of a different genetic profile [27]. In response to this incident, an ad hoc risk assessment was conducted in 2006 by an advisory committee on behalf of the UK Food Standards Agency, which concluded that the risk of infant botulism from powdered infant formulas was low due to the stringent hygiene practices employed during production and a low calculated prevalence of spores [51]. In 2008, the Codex Alimentarius Commission deemed that *C. botulinum* is not considered a hazard in powdered infant formula [52], and in 2014, the International Commission on Microbiological Specifications for Foods recommended that powdered infant formulas and dairy-based ingredients for infant formulas not be tested for *C. botulinum*, but that routine testing for sulfate-reducing clostridia be used to determine adherence to Good Hygiene and Manufacturing Processes [53]. There are currently no clostridial standards for powdered infant formula in the USA or Canada. Retail powdered infant formulas from the United States were previously tested by surveys in 1979 and 1982, which failed to recover *C. botulinum* in 50 and 100 samples tested, respectively [33, 44]. More recently, a 2006–2007 survey from California did not recover *C. botulinum* from nine retail powdered infant formula samples and 30 opened cans from patients' households, although non-pathogenic clostridia were recovered from both retail and home samples [54]. Finally, in 2017, a *C. botulinum* isolate from an opened powdered infant formula was genetically linked to an isolate from the enema fluid of an infant botulism patient from China [55].

Fresh fruits and vegetables are a well-known source of *C. botulinum* spores due to their growth near soil and associations with foodborne botulism outbreaks. Yet, this category of foods can be prepared for infants without cooking. Even the process of boiling vegetables to prepare baby purees may not be sufficient to destroy all heat-resistant group I *C. botulinum* spores that are most often associated with cases of infant botulism. The first two surveys of fruits and vegetables were conducted in 1922 in the United States, which isolated *C. botulinum* from 54/431 (13%) to 33/122 (27%) of samples tested, including carrots (2/18, 11%), string beans (14/44, 32%), peas (3/51, 5%), and tomatoes (2/24, 8%) [21, 22]. A survey in 1975 from Canada isolated *C. botulinum* from all 12/12 (100%) of fresh commercial button mushrooms (*Agaricus campestris*) [56]. Since then, the prevalence of *C. botulinum* in this category of foods has been poorly investigated and limited to the context of foodborne botulism. In 1995, a survey from the United States found that 4/1,118 (<1%) of pre-packaged vegetables tested positive for *C. botulinum*, including one isolate each for shredded cabbage, chopped green pepper, Italian salad mix, and escarole salad mix [57]. A PCR-based screening of raw vegetables used for canned purees in 2001 from France found 0/37 testing positive for *C. botulinum* [58]. A study in 2012 from

France recovered *C. botulinum* type B from 1/128 (0.8%) of raw carrots and 1/188 (0.5%) of raw green beans used for canning [59]. More recently, a high prevalence (24/74, 32%) of *C. botulinum* from both groups I and II and types A, B, E, and F was detected in retail vegetarian sausages by a survey in 2020 from Finland [60]. In the context of food manufacturing, it is simply assumed that viable *C. botulinum* spores may be present in raw fruits and vegetables, and efforts are taken to destroy them or prevent their growth. For infant botulism, this category of foods represents a significant risk that has yet to be fully investigated in recent years.

## Environmental Surveys

The first person to suggest that *C. botulinum* is widely distributed in nature was G.S. Burke in 1919, who recovered isolates from various fruits, vegetables, insects, and other environmental samples in five different locations across California [61]. In 1922, four different landmark surveys were conducted by Meyer and colleagues investigating the prevalence of *C. botulinum* spores in the mainland soils and aquatic sediments of the United States, Canada, Europe, and China [21–25]. At least 73 different environmental surveys from 37 countries have been conducted from 1919 to 2023 and are summarized in Supplementary Table 3. In total, viable *C. botulinum* spores were recovered from 4,655/36,793 (13%) of environmental samples. Mainland soils were extensively tested due to concerns of foodborne botulism from fruits and vegetables grown on cultivated farmlands. Of the 51 surveys conducted on mainland soils worldwide, *C. botulinum* was recovered from 2,498/19,289 (13%) of samples tested. *C. botulinum* was also recovered from 1,314/11,041 (12%) of coastal sediments across 32 surveys and 761/5,151 (15%) of inland sediments across 21 surveys. One study in 1998 from Finland reported a high prevalence of *C. botulinum* type E in sea water (37/42, 88%) and fresh water (34/56, 61%) sampled from the Baltic Sea and Finnish mainland, respectively [62]. Interestingly, the prevalence of *C. botulinum* spores is lower when sampled from indoor household dust. Three small-scale studies in 1979 from California recovered *C. botulinum* from indoor dust in 1/19 (10%), 2/31 (7%), and 1/46 (2%) of samples tested [33, 34, 36]. More recently, a 2023 study from Canada did not detect *C. botulinum* in 963 samples of household vacuum cleaner dust across 13 major Canadian cities [63]. These limited surveys suggest that exposure to *C. botulinum* is less likely indoors unless spores are carried in from an outside source. A survey in 1985 isolated *C. botulinum* from 3/4 (75%) of the outdoor soil samples from the father's worksite and all four samples of dirt from the father's shoes, indicating a likely vector of transfer from outdoors to indoors [64]. Indeed, several cases of infant botulism have been matched by BoNT type

to isolates of both indoor dust and outdoor soil from the surrounding environment [34].

The factors affecting the geographic distribution of *C. botulinum* spores in different environmental niches are poorly investigated, yet some consistent trends have emerged. Overall, mainland soils were most associated with types A (1,062/2,498, 43%) and B (809/2,498, 32%), while aquatic sediments, sea water, and fresh water were most associated with type E (1,851/2,152, 86%). In some instances, types A and B were recovered from aquatic sediments (211/2,075, 10%) and type E was isolated from mainland soils (284/2,498, 11%). *C. botulinum* type F was isolated from both mainland soils (61/2,498, 2%) and aquatic sediments (13/1,314, 1%). Dual-toxin-producing clostridia were only obtained from mainland soil samples, including type Ab (21/2,498, <1%), type Af (26/2,498, 1%), and on one occasion type Bf (1/2,498, <1%). Surveys in 1922 and 1978 from the United States found a clear disparity in the geographic distribution of type A and B spores [21, 65]. Type A spores were predominantly isolated west of the Mississippi river in neutral or alkaline soils with lower organic content. Type B spores were more commonly found in the eastern states in more acidic soils with higher levels of organic content. This distribution is reflected in the BoNT type of *C. botulinum* associated with infant botulism patients in the USA [6]. Worldwide, type A and B spores were more commonly found in the mainland soils of temperate countries, including Argentina (564/2,732, 21%), Brazil (67/314, 21%), China (577/7,378, 8%), Republic of Georgia (40/258, 16%), Taiwan (75/134, 56%), Italy (7/520, 1%), the UK (48/711, 7%), Hawaii, USA (7/19, 37%), and the continental USA (398/2,788, 14%). The psychrotrophic group II *C. botulinum* type E is known as a water-dwelling organism that is often linked to foodborne botulism outbreaks in marine mammals and fish [66]. Overall, *C. botulinum* type E was isolated at a high prevalence from the aquatic sediments of coastlines in northern countries, including Alaska, USA (92/292, 32%), Canada (214/999, 21%), Greenland (18/21, 86%), Denmark (337/629, 54%), Norway (8/12, 67%), Sweden, (236/312, 76%), and Finland (92/137, 67%). Type E spores were also found on the Pacific coastline (224/662, 34%), Atlantic coastline (11/341, 3%), and Gulf coastline (5/717, <1%) of the continental United States.

The original 1922 surveys from the United States found a higher prevalence of *C. botulinum* spores in undisturbed soils that were still in their natural state (150/413, 36%) than in soils that had been disturbed by people through cultivation or urbanization (215/904, 24%) [21, 22]. This was also observed in Canada with undisturbed soils showing a greater prevalence (23/61, 38%) than disturbed soils (4/30, 13%) [25]. However, this finding has not been consistent internationally or regionally within the United States. In 1922, the same authors failed to recover *C. botulinum* from



seven undisturbed soils from the UK, yet were successful for 9/57 (16%) of disturbed soils [23]. A 1946 survey from New York State found that undisturbed soils had a lower prevalence (3/60, 5%) of *C. botulinum* than disturbed soils (26/178, 15%) [67]. In 1980, a survey from Denmark also found a lower prevalence in undisturbed soils (5/38, 13%) than in disturbed soils (13/37, 35%) [68]. Finally, in 2005 a large-scale survey from Argentina found a lower prevalence of spores in undisturbed soils (108/661, 16%) than disturbed soils (256/722, 36%), although this trend was reversed in the central region of Argentina that includes the dense urban city of Buenos Aires, where *C. botulinum* was isolated from 89/397 (22%) of undisturbed soils and 31/88 (35%) of disturbed soils [69]. In total worldwide, 27 studies have isolated *C. botulinum* spores from 484/2,211 (22%) of undisturbed soils and 32 studies have isolated spores from 967/5,140 (19%) of disturbed soils, suggesting that this is not a geographically persistent trend. It is most likely that spores of *C. botulinum* are simply ubiquitous in nature worldwide and may be incorporated into cultivated farmland or urban areas through inadvertent human development.

### Case-Confirmed Associations

Case study investigations, in which isolates of *C. botulinum* from the intestinal contents of patients with infant botulism matched the BoNT type of isolates recovered from ingested food or the local environment, are listed in Supplementary Table 4. Overall, outdoor soil samples were found to contain *C. botulinum* in 39/44 (89%) of associated cases, while indoor dust was positive in 26/156 (17%) of associated cases, and honey was positive in 52/77 (68%) of associated cases. Powered infant formula was associated with two cases, one from the UK in 2005 and one from China in 2017 [26, 55]. A dry rice cereal was associated with one case from China in 2015 [70]. Chamomile herbs were associated with a single case from Portugal in 2012, although ingested honey was also found to contain *C. botulinum* with the same BoNT type [71]. Finally, contaminated well water was associated with one case from Japan in 2014 [72], and water from a pet turtle aquarium was found to contain *C. butyricum* type E associated with two closely related cases from Ireland in 2015 [73]. In some instances, such as a survey conducted in 1979 from the United States, only a small portion of sampled honey (6/17, 35%) or dust (4/85, 5%) recovered *C. botulinum* that matched clinical isolates from the patients residing in associated households [34]. In other studies, such as a survey in 1986 from the United States, all 11 (100%) outdoor soils and 2/3 (67%) of indoor dust samples contained *C. botulinum* that matched the BoNT type of three geographically related infant botulism cases from a small town in Colorado [64].

These targeted surveys and case study investigations demonstrate that, in most instances, the laboratory-confirmed sources of *C. botulinum* exposure are from expected sources. Honey, outdoor soils, and indoor dust accounted for 114/121 (94%) of all verified sources of spores. Of the case studies investigating contaminated honey, 43/49 (88%) occurred before the year 2000, which indicates a collective recognition of honey as a risk factor for infant botulism. The most recent case associated with honey occurred in Israel in 2019 involving a contaminated honey-cake [74]. Outdoor soils were more often found to be a source of exposure (39/120, 33%) than indoor dust (26/120, 22%), which is expected considering that *C. botulinum* is commonly known as a soil-dwelling organism ubiquitous in nature that would need to enter the household via heating/cooling vents or be tracked inside on shoes, clothing, or by pets. One important note is that most of these case associations were established at the level of BoNT type. Comparative genotyping is only rarely performed to confirm these isolates as the definitive source of infection. A study published in 2014 from California performed genetic epidemiological testing at a large scale and found that *C. botulinum* isolates from patients' clinical samples matched to the same genetic clade as 11/14 (79%) of environmental isolates and all 10 (100%) of honey isolates, strongly suggesting that these isolates were the sources of infection [29].

### Additional Factors

A well-known risk factor for infant botulism is the age between three and five months old [7] that coincides with substantial changes to intestinal bacterial taxa after weaning off breast milk onto solid foods [8]. The same shift in microbial ecology is not as substantial in formula-fed infants [75]. Initially the infant gut microbiome has low species diversity and high variability among individuals [76, 77], yet progresses within the first few years of life toward a stable adult profile by a variety of factors, including lifestyle and diet [78]. The first indications that the gut microbiome protects against *C. botulinum* colonization were from studies in mice in 1979 and 1982 demonstrating a susceptibility to infection in germfree mice and mice treated with antibiotics compared to normal mice [79–81]. Interestingly, a study from Utah published in 1980 of 12 infant botulism patients and 87 controls discovered that 20/87 (23%) of control infants were “asymptomatic carriers” of *C. botulinum* in stool samples tested, of which two also contained BoNT [82]. This finding has yet to be replicated. In 1985, a predominance of Enterobacteriaceae was observed in the stools of all seven infant botulism patients investigated [64]. In 2015, a case–control study found that infants with laboratory-confirmed botulism displayed a significantly higher abundance of Proteobacteria, including Enterobacteriaceae, and a reduction in Lactobacilli

and Firmicutes (the phylum that includes clostridia), as compared to infant patients without laboratory confirmation of botulism [83]. Enrichments in Enterobacteriaceae can be triggered by host-mediated inflammatory responses to infection, chemical agents, or genetics, and significantly reduce the abundance of healthy resident bacteria [84]. Recently, the gut microbiota of an infant botulism case with particularly long-lasting fecal excretion of *C. botulinum* and BoNT was characterized, and revealed a persistently high abundance of Bifidobacteria that usually decreases by one year of age following the introduction of solid foods [85]. Interestingly, two bifidobacterial species *B. breve* and *B. bifidum* colonized the gut at later stages of the disease correlating with the clearance of *C. botulinum*. Bifidobacteria are prominent in the neonatal infant gut and have been shown to inhibit colonization of a broad range of enteropathogens [86], as well as prevent BoNT absorption and *C. botulinum* growth in vitro [87, 88]. *Lactobacillus paracasei* subsp. *paracasei* was also shown to inhibit *C. botulinum* growth and toxin production in vitro and its administration prior to infection with *C. botulinum* enhanced survival time in mice [89]. While these preliminary studies suggest a role for probiotics in preventing infant botulism, undertaking a prospective study to evaluate the effect of probiotic supplementation would be impractical due to the rarity of disease. Administration of probiotics after disease onset is unlikely to alter the clinical course or length of hospitalization since the negative effects of toxin on the nervous system have already occurred by the time of diagnosis. Probiotics during recovery could help establish healthy microbiota that may protect against other intestinal pathogens, strengthen the intestinal mucosal barrier, reduce inflammation, and modulate the immune response in a positive manner [90]. Further characterization of the infant gut microbiome could help predict the risk of colonization at a distinct age of susceptibility. It is important to note that, unless another infectious organism is detected, antibiotic use for infant botulism patients, especially aminoglycosides, is not recommended due to lysing of vegetative *C. botulinum* cells that could cause a release of additional BoNT and worsen the patient's paralysis [91].

Breastfeeding was first identified as a potential resilience factor in 1980 when it was found that exclusively breastfed infants developed symptoms at an older age (typically six months or older), while formula-fed infants were hospitalized at younger ages and developed more severe and fulminant illness [92]. This finding was recently confirmed by analysis of data from California spanning 40 years: breastfed patients were more than twice as old at symptom onset as formula-fed patients [38]. The authors suggested that breast milk may influence the composition of the infant gut microbiome to delay *C. botulinum* colonization or prevent the absorption of BoNT through the intestinal epithelium. This effect could be mediated by the predominance of gut bacteria

highly adapted to process milk oligosaccharides, such as Bifidobacteria [7], while formula-fed infants are already exposed to more diverse nutrients and develop a microbial profile more closely resembling the adult microbiome earlier [75]. However, the role of breast milk in infant botulism is controversial. A seven-year case-control study conducted from 1976 to 1983 in California found a greater percentage of infant botulism cases were still being breastfed at symptom onset than age-matched healthy controls [30]. A bivariate analysis identified breastfeeding at birth, breastfeeding at onset of symptoms, and number of breastfeeds per day as risk factors for illness hospitalization compared to healthy neighborhood or county controls. One possible explanation for this unexpected finding is that breastfed infants can survive colonization by *C. botulinum* long enough to be diagnosed in a hospital, while non-breastfed infants succumb to fulminant disease, remain as unrecognized cases, and may be classified as cases of Sudden Infant Death Syndrome [36, 93]. A subsequent multivariate analysis revealed that breastfeeding was only a predisposing factor for infants older than two months of age. This agrees with previous epidemiological studies in 1985 and 2014 that identified breastfeeding as being associated with infant botulism cases [64], and as being a significant risk factor for infant botulism at greater than two months of age [29]. There is no evidence that *C. botulinum* or BoNTs are passed through breast milk to the infant. Therefore, it is generally thought that breastfeeding slows down colonization of the infant microbiome with *C. botulinum*, delays the timing of illness onset, and prevents the progression of life-threatening illness when an infant is under constant exposure to *C. botulinum* spores. Thus, paradoxically, breastfeeding has been associated with an increased risk of infant botulism overall [94], and further investigation is warranted to elucidate the mechanisms that mediate these effects.

## Limitations

There are several limitations to these surveys and case associations. First, the isolation of *C. botulinum* is traditionally determined by the presence of the BoNT in liquid cultures and through neutralization assays using monoclonal antibodies in rodents. The original surveys by Meyer and colleagues in 1922 predate the knowledge of types E (1936), F (1960), and G (1970) [40]. Serological-based identification may also fail due to low concentrations of BoNT in the sample, in which neutralizations would be difficult to distinguish from untreated assays [68, 95]. Overall, a significant percentage of honey isolates (33/174, 19%), other food isolates (68/143, 48%), and environmental isolates (340/4,627, 7%) were of unknown BoNT type. Identification by BoNT type without species determination also precludes the possibility that the organisms identified were neurotoxic clostridia other

than *C. botulinum*, such as *C. baratii* type F or *C. butyricum* type E, which have been linked to previous cases of infant botulism. Furthermore, only rarely were isolates of *C. botulinum* types B or F differentiated as group I or group II by proteolytic digestion of meat pellets in culture media. Traditional intensive heat treatments used for the selection of *C. botulinum* likely destroyed group II spores entirely [96]. Several factors in environmental samples could have also prevented the growth and identification of neurotoxicogenic clostridia, including the presence of bacteriophages, bacteriocins, and competing microorganisms that are common in soil or sediments. In addition, the isolation of *C. botulinum* spores from food matrices such as honey and other syrups can be challenging and requires the use of a suitable method to avoid false negatives [39]. Modern molecular methods of detecting *C. botulinum*, including PCR targeting of BoNT genes from established enrichment cultures allow for highly sensitive and specific epidemiological investigations [60]. Finally, due to the predominant association of *C. botulinum* types A, B, E, and F with human illness, sampling instances of types C, D, and G were not reported in this summary. In 1993, Karen Dodds nicely summarized the food and environmental surveys that have isolated *C. botulinum* types C and D from around the world [40].

## Conclusions

Based on the findings in this summary, we can make several recommendations for future investigations to address gaps in knowledge that could aid in discovering environmental sources of *C. botulinum* spores and propose some simple interventions that could help prevent infant botulism infections in the future. First, it is apparent that *C. botulinum* spores are ubiquitous in nature around the world, yet the prevalence in household dust, where exposure to infants is most likely to occur, is inadequately characterized. Several case associations linking *C. botulinum* isolated from the stool of infant botulism patients to isolates from household dust have demonstrated the apparent risk of infection in this setting. It is likely that geographic areas with a high prevalence of *C. botulinum* spores in outdoor soils also correspond to a high prevalence in indoor dust. Therefore, soil surveys to identify highly contaminated areas that also correspond to a high prevalence indoors in residential neighborhoods could be coupled to public awareness for parents with infants in the high-risk age range to recognize symptoms of illness at early stages. Second, fresh fruits and vegetables that are commonly ingested by infants with minimal processing have not been adequately investigated. This potential source of *C. botulinum* spores represents a diverse group of foods that are widely available to infants and have been previously documented to contain *C. botulinum* spores. A

large-scale survey could identify fresh fruits and vegetables that are more commonly contaminated with spores. Results from such a survey would help inform parents with infants in the high-risk age range to avoid feeding these foods to infants. This same survey would also help identify foods that are at high risk of causing foodborne outbreaks from improper home canning or home jarring. Instances where neurotoxicogenic clostridia are isolated from food or environmental samples should be followed up with characterization by proteolysis from a pure culture (to determine group I vs. group II) or whole-genome sequencing to determine species (*C. botulinum* or otherwise). Third, parents should be aware that when an infant is transitioning from breast milk to formula, or introducing solid foods, a window of vulnerability to infant botulism may exist. At this time in an infant's life, parents should be aware that several days of constipation followed by weakness and poor feeding are the hallmarks of infant botulism, and they should seek medical advice from a pediatrician. In addition, the excretion of BoNT in the feces of recovering patients with IB may represent a risk to other infants in daycare or other settings because of possible contact with fecally soiled material [97]. Parents, caregivers, and clinicians should follow good hygienic practices that include carefully changing and disposing of fecally soiled diapers, disinfecting the changing surface, and thoroughly washing hands. Finally, enhanced communication with public health officials and hospital staff is essential to coordinate the collection and testing of environmental and food samples in cases of laboratory-confirmed infant botulism. Case-by-case investigations could help identify recurring patterns of infection over the course of a long time period and could justify recommendations to avoid any implicated foods commonly fed to infants. In instances where the source of *C. botulinum* spores is soil or dust near the infant's residence, exposure may be unavoidable. Clinicians and parents will need to remain vigilant regarding the symptoms of infant botulism, especially in regions where infant botulism is known to occur more frequently. These future endeavors could identify commonly encountered sources of *C. botulinum* spores that may lead to simple but effective preventive measures against this life-threatening childhood disease.

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## Declarations

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