Spores of *Clostridium botulinum* in Dried Dairy Products

M. Ellin Doyle and Kathleen Glass
Food Research Institute
University of Wisconsin–Madison, Madison WI 53706

Executive Summary

A recent report of presumptive *Clostridium botulinum* spores in whey protein concentrate (WPC) has generated questions about potential risks and strategies for control of spores in dried dairy products. On 28 August 2013, New Zealand's Ministry for Primary Industries reported that additional independent testing confirmed that the isolate was a closely related species, non-toxigenic *Clostridium sporogenes*, and not pathogenic *Clostridium botulinum*. The original reports that the isolate was toxigenic were false positives. Three batches of whey protein concentrate produced in New Zealand had been previously identified to contain clostridia spores, but no toxin and no active cells were found. No illnesses were reported. Regardless, the implicated lots were removed from the food supply out of an abundance of caution.

There are lessons to be learned from this episode and risks put into perspective, particularly with regards to *C. botulinum* and testing. First, bacterial spores are ubiquitous in the environment and in foods. Furthermore, it is not uncommon to find botulinum spores in foods such as honey, vegetables that grow in or near the ground, or smoked fish. Whereas spores in honey or the environment have been associated with infant botulism, consuming the spores of *Clostridium botulinum* alone poses no health risk to children older than 1 year old or to adults with normal microflora.

Spores are known to survive milk pasteurization and other similar thermal processes. Therefore, populations of spores in dried milk products should be minimized through use of good quality milk, temperature control, sanitation of equipment and processing plants, and potentially other processing techniques. The International Commission on Microbiological Specifications for Foods does not recommend routine testing of milk products for *Clostridium botulinum* spores, but testing for other microbes, such as sulfite-reducing clostridia (limit 100 cfu/g) may be useful as indicators of process control and sanitation.

What were the events surrounding the recall of the Whey Protein Concentrate?

According to the supplier's website, three batches of whey protein concentrate (WPC80, 38 tons) that were manufactured May 2012 at a single production site were identified to be presumptively contaminated with spores of *C. botulinum*. No toxin or active cells were present.

Testing of lots in March 2013 first revealed that populations of sulfite-reducing clostridia exceeded a customer's specifications; the presumptive presence of botulinum spores was identified through testing commissioned by the producer in July 2013 but additional independent testing by New Zealand's Ministry for Primary Industries "definitively established that there was no presence of *Clostridium botulinum* in the whey protein concentrate ingredient and the products made using it, including infant formula." Regardless, the source of the sulfite-reducing clostridia contamination was traced back to a non-sterile pipe at the plant concerned.

The lots had been sent to eight customers for use in a variety of products, including infant formula, beverages, and food supplements. All potentially affected products have been contained or recalled. None of the implicated product was imported into the United States. No illnesses have been associated with the implicated products.

Although no *C. botulinum* spores were confirmed, this episode provides an opportunity to review practices and establish procedures to prevent a similar situation in the future and to minimize food safety risks and keep them in perspective.

What are spores?

Certain bacteria, such as *Bacillus* and *Clostridium*, produce spores to survive environmental stresses that would normally kill the vegetative cell. These stresses include drying, high temperature (such as pasteurization), UV irradiation, and chemical damage. These inert spores can break dormancy, germinate, and grow when held under conditions of sufficiently high moisture, low acid, and favorable temperature. Unlike *Bacillus* spp., *Clostridium* spp. also require anaerobic conditions to grow.

Spores, including those of both toxigenic and spoilage species, are found worldwide in soils, sediments, water, dirt, dust, and the digestive systems of animals. Therefore, they can be found in foods and spices obtained from these sources.

Is consumption of *C. botulinum* spores a risk to human health?

Consuming low levels of botulinum spores *per se* rarely poses a health issue to individuals older than one year old, unless they are devoid of intestinal microflora. As a matter of fact, children and adults consume botulinum spores without ill effect when eating foods such as honey, vegetables that grow in or near the ground (such as carrots and potatoes), or smoked fish. Similarly, low numbers of clostridial spores in dried milk products or other dried foods do not present a health risk. A problem could occur if the product is rehydrated and kept anaerobic at non-refrigeration temperatures, allowing the *C. botulinum* spores to germinate, grow, and produce neurotoxins. Hence, low acid foods are protected by canning to kill spores or through proper formulation or strict temperature control to prevent growth/toxin production.

However, infants less than one year old without a well-developed normal intestinal flora could contract infant botulism if they ingest a sufficient number of spores from the environment, honey, or rarely, in infant formula. Children ages between 2 to 32 weeks of age appear to be at greatest risk; 99% of cases occur in children less than 1 year old and 94% of the cases occur in children less than 6 months old. Toddlers, ages one to three, are at no greater risk of contracting intestinal botulism than adults with typical intestinal microflora.

As whey protein is used in infant formula, this recent report of *C. botulinum* spores is of particular concern to manufacturers of infant foods and the dairy plants producing WPC and other dried dairy ingredients because the actual risk to infants is not known. However, the use of the implicated WPC in other properly formulated and stored foods poses no unusual risk to non-infant individuals.

Have any illnesses been associated with *Clostridium* spores found in dried dairy products?

There have been no reports of botulism associated with the implicated WPC from New Zealand. In an unrelated event, one case of infant botulism in the UK in 2001 was linked to infant formula milk powder. In that episode, the numbers of *C. botulinum* spores in infant formula milk powder collected from the patient's home were estimated to be 0.38 per 100 g whereas an unopened can of the same infant formula was reported to contain 0.14 spores/100 g (*7;16*).

C. perfringens was responsible for a 1981 outbreak of gastrointestinal food poisoning affecting 77 school children in the UK who consumed milkshakes made from contaminated powdered milk but which had been stored at abuse (ambient) temperatures for 22 hours before serving (*2*).

What is the infectious dose for infant botulism?

Infectious dose of *C. botulinum* spores for infant botulism is not precisely known, but based on exposures to spore-containing honey, it is estimated to be as low as 10 to 100 spores (*3;15*). Early investigations in infant rodents indicated that the 50% infectious dose (ID_{50}) varied with strain of *C. botulinum* and ranged from 170 to 700 spores/infant mouse. As few as 10 spores were infective to adult germ-free mice (*25*). Similar results were observed in germ-free adult and infant rats. These rodent studies demonstrated that there was a window of about 7 days during infancy when infection with spores could occur. Animals younger than 7 days or older than 13 days were not susceptible (*20*). A later study found the ID_{50} for infant mice was 290 spores/mouse (*26*).

How do spores get into the dairy products?

Bacterial spores are widely present in the environment, including in soils and sediments, both food and feed plant materials, and animal feces (*12*). In addition, there are niches in processing plants where bacteria may accumulate or grow and sporulate. *B. cereus* forms biofilms on surfaces of storage and processing equipment and these can be difficult to remove (*24*). Although not pathogenic, thermophilic spores can accumulate or potentially propagate during production of powdered milk. The primary sites for sporulation were identified as the evaporator and the preheater plate heat exchanger (*22*). Two reviews discuss origins of contaminating spores in foods and methods of control (*9;19*).

C. botulinum spores have also been detected in low numbers in milk and it is believed that these originate from the silage or bedding the animals were exposed to or the feces of infected cattle (*8;11;17*). Effective cleaning of udders prior to milking can significantly reduce spore counts in milk (*18*).

How common are spores in dried milk products?

Spores, including *C. botulinum*, survive pasteurization and drying procedures and can survive for very long periods of time in dried milk products (*21*). *Bacillus cereus* spores are also known to be present in powdered milk and infant foods (*5*). Spores of some species of *Clostridium* and *Bacillus* have been more frequently associated with quality problems in cheese making. Therefore, preventative controls are needed to ensure safety and quality of these products.

C. botulinum can occur but is not a common concern in dried milk products. *Salmonella* spp., *Cronobacter* (*Enterobacter*) *sakazakii*, and staphylococci have been more frequently associated with foodborne illness associated with dried milk and powdered infant formula.

There are very few surveys which describe the frequency, levels or species of clostridial spores in dried dairy products. Following the UK case of infant botulism associated with infant formula, 39 commercial powdered infant formula samples (5 brands) were tested in the United States for the presence of anaerobic spores. Twelve samples contained clostridial spores but no *C. botulinum* or other neurotoxigenic clostridia were identified (*4;23*). Analyses of 7 U.S. commercial cheddar cheese whey samples found that all except one contained mesophilic spores, with highest spore counts detected in winter samples, but *C. botulinum* were not specifically identified (*23*). Of 26 samples of dehydrated dairy ingredients tested in France, three were found to contain *C. botulinum* spores (*10*).

How are foods assayed for *Clostridium botulinum* spores? Should dried dairy ingredients be routinely tested for botulinum spores?

Detection of *C. botulinum* in dried dairy ingredients presents unusual challenges, and the value of routine testing to enhance public health is questionable. A procedure utilizing anaerobic culture followed by multiplex PCR specific for *C. botulinum* neurotoxin genes may provide the most rapid and accurate results (*13*). Confirmation that spores are toxigenic *C. botulinum* should be completed. Although ELISA and other non-animal systems have been evaluated, the mouse bioassay is still considered the standard for toxin confirmation. However, few U.S. laboratories have the capabilities to complete the assay; if *C. botulinum* is isolated from a food, it must be immediately reported to APHIS/CDC via Form 4c; in addition, if the entity is not registered for Tier 1 Select Agent, the isolate must be destroyed and transferred to a registered facility per federal regulations within seven calendar days of identification (http://www.selectagents.gov/FAQ_ReportingForm4.html#se7c1q5).

Nearly all clostridia, including *C. botulinum*, can reduce sulfite to sulfide and methods have been developed for the enumeration of sulfite-reducing clostridia in dried foods. These assays have been used to monitor the quality of dried foods. If counts of *Clostridium* spp. are high, this indicates a lapse in Good Manufacturing Practices and may trigger the additional investigation (*27*). ISO and IDF (International Dairy Federation) published a report in 2009 on "Dried milk—Enumeration of specially thermo-resistant spores of thermophilic bacteria." *C. botulinum* is not a thermophile, but some parts of this publication may be useful.

The Advisory Committee on the Microbiological Safety of Food for the UK Food Standards Agency did not recommend testing for clostridia spores in powdered infant formula (PIF) after investigation of the infant botulism case in that country (*1*). The International Commission on Microbiological Specifications for Foods (ICMSF) does not list finished product specifications for any sporeformers in dried dairy products, infant cereal, and PIF, but instead focuses on aerobic bacteria, enterobacteriaceae, *Salmonella;* PIF has *Cronobacter* sp. as an added specification (*14*).

ICMSF recently concluded that there is not sufficient scientific support to initiate any specifications for botulinum spores in dried dairy products and it does not recommend routine testing for *C. botulinum* in dried dairy powders (http://www.icmsf.org/pdf/ICMSF_Infant_Formula_Testing_27_Aug_2013.pdf). ICMSF further concluded that limits of 100 cfu/g of sulfite-reducing clostridia (SRC) in dried dairy ingredients used in PIF can be useful as an indicator of hygienic practices and conditions which would promote anaerobic clostridia. Continued investigation of normal spore loads and their potential effect on public health should be completed prior to changing specifications in dried dairy products.

How can industry minimize spores in dried dairy products?

Good Manufacturing Practices with careful attention to quality of incoming milk, training of milk suppliers and plant workers, temperature control, and sanitation of equipment and processing plants will significantly reduce contamination with spores. Some specialized processes, such as bactofugation, can remove spores and some spoilage organisms. Resources are available from trade organizations regarding on-farm and in-plant practices impacting spore-formers and some commercial solutions for controlling spore formers.

Where can I get further information on this subject?

Further information on these issues is presented in recent comprehensive review articles on *Clostridium botulinum* and botulism (*12;15*), *Clostridium botulinum* in cattle and dairy products (*17*), and foodborne pathogens in low water activity foods (*6*).

References

1. Advisory Committee on the Microbiological Safety of Food: Ad Hoc Group on Infant Botulism. Report on minimally processed infant weaning foods and the risk of infant botulism. 2006. Accessed 2013. http://www.food.gov.uk/multimedia/pdfs/infantbotulismreport.pdf
2. Anon. 1982. Disease attributed to dairy products. *Brit Med J* 285(6355):1664.
3. Arnon SS. 2004. Infant botulism, p. 1758–1766. *In* Feigen RD and Cherry JD (eds.), "Textbook of Infectious Pediatric Diseases." W. B. Saunders, Philadelphia.
4. Barash JR, Hsia JK, and Arnon SS. 2010. Presence of soil-dwelling clostridia in commercial powdered infant formulas. *J Pediatr* 156:402–408.
5. Becker H, Schaller G, von Wiese W, and Terplan G. 1994. *Bacillus cereus* in infant foods and dried milk products. *Int J Food Microbiol* 23:1–15.
6. Beuchat LR, et al. 2013. Low water activity foods: increased concern as vehicles of foodborne pathogens. *J Food Prot* 76:150–172.
7. Brett MM, McLauchlin J, Harris A, O'Brien S, Black N, Forsyth RJ, Roberts D, and Bolton FJ. 2005. A case of infant botulism with a possible link to infant formula milk powder: evidence for the presence of more than one strain of *Clostridium botulinum* in clinical specimens and food. *J Med Microbiol* 54:769–776.
8. Böhnel H and Gessler F. 2013. Presence of *Clostridium botulinum* and botulinum toxin in milk and udder tissue of dairy cows with suspected botulism. *Vet Rec* 172:397–400.
9. Carlin F. 2011. Origin of bacterial spores contaminating foods. *Food Microbiol* 28:177–182.
10. Carlin F, Broussolle V, Perelle S, Litman S, and Fach P. 2004. Prevalence of *Clostridium botulinum* in food raw materials used in REPFEDs manufactured in France. *Int J Food Microbiol* 91:141–145.
11. Driehuis F. 2013. Silage and the safety and quality of dairy foods: a review. *Agr Food Sci* 22:16–34.
12. Glass K and Marshall K. 2013. *Clostridium botulinum*, p. 371–387. *In* Morris JG and Potter M (eds.), Foodborne Infections and Intoxications. Academic Press, London, UK.
13. Hill BJ, Skerry JC, Smith TJ, Arnon SS, and Douek DC. 2010. Universal and specific quantitative detection of botulinum neurotoxin genes. *BMC Microbiol* 10:267.
14. International Commission on Microbiological Specifications for Foods (ICMSF). 2011. "Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance." Springer, New York.
15. Johnson EA. 2013. *Clostridium botulinum*, p. 288–304. *In* Doyle MP and Buchanan RL (eds.), "Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington D.C.
16. Johnson EA, Tepp WH, Bradshaw M, Gilbert RJ, Cook PE, and McIntosh E. 2005. Characterization of *Clostridium botulinum* strains associated with an infant botulism case in the United Kingdom. *J Clin Microbiol* 43:2602–2607.
17. Lindström M, Myllykoski J, Sivelä S, and Korkeala H. 2010. *Clostridium botulinu*m in cattle and dairy products. *Crit Rev Food Sci Nutr* 50:281–304.
18. Magnusson M, Christiansson A, Svensson B, and Kolstrup C. 2006. Effect of different premilking manual teat-cleaning methods on bacterial spores in milk. *J Dairy Sci* 89:3866–3875.
19. Mettler AE. 1994. Present day requirements for effective pathogen control in spray dried milk powder production. *J Soc Dairy Technol* 47:95–107.
20. Moberg LJ and Sugiyama H. 1980. The rat as an animal-model for infant botulism. *Infect Immun* 29:819–821.
21. Ronimus RS, Rueckert A, and Morgan HW. 2006. Survival of thermophilic spore-forming bacteria in a 90(+) year old milk powder from Ernest Shackelton's Cape Royds hut in Antarctica. *J Dairy Res* 73:235–243.
22. Scott SA, Brooks JD, Rakonjac J, Walker KMR, and Flint SH. 2007. The formation of thermophilic spores during the manufacture of whole milk powder. *Int J Dairy Technol* 60:109–117.
23. Sithole R, McDaniel MR, and Goddik UM. 2006. Physicochemical, microbiological, aroma, and flavor profile of selected commercial sweet whey powders. *J Food Sci* 71:C157-C163.
24. Stoeckel M, Westermann AC, Atamer Z, and Hinrichs J. 2013. Thermal inactivation of *Bacillus cereus* spores in infant formula under shear conditions. *Dairy Sci Technol* 93:163–175.
25. Sugiyama H. 1979. Animal-models for the study of infant botulism. *Rev Infect Dis* 1:683–688.
26. Tabita K, Sakaguchi S, Kozaki S, and Sakaguchi G. 1991. Distinction between *Clostridium botulinum* type-A strains associated with food-borne botulism and those with infant botulism in Japan in intraintestinal toxin production in infant mice and some other properties. *FEMS Microbiol Lett* 79:251–256.
27. Weenk GH, Vandenbrink JA, Struijk CB, and Mossel DAA. 1995. Modified methods for the enumeration of spores of mesophilic *Clostridium* species in dried foods. *Int J Food Microbiol* 27:185–200.

Food Research Institute
University of Wisconsin–Madison 1550 Linden Drive Madison, Wisconsin 53706
Phone: 608/263-7777 Fax: 608/263-1114 Web: fri.wisc.edu/