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Review

Foodborne and Infant Botulism Linkage with the Gut Microbiome's Impact on the Immune System and Mental Function

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ABSTRACT

Food poisoning, in general, is unfortunate, but one type, in particular, is regarded the most dangerous: botulism. The name comes from the Latin bachelors, which means "sausage." It was first identified in processed meat. Foodborne botulism is caused by clostridial organisms that proliferate in the guts of infected people, then produce toxins that are absorbed and have systemic consequences. The toxin acts by interrupting signal transmission in the somatic and autonomic motor systems without affecting sensory signals or mental functions. Mild cases cause drooping eyelids and facial muscles. The smallest amount of this toxin, out of all the actual toxins that exist, would kill the vast majority of people. It is known that the gut microbiome is necessary to keep us healthy and resilient to drastic or harmful infections. Due to babies lacking the same level of immunity as adults, they are vulnerable to infection. They can ingest the spores, grow inside them, and produce the toxin. Thus, it is recommended not to give honey to babies as it commonly contains bacteria. When a baby is born, their bodies are covered in billions of microorganisms from their mother. Special sugars in mother's milk are designed to feed and promote specific microorganisms, act as a decoy for others, and affect the immune system. This system serves as a line of defense against infections transmitted through digestion, such as foodborne and infant botulism, to keep us safe. This study systematically reviewed the data for botulinum toxin which is produced by *Clostridium botulinum* and is deadly even in small amounts through foods, aiming to provide the importance of healthy gut microbiota.

Keywords: Botulinum toxin, Clostridium botulinum, foodborne botulism, food poisoning, gut microbiota, infant botulism

Botulism is a rare and potentially fatal form of food poisoning caused by a neurotoxin produced by the bacterium *Clostridium botulinum* (*C. botulinum*). The bacteria itself is not harmful when put in the right conditions-medical and cosmetic benefits-. It releases botulinum neurotoxin (BoNT) which paralyzes muscles and can lead to death. The five characteristics of human botulism that have been documented since the toxin's emergence are foodborne botulism, wound botulism, infant botulism, adult intestinal colonization, and iatrogenic botulism.[1] *C. botulinum* has the potential to invade the intestine and generate BoNT. Ingestion of *C. botulinum* spores in newborns or early babies up to the age of one year, when the microbiota is not completely established or functioning, can result in bacteria proliferation and synthesis of BoNT in the intestinal tract, and hence infant botulism.[2] The toxin reaches the lymphatic system, therefore the bloodstream, and finally the peripheral nerves after passage from the intestine.[3] This introductory section provides a brief overview of botulism definition, then it goes on to the BoNT complex's organization and functioning as well as the toxin's relationship between the mechanism of influencing the nervous system and the immune function of the intestinal microbiota.

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BIRTH OF *CLOSTRIDIUM BOTULINUM*

In the first half of the 19th century, the medical authority in Stuttgart discerned enlarged deaths due to foodborne poisoning. These views surfaced mainly in the lack of hygienic controls and government finance in the case of Napoleonic warfare (1795-1813). They detected the primary factor of these deaths was smoked blood sausages. The Medical Faculty of the University of Tübingen gathered cases

and made a list of symptoms of alleged "sausage poisoning" like gastrointestinal problems, double vision, and mydriasis. They stemmed from prussic acid which is in the sausage for those cases.[4,5] Between 1817 and 1822 local medical officer Justinus Kerner analyzed some 230 cases[1] and characterized them, which we know botulism here and now. But at that moment he termed it as ''sausage poison'' or ''fatty poison''.[5] He continued to study this toxin with animals, fused toxic sausages and honey to feed them. Experiments showed the motor system is affected by the toxin.[4] Overall, Kerner assumed that low-dose toxins could be used to control hyperextension and hyperflexion as a therapy. German physician Müller (1870) uses the term 'botulism (from the Latin word botulus, meaning ''sausage'')' to refer to sausage poison.[6] In 1895, 24 musicians began to show signs of poisoning-3dead- after they ate raw ham. According to this instance Emile van Ermengem, professor of bacteriology in Belgium, mentioned he named *Bacillus botulinus* (*Clostridium botulinum*) toxin presenting bacteria and clinical findings in his documentary, 1897.[7] Fifty-two years later, Burgen and his colleagues found botulinum toxin blockades neuromuscular gap not operating acetylation enzymes choline and sulphanilamide nor choline esterase.[8] The studies presented provide evidence that botulinum toxin corrupts interneural communication, which is mostly the cause of food contamination.

THE ORGANISM AND ITS TOXINS

Clostridium botulinum is a gram-positive, obligate anaerobe that lacks catalase and superoxide dismutase because oxygen is not the ultimate electron acceptor. As a result, hyperbaric oxygen therapy would be the most effective approach to eradicate Clostridia. Bacteria live in soil, that is, widely all over the world and one of the oldest spore-forming *bacillus*. They don't have cytochromes and rely on economic energy sources like 3-phosphoglycerate instead of ATP. Botulinum spores, a heat-resistant and dehydrated form of a bacterium, have dipicolinic acid as a Ca^{+2} schedule. This gives spores endurance. They produce exotoxins known as BoNTs due to their inhibition to release neurotransmitters on neuron cells. Distinguished with being the most toxic agent in the world.[9] 50 ng neurotoxin adequate to induce human botulism. The quantitatively of this minor lethal dose derives from data being gathered in foodborne botulism experiments on animals with the intake of neurotoxin volume.[10]

Botulinum neurotoxins are 150 kDa proteins consisting of a heavy chain (H_C-100 kDa) and a light chain (L_C-50 kDa) tied with interchain disulfide linkage.[11] The heavy chains have two separable basic functions. Firstly, In peripheral nerve terminals, the C-terminal domain (H_C) targets particular receptors. For instance, BoNT/A serotype binds to N-glycosylated synaptic vesicle (SV) 2A, B, C protein receptors with H_{CN}-H_{CC} side[12,13] and BoNT/B to Synaptotagmin I and II with H_{CC} side.[14,15] Syt and SV2 are membrane proteins that expose their BoNT-binding sites to the synaptic vesicle lumen. Secondly, the N-terminal domain (H_N) is responsible for the light chain translocation into the nerve cell cytoplasm.[11-16] Light chains which are zinc metalloproteases obstacle the most common neurotransmitter release in cholinergic nerves, acetylcholine, as they proteolytically split soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins which lead to exocytosis of neurotransmitters.[17,18]

NEUROTOXIGENIC DIVERSITY OF CLOSTRIDIA: BOTULINUM NEUROTOXINS SEROTYPES AND SUBTYPES

Clostridia standardized into six phylogenetic groups (Table1).[19] *C. botulinum* groups I-IV are categorized by the type of BoNT it produces and groups V-VI part of strains of *Clostridium butyricum* and *Clostridium baratii*. *C. botulinum* Group I (proteolytic *C. botulinum*) is a mesophilic bacterium that produces heat-resistant spores and is a significant source of botulism in humans (foodborne, infant, and wound).[20] *C. botulinum* Group II (non-proteolytic *C. botulinum*) is a dominant contributor of foodborne botulism in humans, and its safe manufacture of slightly heat-processed refrigerated foods is a problem.[21] Group III is a saccharolytic, mesophilic bacterium that looks remarkably like *C. novyi* and *C. haemolyticum* and produces heat-resistant spores, also inducing botulism in animals.[22]

Botulinum neurotoxins are produced by Clostridia and have similarities in structure and function, but it is interesting to have wide heterogeneity on the basis of genome organization with toxin genes cumulation, and especially toxin's amino acid sequence. To better understand the mechanisms of BoNTs and their therapeutic implications, genetic analysis of Clostridial strains studies based on neutralization by specific antisera classified toxins into 9 distinct types.[23] While there are exemplary

cases where other serotypes are also pathogenic, the most pathogenic in humans include BoNT/A, /B, /E, and /F.[24] *In vitro* studies showed antiserum that neutralized BoNT/C (strain Stockholm) was also effective on BoNT/D (South Africa strain).[23] As a result, the two were found to be related. Due to bacterial strains, genetic reshuffling that to mosaic toxins made by BoNT/C L chain and BoNT/D H chain.[25] BoNT/G has been isolated on three out of five people during the autopsy of people who died suddenly 40 years ago, there has been no reliable evidence of deaths from botulism due to serotype tip G.[26] The next two toxin types are called new serotypes because they are not neutralized by the antisera of the classic serotypes of BoNT (A-G). This novel gene derivative toxin is not meant to be expressed by bacteria, as the toxicity function is given by plasmid or chromosomal genes.[27] 'New serotype' BoNT/H was originally isolated from feces that have infant botulism.[28,29] BoNT/H fractions have equitable with BoNT/F5's catalytic domain ≈80%, BoNT/A1's binding domain ≈84%, and produce translocation domain alike to BoNT/F1. So also called BoNT/FA or BoNT/HA.[30] BoNT/X was revealed by the *C. botulinum* 111 sequences. It is not known whether it will show toxicity in different environmental conditions.[27] Belongs to BoNT/X, the quality of Vesicle-associated membrane protein 4/synaptobrevin (VAMP4) and Ykt6 cleaving is impressive. VAMP4 is known to facilitate homotypic endosome fusion but also regulates vesicle fusion between the trans-Golgi network (TGN) and endosomes.[31,32] Ykt6 is soluble N-ethylmaleimide-sensitive factor attachment protein receptor protein but is distinguished from being anchored to the lipid membrane and has critical roles in endocytic pathway's fusion like transformation and formation of ER-Golgi, intra-Golgi, endosome-Golgi, and vacuolar transfer stages, eventually concludes with an autophagosome.[33] To compare BoNT subtypes, it was determined that the amino acid sequence difference should be more than 2.6% as a guideline.[34] Along that line, 41 subtypes have been realeved[11] and 2.6% amino acid difference need to be noted regarding future studies. Although all BoNTs cause loss of motion, most BoNT types and subtypes share a comparative component of activity but contrast by their interactions with various receptors and intracellular SNARE targets that cut at different cleavage locales.[35,36] Table 1 comprehensively documents BoNT serotypes and subtypes including the groups to which they belong.

Clostridia	BoNT serotype	Subtype
Proteolytic C. botulimum group I	A,B,F,H()H/AorH/F)	A1;A2;A3;A4;A5;A6;A7; A8;A9;A10;B1;B2;B3;B5(bA);B6; B7;A(B);Ab;Af;Af84;Bf;F1;F2; F3;F4;F5
Non-proteolytic C. botulinium group II	B,E,F	B4;E1;E2;E3;E6;E7;E8;E9; E10;E11;F6
C. botulinium group III	C,D	C;D;CD;DC
C. ARGENTİNENSE group VI	G	
C. baratii group V	F	F7
C. butyricum group VI	E	E4;E5

Table 1: BoNT diversity of Clostridial species[19]

BOTULINUM NEUROTOXINS PRESERVED THE MECHANISM OF NERVE TERMINALS PARALYSIS

Throughout the life process, BoNTs metalloprotease domain (L chain) gains novel features that make it possible to penetrate neuronal cells' cytosol. By drawing on the concept of BoNTs actions on neurons, empirical studies have been able to based on five stages (Figure 1).[37]

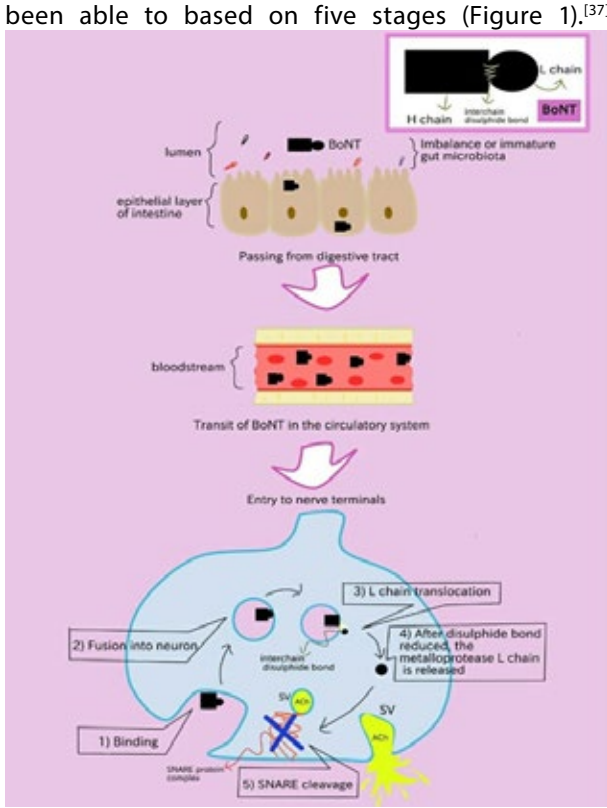

Figure 1: In food-borne botulism, the stages taken by botulinum neurotoxins from the lumen of the digestive tract to the cytosol of the nerve terminal. To cause food-borne botulism, the BoNT must get through the intestinal epithelial barrier. Its trip through the circulation terminates at the nerve terminals, where it blocks the release of acetylcholine.

SV: Synaptic Vesicle, ACh: Acetylcholine (The figure is adapted from Fujinaga[38] and Rossetto et al.[39])

1) Dual receptor binding: Botulinum toxin is not able to enter healthy skin. Botulism is defined as the transmission of botulinum toxin into the bloodstream from a mucosal surface (gut, lung) or a wound.[10] Despite having to pass the blood-brain barrier, botulinum neurotoxins quickly acquire access to the neuromuscular junction forward to joining the lymph and circulatory systems.[40] When the H_{CC} domain of BoNT binds to the presynaptic membrane receptor polysialoganglioside (PSG), internalization of the toxin necessitates secondary interaction to synaptic vesicle proteins on the luminal side.[41,42] Factors that assist this neuron-specific binding are explainable in at least major two respects. First, PSGs have a highly negative oligosaccharide head that extends out of the presynaptic membrane, while BoNTs are electrical dipoles with the positive end quite close to the PSG active domain. Second, the negatively charged presynaptic membrane will reconfigure the BoNT dipole as it approaches the negatively charged membrane, making nearly every collision with the PSG binding fruitful.[39,43]

2) Fusion into nerve terminals: In dual receptor binding, the second synaptic vesicle protein receptor types to which each toxin is associated are: For BoNT/B1, BoNT/DC, and BoNT/G, Synaptotagmin is used; for BoNT/A1 and BoNT/E1, glycosylated SV2 is used. Specific receptors of BoNT serotypes are shown one by one in Table 2l. Resultantly, BoNTs utilize synaptic vesicles like "Trojan horses" to get access to nerve cytosol.[39]

Table 2: Botulinum neurotoxin receptor proteins situated in synaptic vesicles due to serotype.[44-49]

* BoNT/C's protein receptor not yet identified, gangliosides are the only thing it interacts with.[50,51] Syt: Synaptotagmin

3) Due to lumen acidification, botulinum neurotoxin translocation to synaptic vesicle membrane occurs: The proton increasing action of the vacuolar ATPases (V-ATPase) observed on the synaptic vesicle membrane acidifies the SV lumen and the lumen of synaptic endosomes with which SV may have fused after endocytosis is finished.[52] The luminal pH is decreased to a range of almost 5.8 as a result of this activity.[53,54] The action of an ATPase proton pump in the SV membrane, which transforms protons to generate a transmembrane pH gradient that helps the neurotransmitter to be taken from the cytosol to the lumen, reinforces the action of the BoNT in preventing the neurotransmitter from being topped up inside empty vesicles.[39] Once the vesicle lumen becomes acidic, at low pH, the BoNT molecule changes that mentioned above. The H chain breaks the membrane resistance, which will facilitate the translocation process of the L chain, as a result, the L chain moves to the cytosolic side of the SV where it is held in place by the SS bond.[55]

4) Disulphide interchain linkage reduction: Disulfide bonds that become vulnerable after L-domaine translocation, where it is selectively reduced by the NADPH-thioredoxin reductase-thioredoxin redox system (Trx-Tx), which is attached to the cytosolic surface of a synaptic vesicle.[56] Rossetto et al.[57] demonstrated that inhibitors of Trx-Tx induced *in vitro* resistance in human botulism, particularly in infant botulism. Empirical data, on the other hand, suggests that it might be an effective therapy for paralysis.

5) Cleavage of the protein: Normally, the opening of voltage-connected Ca^{+2} channels provides with the fusion of the vesicle laden with the neurotransmitter as the heterotrimeric SNARE formation approaches the synaptic vesicle towards the presynaptic terminal for neurotransmitter release. In the literature, after toxins bind to the SV, the connection between BoNTs and 3 SNARE proteins is referred to: (i)VAMP is a kind of membrane protein found in synaptic vesicles; (ii) SNAP25(synaptosomal-associated protein of 25 kDa); (iii) cytosolic proteins SNAP25 and syntaxin get cleave by the L-chain metalloprotease of toxins in the following order: (i) BoNT/B, BoNT/D, BoNT/F, and BoNT/G; (ii) /A and BoNT/E; (iii) BoNT/C.[56-58] Table 3 lists them individually and the SNARE protein complex is visualized in Figure 2. As a result, these cleavages inhibit the SNARE complex from forming and/or functioning neurotransmitter release leading to neuroparalysis.

Botulinum group	Inhibited SNARE protein subtype
A	SNAP-25
B	VAMP
C	SNAP-25, Syntaxin
D	VAMP
E	SNAP-25
F	VAMP
G	VAMP
X	VAMP

Table 3: Different kinds of botulinum inhibit particular SNARE protein serotypes, preventing acetylcholine exocytosis.[1,39]

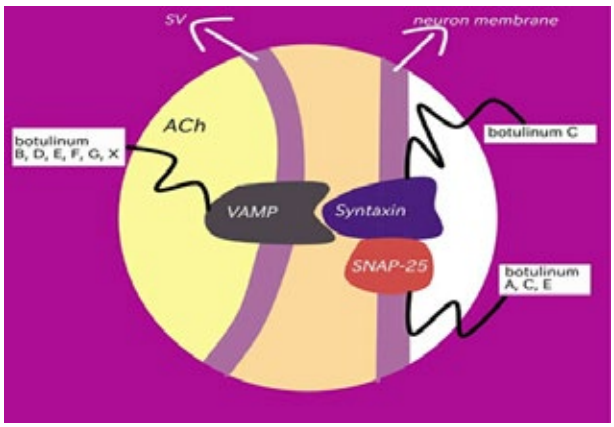

Figure 2: To release acetylcholine (ACh), the SNARE protein complex must be formed. The botulinum toxin that binds to each SNARE protein and blocks acetylcholine release is shown in this diagram. (The figure is adapted from Rossetto et al.[39])

FOODBORNE BOTULISM

Botulism is a type of potential foodborne illness that is actually caused by a toxin produced by bacteria called *C. botulinum*. Toxins are released in food, and eating toxin-containing food can cause conditions. The main symptom is paralysis; usually, the sign of paralysis starts upwards, so the first symptom that is common in people with botulism poisoning usually is dropping of the eyelid and then spreading downwards. It also involves paralysis of breathing muscles. If it spreads to that level, patients often end up in ICU (intensive care unit) setting on ventilators for management of that.[59] The most commonplace that researchers find botulism is in canned goods, where *C. botulinum* bacteria that produces this toxin likes to grow in environments where there's no oxygen. Canning is a process whereby you remove oxygen from the environment, so it likes to describe it in that type of environment. Home-canned or jarred goods are usually the culprit that is found in most outbreaks since there are quite strict standards in place for commercially prepared products that are in place to help protect us from this type of infection.[60] Any cans that appear to be bulging or deformed, especially those that are dented or twisted in any manner, should be avoided since this might indicate a problem with the food within, although sometimes it is unrelated.

Different types of human botulism are identified based on BoNT intake. Foodborne botulism, which is the most prevalent type of botulism in many countries, is caused by ingesting preformed BoNT with infants' vegetative bacteria in food that leads to disease. Foods that have not been acidified, have not been cooked, and have been infected with *C. botulinum* spores are in danger of allowing bacteria to proliferate and produce BoNT during storage.[59,60] The dosed orally BoNT, which is present in contaminated water or food, flows to the intestinal system, penetrates the intestinal barrier, enters the blood and circulation, and spreads to the peripheral nerve system, according to the foodborne botulism framework.[61] BoNT is generated in an advanced molecule (not only known as progenitor toxin) *in vitro* culture or contaminated food by interaction with non-toxic proteins, most especially the non-toxic non-hemagglutinin (NTNHA) and hemagglutinin (HA) proteins.[62,63] This Complex structure protects the BoNT from gastric acid and the proteolytic effect of enzymes in the intestine. NTNHA protects BoNT against gastrointestinal hydrolysis, according to biochemical and functional investigations. Additionally, the structure identifies important BoNT residues that control complex formation in a pH-dependent way.[64] The actual path of penetration of BoNT and/or the whole BoNT group via the intestinal epithelium is yet unknown. Transcytosis includes easy BoNT to pass past intestinal membranes. HAs have also been discovered to attach to intestinal cells, break E-cadherin intercellular connections between enterocytes, and therefore promote the paracellular transit of BoNT complexes. The way HA interacts with E-cadherin depends on the HA serotype and the genetic characteristics. Thus, various BoNT serotypes detect distinct chemicals on neuronal cell surfaces. These findings imply that different botulinum toxin serotypes use different molecular pathways to break through the intestinal barrier of vulnerable animals.[65] Other non-toxic molecules (OrfX1, OrfX2, OrfX3, P47) have not yet been identified as being related to the absorption of BoNT from the gastrointestinal tract (GIT).[66.67] As a result, the transit of BoNT in the circulatory system passing metabolism from the digestive tract appears to be a characteristic of BoNT poisoning.[61] So, the presence of BoNT in bioactive molecules such as serum allows clinical botulism to be confirmed.[68]

DIAGNOSIS AND TREATMENT

Botulism is cured with a combination of treatments, including mechanical breathing and, in severe instances, botulinum antiserum. Adolescent botulism may be treated with equine anti-botulinum sera, whereas infant botulism can be treated with human anti-BoNT/A and anti-BoNT/B immunoglobulins.[69,70] The only particular therapy for botulism is anti-BoNT

antibodies. Anti-BoNT, on the other hand, mostly destroys toxins before they attach to neural cells. Paralysis continues until all toxins are eliminated. According to the World Health Organization (WHO), botulinum toxins are one of the lethal substances known. Symptoms usually occur within 12 to 36 hours after exposure with fatigue weakness and vertigo often followed by blurred vision and difficulty speaking. Early detection of botulism is important for successful treatment. Clinical signs and epidemiological factors are used to identify botulism cases. Laboratory tests, which are necessary for botulism typing, are used to confirm botulism.[71] Food-borne botulism was first verified using experimental animals and BoNT analysis in suspicious food items.[70] BoNT, on the other hand, has been found in serum and blood samples from individuals with systemic botulism. Before 1970, certain papers documented the existence of BoNT in the sera of patients with botulism types B and E, and in severe circumstances, type A botulism. BoNT was found in the sera of 20 human cases of botulism up to 25 days after consumption of contaminated foods in 12 reports from 1905 to 1962.[72] Since 1970, the identification of BoNT in blood samples has been utilized more often to diagnose botulism. Since the quantities of BoNT found in human sera are typically low, a highly sensitive BoNT detection technique is necessary. Almost the majority of the research employed the mouse bioassay, which is the usual technique for detecting a few pg (picogram) of BoNT per ml (milliliter). Antibodies specific to the BoNT type are used to neutralize the BoNT type.[73]

OUTBREAKS OCCUR DUE TO TOXINS AND FOOD TYPE

Since it is not a controlled substance in all countries and the effectiveness of investigating possible outbreaks differs from country to country, the real prevalence of foodborne botulism is likely under-reported.[74] Foodborne botulism outbreaks have occurred to home-prepared meals when recognized security controls have not been adopted. These can be linked to traditional customs or severe economic situations that force people to rely more on home food processing.[2]

Just four outbreaks of type C botulism in humans have been confirmed (eight cases). With the exception of one case of newborn botulism in Japan, where the illness was most likely due to spores from the surroundings because no food contamination was connected to this infant botulism case. All of these cases of human botulism were caused by foodborne botulism. Three fatal instances of botulism were found among the categories C and D human patients.[75] A patient in the United States died as a result of botulism complications. The patient's stomach material included BoNT/C.[76] In France during the Guyana incident, a man who ate sick chickens from his homestead had botulism indications. His egg production farm was found to be infected with botulism type C/D (Both types C and D antisera neutralized BoNT in blood samples were divided into two chickens and one duck).[77]

The far more common foodborne botulism in France is type B botulinum, which is caused by homemade pig meat dishes. The most common causes of toxicity are homemade or small-scale arrangements of raw and salty ham and to a lesser extent pig meat dishes such as "pâté" with little thermal processing.[78] The *C. botulinum* pathogens found in pig products that cause botulism epidemics in people are basically group II *C. botulinum* B4 variants.[79] *C. botulinum* frequency varied by geography. In pig datasets, the prevalence was observed to be 3% in Finland, 24% in Germany, 62% in Sweden, and 80% in Japan.[80-82] Thus, the way pork is prepared and stored has a big impact on the danger of botulism transmitting to humans.

The danger of botulism infection in humans is predicated on the presence of *C. botulinum* spores in meat, milk, and dairy products. During the slaughtering of the animals at the butcher, fecal *C. botulinum* spores might infect the meat. The cow environment is primarily responsible for *C. botulinum* contamination in raw milk. *C. botulinum* is a bacterium that is mostly found in cattle.[83] So, milk and milk products are suitable for toxicity.[84,85] The spores of *C. botulinum* are not inactivated by ordinary pasteurization of milk. Depending on the BoNT type and temperature change, the cohesion of BoNT in milk varies. BoNT/A is inactivated by conventional milk pasteurization (63°C for 30 minutes), while BoNT/B is not.[83] Both BoNT/A and BoNT/B are virtually fully inactivated by the ultra-high temperature procedure (UHT) (72°C, 15 sec).[86] Fortunately, despite the danger, only a few cases of human botulism linked to milk and dairy products have been documented.[85]

INFANT BOTULISM

Though the oldest case (identified retrospectively) is thought to have happened in California as early as 1931, the first diagnosis of baby botulism was made in 1976.[87] Botulism is a problem that attacks babies aged one week to one year, with an average age

of ten weeks. Infant botulism affects up to 95% of children under the age of six months.[88] Long-term constipation and flaccid paralysis are common signs and symptoms. Honey and general oil spills are two forms of spores that have been discovered (e.g. soil, dust). Infection is thought to need between 10 and 100 spores.[21] The intestinal microbiota of infants has not matured, it is more susceptible to all types of infections. While breastfed infants are much more frequently than formula-fed infants to be hospitalized for botulism, some research shows that breastfeeding slows the course of botulism and is thus beneficial.[89-91]

AGGRESSIVE ARMY: GUT MICROBIOTA

Our bodies are home to over 100 trillion microorganisms. These microorganisms are found everywhere and make up microbiomes. Over the course of evolution, microbes have evolved with us and have an important role in the human body, without our microbiome we would not be able to survive. Microorganisms live all over the body but have the highest density in diversity within the gastrointestinal tract. The gut microbiome is vital for digestion, also helps provide vitamins and minerals needed to survive. As well as aiding in digestion, the gut microbiome is thought to play an important role in protecting against disease. An imbalance of microorganisms in the gut is known as microbial dysbiosis.[92] A gut's reduced microbial diversity may make it more susceptible to infection. The microbiome has also been linked to the nervous system and mental health. It is thought that the microbiome is essential to your well-being and there are ways to promote a diverse gut microbiota: eating a varied diet, not using unnecessary antibiotics, and spending more time outdoors are a few ways to promote and maintain a healthy gut.

Gut microbiota (*Lactobacillus* spp. and *Bifidobacterium* spp. are the most common bacteria), the most significant impact on immune system function, resulting in the formation of a robust, well-balanced immune system.[93,94] Due to the gastrointestinal tissues being connected with the biggest and most complicated component of the immune function, the resurrection immune system is essential in human protection versus different infections.[92] The reason for the use of various medications such as antibiotics, the balance of the intestinal flora (microorganisms that normally colonize the body) might alter, increasing the risk of numerous infectious illnesses caused by adaptive pathogens such *Clostridium* spp.[95] Botulism primarily affects many people by infecting infants through honey, and paralysis, vomiting, and difficulty speaking can cause complications such as crying and eating difficulties. Surviving in the GIT is the first role in BoNT infection and foodborne diseases. The poison should next adhere to and enter into the intestinal epithelia, allowing it to travel throughout the body.

In the literature, the use of bacteriocins with probiotic metabolites was examined to strengthen the immune function of the gastrointestinal system against such pathogens.[96] Nisin, a kind of probiotic, is a lanthionine-containing polymer generated by specific *Lactococcus lactis*[97] strains that are commonly utilized as a safe and natural protection in the food industry. Nisin exhibits antibacterial action against a wide spectrum of gram-positive bacteria (e.g. *Listeria monocytogenes* and *C. botulinum*), including numerous foodborne pathogens and bacteria that cause rotting.[98] The protection of nisin varies according to changing ambient conditions (pH, temperature, total amount of spores taken).[99]

In conclusion, *C. botulinum* is an anaerobic, gram-positive, spore-forming type of bacteria that produces a potent neurotoxin. The spores are heat-resistant and can survive in foods that are incorrectly or minimally processed. The severe type of food poisoning is caused by the ingestion of foods containing BoNT during the growth of the organism. The incidence of the disease is low, but it is of considerable concern because of the high mortality rate if not treated immediately and properly. Since the route of contagion is the gastrointestinal system, it is imperative that the gut microbiota be healthy. It takes up to two years, from birth, until a healthy microbe. What is not yet clear is the impact of progression from breastfeeding to supplementary food on changes in gut microbiota protective effect against toxins. Future findings may help us to understand the paradigm that the immune function of the infant's gut flora matures by contact with microbes, but during this interaction even a low dose of botulinum toxin is lethal.

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