https://doi.org/10.17590/20221031-125127

Acute botulism in German dairy herds: human cases of botulism from the consumption of milk and dairy products are very unlikely

BfR Opinion No. 27/2022 issued 20 October 2022

Human botulism is a severe disease caused by toxins called botulinum neurotoxins (BoNTs). They are mainly produced by bacteria of the species *Clostridium* (*C.*) *botulinum* in food and ingested with food. The disease usually leads to specific neurological disorders, e.g. visual disturbances, dry mouth, speech and swallowing disorders, and can be fatal. Animals can also fall ill if they ingest BoNTs with their feed. Among farm animals, cattle are primarily affected. Against this background, the German Federal Institute for Risk Assessment (BfR) has assessed the risk of contracting botulism in Germany from the consumption of milk and dairy products if milk from healthy cows is processed that originates from a farm with acute cases of botulism in the dairy herd.

In the European Union, raw milk must come from animals that are in a good general state of health. They must not show any signs of disease that might result in the contamination of milk. However, on dairy farms with acute cases of botulism, large quantities of *C. botulinum* spores may be excreted in the faeces of healthy as well as sick cattle and get into the tank milk when the healthy cows are milked. However, dilution effects during transport and during processing in the dairy would considerably reduce the spore content. In addition, germination of the spores and the formation of BoNTs is not to be expected in dairy products that are stored refrigerated or frozen (e.g. pasteurised milk, milk ice cream), are highly acidified (sour milk products) or have a low water content (butter, milk powder). That's why cases of botulism from the consumption of these dairy products are very unlikely. Furthermore, the spores of *C. botulinum* can be completely killed by heating to 121 degrees Celsius for at least three minutes or comparable heat treatment processes (e.g. sterilisation, ultra-high temperature). Ultra-high temperature (UHT) processing involves a short-time heating to a temperature of at least 135 degrees Celsius. Therefore, no cases of botulism are to be expected from the consumption of UHT treated milk and dairy products made from it.

So far, no human cases of botulism associated with the consumption of milk and dairy products have been reported in Germany. Individual outbreaks and cases of botulism have occurred worldwide after the consumption of milk and dairy products. However, they were at least partly caused by subsequent contamination and/or faulty storage. Therefore, the BfR assesses the risk of contracting botulism in Germany from the consumption of milk and dairy products as very low. This assessment also applies if milk from healthy cows is processed that originates from a farm with acute cases of botulism in the dairy herd. To protect against botulism, consumers should not store raw milk unrefrigerated, even for the production of dairy products. Furthermore, to protect against food-borne infections, raw farm-gate milk should be boiled before consumption.

BfR risk profile: Consumption of milk from healthy cows from dairy herds with cases of acute botulism and dairy products made from it

A	**Affected persons**	**General population**				
B	**Likelihood of impairment to health from consumption of milk from healthy cows from dairy herds with cases of acute botulism and dairy products made from it**	Very low	Low	Medium	High	Very high
C	**Severity of impairment to health in case of consumption of milk from healthy cows from dairy herds with cases of acute botulism and dairy products made from it**	No impairment	Mild impairment [reversible/irreversible]	Moderate impairment [reversible/irreversible]	Severe impairment [reversible/irreversible]	
D	**Validity of the available data**	High: The most important data are available and are internally consistent	Medium: Some important data are missing or inconsistent	Low: A large volume if important data is missing or inconsistent		
E	**Controllability by the consumer**	Controls not needed	Controllable with precautionary measures	Controllable through avoidance	Not controllable	

Explanations

The risk profile is intended to visualise the risk outlined in the BfR Opinion. The profile is not intended to be used to compare risks. The risk profile should only be read in conjunction with the corresponding Opinion.

FEDERAL INSTITUTE FOR RISK ASSESSMENT (BfR)

Explanations:

Row B - Likelihood of impairment to health

The spores of *C. botulinum* are considerably reduced by dilution effects during transport and during processing in the dairy. In addition, germination of the spores and the formation of BoNTs is very unlikely in dairy products that are stored refrigerated or frozen (e.g. pasteurised milk, milk ice cream), are highly acidified (sour milk products) or have a low water content (butter, milk powder). Furthermore, the spores of *C. botulinum* can be completely killed by a properly performed Ultra-high temperature (UHT) processing.

Row C - Severity of impairment to health

Botulism in humans usually begins with non-specific symptoms like nausea, vomiting and gastrointestinal disturbances. Afterwards, the more specific symptoms appear, such as double vision, pupillary rigidity, speech disorders and later respiratory paralysis and suffocation while fully conscious. Death can occur within 24 hours after the onset of the disease.

Row D - Validity of the available data

In view of the available studies and data, there are uncertainties in estimating the amount of C. *botulinum* spores in bovine faeces and in tank milk from dairy herds with acute cases of botulism and with regard to the multiplication and toxin formation of C. *botulinum* in dairy products. In addition, data on the occurrence of BoNTs must be evaluated cautiously if other methods than the reference method (mouse bioassay) were used for their detection. Nevertheless, there is consensus in the literature that milk and dairy products are very rarely associated with human cases of botulism.

Row E - Controllability by the consumer

To protect against botulism, consumers should not store raw milk unrefrigerated, even for the production of dairy products. Furthermore, to protect against food-borne infections, raw farm-gate milk should be boiled before consumption.

1 Subject of the Assessment

When cases of botulism occur in dairy cows, the question comes up how to deal with the raw milk obtained on the farm from the animals that do not have the disease and the dairy products that are made from it[1]. For this reason, the German Federal Institute for Risk Assessment (BfR) has carried out a risk assessment to answer the question of whether any botulinum neurotoxins (BoNTs) that may be present in the milk are safely inactivated by Ultra-high temperature (UHT) processing.

The Regulation (EC) No 853/2004 lays down health requirements for the production of raw milk and colostrum (Annex III, Section IX, Chapter I)[1]. According to these rules, raw milk and colostrum must come from animals that are in a good general state of health and do not show any symptoms of infectious disease that might result in the contamination of milk and colostrum.

Requirements for UHT processing are set out in Chapter II of Section IX of Annex III to Regulation (EC) No 853/2004, which states that the continuous flow of heat at a high temperature for a short time (not less than 135 °C in combination with a suitable holding time) must ensure that, when kept in an aseptic closed container at ambient temperature, no viable microorganisms or spores capable of growing in the treated product are present.

In addition to *Clostridium (C.) botulinum,* some *C. butyricum* and C. *baratii strains* also possess the ability to form BoNTs. Since the latter are of secondary importance, the BfR limits its assessment to the questionable occurrence of *C. botulinum* and its BoNTs in cattle as well as in cow's milk and dairy products made from it.

The following opinion describes the risk in Germany of contracting botulism from the consumption of milk and dairy products when processing milk from healthy cows that originate from a farm with acute cases of botulism in the dairy herd[2].

2 Results

Botulism in humans is mainly caused by BoNTs A or B, and less frequently by BoNTs E or F. In contrast, botulism in cattle is mainly caused by BoNT C or BoNT D, which have only rarely been associated with human cases of botulism. The symptoms of the disease also depend, among other things, on the amount of BoNTs ingested.

Botulism in cattle can occur when *C. botulinum* cells multiply in the feed, forming BoNT. Settlement and multiplication of *C. botulinum in* the gastrointestinal tract of cattle is very unlikely. However, the spores ingested with the feed are excreted with the faeces and could get into the raw milk via faecal contamination. So far, there is no evidence for a direct secretion of the tox-

1 "Dairy products" means processed products resulting from the processing of raw milk or from the further processing of such processed products (see Annex I to Regulation (EC) No 853/2004).

[2] In this opinion, a dairy herd includes a group of dairy cows including other cattle kept on a holding as an epidemiological unit and, in particular, fed with the same feed.

ins via the udder into the raw milk. Furthermore, since BoNTs are degraded during the passage through the gastrointestinal tract of cattle, it is very unlikely that BoNTs are excreted in the faeces and get into the raw milk through faecal contamination.

A direct entry of *C. botulinum* (vegetative cells and spores) from the udder into the milk can also be ruled out. In a farm with acute cases of botulism in the dairy herd, however, there is the possibility that larger quantities of *C. botulinum* spores are excreted in the faeces of both healthy and sick cattle and get into the tank milk during milking of the healthy cows. This could also include *C. botulinum* spores of types A, B, E or F, which pose a risk to humans. Therefore, it is possible that the tank milk from these farms contains larger amounts of *C. botulinum* spores (estimated levels up to 100 spores/ml) than the milk from farms without acute botulism cases. The estimation of possible spore levels is associated with large uncertainties.

Dilution effects during transport and during processing in the dairy would significantly reduce the spore content. However, due to the high thermostability of the spores, boiling the raw farmgate milk and the usual heat treatment processes for the production of pasteurised milk, sour milk products, butter, milk powder or cheese are not suitable for killing any *C. botulinum* spores present.

The population's risk of disease from BoNTs present in milk depends on the further use of the raw milk and on the types of BoNT. Germination of *C. botulinum* spores and BoNT formation is not to be expected in dairy products that are stored refrigerated or frozen (e.g. pasteurised milk, milk ice cream), are highly acidified (sour milk products) or have a low water content (butter, milk powder). Therefore, cases of botulism from the consumption of these dairy products are very unlikely.

On the other hand, cheese production could include favourable conditions for the germination of spores and the multiplication of *C. botulinum* as well as the formation of BoNTs. In Germany, however, it is to be expected that the anaerobic spore content in cheese-making milk is controlled as part of commercial cheese production or generally reduced by technological processes to avoid cheese defects. Human cases of botulism would therefore be very unlikely. They could only occur and be fatal in extremely rare exceptional situations depending on the toxin formed and the milk product consumed.

Properly performed UHT processing is suitable for complete killing of *C. botulinum* spores. Therefore, no cases of botulism are to be expected from the consumption of UHT treated milk or dairy products made from it. If the spore content in the raw milk is high and UHT processing is not carried out properly and the dairy products are subsequently stored at room temperature for a longer period of time, surviving spores could germinate, multiply and form BoNT. However, aerobic spore formers would also be able to multiply under these conditions and would most likely lead to spoilage of the UHT treated dairy products, so that consumption would be unlikely.

So far, no human cases of botulism associated with the consumption of milk and dairy products have been reported in Germany. Individual outbreaks and cases of botulism have occurred worldwide after the consumption of milk and dairy products. However, they were at least partly caused by subsequent contamination and/or faulty storage. Therefore, the BfR assesses the risk of contracting botulism in Germany from the consumption of milk and dairy products as very low. This assessment also applies if milk from healthy cows from a farm with

acute cases of botulism in the dairy herd is processed. The remaining risk could be further minimised by the following measures:

- Good silage quality
- Good milking hygiene
- Technological reduction and control of anaerobic spore concentration in cheese-making milk
- UHT (Ultra-high temperature) processing of the tank milk from a farm with acute cases of botulism in the dairy herd during the outbreak and for at least one month after its end before further processing.
- No sale of raw milk directly to consumers during the outbreak in the dairy herd and for at least two months after its end.

In addition, the BfR advises to continue the promising work to replace the mouse bioassay as a detection method for BoNTs. This is an important prerequisite for closing existing data gaps through suitable studies.

3 Rationale

3.1.1 Hazard identification

3.1.1.1 *Clostridium* (*C.*) *botulinum* and botulinum neurotoxins (BoNTs)

C. botulinum is a gram-positive, strictly anaerobic growing spore-forming rod bacterium. Its spores can be detected in the soil, in water sediments and in practically all foodstuffs of animal and especially plant origin[2]. It is also found in the gastrointestinal tract of healthy humans and animals.

C. botulinum strains are classified into the metabolic groups I to IV. Of the four metabolic groups, however, only groups I (proteolytic) and II (non-proteolytic) are relevant to food hygiene. Under anaerobic conditions and with sufficient nutrient supply, the bacteria are able to form BoNTs during multiplication. The classical toxins are designated with the letters A to G. *C. botulinum* strains can be named according to the type of toxin they produce (e.g. *C. botulinum* type A for a *C. botulinum* strain that produces the type A toxin).

3.1.1.1.1 Vegetative cells

Group I proteolytic strains multiply at temperatures between 10 °C and 42 °C and under strictly anaerobic conditions starting at a pH of 4.6. Group II non-proteolytic strains' growth is only suppressed below 3.3 °C.[2]. Group III strains mainly occur in botulism of waterfowl and domestic poultry (type C) as well as in other (domestic) mammals (types C and D).[3,4].

The vegetative cells have little resistance to environmental influences and do not survive the presence of oxygen and/or higher temperatures of 50-60 °C[5].
In practice, this is only of minor importance because foodstuffs are predominantly contaminated with heat-resistant spores[6].

3.1.1.1.2 Spores

The spores of *C. botulinum* are very heat-resistant and are only safely killed at temperatures above 100 °C. As a preventive measure, canned food is sufficiently heated during production (so-called “botulinum cook”, 121 °C for 3 minutes). Spores of group I strains are clearly more heat-resistant than those of group II strains. Information on D[3] - and z-values[4] can be found in reviews by Brown[7] and van Asselt et al. as well as in publications by Stone et al. and Diao et al.[8-10]. In the case of non or insufficiently heat-treated foodstuffs, maintaining the cold chain is of particular importance, since the germination of spores can be delayed or prevented by cold storage.[11].

Classic examples of foodstuffs whose consumption led to botulism are canned vegetables or preserved meat produced in private households without sufficient heating. Under strictly an-aerobic conditions, surviving spores can then germinate to cells capable of multiplication, which form BoNTs.

3.1.1.1.3 BoNT

For some years, a further toxin type (H) has been discussed, beyond the classical toxin types (type A-G). For this type, the name BoNT FA is proposed in the literature, since it contains genetic regions with similarity to both BoNT A1 and BoNT F5 and antitoxin against type A leads to neutralisation. In the opinion of some authors, it should therefore not be regarded as an independent toxin type.[12,13].

Sequence differences at the nucleotide and amino acid level within the serotypes A, B, E and F led to a further subdivision of these serotypes into subtypes. Considering the mosaic forms of serotypes C and D, more than 30 subtypes or genetic variants are known[14]. *C. botulinum* is distributed worldwide, whereby toxin formers of different types can occur in different geographical regions. Toxin type E is usually associated with an aquatic environment[15].

The toxins formed are relatively sensitive to heat[16,17]. It can be assumed that BoNTs are inactivated by heating at 80 °C for 20 min or at 85 °C for 5 min.[18].

3.1.2 Hazard characterisation

3.1.2.1 Human botulism

C. botulinum forms neurotoxins whose toxicity is very high. Poisoning caused by these toxins is called botulism. Botulism in humans is mainly caused by BoNT A or B, more rarely by BoNT E or F. BoNT C and BoNT D are often associated with botulism in cattle and poultry. Human diseases with BoNT C or D are only rarely described.

In a review, Rasetti-Escagueil et al. [19] report that publications on type C botulism in humans are available for only eight outbreaks with a total of 15 cases of disease, whereby sometimes

[3] The D-value is the time required to reduce a microorganism population to 10 % at a given temperature.
[4] The z-value is the temperature increase required to reduce the killing time to 1/10.

only one case of disease occurred. Type C botulism was confirmed in only four of these outbreaks (8 cases). The foodstuffs causing or suspected of causing the disease were meat products (paté, poultry products) or could not be identified. One case was infant botulism, which was suspected to have been introduced from the environment. Human cases of disease caused by BoNT D were only reported in one outbreak. Eight people fell ill after eating salted ham produced in private households. Only two of those who fell ill showed symptoms typical of botulism. The other persons showed rather unspecific symptoms, so that it was suspected that they might have fallen ill due to other pathogens. *C. botulinum* type D could be isolated in the ham consumed. The author notes that most of these outbreaks occurred before 1970 and the typing of *C. botulinum* was not well defined at that time[19]. Despite its toxicity, BoNT G has only been associated with human botulism in one report. This report states that in Switzerland, five people whose causes of death were unclear were retroactively analysed and *C. botulinum* type G was detected in all, and BoNT G in three people[20,21].

BoNTs are ingested by humans through the consumption of contaminated food; there is no human-to-human transmission. After a latency period of 12 hours to a few days, the clinical symptoms begin non-specifically with nausea, vomiting and gastrointestinal disturbances. Then, symptoms more specific to human botulism appear, such as double vision, pupillary rigidity, speech disorders and later respiratory paralysis and suffocation with full consciousness. Death can occur within 24 hours after the onset of the disease[15]. BoNTs are neurotoxins that prevent the release of the bound neurotransmitter acetylcholine in the peripheral nervous system and cause paralysis. BoNT A has the highest toxicity in humans. It is the most toxic substance known. The mean lethal dose (LD50) of the toxin is estimated to be 1 µg per kg body weight when ingested orally by humans[15].

In addition, cases of infant botulism can occur in infants up to 12 months of age. In these cases, spores of *C. botulinum* ingested with food germinate in the intestine, multiply due to the still insufficiently developed intestinal flora and form toxins. The main source of infection is honey[2]. In recent years, individual cases of infant botulism have also been reported in Germany[22-33].

Another form of disease is wound botulism. This can occur when *C. botulinum* enters the body via wounds and forms toxins under anaerobic conditions, which then enter the bloodstream. Occasionally, such forms of disease occur in drug-addicted people when the bacteria are transmitted through contaminated needles[33].

In Germany, between 2009 and 2020 a total of 72 cases of human botulism were reported to the Robert Koch Institute (RKI). Of these, 51 diseases were assessed as foodborne. This corresponds to up to nine cases per year[22-33]. At the European level, the annual zoonosis reports give an overview of the occurrence of food-borne diseases and outbreaks[34-40]. For the years 2011 to 2020, a total of 116 outbreaks of disease caused by *C. botulinum* or its toxins were reported to the European Food Safety Authority (EFSA), 74 of which had a strong evidence. In these outbreaks, at least 308 people became ill, at least 242 people were hospitalised and at least six people died. No human botulism outbreaks due to consumption of milk or dairy products were reported to EFSA in the mentioned period.

Only a few publications are available on human botulism outbreaks or individual cases caused by milk or dairy products. A review by Lindström et al.[20] lists a total of 20 outbreaks worldwide, half of which originated in the USA. *C. botulinum* type A was identified in ten outbreaks

and type B in seven outbreaks; no toxin type was specified in three outbreaks. Only in two outbreaks milk is reported as the vehicle. Little is known about these milk outbreaks that occurred in the US in 1920 and 1931, so that contamination of the milk after opening the packaging could also be the cause.

In 1996, an outbreak occurred in Italy after the consumption of mascarpone and products made with mascarpone. Both *C. botulinum* type A and BoNT A were detected in the mascarpone[41,42]. Since all environmental tests at the production site were *C. botulinum* and BoNT negative[41], an entry of *C. botulinum* via the milk or cream used for production was suspected[42]. Furthermore, it was assumed that severe temperature abuse during the production and storage of the cheese could have led to a multiplication of the introduced *C. botulinum* cells/spores with a subsequent toxin formation[20]. From the knowledge of the small number of studies, Lindström et al.[20] conclude, that consumption of dairy products seems to have a risk of botulism, though it seems to be relatively low.

Since 2000, a total of only three milk-associated botulism outbreaks have been reported from the United Kingdom (infant formula), Turkey (home-made yoghurt) and Australia (nachos with cheese)[20].

3.1.2.2 Bovine botulism

Ruminants are susceptible to botulism. BoNTs C and D play the most important role here, and less frequently BoNTs A or B. Cattle react more sensitively to BoNTs than small ruminants (sheep and goats). As botulism in animals is neither a notifiable animal disease nor another reportable animal disease, there are no statistics on its frequency for Germany or Europe. However, botulism outbreaks are usually rare incidences with great significance for individual herds. Botulism is not transmitted from animal to animal. The animals in a herd rather become acutely poisoned within a short period of time by ingesting contaminated feed[5]. Botulism in cattle can occur especially when *C. botulinum* cells multiply in insufficiently fermented silage and form BoNTs.[43].

Outbreaks caused by BoNT B have also been caused by feed other than silage, such as contaminated brewer's grain[44]. In the case of bovine botulism caused by BoNTs C or D, associations with faeces of birds have also been described[4,20,45,46].

In most cases, the symptoms of the disease in cattle manifest themselves in increasing difficulties in taking in feed and water, which are due to paralysis of the tongue, and chewing and pharyngeal muscles. The progressing muscle paralysis can lead to recumbency of the animals and finally to death by respiratory paralysis[47]. In cattle, the mortality rate is between 90 and 95 %[5],which can lead to massive economic losses in the herds. In very rare cases, milder courses occur in which the animals begin to recover after a few days. However, these animals reportedly never returned to full productivity after surviving the disease[48].

In the past, speculations about a new form of botulism ("visceral" or "chronic botulism") were made. The main feature of this supposed toxicoinfection would be a series of unspecific symptoms that are accompanied by a loss of performance. A dysbiosis (= imbalance of the bacterial intestinal flora), which would allow *C. botulinum* to multiply and form toxins in the intestines of affected cows, was supposed to be the cause[49]. Scientifically, however, a connection between the unspecific disease process and *C. botulinum* could never be confirmed. The theory of "chronic botulism" could also not be confirmed in large-scale studies by the University of

Veterinary Medicine Hanover and the Friedrich-Löffler-Institute (FLI). It can be assumed that the clinical picture described is rather a multifactorial disease[50,51].

3.1.3 Exposure assessment and evaluation

For each agent identified as a hazard, the exposure assessment considers its initial concentration in the raw product and any processes that lead to the agent's proliferation or inactivation in the course of food production.

All agents considered in the following exposure assessment (BoNTs, *C. botulinum* cells/spores) can get into raw milk in different ways: Either directly from within the cow's udder during milking or indirectly through entry from the environment (e.g. when milking externally soiled udders).

3.1.3.1 Direct entry of the agents from the udder into the raw milk

To date, there are only two studies that indicate a presence of *C. botulinum* and its BoNTs in milk and/or udders of cows. In 2002, Böhnel et al.[52] detected BoNT B in the udder of a sick cow. However, the animal had fallen sick to a different type of toxin (BoNT C/D) and BoNT B was only found in one single udder quarter that was altered by mastitis. It is therefore unlikely that the BoNT B was secreted into the milk by the diseased animal. It is more likely that this was an external contamination of the udder quarter, or that there was a methodological error. In a second study, Böhnel and Gessler[53] reported the detection of *C. botulinum* and BoNTs (human and animal-pathogenic) in milk and udder samples. This study shows methodological deficiencies: Information on the collection, storage and treatment of the samples is incomplete and information on negative controls is missing entirely. In addition, contrary to the general requirements for performing sound assays[54-57], apparently only one mouse bioassay was performed per sample. In the manuscript, the authors themselves declare their study as unsuitable for the derivation of a risk assessment. In addition, they state that the origin of *C. botulinum* and the BoNTs in the milk and udder samples remains unknown, as they did not collect the disease status of the animals or other relevant data from the samples taken. Therefore, so far there is no tenable evidence that *C. botulinum* or BoNTs are secreted directly into the milk from diseased cows[58,59].

3.1.3.2 Indirect entry of the agents into the raw milk

Since *C. botulinum* spores, vegetative cells or BoNTs can be ingested via feed and enter the digestive tract of cows, the question arises whether these agents could also get into the environment via the faeces of the animals and thus get into the raw milk during the milking process. In order to estimate the quantities of agents that could get into the raw milk via such routes, data on the occurrence of *C. botulinum* and its BoNTs in cattle faeces are relevant.

3.1.3.2.1 Occurrence of the agents in intestinal contents and bovine faeces

In a case study from the Netherlands, in which several dairy farms were affected by botulism caused by BoNT B[44], large quantities of *C. botulinum* type B spores were detected in the cattle's faeces (10^5 -10^7 per gram). Even eight weeks after the outbreak, *C. botulinum* type B spores were still detected in the faeces of healthy cows from these farms, albeit in smaller quantities.

In a Swedish study, faecal samples collected from healthy cattle in slaughterhouses were analysed for the presence of *C. botulinum* types B, E and F[60]. A high detection rate of 73.33 % (44/60) was found for spores of *C. botulinum* type B, with most samples having a low content of less than four spores per gram of faeces. Spores of the other considered *C. botulinum* types E and F could not be detected.

An Egyptian study examined the faeces of healthy cattle, buffaloes, sheep and goats for human-pathogenic *C. botulinum* types[61]. They found *C. botulinum* type A at a very low frequency of 2-5.8% in the faeces samples of all animal species (cattle and buffalo 1/50 each, goats 1/51, sheep 3/52). However, due to the different feeding, husbandry and environmental conditions of dairy ruminants in Egypt, the significance of this study for dairy cattle in Germany is low.

Schmid et al.[62] were unable to detect *C. botulinum* (types A-F) in 292 faecal samples from healthy cattle in Germany.

In a large-scale study by the FLI[51,63] in which 1,388 faecal samples from cattle in Germany were examined, BoNT genes were detected in 7.9 % (109/1,388) of the samples. These genes belonged to the human-pathogenic toxin types A (86/109), B (8/109), E (1/109) and F (5/109) and the animal-pathogenic toxin type D (9/109). All faecal samples were additionally examined for preformed BoNTs, but these were not found in any sample. Investigations have shown that the bacteria in the rumen of cattle and sheep are capable of degrading BoNTs[64] which would explain their absence despite the detection of BoNT genes.

A study from Great Britain examined, among other things, the intestinal contents of cattle suffering from botulism, and *C. botulinum* type D or BoNT D was detectable in 50 % (37/74) of the examined outbreaks[65]. Direct detection of BoNT D was successful in 24 of these outbreaks, with an additional BoNT C being detected in one of the outbreaks. In the remaining outbreaks, *C. botulinum* type D was detected.

Souillard et al.[66] examined the faeces of healthy cattle from France and were able to detect *C. botulinum* type C/D in 4.7 % (3/64) of the samples.

In 1989, Klarmann detected[67] *C. botulinum* in one of 25 faecal samples from cattle but was not able to determine a toxin type.

3.1.3.2.2 Occurrence of the agents in slurry samples

In the context of investigations on the safety of biogas production, Neuhaus et al. examined 24 slurry samples from dairy farms in Germany for the presence of *C. botulinum* types A, B, C, D and E and the associated toxins[68]. They used an ELISA (Enzyme-linked Immunosorbent Assay) to detect the toxins and detected BoNT A or E in one sample each and BoNT D in two samples. In addition, spores of *C. botulinum* type D were detected in two samples and *C. botulinum* type E in three samples. The relevance of this study for the assessment of the contamination risk of raw milk is questionable. Slurry consists of urine and faeces from farm animals and is stored in large slurry silos until further use. Furthermore, feed residues and/or bedding can get into the slurry. Since *C. botulinum* is ubiquitous, entry into the slurry would therefore not only be possible from cattle faeces, but also via the environment. In addition, the time of sampling was not sufficiently specified in the publication. It is therefore conceivable that BoNTs were also first formed during storage in the slurry silo.

3.1.3.3 Influence of milk processing

So far, there is no evidence that BoNTs enter raw milk directly during milking, not even in farms affected by botulism cases. However, an indirect entry of *C. botulinum* spores - especially via faeces - is possible. Estimates of the entry of *C. botulinum* spores into raw milk show that in healthy dairy herds, i.e. herds with low *C. botulinum* spore levels in the faeces, only very small amounts of *C. botulinum* get into raw milk, i.e. less than one spore/cell per litre of milk[69]. However, during an outbreak of botulism in the dairy herd, it is possible that also larger quantities of *C. botulinum* spores could get into the raw milk tank of the affected farm via indirect entry through externally soiled udders during the milking process.

There are only a few studies on the occurrence of *C. botulinum* or its BoNTs in commercial milk. An Italian study[42] detected neither *C. botulinum* nor its toxins in 35 raw milk and 13 pasteurised milk samples. Similarly, Kautter et al.[70] were unable to detect *C. botulinum* or BoNTs in 90 whole milk samples.

For the following exposure estimation in the "raw milk processing" phase, it is therefore primarily relevant whether any *C. botulinum* spores that may be present in small numbers could multiply and form BoNTs during processing.

3.1.3.3.1 Milk collection, storage and transport

In 2020, there were more than three million dairy cows in Germany, producing about 33 million tons of raw milk. The average annual milk yield was 8,457 litres of milk per cow[71]. Dairy cows in Germany produce an average of about 28 litres of milk per day (with peak yields of up to 60 litres per day, depending on the stage of lactation) over a period of about 10 months (about 305 days)[72].

On the farm, the milk is cooled and stored in farm milk tanks as quickly as possible immediately after milking, when it has a temperature of approx. 37 °C. During intermediate storage in the farm milk tank, an initial dilution of any *C. botulinum* spores and/or cells present takes place already, whereby the extent of the dilution depends on the number of cows milked and the size of the farm milk tank. The size of the farm milk tanks ranges from about 300 to 30,000 litres. According to Regulation (EC) No 853/2004, the raw milk must be cooled immediately on the farm to a temperature of max. 8 °C in the case of daily collection or to max. 6 °C in the case of non-daily collection. However, operators' specifications often target a cooling to a maximum of 4 °C within two hours.

The milk is collected daily or at the latest on the day after milking with special milk tankers and transported to the milk collection site or to the processing plant or dairy. The volumes of the milk tankers are about 20,000 to 26,000 litres of milk. During transport, the cold chain must be maintained so that the milk has a maximum temperature of 10 °C when it arrives at the plant of destination. The temperature requirements are intended to ensure that microorganisms do not multiply during the storage and transport of raw milk.

In the dairy, the raw milk is immediately cooled to 4 °C (legal requirement according to Regulation (EC) No 853/2004: max. 6 °C) and stored in silo tanks with a capacity of approx. 100,000 to 500,000 litres at 4 °C (max. 6 °C) until processing.

As a result, the content of the agents (in this case *C. botulinum* spores) introduced into the raw milk is considerably reduced by dilution effects, first in the farm milk tank, then during transport in the milk tankers and finally in the intermediate storage in the dairy. In addition, cooling the raw milk inhibits the germination of the spores and the multiplication and toxin formation of *C. botulinum.*

However, cooling does not lead to the death of *C. botulinum,* nor would it lead to significant inactivation of existing BoNTs[73]. Since the introduction of BoNTs into raw milk can be practically ruled out, this is irrelevant for the possible exposure of humans.

3.1.3.3.2 Heat treatment

Heat treatment processes are used to kill any pathogens that may be present in raw milk and to extend the shelf life of milk. Common processes in dairies are pasteurisation and UHT processing. In pasteurisation, the raw milk is heated to 60-62 °C for about 30 minutes (continuous heating, hardly ever used today) or to 70-72 °C for 15-20 seconds (s) (short-term heating). Any other time-temperature combination with the same effect is legally possible. UHT processing involves heating to a temperature of at least 135 °C for a short time. Another common heat treatment is high pasteurisation, which is used to extend the shelf life of so-called Extended Shelf Life (ESL) milk. This involves heating to 125-138 °C for 2-4 s followed by cooling at max. 7 °C.

Due to the process temperatures, multiplication of *C. botulinum* in the milk during these process steps can be excluded. Instead it can be assumed that any vegetative *C. botulinum* cells present are completely destroyed during both pasteurisation and UHT processing.

Any BoNTs contained are also significantly inactivated by the processes mentioned. A study carried out by Schneider[73] showed that both continuous heating (62 °C for 30 min) and short-term heating of milk (here 72 °C for 20 s) already lead to a significant decrease in toxin levels. However, it was also observed that small amounts of toxin remained detectable after treatment at 72 °C for 2 min. According to the results of a study by Weingart et al.[74] at least 99.99 % BoNT A and 99.5 % BoNT B were inactivated by the common pasteurisation conditions of 72 °C for 15 s.

Nevertheless, this study also showed a pronounced biphasic course of inactivation depending on the process duration. In a study by Rasooly et al.[75] BoNT A could be inactivated by milk pasteurisation at 63 °C for 30 min, but BoNT B could not.

Neither the pasteurisation processes nor the brief boiling common for farm-gate milk are sufficient to kill *C. botulinum* spores (see D- values [7-10] and publication by Deeth and Lewis[76]). Contrary to this, it can be assumed that any *C. botulinum* spores present are completely killed during UHT processing (at least 12D reduction)[76,77].

In summary, it can be stated that pasteurisation, high pasteurisation and brief boiling of raw milk reliably kill vegetative *C. botulinum* cells. However, *C. botulinum* spores can only be reliably killed by UHT processing. All four heat treatment processes mentioned would also be suitable for inactivating existing BoNTs.

3.1.3.3.3 Manufacture of dairy products

In their review, Lindström et al.[20] assessed the possible entry of *C. botulinum* and its survival, multiplication and toxin formation during the production and storage of dairy products. According to their assessment, *C. botulinum* multiplication and BoNT formation are generally not to be expected in dairy products that are stored refrigerated or frozen (e.g. pasteurised milk, milk ice cream), highly acidified (sour milk products) or have a low water content (butter, milk powder). However, cheese production could lead to favourable conditions for the multiplication of *C. botulinum* and the formation of BoNTs.

Nevertheless, in Germany, it can be assumed that the spore content in raw milk is generally controlled and/or reduced by technological processes (e.g. bactofugation, microfiltration) before commercial cheese production. Alternatively, only milk from farms that do not feed silage is used for cheese-making.

This is because, especially with silage feeding, a contamination of the raw milk with so-called cheese-damaging clostridia must be expected. As few as 50 clostridia spores per litre of milk can lead to cheese spoilage through faulty fermentation and other cheese-making problems[78,79].

Since the spores have a higher specific density than skimmed milk and cream, they can be removed from the raw milk using centrifugal force during bactofugation[80]. Manufacturers of so-called bacterial removing separators advertise that the spore content in raw milk can be reduced by up to two log levels with these devices.

3.1.4 Risk characterisation

According to Regulation (EC) No 853/2004, raw milk must come from animals that are in a good general state of health and do not show any symptoms of infectious disease that might result in the contamination of milk. Due to the typical symptoms, it seems very unlikely that cows suffering from acute botulism are not recognised and their milk is used for food production.

Based on the data and information presented in the previous sections, it is also very unlikely that BoNTs get into raw milk through direct secretion or faecal contamination. Furthermore, direct entry of *C. botulinum* (vegetative cells and spores) from the udder into the milk can be excluded. However, also healthy cattle can excrete small quantities of *C. botulinum* spores with the faeces, so that it is basically possible that during milking spores get into the milk via faecal contamination.

It is to be expected that on a farm with acute cases of botulism in the dairy herd, the feed not only contains BoNTs but also larger amounts of *C. botulinum* spores. This also increases the probability of *C. botulinum* spores getting into the raw milk.

If acute cases of botulism occur in dairy cows, there is a very high probability that they are caused by BoNT C or BoNT D. So far however, these toxins have been of almost no importance in human botulism, so that the risk of becoming ill from *C. botulinum* type C or D spores is very low. However, it cannot be ruled out that cows also ingest *C. botulinum* spores of types A, B, E or F with their feed and excrete them with their faeces without showing symptoms of disease and thus having their milk enter the food chain.

Therefore, the consumer risk is characterized below on the basis of three scenarios.

Scenario 1: A farm where acute cases of botulism have occurred in the dairy herd, sells raw milk of healthy cows directly to consumers.

It is possible that the tank milk from this farm contains larger amounts of *C. botulinum* spores (estimated levels up to 100 spores/ml), which would not be killed even by boiling the milk. Health impairments due to the ingestion of the spores are not to be expected for the normal, healthy population. In contrast, the spores could possibly settle in the intestines of infants and people with severely damaged intestinal flora and form BoNT there.

Under certain circumstances, e.g. if large quantities of raw milk are kept unrefrigerated for a long time during further processing, it would also be possible for *C. botulinum* to multiply and to form BoNT in the milk and dairy products made from it.

Therefore, human cases of botulism from the consumption of raw milk from this farm and dairy products made from it are very unlikely. However, individual cases of botulism could theoretically occur and be fatal, depending on the toxin formed and the milk product consumed.

Scenario 2: Raw milk from dairy herds where cases of acute botulism have occurred enters the food chain without further treatment.

It is possible that the tank milk from these herds contains larger amounts of *C. botulinum* spores (estimated levels up to 100 spores/ml). Dilution effects during transport and storage in the dairy would significantly reduce the spore content. However, the common heat treatment processes in the production of pasteurised milk, sour milk products, butter, milk powder or cheese would not be sufficient to kill the *C. botulinum* spores.

Health impairments due to the ingestion of the spores are not to be expected for the normal, healthy population. The health risk for the population depends on the possibility of BoNT formation in the context of further use of the raw milk and on the type of BoNT. A multiplication of *C. botulinum* and BoNT formation are not to be expected in dairy products that are stored refrigerated or frozen (e.g. pasteurised milk, milk ice cream), are highly acidified (sour milk products) or have a low water content (butter, milk powder). Therefore, cases of botulism from the consumption of these dairy products would be very unlikely.

On the other hand, cheese production could lead to conditions that favour *C. botulinum* multiplication and BoNT formation. However, it is to be expected that the anaerobic spore content in the cheese milk is controlled in the context of commercial cheese production or generally reduced by technological processes to avoid cheese-making problems.

Human cases of botulism are therefore very unlikely. They could only occur in extremely rare exceptional situations depending on the toxin formed and the milk product consumed.

Scenario 3: Raw milk from dairy herds where cases of acute botulism have occurred is ultra-high-heated before consumption.

> UHT processing kills *C. botulinum* spores present in the milk. Therefore, no cases of botulism are to be expected from the consumption of UHT treated milk or dairy products made from it.
>
> Theoretically, human cases of botulism can only occur if the temperature-time combinations required for UHT processing are not reached and the raw milk has a high spore content. Due to the killing of vegetative bacteria in the milk and the usually longer-term unrefrigerated storage of UHT treated dairy products, the multiplication of *C. botulinum* and formation of BoNT could be favoured. However, falling below the necessary temperature-time combinations would, due to the simultaneous multiplication of aerobic spore formers, most likely lead to spoilage of the UHT treated dairy products, so that consumption is unlikely.

3.1.4.1 Assessment of the quality of the data

The greatest uncertainties exist with regard to the test methods used for BoNT detection. To date, the gold standard is a mouse bioassay, which, for ethical reasons and in view of the effort involved in keeping laboratory animals, can only be carried out by a few laboratories in individual cases. Immunological methods, such as ELISA, can often only detect larger amounts and not all relevant BoNT subtypes. This leads to the fact that the figures on the occurrence of BoNTs given in the literature should be evaluated cautiously, unless mouse bioassays were used according to the international recommendations for BoNT detection. The possibility of false-negative results in BoNT detection can have a major impact on the risk assessment of botulism from milk and dairy products. However, only a few of the studies assessed in this opinion actually attempted to detect BoNTs, so uncertainties due to unreliable detection are of little relevance. In addition, any pre-formed BoNTs present and not detected in raw milk would undergo severe dilution between the producer and the dairy and would be sufficiently inactivated during pasteurisation.

Available studies show that no BoNTs but spores of *C. botulinum* can be found in the faeces of cattle. There is great uncertainty in estimating the amount of *C. botulinum* spores in cattle faeces and in tank milk from dairy herds with cases of acute botulism.

Furthermore, there are uncertainties regarding the possibility of *C. botulinum* multiplication and toxin formation in dairy products and thus their significance as a trigger of human botulism. Nevertheless, there is consensus in the literature that milk and dairy products are very rarely associated with cases of human botulism.

There is very little uncertainty about the characteristics and pathogenesis of *C. botulinum* or BoNTs, the influence of UHT processing on the survival of *C. botulinum* spores, and the consumption of the foods considered.

3.1.4.2 Research needs

The uncertainties identified and assessed have only a minor influence on the result of the present risk assessment. Therefore, no urgent need for research is derived.

However, the BfR advises to continue the promising work to replace the mouse bioassay as a detection method for BoNTs. This would be an important prerequisite for closing existing data gaps through suitable studies.

3.2 Risk management options, recommended measures

So far, no human cases of botulism associated with the consumption of milk and dairy products have been reported in Germany. Individual outbreaks and individual cases of botulism have occurred worldwide after the consumption of milk and dairy products. However, they were at least partly caused by subsequent contamination and/or faulty storage. Therefore, the risk of contracting botulism in Germany from the consumption of milk and dairy products is assessed as very low. This assessment also applies if milk from healthy cows from a farm with acute cases of botulism in the dairy herd is processed.

The remaining risk could be further minimised by the following measures:

- Good silage quality
- Good milking hygiene
- Technological reduction and control of anaerobic spore concentration in cheese-making milk
- UHT processing of the tank milk from a farm with acute cases of botulism in the dairy herd during the outbreak and for at least one month after its end before further processing
- No sale of raw milk directly to consumers during the outbreak in the dairy herd and for at least two months after its end.

Further information on the BfR website on botulism:

Questions and answers on botulism: https://www.bfr.bund.de/cm/349/questions-and-answers-on-botulism.pdf

Leaflet "Protection against botulism from food (in German): https://www.bfr.bund.de/cm/350/hinweise_fuer_verbraucher_zum_botulismus_durch_lebensmittel.pdf

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