Master's Degree in Medical, Veterinary or Forensic Biotechnological Sciences

University of Perugia
Department of Medicine

BOTULISM

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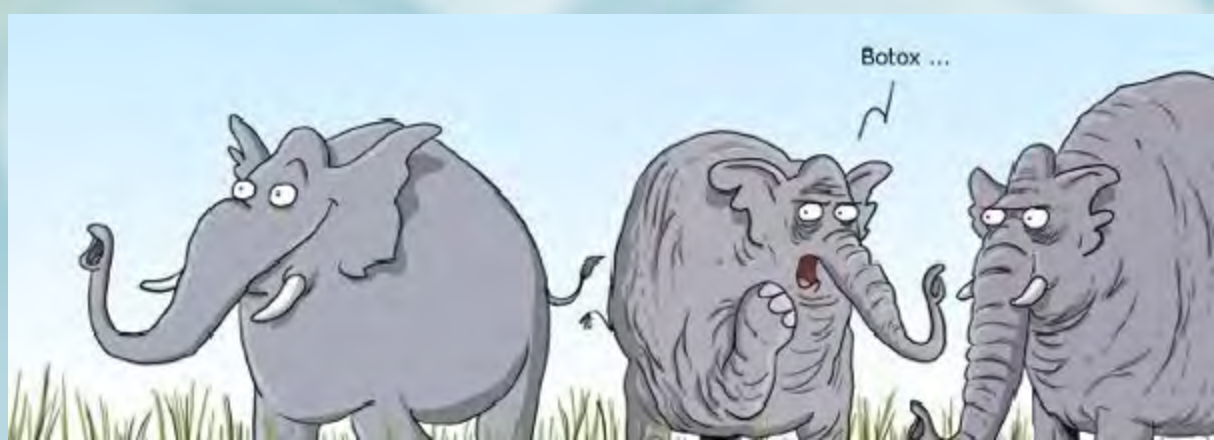
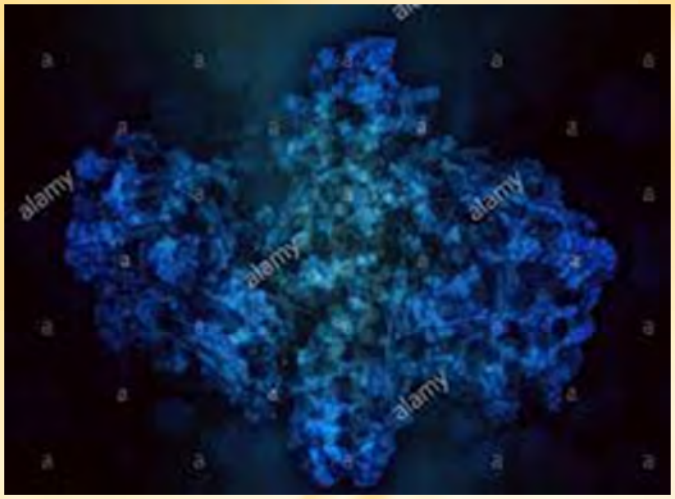
Clostridium botulinum ubliquitary bacterium, obligatory anaerobium, sporogenous, mobile, Gram-positive, catalase negative develops in environments with a very low percentage of oxygen where it germinates producing toxins that are dangerous for human and animals.





Features

Seven antigenic variants of neurotoxins have been identified with different characteristics each capable of processing their own specific toxin or, in some rare cases, they are able to generate two toxins simultaneously, usually one in greater quantity than the other.

For convenience C. botulinum is also divided into four groups according to the metabolic and physiological characteristics and are called proteolytic when they derive the energy necessary for their metabolism from the disintegration of proteins and non-proteolytic when for their metabolism they mainly use sugars:

Group I Proteolytic strain responsible for human botulism the neurotoxins produced are A, B and F

Group II Non-proteolytic strain responsible for human botulism the neurotoxins produced are B, E, and F

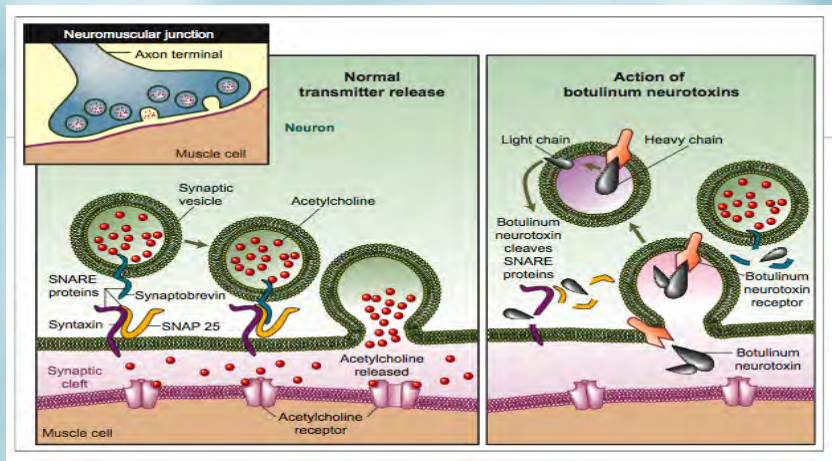
Group III Non-proteolytic strain responsible for animal botulism the neurotoxins produced are D and C and the latter are able to produce simultaneously two different types of neurotoxins defined as C2 and C3 with pathogenic characteristics not yet clarified.

Group IV Strain that produces the neurotoxin G are distinguished from other groups because they do not produce lipases and are completely asaccharolytic.

M.O.A

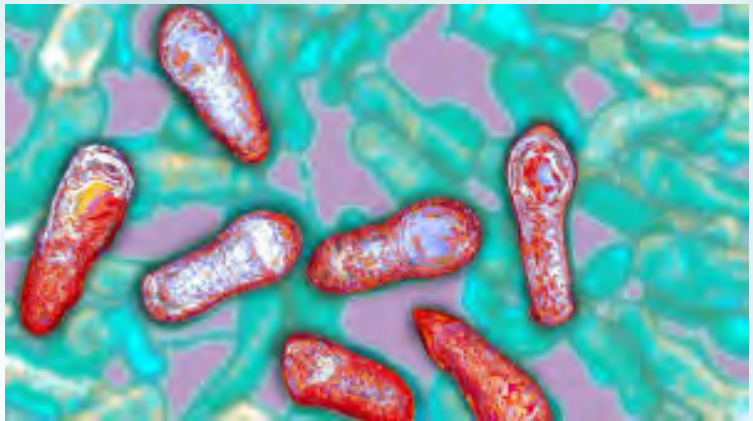
The mechanism of action of botulinum toxin consists in the irreversible pre-synaptic block of the peripheral cholinergic transmission both a
level of the neuromuscular junction both at the sympathetic and parasympathetic levels.

The heavy toxin chain is important for penetration into the axons, a condition to which paralysis is linked. The toxin can then penetrate into neurons way endocytosis. The binding of the heavy chain occurs with the SV2 protein receptor (synaptic vescicle protein 2). The light chain is able to leave the endocytotic vesicles and reach the cytoplasm. The light toxin chain possesses protease activity that proteolytically degrades the SNAP-25 protein, a type of SNARE protein. The SNAP-25 protein is necessary for the release of neurotransmitters from terminal axons.



DESTRUCTION OF SPORES IN FOODS

- Only a few micrograms of the toxin can cause illness in a healthy adult;
- Mortality is high;
- Without the antitoxin and respiratory support, death is likely;
- There are a number of strategies for the control of pathogens;
- Clostridial spores remain viable for long periods of time even when environmental conditions are absolutely unfavourable to their development.



1. Controlling the level of acidity (pH) in the product

2. Controlling the amount of salt or preservatives, such as sodium nitrite

3. Controlling the amount of moisture that is available for pathogenic bacteria growth(water activity)

4. Controlling the introduction of pathogenic bacteria: pasteurization process and after the cooking process performed

5. Managing the amount of time that food is exposed to temperatures that are favorable

6. Use of gamma and x rays

CHLORINE COMPOUNDS

- Among the most used antimicrobials in the food industry
- Calcium hypochlorite, gaseous chlorine, sodium hypochlorite
- antibacterial activity is due to their strong tendency to oxidize, in fact if these are reduced they lose antimicrobial activity
- they destroy the spores by altering the permeability of external structures and eliminating some proteins
- some spores can resist this treatment but it is essential to sensitize them to subsequent treatments such as the use of other chemical treatments or heat treatment.

The resistance of C. botulinum spores to the action of free available chlorine may change depending on strains

TEMPERATURE

- Have to be treated at 121 °C for 3 minutes
- spores are reported to be destroyed at 125 °C
- certain substances in foods (divalent cations, anions of organic acids) may protect toxins from heating consequences
- The vegetative cells of all types of C. botulinum are easily killed by heat. However, C. botulinum is able to produce spores. In this state, the pathogen is very resistant to heat.
- The spores of the proteolytic group are much more resistant

dehydration

mineralization

Thermal adaption

The minim[illegible]wth and toxin formation by C. botulinum type E and nonproteolytic types B and F is 38°F (3.3°C).

For type A and proteolytic types B and F, the minimum temperature for growth is 50°F (10°C).

ALTERNATIVE AND CHEMICAL METHODS

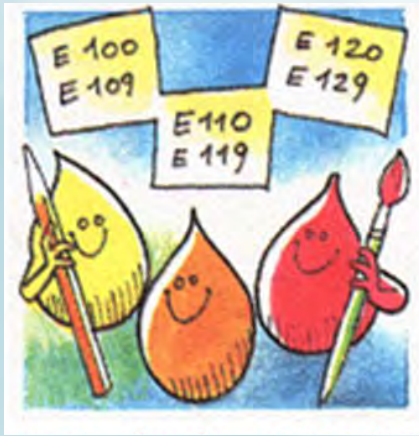
1. Use of ionising radiation gamma rays (produced by radioactive cobalt 60 isotopes) and X-rayscan destroy spores → Destruction Dna

(however, these systems are not used because C. botulinum resistant to allowed irradiation levels for stored food)

2. Ehylene oxide and is used for sterilization treatments of dried foods, or hydrogen peroxide, used in the aseptic packaging of foods

3. In the industry , the use of sodium chloride (NaCl) and sugar can be helpful: refrigerated products can be easily protected with only 5 % of NaCl in the aqueous phase, while products at room temperature would require 10 %.

→ Decrease of water activity

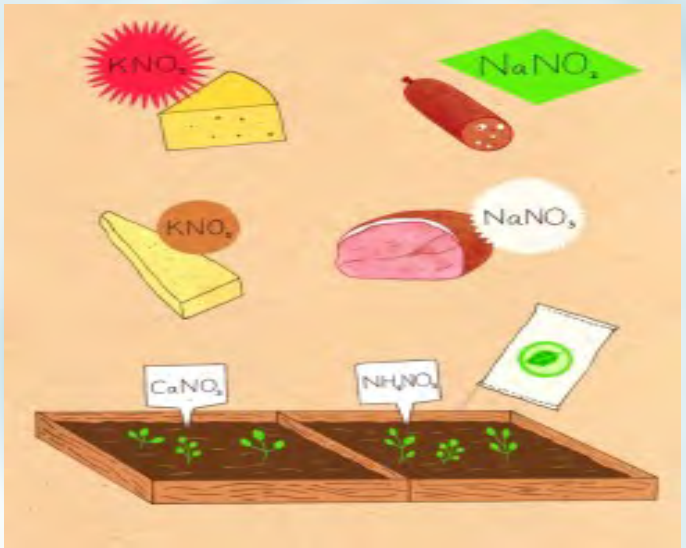


ALTERNATIVE AND CHEMICAL METHODS

4. The Use of Allowed Additives Nitrite is employed in the processing of meat and fish. However, the inhibitory effect of nitrite can be fully enhanced in synergy with other factors (pH, Aw, temperature, etc.).

the possible carcinogenicity and mutagenicity caused by the formation of nitrosamine (produced from the reaction of nitrite with amines).

Research for alternative strategies and substances the final aim should be the reduction or the total replacement of nitrite.



ALTERNATIVE AND CHEMICAL METHODS

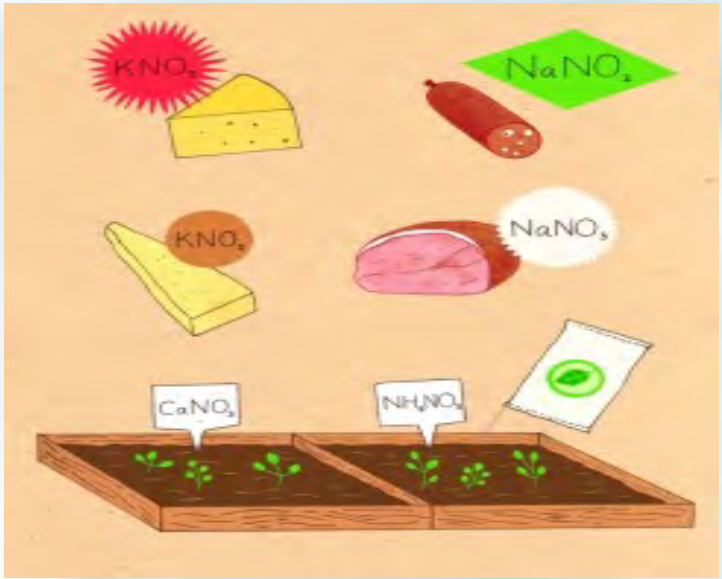
5. Sorbic acid and its salts appear able to delay the growth of C. botulinum toxin production this action is enhanced when pH decreases

Interfer with some enzyme that are necessary for the activity of bacterium

Take place of nitritis but at higher concentration

6. Essential oils (garlic, onion, black pepper, clove, oregano) or alcoholic extracts (nutmeg, garlic, rosemary, thyme and sage) of many aromatic plants are reported to inhibit spore germination or growth of vegetative clostridia

7. Smoke and NaCl combinated



PH

- ph influences growth susceptibility
- acidifying foods helps prolong conservation
- Acid food: Ph ≤ 4,6

Measuring the pH can be used to identify the types of bacteria that can grow

Valori limite di pH per la moltiplicazione di alcuni microrganismi

Specie o gruppo	*pH minimo*	*pH massimo*
Muffe	2,0	11,0
Lieviti	2,0	9,0
Batteri acetici	3,0	9,0
Lattobacilli	3,3	8,5
Enterococchi	3,5	9,6
Lattococchi	4,0	8,0
Micrococchi	4,0	9,3
Staphylococcus aureus	4,0	9,3
Salmonella spp.	4,0	9,6
Bacillus cereus	4,4	9,3
Yersinia enterocolitica	4,5	9,0
Listeria monocytogenes	4,6	9,3
Escherichia coli	4,6	9,0
Clostridium botulinum	4,6	8,5
Pseudomonadaceae	4,6	9,6
Enterobacteriaceae	4,6	9,0
Vibrionaceae	4,6	9,6
Clostridium perfringens	5,0	9,0
Campylobacter jejuni	6,0	9,6

1. Liquid food
2. Solid food
3. Emulsion food

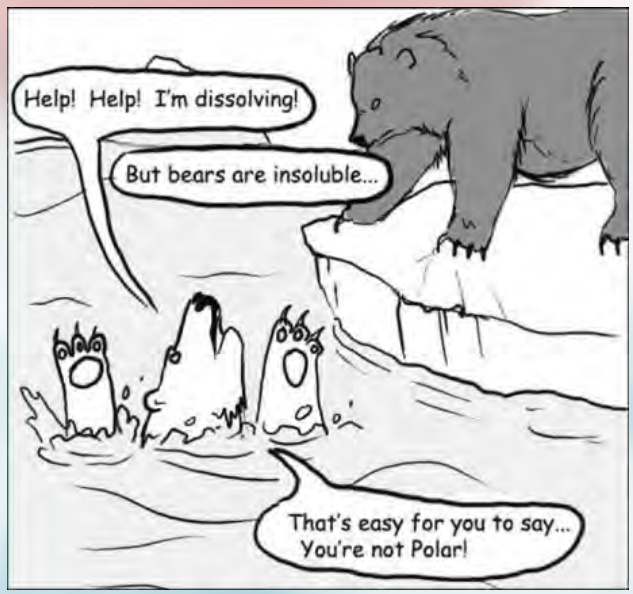
1) Attention to the ph gradients: the non homogeneity of the product could create ph differences that can allow the growth of microorganisms
2) The concomitant spreading of competitors, such as yeasts, moulds and/or bacilli with consequent pH increase ('metabiosis' effect)

LIQUID FOOD

- inserting the electrode and probe directly into the sample
- probe choice based on ph

Solid Food

- The food must be drained
  added distilled water without co2 equal to 10% of the weight (does not change the ph)
- Homogenization with stomacher
  leave to rest on an inclined plane so as to divide the phases



Emulsions

The non-polar phase must be eliminated by means of a separating funnel with ethyl ether or with freezing / thawing cycles

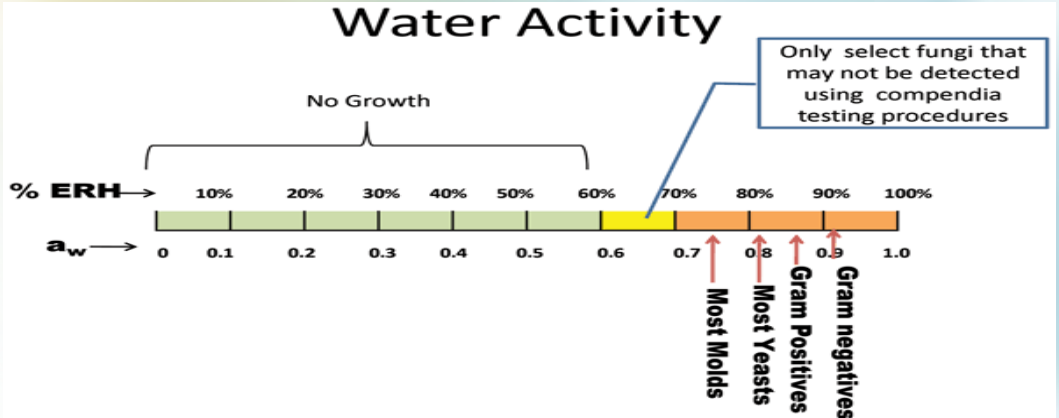
WATER ACTIVITY

Is the ratio of vapor pressure of the food to vapor pressure of pure water at the same temperature.

Amount of water available to the microorganism

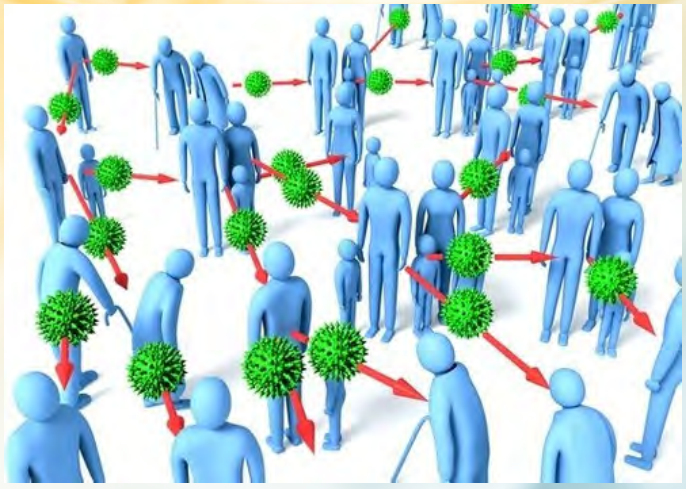
From a purely descriptive point of view, it is an index relating to the quantity of water that, in a given product, is free from particular bonds with other components, therefore, of the quantity of water (expressed in a dimensionless value between 0 and 1) available for chemical and biological reactions.

We can analyze the biological reactions that can occur, when organisms can growth and keep undesirable reactions of product deterioration



ANALYSIS OF FOOD SAMPLES

- When we suspect on infection with botulin is important analyzed the foods that the patient consumed in the days before the symptoms
- It’s necessary the analysis of Original packs and the packs that belonging to the same lot



For avoid the spreading of bacterium (especially if the sample is industrial)

- We must taken at least 250 g of product

And also useful empty containers

Not wash in dishwasher and we mustn't use detergents

The laboratory must guarantee:

1. **receiving samples on holidays**
2. **immediate start of the analysis**
3. **results in 30 hours**

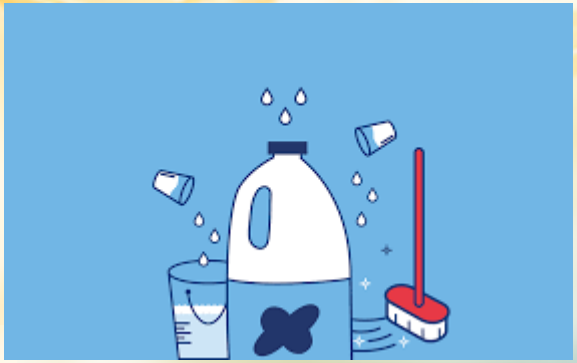
Prophylactic zoo center must warn:

National Center of Reference for Botulism
With PEC

Law January 28, 2009 N° 2 and Law December 17, 2012 N° 221

RISK IN THE LABORATORY

- In the laboratory the operator keep in mind that small concentration of toxin can be lethal
- The level of risk is II



- For avoid contamination and made a good sterilization we can use sodium hypochlorite 1g/l
- For 15/20 Min.

LABORATORY DIAGNOSIS AND METHODS OF ANALYSIS

- The laboratory diagnosis of clinical suspects, in addition to excluding any other central nervous system diseases that fall into differential diagnosis with botulism(for exemple GUILLAIN- BARRè SYNDROME) is classification of the case for epidemiological purposes, as required by the decision.
- This diagnosis is carried out by demonstrating the presence of botulinum toxins in biological samples such as serum, vomit, gastric contents, faeces, or in food residues consumed by subjects with a characteristic symptomatology.
- Generally, clostridia producing botulinum toxins are more persistent, in all types of samples(in the blood, because botulism does not cause bacteraemia).
- For this reason, the finding of these microbial agents in biological samples taken from subjects presenting a characteristic symptomatological pattern, constitutes a valid laboratory criterion for the purpose of confirming the clinical suspicion.

SAMPLING

CLINICAL SAMPLES

- The collection of clinical samples should be performed immediately after the diagnostic suspect formulation and in any case before the administration of the antidote.
- All samples useful for diagnostics and in any case before the administration of the antidote.
- All samples useful for laboratory diagnosis must be stored until they are sent to the laboratory under refrigeration conditions (in the refrigerator) and not frozen.
- All samples must be sent to the laboratory with the international identification code UN3373 - organic matter, category B.
- Particular attention must be paid to packaging the package especially if there are glass jars that could break during transport.

IN THE CASE OF FOOD BOTULISM, THE SAMPLES USEFUL FOR LABORATORY DIAGNOSIS ARE:

Serum

Feces

rectal swabs and washing in the rectal ampoule

gastric contents and vomiting

SERUM

- Serum is the "gold standard" sample for laboratory diagnosis, however it may provide conclusive results only if taken within 4-5 days of the onset of clinical symptoms / signs of the disease.
- After this period, in fact, it is extremely rare to find botulinum toxins circulating in the blood stream. Serum and not whole blood should be sent to the laboratory. For the collection of the serum, test tubes with no additives must be used, which usually have a stopper of one of the following colors: white, red, orange, yellow.

- The collection must be carried out before the antidote therapy begins. The ideal amount of serum to be sent to the laboratory is 10-15 ml, while the minimum is 3 ml. Minor quantities can be analyzed but they could provide inconclusive results. In botulinum it is only possible to search for botulinum toxins as botulism does not cause bacteremia.

FECES

The faecal sample generally provides conclusive analytical results for longer times than serum. Both the botulinum toxins and the clostridia producing botulinum toxins should be sought in the faecal sample. In the case of the determination of clostridia producing botulinum toxins and not botulinum toxins, the laboratory criterion is only satisfied in the presence of a symptomatic picture suggestive of botulism.

The ideal quantity of faeces to be analyzed is 25-50 g, but it is also possible to analyze minimum quantities (<1 g). Since constipation is a clinical sign of botulism present in most cases, the faecal sample may not be available. As an alternative to the faecal sample it is possible to analyze rectal swabs and rectal ampoule (enema).

RECTAL SWABS AND WASHING IN THE RECTAL AMPOULE

As an alternative to the faecal sample, both rectal swabs and rectal ampoule should be collected and sent to the laboratory. First the rectal swabs must be performed and then the washing. Since rectal swabs generally contain minimal amounts of sample, they must be collected in an amount not less than 4, preferring dry buffers to those containing the transport medium.

The rectal ampoule must be washed with 30 ml of non-bacteriostatic sterile water or alternatively with sterile saline solution. After infusing the liquid, wait at least 30-60 seconds before aspiration. The collected liquid must be placed in a sterile container.

In the rectal swabs it is only possible to determine the clostridia producing botulinum toxins, while botulinum toxins should also be sought in rectal ampoule washing.

GASTRIC CONTENTS AND VOMITING

- These samples are analyzed with

a much lower frequency than the previous

ones, however they can provide conclusive results.

Both botulinum toxins and botulinum-producing

clostridia should be sought in gastric contents and vomit.

In the case of the determination of clostridia producing botulinum toxins and not botulinum toxins, the laboratory criterion is only satisfied in the presence of a symptomatic picture suggestive of botulism.

The ideal quantity of sample to be analyzed

is equal to 25-50 g, however it is possible

to analyze

even minimum quantities (<1 g).

EPIDEMIOLOGICAL INVESTIGATION

What is?

- collection and interpretation of data carried out in an emergency
- element of epidemiological surveillance
- prevention tool

Relevant, correct and accurate data collection, that allow rapid identification of vehicle intoxication and prompt public health interventions.

FUNDAMENTALS OF EPIDEMIOLOGY OF FOOD BOTULISM IN ITALY

Italy registers one of the highest prevalence rates in Europe, about 20 cases are confirmed in the laboratory each year, compared to about 50 reports of clinical suspicions.

In most cases the outbreaks involve family groups that consume canned domestic production.

From the analysis of the epidemiological data collected by the National Center of Reference for Botulism, it is observed that the food responsible for the intoxication is identified by laboratory analysis only in 41% of confirmed cases. In the remaining cases it is not possible to identify the toxic food.

Foods of industrial production are involved in about 27% of botulism cases and these are the most worrying public health concerns.

Vegetable preserves in oil or in water / brine are the types of food most frequently involved in botulism accidents.

PLAUSIBILITY OF SUSPECTED FOODS TO BE INVOLVED IN CASES / OUTBREAKS OF FOOD BOTULISM

The epidemiological survey involve the timely collection of all food consumed during the 8 days preceding the appearance of clinical symptoms to identify foods that by their nature are at risk and to allow to trace back to possible other responsible food (as preservatives with defect of sterilization).

A product is at risk when:

- the raw materials must be contaminated with the clostridia;
- the chemical-physical characteristics of the food must be favorable to the development and toxinogenesis of the clostridia;
- the product must undergo ineffective sanitization processes for the destruction of botulinum-producing clostridia;
- The food must be consumed without being cooked or the heat treatment applied has not been sufficient to deactivate the toxin that may be present.

At risk botulism there are:
- preserves and semi-preservatives that are not acidic or that have undergone ineffective acidification treatments;
- preserves in brine which show aw> 0.93;
- products REPFED if the cold chain has not been appropriately maintained;
- creams and sauces not suitably acidified / processed;
- some macrobiotic products (seitan / tofu);
- products stored under vacuum with equipment available at the domestic level.

Products that are not at risk:

-fresh products;
-products properly cooked before consumption;
-frozen / frozen products / ice creams;
-jams and jams containing appropriate quantities of sugar and acidifying agents;
-sterilized meat or fish preserves or containing acidifying agents and preservatives (nitrates, brine);
-preserves in brine with aw> 0.93.

Generally the products contaminated with botulinum toxins are presented with altered sensory characteristics.

For example, the palatability of a food can be influenced by the presence of spices or other ingredients that can partially or totally mask any sensory changes.

Many products are subjected to minimal processes in order to preserve their nutritional properties and sensorial quality as much as possible. On the contrary, these processes destroying the microbial communities naturally present in the food and create the optimal conditions, in case of microbial development, for culture as well (new food formulas and creams / sauces used in commercial restaurants).

These latter products can be a risk factors if they are used in association with others that altering the final pH of the new compound, allowing the growth and toxinogenesis of clostridia producing botulinum toxins.

Honey should not be considered a food at risk of food botulism, as its chemical-physical characteristics do not support the development and toxinogenesis of clostridia producing botulinum toxins.

However, it can be a vehicle of spores and this is often related to infant botulism which affects only infants under the age of one year (the spores eventually swallowed with honey could multiply in the intestinal lumen producing in situ the toxins responsible for botulism)

Instead it does not pose any health risk in children older than one year and for adults.

METHOD OF PREVENTION AND CONTROL OF FOOD BOTULISM

- The systems for the prevention and control of food botulism, both at industrial and domestic level, consist in the destruction of spores possibly present in the raw materials, or in the control of the growth and toxinogenesis of the botulinum-producing clostridia.
- Temperature is a very important control parameter. Clostridia producing botulinum toxins are capable of developing in a very wide temperature range (3-38° C), whereby food storage outside this range is a suitable prevention and control system.
- The safety of low-acid preserves (pH> 4.6), whose ph allows the growth of botulinum-producing clostridia, is ensured by the industrial sterilization treatment.

- For acid preserves the applied heat treatments are generally milder because these ph values do not allow the germination and toxinogenesis of the clostridi spores producing botulinum toxins.
- The heat treatment is carried out in order to eliminate microbial populations that could cause variations in pH up to values compatible with the germination of clostridia producing botulinum toxins.

Jams and fruit jams are generally acidic, but the control of botulism risk in this kind of products is assured by the high sugar content that allows you to check the values of water activity, keeping them below 0.93.

- Products in brine are generally considered safe as long as the concentration of brine sodium chloride is at least 10%. This salt concentration allows to control the water activity, keeping it at a minimum value of 0.93.
- In the case of olives in brine, for example, the salt level selects halophilic microorganisms capable of fermenting the product by acidifying it until it reaches a pH capable of inhibiting the development of clostridia producing botulinum toxins.

In recent years the demand for products that undergo mild thermal treatments has grown strongly and are lacking or almost preservatives like REPFED. For this type of products the control of botulism risk is carried out through a combination of different factors, such as a pasteurization treatment, the addition of minimum quantities of additives or preservatives and the preservation for relatively long times at the refrigeration temperature.

In all cases of botulism associated with the consumption of this type of products, the interruption of the cold chain has been identified as the cause that has allowed the development and toxigenesis clostridia.

Chilling or freezing are very effective methods. Freezing prevents sprouting, growth and toxigenesis. Moreover it has a lethal action on the vegetative forms, but it has no effect on the toxin or on the spores.

Other control methods include the use of additives such as nitrite and nitrate, potassium sorbate, ascorbic acid, sodium lactate and nisin.

Nitrite (E249, E250) and nitrate (E251, E252) are naturally occurring substances in animal, plant and water food.

They are added as additives to sausage, hams, canned meat and other meat products.

Nitrites and nitrates are used for:

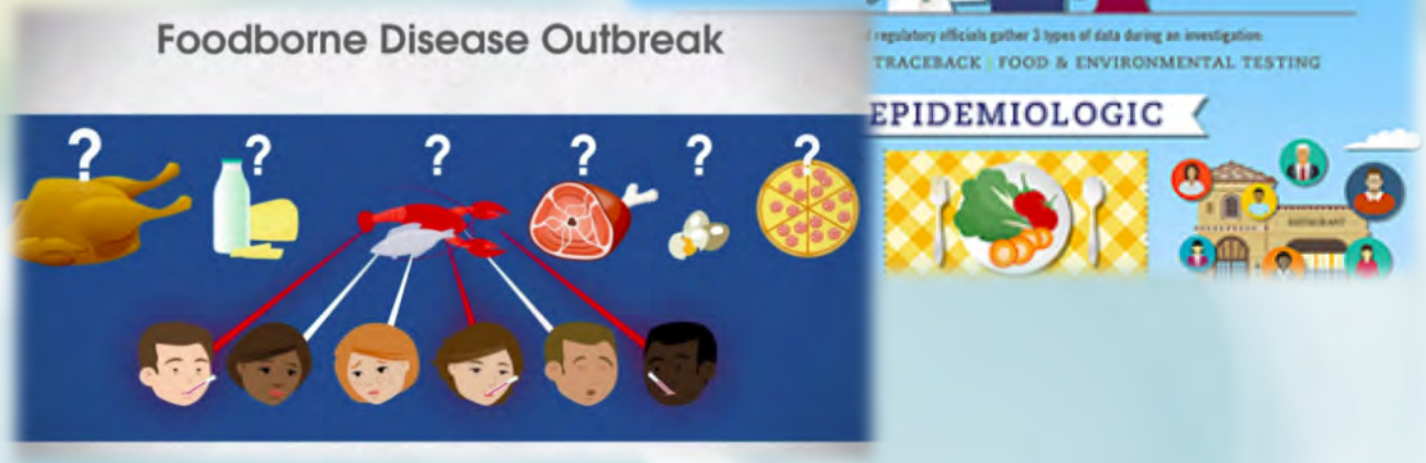
- Keep the red color of the meat;
- favor the development of the aroma acting selectively towards the microorganisms that determine the curring of the cured meats;
- they perform antimicrobial and antiseptic action.

CONDUCTION OF THE EPIDEMIOLOGICAL INVESTIGATION

After the clinical diagnosis, the doctor who has placed the suspected diagnostic must notify the case to the Department of prevention of the ASL for the immediate activation of the epidemiological investigation.

The purpose of this investigation is to:
- identify the **cause**, the **risk factors** and the **source of intoxication**;
- organize public health interventions to prevent further cases.

At the same time environmental investigations, food and laboratory confirmation analyzes should be conducted.



ENVIRONMENTAL INVESTIGATIONS ON FOODS / INGREDIENTS

Simultaneously with epidemiological investigations, environmental investigations must be carried out to identify:

-the place where the food has been contaminated;

-the factors that did not allow the proper control of botulism risk;

-corrective actions.

Is fundamentale the costant communication with the competent authorities involved in the management of the incident to activate all measure to protect public health.

At the end of the activities a final report on the incident must be carried out, with a chronological summary of the activities carried out and recommendations on future actions.


THANK YOU FOR YOUR ATTENTION!
YOU MAY NOW CLAP!
IF YOU HAVE ANY QUESTIONS,
ASK KEVIN!