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## ΠΡΟΓΡΑΜΜΑ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΑΕΙ ΓΙΑ ΤΗΝ ΕΠΙΚΑΙΡΟΠΟΙΗΣΗ ΓΝΩΣΕΩΝ ΑΠΟΦΟΙΤΩΝ ΑΕΙ (ΠΕΓΑ)

*«Οι σύγχρονες τεχνικές βιο-ανάλυσης στην υγεία, τη γεωργία, το περιβάλλον και τη διατροφή»*

# APPLIED FOOD MICROBIOLOGY

## FOOD POISONING

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# FOOD INTOXICATIONS

A bacterial food intoxication refers to food-borne illness caused by the presence of a bacterial toxin formed in the food.

The major food intoxications are caused by *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum*, *Bacillus cereus* and mycotoxin-producing moulds.



# STAPHYLOCOCCUS AUREUS

is a nonmotile, non-sporeforming Gram+ coccus.

In liquid culture media or suspended on a slide *S. aureus* arranges itself in grapelike clusters, in small groups or in pairs.

There are 8 serotypes, depending on the type of enterotoxin that is produced : A,B,C<sub>1</sub>,C<sub>2</sub>,C<sub>3</sub> and E.

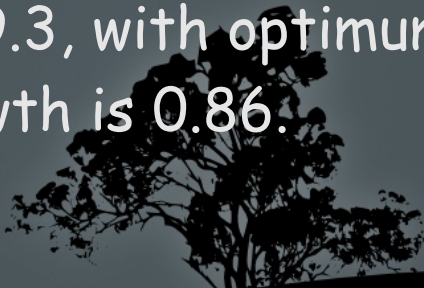
Types A and D cause food intoxications to human beings.

It is an aerobic or facultative anaerobic bacteria.

The optimum T° for growth is 35-37°C with a min. t° 5-6°C and max. t° = 45°C.

*S. aureus* has a low heat-resistance, and hence it is killed by pasteurisation.

Growth occurs in a pH range between 4.5 and 9.3, with optimum pH for growth being 7.0 to 7.5. Min. a<sub>w</sub> for growth is 0.86.



# Staphylococcus aureus enterotoxines (SE)

These toxins are proteinaceous and have a M.W. between 26.000 and 30.000.

Furthermore, they have an antigenic structure which means that specific antitoxins may be produced.

In food products containing glucose, the production of SE will be inhibited by growth of lactic acid bacteria, that lower the pH of food products as a result of acid formation (pH < 5.0).



# Factors that influence enterotoxin production

Factor	Optimum	Limits
$a_w$	0,99	0,93-0,99(x)
pH	6-7	4,5-9,8
$T^\circ$	40-50	10-46
% NaCl	0,0	0,0-10,0
$O_2$	aerobic	aerobic-anaerobic

(x) Remark : min.  $a_w$  for toxin production is higher than min.  $a_w$  for growth of *S. aureus* (0.86).



# Staphylococcus aureus enterotoxines (SE)

are single polypeptide chains that contain relatively large amounts of lysine, aspartine and glutamic acid and tyrosine.

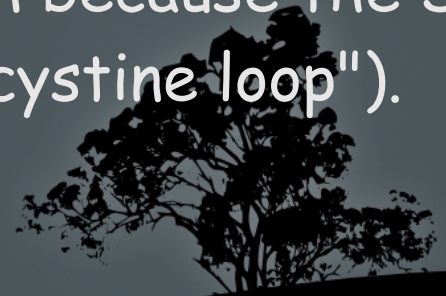
The amino acid composition of SEB and SEC are similar, but then again differ from those of SEA and SED.

They all have two residues of half-cystin and one or two tryptophan residues.

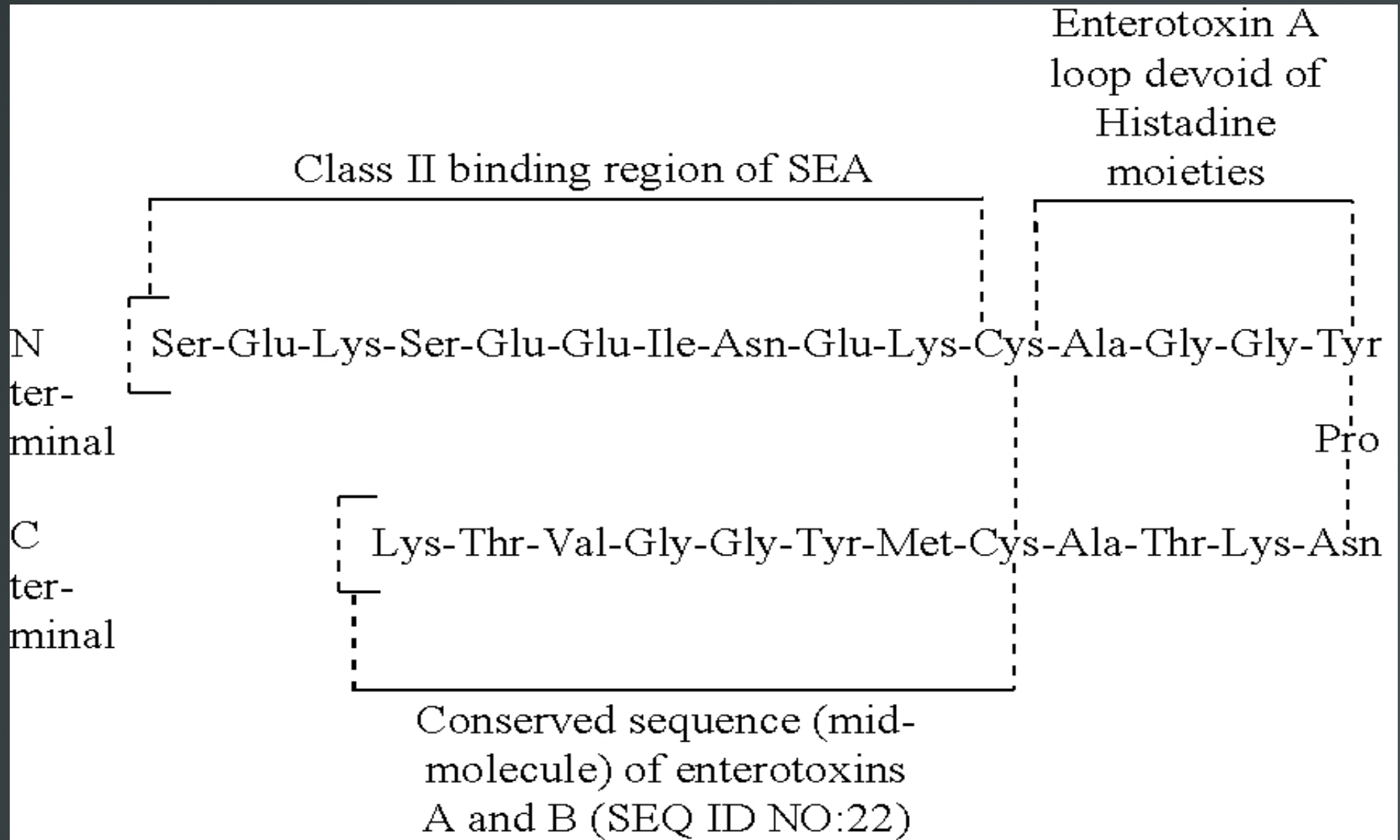
SEB has 239 amino acids.

The two residues of half-cystin are situated close to the centre of the chain, mainly near position 92 and 112.

These 2 residues create a loop in the chain because the SH-group of these amino acids is missing ("cystine loop").



# The cystine loop





# The cystine loop

is common to all *S. aureus* enterotoxins, although the sequence of amino acids is not the same for the different enterotoxins.

Both in the loop of SEA as in the one that of SEB a sequence of 7 amino acids occurs, that is the same for both, namely :  
-Cys-Met-Tyr-Gly-Val-Thr-Leu-His-.

This segment causes the toxicity of the molecule and the antigenic property is associated with the free terminal nitrogen group.

As it is the case for all active proteins, the environment is an essential factor for biological activity of the enterotoxin.



# Stability of enterotoxin

The *S. aureus* enterotoxins are in many respects more stable than other proteins.

They are resistant to proteolytic enzymes such as trypsin, chymotrypsin, rennin and pepsin.

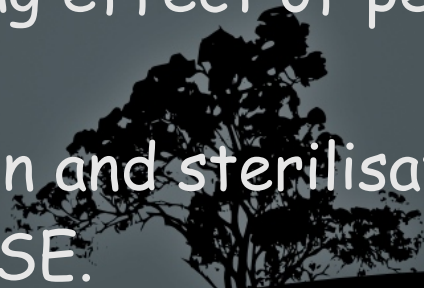
However, pepsin will inactivate SEB at  $\text{pH} < 2$ .

At higher pH values the effect of pepsin fails.

This resistance to proteolytic enzymes explains why enterotoxins are still active after ingestion.

The pH of the stomach increases as the amount of ingested food increases and hence the inactivating effect of pepsin disappears.

Irradiation doses applied for pasteurisation and sterilisation of food are not sufficient to inactivate SE.



# Stability of enterotoxin

Enterotoxines are remarkably resistant to heat-inactivation.

The activity of the SE drops by 50 % when heated during 50 minutes.

The higher the concentration of SE, the more heat is required to inactivate the enterotoxin.

A major loss in activity of the SE occurs after pasteurisation (700 bar, 15 sec) of milk, skimmed milk and cream.

Air drying of milk only slightly influences the toxicity of the present SE.

Common boiling process is not sufficient to inactivate the toxin.

The T/t combination that are applied for treatment of canned food are sufficient to denaturate the amounts of toxin usually present with food intoxication (< 0.5 to 10 mg/100 g).



# Conditions for intoxication

The food has to be contaminated with an enterotoxin producing *S. aureus* strain.

The food has to be an appropriate substrate for growth and toxin production.

The temperature needs to be sufficiently high and the period sufficiently long for toxin production : sufficient amounts of toxin are produced if the total count reaches  $10^5$ - $10^9$ /g food.

The enterotoxin has to be absorbed.



# Disease

Symptoms= after 3 to 5 hrs, vomiting, nausea, stomach cramps, diarrhoea and headache. Usually no fever. The symptoms last 2 to 3 days and recovery is complete. Mortality is very low.

Diagnosis= by tracing *S. aureus* in the suspect food product. This can also be demonstrated by means of serological methods. If the food product was heated after growth and toxin production, causing death of *S. aureus* without destruction of the thermoresistant SE, the thermoresistant DNase or thermonuclease (TNase) can be traced, and the SE-production becomes evident.

Immunity= Sensitivity can differ considerably from one individual to another.

# Sources of contamination

The major are humans and animals.

*Staphylococcus aureus* can be found in the nose (50 %) and on hands (5-30 %) of healthy persons.

They also occur in hair, eyes, throat, and intestines.

Human beings and animals spread *S. aureus* in the air and contaminate clothing and equipment.

This way food products are contaminated.

Contamination of raw food products has few risks because of the antagonistic activities of the initial flora.

Possibility of growth and toxin production by *S. aureus* is larger in heated and cured food products than in raw foods because of the reduced or even totally disappeared antagonistic activity of the accompanying microflora.

Food intoxication caused by *S. aureus* is mostly due to bad preparation techniques in kitchen at home and in industrial kitchens.



# Sources of contamination

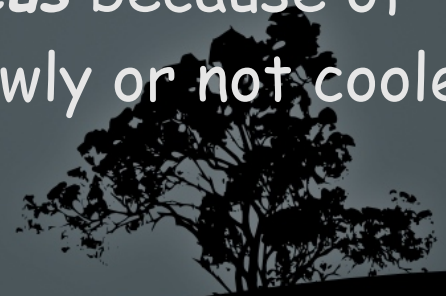
They especially occur in prepared food products that are heated first and then contaminated with *S. aureus*.

By slow and insufficient cooling large amounts of *S. aureus* will develop, with SE production in the food.

Heating causes cell death of *S. aureus* whilst thermoresistant SE will survive and may cause food intoxication.

These bad methods of preparation may also occur on food industry.

The most sensitive groups of food products are the heated food products that are contaminated with *S. aureus* because of further processing and that are cooled too slowly or not cooled at all.



# Physiological properties of *S. aureus*

Enterotoxin producing strains produce a coagulase.

Not all coagulase-positive strains produce enterotoxin.

Under the influence of coagulase, the soluble fibrinogen present in blood plasma is converted into the insoluble fibrin (coagulation).

In addition to this fibrinolysin is released and liquefies fibrin.





# Physiological properties of *S. aureus*

The enterotoxin producing strains are able to produce a thermostable extracellular nuclease during their growth, the so called deoxyribonuclease (DNase), a phosphodiesterase that cleaves extracellular nucleic acids.

There is a correlation between the thermostable DNase or TNase and toxin production.

TNase can be traced in suspected reheated foods in which the *S. aureus* cells are killed.

Presence of TNase is an indicator for growth of *S. aureus*.

*S. epidermis* and many micrococci also produce a thermolabile nuclease.

Some *Streptococcus faecalis* strains produce TNase with optimal activity at pH=6.7; whereas the optimal activity of staphylococci TNase is at pH = ± 9.

# Physiological properties of *S. aureus*

Another enzyme produced by *S. aureus* is hyaluronidase. This enzyme affects hyaluronic acid, which is an essential element of the connective tissue. The influence of the enzyme lowers the viscosity of the connective tissue, and as a result the staphylococci spread in the connective tissue.



# Measures to prevent *S. aureus* intoxication

- a) Prevent contamination by respecting the rules of personal and general hygiene (cleaning and disinfection).
- b) Inhibit multiplication by storage under refrigeration or by reducing pH.
- c) Kill *S. aureus* by heating or by irradiation with g-rays.



# CLOSTRIDIUM PERFRINGENS

is a Gram+ non motile spore-forming rod, that only occurs in couples or in chains.

There are 6 serological types, namely A,B,C,D,E and F.

Types A and C may lead to food intoxication ; but type A is the major causative agent.

*C. perfringens* type A is responsible both for "gas gangrene" as for food poisoning. There is a substantial difference between strains causing "gas gangrene" and strains causing food intoxications.



## *C. perfringens* type A strains

<i>Gas-gangrene</i>	<i>Food intoxication</i>
High $\alpha$ -toxin production (a)	Low $\alpha$ -toxin production
Always Q-toxin production (b)	Seldom Q-toxin production
Always k-toxin production (c)	Variable k-toxin production
Heat-sensitive spores	Thermoresistant spores (6hrs/100°C)

(a)  $\alpha$ -toxin is a phospholipase with haemolytic and lecithinase activity. Breakdown of lecithine leads to the formation of stearyldiglyceride and phosphorylcholine

(b) Q-toxin has a haemolytical activity.

(c) k-toxin is a collagenase that breaks down connective tissue.



# growth of *C. perfringens*

optimal temperature is 46°C.

min. and max. are 15°C and 55°C respectively.

pH-range pH = 5 - 9.

min.  $a_w$  0.97 and 0.95.

*C. perfringens* isn't as strickly anaerobic as *C. botulinum*,  
Eh varies from +310 mV at pH = 7.7 to +230 mV at pH = 6.0.

optimum Eh for growth is -200 mV.

Growth is not inhibited by the presence of oxygen,

*C. perfringens* is capable of growth in foods that are not  
anaerobically packed.



## *C. perfringens* enterotoxin (CPE)

is a protein with a molecular weight of 36.000.

contains 19 amino acids, of which mostly are: aspartic acid, serine, leucine and glutamic acid.

is inactivated by pronase and protease (produced by *B. subtilis*)

but not by chymotrypsin, papain, bromelain or carboxypeptidase.

CPE is thermolabile with  $D_{60} = 4$  min.

minimal lethal dose (MLD) for mice (intravenous) amounts to 2.000 mg N.

toxin activity is within an optimum pH between 5.0 and 9.0.



# *C. perfringens* enterotoxin (CPE)

has an antigen structure,

means that a specific antibody can be formed, that will not

react with other toxins produced by *C. perfringens* type A.

usually is produced in the intestines, seldom in food products.

CPE production takes place at the moment of sporulation.

sporulation in food products and in laboratory media is

extremely difficult,

*C. perfringens* sporulates easily in the intestine.





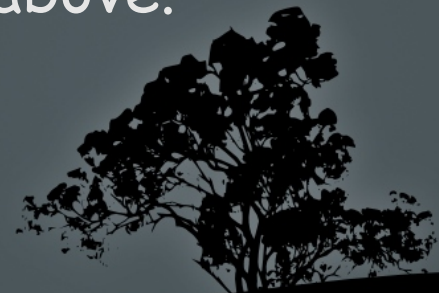
# food intoxication caused by *C. perfringens*

- 1.consumption of food contaminated with of *C. perfringens*;
- 2.multiplication and sporulation in the intestine ;
- 3.CPE production ;
- 4.release of CPE after lysis of the cells ;
- 5.diarrhoea.



# Conditions for CPE intoxication

- 1) The food product needs to be contaminated with heat-resistant spores of *C. perfringens* type A.
- 2) The spores have to survive the heat process and the food product has to be insufficiently chilled and not stored under refrigeration, so that spores are able to germinate, resulting in growth of vegetative cells.
- 3) The food product has to be consumed after insufficient heating, and this way high numbers of *C. perfringens* end up in the intestine, where they may cause food intoxication according to the mechanism mentioned above.



# Disease

Symptoms= after 8 to 24 hrs., diarrhoea, stomach cramps (usually without vomiting). The disease may be lethal for weak persons.

Diagnosis= by isolating *C. perfringens* from the suspect food product and mainly from the faeces of the patient. Both isolated strains have to be similar

Immunity= the CPE will be neutralized by the specific antitoxin.



# *Contamination sources*

Human are a major source of contamination with *C. perfringens*.

Human faeces contain *C. perfringens* (2 to 30 % contain heat-resistant strains) Heat-resistant strains were isolated from faeces of pigs, cattle, chicken, sheep and rats.

Muscles and viscera of animals that suffered stress could be contaminated with *C. perfringens*.

Hence the importance of 24 to 48 hrs of rest prior to slaughter.

Soil and dust are also important sources of contamination.

Flies, on the other hand, only play a minor role.



# Measures to prevent *C. perfringens* intoxication

- a) Chill heated food products rapidly and efficiently
- b) Store heated products above 70°C
- c) Reheat the remains of a meal
- d) Personal and general hygiene.



# CLOSTRIDIUM BOTULINUM

is a Gram+ motile (peritrichous flagella), spore-forming (sub-terminal) rod, that occurs alone, in couples or in chains. strains of *C. botulinum* are classified into 7 serological types, deduced from the different antigen structures of the formed toxins.

Following serotypes are recognized : A,B,C,D,E,F and G. Types A,B,E,F and G cause disease in humans.



# *C. botulinum*

grows in a temperature range  $10^{\circ}\text{C}$  -  $48^{\circ}\text{C}$ . optimum for growth is  $35^{\circ}\text{C}$ .

The minimum temperature for germination is  $10^{\circ}\text{C}$ .

Exceptions are *Clostridium botulinum* type E, the spores of which germinate and grow at min. temperature of  $3.3^{\circ}\text{C}$ .

Non-proteolytic strains of type B are also psychrotrophic and are able to grow at temperatures  $> 3.3^{\circ}\text{C}$ .

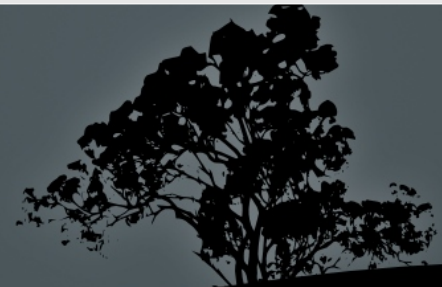
min. pH for growth is 4.6.

type E is less acid sensitive than the other types, which presents a risk for fish marinades, since type E mainly occurs in fish.



# Min. $a_w$ values for growth and toxin-production of *C. botulinum*

	<i>Type</i>	<i>Min. <math>a_w</math></i>
Growth of vegetative cells	A	0.93-0.94
	B	0.93-0.94
	E	0.965
Sporulation and toxin-production	A	0.95
	B	0.94
	E	0.97





# *Clostridium botulinum*

is a strictly anaerobic bacterium and will grow at low Eh ( $< +150$  mV).

Spores of *Clostridium botulinum* are heat-resistant. spores of type A resist 20 minutes heating at  $110^{\circ}\text{C}$  and spores of type B survive 30 minutes heating at  $80^{\circ}\text{C}$ .



# Botulinal toxins

Botulinal toxins consist of two components, one of them being toxic. The toxic component is a neurotoxin.

The non-toxic component protects the toxic component from inactivation.

At  $\text{pH} > 7.5$  the toxin dissociates into 2 components

Since in most food products  $\text{pH} < 7.0$ , the botulinal toxin will be present in its most stable form.

But if potable water has  $\text{pH}$  above 7, dissociation occurs and the toxic component loses biological activity.

there are no substances that influence toxicity in food products (e.g. salts or acids)

botulinal toxins are thermolabile at  $80^{\circ}\text{C} / 10 \text{ min}$  or at  $86^{\circ}\text{C} / 1 \text{ min}$ .



# botulinal neurotoxin

is neutralized by type-specific antitoxins.

there are cross-reactions between D and E, C and D.

there are no cross-reactions between A,B and E.

minimal lethal dose (MLD) is the amount of toxin causing death  
of 20 g weighing mouse in 24 hrs ;

1 mg botulinal toxin type E is  
4.800.000 MLD for guinea pigs,  
31.000.000 MLD for mice and  
10.000 MLD for human beings.

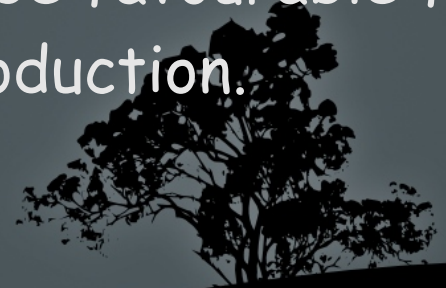


# Conditions for botulism

1. The food product has to be contaminated with spores of *C. botulinum* type A,B,E, F or G.
2. Spores have to be able to germinate, so that vegetative cells will multiply and produce toxin.
3. The food product has to be heated insufficiently to inactivate toxin.
4. The food has to be absorbed.

For canned foods, spores have to survive because of insufficient heating.

Moreover, the storage conditions have to be favourable for germination, multiplication and toxin production.



# Disease

Symptoms= incubation period 12 - 36 hours.


1st phase of the disease is characterized by gastrointestinal symptoms such as nausea, vomiting and diarrhea.

this results from resorption of toxin in the gastrointestinal tract.

After botulinal toxins are absorbed into the bloodstream, they enter into the peripheral nervous system where they affect nerves.

2nd stage of the disease, paralysis phenomena occur : paralysis of muscles, double vision and finally, respiratory failure and death.

Diagnosis= is traced in the suspect food product, both in vivo (mice) as in vitro (immunological tests).



# Disease

Immunity= Active immunity after consumption of a food product contaminated with botulinal toxin, cannot be obtained, because the dose of toxin causing clinical phenomena is too low to stimulate antitoxin production.

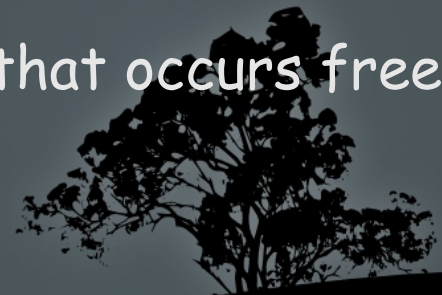
Botulinal toxoid can be administered to persons who come regularly in contact with botulinal toxin.

The toxoid is obtained by treating the toxin with chloroform, and has a high antigenic activity.

Immunity for ca 10 years can be obtained by vaccination.

In addition to this, antitoxin can be administered to persons who have consumed foods contaminated with botulinal toxin and who do not show the symptoms yet.

The antibotulinal toxin only neutralises the toxin that occurs freely.



# Sources of contamination

*C. botulinum* mainly occurs in the soil and this way it ends up in food products (especially vegetables) and feed.

*C. botulinum* type E mainly occurs in seawater and thus in fish.

Meat is contaminated by faecal contamination during slaughter.

Mainly type B, pathogenic to human beings, is found regularly in meat.



# Measures to prevent botulism

- a) Canned food products need to be sufficiently sterilised.
- b) Swollen cans have to be removed.
- c) Change in colour, odour or flavour may not occur.
- d) Consumption of heated foods that are stored at high temperatures (room t°) must be refused.
- e) Suspect canned foods have to be boiled for 15 minutes.





# Special attention

should be paid to smoked vacuum-packed fish.

Fish can be smoked at low temperatures (28°C) or at high temperatures (70°C).

*C. botulinum* spores will survive this process.

After the smoking process, the fish has to be packed rapidly and stored under refrigeration (< 3°C).

The same measures apply for prepared chilled meals with extended shelf-life.



# BACILLUS CEREUS

is a Gram-positive, motile, spore-forming (central, ellipsoid) rod with a granular internal structure.

optimum temperature for growth is  $28^{\circ}\text{C}$  -  $35^{\circ}\text{C}$  with min.  $T^{\circ} = 4^{\circ}\text{C}$  and max.  $T^{\circ} = 50^{\circ}\text{C}$ .

grows between  $\text{pH} = 4.9 - 9.3$ .

min.  $a_w$  for growth = 0.90, which corresponds to 15 % NaCl.

Spores are thermoresistant.

Spores germinate at  $50^{\circ}\text{C}$



## *B. cereus* enterotoxin

is a protein with an antigenic structure, that is sensitive to trypsin and pronase.

It is stable in a pH-range between 5 and 10.

is thermolabile and is inactivated if heated at 56°C during 5 minutes.



# Conditions for intoxication caused by *B. cereus*

1. The food product has to be contaminated with spores of *B. cereus*.
2. The food product must have been insufficiently heated.
3. The food product has to be a suitable substrate for germination of spores, growth and toxin production.
4. The time/temperature combination has to be appropriate.
5. The food product (containing enterotoxin) needs to be absorbed.



# Disease

Symptoms= Intoxication can be divided into 2 groups, "diarrhoea syndrome" food poisoning and "emetic syndrome" food poisoning.

Intoxication is not lethal and recovery is quick and complete.

Diagnosis= by tracing *B. cereus* in suspect food.

Immunity= Little or no immunity can be obtained, depending on the individual.



# The 2 types of symptoms of *B. cereus* intoxication

	<i>diarrhoea syndrome</i>	<i>emetic syndrome</i>
Incubation	8-16 hrs	1-5 hrs
Diarrhoea	general	regularly
Vomiting	occasionally	general
Duration	12-14 hrs	6-24 hrs
Food product	meat, soup, sauces, vegetables, puddings	fried rice



# Sources of contamination

*B. cereus* is a frequently occurring microorganism that is mainly found in air, soil, water and all kinds of waste matter. Food products are contaminated via these sources.



# Physiological characteristics

*B. cereus* produces a number of extracellular metabolites such as protease,  $\beta$ -lactamase, specific peptide antibiotics a phospholipase and a haemolysine.

The last ones are 2 metabolites that exercise a joint action comparable to that exercised by the  $\alpha$ -toxin, produced by *C. perfringens*.





# Measures to prevent *B. cereus* intoxication

Since *B. cereus* commonly occurs in nature it is evident that it sometimes is found in foods.

Small numbers are not harmful, but germination of spores and multiplication of vegetative cells should be prevented.

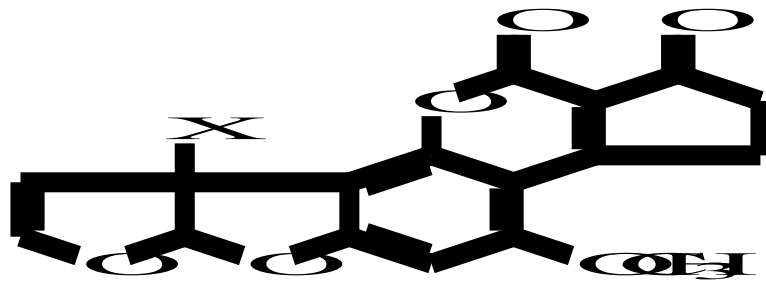
In practice, this means that foods have to be consumed immediately after heating, or otherwise they have to be cooled rapidly to  $T^{\circ} < 10^{\circ}\text{C}$ .



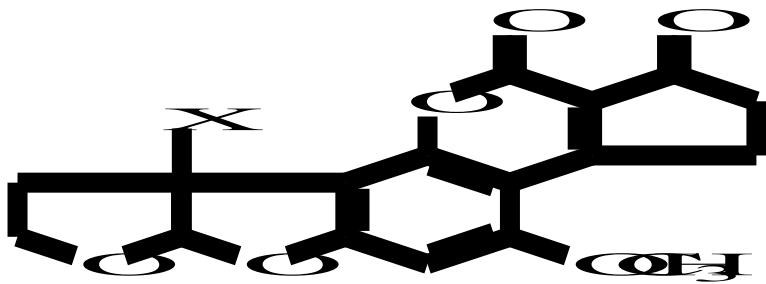
# Mycotoxins

Numerous moulds are capable of producing toxic metabolites in food products.

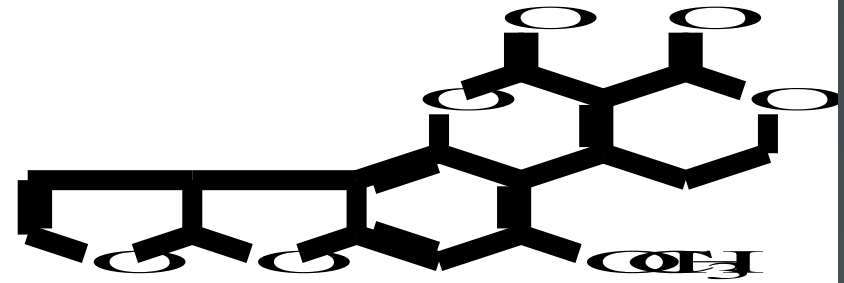
The major group is the aflatoxin-group, produced by some strains of *Aspergillus flavus* and *Aspergillus parasiticus*.



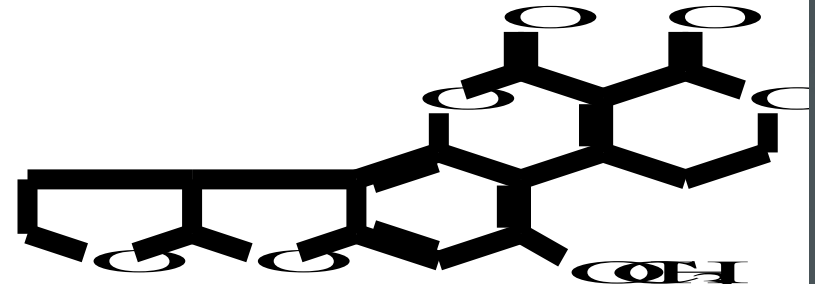
B<sub>1</sub>XH  
HXB  
HXBH



B<sub>2</sub>XH  
HXB  
HXBH



G



G

# Chemical characteristics

Aflatoxins are coumarin derivatives. 4 types are formed : aflatoxin B<sub>1</sub> and aflatoxin B<sub>2</sub> (blue fluorescent) and G<sub>1</sub> and G<sub>2</sub> (green fluorescent).

The structure of these metabolites is as follows :

The aflatoxins B<sub>1</sub> and B<sub>2</sub> are converted by hydroxylation in milk to M<sub>1</sub> and M<sub>2</sub>.

Aflatoxins are soluble in methanol and chloroform and a slight water-solubility.



# Substrate

Food products on which *Aspergillus flavus* and *Aspergillus parasiticus* can grow and produce toxins.

Especially dry food products (still having  $a_w > 0.85$ ) are qualified.

One strain can only form 2 or 3 aflatoxins, at least one of them being  $B_1$ .

The most sensitive food products are cereals and nuts.

In milk  $B_1$  and  $B_2$ , that may be present in feed as a result of mould growth, are converted into  $M_1$  and  $M_2$ .



# Biological activity

## LD<sub>50</sub>-values of aflatoxins

Type	Ld <sub>50</sub> in mg/kg body weight
B <sub>1</sub> and M <sub>1</sub>	0,36
G <sub>1</sub>	0,79
B <sub>2</sub> and M <sub>2</sub>	1,8
G <sub>2</sub>	3,45

Aflatoxins are carcinogenic.

They are hepatotoxic and hepatic carcinogenic.

The pathological implications for human beings are characterised by

1) bleedings of kidneys, lungs, intestines and

2) a chronic stage during which hepatomas and sarcomas are formed.



# Other mycotoxins

Toxin	Microorganism	Food product
Zearalenone	Fusarium roseum	Cereals
	Fusarium tricinctum	(maize)
Ochratoxin A	Aspergillus ochraceus	Cereals, beans, nuts,
	Penicillium viridicatum	poultry, pork meat
	Penicillium citrinum	Cereals
Citrinin	Penicillium viridicatum	
	Penicillium spp.	Cereals, beans
Penicillic acid	Aspergillus spp.	
	Penicillium expansum	Apples, cider,
Patulin	Penicillium urticae	applejuice
	Aspergillus versicolor	Wheat, green
	Aspergillus viridulans	coffeebeans
Sterigmatocystin	Aspergillus rugulosus	
	Bipolaris spp.	
	Penicillium islandicum	
	Fusarium	Rice
Luteoskyrin	Trichothecium	Maize
Trichotricene	Various moulds	
	Penicillium spp.	Cereals
A.T.A. (alimentary toxic aleukia)		Bleu cheeses
Roquefortine		

# Measures to prevent mycotoxicosis

- a) Prevent fungi growth and toxin production by dry storage and use of fungicides.
- b) Detect moulds and toxins in foods.
- c) Destroy present mycotoxins if possible.

This is usually hard to accomplish because of the high resistance to various physical and chemical treatments. Aflatoxins do not have an antigenic structure, and as a result the body does not form antibodies will not be produced.



# FOOD INFECTIONS

A bacterial food infection refers to food-borne illnesses caused by the entrance of bacteria into the body through ingestion of contaminated foods and the reaction of the body to their presence or their metabolites.





# SALMONELLA

belongs to the family of *Enterobacteriaceae*.

So far, approximately 2000 serotypes have been isolated and identified, based on the antigenic properties of *Salmonella* spp.

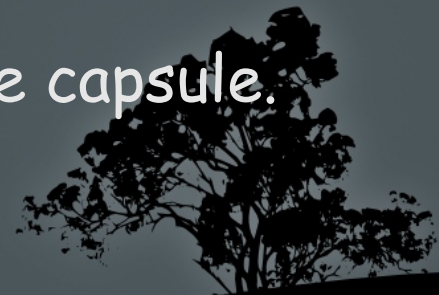
*Salmonella* can have 4 types of antigens.

O-antigens (somatic or cell bound) which are thermostable. 64 O-antigens.

H-antigens (flagellar bound) which are thermolabile. 51 H-antigens

K-antigens (capsular antigen) and

Vi (Virulence antigens) that is linked to the capsule.



# Salmonella

is a Gram-negative, motile (peritrichous flagella) non-spore-forming rod.

optimum temperature for growth is  $35^{\circ} - 37^{\circ}\text{C}$  (min-max  $5^{\circ}\text{C} - 47^{\circ}\text{C}$ )

Heat-sensitive, killed at  $66^{\circ}\text{C} / 12$  minutes.

survive freezing and chilling temperatures.

pH range of 4.5 - 9.0, optimum 6.5 - 7.0,  $< \text{pH} = 4$  and  $> \text{pH} = 9.0$  will die.

min.  $a_w$  for growth is 0.95 but *Salmonella* survives in dry foods.

is an aerobic or facultative anaerobic bacterium.



# The species name of *Salmonella*

refers to

the city or place where it first was isolated (*S. newport*, *S. panama*, *S. dublin*)

or to the disease it has caused (*S. typhi*, *S. typhimurium*, *S. enteritidis*)

or to the host (*S. pullorum*, *S. gallinarum*).



# Conditions for infection

The food product has to be contaminated with living *Salmonella* cells.

The food has to be a suitable substrate for growth, in order to attain the infectious dose.

Enough time must be allowed and the temperature must be favourable for growth.

The amount of ingested food must be sufficient to cause infection. Also the acid pH of the gastric juice, that has a bactericidal effect (amongst others against *Salmonella*) has to be taken into account.



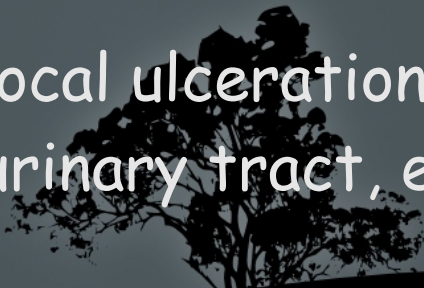
# Symptoms=

Gastro-enteritis, incubation 8 to 72 hrs, bloody, slimy diarrhoea, abdominal pain, nausea and vomiting (during the first few days) and fever (38-39°C).

Typhoid fever occurs as a result of sepsis by *S. typhi*, incubation 3 to 28 days, fever, combined with malaise and headache followed by abdominal pain, general pain and weakening, anorexia and delirium.

Some serotypes, (*S. paratyphi* A, B, C, *S. enteritidis*, *S. cholerae-suis*) may cause paratyphoid fever (gastro-enteritis followed by sepsis).

Local infections: appendicitis, peritonitis, local ulceration, pneumonia, meningitis, infection of the urinary tract, et al.



# Diagnosis=

in case of gastro-enteritis, *Salmonella* is traced in the faeces,  
in case of typhoid fever, *Salmonella* is traced in the blood.  
in case of paratyphoid fever, *Salmonella* is usually traced in  
the blood, and sometimes in the faeces.

$10^6$  to  $10^9$  salmonellae/g faeces may be present during the  
acute stage of diarrhoea.

An infected person may still be carrier of *Salmonella* for 2 to  
4 weeks after his recovery.

Hence, it is important to control people working in food  
industry regularly.

- Immunity= Immunity can be obtained by vaccination (dead cells).



# Sources of contamination

Human beings and animals are the major sources.

Human and animal faeces will contaminate various types of vegetables via liquid manure, and oysters and mussels via waste water that ends up in estuaries.

eggs may be contaminated with *Salmonella*.

If an omelette is made of such eggs, *Salmonella* may be present in the omelette.

Food products may be contaminated with *Salmonella* via vermin and insects.

Meat and meat products, poultry, dairy products, fish and especially shrimps and frog legs are risk-bearing products.



# Measures to prevent salmonellosis

- a) Avoid food products being contaminated with *Salmonella* bacteria. This can be obtained mainly by respecting the general rules of hygiene.
- b) Check raw materials and ingredients regularly.
- c) Destroy *Salmonella* by pasteurisation, sterilisation and  $\gamma$ -irradiation.
- d) Store food products under refrigeration to prevent growth of *Salmonella*.





# SHIGELLA

belongs to the family *Enterobacteriaceae*,  
group A consists of 10 types of *S. dysenteriae*  
group B consists of 6 types of *S. flexneri*  
group C consists of 14 types of *S. boydii* and  
group D that consists of 1 type of *S. sonnei*.



# Shigella

is a Gram-negative, nonmotile, non-spore-forming rod.

optimum growth temperature is 37°C, min-max 10° - 40°

is heat-sensitive, common pasteurisation temperatures can kill the bacterium.

grows in a relatively narrow pH-range (6.6-8.8) with optimum pH = 7.8.

min.  $a_w$  for growth is 0.95

growth occurs both under aerobic and anaerobic conditions.

*Shigella* has a limited life in food products



# Toxins

If *Shigella* ends up in the intestine (colon) it will grow, and produce toxin.

A thermostable endotoxin (an O-antigen) is formed.

This toxin is enterotrophic and has a glucido-lipido protein structure.

Another toxin that is formed is a neurotrophic, thermolabile toxin, which has a protein structure.



# Conditions for Shigellosis

Conditions for shigellosis are the same as those for salmonellosis.

## Disease

Symptoms= incubation period 4 days (1-7 days), stomach-ache diarrhoea and high fever, followed by intoxication phenomena, (bloody slimy stools and loss of weight).

Diagnosis= by tracing *Shigella* in faeces rapidly.

Immunity= a very small immunity can be obtained, vaccino- and serotherapy cannot be applied.



# Sources of contamination

*Shigella* is not a saprophyte and consequently cannot live long in food products.

Survival depends on temperature:

long at  $-20^{\circ}\text{C}$ , relatively long at  $0^{\circ}\text{C}$ , short at  $20^{\circ}\text{C}$  to  $30^{\circ}\text{C}$ .

The major source of contamination are human beings, who may be carriers of the pathogen even up to three months after the symptoms of dysentery.



# Measures to prevent shigellosis

The measures to prevent shigellosis are similar to those to prevent salmonellosis.



# ESCHERICHIA COLI

The genus *Escherichia* is also classified in the family *Enterobacteriaceae*.

*Escherichia coli* is a lactose-fermenting faecal microorganism. *E. coli* is a Gram-negative, motile (peritrichous) or sometimes non-motile, non-spore-forming rod.



# *E. coli*

grows between 10°C and 50°C with optimal growth at 37°C.

faecal coli grow well at 44°C, property used to assess *E. coli* in food products.

has also thermoresistance, property applied to control pasteurisation, survival indicates insufficient pasteurisation.

is extremely tolerant of pH.

min.  $a_w$  is 0.95

is a facultative anaerobic

Resistance to phenols, dyes and bile salts is typical, components frequently used in selective media for isolation and enumerating of *E. coli*.





# Toxins and other metabolites

- a) *E. coli* produces the following toxins in the intestine :  
an exotoxin which is thermolabile and causes paralysis ;  
an endotoxin which is thermostable and affects the intestinal tract.
- b) Other metabolites: *E. coli* produces colilysine (haemolysine). Colilysines may have an antagonistic effect on *Salmonella* and *Shigella* in the intestine
- E. coli* is also capable of producing lactamase which will inhibit the effect of lactam antibiotics.



# Conditions

The conditions are similar to those required for infection caused by *Salmonella*, but only clearly defined serotypes of *E. coli* are pathogenic.



# Disease

Symptoms= short incubation period (2-14 hrs), stomach-ache, headache, nausea, diarrhoea and fever. Diarrhoea is serious for babies and adults with weak immune system. Adults may develop urinary tract infection.

Diagnosis= can be traced in a sample of the suspect food product and in a sample of faeces of the patient. Both samples must be of the same serological types.

Immunity= cannot be obtained, vaccino- and serotherapy cannot be applied.



# Sources of contamination

*E. coli* occurs as commensal in men and animals and can mainly be found in faeces.

It ends up in nature via faeces and it also occurs in water and food products.

Thus, presence of *E. coli* always indicates faecal contamination.



# Measures to prevent E. coli infection

Similar measures as those to prevent salmonellosis are required.



# VIBRIO CHOLERAE

is a Gram-negative, comma-like, motile (polar), non-spore-forming bacterium.

optimum temperature for growth is 30°C-35°C, min-max 15°C - 42°C respectively.

is heat sensitive and is killed if heated at 55°C / 15 minutes.

growth occurs in a pH-range 6.5 - 9.6, optimum growth at slightly alkaline pH (7.6-8.6).

min.  $a_w$  is 0.95, but is extremely sensitive to dryness.

is an aerobic or facultative anaerobic bacteria.



# Toxin

cells multiply in the intestinal tract and produce an endotoxin with protein-structure (MW = 84.000), that consists of 2 components.

component A has a MW of 28.000, is biologically active and causes hypothermia (cold fever).

component B has a MW of 56.000 and its toxicity is based on interaction with the cells of the intestine wall, causing cholera.

The endotoxin is thermolabile and is destroyed if heated at 56°C.



# Conditions for cholera infection

The conditions required for cholera infection are similar to those required to cause salmonellosis.





# Disease

Symptoms= incubation period of 2 days (1-5 days), vomiting, liquid diarrhoea, resulting in dehydration (muscle cramps, loss of weight, anuria). Cholera can be lethal (after 2 to 4 days).

Diagnosis= by tracing *V. cholerae* in the patient's faeces.

Immunity= by vaccinating death cells (vaccinotherapy). Each year 2 injections are administered. Antibiotics cannot be administered curatively, because the cells are lysed before the endotoxins that caused cholera are released. Vaccine and sulphonamides are administered simultaneously.



# Sources of contamination

The major source of contamination is water, and subsequently numerous food products can also be contaminated.



# Measures to prevent cholera

Cholera can be prevented if the same precautions are taken as the ones taken to prevent salmonellosis.



# VIBRIO PARAHAEMOLYTICUS

is a Gram-negative, straight or curved, motile, non-spore-forming, rod.

peritrichous flagella are formed on solid substrates, and in liquid substrates polar flagella are formed.

grows between 4°C and 42°C with optimum temperature 30°C - 35°C.

is killed if heated at 60°C / 15 minutes,

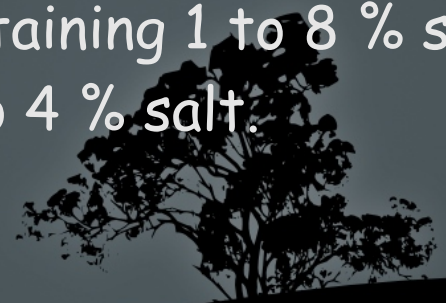
growth occurs in a pH 5.6 - 9.6 with optimal growth at pH = 7.6 - 8.6.

is acid sensitive.

is a facultative anaerobic,

min.  $a_w$  for growth amounts to 0.94.

is halophilic bacterium, grows in a medium containing 1 to 8 % salt, optimum growth in a medium containing 2 to 4 % salt.



# Toxicity

*parahaemolyticus* strains, isolated from patients are haemolytic, whereas strains isolated from fish and water are non-haemolytic.

This phenomenon is termed the Kanagawa phenomenon.

Kanagawa-positive strains cause infections, produce 2 toxins: toxin a and toxin a'.

They have different chemical structures and different immunological properties

but they have similar haemolytic activities and similar toxicity in laboratory animals.



# *V. parahemolyticus*

strains produce haemolysins that can be divided into 4 groups:

1. Thermolabile haemolysin, produced by all strains and not inactivated by trypsin, and not toxic to mice.
2. Thermostable haemolysin, occurring in the lipid fraction of all strains.
3. Haemolysins, occurring in the supernatant of liquid cultures of some strains, and associated with phospholipase activity.
4. A haemolytic fraction that is only present in the supernatant of cultures of Kanagawa-positive strains but not in that of Kanagawa negative strains, causes gastro-enteritis and has an haemolytic effect on human blood (but not on horse blood).



# Conditions for *V. parahaemolyticus* infection

Similar conditions as those to cause salmonellosis are required.



# Disease

Symptoms= relatively short incubation period of 12 hrs (2-48 hrs), stomach-ache, diarrhoea, nausea and vomiting.

Mortality is low.

Diagnosis= is traced both in the suspect food product as in the faeces.

Immunity= Immunity cannot be obtained.





# Sources of contamination

The major sources of contamination are seawater, sediment and plankton, mainly of warm coastal waters. This way fish and fish products (slightly salted) are contaminated.



# Measures to prevent *V. parahaemolyticus* infections

The measures are similar to those taken to prevent salmonellosis.



# CAMPYLOBACTER

Two species of *Campylobacter* spp. may cause food infections:  
*Campylobacter jejuni* and  
*Campylobacter coli* (to a smaller degree).



# *Campylobacter*

is a small non-spore-forming, Gram- rod, curved or spiral shaped bacterium.

is strictly microaerophilic and will therefore grow at reduced oxygen level.

growth occurs optimally in an environment of 5 % O<sub>2</sub>, 10 % CO<sub>2</sub> and 85 % N<sub>2</sub>.

temperature for growth of *C. jejuni* strains ranges 32°C - 45°C, optimal growth at 42°C - 45°C.

HTST treatment is sufficient to kill *C. jejuni*.

optimal growth at pH=6 and pH=8.

*C. jejuni* is acid-sensitive and dies at pH=5.



# *C. jejuni* is sensitive to drying.

cell death occurs more rapidly if dried at 25°C than at 4°C.  
Best survival is at R.H. = 14 % and the lowest at R.H. = 59 %.

*C. jejuni* can survive at refrigerated temperatures and an appropriate humidity (chilling of poultry).

*C. jejuni* survives better if dried in Brucella broth than dried in skimmed milk.



# Disease

incubation period ranges from 2 to 11 days, stomach-ache, diarrhoea, fever, headache, qualm and delirium.

symptoms may last 40 to 72 hrs, but sometimes the disease may last several weeks. death is exceptional.

*C. jejuni* cells can remain for 4 to 7 weeks in the patient's intestines after the infection.

Some people may still be carrier of *C. jejuni* up to one year.

The mechanism of pathogenesis has not been elucidated completely yet.

There are usually polymorphic nuclear leukocytes in the faeces of the infected persons.



# Sources of contamination

*C. jejuni* and *C. coli* may occur in the intestines of animals such as poultry, cattle, pigs, sheep, dogs and cats.

*Campylobacter* is also found in the secretions of healthy rodents.

in developing countries human beings can play a role in transmitting *Campylobacter*.

Water and soil, contaminated with animal faeces containing *Campylobacter*, can also be a source of contamination although survival in such substrates is relatively low, depending on temperature (the lower, the longer the survival).



# Measures to prevent *Campylobacter* infection

The measures are similar to those taken to prevent salmonellosis.





# LISTERIA MONOCYTOGENES

is a small, short Gram+ non-spore-forming rod, occurs both among human beings and animals as in the environment.

temperature growth range  $-0.1^{\circ}\text{C}$  -  $44^{\circ}\text{C}$ , optimum at  $30^{\circ}\text{C}$  -  $37^{\circ}\text{C}$ .

survives deep-freeze temperatures but the cells are sublethally damaged.

more heat-resistant than many other non-spore-forming spoilage organisms.

capable of growth in 10 % NaCl-solutions ( $a_w = 0.94$ ) and of survival in 20 % NaCl-solutions ( $a_w = 0.88$ ).

*L. monocytogenes* is facultative anaerobic



# Minimum pH for growth of *L. monocytogenes*

is determined by the type of acid it is acidified with.

In media acidified with HCl min. pH for growth is 4.39,  
but in cabbage juices, whose pH is controlled by lactic acid,  
min. pH for growth is 4.80.

some organic acids have antimicrobial activity on top of their  
pH-reducing effect.

min. pH for growth differs, depending on type and acid  
concentration applied to obtain the desired pH.



# Infection

The number of *L. monocytogenes* cells required to cause listeriosis is still unknown.

in certain groups of food products presence of low numbers of *L. monocytogenes* can be tolerated, total absence of *L. monocytogenes* is not realistic, e.g. in raw food products, raw fermented meat products and accidentally recontaminated foods.



# Conditions for infection

The food product needs to be contaminated with living *L. monocytogenes* cells.

The infective dose has to be present or the medium has to be a suitable substrate so that the infective dose can be obtained.

The period has to be long enough and temperature needs to be favourable for growth.

The amount of consumed food has to be high enough to cause infection.



# Disease

*L. monocytogenes* is a facultative, intracellular pathogen.

The bacterium enters the epithelial cells of the intestine and subsequently they migrate to the liver and the spleen.

Macrophages in the blood do not destroy the bacteria, so that they not only survive but also multiply by the formation of haemolysine.

By formation of actine the bacterium migrates from one macrophage to another and this way the bacterium withdraws from the immune system.



# Disease

Bacteria will enter epithelial cells of the intestine and are subsequently taken up by macrophages.

This process may be accompanied by transitory flulike symptoms that may include general malaise, diarrhoea and mild fever.

Virulent *L. monocytogenes* strains are capable of multiplying in macrophages, disrupting these cells and consequently end up in the bloodstream (septicaemia).

In this stage the organisms have access to other parts of the human body which may involve the central nervous system, the heart, the eyes, and the foetus of pregnant women.



# Disease

The stage of pregnancy when invasion occurs determines the outcome of the disease : abortion, stillbirth or prenatal sepsis, resulting in death within a week.

The infected baby may also be born without disease symptoms and subsequently develop acute meningitis within 3 weeks.

In one of two thirds of the cases the disease occurs during pregnancy or during the prenatal period.



# Disease

Elderly persons and liver patients and in general the immunocompromised (e.g. alcohol- and drug addicts) belong to the high-risk group.

Also AIDS patients and cancer patients who get chemotherapy are more sensitive to listeriosis.

Mortality of persons in the high risk groups amount to 30 % or more.

It is assumed that human beings obtains immunity after uptake of the bacterium, except for babies and immunocompromised persons.





# Sources of contamination


It has been demonstrated 4.2 to 58 % of the samples of raw milk were contaminated with *Listeria* spp. and 1.3 to 45 % were contaminated with *L. monocytogenes*.

*Listeria* mainly ends up in milk via the faeces. It may also be secreted in the milk of cows contaminated with *Listeria*.

The main source of contamination of cheeses is post-contamination as a result of lack of hygiene, mainly in the ripening rooms.

Raw meat and poultry may be contaminated with *L. monocytogenes*, and consequently meat products may also be contaminated with *L. monocytogenes*.

Presence of *L. monocytogenes* in heated food products indicates insufficient pasteurisation and/or post-contamination as result of insufficient general and/or personal hygiene.



# Sources of contamination

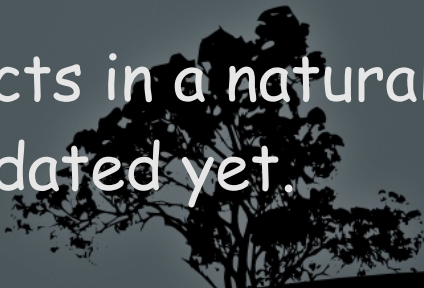
Vegetables are usually contaminated by animal faeces, contaminated with *L. monocytogenes*.

This is mainly important for lettuce and cabbages, since those vegetables are stored under refrigeration for a relatively long period, and then eaten raw.

*Listeria* has been isolated from deep-frozen fish and shellfish: 46% of the positive samples were contaminated with *L. innocua* and 28% with *L. monocytogenes*.

Data concerning the degree of contamination of seawater are not available at the moment.

Whether *Listeria* ends up in those products in a natural way or during processing has not been elucidated yet.



# Measures to prevent listeriosis

Listeriosis can mainly be prevented by avoiding food products to be contaminated with *L. monocytogenes* bacteria.

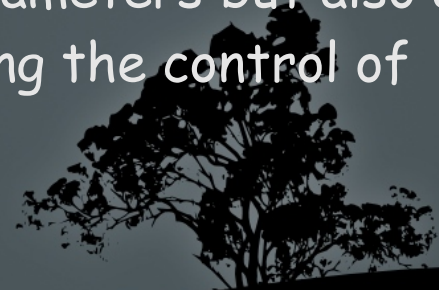
This can be obtained if the general rules of hygiene are respected, and "Good Manufacturing Practice" is pursued.

If *L. monocytogenes* bacteria are present, they can be killed by an efficient heat treatment or by irradiation of the food product with gamma-rays.




## In practice, following measures should be taken:

- a) use of recycled products possibly contaminated with animal or human waste must be avoided during production of food products ;
- b) food processing plants must be situated in a bacteriologically clean environment ;
- c) all equipment used to transport raw materials to the processing plant must be properly cleaned and disinfected ;
- d) food processing plants must be designed to prevent the entrance of animals, birds, insects and dust ;
- e) finished products must be separated from raw products to avoid cross-contamination ;
- f) food processing plants must have a comprehensive quality control program that addresses not only processing parameters but also all aspects of product environment control, including the control of personnel.



# Certain areas in the plant should receive special attention :

- a) floor and walls should receive regular sanitising, with special attention to floor drains ;
  - b) air conditioners have the potential to create aerosols that may contain *Listeria* ;
  - c) elevated cold surfaces may provide contamination through condense dripping onto food contact surfaces or into the product ;
  - d) used water or even starter cultures used in the manufacturing process of fermented food products should be routinely examined ;
  - e) disassembly and thoroughly cleaning and disinfecting tubes, valves and pumps are also required.
- 

# LESS FREQUENTLY OCCURRING FOOD INFECTIONS

Other bacteria may sporadically cause food infections, e.g. *Yersinia enterocolitica*, *Arizona*, *Proteus*, *Providencia*, *Klebsiella*, *Enterobacter* and *Pseudomonas aeruginosa*.



# VIRAL FOOD INFECTIONS

Food infections may also be caused by viruses.

The following groups play here a role:

enteroviruses, reoviruses, parvoviruses, adenoviruses and hepatitis A virus.



# Enteroviruses

are found in the intestines of human beings where they multiply and, consequently can be found in large numbers in faeces.

As a result of faecal contamination these viruses may end up in food products.

If these food products are consumed raw (vegetables) or insufficiently heated (minced meat), infection may occur.





# Enteroviruses

Polioviruses may cause infantile paralysis.

Polio-vaccination is given prevently.

Cocsacki viruses will damage the respiratory tract.

Vaccination and chemotherapy are without success.

Echoviruses affect also the respiratory tract.



# Reoviruses

Reoviruses are viruses of the bovine type and can contaminate human beings via beef and this way cause gastro-enteritis.



# Parvoviruses

Parvoviruses cause gastro-enteritis to human beings.



# Adenoviruses

Adenoviruses cause infections of the respiratory tract and of the eye membrane, and this way they can end up in food products.

Eating raw or insufficiently heated contaminated food products may cause gastro-enteritis.



# Hepatitis A virus

A person infected with hepatitis A may cause faecal or oral contamination of food products.

By consuming such contaminated product, hepatitis may occur after an incubation period of 15 to 50 days (an average of 28 days).

Hepatitis is an inflammation of the liver, characterised by fever, swollen liver and sometimes yellow skin colour.

Symptoms disappear after 2 to 4 weeks.

After this period the infected person is no longer carrier of the virus.

An infected person is a potential carrier from 7 days prior to the appearance of the symptoms.

