

# The *Listeria monocytogenes* contamination of meat products

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## Abstract

The *Listeria monocytogenes* (*Lm*) is repeatedly identified in ready-to-eat foods. Analyses performed on samples taken during state inspections showed unsatisfactory results in 1.8% of cases. Totally 2% of analyses performed on samples taken by food industry operators showed unsatisfactory results. The greatest contamination was found in sea fish (10.8%), followed by delicatessen products (3.4%), meat products supporting the growth of *Lm* (3%) and fermented meat products (1.6%). Frozen vegetables show consistently high contamination (2.5%). The contamination of raw meat is a significant factor. The *Lm* was detected in raw meat from sows (36.4% positive samples) and meat from cows (4.8% positive samples), in 1988. Positive results were found in 1.1% of samples taken from the surface of pork shoulder, 1.8% of samples from the surface of leg of pork and 0.6% of samples of mesenteric lymph nodes.

*Food contamination, Listeria monocytogenes, meat products, raw meat*

## Introduction

*Listeria monocytogenes* (*Lm*) is the causative agent of a disease (listeriosis) that is associated largely with the consumption of contaminated foods. This disease is diagnosed both in animals and in people of all age groups. High levels of *Lm* contamination are found in all kinds of foods. It is transmitted most commonly through milk, cheeses, vegetables, salads and meat and meat products.

The *Lm* is frequently an enormous complication for food business operators, including operators of storage facilities and points of sale. The reason for this is the ability of *Lm* to multiply at a wide range of temperatures from 1.5 to 45 °C (growth has even been confirmed at minus 0.2°C) and in an environment with a pH from 4.39 to 9.40 and a salt concentration of as much as 10%, as stated by, for example, Guillier et al. (2005). It must be noted that other authors have reported *Lm* resistance over even a wider range of pH and salt content in particular. The ongoing “Report on the occurrence of selected infectious diseases in the Czech Republic” over the last ten years indicates that the situation relating to the occurrence of listeriosis has stabilised in recent years and that fortunately no mass outbreak of the disease has occurred.

Table 1. Selected foodborne illnesses in the Czech Republic in the years 2005 – 2014 (number of reported cases)

Disease / Year	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Listeriosis	15	78	51	37	32	26	35	32	35	37
Salmonellosis	32 927	25 102	18 204	11 009	10 805	8 622	8 752	10 507	10 280	13 633
Shigellosis	278	289	349	229	178	450	164	266	257	92
Campylobacteriosis	30 268	22 713	24 254	20 175	20 371	21 164	18 811	18 412	18 389	20 902

Listeriosis presents a particularly serious risk to pregnant women and their foetuses and to newborn children (De Luca et al. 2015). Pregnancy is mentioned in more than 14% of all cases of this disease in humans which is around ten times more than for other forms of illness, e.g. encephalitis, etc. A higher illness rate is also seen in certain ethnic groups.

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In pregnant Hispanic women, for example, the occurrence of the disease is as much as 24 times higher than that in the general population. As the authors state, the occurrence of the disease is frequently associated with contamination of foods to a level of  $10^4 - 10^6$  CFU  $g^{-1}$ . The incubation period is usually long (2 – 6 weeks), though it may be extremely short in sick people, the older population, etc. The incubation period may also be shortened by certain drugs – antibiotics, H2 blockers, antacids, laxatives, etc.

In contrast to its systemic invasive form, gastroenteritis caused by *Lm* has a relatively short incubation period, from a few hours to 2 – 3 days (FDA 2012).

The infection gateway is most commonly the gastrointestinal tract. After penetrating the protective barriers of the intestine, *Lm* gets into the blood and is then carried to the target organs – primarily the CNS and the placenta.

Little attention is drawn to the high-risk groups of patients receiving treatment for diabetes, diseases of the liver, ulcer disease, etc. In contrast to other bacterial diseases that are associated with consumption of contaminated foods, listeriosis is characterised by the pronounced onset of symptoms associated with the subsequent need for hospitalisation and intensive treatment. The greatest complication of listeriosis is its high mortality rate (20 – 30%). Hospitalisation is necessary in around 95% of cases which results in high financial demands on treatment. It has been calculated that acute listeriosis in the USA in 1993 resulted in a financial cost of treatment of 61.7 – 64.8 million dollars (Economic Research Service/USDA). The treatment of all bacterial diseases associated with food consumption, meanwhile, resulted in an annual cost of around 2.9 – 6.7 billion dollars in the USA (Buzby et al. 1996).

As stated by Oliver et al. (2005), the financial costs of the treatment of foodborne diseases are increasing rapidly. In 2000, around 1.2 billion dollars was expended in the USA on diseases caused by the *Campylobacter* genus (all serotypes) and 2.4 billion on the treatment of salmonellosis, 0.7 billion was needed for treating illnesses caused by *E. coli* O 157:H7, another 0.3 billion for other strains of *E. coli* producing Shiga toxin, while the costs of treating *Listeria monocytogenes* amounted to 2.3 billion dollars.

In the Czech Republic, the average occurrence of listeriosis was 0.32 cases per 100 000 population in 2012. The European average was 0.41 (2012). The highest level of occurrence of human listeriosis in 2012 was reported by Finland (1.13), Spain (0.93) and Denmark (0.90 per 100 000 population).

Table 2. The mortality rate associated with *L. monocytogenes*

Country	Year	Mortality rate [%]
USA	2009 – 2011	17.6
China	1964 – 2010	26
Denmark	1994 – 2003	21
Spain	2011	20 – 30
Majorca	2002 – 2012	25
Madrid	1986 – 2007	24.3
Barcelona	2011	14

The EFSA annual report published in 2014 shows that the mortality rate for this disease was extremely high, i.e. 12.7% in 2011 and even higher a year later in 2012 (17.8%). The mortality rate for other zoonoses was much lower. In the EU (2011), e.g., the mortality rate was 0.12% for salmonellosis, 0.04% for campylobacteriosis and 0.75% for infection Verotoxigenic *E. coli* (VTEC). The *Lm* serotypes most frequently detected in 2012 were 1/2a (46.8%) and 4b (41.7%), followed by 1/2b (8.5%), 1/2c (2.7%) and 3a (0.3%).

A quite comprehensive overview on the numbers of cases of disease and the associated mortality is provided by, e.g., Hernandez-Milian and Payeras-Cifre (2014) (Table 2).

### Modes of transmission of *Listeria monocytogenes*

As has been said above, the transmission of *Lm* and the subsequent onset of the disease in human is most commonly associated with consumption of contaminated food, though this theory was not fully accepted until the 1980s. Until that time, there was only sporadic information on transmission by food. We must also not forget the proven vertical transmission from mother to foetus/child, contact with animals, contact with sick people and transmission through contaminated materials.

It has been shown that around 0.6 – 3.4% of healthy people excrete *Lm* in the faeces without known prior contact or source of contamination (FDA/USDA/CDC 2003). Other studies show between 1% and 10% of people to be germ carriers (Hernandez-Milian and Payeras-Cifre 2014). Elischerová et al. (1979) studied the occurrence of *Lm*-positive workers in meat processing plants in Slovakia. The laboratory results for, e.g., butchers were rather surprising. The highest percentages of positive results were found in workers in smoked-meat production (14.5%) and the cutting room (8.9%). Tests were also performed on 294 rectal swabs taken from sales staff in retail meat outlets, with the results showing that 2.4% of men and 3.5% of women were positive for *Lm*. There are no similar contemporary studies in this country.

This source of *Lm* should not, however, be overestimated, though certainly not underestimated as the secondary contamination of foodstuffs is not mere theory, but undeniable reality. The aim of this work is to summarise the results of analyses performed by the State Veterinary Administration of the Czech Republic (SVA CR) in 2014.

### Materials and Methods

The samples (Table 3) were taken by inspectors from the SVA CR or (in the case of commercial testing) by food business operators. Laboratory processing and assessment of the results were performed in accordance with the Commission Regulation (EC) No. 2073/2005, as amended.

The samples, approximately 250 g slices of meat from the leg or shoulder (Table 5), were taken by SVA inspectors in a processing plant exporting hams to the USA. Testing was performed according to the methodology provided by the FSIS USA in 1987 within the framework of the Meat Inspection – FSIS *Listeria* Monitoring Program.

### Results and Discussion

*Lm* occurs frequently in ready-to-eat foods. The food contamination found in the Czech Republic is given in Table 3 which was drawn up by the National Reference Laboratory for *Listeria monocytogenes*.

Only commodities associated with the production of meat products and deli products are given in the table 3. Laboratory testing was performed by the *Lm* detection method. There are at least two viewpoints that can be taken in the assessment of the results given above. The first may defend the position that the situation is extremely good and that the occurrence of *Lm* in food is under control. The merely sporadic occurrence of illness and the number of cases of listeriosis certainly support this viewpoint.

On the other hand, it could be only a matter of time as to when a mass outbreak will occur. Evidence for this is provided by a recalculation of the number of unsatisfactory laboratory results to the number of samples taken (rather than the number of analyses performed). For fermented products, the number of unsatisfactory samples would change from an original 1.6% to 7.2%. The corresponding change in the case of heat-treated meat products made from poultry would be from 1.8% to 6%, and for heat-treated meat products made from red meat from 2.1% to 4.5%. These samples were taken shortly after production, so it is unknown how high the contamination on the sales counter is.

Commercial Samples – 1.6% of the total number of analyses performed were unsatisfactory. If we apply the results determined to the number of samples taken, then 4.6% of samples

Table 3. Overview of tests for *Listeria monocytogenes* in foods (Commercial + Service purchase) (SVI 2014)

Commodity 2014	Commercial				Service purchase			
	n – samples	n – analyses detection	n – unsatisfactory analyses detection	Percentage	n – samples	n – analyses detection	n – unsatisfactory analyses detection	Percentage
Meat products supporting growth – poultry (HTMP)	1 625	5 533	97	1.8	57	267	8	3.0
Meat products supporting growth – red (HTMP)	245	521	11	2.1	1 157	5 597	95	1.7
Meat products (fermented)	14	62	1	1.6	26	122	2	1.6
Meat ready-to-cook foods	65	85	2	2.4	3	11	0	0
Delicatessen products	6	18	0	0	25	117	4	3.4
Freshwater fish	55	63	0	0	9	45	0	0
Sea fish	73	93	10	10.8	16	72	1	1.4
Vegetables	1 327	1 511	37	2.5	0	0	0	0
Total	3 410	7 886	158	2.0	1 293	6 231	110	1.8

Service purchase – sampling performed by the SVA CR; CO – sampling paid by the owner; HTMP – heat treated meat products

were unsatisfactory for the detection of *Lm*. The number of unsatisfactory samples is not particularly dramatic, though this number may be considerably higher in reality. Why?

When answering this question, we are confronted with a number of paradoxes. The first paradox results from the number of products tested and the number of registered food business operators. It also involves the selection of representative products for this testing. For testing, manufacturers generally try to send products that they are convinced to be safe and will prove satisfactory. A satisfactory result is then applied to production as a whole – the second paradox. The situation is rather different once the presence of *Lm* is demonstrated in a product and the product is adjudged to be unsatisfactory. Here the unsatisfactory result applies merely to the given batch produced – the third paradox.

There is still a prevailing opinion among a number of food business operators that the rules relating to the production of safe food and health protection are restrictive and represent barriers to their business activities. They consider the money expended on the laboratory inspection of products and HACCP verification to be money thrown away.

In view of the favourable situation relating to proven cases of listeriosis in man (Table 1), we may get the impression that there is nothing to be worry about, that we are being frightened unnecessarily. We recently saw problems with salmonellosis. If the same number of people were to fall ill with listeriosis, considering the mortality rate, the situation would be extremely serious indeed.

Service purchase – 1.7% of the total number of analyses performed were unsatisfactory. If we relate the results determined to the number of samples taken (rather than the number of analyses performed), then a total of 8.5% of samples were unsatisfactory in relation to the detection of *Lm*. Here, the numbers of samples taken in comparison with the number of registered producers may also seem something of a paradox.

Determination of the number of *Lm* – during the inspection of products in relation to determination of the number of *Lm*, totally 2 454 samples were tested, on which 6 726 analyses were performed. All the results were satisfactory.

In defence of producers, we must note that the risk of infection lies not merely in what we bring home with us in our shopping bag. We also have to transport the food we buy quickly and store it appropriately at home.

An extensive study aiming to uncover sources of the disease has shown that *Lm* was proven in around 33% of households from swabs taken from patients' household refrigerators (Economic Research Service/USDA). Similar results have been shown by, e.g., Meldrum et al. (2010), who presented the following figures on food contamination in Wales. Contamination was found at a level of 4.1% in crustaceans ( $n = 147$ ), 6.7% in smoked fish ( $n = 178$ ), 2.0% in sushi ( $n = 50$ ) and 0.9% in green salad ( $n = 335$ ). The EFSA annual report (2014) also shows the occurrence of *Lm* in food (detection method). Fish products dominate significantly (10.3%), with considerably lower occurrence in meat products (2.1%) and cheeses (0.47%).

The limit of  $100 \text{ CFU} \cdot \text{g}^{-1}$  was exceeded at the end of the use-by period in samples tested for the number of *Lm*, particularly in smoked fish (1.7%), meat products (0.43%) and hard cheeses (0.06%) (FSANZ 2009). The best way of ensuring the microbiological safety of food is proper heat treatment. The heat treatment of milk at  $69 \text{ }^\circ\text{C}$  for 16.2 seconds has been proven to reliably destroy *Lm*. For meat, an inactivation temperature of  $70 \text{ }^\circ\text{C}$  for 2 minutes is required. Exposing *Lm* to sub-lethal temperatures ( $44 - 48 \text{ }^\circ\text{C}$ ) leads to the increased thermotolerance (Farber and Peterkin 1991).

There is, unfortunately, a myth that freezing is an adequate and appropriate pasteurisation or sterilisation process for foods. We come across this myth, and even the freezing of fermented meat products for the purpose of inactivating *Lm*, relatively frequently. Although bacteria cannot multiply when products are frozen, they are well able to survive (starter cultures are supplied in a lyophilised state). Salmonella, for example, has been found to survive in ice cream for 7 years at a temperature of  $-23 \text{ }^\circ\text{C}$ .

Beverly (2004) states that the critical period for the survival of *Lm* in ready-to-eat frozen poultry meat products ( $-18 \text{ }^\circ\text{C}$ ) is the first 30 days. A reduction to the number of *Lm* of  $0.2 - 0.8 \log \text{ CFU} \cdot \text{g}^{-1}$  of the original contamination may occur, with the method of packing and the type of meat being extremely important. A reduction to the number of *Lm* is evident in products packed in a vacuum and stored at  $-18 \text{ }^\circ\text{C}$ . Decline in products stored without a vacuum is insignificant even after 90 days.

The figures reported by Porto et al. (2004) contrast to the above study, with the freezing of frankfurters (pork/beef) at  $-18 \text{ }^\circ\text{C}$  having no significant effect in reducing the number of *Lm*.

During freezing, intracellular and extracellular ice crystals may cause the death of bacterial cells. This process is, however, accompanied by damage to the structure of meat products. Rapid freezing has a considerably smaller destructive effect on food, though it also does not destroy bacteria. Freezing has no reductive effect on bacterial toxins and enzymes that may cause food spoilage. We must also give a mention to psychrotrophic moulds (black stains, white stains) that also grow at  $-5 \text{ }^\circ\text{C}$  to  $-10 \text{ }^\circ\text{C}$  and cause the spoilage of frozen foods.

Freezing increases the sensitivity of *Lm* to the action of the lysozyme and lipases that occur naturally in foods and food ingredients. On the other hand, for example, certain components of milk and dairy products may provide bacteria with additional protection, first and foremost lactose, casein and milk fat. This protection is not, however, the only reason for positive findings of listeria in ice cream and other products even after long periods of storage (Beverly 2004). *Lm* is, similarly to other pathogenic bacteria, genetically well equipped for this process of cold treatment.

Other cryoprotective substances are also well known in practice. Glycerol (E422), for example, protects bacteria during freezing. It makes up part of fat molecules. It is used as a sweetener, a solvent, etc. in foods. It can also be used to treat the surface of meat

and cheeses during processing, as a solvent in alcoholic and non-alcoholic drinks, bakery products and smoked products, or as a humectant in treating the surface of confectionary, icings and grated coconut against drying. Thanks to glycerol, foods may contain a large amount of water without spoiling because the water bound to the glycerol is unavailable to bacteria (El-Kest and Marth 1991). The content of intramuscular fat and the content of fat in meat products also plays a considerable protective role. The reason for this is again the content of glycerol in fat and fat molecules.

#### The infectious dose of *Listeria monocytogenes*

The infectious dose cannot be determined precisely. It is different for pregnant women, newborns, older people and ill people, etc. According to figures from the FSANZ (2009), an infectious dose within a range of  $1.31 \times 10^8$  to  $5.01 \times 10^{11}$  has been determined. There is, of course, a significant difference between individual serovars, and the infectious dose greatly depends on the application of the individual virulence factors of individual isolates.

Experts in the USA estimate that around 0.2% of the total number of 2 500 cases of human listeriosis are caused by the consumption of food containing  $Lm < 100 \text{ CFU} \cdot \text{g}^{-1}$ . In contrast, more than 80% of cases are caused by contamination of foodstuffs with  $> 1.10^6 \text{ CFU} \cdot \text{g}^{-1}$ . Another high-risk group are people with AIDS who are 850 times more sensitive to *Lm* infection than healthy individuals.

As has already been said, *Listeria monocytogenes* is a significant pathogen for animals and humans and does not require any particular specificity from the individual attacked. The infection develops in a number of steps (Jemmi and Stephan 2006):

- The entry of the bacteria into the host cell
- Lysis of the phagosomal vacuole
- Reproduction in the cytosol of the host cell
- Direct entry to neighbouring cells by means of motion exploiting host cell actin

Ribet and Cossart (2015) note that the respiratory, digestive and urogenital systems represent a total mucosal area of 300 to 400 m<sup>2</sup> which is 200 times larger than that provided by the skin. This area is exposed to continual attack from saprophytic and pathogenic bacteria. These bacteria must overcome the natural barriers of the mucosa, following which they adhere to the surface of cells and penetrate into the body. In order for the intestinal barrier to be overcome and for listeria to penetrate into the organism, they must be present at a concentration exceeding  $10^6 \text{ CFU} \cdot \text{g}^{-1}$  of food. Infection may also occur through the skin, for example in vets coming into contact with infected birth canals or in butchers coming into contact with contaminated material (Duben 2007).

#### The reservoir of *Listeria monocytogenes*

External – livestock animals, hunted game and game meat, and domestic pets are all a significant reservoir and source of listeria. In its final report, the EFSA (2014) states figures for *Lm* positivity in the faeces of cattle showing that 1.5% of samples were positive. *Lm* is also found in soil and fresh vegetables – it is an ubiquitous bacteria that may occur anywhere around us and that cannot be eliminated. It has long been known that *Lm* may be spread in the environment by wild birds. Significantly greater seroprevalence has been found in birds that feed on scraps from waste dumps and other sources of waste than in birds living in the natural landscape (Hellström et al. 2008). Insects tend to be carriers rather than a reservoir of *Lm* (OIE Terrestrial Manual 2014).

Internal – *Lm* may colonise the gall bladder without significant restriction and get into the intestine within 5 minutes of a signal for the excretion of bile. Bile supports the growth of certain bacteria, including *L. monocytogenes*, *Lactococcus lactis*, *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. The number of *Lm* and *Listeria innocua* doubles in porcine bile in around 52 minutes at 37 °C. Bile is not

a significant stress factor for listeria (Dowd et al. 2011). *L. monocytogenes* has been isolated from the faeces of healthy people, and has also been shown to cause cholecystitis in man. *Listeria monocytogenes* has, for example, been isolated from a pure culture from the gall bladder of a dog in connection with liver disease (Marien et al. 2007).

#### Contamination of raw meat

Duffy and Danaher (2014) have presented figures on the contamination of raw beef meat with *Lm*. A total of 0.8% of samples contained *Lm* at an amount  $< 100 \text{ CFU} \cdot \text{g}^{-1}$  and another 0.2% at  $> 100 \text{ CFU} \cdot \text{g}^{-1}$ . Khen et al. (2015) state that *Lm* contamination on beef hide in Ireland in 27% of samples. The meat surface was contaminated in 14% of cases, while 29% of samples of minced meat on the retail network were positive (highest values  $100 - 200 \text{ CFU} \cdot \text{g}^{-1}$ ).

Different figures were found in Poland (Wieczorek and Osek 2010) where *Lm* was detected on beef hide in 10.8% of samples and 2.5% of samples of beef meat were positive. Contamination was dominated by serotype *1/2a* (87%), with serovars *1/2c* and *4b* being less dominant (both serovars were found in only 2 of the 31 samples tested). As far as the legislation is concerned, the cleanliness of hide is covered by Regulation (EC) No. 853/2004 which states that animals for slaughter must be clean. There is no unambiguous answer to the question as to whether there is a correlation between soiled hide and the occurrence of pathogens – VTEC, for example. A positive relationship has, in contrast, been demonstrated between clean hide and a lower level of contamination of the surface of the carcass (Duffy and Danaher 2014).

Intervention leading to reduced microbial contamination of the carcass (Duffy and Danaher 2014) is based primarily on treating the carcass surface – washing with hot or cold water, spraying with organic acids, etc. The Commission Regulation (EU) No. 101/2013 permits the use of lactic acid to reduce surface microbial contamination of cattle carcasses. Spraying or misting with a 2 – 5% solution of lactic acid in drinking water at a maximum temperature of 55 °C is permitted. Controllable and verifiable conditions and quality system integration (HACCP) are required for use.

The prevalence of *Lm* in raw meat can differ greatly. The number of positive samples in various countries is given in Table 4 (Jemmi and Stephan 2006).

Table 4. The prevalence of *Listeria monocytogenes* in raw meat (Jemmi and Stephan 2006)

Country	Meat	n	Positive [%]
Italy	Species not known	113	8
Belgium	Poultry	772	38
UK	Poultry	100	60
Denmark	Minced meat	67	28
France	Species not known	112	17
	Minced beef	34	21
Japan	Minced meat	400	11

n – number of samples

The given positive results are significantly higher than those found in the laboratory in 1988 (Table 5).

Bacteria (pathogenic bacteria and spoilage agents) are present to a large degree in cut meat. They get onto the surface of the meat during handling and carcass processing at slaughterhouses, cutting rooms and cold stores during any kind of action in which knives and other equipment get into the depth of the meat where the bacteria may reproduce. Unfortunately, the presence of pathogenic bacteria cannot be directly evaluated from the total number of bacteria. Animals for slaughter themselves represent another reservoir of

Table 5. Results of tests on raw meat in 1988 (SVI, Jihlava)

Sample	n	<i>Lm</i>	[%]	<i>L. innocua</i>	[%]
Shoulder of pork	89	1	1.1	5	5.6
Leg of pork	106	17	1.8	64	6.6
Sow meat	11	4	36.4	3	27.3
Lymph node	164	1	0.6	0	0
Cow meat	42	2	4.8	2	4.8
Calf meat	13	0	0	0	0
Frozen beef meat	17	1	5.9	10	58.8
Ham	118	0	0	0	0

n – number of samples

*Lm*, in particular pig tonsils. As is stated in the literature, contamination of tonsils may fall within a range of 12 – 14% (in Finland, for example). A higher incidence has been found in young pigs for slaughter (22%) than in sows (6%). Pigs from certain farms in Germany showed tonsil contamination of 35 – 45% (Autio et al. 2000).

### Stressors in food production

Stress factors that are routinely applied in the processing, production and storage of foodstuffs (Table 6) are a significant hurdle to the survival and growth of bacteria in ready-to-eat foods.

Table 6. Factors directly influencing the survival and growth of *Lm* (Ocete del Pino and Gómez Rojo 2015)

Factor	Growth			
	Lowest limit	Optimum	Higher limit	Survives
Temperature [° C]	-1.5 – 3.0	30 – 37	45	-18
pH	4.2 – 4.3	7	9.4 – 9.5	3.3 – 4.2
Salt [%]	< 0.5	0.7	12 – 16	> 20
$a_w$	0.9 – 0.93	0.99	> 0.99	< 0.90
Atmosphere	Survives the presence and absence of oxygen, facultative anaerobic bacteria			

### Conclusions

The *Lm* is repeatedly detected in ready-to-eat foods. Food business operators do not frequently perform laboratory analyses of products in such a way that would represent individual production groups (small meat products, soft salamis, durable and special meat products, etc.)

Food business operators do not perform shelf-life studies in accordance with Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foods, as amended. A mistake by somebody or other always lies behind each case of human illness (or death) connected with contaminated food.

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