## LISTERIA MONOCYTOGENES AND HYGIENIC QUALITY OF RAW MEAT

Alexandra Esteves, Cristina Saraiva, Paulo Ribeiro, Luis Patarata & <u>Conceição Martins</u> Universidade de Trás-os-Montes e Alto Douro, P-5001 Vila Real, Portugal

Keywords: Listeria spp; Listeria monocytogenes; hygienic quality; raw meat

### Abstract

42 samples of 6 types of prepacked and refrigerated raw meat; chicken, turkey, pork, beef, minced beef and pork spit<sup>\*</sup> were obtained in local market and monitored for *Listeria spp.* and *Listeria monocytogenes*. Total count of mesophilic microorganisms, search for coliform bacteria, *Escherichia coli* and sulfite reducing *Clostridium* were made. *Listeria spp.* occured in 69% of the samples, and *Listeria monocytogenes* was found in 17%. No relationship was found as to the presence of *Listeria* either in relation to the total plate counts of mesophilic microorganisms, or to the level of contamination with *Escherichia coli* and sulfite reducing *Clostridium*.

#### Background

Listeria monocytogenes arises as one of the new "enigma" in food microbiology, largely spread in the environment. It was isolated from a great variety of animal origin food products. Only in 1953 was it proved by Potel for the first time, that there was a direct connexion between the animals and the human listeriosis of food origin. Although most listeriosis cases caused by food are related to dairy products, some sporadic cases associated with meat and meat products have been recently reported (Farber, 1991). The high rate of mortality listeriosis causes justifies our concern, specially because we are dealing with an ubiquitous microorganism, wich can contaminate food products at any critical moment of its production (Fernandes & Sol, 1990). The use of new manufacturing technologies, along with the use of chilling networks at different moments of the food circuit, allows bacteria such as *Yersinia* and *Listeria*, which are capable of reproducing at low temperatures to cause more frequent infections (Freire, 1991). Studies carried out in other countries indicate a relatively high level of contamination with *Listeria spp*. in all type of fresh meats. According to Bernardo (1994), about 30% of the poultry and beef meat comercialized in Portugal analyzed in his work had *Listeria monocytogenes* in 25g.

Pork spit: pieces of raw pork and vegetables (pepper, onion), pierced on a wood spit, ready to grill.

#### **Objectives**

Determination of the contamination with *Listeria spp* and *Listeria monocytogenes* in fresh meat. Due to its specific ecology, the relationship between the presence of *Listeria* in the samples and the higienic quality of those samples was also analyzed.

#### Methods

Samples: Six types of prepacked and refrigerated raw meat, chicken and turkey, pork, beef, minced beef and pork spit in a total of 42 samples, were obtained in the local market.

Detection of *Listeria* in 25g: Pre-enrichment: *Listeria* enrichment broth (Oxoid 862) 24 h at 30°C; Enrichment: *Listeria* selective enrichment broth (Oxoid 862, SR 141) 24 h at 30°C; Isolation: Oxford agar (Merk 7004, 7006) 24-48 h at 37°C; Identification: typical colonies with black zones around on Oxford agar; blue to blue grey colour under Henry's illumination; catalase positive; oxidase negative; Gram positive; fermentation of rhamnose but not xylose; motility giving a typical umbrella like growth pattern;  $\beta$  heamolitic activity; the identified strains were confirmed using API *Listeria* system (BioMérieux 10300; France).

Total count of <u>mesophilic microrganisms</u>, <u>coliform bacteria</u>, <u>Escherichia coli</u> and <u>sulfite reducing Clostridium</u> were performed according to Portuguese standard methods NP1995 (1982); NP2164 (1983); NP2308 (1986) and NP2262 (1986) respectively.

The Student Newman Keuls test was used to evaluate the significance of the differences among the results of the counting of total mesophilic microorganisms.

#### **Results and Discussion**

It was observed that 31% (13 out of samples) were absented of *Listeria*. Only 17% of the samples (7 out of 42) presented *L.* monocytogenes in 25g, wich might, eventually, represent a risk for the consumer's health. However, according to the majority of the authors, the absence of *L monocytogenes* in 25 g of raw meat is an exigence not practicable, so, they propose an acceptable level of 100 c.f.u./g of this bacteria (Moreno & Garcia, 1993). The presence of *L.spp.* and *L. monocytogenes* do not appear to be related with the type of meat, as in all of them it was found a high number of samples contaminated with these microorganisms. Curiously, it was in the products subject to higher manipulation (minced beef and pork spit), that less number of samples with *L.spp.* and *L. monocytogenes* in 25g were found.

The average counting of mesophilic microorganisms and the number of samples of each type of meat that exceed the established limit for coliform bacteria, *Escherichia coli*, sulfite reducing *Clostridium*, *Listeria spp* and *Listeria monocytogenes* are presented on table 1. The

mean values of total counting of mesophilic microorganisms varied from  $6.9\pm0.4 \log c.f.u./g$  to  $8.3\pm1.1 \log c.f.u./g$ . No significant differences were found among the different types of meat for this counting, indicating that all samples had a similar contamination. However, it is possible to observe that the smallest counting was found on minced beef, in contrast to the expected results, as these are the samples wich had more risk of contamination. No relationship between the level of mesophilic microorganisms and the ocurrence of *Listeria* was found on the analyzed samples. These results agree with the statement of Holland (1979): "The aerobic plate counts are usually a poor way to predict the probability of a food to contain pathogenic microorganisms" (quoted by Vorster *et al.*, 1993). However, Vorster *et al.* (1993), working on meat products, concluded that meat products with total counts between 5 and 7 log c.f.u./g, appear to be at greater risk of contamination with *Listeria* than those with less or higher contamination. These authors concluded that samples with low total counts have less chance of being contaminated with this pathogen, and the absence of *Listeria* in the samples with higher countings could be explained by competition.

As it is possible to observe in Table 1, these samples had a high level of coliform bacteria, *Escherichia coli* and sulfite reducing *Clostridium* indicating a low higienic quality. It was an objective of this work to obtain a relation between the presence of *Listeria* and the source of contamination, namely faecal, indicated by the presence of a high level of *E. coli*, and teluric, by the presence of sulfite reducing *Clostridium*, once *Listeria monocytogenes* is a foodborne pathogen that can be isolated from diverse environments including soil, water, animal feed and faeces (Ryser and Marth, 1991).

Regarding the individual results of each sample it was observed that only in 14 out of the 29 samples it was possible to obtain a relation between the presence of *Listeria spp.* and the source of contamination. Thus, in 11 samples with *L.spp.* it is possible to suspect that the contamination could have been of faecal origin, as these samples presented a high level of *E.coli*, (present in more than 0.01g) with a moderate contamination by sulfite reducing *Clostridium*. In 3 samples the source of contamination might have been environmental, because a high number of sulfite reducing *Clostridium* (present in more than 0.01g) was found in samples showing a reduced presence of faecal contamination indicators. In the remaining 15 samples no relationship was found.

## Conclusion

17% of the analyzed samples presented *Listeria monocytogenes* in 25g. That prevalence is in agreement with the results obtained by other authors. The majority of the samples presented a low hygienic quality regarding the number of samples that exceed the established limit for coliform bacteria, *Escherichia coli*, sulfite reducing *Clostridium*. No relationship between the presence of *Listeria* and the total count of mesophilic microorganisms was found, and it was difficult to obtain a relationship with the source of contamination, namely faecal or teluric. The reduced number of samples analysed in this work do not lead us take conclusions about the real incidence of these microorganisms in fresh meats, so it is necessary to carry more research viewing obtain a real evaluation of the situation.

# References

Bernardo, F. M. A., 1994. A carne: Processos de obtenção e qualidade. In Meeting: A indústria de carnes - Uma perspectiva integrada. pp 46. Farber, J. M., 1991. *Listeria monocytogenes*. J. Assoc. of Anal. Chem., **74** (4): 701-704

Fernandes, M. C. L. F. & M. M. Sol, 1990. Novas perspectivas analíticas no âmbito da Microbiologia Alimentar. LNETUDTIA, 1-24. Freire, L., 1991. Yersinia e Listeria - Métodos de Isolamento e Riscos Alimentares. Revista Portuguesa de Medicina Veterinária, 39 (4): 31-33. Moreno, b. & M. C. Garcia, 1993. Listeria monocytogenes en Carne y Produtos Cárnicos: possibles normas microbiológicas. Cárnica 2000: 114 (Jun): 41-54.

Norma Portuguesa 1995, 1982. Microbiologia Alimentar. Regras gerais para contagem de microrganismos a 30ºC.

Norma Portuguesa 2164, 1983. Microbiologia Alimentar. Regras gerais para pesquisa de bactérias coliformes.

Norma Portuguesa 2262, 1986. Microbiologia Alimentar. Regras gerais para pesquisa de esporos de clostrídios sulfito-redutores.

Norma Portuguesa 2308, 1986. Microbiologia Alimentar. Regras gerais para pesquisa de Escherichia coli.

Ryser E. T. & E. H. Marth, 1991. Listeria, Listeriosis and Food Safety. Marcel Dekker, Inc., New York, NY. pp. 22-32

Vorster, S. M. R. P. Greebe & G. L. Nortjé, 1993. The incidence of *Listeria* in processed meats in South Africa. Journal of Food Protection, 56 (2): 169-172

 Table 1 - Average of counting of mesophilic microorganisms and number of meat samples that exceed the established limit for coliform bacteria, Escherichia coli, sulfite reducing Clostridium, Listeria spp and Listeria monocytogenes.

Samples	N	Mean of counting of mesophilic microorganisms log c.f.u./g	Coliforms 0.001 g	<i>Escherichia</i> <i>coli</i> 0.01 g	sulfite reducing <i>Clostridium</i> 0.01 g	Listeria spp 25 g	Listeria monocytogenes 25 g
Chicken	7	8.2 <sup>a</sup> +1.2	4	6	1	5	1
Turkey	7	$8.3^{a} + 1.1$	7	5	1	4	2
Pork	7	$7.4^{a} + 1.4$	4	4	3	6	2
Beef	7	$7.6^{a} + 1.4$	6	5	2	6	2
Mince Beef	7	$6.9^{a} + 0.4$	6	1	4	5	0
Pork spit	7	8.2 <sup>a</sup> ±1.0	7	7	4	3	0

-means followed by similar letters do not differ significantly (P>0.05)