Prevalence and characterization of Salmonella and Listeria monocytogenes in french raw pork meat at the distribution level

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Abstract

This study was undertaken in 2010 to estimate the occurrence of Listeria monocytogenes in raw pork meat at the distribution level in France.

A total of 320 samples (minced pork meat, pork chop, fillet and roast, and other various pieces) have been collected at the distribution level in various geographical areas throughout France. Sampling was done over the year. Detection and enumeration were conducted as described in ISO methods. All isolates were serogrouped by PCR and genotyped by a standardized PFGE method.

L. monocytogenes was detected in 12.8% of the samples (41 on 320). Eight samples could be numerated and number of ufc/g varied from 10 to 730. Minced pork meats were particularly contaminated (25%). The 159 isolates of L. monocytogenes were serogrouped by PCR; 57, 11, 75, 16 isolates were respectively from serogroup IIa, IIb, IIc and IV. PFGE, after ApaI and AscI restriction, generated 33 and 23 PFGE types respectively. Most often, isolates of L. monocytogenes from a same sample highlighted the same serogroup and the same PFGE pattern except one sample from which the 8 isolates were distributed in 3 serogroups and 5 PFGE patterns. Diversity of genotypes was higher among isolates from minced meat with 20 combined profiles. Few genotypes were common between minced meats, cut meats and others pieces.

This study provided recent valuable information on the occurrence of L. monocytogenes (13.3%) in raw pork meat at the distribution level. Minced pork meats were particularly contaminated and various genotypes of L. monocytogenes were found indicating that various source of meats and transformation of meat increase the risk to contaminate minced pork meat.

Introduction

The facultative intracellular bacterium Listeria monocytogenes is capable to cause a severe invasive illness in human (listeriosis). This ubiquitous bacterium is widely distributed in the environment and infection occurs through ingestion of contaminated food (Schlech, et al.,, 1983). In France, the joined efforts of the government and food producers have led to decrease significantly the incidence of listeriosis in the past 20 years and thereby the number of epidemics. However, the recent observation of increasing number of listeriosis cases (Goulet, et al.,, 2008) in most of the industrialized countries calls up to reinforce the epidemiological surveillance. Pork meat and processed pork products have been the sources of outbreaks of listeriosis in France and in other European countries during the past decade (Jacquet, et al.,, 1995; Jay, 1996; Loncarevic, et al.,, 1997; Goulet, et al.,, 1998). So, the aim of this study was to estimate for 2010 the occurrence of Listeria monocytogenes in raw pork meat and to characterize isolates. Several methods have been used to differentiate L. monocytogenes strains (Liu, 2006). We chose here to characterize the isolates by PCR (Kerouanton, et al., 2010) and by pulsed-field gel electrophoresis (PFGE), considered accurate for epidemiological investigations and of help for surveillance and control of listeriosis (Kerouanton, et al.,, 1998).

Material and Methods

Samples

A total of 320 samples (112 minced pork meats, 120 cut meats (pork chop, fillet and roast), and 88 other various pieces) have been collected at the distribution level in various geographical areas distributed on all France. Sampling was done over the year 2010.

Detection and enumeration of L. monocytogenes

Detection and enumeration were conducted with adaptation of the NF EN ISO 11290-1, NF EN ISO 11290-1/A1 and

NF EN ISO 11290-2 methods. Typical isolates on ALOA were subcultured on TSA-YE plates and stored at -80°C in glycerol peptone broth.

Molecular serotyping

DNA extraction was performed from fresh bacterial cultures on TSA-YE plates using Instagene kits (BioRad, France). The multiplex PCR assay method was performed following the recommandation of Kerouanton et al. (Kerouanton, et al., 2010). Listeria genus recognition was ensured by detection of the prs gene. The amplification mix of 25µl, with 0,5µl DNA, consisted of 1U of Taq DNA polymerase (Faststart, Roche); 1x fast-start Buffer without MgCl2, 2mM MgCl2, 0.2mM dNTPs, 0.4µM of each of the following primers: lmo0737 1 and lmo0737 2; lm31118 1 and lm31118 2; orf2110 1 and orf2110 2; and 0,2µM of the other primers prs1 and prs2, lip1 and lip2a. The cycling program consisted of initial denaturation for 3 min at 94°C, followed by 35 cycles of denaturation at 94°C (40s), annealing at 53°C (45s), extension at 72°C (1min15s) and a final extension at 72°C (7 min).
Amplified PCR fragments were separated by 2% agarose gel electrophoresis in 1x Tris borate EDTA buffer and visualised by GelRed staining (Interchim, France))

PFGE Typing

DNA plugs were prepared from fresh bacterial cultures on TSA-YE plates. PFGE was performed according to the CDC PulseNet standardized procedure for typing L. monocytogenes (Graves, 2001). DNA was digested at 37°C for 4 h with two different macrorestriction enzymes, ApaI or AscI. Restriction fragments were separated in a 1% SeaKem Gold agarose gel, using the CHEF method in a CHEF-DRIII apparatus. The following electrophoresis conditions were used: voltage (6 V cm) 1; initial switch time, 4 s; final switch time, 40 s; run time, 21 h. XbaI-digested DNA from Salmonella Branderup H9812 was included, as a reference, in all PFGE gels (Hunter, et al.,, 2005).
Electrophoretic patterns were compared using BioNumerics® (Applied Maths, Sint-Martens-Latem, Belgium). Similarities between profiles were determined by calculating the Dice correlation coefficient, with a maximum position tolerance of 1%. A dendrogram based on the combined results for ApaI- and AscI -digested DNA (KS) was constructed. Strains were clustered by the unweighted pair-group method using the arithmetic mean (UPGMA) (Struelens, 1996). The Simpson's index (D) was determined as described by Hunter (Hunter, 1990).

Results

L. monocytogenes was detected in 12.8% of the samples (41 on 320). The prevalence was significantly more important in minced pork meat (25%) (Table 1). Eight samples could be numerated and number of ufc/g varied from 10 to 730.

Table 1. Prevalence of L. monocytogenes in pork meat

Pork meat	Number of sample			
	Positive	(%)	Negative	Total
minced pork meat	28	25	84	112
pork chop, fillet and roast (cut meat)	5	5	115	120
other various pieces	8	9	80	88
Total	**41**	**12.8**	**279**	**320**

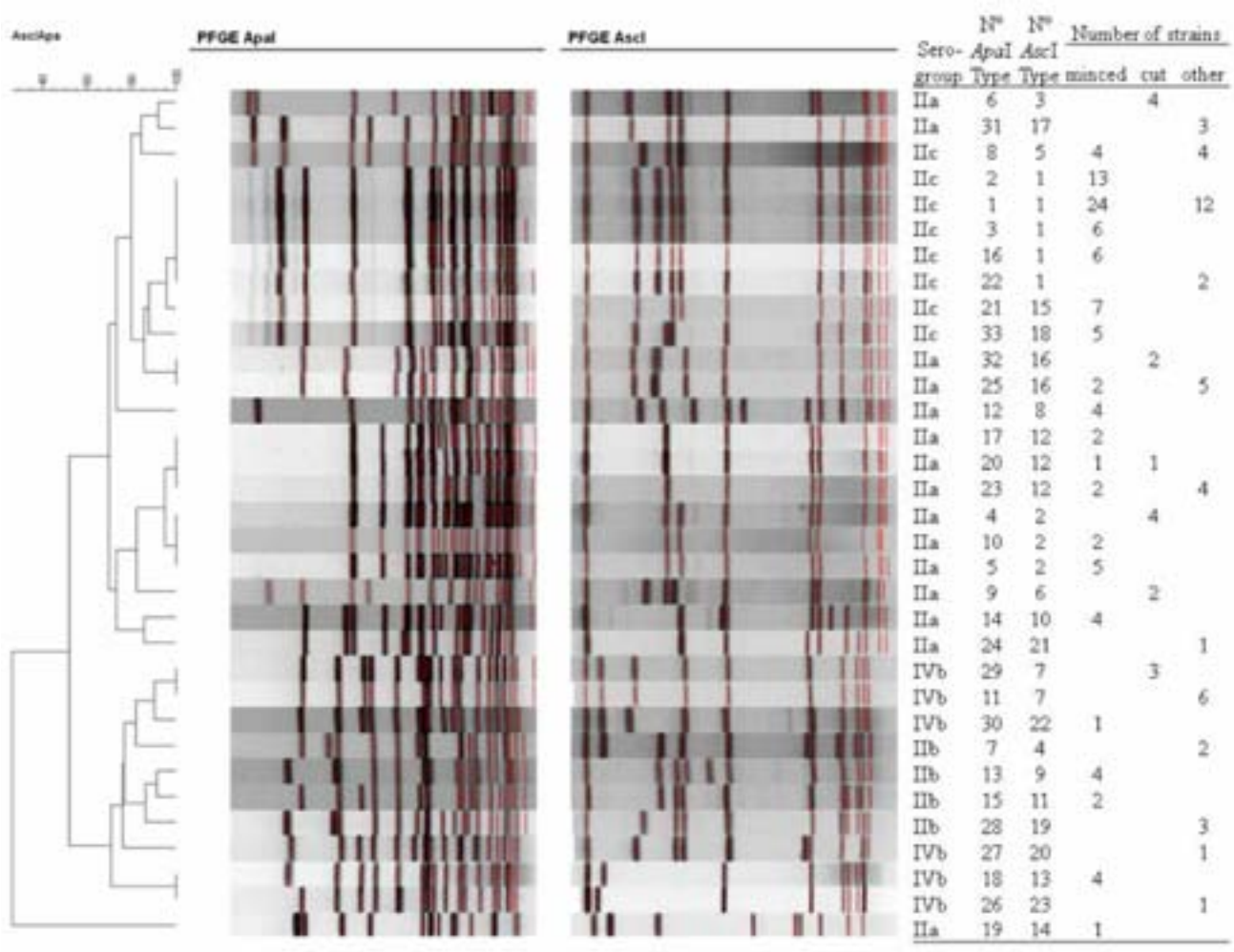
By multiplex PCR, 159 isolates have been confirmed as L. monocytogenes. They were distributed in four serogroups (Table 2). The major serogroup identified was IIc (serotype 1/2c or 3c) with 75 isolates (47%). Others serogroups were IIa (57 isolates), IIb (11 isolates) and IVb (16 isolates). Serogroups IIa and IVb were found whatever the type of meat.

Table 2. Distribution of the isolates according to their serogroup and source.

	PCR serogroups				Total
	IIa	IIb	IIc	IVb	
minced pork meat	27	6	61	5	99
pork chop, fillet and roast (cut meat)	13	-	-	3	16
other various pieces	17	5	14	8	44
Total	57	11	75	16	159

PFGE after ApaI and AscI restriction generated 33 and 23 PFGE types respectively (figure 1). The discriminatory index of the method for the combined results was of 0.93.

Figure 1. Distribution of the isolates according their PFGE patterns and source



Sero-group	N° ApaI Type	N° AscI Type	Number of strains minced	cut	other
IIa	6	3		4	
IIa	31	17			3
IIc	8	5	4		4
IIc	2	1	13		
IIc	1	1	24		12
IIc	3	1	6		
IIc	16	1	6		
IIc	22	1			2
IIc	21	15	7		
IIc	33	18	5		
IIa	32	16		2	
IIa	25	16	2		5
IIa	12	8	4		
IIa	17	12	2		
IIa	20	12	1	1	
IIa	23	12	2		4
IIa	4	2		4	
IIa	10	2	2		
IIa	5	2	5		
IIa	9	6		2	
IIa	14	10	4		
IIa	24	21			1
IVb	29	7		3	
IVb	11	7			6
IVb	30	22	1		
IIb	7	4			2
IIb	13	9	4		
IIb	15	11	2		
IIb	28	19			3
IVb	27	20			1
IVb	18	13	4		
IVb	26	23			1
IIa	19	14	1		

Diversity of genotypes was higher among isolates from minced meat with 20 combined profiles. Most often, combined PFGE was specific to one type of pork meat. Sixteen were only found for strains isolated from minced pork meat, 6 for strains isolated from cut meats (pork chop, fillet and roast), and 7 for strains isolated from other various pieces. Six of the seven combined profiles found among cut meat isolates were specific to this origin. Thus, only 3 profiles were common to minced pork meat and other various species of meat and 1 was common to minced and cut meats. None was common to the 3 origins. Most often strains from a same sample highlighted the same serogroup and the same PFGE pattern. Only 4 samples showed diversity of isolates and 1 was particularly poly-contaminated; the 9 isolates studied from this sample were distributed in 3 serogroups and 5 PFGE patterns.

Discussion

Occurrence of L.monocytogenes observed in this study is high (12.8%) with 41 positive samples on 320. But only 8 of the 320 samples could be enumerated for the presence of this pathogen which means that 33 samples were with less than 10 UFC/ g of meat.

Most of isolates (47.1%) in our study were from serogroup IIc (1/2c or 3c strains). These serotypes have been recently associated to pork meat in a Japanese study (Ochiai, et al.,, 2010).The percentage of isolates with serotype 1/2c was previously shown to be higher on pork products (Thevenot, et al.,, 2006). Furthermore, the serotypes found in this study are identical to those usually involved in human listeriosis in Europe (Goulet et al. 2008).

The isolates displayed a very high level of genetic diversity; 33 combined PFGE patterns were obtained, However only 8 PFGE combined ApaI/AscI patterns have been highlighted for the 75 IIc isolates; these serotypes 1/2c or 3c are known

to be genetically homogeneous (Ragon, et al.,, 2008).
Minced pork meats were particularly contaminated and various genotypes of L. monocytogenes were found; different sources of meat and transformation could explained this result.

Conclusion

This study provided recent valuable information on the occurrence of L. monocytogenes in raw pork meat at the distribution level (12.8%). Minced pork meats were particularly contaminated and various genotypes of L. monocytogenes were found indicating that various source of meats and transformation of meat increase the risk to contaminate minced pork meat. However, the predominance of serogroup IIc isolates, rarely associated with listeriosis, could suggested than pork meat is not the major food source of listeriosis.

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