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CHARACTERISATION OF LISTERIA MONOCYTOGENES FROM PIGS AND PORK AT A SLAUGHTERHOUSE USING RANDOM AMPLIFICATION OF POLYMORPHIC DNA; A PILOT STUDY

A.M.G. VAN DEN ELZEN, C.C.M. KLAASSEN, P. VOSKAMP AND J.M.A. SNIJDERS

Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.175, 3508 TD Utrecht, The Netherlands. Tel. 00-31-30535367, fax 00-31-30532365.

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Introduction

Listeria monocytogenes is a human pathogen, ubiquitair in nature, which recently caused an outbreak of listeriosis in France associated with pork tongue in jelly. L. monocytogenes is frequently found in high numbers on raw foods of animal origin (Jansen et al. 1993). Although faeces and skin are considered sources of L. monocytogenes contamination, lately the slaughterhouse environment has more often been implicated as an important source. Recent studies showed a much higher incidence of L. monocytogenes in the environment of the cutting room as well as on the primal cuts produced in this slaughterhouse area compared to the incidence in previous stages of pig slaughtering (Wendtland and Bergann, 1994; Van den Elzen and Snijders, 1993). These studies indicate the high potential of meat contamination by environmental sources in the cutting room. This pilot study was undertaken to characterise the L. monocytogenes strains isolated from different pig/pork samples with Random Amplification of Polymorphic DNA analysis (RAPD). RAPD has proven to be a valuable tool in studies on epidemiology of L. monocytogenes (Mazurier et al., 1992; Mazurier and Wernars, 1992) and was used to discriminate between L. monocytogenes strains isolated from faeces and skin in the lairage and from primal cuts in the cutting room area of a Dutch pig slaughterhouse.

Materials and methods

Sampling procedures; In a Dutch pork slaughterhouse, 198 samples were taken on two consecutive days. On both days, samples were taken from faeces and skin of pigs in the lairage and from hams, bellies and shoulders in the cutting room area. Faeces were collected rectally, by means of a sterile stomacher bag. Twentyfive gram portions were diluted in 225ml buffered pepton water and macerated in a stomacher for 2 minutes. Surface samples were taken from skin, hams, bellies and shoulders as described by Van den Elzen and Snijders (1993).

Isolation and identification of L. monocytogenes; Isolation principally followed the revised USDA-FSIS protocol (1989), except that 10ml of the surface sample homogenisate and 25ml of the faeces sample homogenisate were added to 10ml and 25ml respectively of double-strength UVM broth. For identification the same comfirmation tests were used as described by Van den Elzen and Snijders (1993). Whenever possible, ten strains of L. monocytogenes were isolated from each sample. Suspected strains were also confirmed serologically using polyvalent (Difco#2302-50), serogroup 1 (Difco#2300-50) and serogroup 4 (Difco#2301-50) antisera. Strains positive for L.monocytogenes were stored at -80°C.

RAPD analysis; L. monocytogenes strains were subcultured in Brain Heart Infusion overnight at 37°C. RAPD analysis was performed as described by Mazurier and Wernars (1992). Primers HLWL74 (5'-ACGTATCTGC-3') and HLWL82 (5'-CGGCCTCTGC-3') were used in the PCR reactions.

Results and discussion

In total, 61 samples were positive for L. monocytogenes and provided 500 isolated strains. Only 1 (1,3%; 6 strains) faeces sample and 1 (5,5%; 4 strains) skin sample were positive for L. monocytogenes. The high incidence of L. monocytogenes on the primal cuts are in accordance with the results of previous studies (Wendtlan and Bergann, 1994; Van den Elzen and Snijders, 1993). Of shoulders, bellies and hams, 79%, 67% and 14% respectively were contaminated with L. monocytogenes. In addition to serogroup 1 isolates, isolated

TABLE 1 Listeria monocytogenes on pigs and pork in certain areas of a slaughterline; their incidence, serotype and RAPD profile (obtained with primer HLWL74 and HLWL82)

Area	Sample	Number samples	Positive samples for Listeria spp	Positive for L.mon.	Serotypes	HLWL74[a]	HLWL82[b]
Lairage							
	Faeces	80	14	1	1	D	XXI
	Skin	18	4	1	1	A,B	-
Cutting room							
	Shoulders	33	26	26	1,4[c]	C,D,F,G,J,L, M,N,Q,R,T,V	I,II,III,V,VI,VII,VIII,IX,X, XII t/m XX,XXII,XXIII, XXVI,XXVII
	Hams	22	4	3	1,4	C,D	I,V
	Bellies	45	30	30	1,4	C,D,E,F,G,H,I, J,K,O,P,U,W,Z	I,III,IV,V,VIII,IX,X,XI, XII,XIII,XXII,XXIII, XXIV,XXV

[a] HLWL74 is only used when serotype 1 was obtained with serotyping

[b] HLWL82 is only used for RAPD typing of D types, obtained with HLWL74

[c] Serotypes 1 and 4 were both isolated from samples of these primal cuts

from faeces, skin and primal cuts, serogroup 4 strains were only isolated from the primal cuts. When all of the serogroup 1 strains (in total 424 strains) were subjected to RAPD analysis with random primer HLWL74, 24 different RAPD profiles (A to Z) were obtained. The skin isolates could be distinguished from the other serogroup 1 isolates with this primer (profile A and B versus C to Z). Profile D was obtained from the isolates, found in faeces as well as those found on the primal cuts. Primer HLWL82 yielded discriminatory profiles within the D typed strains (profiles I till XXVII) and showed that the faeces strains (XXI) were distinguishable at molecular level from the other D typed strains. Both primers generated profiles that were not well-marked, due to the faintness of some bands. It's remarkable that at least 49 (23 and 26) polymorphic different L. monocytognes strains were found on the primal cuts.

Conclusion: The results indicate that the contamination on primal cuts might originate from many different sources in the cutting room area. Although only ten strains isolated from pigs were typed, it is tempting to assume that L. monocytogenes strains originating from pigs do not account for the contamination of the primal cuts. This is supported by Boerlin and Piffaretti (1991) who found different types of L. monocytogenes on live pigs and at the beginning of slaughtering compared to those found on the meat at the end of the slaughterline.

References

Boerlin, P., & Pifaretti, J.C., (1991), Typing of human, animal, food and environmental isolates of Listeria monocytogenes by Multilocus Enzyme Electrophoresis. Appl. Environ. Microbiol. 57, 1624-1629.

Jansen, J., Mossel, D.A.A., De Boer, E., & Van der Zee, H. (1993), A rational approach to praticable safety criteria for Listeria monocytogenes in food products. Food Micro; 15th Int. Symp. The Int. Committee on Food Microbiol. and Hygiene, p.61

Mazurier, S.-I., & Wernars, K. (1992), Typing of Listeria strains by random amplification of polymorphic DNA. Res. Microbiol., 5, 143, 499-505.

Mazurier, S.-I., Audurier, A., Marquet-Van der Mee, N., Notermans, S., & Wernars, K. (1992), A comparative study of randomly amplified polymorphic DNA analysis and conventional phage typing for epidemiological studies of Listeria monocytogenes isolates. Res. Microbiol., 143, 507-512.

United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS), (1989), Lab Communication #57, Beltsville, MD, USA.

Van den Elzen, A.M.G., & Snijders, J.M.A. (1993), Critical points in meat production lines regarding the introduction of listeria monocytogenes, Vet. Quart. 15, 4, 143-145.

Wendtland, A., & Bergann, T., (1994), Listeria monocytogenes; Occurence in a factory for slaughtering, carving and meat processing. Fleischwirtsch. 74, 1329-1331.