

ISOLATION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP. FROM PIGS AT SLAUGHTER IN ITALY.

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Introduction

The hazard of pork carcass contamination by zoonotic bacteria during swine slaughter process cannot be completely eliminated. In fact, sources of contamination during swine slaughter are both pig-related, such as faecal and pharyngeal, and environmental. Potentially pathogenic micro-organisms such as *Listeria monocytogenes*, *Salmonella enterica* and *Yersinia enterocolitica* are spread to the carcass mainly from the carrier animal (de Boer and Nouws, 1991; Sorensen *et al.*, 1999; Autio *et al.*, 2000). In particular, *L. monocytogenes* from tongue or tonsils may contaminate the equipment at the abattoir and consequently spread to the carcass. Therefore, following good manufacturing practices and efficient cleaning and disinfection procedures at the abattoir is of the utmost importance to prevent slaughtering equipment being contaminated with the micro-organism (Autio *et al.*, 2000). In fact, *L. monocytogenes* and *Listeria* spp. can be endemic in the abattoir environment and therefore controlled only by proper cleaning and disinfection (Borch *et al.*, 1996).

Moreover, *L. monocytogenes* can be considered one of the most difficult bacteria to eliminate from food processing plants, due to its ubiquity in the environment (Lar-pent, 1995).

L. monocytogenes human infection frequently results in meningitis, with or without septicaemia, or in septicaemia alone. Immuno-compromised people, infants and elderly are particularly vulnerable. In pregnant women listeriosis may produce a flu-like illness, with the infection spreading to the foetus and resulting in miscarriage, stillbirth or prematurely birth of an ill child (SCVM, 2000). As *L. monocytogenes* is recognized as a significant foodborne pathogen and it is a major concern in food industries (Farber and Peterkin, 1991), major goal of this study was to investigate the carriage rate of this micro-organism by slaughtered pigs and the prevalence of pork carcass contamination during slaughter.

Materials and methods

Between December 1999 and December 2000, 150 finishing pigs (150-180 kg weight) at slaughter were randomly selected in two abattoirs of Emilia-Romagna

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region, northern Italy. The two slaughterhouses had a capacity of 300 swine per hour, *i.e.* 12.000 per week. A total of 300 specimens were collected during 23 sampling visits. From each animal, caecal material was aseptically collected immediately after slaughter and placed in separate sterile containers. Carcass swabs were taken from the sternal region and the throat region. The sampling areas were 20 x 20 cm wide and each carcass was swabbed with only one sterile cotton swab, placed after swabbing in a sterile tube containing 2 ml Ringer ¼ strength solution (LAB M, Bury, United Kingdom). The specimens were transported at 4° C to the laboratory within 2 h after collection and stored at 4° C overnight before examination. The sample size was defined to give a 95% probability of detecting one positive animal/carcass if the carriage/contamination rate was $\geq 2\%$ (Cannon and Roe, 1982). The pigs came from 27 different farms located in three neighbouring regions (Emilia-Romagna, Lombardia, Veneto) of northern Italy.

For *L. monocytogenes* detection a double enrichment method was employed: 10 g of faeces and 1 ml of the Ringer ¼ strength solution used as surface swabs transport medium, together with the cotton swab itself, were suspended 1:10 in 90 ml and 9 ml of *Listeria* Enrichment Broth UVM I (LEB; Oxoid, Basingstoke, United Kingdom), respectively. The primary enrichment broth was incubated at 30° C for 22 ± 2 h and 0.1 ml portion of the UVM I broth was transferred into 10 ml of LEB UVM II medium (Oxoid). After incubation at 30° C for 22 ± 2 h, a 10 µl loopful of the secondary enrichment broth was streaked onto Oxford Agar (Merck, Darmstadt, Germany) plates, incubated at 37° C for 48 h. Suspect *Listeria* colonies, which are small, brown, and surrounded by black halos, were selected for confirmation. They were streaked onto Tryptone Soya Yeast Extract Agar (TSYEA; Biokar Diagnostics, Beauvais, France) plates and incubated 24 h at 37° C to obtain well-isolated pure colonies. All cultures were tested for Gram-staining, catalase reaction, and haemolysin production. The CAMP test was performed according to ISO 11290 (1996) procedure. Gram positive, catalase positive, motile, β-haemolytic cultures were tested for biochemical properties with the API *Listeria*® (bioMérieux, Marcy l'Etoile, France) microsubstrate system. Gram-positive, catalase positive, non-haemolytic isolates were tested for *Listeria* spp. identification. *L. monocytogenes* isolates were subjected to slide agglutination with O antiserum type 1 and O antiserum type 4 (Difco Laboratories, Detroit, USA).

Results

Only two faecal samples were contaminated by *L. monocytogenes* (1.3%). The isolates belonged to serotype O1 and O4, respectively. *L. innocua* was detected in 22 out of 150 specimens (14.7%; C.I. 95% 9.0% - 20.3%), *L. gray* in two (1.3%) and *L. seeligeri* in one (0.7%).

L. monocytogenes serotype O1 was isolated from one of 150 pork carcasses (0.7%); no other *Listeria* spp. were detected from the carcass swabs.

Discussion

This survey indicated that a very low proportion of finishing pigs (two out of 150,

i.e. 1.3%) slaughtered in two abattoirs of northern Italy were intestinal carriers of *L. monocytogenes*.

The carriage rate of *L. monocytogenes* in healthy pigs was lower than the prevalence we detected in the faecal matter of 50 slaughtered pigs (6.0%) in a 1997 survey (Bonardi *et al.*, 1997). In the present investigation, *L. monocytogenes* positive faecal samples were collected during cold months, and exactly from January to March, and those examined in 1997 and found to be contaminated by *L. monocytogenes* were collected during the winter season too. We therefore detected a higher prevalence of *L. monocytogenes* healthy carriers among finishing pigs during cold months.

In this study, the contamination rate of swine carcasses with *L. monocytogenes* was very low (0.7%). Nevertheless, *L. monocytogenes* contamination rate of pork carcasses at slaughter can be higher in abattoirs where environment and equipment contamination occurs. As demonstrated by Autio *et al.* (2000), 12% of pork carcasses were positive for *L. monocytogenes* in slaughterhouses where the mechanical saws used for brisket and back splitting were contaminated with the micro-organisms.

According to Borch *et al.* (1996), *L. monocytogenes* transmission to the pork carcasses does not occur primarily *via* the animal, but is mainly linked to the slaughterhouse environment. In fact, the bacterium has been isolated from food contact surfaces, floors and hand-wash basins at the abattoir (Luchansky and Doyle, 1991; Sammarco *et al.*, 1997), as well as from chill rooms (Graham and Collins, 1991). As *L. monocytogenes* may persist in pork processing environments, such as slaughterhouses, chilling rooms and cutting rooms, the efficiency of cleaning and disinfection procedures are of the utmost importance (Giovannacci *et al.*, 1999).

Therefore, keeping in mind that both *L. monocytogenes* and *Listeria* spp. may be useful indicators of the hygienic status of pig slaughterhouses, with no doubt the plants we selected for our investigation were characterized by high levels of hygiene.

Identification and control of the critical operations at slaughter are essential to reduce the hazard of pork carcasses contamination. Circumcising of the rectum, removal of the pluck set (tongue, larynx, trachea, lungs, heart and liver) and removal of the intestinal tract are the procedures which may give pathogenic bacteria the opportunity to spread to the pork carcass from the gut and the oral cavity (Borch *et al.*, 1996). To avoid carcass contamination after loosening of the rectum, a plastic bag to seal off the rectum should be used (Nielsen and Wegener, 1997). Another critical operation at swine slaughter is the removal of tonsils. In fact, tonsils portions not completely removed from the pharyngeal cavity can be the cause of spreading pathogenic micro-organisms from the lymphatic niche to the adjacent muscular tissue. Healthy carriers, in particular pharyngeal carriers, are very difficult to identify before slaughter and, as demonstrated by several authors, *L. monocytogenes* is often detected in the tonsils of slaughtered pigs (Buncic S., 1991; Autio *et al.*, 2000; Cantoni *et al.*, 2002).

In order to reduce healthy risks for the consumer, some preventive measures at slaughter, such as implementation of codes of good manufacturing practices (GMP), increased care during evisceration, and proper cleaning and disinfection procedures should be encouraged. Anyway, other critical points concerning *L. monocytogenes* contamination of pork meat are to be found in the processing environment of the

chilling and cutting rooms. In fact, the incidence of *L. monocytogenes* in pork cuts often increases after chilling and cutting (van den Elzen and Snijders, 1993).

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Key words: *L. monocytogenes*, *Listeria* spp., pig, slaughterhouse

Mots clés: *L. monocytogenes*, *Listeria* spp., cochon, abattoir

Parole chiave: *L. monocytogenes*, *Listeria* spp., suino, macello

SUMMARY - From December 1999 to December 2000, a total of 150 slaughtered pigs were randomly selected in two abattoirs with a capacity of 12.000 swine per week. 150 caecal material samples and 150 carcass swabs were collected and examined for *Listeria monocytogenes* and *Listeria* spp.

L. monocytogenes was detected in the caecal content of two (1.3%) slaughtered pigs. The strains isolated from the faeces belonged to serotype O1 and O4. *L. innocua* was isolated from 22 (14.7%) out of 150 faecal samples, *L. gray* from two (1.3%) and *L. seeligeri* from one (0.7%). From one (0.7%) carcass *L. monocytogenes* serotype O1 was isolated. No other *Listeria* spp. micro-organisms were detected on the pork carcasses.

RÉSUMÉ - Que de décembre 1999 à décembre 2000, un total de 150 cochons ont été sélectionnés casuellement dans deux abattoirs avec une capacité de 12.000 cochons par semaine. 150 échantillons des fèces et 150 tampons de la carcasse ont été examinés pour *Listeria monocytogenes* et *Listeria* spp.

L. monocytogenes O1 et O4 a été détecté dans les fèces de deux (1,3%) cochons. *L. innocua* a été isolée de 22 (14,7%) échantillons des fèces, *L. gray* de deux (1,3%) et *L. seeligeri* d'un (0,7%). D'une carcasse *L. monocytogenes* O1 a été isolée. Aucuns autres *Listeria* spp. micro-organismes n'ont été détectés sur les carcasses des cochons.

RIASSUNTO - Nel periodo compreso tra Dicembre 1999 e Dicembre 2000, sono stati prelevati 300 campioni da 150 suini macellati in due stabilimenti di macellazione caratterizzati da una capacità operativa di 12.000 suini alla settimana. I campioni, rappresentati da 150 campioni di materiale cecale e 150 tamponi eseguiti sulla carcassa, sono stati sottoposti ad analisi microbiologica per la presenza di *Listeria monocytogenes* e *Listeria* spp.

L. monocytogenes è stata isolata dal materiale cecale di due suini (1,3%). I ceppi isolati dalle feci appartenevano ai sierotipi O1 e O4. *L. innocua* è stata isolata da 22 (14,7%) campioni di feci, *L. gray* da due (1,3%) e *L. seeligeri* da uno solo (0,7%). Da una carcassa (0,7%) è stata isolata *L. monocytogenes* sierotipo O1. Nessun altro microrganismo appartenente al genere *Listeria* è stato isolato dalle restanti carcasse.

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