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Pathogenic bacterial contamination of carcasses from outdoor organically reared pigs: preliminary results from a survey in Northern Italy

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Abstract. Organic pigs often are reared and fattened outdoor; this production system normally affects skin cleanliness especially during wet season. The slaughtering of very dirty pigs may increase the risk of carcass contamination. The following survey investigates the presence of several pathogenic bacterial for human foodborne such as *Listeria monocitogenes*, *Salmonella* spp., *Escherichia coli* O157, *Yersinia enterocolitica* and *Campylobacter* spp. Different samples (skin swab sponges performed before and after slaughtering, faeces, tonsils and rind) from risky areas for contamination have been collected from 31 fattened pigs coming from 7 organic farms and slaughtered in 3 small abattoirs. None of those samples were positive for *Salmonella* spp. *Campylobacter* spp., was found on 64.5 % of samples (77% of samples were positive for *Campylobacter coli*). *Listeria monocitogenes* and *Yersinia enterocolitica* were detected on 13% and 6.5% of samples respectively; *Escherichia coli* O157 was found only on 3.2% of considered samples. Preliminary results on the level of carcass contamination show similar figures as found in literature for *Campylobacter* spp., *Listeria monocitogenes* and *Yersinia enterocolitica*, both for the outdoor organic pigs and the conventional ones. The high presence of *Campylobacter* spp. in faeces suggests to pay particular attention during evisceration to prevent carcass contamination.

Keywords. Pig – Organic – Pathogens – Carcass.

Contamination de carcasses de porcs élevés en système biologique en plein air par des bactéries pathogènes: résultats préliminaires d'une étude réalisée en Italie du Nord

Résumé. Souvent élevés en plein air, les porcs bio, au moment de l'abattage, en particulier pendant la saison des pluies, se présentent très sales, ce qui peut représenter un risque accru de contamination de la carcasse. Dans cette étude a été évaluée la présence de certains pathogènes périlleux pour la consommation de viande: *Listeria monocitogenes*, *Salmonella* spp., *Escherichia coli* O157, *Yersinia enterocolitica* et *Campylobacter* spp. Les matrices qui ont été soumises à l'analyse (tampons cutanés avant et après l'abattage, fèces, amygdales et couenne) ont été prélevées sur 31 porcs provenant de 3 abattoirs et élevés dans 7 fermes. Les données obtenues ont démontré l'absence de *Salmonella* spp. dans tous les échantillons. La présence de *Campylobacter* spp. a été relevée dans 64,5%, avec une prévalence de *Campylobacter coli* (77%). 13% des porcs se sont avérés positifs pour *Listeria monocitogenes*; *Yersinia enterocolitica* était présente dans 6,5% des sujets analysés; pour les deux la couenne a été la matrice la plus contaminée. *Escherichia coli* O157 a été relevée seulement dans 3,2% des échantillons. Les niveaux de contamination obtenus pour *Campylobacter* spp., *Listeria monocitogenes* et *Yersinia enterocolitica* se sont montrés compatibles avec les données citées dans la littérature par rapport aux fermes conventionnelles. La présence consistante de *Campylobacter* spp. dans les fèces nous impose de prêter une attention particulière à l'éviscération, en tant que facteur de risque de contamination des carcasses.

Mots-clés. Porc – Biologique – Pathogènes – Carcasse.

I – Introduction

Organically reared pigs in Italy has been growing during the last five years due to the increasing of consumer demand for organic products, and for a regional plans that support the use of local-autochthonous pig breeds. However, in Veneto Region (north-east of Italy) organic pig farms

are still limited in number and small in size (about one hundred fattened pigs/year/farm), if compared with conventional ones. Differently from conventional, organic pigs are often reared in outdoor systems, using grazing areas with different type of soil, cover crops (grass, corn, sorghum, etc.) or bushes, and slaughtered mainly during wet and cool season. At slaughtering normally pigs are quite dirty and skin is often covered with mud and faeces; this may increase the risk of carcass contamination especially when slaughtering is performed in a small abattoir, often used in local organic production chain. The presence of foodborne pathogens, as well as the related contamination of carcasses and processing facilities, could dramatically increase the health risk, especially if carcasses are deboned and processed at farm level for traditional fermented salami production. Representing a high risk for human foodborne diseases, meat contamination from pathogenic bacteria such as *Listeria monocitogenes*, *Salmonella* spp., *Escherichia coli* O157, *Yersinia enterocolitica* and *Campylobacter* spp. are under continuous investigation by local health authorities.

A previous investigation carried out on the same area (Schiavon, *et al.*, 2006) has shown a similar (*Campylobacter* spp.) or higher (*Yersinia enterocolitica*) contamination of outdoor organic pork carcasses compared to the conventional ones. In this second investigation different farms and small abattoirs were included, with the aim of improve the knowledge on the presence of critical foodborne pathogens, in order to suggest different or more careful management of organic outdoor pigs.

II – Materials and methods

1. Sampling

The research has been carried out by sampling 31 pigs produced in 7 outdoor organic farms located in the Veneto region (plane and low mountain areas); animals have been randomly chosen at farm level and slaughtered in 4 different abattoirs, during winter season (from November to February).

Five samples have been collected from each animal: skin swab before slaughtering, skin swab post-slaughtering (at the end of slaughtering line before carcass cooling), faeces, tonsils and a portion of rind (a strip about 5 x 15 cm length on belly and thorax area).

The skin swabs before and after slaughtering has been performed through sponges BS10-BPW (Qualicum Scientific Limited) on dorsal, flank and jaw regions, using one sponge on the three regions.

All samples has been microbiologically investigated for presence of *Salmonella* spp., *Yersinia enterocolitica*, *Listeria monocitogenes*, *Campylobacter* spp. and *Escherichia coli* O157, apart tonsil that were investigated for *Yersinia enterocolitica* only.

2. Analytical methods

***Salmonella* spp.:** Samples were suspended in buffered peptone water (ratio 1:10), incubated overnight at 37°C, then inoculated on MRSV (semisolid) medium and incubated overnight at 42°C. Positive samples were streaked into BGA and XLD media and incubated at 37°C overnight. In case of suspect, colonies were identified through biochemical reaction and confirmed with serotyping.

***Campylobacter* spp.:** Samples were suspended in Preston broth (ratio 1:10) and incubated overnight at 42°C. Then they were streaked on Karmali agar plates and incubated at 42°C for 24-72 under modified air. After Gram staining, the suspected colonies have been identified through PCR.

***Yersinia* spp.:** Samples were homogenized in bile-sorbitole broth (ratio 1:10) and incubated at 4 °C for 10 days. After this, the samples were st reaked into selective CIN agar and incubated at

37°C overnight; suspected colonies were identified by biochemical methods and confirmed using commercial API 20E system (BioMérieux Italia SpA).

Listeria spp.: Samples were homogenated with Half Fraser Broth (ratio 1:10) and streaked on agar blood plate and were incubated for 24 hs at 37°C. From HFB samples were inoculated into solid media (Oxford agar and Aloa agar) incubated 48 hs at 37 °C and suspected colonies were identified using biochemical method (API test).

E. coli O157: Samples were suspended in Tryptone soy broth (ratio 1:10) and incubated overnight at 41,5°C. After this, *E. coli* O157 is concentrated using magnetic immunoseparation method, streaked on sorbitol McConkey agar and incubated at 37°C overnight, and confirmed using biochemical and serological methods.

III – Results

All data are shown in Tables 1 and 2.

Table 1. Presence of pathogens in samples: % prevalence and absolute numbers

Pathogen samples	<i>Salmonella</i> spp.	<i>Listeria monocitogenes</i>	<i>Yersinia enterocolitica</i>	<i>Campylobacter</i> spp.	<i>Escherichia coli</i> O157
Pre-slaughtering swab	0	6.5 (2/31)	0	9.7 (3/31)	0
Post-slaughtering swab	0	3.2 (1/31)	0	9.7 (3/31)	0
Pork-skin	0	9.7(3/31)	6.5 (2/31)	12.9 (4/31)	0
Tonsils	-	-	3.2 (1/31)	-	-
Faeces	0	0	0	51.6 (16/31)	3.2 (1/31)

Table 2. Presence of pathogens in samples of each farm

Farm	Pigs tested (n)	<i>Salmonella</i> spp.	<i>Listeria monocitogenes</i>	<i>Yersinia enterocolitica</i>	<i>Campylobacter</i> spp.	<i>Escherichia coli</i> O157
1	7	neg	pos	neg	pos	neg
2	5	neg	neg	neg	pos	neg
3	6	neg	pos	neg	pos	pos
4	6	neg	neg	neg	pos	neg
5	1	neg	neg	neg	neg	neg
6	2	neg	neg	neg	pos	neg
7	4	neg	neg	pos	pos	neg

None of the 31 pigs sampled has shown the presence of *Salmonella* spp.

Campylobacter spp, was found in 20 animals (64,5%) with higher prevalence on faeces as expected from previous experiences. On 26 strain isolated, 20 were identified by PCR as *C. coli* and 6 as *C. jejuni*.

Listeria monocitogenes was found in 4 pigs (13%), only on skin.

Yersinia enterocolitica was found only in 2 pigs (6,5%); one presented tonsils (both) and skin contaminated, the other one the skin only.

Escherichia coli O157 was found only on one pig's faeces (3,2%).

IV – Discussion

The analysis of the data underlines a possible unfriendly situation, depending from the bacterial strains under investigation.

No *Salmonella* strains were isolated, but the low number of samples analyzed could not guarantee the absence of *Salmonella* in the farms. These findings may suggest that the bio-safety level could be considered quite good on the investigated farms. However, it seems reasonable that in outdoor organic pig production, the source of possible contamination of *Salmonella* has to be carefully investigated, in relation to rearing system and the possibility of contact between pigs and wild animals.

Listeria monocytogenes was found in 13% of the slaughtered pigs, but only on their skin; moreover only pigs from two farms were contaminated. This could suggest a cross-contamination between pigs and ground. According to some literature findings, our data confirm that *Listeria monocytogenes* derive more from the environment (farm or processing environment) than from the animals (Samelis and Metaxopoulos, 1999). This confirms the importance of monitoring the presence of *L. monocytogenes* in the environment where the pigs live, on transport vehicles, on abattoir lairage and on processing plant.

Campylobacter spp. is the most common pathogen isolated. It was found in 64.5% of the slaughtered pigs coming from 6 farms. According to literature and as for conventional pigs, its prevalence is higher in faeces (51,6%) than on skin, and *C. coli* prevalence is higher compare to *C. jejuni* (Schiavon, *et al.*, 2006; Farzan *et al.*, 2010). It seems enough clear that to lower as much as possible the prevalence of *Campylobacter* spp. on pig carcasses, the main critical point should be to guarantee a higher level of hygiene (expertise of operators, knives sterilization, etc.) during the evisceration process.

The prevalence of *Yersinia enterocolitica* is very low, and similar to as reported in literature for wild boar. Only pigs coming from one farm were contaminated. Tonsils, known as a target organ for this bacteria, present a very low contamination level. However, a careful processing technique has to be implemented in order to minimize the risk of cross contamination during processing and foodborne associated to pork consumption.

The prevalence of *E. coli* O157 is very low, too; it was found into the faeces (on one pig only) similar to investigation reported by Ercolini *et al.*, (2007) for wild boar.

Our findings confirm that the risk associated with carcasses from outdoor organic pigs are similar to which of intensive reared pigs. The most prevalent foodborne pathogen was *Campylobacter coli*, with less prevalence of *Campylobacter jejuni* and *Listeria monocytogenes*. *Yersinia enterocolitica* and *E. coli* O157 were very low in prevalence, and *Salmonella* spp. was absent.

Related risk can be minimized using appropriate processing methods; furthermore it is important to investigate foodborne risk associated to outdoor organic reared pigs, as the market of this product is increasing. The low level of main investigated foodborne pathogens, in the majority of farms, indicates that it could be possible to obtain safe pig carcasses from controlled outdoor organic production system.

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