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Original Contribution

PREVALENCE OF *CAMPYLOBACTER SPP.* IN PIG SLAUGHTER CARCASSES DURING THE PROCESSING

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ABSTRACT
It was performed a study concerning the prevalence of *Campylobacter spp*. in pig carcasses to estimate the slaughterhouses contamination risk during the slaughter process of this type productive animals and technology. Samples from 84 carcasses were collected after evisceration and after dressing at the time of the chilling because these stages in the slaughter process have deep impact on the contamination levels in carcasses during the entire process. To find out the cross – contamination risk, another environmental samples of the slaughter process concerning different slaughter areas and instruments closely involved in the processing, were also collected and investigated. For the purpose of the study standard microbiological method for detection and enumeration was used as well as immunoassay. The results indicated a low level of *Campylobacter spp*. contamination among slaughter carcasses during the slaughter process – about 13%. It also revealed a contamination reduction after the end of the dressing procedures at the time of the chilling in levels that exclude the risk of food-born infection to the consumers.

Key words: Campylobacter spp. slaughterhouses, pig carcasses

INTRODUCTION

In the last few decades, it has been found, the microorganisms known as *Campylobacter spp*. are one of the most frequent causative agents of gastroenteritis among human population. The European Food Safety Authority reported that a source of *Campylobacter* contamination with significant social impact on human health can be meat and meat products (1, 2, 3). The data reveals poultry meat as an essential source of *Campylobacter* pathogens, but it is important to be signed out that these enteric disease-related microorganisms has been also isolated from other productive animals meat samples as pork, lamb and beef rarely. It is stated that the four thermophilic species representing *Campylobacter genus (C. jejuni, C. coli, C. lary and C. upsaliensis)* can be transmitted to humans through food stuffs. Main attention is paid to *Campylobacter jejuni* and *Campylobacter coli* because they are the two enteric pathogens recognized as the leading cause for human enteric pathology (4). The European researches present statistic data according to that, the prevalence of *Campylobacter jejuni* is found to be most significant factor of infection in poultry, beef and lamb, while *Campylobacter coli* is more common finding in pork meat samples (1, 2, 3).

The hygiene surveillance of the slaughter process in the slaughterhouses and the further meat manufacturing in the retailed meat plants is conducted under the requirement, stated in Regulation EU 2073/2005, 1441/2007 and Ordinance No 5/2006 (5, 6, 7) concerning the hygiene of foods. The main hygiene criteria of the technology process concerning slaughter carcasses and retailed meat (mechanically separated, mince meat and cuttings) are the enumeration of microorganisms at 30^{o} C and Enterobacteriaceae, presence of Salmonella spp. and *E. coli*. Although their evident significance to human health, the presence of

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Campylobacter spp. is not established as a hygiene processing criterion in the slaughterhouses and retailed meat plants yet. Concerning these enteric pathogens important role as a main cause for human gastroenteritis infections, our laboratory carried out a research to estimate the prevalence of *Campylobacter spp*. in the pig slaughter carcasses during the different stages of the slaughter process.

MATERIALS AND METHODS

To perform our study, 168 swab pig carcasses samples, as well as 20 meat samples were collected and tested for presence of *Campylobacter spp*. We used contaminant – free, ready-to-use swabs in charcoal medium (Himedia) and contaminant – free containers to collect the required specimens for the investigation. The meat samples were collected from the median part of each carcass half. The swab samples were obtained from the lateral part of the carcasses.

Intestinal content from pigs representing different lots were also gathered to check the availability of the infection among the animals. The number of these samples is 20 and they are presented in table number 1 given in the reference. These samples were taken from the pigs' ceacum and ileum.

Also swab samples were gathered from different processing surfaces to investigate the hygiene of the slaughter process concerning mainly the presence of *Campylobacter spp.*

Method of investigation

Swab and meat samples were tested according to the standard procedures described in ISO 10272 -1 and 2/2006 – Microbiology of food and feed. Horizontal method for detection and enumeration of *Campylobacter spp*.(8) The procedures include the next stages of investigation:

1. Enrichment in liquid selective medium
2. Isolation on two solid selective media
3. Subculture the characteristic colonies from the solid selective media on non – selective blood containing medium
4. Conformation and species identification of *Campylobacter spp.* isolates
   - morphology and motility by using microscope observation
   - growth at 25^{o} C in microaerobic atmosphere
   - growth at 41^{o}C in aerobic atmosphere
   - oxidase activity
   - catalase activity
   - indoxil acetate hydrolysis
   - hypurate hydrolysis
   - resistance to Nalidix acid
   - resistance to Cefalotine

The intestinal content samples were investigated following the guideline procedures of the second part of ISO 10272/2006. The enumeration of bacteria was performed in one milliliter intestinal substance, after preparation of initial dilution 1x10 and the following decreasing dilutions to 10^{-6} . The broth culture was inoculated in 90 mm petry dishes with solid selective medium mCCD (Merck).

The following media was handled.

1. Liquid selective broth – Bolton (Merck)
2. Solid selective medium – mCCD agar (Merck)
3. Solid selective agar with blood – Scirow (Merck)
4. Nonselective Columbia agar with 5% blood

We have also used immunoassays for detection of *Campylobacter spp*.:

1. Enzyme Linked Flourescent Assay (ELFA) performed by the VIDAS instrument with VIDAS Campylobacter kits (BioMerieux)
2. Campylobacter visual immunoassay (ELISA - Tecra)

Preparation of samples for VIDAS Campylobacter assay

The swab samples were incubated in microaerobic conditions for 40 – 48h in tubes with 10 ml Bolton broth at 41,5^{o}C. Afterward 1-2 ml of the culture was transferred into another tube and heated for 15 minutes in boiling bath. After cooling to room temperature, 0,5 ml from the second tube was transferred into VIDAS strip and performed the assay in the VIDAS instrument.

Preparation of samples for Tecra – ELISA Campylobacter assay

We used the same procedure with Bolton broth enrichment selective medium. After enrichment 1ml from the broth culture was transferred into a tube, mixed with 50µl of Sample Additive and heated for 15 minutes in a boiling water bath. After cooling the sample, 200 µl from the mixture was transferred into Tecra-plate well and further according to the Tecra protocol was handled.

To control the reliability of test procedures the following referent strains were used: *Campylobacter jejuni* ATCC 33560, *Escherichia coli* ATCC 25922, *Staphilococcus aureus* ATCC 3403.

RESULTS

Intestinal content samples results

Twenty samples intestinal content were examined for presence of *Campylobacter spp*. as well as enumeration and identification of the positive ones were performed. Among these tested samples we found ten positive for *Campylobacter spp*. or 50% positive. From the positive samples, seven were identified as *Campylobacter coli* and three were registered as *Campylobacter spp*. because the biochemical identification did not distinguish them to species level. The number of the colony forming units varied between 10^6 to 10^7 in the positive samples **(Table 1)**.

Table 1. *Campylobacter spp. strains derived from intestinal contents*

Sample No		Number of cfu/ml
1	Negative	
2	Negative	
3	Negative	
4	Negative	
5	C. coli	$1,5x10^7$
6	Campylobacter spp.	$1,5x10^6$
7	C. coli	$1,1x10^7$
8	C. coli	$3,8x10^7$
9	C. coli	$1,8x10^6$
10	Campylobacter spp.	
11	Negative	
12	Negative	
13	Negative	
14	Negative	
15	C. coli	$1.8x10^6$
16	Negative	
17	Campylobacter spp.	1.4x106
18	C. coli	$2.2x10^6$
19	C. coli	$1.4x10^7$
20	Negative	

Carcasses swab samples results

The swab samples from carcasses were divided into two groups. In the first 84 swab samples, collected after the evisceration we registered 7 positive *Campylobacter spp.* results. The prevailing species was found to be *Campylobacter coli*. In fact four *C. coli* strains, one – *C. jejuni* and two unspecified isolates were detected **(Table 2)**. In the second group of swab samples, collected after dressing and during the process of chilling, was detected only one positive sample from 84, that was specified as *Campylobacter coli* **(Table 2).** We should state, we registered decrease in the positive *Campylobacter spp* findings in carcass swamp samples after the dressing and during the chilling process.

Table 2. *Campylobacter spp. derived from swab samples collected from carcasses*

After evisceration 100 cm^2				
Number of tested sample	Positive	Campylobacter jejuni	Campylobacter coli	Unspecified
84	7	1	4	2
After dressing, during chilling 100 cm^2				
Number of tested sample	Positive	Campylobacter jejuni	Campylobacter coli	Unspecified
84	1	0	1	0

Meat carcasses samples results

In the meat samples, collected after the dressing and during the chilling process were found two positive for *Campylobacter spp.* specimens. One of them was specified as Campylobacter coli and the other couldn't be identified by the biochemical tests, we used. The data points out that the possibility to find vital forms of *Campylobacter spp*. is greater in meat samples than in swabs.

Table 3 reveals the positive *Campylobacter spp.* findings registered in the six different sampling procedures during the different stages of the slaughter process at the time of the research. The last two columns in the table present the data from the last two times when the swab samples were collected before the beginning of the slaughter process and during the process, while in the previous sampling procedures the samples were collected during the slaughter process only. As it is clear from the data, the greatest amount positive results were registered among swab samples collected from the offal processing surface. Positive results were found also among knife swab samples and saw swab samples collected during the slaughter process. It is important to be stated that during the processing, the positive *Campylobacter spp.* findings among the swab samples collected from the head processing surface weren't detected. This fact indicates an absence of cross contamination between the different work areas in the slaughterhouse **(Table 3)**.

Table 3. *Results from swab samples collected from processing surfaces and instruments*

Number of sampling / Sampling places	First sampling	Second sampling	Third sampling	Fourth sampling	Fifth sampling	Sixth sampling
Surface for head handling	-	-	-	-	-	-
Surface for offal handling	+	+	+	+	Before (-) During(-)	Before(-)\ During(+)
Knife (evisceration)	+	+	-	-	Before (-) During(-)	Before(-)\ During(-)
Mechanical saw	+	+	-	+	Before(-) During(+)	Before(-) During(-)

DISCUSSION

The data world-wide reveals, the predominant *Campylobacter* species in pig population is found to be *Campylobacter coli*, 60 – 90% from the *Campylobacter* isolates (9, 10, 11). From the other hand, other scientific publications pointed out a *Campylobacter jejuni* prevalence in the tested pig samples (12). Our intestinal content samples results reveal *Campylobacter coli* as predominant species in the positive samples. This information is in correlation with the official EFSA data published in the annual journal. In the majority of the European countries where similar researches have been performed, is found that *Campylobacter coli* is the prevailing species in the examined groups of pigs.

Data concerning the *Campylobacter spp*. prevalence in slaughterhouses where pigs are handled is submitted in several research notes (13, 14, 15). The authors have found in the pig intestinal content samples collected from different pigs' slaughter batches high percent positive results. In fact it reaches almost 100%. During the slaughter process significant percent positive specimens have been registered among pig carcasses after the process of evisceration 9 - 33%. Our results reveal 8% positive findings among the tested pig slaughter carcasses after the evisceration. The free author research groups declare that the dressing and chilling are very important processes leading to reduction of Campylobacter spp. contamination in slaughter pig carcasses. It has been registered a decrease and even complete lack of positive findings after the chilling process. At the same time our research found out a relationship between the method of collecting samples for microbiological testing and the frequency of the positive findings. It is found that greater percent positive results was obtained among samples collected by using the destructive sampling method (meat matrix) than the swamp samples, collected from the surface of the slaughter carcasses. All this data can be explained with the physiological characteristics of *Campylobacter spp*. bacteria that are very sensitive to the environmental conditions (temperature and drying) (4).

The analysis of the working surfaces and instrument swamp samples results reveals that the higher contamination rate was detected among samples from offal processing surface, while among samples from head processing surface were not found any positive. This should be pointed out because the data underlines the lack of cross-contamination between these two separated in the process of slaughtering working surfaces. This is a good attestation for the process hygiene in the slaughterhouse. At the same time among swamp samples collected from knives and saw we found positive specimens. That's why during the fifth and sixth sampling procedures the samples were collected before the initiation of slaughtering and in the process of slaughtering. There were no positive findings before the initiation of manipulations with the instruments. It is another good attestation for the hygiene practice performance during the slaughter process and confirmed one of the *Campylobacter spp*. characteristics, their sensitivity to the environmental factors, that do not allow them of keeping vitality on the instrument surfaces long time after their mechanical cleaning and disinfection. The similar sampling performance was done with the offal processing surfaces. The positive results were not registered before the initiation of the slaughter process too.

CONCLUSIONS

1. It was found 50% *Campylobacter spp*. presence among pigs for slaughtering (Results of intestinal swab samples)
2. The predominant species among pigs was found to be *Campylobacter coli*
3. After the evisceration, it was found low (5.8%) percent positive samples
4. The dressing and chilling processes lead to reduction of contamination among pig slaughter carcasses
5. The hygiene procedures (cleaning and disinfection) as well as the slaughter processing do not allow dissemination of *Campylobacter spp*. in the slaughterhouse affecting the final contamination of carcasses

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