

## THE IMPORTANCE OF PIG TONSILS REMOVAL FOR THE FINAL ASSESSMENT OF THE CARCASSES' HYGIENE QUALITY

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### Abstract

Although the tonsils removal at pigs is mandatory in conformity with the European Union Regulation 854/2004, there are slaughtering units that do not respect this rule. The importance of their complete removal is shown in this study through a thorough assessment of the bacteria load found in tonsils and afterwards in the carcasses where the tonsils have not been removed. For the isolation of these bacteria the classical method was used and the confirmation being performed through biochemical microtest systems (API) and molecular methods (simplex PCR). Also, there were two other automatic ways of bacteria identification: the microscan WALKAWAY system and the Trek system. The results revealed a polymorphic microflora, with a predominance of Gram negative bacteria in the majority of the tonsil samples examined. The bacteria prevalence in the pharyngeal tonsils was represented by: Gram negative bacilli (32.1%), diplococcic (19.75%), streptococci (16.05%), Gram positive bacilli (13.58%), polymorphic non spore forming Gram positive bacilli (8.64%), spore forming Gram positive bacilli (1.24%) and Candida (8.64%). Following the bacteriological exam, a large variety of bacteria species were revealed at the carcasses also, worth mentioning are Staphylococcus, Proteus, Streptococcus, Listeria and Salmonella and the prevalence of these species was significantly higher ( $p < 0.05$ ) during the warm season than the cold one in both units studied. The pharyngeal tonsils at pig represent a deposit area for some pathogen bacteria (*Yersinia enterocolitica*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp.), that can contaminate the carcasses during slaughtering and can jeopardize the consumers' health.

**Keywords:** carcasses, hygiene, tonsils, quality.

### INTRODUCTION

The lymphoid tissue of the pharyngeal tonsils plays a very important role in the immune response against oral and nasal cavity bacteria (Belz and Heath, 1996; Horter et al., 2003). A large number of studies have high lightened an asymptomatic prevalence with commensal bacteria like *Actinobacillus pleuropneumoniae*, *Actinobacillus suis*, *Streptococcus suis*, *Haemophilus*

*parasuis* or *Mycoplasma hyopneumoniae* and some viruses: the respiratory and reproductive syndrome virus, the classical swine fever (Marois et al., 2008). The pharyngeal tonsils can represent the focus of some pathogen bacteria like *Salmonella spp.*, *Campylobacter spp.*, *Listeria monocytogenes*, *E. coli* or *Yersinia enterocolitica* (Swanenburg et al., 2001; Bucher et al., 2008). The purpose of this study was to make a complex evaluation of the bacteria prevalence through bacterioscopic and bacteriologic methods combined also with molecular ones for accurate confirmation in pigs' pharyngeal tonsils and to correlate them to the hygiene quality of the carcasses obtained.

## **MATERIALS AND METHODS**

The study was conducted on 81 pig pharyngeal tonsils samples collected from two slaughterhouses ("A" and "B") found in Maramureş county and respectively 40 samples taken from the carcasses obtained in the same units. The methodology of examination followed the steps stated in the National and Community legislation, respectively Regulation (CE) no.2073/2005 regarding the microbiological criteria for food products modified with Regulation. (CE) no.1441/2007. The cultural and morphological aspects of the bacteria were characterized on regular and blood media. The morphological characters were revealed on smears made from broth test tubes BHI stained by Gram method. For differentiation, colonies developed on broth were passed on differentiation on selective media: Chapman or Baird-Parker for staphylococci, MacConkey and XLD for Enterobacteriaceae. The colonies were incubated for 24 hours at 37° C. for confirmation the following API galleries were used: API 20 STAPH, API 20 STREP, API 20 E and ID32 E.

The bacteria isolation was performed also by using the automatic system WALKAWAY which identifies the susceptibility patterns *in vitro* of the isolated bacteria from the clinical specimens. It is used along with the Microscan dilution plates that contain media, antimicrobials in serial dilution and selected chemical reagents. The confirmation was performed also by molecular testing (PCR) following the protocol described previously by Lăpuşan A. (2012). The reaction was performed in a 25µl in which it was added: 12,5 µl MyTaq (Bioline), 1µl primer Forward; 1µl primer Reverse, 4 µl DNA and 6,5 µl pure grade water PCR (Sigma). In order to confirm the *Listeria* bacteria we used the *Listeria* Genus Primer Set (BioScientific) which contains the Forward and Reverse primers common for all the *Listeria*

species. For the confirmation of *Salmonella* spp. the following primers previously used by Malorny et al. (2004), were selected:

F: 5'- CTCACCAGGAGATTACAACATGG -3

R: 5' – AGCTCAGACCAAAAGTGACCATC – 3'

The *E.coli* confirmation was performed using the following sequences that amplify the common region (23S) of all *E.coli* strains, having a molecular weight of 736 bp.

F: 5' – AAGGAATCACCTTGCAGATAAACTC – 3'

R: 5' – TTTCCGAGTACATTGGCATCGT – 3'

The amplification protocol was: Hot start: 95°C–5min.; Denaturation: 94°C – 00.45''; Alignment: 55°C (*Listeria* spp.); 62°C (*Salmonella* spp.); 57°C (*E.coli*) – 00.45''; Elongation: 72°C – 1min. These cycles were repeated 35 times after which the final elongation was made at 73°C for 4 minutes. The statistic analysis was performed in Windows 7, program Origin 8.5, ANOVA test.

## RESULTS AND DISCUSSIONS

Following the bacterioscopic exam of the 81 smears made from the analyzed tonsils the following prevalence was revealed: Gram negative bacilli (32.1%), dyplococci (19.75%), streptococci (16.05%), Gram positive bacteria (13.58%), Gram positive bacilli non-sporulated (8.64%), sporulated Gram positive bacilli (1.24%) and *Candida* yeasts (8.64%). Results are shown also according to the unit in figure 1:

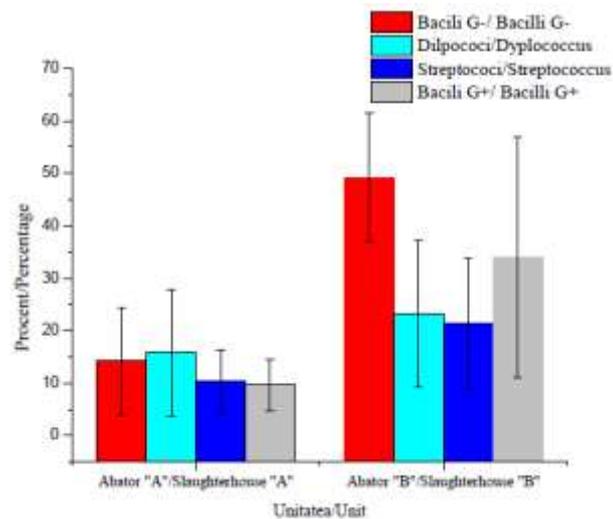


Figure 1: The bacteria prevalence in the units studied



Figure 2: The electrophoresis profile of the 340 bp fragment corresponding to *E.coli*

Along the *E.coli* strains identified we had samples where we found *Streptococcus* spp. associated with *Klebsiella pneumoniae*. In the samples harvested from the small unit "B" in 16 of them there were identified germs belonging to *Staphylococcus* genus. With the help of Walkaway system it was confirmed with a certainty of 85% that the strain identified was *Staphylococcus aureus* in 3 of the samples tested (18%). The rest of the positive samples belonged to *S. chromogenes* (44%), *S. Intermedius* (22%), *S. Wernerii* (16%).

It is well-known that the palatin tonsils are an entrance gate and a multiplication area for a number of microorganisms (Salles and Middleton, 2000). In this study the number of bacteria and especially *E.coli* was very high. In the samples harvested from unit "B", the bacteria load was the highest, and the percent of *E.coli* reported a prevalence of 76%. Some of these samples (12%) were confirmed also for *Staphylococcus* spp. The

*E.coli* prevalence in this study is much higher from the one previously reported by Salles and Middleton (2000).

Most of the bacteria strains isolated from the tonsils were identified also at the carcasses samples examined. The prevalence was again revealed for *E.coli* (56%) and *Staphylococcus* spp (34%). In previous studies concerning pork carcasses obtained in different slaughtering units the Enterobacteriaceae bacteria were revealed in a high percent (75%) (O'Brien et al., 2007, Quirke et al., 2001). Similar results have been reported by other authors like Dorsa et al., 2000, Gill et al., 2000, Pearce et al.,2005).

Another study made on the prevalence of *Salmonella* spp. in three processing steps of pig slaughtering (after the bleeding, at hygiene and refrigeration) has shown that from a total of 182 positive samples, 24% were confirmed as *Salmonella* spp. after bleeding and 3% after refrigeration (Bouvet et al., 2003). The most common serovars were *S. Typhimurium* (27%) and *S. Dersy* (40.5%).

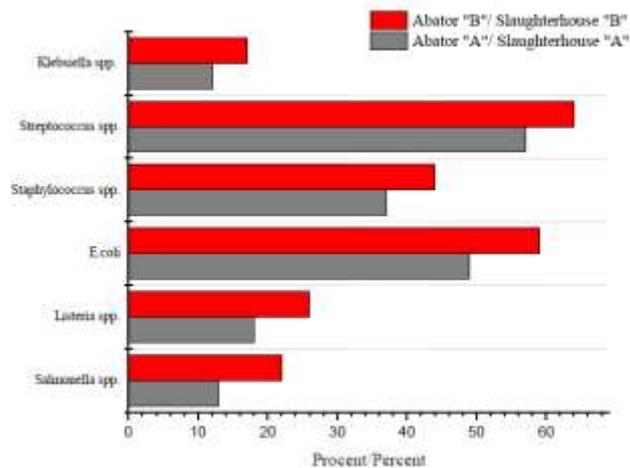


Figure 3: The comparative analysis of the bacteria prevalence in tonsils and carcasses between the two units studied

The highest prevalence of bacteria in tonsils and carcasses was found in the small unit “B” where it was seen at a risk analysis performed that the tonsils are not being removed completely or not at all. It was not surprising the fact that in this slaughterhouse the hygiene quality of the carcasses was much lower as it can be seen in figure 3. Concerning for public health is the presence in both units of the *Salmonella* spp. and *Listeria* spp. at the

carcasses analyzed which represent a great risk for food poisonings. All the *E.coli* strains identified were non-pathogenic.

## CONCLUSIONS

In the small capacity unit, the bacteria prevalence was significantly different ( $p < 0.05$ ) than in the large unit, identifying strains that were present also in the carcasses.

The pharyngeal tonsils at pig, represent a deposit area for some pathogenic bacteria (*Yersinia enterocolitica*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp.) that can contaminate the carcasses during the slaughtering process if the tonsils' removal process is not correctly performed and can jeopardize the consumer's health.

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\*\*\*Regulation (CE) no.2073/2005

\*\*\*Regulation. (CE) no.1441/2007.