

**EFFECT OF SOW SKIN DISINFECTION ON *ENTEROBACTERIACEAE* COUNTS
WITH EMPHASIS ON *ESCHERICHIA COLI*****Marina Mikulić, M. Ostović, Ž. Pavičić, Andrea Humski, Gordana Gregurić Gračner,
Anamaria Ekert Kabalin, S. Menčik, Dinka Milić, M. Samardžija, Ž. Cvetnić****Summary**

The aim of the present study was to investigate the impact of sow skin disinfection in commercial breeding conditions on *Enterobacteriaceae* and *Escherichia coli* counts. The study included 30 sows from a pig-breeding farm. The ventral part of the sow trunk with mammary glands was disinfected before placing the piglets under the sow. Skin swabs were obtained by use of cellulose sponge and quantitatively analyzed according to the ISO 21528-2:2004 and ISO 16649-2:2001 standard methods. The mean *Enterobacteriaceae* and *Escherichia coli* counts were significantly lower after disinfection ($P < 0.001$), resulting in 98.4% and 98.8% reduction, respectively.

Key words: pig, mammary gland, bacterial reduction, skin swabs, ISO standards.

Introduction

The conditions of intensive pig breeding with a large number of animals accommodated in a relatively small area and the increasing demands upon animal productivity impose a number of animal health care problems. Therefore, in pig-breeding industry, prophylactic measures have been increasingly emphasized (Vučemilo, 2007), with the aim to prevent the inception of diseases from outside and to reduce the undesired microflora inside the farm (Novak et al., 2001).

Unlike dairy farming, disinfection of the sow mammary glands during lactation, as a prophylactic procedure, is not a routine in commercial pig production, although it has been demonstrated to significantly reduce total bacterial count on the sow mammary glands (Ostović et al., 2010), thus decreasing the piglet death rate due to alimentary infections (Pavičić et al., 2011). However, at many pig farms, sows are now washed with warm water or soap suds and then disinfected, or are just washed with water before entering the farrowing unit. Thus, the sow skin is cleaned and the possibility of piglet exposure to microorganisms during sucking is reduced (Meyerholz and Gaskin, 1987; van der Vinne, 2009). Furthermore, at some farms sows undergo washing before entering the farrowing unit, whereas disinfection of mammary glands is performed in the farrowing unit, immediately prior to placing piglets under the sow, as in the present study.

As *Enterobacteriaceae* and in particular *Escherichia coli* are known to be among the most important agents causing mammary gland related diseases in sows and piglets (Dewey et al., 1995; Francis, 2002; Gerjets and Kemper, 2009), the aim of the study was to assess the rate of their reduction upon disinfection of the sow mammary glands.

Material and Methods

The study was carried out in commercial pig breeding conditions in the farrowing unit of a pig-breeding farm in the northwest part of Croatia. The study included 30 own-bred Swedish Landrace sows in third parity. Each sow was accommodated in a farrowing crate's restriction, 1.80x0.56 m in size, with partially slatted concrete floor. The farrowing unit contains 80 crates.

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Temperature, relative humidity and airflow rate in the farrowing unit were 21.4°C, 62.7% and 0.08 m/s, respectively. Sows were fed periodically, six times daily, with concentrated feed containing 17.96% crude protein, 4.65% crude fat, 4.27% crude fiber and 13.12 MJ/kg of metabolic energy, with drinking water *ad libitum*.

A week before the expected farrowing, the sows were placed in the clean and disinfected farrowing unit according to the "all in-all out" principle. Before entering the farrowing unit, the sows were washed with warm water, whereas disinfection was performed in the farrowing crate immediately before placing the piglets under the sow, according to the usual management practice at the farm. Namely, piglets are kept in a box under the heating source until the last piglet is brought forth, where they undergo the usual post-farrowing procedures prior to being placed under the sow. Ventral aspect of the sow trunk with mammary glands was washed with antiseptic and wiped with disposable paper napkin. The commercial chlorine-based antiseptic (D-V, Genera Inc., Croatia), a concentrated udder disinfectant, was used for disinfection at 1:100 dilution, according to the manufacturer's instructions.

In each sow, a 100 cm² skin surface in the ventral median line area between posterior mammary glands was marked using a sterile plastic template (10x10 cm). Samples were collected aseptically by rubbing the cellulose sponge premoistened with 10 ml of buffered peptone water (Hydrasponge[®], Biotrace, USA) approximately ten times in vertical and then ten times in horizontal direction. Upon sow disinfection and drying, the procedure of swabbing was repeated cranially adjacently to the previous sampling site. Swabs were transported to the laboratory in chilled containers and examined on the same day. Upon receipt at the laboratory, they were homogenized by stomaching. *Enterobacteriaceae* counts were determined by the ISO 21528-2:2004 method, whereby enumeration of colonies was carried out in a solid medium after incubation at 37°C. Enumeration of *Escherichia coli* was performed according to the ISO 16649-2:2001 standard determining the number of β -glucuronidase positive *Escherichia coli* at 44°C on a solid medium containing a chromogenic ingredient for detection of the β -glucuronidase enzyme. The testing laboratory has accreditation certificates for both methods and has been accredited according to the ISO 17025 standard.

Enterobacteriaceae and *Escherichia coli* counts were expressed as CFU/cm². Statistical analysis was performed using the reference Statistica v.10 (Statsoft Inc., 2011) software. The values of study parameters were expressed as mean and standard deviation, also stating median (min-max) because some parameters did not follow normal distribution. The significance of bacterial count reduction after disinfection was analyzed by the nonparametric Sign Test for dependent samples.

Results and Discussion

According to the Commission Regulation 2005/2073/EC in European Union and regulations in force in Croatia (Anonymous, 2005; 2008), *Enterobacteriaceae* counts are obligatorily determined on slaughterhouse pig carcasses as an indicator of their microbiological contamination during slaughter and processing. However, determination of *Enterobacteriaceae* counts in pig housings and pigs during breeding period is rarely reported (Mannion et al., 2007; Poletto et al., 2008).

In the present study, *Enterobacteriaceae* and *Escherichia coli* counts were determined on the sow skin, that is mammary glands before and after their disinfection. Pre-disinfection counts are shown in Figure 1. Before disinfection, *Escherichia coli* accounted for 44.4% of the overall *Enterobacteriaceae* counts. This percentage can be related to the study conducted by Schierack

et al. (2007), where *Escherichia coli* was the most common member of intestinal *Enterobacteriaceae* populations in healthy domestic pigs. After disinfection, the rate of *Escherichia coli* in the overall *Enterobacteriaceae* counts was 30.7%, pointing to a reduction from the pre-disinfection percentage; this finding might also be explained by the lower *Escherichia coli* resistance to disinfection (Rivera et al., 2010).

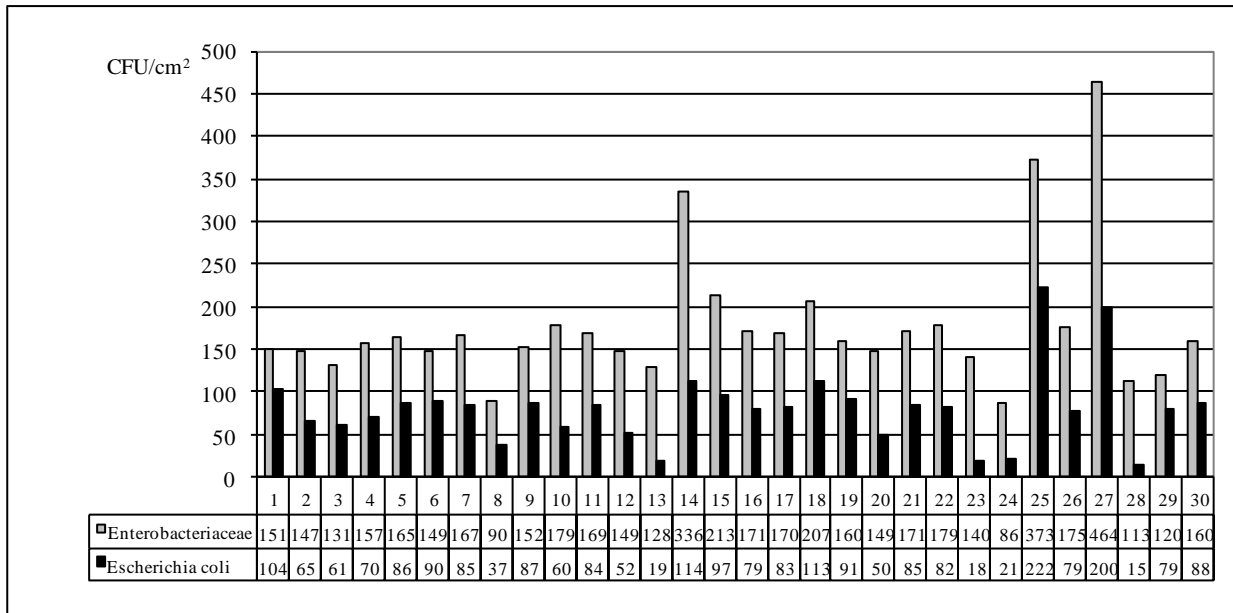


Figure 1. – *ENTEROBACTERIACEAE* AND *ESCHERICHIA COLI* COUNTS OBTAINED BY SKIN SWABBING IN 30 SOWS PRIOR TO DISINFECTION

The mean *Enterobacteriaceae* counts of 177.4 ± 79.4 CFU/cm² (median = 160.0 CFU/cm²) before disinfection decreased significantly ($P < 0.001$) to 2.9 ± 1.7 CFU/cm² (median = 3.0 CFU/cm²) after disinfection, yielding a 98.4% reduction (Table 1). The mean *Escherichia coli* count before disinfection was 80.5 ± 44.7 CFU/cm² (median = 82.3 CFU/cm²), also having decreased significantly ($P < 0.001$) to 1.1 ± 1.0 CFU/cm² (median = 1.0 CFU/cm²) after disinfection, yielding a 98.8% reduction (Table 1).

Table 1. – *ENTEROBACTERIACEAE* AND *ESCHERICHIA COLI* COUNTS ON SOW SKIN SWABS COLLECTED BEFORE AND AFTER DISINFECTION AND REDUCTION PERCENTAGE

Bacteria	Bacterial counts (CFU/cm ²)				P-value	Reduction (%)
	Before disinfection		After disinfection			
	x±SD	Me (min-max)	x±SD	Me (min-max)		
<i>Enterobacteriaceae</i>	177.4±79.4	160.0 (86.4-463.6)	2.9±1.7	3.0 (0-5.0)	<0.001	98.4
<i>Escherichia coli</i>	80.5±44.7	82.3 (14.6-221.8)	1.1±1.0	1.0 (0-4.0)	<0.001	98.8

N = 30 sows, x = mean, SD = standard deviation, Me = median, min = minimal value, max = maximal value

Recovery of bacteria from the surface depends on the sampling method used. The nondestructive method of sponge swabbing was employed in the present study, suggesting a probably higher actual bacterial count on the sow skin, as demonstrated by studies comparing the methods of carcass sampling at slaughterhouses (Pearce and Bolton, 2005). In addition, when using the ISO 16649-2:2001 horizontal method for enumeration of β -glucuronidase positive *Escherichia coli*, the actual number of this bacterial species is expected to be higher due to the method limitations because β -glucuronidase negative strains such as *Escherichia coli* O157 and strains that do not grow at 44°C will not be detected.

Conclusions

The investigated procedure of disinfection of the sow mammary glands, which is routinely performed at the farm, is considered justifiable for resulting in considerable reduction in the number of bacteria from the family *Enterobacteriaceae*.

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UČINAK DEZINFEKCIJE KOŽE KRMAČA NA BROJ ENTEROBAKTERIJA S NAGLASKOM NA BAKTERIJU *ESCHERICHIA COLI*

Sažetak

Cilj ovog rada bio je istražiti utjecaj dezinfekcije kože krmača držanih u komercijalnim uzgojnim uvjetima na broj bakterija porodice *Enterobacteriaceae* i vrste *Escherichia coli*. Istraživanjem je bilo obuhvaćeno 30 krmača na svinjogojskoj farmi. Ventralni dio trupa krmača, s mliječnim žlijezdama, dezinficiran je neposredno prije stavljanja prasadi pod krmače. Obrisici površine kože dobiveni su korištenjem celuloznih spužvica i kvantitativno analizirani prema standardnim metodama ISO 21528-2:2004 i ISO 16649-2:2001. Prosječni broj enterobakterija i bakterija *Escherichia coli* bio je značajno manji nakon dezinfekcije ($p < 0,001$), koja je rezultirala smanjenjem broja bakterija za 98,4%, odnosno 98,8%.

Ključne riječi: svinja, mliječna žlijezda, smanjenje bakterija, obrisci kože, ISO standardi.

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