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THE PRESENCE OF *SALMONELLA ENTERICA* AND *LISTERIA MONOCYTOGENES* ON POULTRY SLAUGHTER PLANT EQUIPMENT AFTER SANITATION PROCEDURES

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Abstract

The persistence of *Salmonella enterica* and *Listeria monocytogenes* on poultry slaughter plant equipment and food contact surfaces is an important factor for food contamination. Both bacterial foodborne pathogens can form biofilms and survive stress caused by sanitation procedures. The aim of this study was to evaluate the degree of contamination of poultry slaughter plant equipment by *Salmonella enterica* and *Listeria monocytogenes* after cleaning and disinfection. **Samples were taken from** four areas of the poultry slaughter plant (i.e., slaughter line, cutting line, meat processing, and packing area) and evaluated using real-time quantitative PCR. In total 32.5% of samples were positive for *Salmonella enterica,* while 37.5% were positive for *Listeria monocytogenes*. Both *Salmonella enterica* and *Listeria monocytogenes* were mostly detected on the slaughter line, mostly after evisceration. Evisceration, or removal of the birds' internal organs, may present a high-risk step of cross-contamination due to gut breakage and transfer of the contents to the skin, other carcass surfaces, and on equipment surfaces. This study indicates that poultry slaughter plant equipment may harbor foodborne pathogens like *Salmonella enterica* and *Listeria monocytogenes* after applying standard sanitation procedures.

Key words: foodborne pathogens, cross-contamination, real-time quantitative PCR, food contamination

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PRISUSTVO *SALMONELLA ENTERICA* I *LISTERIA MONOCYTOGENES* NA OPREMI U POGONU ZA KLANJE ŽIVINE NAKON SPROVEDENE PROCEDURE SANITACIJE

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Kratak sadržaj

Perzistentnost organizama *Salmonella enterica* i *Listeria monocytogenes* na opremi u pogonima za klanje živine kao i na površinama koje su u kontaktu sa hranom predstavljaju značajan faktor u pogledu kontaminacije hrane. Oba pomenuta bakterijska patogena mogu da formiraju biofilmove i da prežive na površinama nakon procesa sanitacije. Cilj ovog istraživanja bio je da se proceni stepen kontaminacije opreme u pogonu za klanje živine bakerijama *Salmonella enterica* i *Listeria monocytogenes* nakon čišćenja i dezinfekcije. Uzorci su sakupljeni na četiri lokacije u pogonu za klanje (linija klanja, linija rasecanja, zona za obradu mesa i zona za pakovanje) i analizirani pomoću real-time kvantitativne PCR metode. Ukupno 32,5% uzoraka bilo je pozitivno na *Salmonella enterica,* a 37,5% na *Listeria monocytogenes*. Obe bakerije, *Salmonella enterica* i *Listeria monocytogenes* su najčešće detektovane na liniji klanja, uglavnom nakon evisceracije. Evisceracija odnosno uklanjanje unutrašnjih organa, može predstavljati korak visokog rizika za unakrsnu kontaminaciju zbog pucanja creva i prenosa crevnog sadržaja na kožu i ostale površine trupa, kao i na površinu opreme. Ova studija je pokazala da se na opremi u pogonima za klanje živine i nakon primene standarnih procedura sanitacije mogu zadržavati patogeni poreklom iz hrane poput *Salmonella enterica* i *Listeria monocytogenes*.

Ključne reči: patogeni hrane, unakrsna kontaminacija, kvantitativni PCR u realnom vremenu, kontaminacija hrane

INTRODUCTION

Poultry meat is one of the primary animal protein sources in human nutrition. Due to the short production period, poultry meat production is highly

economical (Uzundumlu and Dilli, 2023). The modern poultry meat industry has achieved great progress in the field of food safety and hygiene of the production process by applying good production and hygiene practices, Hazard Analysis Critical Control Points (HACCP), and other food safety systems. Despite all that, it still faces the problem of foodborne pathogenic bacteria control, such as *Salmonella enterica* subspecies *enterica*, and *Listeria monocytogenes*. These foodborne pathogenic bacteria, in addition to being responsible for a large number of hospitalized people worldwide, also affect the shelf life of poultry meat and meat products (Redmond and Griffith, 2006).

According to Zoonoses Directive 2003/99/EC, foodborne outbreaks caused by *Salmonella* and *Listeria monocytogenes* are mandatory for reporting in Europe. *Salmonella enterica* is the etiologic agent of salmonellosis, while *Listeria monocytogenes* causes listeriosis (EFSA and ECDC, 2021). The risk groups for both foodborne diseases are mostly people with compromised immune systems, elderly, and children. Pregnant women are at an increased risk of developing listeriosis (Sereno et al., 2019). *Salmonella enterica* is a Gram-negative bacterium that can survive at pH 4.0 - 9.0, water activity > 0.94, and with temperature growth range 5 - 45 °C (Gast and Porter, 2020), while *Listeria monocytogenes* is a Gram-positive bacterium, which can survive at pH 4.6 - 9.5, water activity > 0.92, and with temperature growth range from a few degrees below 0 °C to 45 °C (Carpentier and Cerf, 2011).

Microbial contamination of poultry meat can occur during the entire poultry production chain with bacteria from the digestive tract, skin, and feathers, especially as a result of wrong techniques during slaughtering. Evisceration is a critical point of carcass contamination due to the presence of many bacteria in the gastrointestinal tract of broilers. Additionally, microbial contamination may occur from water, air, and equipment surfaces (Rouger et al., 2017).

On equipment and machine surfaces, such as stainless steel and polystyrene, at poultry slaughterhouse plants, *Salmonella enterica* and *Listeria monocytogenes* can easily adhere and form microcolonies. Further bacterial reproduction leads to biofilm formation (Vidaković Knežević et al., 2021), an association of microorganisms firmly attached to the surface, encased within an extracellular polymeric substance matrix. Biofilm acts as the constant source of microbial contamination in food processing environments (Abebe, 2020).

Real-time PCR is a valuable and sensitive method for investigating foodborne outbreaks and identifying pathogens. This method provides fast results, contrary to conventional culture methods that require at least five or more days to confirm the identification of *Salmonella enterica* and *Listeria monocytogenes* in positive samples. Besides replacing conventional time-consuming

methods, the advantages of the real-time PCR method are minimized cross-contamination and reduced manpower (Bohaychuk et al., 2007).

This study was conducted at a slaughterhouse plant to evaluate the contamination rate of equipment after sanitation and disinfection with *Salmonella enterica* and *Listeria monocytogenes,* using a real-time PCR method.

MATERIALS AND METHODS

Sample collection

The study was performed at a small-scale poultry slaughter plant in the Srem district, Republic of Serbia. This poultry slaughter plant has implemented a Hazard Analysis and Critical Control Point (HACCP) system and Good Hygiene Practice (GHP). Its main products are fresh meat in the form of whole, half, or quarter carcasses or smaller meat cuts. Further, this poultry slaughter plant processes fresh poultry meat into cured, smoked, canned, and other meat products. The sampling protocol applied in the poultry slaughter plant included sampling the equipment with cotton swabs under SRPS EN ISO 18593:2018 within 2 hours after cleaning and disinfection. Samples were taken from four different areas of the poultry slaughter plant, including the slaughter line (19 samples), the cutting line (11 samples), the processing area (8 samples), and the packing area (2 samples). A total of 40 samples were collected. All samples were transported to the laboratory under cooled conditions and processed the same day.

DNA extraction and real-time PCR

Genomic DNA extraction from swabs was performed using the Genesig Easy extraction kit (Primedesign™ Ltd, Eastleigh, United Kingdom) through a six-step process (Žekić Stošić et al., 2021). The detection and quantification of *Salmonella enterica* and *Listeria monocytogenes* genomes were performed using the commercial genesig real-time PCR kits (*Salmonella enterica* Genesig® Easy kit, and *Listeria monocytogenes,* Primedesign™ Ltd, Eastleigh, United Kingdom). Both kits contain primers representing 100% homology with over 95% of the National Center for Biotechnology Information Database reference sequences. The *Salmonella enterica* kit targets the invA gene, while the *Listeria monocytogenes* kit contains specific primers for quantifying all *Listeria monocytogenes* and does not detect other *Listeria* species. Under optimal PCR conditions, detection kits have very high priming efficiencies of > 95% and

can detect less than 100 copies of the target template. In both of these qPCR protocols, there are 50 cycles. Every cycle consisted of enzyme activation at 95 °C for two minutes, followed by denaturation at 95 °C for 10 seconds and data collection at 60 °C for one minute. Genomic DNA from *Salmonella* Enteritidis ATCC 13076 and *Listeria monocytogenes* ATCC 19118 served as positive controls, while sterile water was a negative control.

RESULTS

According to the preslaughter documentation, the flock slaughtered on the sampling day had an official *Salmonella enterica* negative status. In total, 40 samples (Table 1.) were collected from four areas of the poultry slaughter plant after cleaning and disinfection. Thirteen samples were *Salmonella enterica* positive (32.5%), and fifteen were *Listeria monocytogenes* positive (37.5%) by qPCR method. Eleven samples were positive for both *Salmonella enterica* and *Listeria monocytogenes* (27.5%). Along the slaughter line, both *Salmonella enterica* and *Listeria monocytogenes* were first detected in the evisceration line. From the vent cutter drip tray to the giblet sorting table, only the foot loop of the eviscerator and the neck skin cutter were free from *Salmonella enterica.* Similarly, *Listeria monocytogenes* was detected throughout the evisceration line, starting from the vent opener cutter assembly (blade). In total, 12 (63.16%) and 13 (68.42%) of the 19 samples taken from the cleaned and disinfected slaughter line were *Salmonella enterica* and *Listeria monocytogenes* positive, respectively. *Salmonella enterica* and *Listeria monocytogenes* positive samples were taken from the slaughter line (57.89%).

Table 1. Distribution of *Salmonella enteric* and *Listeria monocytogenes* on poultry slaughter plant equipment surfaces.

No.	Areas of poultry slaughter plant	Surfaces	*Salmonella enterica*	*Listeria monocytogenes*
1	Slaughter line	Live bird hang-on area	Negative	Negative
2		Waterbath Stunner	Negative	Negative
3		Shackle of the slaughtering conveyor	Negative	Negative
4		Rubber fingers of plucker	Negative	Negative
5		Vent cutter assembly (blade)	Negative	Negative
6		Vent cutter drip tray	Positive	Negative
7		Vent opener cutter assembly (blade)	Positive	Positive
8		Vent opener drip tray	Positive	Positive
9		Evisceration spoon	Positive	Positive
10		Foot loop of eviscerator	Negative	Positive
11		Wing grid of eviscerator	Positive	Positive
12		Breastplate of eviscerator	Positive	Positive
13		Frame (base of the machine) of eviscerator	Positive	Positive
14		Glaze wheel of eviscerator	Positive	Positive
15		Cropping machine cleaning mandrel	Positive	Positive
16		Neck skin cutter	Negative	Positive
17		Throat slitting machine stand	Positive	Positive
18		Giblet harvesting	Positive	Positive
19		Giblet sorting table	Positive	Positive

No.	Areas of poultry slaughter plant	Surfaces	*Salmonella enterica*	*Listeria monocytogenes*
20	Cutting line	Shackle	Negative	Negative
21		Whole wing cutter	Negative	Negative
22		Saddle cutter	Negative	Negative
23		Drum and thigh cutter	Negative	Negative
24		Breast cap cutter	Positive	Negative
25		Portioning spindle (cone)	Negative	Positive
26		Chicken breast filleting machine conveyor belt	Negative	Negative
27		Sloped belt conveyor for boneless chicken breasts	Negative	Negative
28		Planed belt conveyor for boneless chicken breasts	Negative	Positive
29		Whole wings conveyor belt	Negative	Negative
30		Drums and thighs conveyor belt	Negative	Negative
31	Processing area	Wolf meat grinder machine No. 1	Negative	Negative
32		Wolf meat grinder machine No. 2	Negative	Negative
33		Baader Meat Separator	Negative	Negative
34		Meat Filling Machine	Negative	Negative
35		Cutter	Negative	Negative
36		Tumbler	Negative	Negative
37		Meat cart	Negative	Negative
38		Floor drain	Negative	Negative
39	Packing area	Meat slicer knife	Negative	Negative
40		Meat slicer conveyor belt	Negative	Negative

One more *Salmonella enterica* positive sample was collected in the cutting line from the knife of the breast cap cutter machine. This sample was negative for *Listeria monocytogenes*. However, *Listeria monocytogenes* positive samples in this area of the poultry slaughter plant were portioning spindle (cone) and the planed belt conveyor for boneless chicken breasts.

Both the processing and packing area tested negative for *Salmonella enterica* and *Listeria monocytogenes.*

DISCUSSION

At the four stages of the poultry production chain investigated (the slaughter line, the cutting line, the processing area, and the packing area), the prevalence of *Salmonella enterica* ranged from 0% to 63.16%, while the prevalence of *Listeria monocytogenes* ranged from 0% to 68.42%.

As we only looked into one specific poultry slaughter plant, the prevalence found at the various production stages cannot necessarily be generalized. Besides, PCR may be too sensitive and detect microorganisms that are present at non-pathogenic levels (Mackay, 2004). However, the result was expected because the area with the highest prevalence is also the dirtiest in the poultry slaughter plant. The observed difference in prevalence could also be explained by the very different number of samples or by the variable efficiency of sanitation practices. Earlier studies show differences in cleaning and disinfection when performed by the slaughterhouse staff and external professional cleaning firms (Zeng et al., 2021). In our study, cleaning and disinfection are performed by the staff working in the specific poultry slaughter plant area. The difference in cleaning and disinfection results could be caused by the lack of food safety awareness and the level of training, motivation, etc. Besides that, the accessibility of surfaces for cleaning and disinfection plays an important role in *Salmonella enterica* and *Listeria monocytogenes* presence. Additionally, the positive rate of swabs at the slaughter and evisceration line is because *Salmonella enterica* and *Listeria monocytogenes* are commensally pathogens of the gut for many animals, including poultry, from where they can contaminate equipment surfaces during the evisceration and enter into the food chain. It has been previously suggested that *Salmonella enterica* ***serovar*** Enteritidis, *Salmonella enterica* ***serovar Typhimurium***, and *Listeria monocytogenes* could persist in a meat processing environment by attaching firmly to stainless steel and plastic surfaces and forming biofilms (Vidaković Knežević et al., 2021; Vidaković Knežević et al., 2023). Forming biofilms is an important survival strategy for bacteria due to unfavorable conditions. Adhered cells tend to be more resistant to cleaning and disinfection than suspended bacteria. Once established, biofilms act as permanent sources of contamination and dispersal in the environment, leading to cross-contamination (Alvarez-Ordóñez et al., 2019).

The total elimination of *Salmonella enterica* and *Listeria monocytogenes*

from food processing environments is almost impossible because of their ubiquitous presence in nature and the many potential avenues for entry into these processing environments (Duze et al., 2021). Additionally, the constant use of the same disinfectants and the sub-lethal concentrations may lead to resistance of these foodborne pathogens to the antibacterial agent applied (Langsrud et al., 2003). Therefore, controlling *Salmonella enterica* and *Listeria monocytogenes* in poultry slaughterhouses is complex.

CONCLUSION

The prevalence of *Salmonella enterica* and *Listeria monocytogenes* in the poultry slaughter plant studied was very high, with up to over 60% of equipment surfaces. The most critical point is the stage of evisceration. The complex machines, containing many small and hard-to-reach parts, make cleaning and disinfection processes challenging. In order consider these results as food safety and health risks, it is necessary to isolate and identify *Salmonella enterica* and *Listeria monocytogenes* because those methods are irreplaceable as confirmatory methods in the microbiology of food. Elementary principles for hygiene must be respected, including effective mechanical cleaning, the recommended concentrations of antibacterial agents, contact time, rinsing, and drying. All workers involved in hygiene must be well-trained.

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Author's Contribution

SVK basic idea, conception, and design, drafting the manuscript; SVK and MP sampling; MŽ and SS examination; SK and JV interpretation of results and manuscript revision; DLJP conception and design. All authors read and approved the final version of the manuscript.

Competing interest

The authors declare that they have no competing interests

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