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ASSESSMENT OF THE HYGIENIC CONDITION OF THE SLAUGHTERHOUSE BASED ON THE EVALUATION OF MICROBIOLOGICAL SWABS

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ABSTRACT

This study objective was to evaluate the hygienic condition of surfaces by microbiological swabs and the effectiveness of disinfectant Virkon S. Level of hygiene was evaluated in a small-capacity slaughterhouse located in the Košice region with a maximum weekly capacity of 5 large livestock units. Microbiological swabs were taken from an area of 10 cm^2 before the process of slaughtering, during the process, and after disinfection. Disinfectant Virkon S was used in a 1% concentration during an exposure time of 30 minutes for disinfection of monitored surfaces. Disinfectant was effective on surfaces of cage, wall, floor, and ($p<0.0001$), where was determined significant decrease of microorganisms, but we recorded 1.0 x 10^4 colony forming units (CFU)/10 cm^2 of the total count of bacteria (TCB) on the cage, 7.5 x 10^3 CFU/10 cm^2 of TCB on the wall and 1.5 x 10^4 CFU/10 cm^2 of TCB on the floor after disinfection, which indicates an insufficient level of disinfection. From the achieved results, it is clear, that the disinfection provided by the disinfectant Virkon S was not effective, as the evaluated disinfectant did not achieve a decrease in the number of microorganisms and thus did not ensure a sufficient level of hygiene. We concluded that it is crucial to effectively disinfect products using the right disinfectant at the right exposure period since it helps to stop the spread of several germs that could contaminate products and have a negative impact on consumers' health.

Keywords: disinfection, hygiene, microbiological swabs, slaughterhouse

INTRODUCTION

Slaughterhouse is a key source of bacterial contamination in meat and its products, which is a major health and economic concern for several public authorities **(Klaham *et al.*, 2022).** The key hygiene controls at each stage are highlighted in the general principles of food hygiene, which represent hygiene practices from basic production through to final consumption. Food contamination can happen in a variety of ways and at any point throughout production, distribution, and storage **(McBain *et al.*, 2000)**. Slaughtering procedures in slaughterhouses play a significant influence in the transmission of foodborne microorganisms **(Shang *et al.*, 2019; Rasschaert *et al.*, 2008)**. Slaughterhouse is a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, processing, effective preservation, and storage of meat products for human consumption (**Alonge, 1991)**. Their general hygiene requirements are set out in Regulation of the European Parliament and the Council (EC) No. 853/2004 and Commission Regulation (EC) No. 2023/2006. A slaughterhouse is an establishment used for the slaughter and slaughtering of animals whose meat is intended for human consumption, must be equipped with equipment for disinfecting instruments with hot water (minimum temperature is 82 °C) **(Lagin & Lopašovský, 2004).**

The slaughtering of animals should take place under veterinary supervision and complete and appropriate hygienic precautions since this is the most crucial aspect in ensuring the production of meat products of high quality **(Serda *et al.*, 2015; Zailani *et al.*, 2016)**.Bacterial contamination can occur on the surface of the meat during meat preparation, carcass cutting, manufacturing of meat products, packing, during transportation and storage until it reaches the consumer **(Heyndrickx *et al.*, 2002; Ananchaipattana, 2003).** This contamination can also occur during primary production at the farm from the first skin incision made to remove the blood, especially if the tools used by the operator are not sterile. In most slaughterhouses, carcasses are not skinned, but they are subjected to several steps that result in the skin being visibly clean and free from hair. Despite this, the carcasses may be heavily contaminated with microorganisms. The carcasses may be further contaminated during the next steps of evisceration and cutting **(Gill *et al.*, 2000)**. Bacterial hazards represent a major concern in the production of food of animal origin. The major contamination points during slaughter are animal-related, such as faecal and pharyngeal, and environmental. Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practice (GMP) must be focused on maximal limiting this spread which leads to the prevention of microbial carcass contamination to ensure the health protection and meat safety **(Lindblad & Berking, 2013).** Because slaughter is an open process, there are several chances for the carcass to become contaminated with potentially harmful microorganisms. The procedure has some steps where the amount of bacteria may be decreased, but it doesn't include any steps where risks are completely removed. Only partial control can be obtained for the critical control points (CCPs) indicated for slaughtering techniques, and there is limited scope for risk prevention **(Bakri *et al.*, 2017).** Some CCPs stand for control points (CPs) that GMP controls (Fig. 1). The general mechanisms of spread of the harmful bacteria differ. The main source of contamination for *Yersinia enterocolitica*, *Campylobacter* spp., and *Salmonella* spp. is the contamination of carcasses, which can be restricted if only stringent slaughtering protocols are followed. In the processing environment, other organisms such *Aeromonas* spp. and *Staphylococcus aureus* may be endemic, but they may be managed with proper cleaning and disinfection **(Brown *et al.*, 2000; Zweifel *et al.*, 2008).** These microorganisms serve as indicators for the GMP regulations.

Flawless hygienic production conditions are ensured by sanitation. The basic elements of a comprehensive sanitation activity are cleaning and disinfection. Thorough cleaning must be performed before applying disinfection. This step is essential for the disinfection to be as effective as it can be to remove organic and other undesirable matter, like biofilm, from the surfaces that may protect the microorganisms from the effects of the disinfectant **(Dvorak, 2005).** The goal of disinfection is to destroy microorganisms; this does not automatically mean killing all microorganisms but reducing the number to a level that is not normally harmful to health **(Skaarup, 2011).** To achieve effective disinfection the surfaces subject to disinfection must be thoroughly wet, and the disinfectant must be applied in the correct concentration. It must also be allowed to stay on the surfaces for the appropriate contact time **(Dvorak, 2005).** Inappropriate use but also overuse of biocides in different areas such as food industries, hospitals, and homes may lead to the emergence of resistance to various biocides (e.g.: quaternary ammonium compounds, triclosan, chlorhexidine or trisodium phosphate) **(Brauodaki & Hilton, 2005; Romanova *et al.*, 2002; Yuk & Marshall, 2006).** The selective

pressure exerted by biocides is responsible for cross-resistance between antibiotics and biocides **(Davin-Regli & Pagès, 2012)** since the use of low concentrations of biocides may increase the risk of selection of resistant microorganisms.

Table 1 Hygienic aspects and preventive actions with respect to bacterial hazards at the slaughterhouse

Process step	Hygienic aspect	Preventive actions	CP/CCP
Lairage	Contamination between animals	Cleaning & disinfection	CP
↓			
Stunning			
↓			
Killing	Contamination from tools	Cleaning & disinfection	CP
↓			
Scalding	Reduction of bacterial levels	Time/Temperature	CP
↓	Contamination of lungs		
Dehairing	Contamination from machines	Cleaning & disinfection	CP
↓			
Flaming	Reduction of bacterial levels	Time/Temperature	CP
↓			
Polishing	Contamination from machines	Cleaning & disinfection	CP
↓			
Evisceration	Contamination from intestines	Enclosure of rectum	CCP
↓	Contamination from the tongue, pharynx and tonsils	Working instructions Disinfection of tools	
	Contamination from tools		
Splitting	Contamination via splitter/saw	Line-speed	CP
↓		Water temperature	
Meat inspection	Contamination from inspection	Disinfection of tools	CCP
↓			
Deboning of head	Contamination from head	Working instructions Disinfection of tools	CCP

Legend: CP - **control point, CCP – critical control points**
Source: **Borch *et al*. (1996)**

Evaluation of the disinfection process is one of the stages of disinfection. It should be done both during and after the process. Microbiological control is the most impartial way of disinfection evaluation. This technique shows whether the disinfection was effective. The total bacterial count or the presence of indicator bacteria are employed as a substitute for the arduous and unreliable process of detecting pathogenic germs **(Tuladhar *et al*., 2012).** Microbiological swabs are taken after the correct exposure time of the disinfection. A sufficient number of swabs according to the size of the disinfected area should be taken from different equipment and surfaces (**Griffith, 2016)**.
The objective of the study was to evaluate the hygienic condition of monitored surfaces by microbiological swabs and the effectiveness of disinfectant Virkon S used in a 1% concentration during an exposure time of 30 minutes.

MATERIALS AND METHODS

Characteristics of slaughterhouse and disinfectant

The study was performed in a small-capacity slaughterhouse with a maximum weekly capacity of 5 large livestock units. Slaughterhouse was located in the Košice region. The slaughter was divided into 3 parts - part I (slaughter and bleeding); part II (steambath and removal of bristles) and part III (evisceration) (Figure 1). In terms of slaughterhouse structure, the slaughterhouse in our experiment was a closed-system building and it had separated slaughtering lines for clean and unclean areas. Workers were assigned to a single location and did not rotate across other regions of the slaughterhouses. The equipment is rigorously confined to the authorized regions and is not combined within the designated zones. Workers donned safety gear, including boots and jackets, and cleansed their hands before going through the slaughtering line. The temperature of the scalding water was regulated, and the slaughtering and cutting knives were sterilized. To avoid cross-contamination between carcasses and the slaughtering floor, the slaughterhouse utilized hanging apparatus. For the purposes of the butchering, they used tap water. Every carcass treated in slaughterhouses had its internal organs removed.
For disinfection of different surfaces in evaluated slaughterhouses detergents and disinfectants were used. The detergent used for mechanical cleaning of surfaces was 2% Fint used as a degreaser by heating 50 – 60 °C with an exposure time. Disinfectants used for disinfection of surfaces were Fink – FC 21 and Virkon S which was evaluated. Fink FC 21 is a disinfectant used in liquid form, which is a high-foaming and cleaning agent, alkali, containing active chlorine. The disinfectant was applied by spraying at 2% concentration while being heated to 50 °C for 20 minutes of exposure. Virkon S was applied as a liquid in a 1% concentration by spraying, without being heated. The exposure lasted for thirty minutes. Oxone (potassium peroxymonosulfate, used as an oxidizing agent), sodium dodecylbenzene sulfonate (anionic surfactant), sulfamic acid, and inorganic buffers are all components of this multipurpose disinfectant. It is a balanced, stabilized blend of peroxygen compounds, surfactant, organic acids, and inorganic buffer. This disinfectant is recommended for use as a hard surface disinfectant in livestock production and transportation facilities. Fink Kanalreiniger was used for disinfection of the channel, it is powder. Disinfectants contain alkalis and anionic surfactants. It was used 200 grams in 0.5 liters of water with an exposure time of 30 minutes. Detergents and disinfectants used in the slaughterhouse were rinsed with water at the end of the processes of mechanical cleaning and disinfection.

Figure 1 From left: Division of slaughterhouse - part II (steambath and removal of bristles) and part III (evisceration)

Microbiological swabs

Assessment of the hygienic condition of the slaughterhouse was carried out by microbiological swabs. Swabs were taken from monitored places, from part I (slaughter and bleeding) before and during slaughtering and bleeding and after disinfection. Microbiological swabs were collected from 10 cm2 areas of the cage, wall, floor, and lift that were under evaluation (Figure 2). Six swabs were obtained from each location, totaling 24 samples for analysis. A sterile tube containing 10 ml of sterile saline solution and swabs was used. 0.1 ml of this mixture was put to the various agar plates. After being incubated in a thermostat, plates were used to analyze the colonies that had grown. Endo agar was used for coliform bacteria (CB), meat peptone agar was used for the total count of bacteria (TCB), and Sabouraud agar was made from molds. After 24 hours at 37 °C, the findings from Meat Peptone Agar and Endo Agar were obtained. After 3 to 5 days of incubation at room temperature, the findings from Sabouraud agar were obtained. For the determination of coliform bacteria, the total count of bacteria, and molds, the procedures according to the applicable ISO standards were used (ISO 18593; ISO

21257; ISO 4832). Numbers of microorganisms were expressed in CFU (colony forming units).

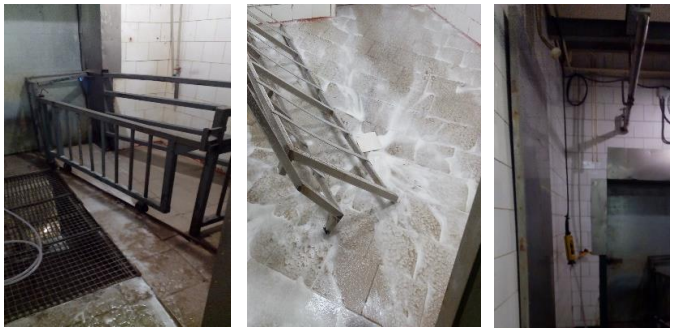

Figure 2 From left: Evaluated places for microbiological evaluation - cage, wall, floor, lift, and way of application of disinfectant

Statistical analysis

The results were statistically processed using descriptive statistical analysis of data and statistical method of the Student's t-test **for paired comparisons**. The differences in the numbers of the total count of bacteria, coliform bacteria, and molds were calculated between conditions before the process of slaughtering and after disinfection. The continuous variables were represented using mean (M) standard deviation (SD), whilst the categorical variables were described over the average of 5 samples from chosen surfaces (CFU/10 cm2). 0.05 was the threshold for significance (p)**.**

RESULTS AND DISCUSSION

The effectiveness of disinfectants for microorganisms depends on many factors. On the one hand, these are the properties of the microorganisms themselves, on the other hand, the chemical and physical properties of the external environment **(Vargová *et al.*, 2022).** The concentration of the disinfectant, exposure time, pH, temperature, the presence of organic contaminants, such as blood, serum, or other body fluids, the microorganism or agent itself, their type (prions, viruses, gram-negative, gram-positive bacteria, microscopic fungi, protozoa, or spores), as well as their number and location, are factors that affect disinfection efficiency **(Štefkovičová, 2007)**. Failure of the disinfection may be because of an ineffective disinfectant chosen, insufficient exposure time, incorrect use of the disinfectant, or because of the effect of an environmental factor **(Simões *et al.*, 2010)**. The insufficient exposure time was the reason for the failure of disinfection in our study.

The detection of pathogenic microorganisms in the outdoor environment is difficult and not sufficiently reliable, therefore, as part of the microbiological control of the effectiveness of disinfection, we determine the total count of bacteria or the presence of indicator bacteria – coliform bacteria **(Vargová *et al.*, 2022).** *Escherichia coli* is an example of a culturable coliform bacterium that can be utilized as a microbial surrogate for surface quality monitoring since it can be used to detect the presence of fecal material from warm-blooded animals. These bacterial species are part of the normal microflora that live in warm-blooded animals' intestines, and their presence on surfaces indicates the presence of bacterial pathogens. There are four different indicators of fecal contamination: total coliform, fecal coliform, *E. coli*, and *enterococcus* **(Byappanahalli *et al.*, 2012)**.

In Tables 2, 3, 4, and 5 is shown the effect of disinfectant Virkon S used at 1% concentration during exposure time 30 minutes on the evaluated surfaces - cage, wall, floor, and lift before the process of slaughtering, during the process of slaughtering and after disinfection.

Table 2 Effect of disinfectant Virkon S on monitored microorganisms present in the cage before the process of slaughtering, during the process, and after disinfection

	Cage (CFU/10 cm^2)			**P**
	before	**during**	**after**	
TCB	1.5 x 10^6	2.4 x 10^4	1.0 x 10^4	p<0.0001
p-value			before vs after p<0.0001	
CB	2.5 x 10^4	3.8 x 10^3	1.8 x 10^2	p<**0.0001**
p-value			before vs after p<0.0001	
Molds	1.5 x 10^2	1.9 x 10^4	8.4 x 10^1	p<**0.0001**
p-value			before vs after p = **ns**	

Legend: CFU - colony forming units; TCB - total count of bacteria; CB - coliform bacteria. A level of 0.05 was considered significant (p), ns – not significant

Finding the total count of bacteria up to 10^3 on the floor and up to 10^2 on the other monitored surfaces is permissible and disinfection is considered effective. In Table 2, the number of TCB after disinfection was 1.0 x 10^4 CFU/10 cm^2 of TCB, which indicates insufficient disinfection **(Sasáková *et al.*, 2020)**. According to **Ondrašovičová *et al.* (2013),** the effectiveness of preventive disinfection is satisfactory if the number of indicator bacteria is within 10% of the original number. The numbers of CB on the cage after disinfection did not exceed 10% of their original number, which is considered a good result of disinfection.

Table 3 Effect of disinfectant Virkon S on monitored microorganisms present on the wall before the process of slaughtering, during the process, and after disinfection

	Wall (CFU/10 cm^2)			**P**
	before	**during**	**after**	
TCB	3.5 x 10^4	1.4 x 10^4	7.5 x 10^3	p<0.0001
p-value			before vs after p<0.0001	
CB	>1.0 x 10^1	1.1 x 10^2	>1.0 x 10^1	p<**0.0001**
p-value			before vs after p = **ns**	
Molds	1.0 x 10^3	2.3 x 10^3	1.1 x 10^1	p<**0.0001**
p-value			before vs after p>0.0001	

Legend: CFU - colony forming units; TCB - total count of bacteria; CB - coliform bacteria. A level of 0.05 was considered significant p)

Table 4 Effect of disinfectant Virkon S on monitored microorganisms present on the floor before the process of slaughtering, during the process, and after disinfection

	Floor (CFU/10 cm^2)			**P**
	before	**during**	**after**	
TCB	1.5 x 10^6	2.7 x 10^4	1.5 x 10^4	p<0.0001
p-value			before vs after p<0.0001	
CB	1.1 x 10^4	1.5 x 10^4	1.4 x 10^2	p<**0.001**
p-value			before vs after p<0.0001	
Molds	>1.0 x 10^1	2.2 x 10^3	>1.0 x 10^1	p<**0.0001**
p-value			before vs after p = **ns**	

Legend: CFU - colony forming units; TCB - total count of bacteria; CB - coliform bacteria. A level of 0.05 was considered significant (p).

The bacterial contamination originates from the external animal surface, from the internal animal environment - from the gastrointestinal tract, as well as from the environment, including air, soil, water, equipment surfaces, and also humans. Control of the presence of pathogenic microorganisms on surfaces is based on the approaches of minimizing surface contamination through proper sanitation which includes mechanical cleaning and disinfection and the application of decontaminating procedures **(Gebel *et al.*, 2013).**

In Table 3 is shown the effect of disinfectant on evaluated microorganisms present on the wall. Virkon S was not effective enough on the monitored surface, because after disinfection we recorded 7.5 x 10^3 CFU/10 cm^2 of TCB, which indicates an insufficient level of disinfection. In Table 4 is shown the effect of disinfectant on the presence of TCB, CB, and molds situated on the floor. In a comparison of several monitored microorganisms before the process of slaughtering and after disinfection, was recorded with a significant decrease in the number of TCB, CB, and molds (p<0.0001). Disinfectant Virkon S was effective against CB and molds, except TCB, where after disinfection we recorded 1.5 x 10^4 CFU/10 cm^2.

Table 5 Effect of disinfectant Virkon S on monitored microorganisms present on the lift before the process of slaughtering, during the process, and after disinfection

	Lift (CFU/10 cm^2)			**P**
	before	**during**	**after**	
TCB	1.1 x 10^4	1.3 x 10^2	1.0 x 10^2	p<0.0001
p-value			before vs after p<0.0001	
CB	5.7 x 10^2	8.4 x 10^2	>1.0 x 10^1	p<0.0001
p-value			before vs after p<0.0001	
Molds	1.3 x 10^2	8.5 x 10^3	>1.0 x 10^1	p<**0.0001**
p-value			before vs after p<0.0001	

Legend: CFU - colony forming units; TCB - total count of bacteria; CB - coliform bacteria. A level of 0.05 was considered significant p).

In Table 5 is shown the effect of disinfectant on TCB, CB, and molds present on the lift. Virkon S was effective on the monitored surface where the number of evaluated microorganisms was permissible after disinfection and a significant decrease in microorganisms were obtained (p<0.0001).

In our experiment, disinfectant Virkon S caused a significant decrease of evaluated microorganisms but wasn´t effective enough on each of the evaluated surfaces which leads to the conclusion that this disinfectant used at 1% of concentration during exposure time 30 minutes is not suitable for disinfection of surfaces in a slaughterhouse. However, according to a study by **Vargová *et al.* (2021)** Virkon S

used in a 1% concentration during an exposure time of 60 minutes was suitable for disinfection on the premises of slaughterhouses.

The finding of some of the monitored microorganisms on evaluated surfaces after disinfection is related to insufficient exposure time (30 minutes) and in some cases - Table 2: 1.0 x 10^4 CFU/10 cm^2 of TCB; Table 3: 7.5 x 10^3 CFU/10 cm^2 of TCB and Table 4: 1.5 x 10^4 CFU/10 cm^2 of TCB exceed the limit for preventive disinfection. Therefore, ensuring an appropriate contact time = exposure time can influence whether a pathogen is inactivated, killed, or unaffected. Contact times are usually dependent on the material of the surface and the concentration used.

CONCLUSION

We confirmed that the disinfectant Virkon S used in 1% concentration during an exposure time of 30 minutes was not effective enough against the total count of bacteria, coliform bacteria due to the insufficient exposure time. Our findings regarding the microbiological contamination of surfaces in the slaughterhouse may help hygienists in comparable settings establish appropriate hygienic practices for the prevention or reduction of microbiological contamination of surfaces.

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