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Analysis of microbiological composition of deep-frozen meet of selected game animals stored in the refrigeration

Abstract

Game meat, similarly to meat from farm animals, may pose a serious threat to consumer health and lead to infections and food poisoning, as it is a food product sensitive to microbiological contamination. The aim of the study was to analyse the microbiological composition of selected types of game meat, i.e. wild boar, roe deer, and red deer, using microbiological methods involving the determination of the total number of microorganisms (L) in the meat during storage under refrigerated conditions (4°C) for nine days, as well as the control of the presence of pathogenic bacteria of the genera *Salmonella*, *Yersinia*, *Escherichia*, and *Listeria*. The study showed that the total number of microorganisms in all meat samples increased with the duration of storage. After nine days, the lowest total number of microorganisms was observed in red deer meat, and the highest in roe deer meat. In all tested samples, the total number of microorganisms (L) did not exceed the permissible microbiological limits, indicating that the meat meets the current quality and safety standards for sale and consumption. The conducted analysis in the field of microbiological control confirmed the presence of pathogenic bacteria of the genera *Escherichia* and *Yersinia* in all tested samples.

Keywords: bacteria, game, microbiological quality, selective microbiological media

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Introduction

Meat is a food product that is highly susceptible to oxidative processes and microbiological contamination. It should be noted that meat products can serve as a source of diseases and infections caused by various microorganisms, such as bacteria, fungi, or protozoa. This results from the fact that meat constitutes an excellent medium for microbial growth due to its high protein content and nearly neutral pH (Libudzisz, Kowal, 2000; Sofos, 2008). Several factors further contribute to meat spoilage, including a redox potential (Eh) favourable to most microorganisms, high water content and water activity, rich chemical composition, release of cellular enzymes, and the surface being exposed to light and atmospheric oxygen (Hac-Szymanczuk, 2012). In addition

to bacterial infections, meat consumption may also carry the risk of parasitic infections. Eating raw or undercooked meat typically pork or game – raises the risk of infection with nematodes (roundworms), such as *Trichinella spiralis* Owen (Gołąb, Sadkowska-Todys, 2003).

Microorganisms present on the surface of slaughtered animal carcasses belong to various taxonomic groups. Bacteria found in meat usually represent the following genera: *Pseudomonas*, *Alcaligenes*, *Escherichia*, *Streptococcus*, *Bacillus*, *Clostridium*, *Micrococcus* and *Proteus*. The occurrence of zoonotic pathogens in raw meat is variable, although most often it is between 1% and 10%, depending on the organisms, geographical factors, agricultural practices and/or meat production methods (Nørrung. Buncic, 2008). Microorganisms present on the surface of animal carcasses represent a significant microbiological hazard. Their presence results from the fact that both farmed and wild animals, in their natural environment, come into contact with numerous sources of contamination. Additionally, irregularities that may occur at any stage of the food production chain – such as transport, processing, and carcass storage – increase the risk of meat contamination. One particularly critical control point is the evisceration process. Improper evisceration poses a high risk of transferring intestinal bacteria to the surface of carcasses. Furthermore, inadequate hygiene and improper storage conditions during meat processing promote the further proliferation of pathogenic microorganisms, significantly increasing the risk of microbiological contamination (Rouger et al., 2017; Altissimi et al., 2023; Nakamura et al., 2023). Microorganisms present on carcass surfaces, such as *Pseudomonas* spp., may also cause undesirable organoleptic and physicochemical changes, such as off-odours, slimy surface texture, discoloration, and altered meat consistency (Doulgeraki et al., 2012; Luong et al., 2020).

The most common zoonotic diseases in humans are infections with bacteria of the genera *Campylobacter* and *Salmonella* (*European Food Safety Authority*, 2016, 2018, 2021). Campylobacteriosis is caused by thermophilic *Campylobacter* spp., of which 90% of infections are caused by *Campylobacter jejuni* (Jones & Little) Veron & Chatelain and *C. coli* (Doyle) Véron & Chatelain (Bruś-Chojnicka et al., 2011). The most common symptoms of this disease include diarrhoea (often bloody), abdominal pain, fever, headaches and nausea. Infections are usually self-limiting and last a few days. Salmonellosis in humans, on the other hand, is usually characterised by fever, diarrhoea, abdominal pain and nausea. Symptoms are typically mild, with most infections resolving spontaneously within a few days. Yersiniosis caused by *Yersinia enterocolitica* Schleifstein & Coleman causes abdominal pain, associated diarrhoea and fever (Janowska et al., 2012). This disease usually has a mild course. *Escherichia coli* produces Shiga toxin (STEC), which causes severe abdominal cramps, diarrhoea, bloody stools, fever, flatulence, and abdominal pain (Díaz-Sánchez et al., 2013). These bacteria are the main cause of bacterial food poisoning in humans worldwide. Listeriosis is a disease caused by infection with

Listeria monocytogenes (Murray et al.) Pirie. Symptoms of listeriosis can range from mild flu-like symptoms and diarrhoea to life-threatening forms characterised by sepsis and meningitis. This disease is rare in humans but has a severe course.

Food safety regulations define standards for producers and distributors, following hygiene rules is equally important for minimising the risk associated with pathogens present in raw meat. The likelihood of contamination largely depends on the health status of individuals handling food, their personal hygiene, knowledge, and compliance with food hygiene practices (Mama, Alemu, 2016). A fundamental condition for reducing the risk of infection is maintaining hygiene in the kitchen. The *World Health Organization* (WHO) recommends following five key food safety rules: maintaining cleanliness (washing hands, surfaces, and kitchen utensils), thorough cooking (achieving an internal temperature ≥70°C), keeping food at safe temperatures (below 5°C for refrigeration and above 60°C for reheating), and using safe water and raw materials (*WHO*, 2006). Bacteria such as *Listeria monocytogenes* and *Yersinia enterocolitica* can be present on the surface of raw meat; therefore, avoiding contact between these products and other food items or kitchen utensils is crucial to minimize the risk of cross-contamination (Guron et al., 2024). Storage temperature is one of the key factors influencing the microbiological spoilage of fresh meat, and improper temperature selection significantly accelerates this process (Zhao et al., 2022). Applying thermal treatment of meat to a minimum internal temperature of 70°C allows for the effective elimination of pathogenic microorganisms (Han et al., 2023). Thus, maintaining hygiene and implementing proper culinary practices in the home environment play a vital role in the prevention of foodborne illnesses.

The aim of the present study was to analyse the microbiological composition of selected types of game meat, namely wild boar, roe deer, and red deer, using classical microbiological methods. The scope of the research included the determination of the total number of microorganisms developing during the storage of game meat for nine days at 4°C (refrigeration conditions) and the identification of the presence of pathogenic bacteria from the family Enterobacteriaceae Rahn, including the genera *Salmonella* Lignieres, *Yersinia* van Loghem, *Escherichia* Castellani et Chalmers, as well as from the family Listeriaceae Garrity et al. – specifically *Listeria* Pirie.

Material and methodology

The research material consisted of deep-frozen meat of three game species: wild boar, roe deer and deer, purchased at retail in one of Rzeszów supermarkets (Southeastern Poland). Before preparing the samples for analysis, the meat was thawed and stored at refrigeration conditions at 4°C for 9 days.

Preparation of buffered peptone water
Buffered peptone water is a non-selective medium recommended as a pre-enrichment broth for food samples suspected of containing *Salmonella* spp. A quantity of 8.028 g of buffered peptone water powder was weighed and dissolved in 400 ml of distilled water. The prepared medium was used as a diluent for the tested samples.

Preparation of samples for analysis
Five grams (± 0.05 g) of each of the three types of game meat were weighed on a laboratory scale (*Medicat 1600C*, Poland) and placed into sterile containers. Subsequently, 45 ml of buffered peptone water was added to each sample. The prepared samples were subjected to shaking in a laboratory shaker (*Heidolph Unimax 1010*, Germany) at 250 rpm for 30 minutes to obtain meat extracts.

Microbiological media used for research
Plate Count Agar (PCA)
This medium is a solid, non-selective culture medium used for the enumeration of the total aerobic microorganisms (bacteria and fungi) on tested surfaces. Its composition includes enzymatic casein hydrolysate, yeast extract, glucose, and agar. A quantity of 9.4 g of Plate Count Agar (PCA) medium was weighed and dissolved in 400 ml of distilled water with stirring. Subsequently, the medium was sterilised by autoclaving. After cooling, approximately 20 ml of the medium was aseptically poured onto Petri dishes and left to solidify.

Violet Red Bile with Lactose (VRBL)
This is a selective medium used for the detection and enumeration of lactose-fermenting bacteria, as well as for differentiating bacteria of the coli genus or the aerogenes group from non-lactose-fermenting organisms. The enzymatic hydrolysate of animal tissues is a source of carbon, nitrogen, vitamins and minerals. 16.6 g of Violet Red Bile with Lactose (VRBL) substrate was weighed and 400 ml of distilled water was added and stirred until dissolved. The further procedure was analogous to the PCA medium.

Listeria acc. to Palcam and Chromogenic Listeria SET Supplement
It is a selective differential medium for the isolation and detection of *Listeria monocytogenes* species and other bacteria of the genus *Listeria*. It allows easy differential diagnosis of *L. monocytogenes* using a dual indicator system: esculin/iron and mannitol/phenol red. Chromogenic Listeria SET Supplement was used in two variants – L-α-phosphatidylinositol 1.0 g/500 ml and distilled water 20.0 g/500 ml and nalidixic acid 10.0 g/500 ml, ceftazidime 10.0 g/500 ml, amphotericin B 5.0 g/500 ml, polymyxin B 38350 IU. 27.6 g of Listeria acc. to Palcam medium was weighed and 400 ml of distilled

water was added, mixing thoroughly. Then, after sterilisation, 1 vial of Chromogenic Listeria SET Supplement was introduced. Further procedures were the same as for the PCA medium.

Yersinia Selective and Yersinica CIN Selective Supplement

It is a selective and differentiating preparation used to isolate *Yersinia* bacilli, especially *Yersinia enterocolitica*. Mannitol is a fermentable carbohydrate. Fermentation of mannitol in the presence of neutral red results in the formation of a characteristic "bull's eye" colony, colourless with a red centre. Yersinica CIN Selective Supplement consisted of – novobiocin 1.25 mg/ 500 ml, cefsulodin 7.5 mg/ 500 ml and irgasan 2.0 mg/ 500 ml. 23.2 g of Yersinia Selective LAB-AGAR BASE was weighed, and 400 ml of distilled water was added and mixed. After sterilisation, 1 vial of Yersinia CIN Selective Supplement was added. Further, the procedure was the same as for PCA medium.

ENDO

This is a culture medium used for confirmation, detection, isolation, and enumeration of the total number of *Escherichia coli* bacteria from milk, dairy products, and food (Seham et al., 2010). Fuchsin is employed in the medium to differentiate between lactose-fermenting and non-fermenting bacteria. The production of acetaldehyde by lactose-fermenting organisms such as *E. coli* results in characteristic colonies ranging in colour from dark pink to reddish with an opalescent greenish metallic sheen, accompanied by a similarly coloured medium. ENDO agar is also used as an indicator for the presence of non-lactose-fermenting bacteria, including *Salmonella* spp., which form uniformly colourless or pale pink colonies. A quantity of 16.6 g of ENDO Agar was added to 500 ml of distilled water and mixed. Subsequent procedures were analogous to those applied to the other media.

Surface Plating

For each type of tested meat, 100 µl of suspension from each dilution level was taken using a sterile pipette tip and surface-plated onto previously prepared media in Petri dishes. Four replicates (n = 4) were performed for each dilution to ensure the reliability of microbiological results. To determine the total number of microorganisms, after appropriate spreading of the sample onto the non-selective Plate Count Agar medium, the plates were incubated at 30°C for 72 hours. The presence of selected bacteria was checked using appropriately chosen selective-differential media. After performing the surface plating, the plates were incubated as follows: on VRBL and Listeria acc. to Palcam medium supplemented with additives for 24 hours at 37°C, on Yersinia Selective medium for 24 hours at 25°C, and on ENDO medium for 24 hours at 35°C. The study were performed on the 1st, 3rd, 6th and 9th day of refrigerated storage

of selected game meat, using the same microbiological media and incubation conditions at all time points.

Determination of the total number of microorganisms
Colonies of bacteria grown on Petri dishes from surface plating were counted, and then the total number of microorganisms (L) was calculated using the following formula:

$$L = \frac{C * d * a}{(N_1 + 0{,}1N_2)}$$

where:
C – sum of colonies on all plates selected for counting
N_1 – number of plates from the first counted dilution
N_2 – number of plates from the second counted dilution
d – dilution index corresponding to the first (lowest) counted dilution
a – factor of seeded amount of material, with culture of 100 µl, a = 10

The results as the mean of four replicates with standard deviation are tabulated in colony forming units – colony forming units CFU/g or log CFU/g.

Statistical analysis of the results
The results are given as averages, along with the standard deviation (±SD). A one-way ANOVA and Tukey's post hoc test (for $n = 4$, with $p \leq 0.05$) were used to determine the differences between the obtained mean values of the total microbial count. Statistica 13.3 software was used for these calculations.

Results

Comparison of the averaged total number of microorganisms on PCA medium in all tested meat samples stored under refrigeration conditions (4°C) showed that with the extension of incubation time, the total number of microorganisms increased in all samples (Tab. 1, Fig. 1A – Appendix 1). On the first day of incubation, the highest number of microorganisms was found in wild boar meat samples (2.01 log CFU/g), but from the third day of incubation onwards, the highest number of microorganisms was recorded in the venison roe deer meat samples, and this trend persisted until the end of the experiment, i.e., the ninth day of incubation (2.12–2.26 log CFU/g). Throughout the incubation period, deer meat samples had statistically significantly the lowest microorganism count.

Control of the suspensions of the analysed game meat samples on various media showed that starting from the first day of incubation, bacteria *Escherichia coli* appeared in suspensions from all three types of meat on VRBL medium (Fig. 1B – Appendix 1) as well as on ENDO medium. Similarly, at the same time, bacteria of the *Yersinia* genus were observed on the Yersinia Selective medium (Tab. 2; Fig. 2A – Appendix 1).

Tab. 1. Comparison of total number of microorganisms (L) [CFU/g] in colonies on PCA medium in all meat samples tested; mean values from four replicates (x ±SD) marked with different letters in the rows are significantly different according to Tukey's test, $p \leq 0.05$; the highest values on the logarithmic scale are highlighted in grey (L) [log]

Meat sample	Wild boar			Roe deer			Deer		
Day of incubation	x	±SD	log	x	±SD	log	x	±SD	log
1	101.25^{a}	±0.37	2.01	92.15^{b}	±0.50	1.96	10.10^{c}	±0.85	1.00
3	120.88^{b}	±1.08	2.08	133.00^{a}	±1.25	2.12	35.78^{c}	±0.56	1.55
6	156.18^{b}	±0.60	2.19	166.20^{a}	±0.75	2.22	59.38^{c}	±0.92	1.77
9	170.98^{b}	±0.90	2.23	180.35^{a}	±1.12	2.26	70.65^{c}	±0.71	1.85

Tab. 2. Control of the presence of bacteria from suspensions of wild boar, roe deer and red deer meat samples on the used substrates.

Meat sample Incubation day	Wild boar	Roe deer	Deer
	Escherichia coli on VRBL medium		
1	+	+	+
3	+	+	+
6	+	+	+
9	+	+	+
	Salmonella gallinarum on VRBL medium		
1	–	–	–
3	–	–	–
6	–	–	–
9	–	–	–
	Listeria sp. on *Listeria* acc. Palcam medium		
1	–	–	–
3	–	–	–
6	–	–	–
9	–	–	–
	Yersinia sp. on *Yersinia* Selective medium		
1	+	+	+
3	+	+	+
6	+	+	+
9	+	+	+
	Salmonella typhimurium on ENDO medium		
1	–	–	–
3	–	–	–
6	–	–	–
9	–	–	–
	Escherichia coli on ENDO medium		
1	+	+	+
3	+	+	+
6	+	+	+
9	+	+	+

On Listeria acc. Palcam medium, no *Listeria* bacteria appeared (Fig. 2B – Appendix 1). In none of the samples on VRBL and ENDO media (Fig. 1, 3 – Appendix 1) *Salmonella* bacteria (*S. gallinarum, S. typhimurium*) were detected.

Discussion

The safety of consuming game meat is closely linked to the hunting process and hygiene – primarily the temperature during subsequent processing and transportation of carcasses. Ensuring hygiene and safety standards for game meat is crucial for consumers in order to reduce the potential risk of food poisoning. The microbiological quality of food directly determines its safety for consumers, making it a critical aspect (Gill, 2007; Paulsen et al., 2012; Battaglia Richi et al., 2016).

Determining the total microbial count during storage of the tested meat samples allowed for the observation of its increase with prolonged incubation. Among the samples analysed, the highest number of microorganisms on the 9th day of storage was found in roe deer meat (Tab. 1). For example, compared to the study by Daszkiewicz et al. (2011), which investigated changes in the quality of roe deer meat during refrigerated storage under vacuum and modified atmosphere conditions, the total microbial count after seven days of storage was significantly higher (6.10 to 6.20 log CFU/g) than in the sample analysed here after nine days of incubation (2.26 log CFU/g – Tab. 1).

The lowest total microbial count per gram of product was recorded in red deer meat – this value was more than twice lower than in the case of the other meat samples tested (Tab. 1). Research conducted by Kunová et al. (2022) showed that on the fifth day of refrigerated storage of red deer meat, the total microbial count reached 3.38 log CFU/g, which is considerably higher than in the red deer meat sample analysed on the ninth day of incubation in this study (1.85 log CFU/g – Tab. 1).

The total microbial count per gram of wild boar meat on the final day of analysis was 2.23 log CFU/g (Tab. 1). This value is higher than the findings of Borilová et al. (2016), where the microbial count in wild boar leg meat stored under aerobic conditions at 0°C for seven days was approximately 1.8 log CFU/g.

Overall, based on the conducted analysis, all tested meat samples met the acceptable average count of aerobic bacteria as defined by the *International Commission on Microbiological Specifications for Foods* (ICMSF). The relatively low total microbial counts in the tested samples may indicate high hygiene standards and appropriate storage conditions at all stages of processing, or, unfortunately, could be the result of preservatives used during storage.

The studies conducted to detect the presence of pathogenic bacteria showed that no *Salmonella* was found in any of the tested meat samples (Tab. 2). These findings are consistent with studies carried out by Italian and Japanese researchers. In research conducted

in Italy (Orsoni et al., 2020) and Japan (Asakura et al., 2017), *Salmonella* was not detected in any of the wild boar meat samples analysed. Similarly, in the study by Avagnina et al. (2012), no *Salmonella* was found in red deer or roe deer meat. Spanish research on the occurrence of pathogenic bacteria in large game animals also showed a low incidence of *Salmonella* in roe deer and red deer carcasses – only 0.3% (Díaz-Sánchez et al., 2013).

Another group of microorganisms important in the microbiological analysis of meat are *Yersinia* bacteria. Studies by Bancerz-Kisiel et al. (2015) revealed a high level of contamination with *Y. enterocolitica* in carcasses of large game animals stored in cold rooms. The presence of this bacterium was found in 55% of wild boar carcasses, 60% of roe deer carcasses, and 44% of red deer carcasses. These results are consistent with the present study, in which an increase in colonies of *Yersinia* was observed in wild boar, roe deer, and red deer samples grown on microbiological media (Tab. 2).

The analysis for the presence of *Listeria* showed that these bacteria were absent in the meat of wild boar, roe deer, and red deer (Tab. 2). Studies by Stella et al. (2018) and Peruzy et al. (2019) also reported no detection of *L. monocytogenes* in swabs taken from wild boar carcasses. However, French studies assessing bacterial contamination levels in game meat across Europe detected *Listeria* in roe deer and red deer meat, although at very low levels (Membré et al., 2011).

Microbiological analysis also revealed the growth of *Escherichia* bacteria in all tested meat samples (Tab. 2). Scientific research confirms the presence of these bacteria in game meat. Díaz-Sánchez et al. (2013) reported the detection of *E. coli* in 18% of wild boar carcass samples. *E. coli* was also found in red deer and roe deer meat samples collected from a game meat processing facility in France (Membré et al., 2011). Unfortunately, bacteria of this genus are considered potentially dangerous and may cause foodborne illness, as previously mentioned.

Game meat, compared to meat from domestic animals, contains higher levels of minerals and vitamins (including high levels of sodium, potassium, calcium, magnesium, and phosphorus – especially in roe deer meat – and iron in wild boar meat) (Werpachowski, Zalewski, 2012). It can certainly be a valuable component of the human diet due to its high content of good-quality protein, favourable profile of unsaturated fatty acids, relatively low energy value, and moderate cholesterol content (Kunachowicz et al., 2003; Pereira, Vicente, 2013; Czarniecka-Skubina, Hamulka, 2016; Królikowski, Depłuta, 2020). However, to ensure these health benefits, proper processing and storage conditions must be maintained, as previously mentioned.

Conclusions

The total number of microorganisms (L) in all tested meat samples increased with the time of their storage in refrigerated conditions at 4°C. The microbiological analysis

showed that among the tested samples after ninth day of storage, the highest average L value was found in roe deer meat, and the lowest in red deer meat. In all tested samples, the total number of microorganisms (L) did not exceed the permissible microbiological limits, indicating that the meat meets the current quality and safety standards for sale and consumption. The microbiological analysis showed the presence of bacteria of the genera *Escherichia*, *Yersinia* in all tested samples. However, no presence of bacteria of the genera *Listeria* and *Salmonella* was observed in the tested samples. The detected bacteria may be a potential source of infections of the digestive system.

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Conflict of interest

The author declare no conflict of interest related to this article.

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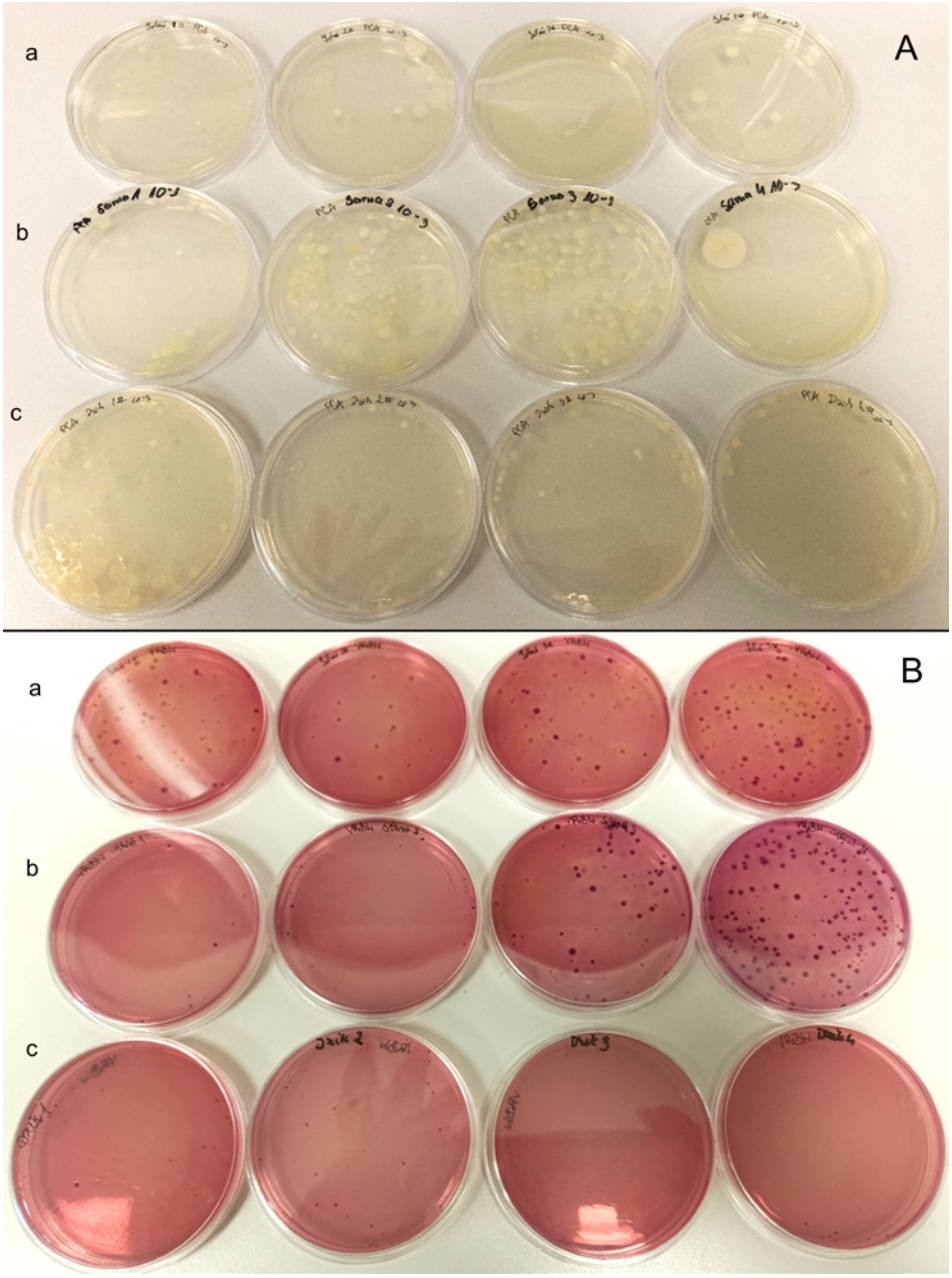

Fig. 1. Bacterial colonies on the 3rd day of incubation from suspensions of red deer (a), roe deer (b), wild boar (c) meat on PCA medium – A; bacterial colonies on the 3rd day of incubation from suspensions of red deer (a), roe deer (b), wild boar (c) meat on VRBL medium – B (Photo. M. Rak)

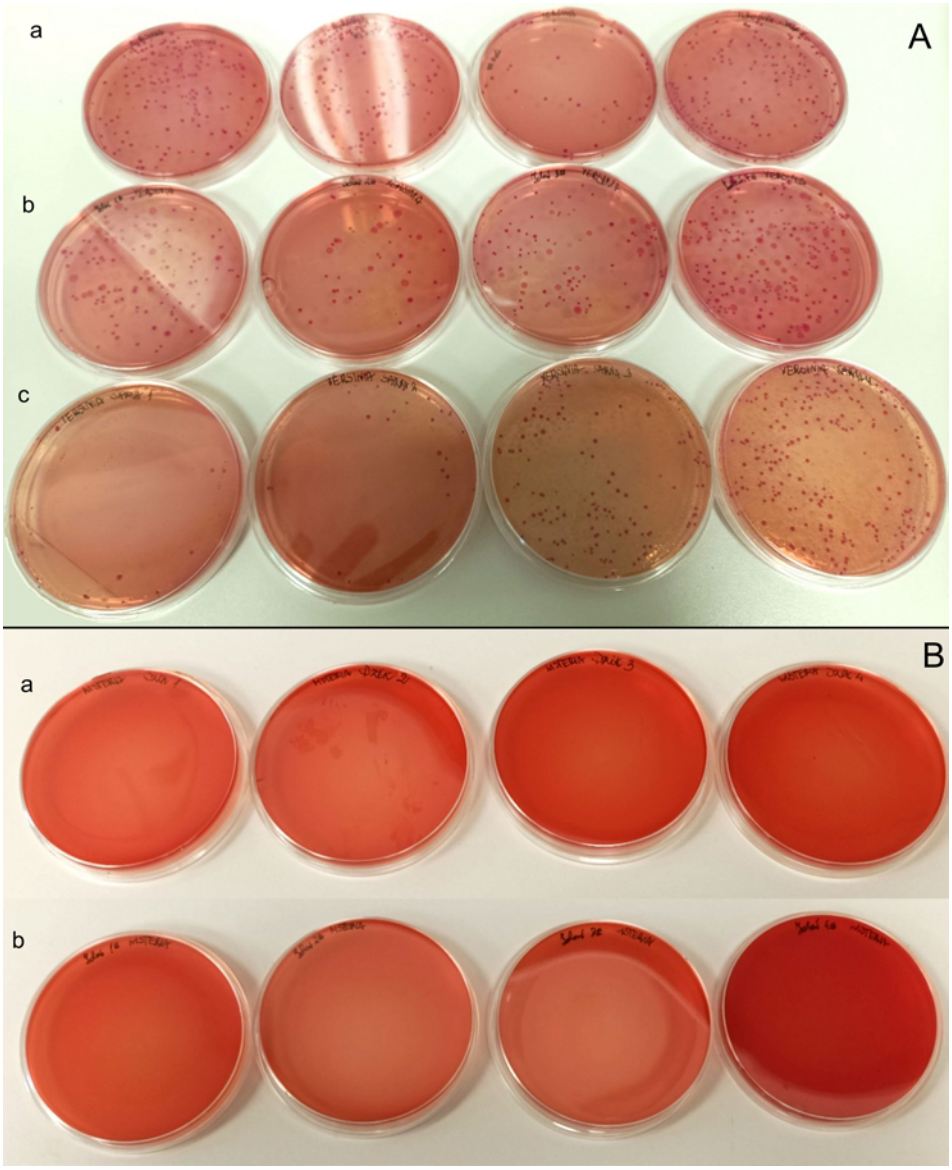

Fig. 2. Bacterial colonies on the 3rd day of incubation from a suspension of wild boar (a), red deer (b), roe deer (c) meat on Yersinia Selective medium – A; bacterial colonies on the 3rd day of incubation from a suspension of wild boar (a), red deer (b) meat on Listeria acc. to Palcam medium – B (Photo. M. Rak)

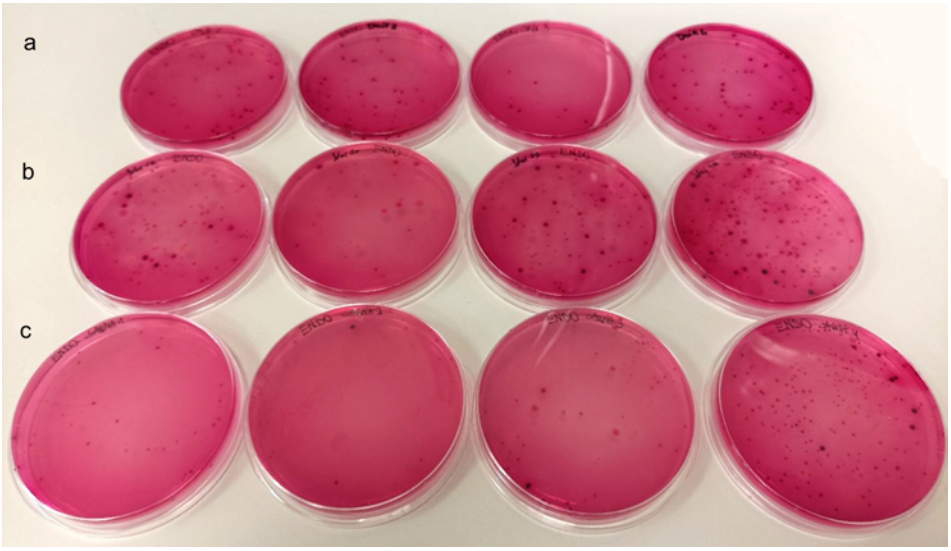

Fig. 3. Bacterial colonies on the 3rd day of incubation from the suspension of wild boar (a), red deer (b), roe deer (c) meat on ENDO medium (Photo. M. Rak)

Analiza składu mikrobiologicznego głęboko mrożonego mięsa wybranych zwierząt łownych przechowywanego w warunkach chłodniczych

Streszczenie

Dobrym surowcem mięsnym może być dziczyzna charakteryzująca się bardzo wysoką jakością odżywczą, korzystnym profilem kwasowym, większą zawartością witamin i składników mineralnych, mniejszą ilością tłuszczu oraz mniejszą zawartością cholesterolu, w porównaniu z powszechnie spożywanymi rodzajami mięs. Jednak mięso zwierząt łownych, podobnie jak i hodowlanych, może prowadzić do zakażeń i zatruć pokarmowych, ponieważ jest produktem spożywczym wrażliwym na zanieczyszczenia mikrobiologiczne. Celem badania była analiza składu mikrobiologicznego wybranych rodzajów mięsa zwierząt łownych tj. dzika, sarny i jelenia, przy użyciu klasycznych metod mikrobiologicznych, polegających na oznaczeniu ogólnej liczby drobnoustrojów (L) podczas przechowywania w warunkach chłodniczych oraz kontrolę obecności patogennych bakterii z rodzaju *Salmonella*, *Yersinia*, *Escherichia* oraz *Listeria* w badanych próbach. Badania wykazały, że ogólna liczna drobnoustrojów we wszystkich badanych próbach mięs rosła wraz z upływem czasu obserwacji. Po dziewięciu dniach przechowywania, najniższą ogólną liczbą drobnoustrojów charakteryzowało się mięso z jelenia, najwyższą zaś mięso z sarny. We wszystkich badanych próbkach ogólna liczba drobnoustrojów nie przekraczała dopuszczalnych norm mikrobiologicznych, co wskazuje, że mięso spełnia obowiązujące kryteria jakości i bezpieczeństwa przeznaczone do sprzedaży oraz konsumpcji. Przeprowadzona analiza w zakresie kontroli potwierdziła obecność patogennych bakterii z rodzaju *Escherichia*, *Yersinia* we wszystkich badanych próbkach oraz wykazała brak obecności bakterii z rodzaju *Listeria* i *Salmonella*. Wykryte bakterie potencjalnie mogą stanowić źródło zakażeń układu pokarmowego.

Słowa kluczowe: bakterie, dziczyzna, jakość mikrobiologiczna, selektywne podłoża mikrobiologiczne

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