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DETECTED MICROBIOLOGICAL ISOLATES IN MEAT AND MEAT PRODUCTS WHEN TAKING SAMPLES FOR ANALYSIS BY FOOD OPERATORS AND OFFICIAL VETERINARIANS IN THE REPUBLIC OF NORTH MACEDONIA FOR THE PERIOD FROM 2016 TO 2020

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ABSTRACT

Meat and meat processing industry make great efforts on a daily basis to improve technological processes and hygienic conditions. Competent institutions take appropriate measures to prevent placing of unsafe product on the market. However, we witness a daily global increase in the number of people suffering from foodborne diseases. For the period 2016-2020, the Food and Veterinary Agency of the Republic of North Macedonia received a total of 686 reports from laboratories about the detected presence of an isolate (positive finding) from samples taken by official veterinarians, food operators, and consumers. Isolates (positive findings) in meat and meat products were detected in 288 or 41.98% of the total analyzed samples. Campylobacter spp., Listeria monocytogenes, Escherichia coli and Salmonella spp. are the most frequent potential pathogens. For the five-year analyzed period, the presence of an isolate of Salmonella spp. was determined in 164 samples of meat and meat products. The bacterium Essherichia coli is the second most frequently detected bacterium in meat and meat product samples, after Salmonella spp., with a total of 48 samples with a positive finding for the analyzed period. The bacterium Listeria monocytogenes was detected in 39 samples of meat and meat products. An isolate of Campylobacter spp. was determined in a total of 28 samples of meat and meat products. By the Food and Veterinary Agency according to the annual food safety monitoring program were aimed at preventing the development of foodborne diseases.

Key words: meat, meat products, *Listeria monocytogenes*, *Campylobacter spp*., *Yersinia enterocolitica*, *Ecsherichia coli*.

INTRODUCTION

People have been getting sick from foodborne diseases since the beginning of time. Even today, in the XXI century, even though science and technology in food production have advanced immensely, food poisoning is a very current health problem of the world population.

Alimentary zoonotic diseases (including foodborne diseases) in humans are a global public health problem - in industrialized countries, one tenth of the population suffers from them annually (Käferstein & Abdussalam, 1999; Schlundt et al., 2004). Therefore, food safety is one of the key factors for the public health safety of the population.

The increasing number of incidents caused by food poisoning poses a global threat to regulatory authorities, reinforcing the need for governments, the food industry, and individuals to contribute to the production of safer food and thereby prevent the development of foodborne diseases (Bari & Yeasmin, 2018).

Food safety is a shared responsibility of all parties involved in the food chain. That is why it is necessary to have a continuous cooperation with the respective national government. It will help in establishing and implementing food security strategies and policies, thus ensuring safe food for both the population of the respective country and the world's population.

Meat is food of animal origin. It is a product necessary for proper development and health of human beings. It contains a large number of nutrients, among which essential amino acids, B vitamins, iron, zinc, which are of particular importance (Higgs and Mulvhill, 2002). Considering its characteristics, meat is a nutritional substrate with optimal conditions for the reproduction of bacteria that cause meat spoilage and human poisoning (Hammes, et al., 2008). It is for these reasons that meat is one of the most important sources of foodborne diseases (Berends et al., 1993; Pointon et al., 2006).

Zoonotic and biological agents, pathogenic microorganisms such as: non-typhoidal *Salmonella spp.*, thermophilic *Campylobacter spp*. and *Yersinia enterocolitica*, the verocytotoxic *Ecsherichia coli* and *Listeria monocytogenes*, as well as parasites, including *Toxoplasma gondii* (EFSA, 2011) are the most common causes of food poisoning in humans. Nørrung and Buncic (2008) point out that meat and meat products are main sources of these biological hazards, while some of them are their only source.

No one process can guarantee the complete absence of microorganisms or even potential pathogens for humans while obtaining meat from live animals to carcasses. Live animals are the main source of primary contamination of meat with microorganisms, while secondary contamination of meat occurs due to improper handling, unhygienic conditions, poor hygiene of employees and working surfaces, inadequate storage temperature, etc. Therefore, a greater emphasis should be put on prevention and control of contamination during all aspects of production of meat and its further processing: animal breeding, transportation, slaughtering and processing, cooling and storage of carcasses and half-carcasses. Slaughterhouses have a very important role for the biological safety of meat because they represent one of the key points that lead to the contamination of meat with numerous biological hazards, but at the same time it is possible to implement effective measures to reduce the biological zoonotic alimentary risks for humans. However, in addition to slaughterhouses, meat processing plants also play an important role in the biological safety of meat products.

The special requirements for safety of meat and meat products in relation to the microbiological criterion are prescribed in the Rulebook for Special Requirements Relating to Microbiological Criteria for Food ("Official Gazette of the Republic of Macedonia" no. 100/13, 145/14, 37/17, 173/18, 229/20). This rulebook complies with Commission Regulation number 2073 of November 15, 2005 on microbiological criteria for food (Celex number 32005P2073). For this purpose, operators of food of animal origin (slaughterhouses, meat cutting plants, meat processing plants, etc.) need to take measures to ensure that food products meet the appropriate microbiological criteria for food safety, according to the criterion for food safety and the criterion for hygiene during the process.

Operators of food of animal origin should establish appropriate sampling plans for testing the specified microbiological criteria and implementing corrective actions. The sampling plan for laboratory tests from slaughterhouses, meat cutting plants, meat preparations plants, and deboning plants must contain the number of samples and the time interval at which the samples are taken per certain product categories. The sampling plan is prepared according to the scope of work and the frequency given in Annex 1 in the annual orders for the implementation of veterinary measures as well as the controls for protection of public health from contaminants or residues transmitted by animals or products of animal origin, passed by the director of the Food and Veterinary Agency, pursuant to Article 57, Paragraph 1, Point 2 of the Law on Veterinary Health ("Official Gazette of the Republic of Macedonia" No. 113/07, 24/11, 136/11, 123/12, 154 /15 and 53/16).

Operators of food of animal origin, in addition to the above-mentioned obligations in relation to sampling plans for testing the specified microbiological criteria, should also take samples for laboratory testing of the product, as well as to take swabs for laboratory testing from the processing plants and from the equipment, in order to fulfill the special requirements for food safety for *Listeria monocytogenes*, in terms of microbiological criteria, and in accordance with the Rulebook for Special Requirements Relating to Microbiological Criteria for Food ("Official Gazette of the Republic of Macedonia" no. 100/13, 145/14, 37/17, 173/18, 229/20). The sampling frequency for *Listeria monocytogenes* is determined by the food operator depending on the volume of work, in accordance with the frequency given in Annex 1 of the annual orders for the implementation of veterinary measures and controls for the protection of public health from contaminants or residues transmitted by animals or products of animal origin, passed by the director of the Food and Veterinary Agency pursuant to Article 57, Paragraph 1, Point 2 of the Law on Veterinary Health ("Official Gazette of the Republic of Macedonia" No. 113/07, 24/11, 136 /11, 123/12, 154/15 and 53/16). It is necessary to take samples at least once a month.

When the results of the performed tests are unsatisfactory, food operators should take corrective measures, in accordance with the Rulebook for Special Requirements Relating to Microbiological Criteria for Food and other activities necessary to protect the health of consumers prescribed in their own operating procedures, as well as to notify the Food and Veterinary Agency.

Pursuant to Article 70 Paragraph (5) of the Law on Food Safety ("Official Gazette of the Republic of Macedonia" No. 157/10, 53/11, 1/12, 164/13, 187/13, 43/14, 72/ 15, 129/15 and 213/15), every year the Government of the Republic of North Macedonia develops a program for monitoring food safety in the Republic of North Macedonia. In accordance with the Law on Food Safety and the recommendations of the European Union for monitoring harmful agents in food, and with the aim of producing, selling, and placing safe food on the market, as well as protecting the health of consumers, this program pays special attention to determining food safety in terms of microbiological criteria.

MATERIAL AND METHOD

The research was carried out in February 2021. The data for this research were obtained from the Food and Veterinary Agency of the Republic of North Macedonia, after submitting an electronic request for free access to information of a public nature, archived with number 03-312/1 dated 5.2.2021, by the Food and Veterinary Agency.

The submitted data for the period 2016-2020 referred to:

- The number of total reports received from laboratories for isolates (positive finding) of samples taken by official veterinarians, food operators and consumers.
- The number of total reports received from laboratories for isolates (positive finding) of samples taken from meat and meat products and the determined type of isolate.

To describe the analyzed data, we applied statistical analysis. First, a tabular presentation of data was made. The next step was summarizing the data and their statistical processing, supported by the creation of models in Microsoft Excel, based on scientific methods. As the last step, the results were presented in an analytical and graphical form using diagrams and tables.

RESULTS AND DISCUSSION

The Department for State Veterinary Inspection in the Veterinary Public Health Section at the Food and Veterinary Agency of the Republic of North Macedonia receives reports from laboratories about isolates (positive findings), in accordance with the Law on Amendments and Supplements to the Law on the Protection of the Population against Infectious Diseases

("Official Gazette of the Republic of Macedonia" No. 149/14), for the samples taken by official veterinarians, food operators and consumers. After receiving a positive finding, an extraordinary inspection is carried out by official veterinarians, in accordance with their competences, and all activities from the aspect of veterinary public health are undertaken.

Table 1 shows the number of reports received from laboratories for determined isolates (positive findings) to the Food and Veterinary Agency for the period 2016-2020. Samples are taken by official veterinarians, food operators and customers. In doing so, it can be stated that a total of 686 determined isolates (positive findings) were received for the analyzed period. In the samples that were taken by food operators and consumers, a positive finding was observed in 425 or 61.95% of the total samples taken, compared to 261 or 38.05% of the total samples taken by the official veterinarians. The highest number of determined isolates (positive findings) was determined in 2018, 177 samples, compared to the lowest determined number of 107 samples in 2017. The average number of total positive findings for the analyzed period was ascertained in 137 samples. From Table 1 it can be observed that the average rate of change, from year to year, records an increase of 2.61% in the number of samples with a positive finding.

Table 1. An overview of the number of reports received from laboratories to the Food and Veterinary Agency for isolates (positive findings) for the period 2016-2020

Year		Number of positive samples, taken from		
		Official veterinarians	Food operators and consumers	Total
2016		31	107	138
2017		19	88	107
2018		66	111	177
2019		38	73	111
2020		107	46	153
Total		**261**	**425**	**686**
Average 2011-2020		**52**	**85**	**137**
Variation interval	**Min**	**19**	**46**	**107**
	Max	**107**	**111**	**177**
SD		**31**	**24**	**26**
CV (%)		**60.26**	**28.01**	**19.10**
Average rate of change (%)		**36.30**	**-19.03**	**2.61**

Source: Food and Veterinary Agency of the Republic of North Macedonia

After all reports received from laboratories for isolates (positive findings) by the Department for State Veterinary Inspection in the Veterinary Public Health Section in the Food and Veterinary Agency of the Republic of North Macedonia, extraordinary inspections were carried out by official veterinarians, in accordance with their competences and all activities were undertaken for incorporation of veterinary public health activities, thus preventing the possibility of unsafe food being placed on the market.

Table 2 shows the number of reports received from laboratories about isolates (positive findings) for the period 2016-2020, for samples taken from meat and meat products in which the presence of isolates (positive findings) was detected. The lowest number of isolates in meat and meat products was detected in 2017, 24 or 22.42% of the total detected isolates (Figure 1), compared to the following year 2018, when the highest number of isolates was detected, as high as 93, i.e. 52.54% of total detected isolates. The highest percentage of 54.90% of detected isolates (positive finding) in samples of meat and meat products was confirmed in 2020, compared to the total detected isolates (positive finding) in samples per year for the period 2016-2020.

Table 2. An overview of detected isolates in meat and meat products for the period 2016-2020

Detected isolates	Year				
	2016	**2017**	**2018**	**2019**	**2020**
Campylobacter spp.	1	4	7	9	7
Escherichia coli	8	2	33	1	4
Listeria monocytogenes	13	8	5	5	8
Salmonella spp.	15	10	48	31	60
Salmonella spp. and Campylobacter spp.	0	0	0	0	5
Escherichia coli and Salmonella spp.	3	0	0	0	0
Listeria monocytogenes and Escherichia coli	1	0	0	0	0
Total	**41**	**24**	**93**	**46**	**84**

Source: Food and Veterinary Agency of the Republic of North Macedonia

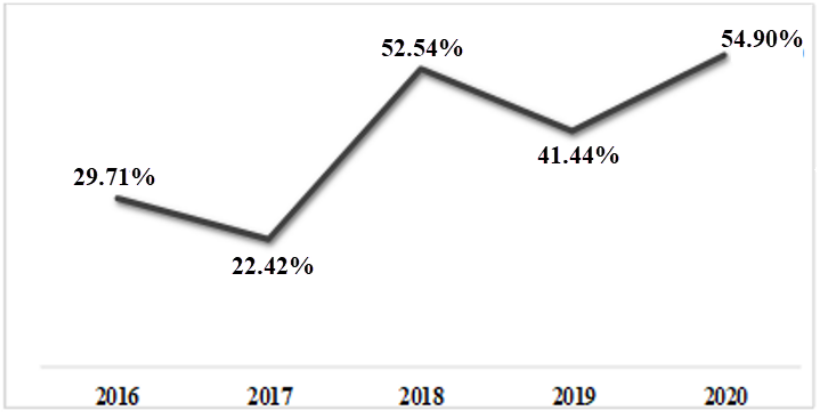

Figure 1. Graphic overview of the percentage representation of detected isolates (positive finding) in samples of meat and meat products per year, in relation to total detected isolates (positive finding) in samples per year for the period 2016-2020

It can be seen table 2 that *Campylobacter spp*., *Listeria monocytogenes*, *Escherichia coli* and *Salmonella spp*. are the most common bacteria detected in the tested samples of meat and meat products. According to EFSA reports (2012 and 2013), starting from the 1990s until today, the bacteria from the genera *Salmonella*, *Campylobacter*, *Listeria* and *Escherichia* are the most common bacteria that cause foodborne diseases.

The bacterium *Salmonella spp*. was detected in 164 samples of meat and meat products (Table 3). It can be noted that in 2020 the presence of *Salmonella spp*. was detected in as many as 60 samples, compared to 2017 when the bacterium was detected in 10 samples. *Salmonella spp*. was detected in 33 samples on average during the analyzed period. The average deviation from the average number of samples in which an isolate of *Salmonella spp*. was detected is 19 samples for the analyzed period. Regarding meat and meat products, it can be stated that the annual average rate of change for the period 2016-2020 observes a trend of increase of 41.42% of detected isolates of the bacterium *Salmonella spp.*

Table 3. An overview of detected isolates of bacterial species in meat and meat products for the period 2016-2020

Year		*Campylobacter spp.*	*Escherichia coli*	*Listeria monocytogenes*	*Slamonella spp.*
2016		1	8	13	15
2017		4	2	8	10
2018		7	33	5	48
2019		9	1	5	31
2020		7	4	8	60
Total		**28**	**48**	**39**	**164**
Average 2011-2020		**6**	**10**	**8**	**33**
Variation interval	**Min**	**1**	**1**	**5**	**10**
	Max	**9**	**33**	**13**	**60**
SD		**3**	**12**	**3**	**19**
CV (%)		**50.00**	**124.41**	**37.51**	**58.02**
Average rate of change (%)		**62.66**	**-15.91**	**-11.43**	**41.42**

Source: Food and Veterinary Agency of the Republic of North Macedonia

Danev (1999) points out that bacteria of the genus *Salmonella* are considered to be major meat poisoners. They belong to the family *Enterobacteriaceae*. More than 2000 strains are known, while more than a hundred species are pathogenic for humans. The gastrointestinal tract of mammals and birds are major reservoirs of *Salmonella spp*. The bacterium is mostly found in poultry (in all stages of production), less often in pork, and even less often in beef. The contamination of raw meat with *Salmonella spp*. is a consequence of direct or indirect (cross) faecal contamination during slaughtering and carcass processing (Borch et al., 1996, Botteldoorn et al., 2003).

Regarding the analyzed period, the bacterium *Ecsherichia coli* is the second most frequently detected bacterium in meat samples and meat products, after *Salmonella spp*. The bacterium was detected in 48 samples of meat and meat products in the period from 2016 to 2020. In 2018, the largest number of isolates of the bacterium *Escherichia coli* was detected, as many as 33 samples, while in 2019, an isolate of the bacterium *Escherichia coli* was observed in only one sample. The bacterium *Escherichia coli* was detected on average in 10 samples for the analyzed period. The average deviation from the average number of samples in which isolates were detected is 12 for the analyzed period. The variability of the detected samples is very high. When it comes to meat and meat products, it can be stated that the average annual rate of change for the period 2016-2020 shows a decreasing trend of -15.91% of the detected isolate of the bacterium *Escherichia coli*.

The presence of *Escherichia coli* in food is an indicator of fecal contamination because *Escherichia coli* is most abundant in the intestines of humans and animals. It can adapt to very harsh conditions outside its host, which makes it very resistant and flexible (Blount 2015).

Raw and undercooked food contaminated with *Esherichia coli* is often the cause of a large number of food poisonings (Marinculić et al., 2009). Fresh beef and chicken are the most common source of enteropathogenic strains of *Escherichia coli*, but also food exposed to human contamination with faecal potential sources of infection (Feng et al., 2017; Ray and Bhunia, 2013).

It can be Table 3 noted that positive findings for the presence of *Listeria monocytogenes* were detected in a total of 39 samples for the analyzed period, of which the largest number of positive findings, 13, were determined in 2016. In 2018 and 2019, an isolate of the bacterium *Listeria monocytogenes* was detected in 5 samples. The bacterium *Listeria monocytogenes* was detected on average in 8 samples for the analyzed period. The average deviation from the average number of samples in which isolates were detected is 3 for the analyzed period. The variability of the detected samples is moderate. It can be concluded that in meat and meat products the average rate of change for the analyzed period has a decreasing trend of -11.43% per year.

The bacterium *Listeria monocytogenes* is a species of the genus *Listeria*, which is considered a human pathogen (Chlebicz & Śliżewska, 2018). It is isolated in a wide variety of foods, but it is mostly present in beef and pork (Johansson et al., 1999). Shoukat et al. (2017) state that *Listeria monocitogenes* can contaminate different types of meat and meat products. Lowry & Tiong (1988) found that 48% of the samples taken in the examination of beef were contaminated with *Listeria monocitogenes*. Bencić (1991) points out that freshly- ground meat can often be contaminated with *Listeria monocitogenes.*

Gandhi & Chikindas (2007) state that meat processing plants are often contaminated with the bacterium *Listeria monocytogenes*. Floors, walls, doors, door handles, toilets, shoes, trucks, equipment, work surfaces, ice machines, packaging machines, etc are the most common places where this bacterium can be found and cause food contamination (Norton et al., 2001). Bacteria can remain in meat processing plants for a longer period of time, especially if the temperature is low and if the organism is protected and supplied with organic substances from food (Gómez et al., 2015).

An isolate of *Campylobacter spp*. was determined in a total of 28 samples of meat and meat products in the period from 2016-2020 (Table 3). In 2016, the presence of a positive finding of the bacterium *Campylobacter spp*. was confirmed in only 1 sample, compared to 2019 when 9 positive findings were determined. On average, the bacterium *Campylobacter spp*. was detected in 6 samples for the analyzed period. In the case of meat and meat products, it can be stated that the average rate of change for the analyzed period, annually, shows an increasing trend of 62.66%.

The species *Campylobacter jejuni* and *Campylobacter coli* of the genus *Campylobacter* are the most common causes of foodborne diseases in Europe and the USA (EFSA, 2015). These two species of bacteria are mostly isolated from poultry and much less from other types of animals. For these reasons, contaminated poultry is one of the most common causes of foodborne illness (Humphrey et al., 2007). Poultry is contaminated during the slaughtering and processing of poultry carcasses in the slaughterhouse. Carcasses are commonly contaminated by the contents of the gastrointestinal tract during evisceration. The processing of the carcass is complex, fast and largely automated, which provides an excellent opportunity for contamination of the carcass with *Campylobacter* (Wieczorek et al., 2015).

CONCLUSION

Based on the obtained results of this research, the following conclusions can be made: microbiological contamination of meat and meat products is dominant compared to other parameters. For these reasons, it is necessary for slaughterhouses, meat cutting plants, meat preparations plants, and deboning plants to regularly implement their plans for taking samples for laboratory examination to test the hygiene during the process and the safety of the product. This should be performed according to the annual orders relating to the execution of veterinary measures and controls for the protection of public health from contaminants or residues transmitted by animals or products of animal origin; pursuant to the Law on Food Safety and the recommendations of the European Union for monitoring harmful agents in food, and with

the aim of producing, selling and placing safe food on the market, as well as protecting the health of consumers, this program pays special attention to determining food safety in relation to microbiological criteria, compliance and implementation of the annual food safety monitoring program in the Republic of North Macedonia by the Food and Veterinary Agency and meat and meat product operators, together with competent institutions, should strive to deliver safe meat and meat products to the market and thereby reduce the development of acute foodborne diseases.

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