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THE IMPORTANCE OF HYGIENE SYSTEMS IN QUALITY ASSURANCE IN THE MEAT INDUSTRY

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1. BACKGROUND AND OBJECTIVES OF RESEARCH

During our preparatory studies, my colleagues and I assessed the hygienic conditions of slaughterhouses, meat processing and additive producing plants. We put special emphasis on the detection of the presences of such microbial communities as *Listeria* and *Salmonella,* which are of particular concern regarding food and nutritional health. We were carrying out observations during the process of production, as well as after cleaning and disinfection. Besides detecting pathogens, in certain cases we also estimated total plate count, coliform number and *Escherichia Coli* number.

Despite the regular previous inspections by the Animal Health And Food Control Station, there has not been enough information provided about so-called 'base-line studies' so far, however, they are well-known in international literature. Without results of such studies, it is hardly imaginable to further improve the microorganism-focused and general hygiene conditions of meat processing plants.

Therefore, our objective is to conduct the abovementioned studies on microbial communities such as *Listeria* and *Salmonella*, which are of utmost importance concerning healthcare.

Base-line studies serve to assess the level of microbiological contamination. Their results show what can be regarded as a 'base line' in connection with a certain plant or industry, that is, they set the average microbiological contamination level.

Our objective is to provide a reliable production basis through the results of studies in Hungary. In the light of the results, the data can be

adapted in various ways, they can be put to use in the circumstances of Hungary as well.

Firstly, the results can reveal the weak points of a certain plant, technology or product; that is, it is an opportunity to detect the gaps through which contamination types hazardous to final products and thus to consumers can sneak into the system.

Secondly, due to the results, sanitation technology, procedures and the overall hygiene system can be optimized and made cost-effective.

Objective of the Thesis

In the field of meat industry, an immaculate final product that is microbiologically safe for the consumer can be produced only from proper raw material with adequate technology and impeccable personnel.
However, in the procedures of slaughtering, processing and storage, several incidents might occur for contamination. Consequently, the microbiological safety of the products can be at risk. Therefore, special attention must be paid to the disinfection and cleaning of slaughterhouses and meat processing plants, and also to the control and maintenance of their hygiene conditions.

The present thesis intends to provide a comprehensive overview of the methods that can be employed to manufacture safe meat products.

2. MATERIALS AND METHODS

2.1 Location and Time of the Studies

Among the slaughterhouses and meat producing plants of Hungary, we decided to study two large-scale plants*:* GyulaiHúskombinát RT. (the Meat Combine of GyulaPlc) and Debrecen 2000 KFT (Debrecen 2000 Ltd). We assessed their microbiological and hygienic conditions, with particular emphasis on the presence of microbe communities such as *Listeria, Salmonella, Escherichia coli* and *Escherichia coli 0157*, all of which microbes mean hazards in terms of food and nutritional health. We carried out our studies during the whole process of the production, both in a summer and in a winter period, between 2004 and 2006.

2.2 Sampling

Samples were collected with sterile swabs in the animal shelter, the slaughterhouse, the cold storage room and the processing departments. The swabs were pre-moistened with physiological saline solution, then, a 100-cm2 area was covered for sampling. We took further samples from the hands of the workers, from tools, the surfaces of equipment and also from the surface of cleaned and cut pork meat. In compliance with the regulation in force, we based our final product studies on a 25g sample.

2.3 Detection and Identification Methods

2.3.1, Detection of *Listeria monocytogenes*

Swab samples were incubated in FRASER broth at 37°C for 48 hours, then were subcultured onto Listeria selective agar (OXFORD, RAPID L'MONO, OCLA, LIMONO-IDENT). From the selective agars, we tested suspect colonies in accordance with the prevailing Listeria standards (MSZ EN ISO 11290-1).

2.3.2, Detection of *Salmonella*

At the first stage, swab samples were inoculated in a selenite cystine enrichment broth and incubated at 37 °C 24 hours, then plated out onto *Salmonella* selective HEKTOEN enteric and RAMBACH agars. From the selective agars, we tested the presumptive colonies in accordance with the prevailing *Salmonella* standards (MSZN EN 12824).

Confirmatory testing was made using Enteroclon Anti-Salmonella A67 omnivalent.

2.3.3, Detection of *Escherichia coli*

Swab samples were enriched in LMX broth for 18 hours, then were plated out onto Fluorocult ECD agar, finally, typical colonies were identified.

2.3.4, Detection of *Escherichia coli 0157*

Samples were enriched in a modified E coli broth (mEC) supplemented with novobiocin for 6 hours, then we applied immuno-magnetic separation (IMS). The isolated material was plated out onto sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC). The plates were incubated for 24 hours and, finally, we used *E. coli 0157* immunoassay for the final confirmatory testing of the typical colonies.

3. Results and Evaluation

3.1, The Year 2004

We tested a total of 292 samples taken from the plant in order to detect the presence of *Listeria, Salmonella, E. coli* and *E. coli* 0157. 200 samples were taken in the summer period (May and June) and 92 samples were taken in a colder period (October).

The microbiological results and the distribution of the samples taken during the summer. Out of 200 examined samples, 13 (6,5%) yielded *Listeria*; however, we could detect *Listeria monocytogenes* in one case only, and *Salmonella* spp in another incidence, but in both cases the samples were obtained from the animal shelter. The frequency of *E. coli* incidence was higher, 35,5 %, most positive samples were isolated from the animal shelter again, while the fewest samples (9,37%) were from the cold storage room (*Table 1*).

16 samples were collected from the slaughter line, 80 from the boning rooms, 64 from the cold storage rooms and 40 from the animal shelter. All *Listeria* isolates derived from the summer sampling of the animal shelter.

Indicate the microbiological results and the distribution of the samples obtained during the colder period (October). Out of 92 analysed samples, 16 (17,4%) proved positive for *Listeria,* however, in no cases were *Listeria monocytogenes* or *Salmonella spp* detected.

8 samples were collected from the slaughter line, 40 samples from the boning room, 32 from cold storage rooms and 12 from the animal shelter. *Listeria* could be detected at all the sampling sites, most isolated microbes derived from the boning room sampling in the colder season.

Compared to the summer sampling, the occurrence of E. coli dropped significantly, to 9,8%; again, most positive samples were the ones taken from the animal shelter (41,6%), and the fewest (3,1%) from the cold storage room.

Based on the results of our *Listeria* tests, we can conclude that there is a difference between the samples of the two seasons (*Table 1*). While in summer the occurrence of *Listeria* was only 6,5%, in the colder month the frequency of its incidence was 17,4%. Frequency regarding all the samples (9,93%) was 0,5% lower than the result of the previous year (10,53%). Slight difference between the testing periods had also appeared the year before. As in 2004, the frequency of *Listeria* was also

higher in the colder period, which phenomenon can be explained with the high cold tolerance quality of *Listeria*. Then, with a similar amount of samples (342), the difference between the acquired values was not more than 3%. Compared to the data of the previous year, the frequency of *Listeria* incidences had slightly decreased.

Table 1. Incidence of *Listeria* strains; figures regarding *Listeria monocytogenes* are shown in brackets (2004)

	Number of Samples	**Positive samples**	**Positive frequency %**
slaughterhouse	24	3	12,5
boning room	120	8	6,6
cold storage room	96	2	2,1
animal shelter	52	16	30,8
Total	***GyHK. RT.***	**29 (1)**	**9,93 (0,3)**

	winter months			**summer months**		
	Number of samples	Positive Samples	Positive Frequency %	Number of Samples	Positive Samples	Positive Frequency %
slaughterhouse	8	3	37,5	16	0	0
boning room	40	8	20	80	0	0
cold storage room	32	2	6,25	64	0	0
animal shelter	12	3	25	40	13	32,5
total	**92**	**16**	**17,4**	**200**	**13 (1)**	**6,5 (0,5)**

The occurrence of *E. coli* was more frequent (approx. 35%) in the summer months (*Table 2*), but its occurrence decreased significantly (9,8%) in the colder months. *E. coli 0157* microbes were not detected in the *E. coli* positive samples.

The result of the *Salmonella* test was also promising, as *Salmonella* occurred only once, in a sample from the animal shelter.

Table 2. Incidence of *E. coli* at the plant (GyulaiHúskombinát RT.2004)

	Number of Samples		**Positive Samples**		**Positive Rate %**	
GYHK. RT.						
slaughterhouse	24		8		33,3	
boning room	120		21		17,5	
cold storage room	96		7		7,3	
animal shelter	52		26		50	
Total	**292**		**62**		**21,2**	
	Winter Months			**Summer Months**		
	Number of samples	Positive Samples	Positive Rate %	Number of Samples	Positive Samples	Positive Rate %
slaughterhouse	8	1	12,5	16	7	43,75
boning room	40	2	5	80	19	23,75
cold storage room	32	1	3,1	64	6	9,37
animal shelter	12	5	41,6	40	21	52,50
Total	**92**	**9**	**9,8**	**200**	**53**	**35,3**

3.2, The year 2005

In the year 2005, I continued the hygiene assessment of the slaughterhouse and the processing plant; that is, we carried out the identification of *Salmonella, Listeria monocytogenes, Escherichia coli* and coliform microbes. We also examined heat-treated, vacuum-packed final products and sausages from a microbiological point of view, focusing on *Listeria monocytogenes* and other pathogens specified by the relevant regulation.

The studies of the selected plants were conducted at pre-determined sampling sites. Sampling was targeted to areas such as the animal shelter, the processing department,final products and the tools of the personnel.

In Gyulai Húskombinát RT, we have examined a total of 145 samples for the presence of *Listeria, Salmonella, E. coli* and coliform bacteria. The samples were obtained during the summer season, after that, slaughter terminated in the plant.

Distribution and microbiological results of the summer samples are shown in *Table 3.* The plant in question is several hours from the institute, thus we often received the swabs a day after sampling. Therefore we expanded our methods to a new swab study which contained transport medium so that we could detect the pathogens that otherwise could die during transport. From the samples analysed, 7 (11,3%) yielded *Listeria* positive by pre-moistened swab studies, while from the swabs containing transport medium 23 (27,7%) tested positive, that is, more than twice (*Table 3*).

14 samples were obtained from the slaughter line, 70 from the boning room, 56 from cold storage rooms and 5 from the animal shelter. In the previous year, all isolated *Listeria* came from the animal shelter sampling in the summer season. In 2005, no positive samples were from the shelter, which can be due to the small amount of samples.

Table 3. Occurrence of Listeria strains in *Gyulai Húskombinát* . 2005

	Total Number of Samples			**Total Positive Samples**		**Positivity Rate %**
GYHK. RT.						
slaughterhouse	14			1		7,1
boning room	70			17		24,3
cold storage room	56			12		21,4
animal shelter	5			0		0
Total	**145**			**30**		**20,7**
	pre-moistened swabs			**transport medium swabs**		
	Number of Samples	Positive Samples	Positivity Rate %	Number of Samples	Positive Samples	Positivity Rate %
slaughterhouse	6	0	0	8	1	12,5
boning room	30	5	16,6	40	12	30
cold storage room	24	2	8,3	32	10	31,3
animal shelter	2	0	0	3	0	0
Total	**62**	**7**	**11,3**	**83**	**23**	**27,7**

14 samples were obtained from the slaughter line, 70 from the boning room, 56 from cold storage rooms and 5 from the animal shelter. In the previous year, all isolated *Listeria* came from the animal shelter sampling in the summer season. In 2005, no positive samples were from the shelter, which can be due to the small amount of samples. Positive isolates were from the pig slaughter room, the boning room and from the cold storage room; percentage of their distribution is also indicated in *Table 4.*

Salmonella was not detected from any of the samples using either method.

The occurrence of *E. coli,* when tested with swabs, turned out to be 17,7%. When tested using swabs with transport medium, the result was 32,5 %; in case of employing transport medium, most positive swabs (100%) were obtained from the animal shelter and the lowest percentage (15%) from the boning room (*Table 4*).

Table 4. Occurrence of E. coli at GyulaiHúskombinát Rt. 2005

E. coli	**Total Number of Samples**			**Total of Positive Samples**			**Positivity Rate %**		
GYHK. RT.									
slaughterhouse	14			6			30		
boning room	70			11			17,3		
cold storage room	56			18			5,8		
animal shelter	5			3			50		
Total	**145**			**38**			**20**		
	pre-moistened swabs			**transport medium swabs**					
	Number of Samples	Positive Samples	Positive Rate %	Number of Samples	Positive Samples	Positive Rate %			
slaughterhouse	6	1	16,6	8	5	62,5			
boning room	30	5	16,6	40	6	15			
cold storage room	24	5	20,8	32	13	40,63			
animal shelter	2	0	0	3	3	100			
Total	**62**	**11**	**17,7**	**83**	**27**	**32,5**			

When traditional swab samples (62 pieces) were employed,the frequency of *Listeria* occurrence was 11,5% in 2005, which is slightly higher than the results of the previous years, 9,44% in 2004 and 10,53% in 2003. When transport medium swabs were used (83 pieces), the frequency was 28%.

The incidence rate for *E. coli* in 2004 was 20%, while in 2005 using the previous year's method, we observed a similar value, 18%. When we

employed transport medium swabs, the frequency went much higher, to 32,5%.

The incidence rate for coliform microbes using traditional swabs resulted in 37,1%; while using transport medium swabs, it resulted in 72,3% (*Table 5*).

Table 5. Occurrence of Coliform at *GyulaiHúskombinát Rt.* 2005

Coliforms	**Number of Samples**		**Positive Samples**		**Positive Rate %**	
GYHK. RT.						
slaughterhouse	14		6		30	
boning room	70		44		62,85	
cold storage room	56		29		51,8	
animal shelter	5		4		80	
Total	**145**		**83**		**57,2**	
	pre-moistened swabs			**transport medium swabs**		
	Number of Samples	Positive Samples	Positive Rate %	Number of Samples	Positive Samples	Positive Rate %
slaughterhouse	6	1	16,6	8	5	62,5
boning room	30	13	43,3	40	31	77,5
cold storage room	24	8	33,3	32	21	65,6
animal shelter	2	1	50	3	3	100
Total	**62**	**23**	**37,1**	**83**	**60**	**72,3**

3.3, The year 2006

In the year 2006, I also carried out hygiene assessment, I was collecting samples during production throughout a longer period but from the same locations in order to determine the hygienic conditions of the plant, and, if needed, to make suggestions for development and improvement. Based on my assessment, I analysed certain parts of the

hygiene system and also modified it when it was needed. With my colleagues, we studied the presence of *Salmonella, Listeria monocytogenes*, *Escherichia coli* and *Escherichia coli 0157*. Heattreated, sliced, vacuum-packed final products and sausages were studied constantly. We broadened our studies to detect *Listeria* and other pathogens.

In 2006, we conducted the above tests, that is, we detected *Salmonella, Listeria monocytogenes, S. aureus, Escherichia coli* and coliform microbes in a plant and also identified *Listeria* and other pathogens in heat-treated, vacuum-packed final products and sliced, packed dry goods.

Researches were made in pre-determined sampling sites of the specified plants (a slaughterhouse and a processing plant). Sampling was targeted to different parts of the processing plant, the final products, the tools and hands of the personnel. As for the slaughterhouse, samples were muscle tissues from pork and beef carcasses. Throughout the year, 338 swab and meat samples were tested for *Salmonella* and we could detect its prevalence in 8 cases, from swab samples obtained from one the plants and from purchased meat. The examined tissue samples yielded no detectable *Salmonella.*

Compared to the previous years, the incidence rate for both *Listeria* and *L. monoytogenes* had increased (51,6% compared to 11, 3% in 2005). However, being supported by our previous experience, in 2006 we collected the samples from critical points which can explain the higher frequency. Also, in case of too many suppliers (14), there is a higher chance of *Listeria* and *Salmonella* contamination as the suppliers are examined repeatedly.

39,6% of the tested swabs and 51,1% of the meat samples were contaminated with *S. aureus.* These figures correlate with the staphylococcus contamination of the observed half carcasses.

The occurrence of *E. coli* (53,7%) was similar to that of the previous year. All the muscle tissues obtained from the slaughter line (30 samples) tested negative for *Salmonella.*

Taken into account the set values of the regulation No 2073/2005, the 5-unit samples exceeded the limit three times: in one case the total plate count, and twice the *Enterobacteriaceae* number.
A basic objective would be to further reduce the pathogenic contamination of all meat plants, that is, further monitoring and control measures are needed. In addition, in the light of microbiological results, the efficiency of interventions and hygiene control action (cleaning and disinfection) can be judged as well.

3.4 Results

We assessed the presence of *Listeria, L. monocytogenes, Salmonella, S. aureus, coliforms* and *E. coli* during processing at nine specified areas (which we had chosen according to the results of the previous year) on a weekly basis; in case of presumption, swab sampling was made on further areas.

In light of the results, we came to the conclusion that raw materials are main source of contamination in the processing line. Therefore we analysed the microbial contamination of primary materials. In the plant there is no slaughter, instead, Hungarian and foreign meat is purchased and processed. Samples were taken with pre-moistened swabs and muscle tissue samples were collected as well. The samples were tested within an hour.
Out of the 300 examined swabs and meat samples (*Tables 6*), 155 yielded positive result for *Listeria* (51,6%). Result for *L. monocytogenes* contamination was similar, 3 meat samples (3,6%) and 8 swabs (3,7%) were positive.
Table 6. *Listeria monocytogenes* contamination rate for swab and meat samples at GyulaiHúskombinát Rt.
The occurrence of *E. coli* was also high, 53,7%, in case of meat samples their frequency was even slightly higher, 59,5%. Sampling sites and the results are shown in *Tables 6*.
9 swab samples (3,9%) and 3 meat samples (3,8%) were positive for *Salmonella*, which reveals similar frequency- When employing swab

sampling, we could detect *Salmonella* from the slaughter line (3 positive), the meat processing tables, the cut resistant

Table 6.Surface and meat samples at GyulaiHúskombinát Rt in 2006.

Sample Type	Listeria strains	Listeria monocytogenes	Listeria positivity %	Listeria monocytogenes positivity %
Swab Sample	221/122	221/8	55,2	3,6
Meat Sample	79/33	79/3	41,7	3,7
Total	300/155	300/11	51,6	3,6

gloves, the meat shovel and the sausage stuffing table; it means that pathogens might spread through the whole production line. Therefore we drew the conclusion that, instead of regular swab testing, it was more effective to examine pork carcasses and other meats (certainly, parallel with all hygienic and other, specific examinations after the cleaning and disinfection process). This way, more useful data regarding the processed products could be gained.

Item-monitoring of the suppliers helps the intensive control of the items liable to Salmonella. It also helps to choose the circumstances of the maturation process carefully as well as the convincing analysis of chemical and microbiological parameters of the final product.

In case of meat inspection for *S. aureus*, not only pure detection of contamination is essential, but also the identification of the level/quantity of contamination. 10^2-10^3/TKE/g or higher

***Staphylococcus* contamination of pork cuts results in loss of the quality of the final product, and *S. aureus* number in sausages will not decrease significantly even if the product undergoes further drying.** 39,6% of the tested swabs and 51%of the meat samples were *Staphylococcus* contaminated, which result is close to the *Staphylococcus* contamination of the examined half-carcasses.

The suppliers who regularly deliver their products with such plate count should be informed of the problem first, then, if they cannot improve the quality of their meat, for food-safety reasons, they should be excluded from the range of suppliers. This also applies to the suppliers who regularly deliver *Salmonella* contaminated meat.

5. New and Novel Scientific Results

1. In case of meat inspection for *S. aureus*, not only pure detection of contamination is essential, but also the identification of the level/quantity of contamination. 10^2-10^3/TKE/g or higher *Staphylococcus* contamination of pork cuts results in loss of final product quality, and *S. aureus* number in sausages will not decrease significantly even if the product undergoes further drying.

2. *Listeria* incidence of the winter months is nearly 3 times higher than that of the summer months. Therefore we can draw the conclusion that a plant is protected against particulates more effectively than against the mud which is introduced into the plant from the skin of the animals. The data highlights the importance of animal shelter, supplier and transport vehicle hygiene. Frequent change of plucking tub water and intensive rinse of the plucking machine can add to lowering the incidence of *Listeria,* however, it is much less cost-effective than demanding proper animal and transportation hygiene towards the suppliers. The risk of contamination from the mud and faeces on the animal skin, which is due to the low temperature of colder months, can be decreased by always providing clean and dry animal bedding.

3. Chances for the survival of *Listeria* are the highest in the pharynx, as neither the plucking tub nor the singeing machine can raise oral cavity temperature so high as to kill the bacteria. At home slaughtering, I could observe that the gas torch in all cases was directed

to both the open oral cavity and the nasal cavity of the animal. Also at home slaughtering, the pharynx is cooked in boiling water which is a satisfactory type of heat treatment. In a plant, the splitting saw means a high risk as it can spread the contamination throughout the whole carcass. Consequently, attention should be paid to the proper hygiene and disinfection of the splitting saw. A burner suitable for singeing the pharynx should be set up. Unfortunately, it is technologically impossible due to the different carcass sizes and the water dripping from the carcass. This step is only manual work with resolve. The solution costs money but the product microbiological safety increases significantly. It's a worthwhile investment.

4. The data processed in the dissertation show that in large-scale processing, the splitting saw presents a high risk. Because it can smear the dirt. Therefore, the disinfection of the splitting saw must be very carefully monitored and regulated in detail in the GHP system. The HACCP system should always be regulated as a critical point.

5. Based on the results of the dissertation, supplier control is warranted even when documented healthy stock is processed. Some of the tons of half-carcasses, some infected pigs, were a major problem in the quality of the finished product during the processing process. Therefore, regular monitoring of suppliers should be regulated in the GHP system and strictly enforced.

6. The results gained in our studies are supported by other experts' observations. Hence we state that *Listeria* can be transferred to a meat processing plant by animals. It can settle and grow in the plant and contaminate all the products. However, in case of proper heat treatment, *Listeria* is killed and does not cause any problems. On the other hand, products are at risk of re-contamination during slicing and packing processes. Furthermore, the bacteria can also reproduce during the cold

storage stage which can result in infection or even in fatal illnesses. Therefore, not only the purity of the technical environment, but also the purity of the raw material should be addressed when designing the quality system.

7. Maintaining an effective HACCP and GMP systems (of which adequate sanitation and disinfection is an essential part) helps to lower the risk of pathogen occurrence. Our results support the fact that slight fluctuations can occur even if all regulation are observed. Regular assessments and inspections help the plants to further improve their hygiene standards.

6. Summary

For several years, we selected certain manufacturing sites where we were performing hygiene assessments. To determine the hygienic status of a certain site, we collected samples regularly, on a daily basis, from the same places, during working hours. When needed, we proposed ways of improvement and development.

We assessed the presence of *Salmonella, Listeria monocytogenes*, *Escherichia coli* and *Escherichia coli 0157*. Heat-treated, sliced, vacuum-packed final products and sausages were regularly tested microbiologically, including the detection of *Listeria* and other foodborne pathogens.

The above mentioned studies were conducted between 2004 and 2006, that is, we evaluated the presence of *Salmonella, Listeria monocytogenes, S. aureus, Escherichia coli* and *coliform microbes* at the plants and in heat-treated, vacuum-packed final products and sliced, packed dry food.

In the selected manufacturing sites (a slaughterhouse and a processing plant) we took samples from pre-determined areas. In the processing plant the samples were taken from different pointsof the site, from final product, as well as from the tools and hands of the workers. In the

slaughterhouse, the samples were collected from the pork and beef muscle tissues.

We selected swab and meat samples to detect *Salmonella* which proved positive in some cases, at one of the sites *Salmonella* was detected in the swab sample as well as in the purchased meat. All tissue samples were *Salmonella* negative.

The number of incidence of both *Listeria* and *L. monocytogenes* had increased year by year, but sampling was made from the critical points identified during the previous years, which can be an explanation for the increase in incidence in the swab samples.

The high number of suppliers is not ideal either. The chances of *Listeria* and *Salmonella* contamination are higher at the suppliers who were investigated many times.

39,6% of the swab samples and 51,1% of the meat samples were contaminated with *S. aureus* (2006). These figures are in line with the *Staphylococcus* contamination level of the half carcasses.

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