Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznań, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wrocław.

ELECTRONIC
JOURNAL
OF POLISH
AGRICULTURAL
UNIVERSITIES

1998
Volume 1
Issue 1
Series
ENVIRONMENTAL
DEVELOPMENT

PALUSZAK Z., OLSZEWSKA H., SZEJNIUK B. 1998. EXAMINATION OF HEALTH-HYGIENIC CONDITION OF A SELECTED UTILISATION PLANT. **Electronic Journal of Polish Agricultural Universities**, Environmental Development, Volume 1, Issue 1.
Available Online http://www.ejpau.media.pl

EXAMINATION OF HEALTH-HYGIENIC CONDITION OF A SELECTED UTILISATION PLANT

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ABSTRACT

The environment microbiological assessment concerning a utilisation plant was conducted, basing on the occurrence of Salmonella spp., E. coli and feces streptococci in it. Most of the examined microorganisms were isolated in summer time in the septic part of the plant, that is on the unloading platform, in the dissection room, in the offal container, and in the load-carrying body and tyres of cars transporting carcass. Salmonella spp. occurred 3 times in insignificant quantities. In the non-septic part of the plant the fecal bacteria occurred in the mill room, more infrequently in the sift room and in the engine room. The meat-and-bone meal did not raise any

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objections in the health-hygienic respect. Among numerous faults, the lack of proper room separation of septic and non-septic parts of the plant calls for particular attention.

Key words: utilisation plants, hygiene, Salmonella, E.coli

INTRODUCTION

Utilisation plants processing carcass and animal offal play an important role in the realm of non-specific preventive treatment [2,4]. They should be numbered among the specially burdensome plants, both in respect of labour conditions and their influence on the surroundings [6,7]. Both epizootic and economic considerations (94% of recycling) are the reasons for growing interest in the proper functioning of this branch [14]. A special role for the microbiological purity of the final product, i.e. meat-and-bone meals is fulfilled by accurate room separation of the clean (non-septic) and dirty (septic) part of the plant. In spite of detailed regulations one can observe numerous irregularities in this respect.

The aim of the conducted examination was the assessment of health-hygienic condition of a utilisation plant producing meat-and-bone meal on the basis of occurring selected fecal bacteria in it. As for the size and production mode the plant can be treated as a representative one for the region of North Poland.

MATERIALS AND METHODS

The subject of the examination was the environment microbiological assessment of a utilisation plant as for the occurrence of microorganisms such as Salmonella, Eschericcchia and feces streptococci. The research included the bacteriological examination of the septic and non-septic part of the plant. In the septic part of the examination the samples were taken by the use of swabs from the unloading platform, the dissection room wall, the offal container, the load carrying body and tyres of a car used to transport carcass. In the non-septic part of the plant the material meat-and-bone pulp was examined (after sterilisation under the pressure of 3 atm. for 30 min.), pressed meat-and-bone pulp and the final product - milled meat-and-bone meal. Besides, swabs were taken by the use of tampons from the following places: the wall and floor of the mill room, the engine room, the sift room and from the mill. The investigations lasted 10 months, while the samples for the microbiological assessment were taken 5 times with two months' intervals.

1. Sampling

Samples for the bacteriological examinations were taken by the use of tampon swabs, assigning 4 tampons for each sample. The gauze tampons and moulds were made according to PN-82/A-86032. The swabs were taken from a surface limited by the mould of 5 x 5 cm size of the inside opening. In case of an examination from dry surfaces, the tampon was wetted with physiological fluid directly before sampling. Until performing the bacteriological analysis the samples were stored in a fridge in 0-40C temperature. Sterilised meat-and-bone pulp, pressed meat-and-bone pulp and meat-and-bone meal for the bacteriological examination was taken in the quantity of 1000 g.

2. Quantitive determination of Escherichia coli microorganisms

In the first phase, from each sample prepared in 3 repetitions, slurry of 1ml and 10ml volume was taken (tampon swabs) or 1g and 10g of meat-and-bone pulp and meat-and-bone meal, and they were mixed respectively with 9 and 90 ml of liquid base according to Mac Concey. Out of 1g or 1ml of slurry samples, stock dilutions were prepared from 10^{0} to 10^{-9} . The samples

were incubed in the temperature of 37^0C for 24 hours, and then, from particular dilutions, inoculation was performed with a sterile eza onto a liquid base with TTC tergitol (2,3,5-Trisphenyl - Tetrazolium Chloride), being a selective solid base for Escherichia coli. The plates were incubed for 24 hours in the temperature of 37^0C. Positive increase was characterised by yellow colouring of the colonies, and base decolouring.

3. Quantitative determination of Salmonella spp.
The quantitative determination of Salmonella spp. was conducted on a liquid base (peptone water) preparing dilutions (10^0 -10^{-6}) in three repetitions. After 24 hours of incubation at 37^0C, 0.1 ml of the sample was shifted to a liquid Rappaport base (43^0C 24h). In the next determination phase the material was sifted with eza onto a selective BPL-Agar base (according to Kauffmann), which was also incubed for 24 hours at 37^0C. The positive increase was confirmed by the occurrence of pale-pink colonies, while - at the same time - the base colour was changed from green-like to pale-pink colour. In the final phase of the determination the serological test was used - serum for agglutination in a HM antigen drop.

4. Quantitative determination of group D streptococci
As a liquid base for a selective increase of fecal D-streptococci, glucose and azide stock was used. After 48 hours of incubation at 370C the appearing opacity caused suspection of group d streptococci occurrence in the examined sample. The lack of opacity testified univocally that the result was negative. From the positive samples the material was shifted onto a solid base, i.e. agar with esculine and azide (37^0C for 48 hours). Fecal streptococci grew in the form of dark colonies, around which a darkly-coloured base appeared. The final identification of group D streptococci consisted in using a serological test (Phadabac - test). After establishing the characteristic number, NPL was specified for particular microorganisms, using Mac Crady charts [10].

RESULTS

1. Septic part of the plant
The septic part of the plant having a direct contact with the utilisation material was characterised by a high degree of bacteriological contamination. Most of the examined microorganisms were isolated from the unloading platform surface, the dissection room and the offal container. Table 1 shows that the coli quantity in samples taken from the unloading platform was within the range of 2.0×10^0 to 1.5×10^7 microorganisms on $1cm^2$ of examined surface during the whole investigation period. The highest contamination was noted in the summer period, i.e. July - August, and the lowest one - in autumn. The number of fecal streptococci in this measurement point was quite even during all the examination period and ranged from 1.6×10^2 to 2.5×10^4 of bacteria on 1 cm^2 (tab.2). A similar quantity of microorganisms was noted on the walls of the dissection room, where carcass was was prepared to be processed. Particularly high contamination was noticed near the shredder. In the summer period 4.5×10^6 of E.coli and 1.5×10^4 of fecal streptococci were isolated from $1cm^2$. In October and December their number decreased significantly, and later increased again. Subsequently, swabs were taken from the offal container reserved for storing products unfit to eat and non-edible parts obtained in the meat industry. The highest number of E.coli and fecal streptococci occurred here in August, 2.5×10^7 and 4.5×10^5 of bacteria in $1cm^2$ respectively. In later examination period the bacteriological contamination of the investigated surfaces was quite even, since the number E.coli did not exceed 1.5×10^4, and the number of fecal streptococci – 1.6×10^4 of bacteria on $1cm^2$ of surface (tab. 1 and 2). Improperly disinfected utilisation cars may play a significant role in pathogen propagation. The research

proved that in spite of disinfection conducted after each transport, one could observe fecal bacteria both on load-carrying bodies and tyres. On 1cm^2 of car load-carrying bodies one could observe from 0.9 x 100 to 1.5 x 10^3 of E.coli and from 4.5 x 10^0 to 2.0 x 10^5 of fecal streptococci. The Salmonella research conducted in the septic part of the plant demonstrated that it occurred 3 times in very insignificant quantities (tab.3). In the summer period (July - August) 2.5 x 10^1 of microorganisms were isolated from1cm^2 from the unloading platform, and as for the car load-carrying body and the dissection room wall, the number amounted to 0.4 x 10^{-1} and 4.5 x 10^0 of bacteria on 1cm^2 respectively.2. Bacteriological research results of the non-septic part of the plant.

Table 1. The number of *E.coli* particles (cfu/cm^2 on the surface of the examined element in the septic part of the plant

Place of sampling	Period of investigations				
	July August	September October	November December	January February	March April
Unloading platform	1.5 x 10^7	2.0 x 10^0	4.5 x 10^4	1.5 x 10^5	2.0 x 10^4
Dissection room	4.5 x 10^6	0.4 x 10^0	2.5 x 10^0	4.5 x 10^3	3.0 x 10^4
Offal container	2.5 x 10^7	2.0 x 10^0	9.5 x 10^3	4.5 x 10^3	1.5 x 10^4
Load carrying body (truck)	1.5 x 10^3	9.5 x 10^1	0.9 x 10^0	9.5 x 10^1	1.6 x 10^2
Tyre (truck)	4.5 x 10^1	4.5 x 10^1	2.5 x 10^0	n.d.	7.5 x 10^1

n.d. - not detected

Table 2. The number of group D sheptococci on the surface of the examined element in the septic part of the plant

Place of sampling	Period of investigations				
	July August	September October	November December	January February	March April
Unloading platform	7.5 x 10^3	1.6 x 10^2	2.0 x 10^3	2.5 x 10^4	7.5 x 10^3
Dissection room	1.5 x 10^4	9.5 x 10^0	2.5 x 10^2	1.5 x 10^4	2.5 x 10^3
Offal containerl	4.5 x 10^5	1.6 x 10^2	1.5 x 10^4	9.5 x 10^3	1.6 x 10^2
Load carrying body (track)	4.5 x 10^0	2.0 x 10^5	2.0 x 10^2	4.5 x 10^2	1.5 x 10^2
Tyre (truck)	2.5 x 10^3	1.6 x 10^2	4.5 x 10^0	2.5 x 10^0	7.5 x 10^0

Table 3. The number of Salmonella spp. (cfu/cm^2) on the surface of the examined element in the septic part of the plant

Place of sampling	Period of investigations				
	July August	September October	November December	January February	March April
Unloading platform	2.5 x 10^{-1}	n.d.	n.d.	n.d.	n.d.
Dissection room	n.d.	n.d.	n.d.	4.5 x 10^0	n.d.
Offal container	n.d.	n.d.	n.d.	n.d.	n.d.
Load carrying body (track)	n.d.	0.4 x 10^{-1}	n.d.	n.d.	n.d.
Tyre (truck)	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. not detected

2.1 Apparatuses and rooms

The bacteriological research results of elements included in the non-septic part are shown in tab. 4,5 and 6. Among the examined microorganisms the fecal streptococci occurred quite often, especially in the mill room (tab.5). Once they occurred there in the number of 1.5 x 10^4 of microorganisms on 2 cm^2, and in the remaining cases their quantity ranged from 1.5 x 10^0 to 9.5 x 10^1 of bacteria. Sporadically, the fecal streptococci also occurred in the sift room, engine room and on the mill surface (from 3.5 x 10^0 to 3.0 x 10^2 of microorganisms on cm^2). E.coli of fecal origin were isolated in smaller qantity more infrequently (tab.4). During the conducted research they were detected twice in the mill room (from 0.9 x 10^0 to 2.5 x 10^2 of bacteria on 1cm^2), and twice in the engine room (1.5 x 10^0 to 2.5 x 10^0 of microorganisms on 1cm^2). It should be indicated that the presence of Salmonella spp. was not proved in any research point.

Table 4. The numer of *E.Coli* on the surface of the examined element in the non-septic part of the plant

Place of sampling	Period of investigations				
	July August	September October	November December	January February	March April
Mill	n.d.	n.d.	n.d.	n.d.	n.d.
Mill room	n.d.	0.9 x 10^0	2.5 x 10^2	n.d.	n.d.
Sift room	n.i.	n.i.	n.i.	n.d.	n.d.
Engine room	n.i.	n.i.	n.i.	1.5 x 10^0	2.5 x 10^0

n.d. - not detected, n.i. – not investigated

Table 5. The number of group D streptococci (cfu/cm^2) on the surface of the examined element in the non-septic part of the plant

Place of sampling	Period of investigations				
	July August	September October	November December	January February	March April
Mill	3.0×10^2	3.5×10^0	n.d.	n.d.	n.d.
Mill room	1.5×10^4	4.5×10^0	4.5×10^0	2.5×10^0	7.5×10^0
Sift room	n.i.	n.i.	n.i.	1.5×10^0	1.5×10^1
Engine room	n.i.	n.i.	n.i.	1.5×10^2	9.5×10^1

n.i. not investigeted

Table 6. The number of *Salmonella* spp (cfu/cm^2) on the surface of the examined element in the non-septic part of the plant

Place of sampling	Period of investigations				
	July August	September October	November December	January February	March April
Mill	n.d.	n.d.	n.d.	n.d.	n.d.
Mill room	n.d.	n.d.	n.d.	n.d.	n.d.
Sifter room	n.d.	n.d.	n.d.	n.d.	n.d.
Engine room	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. not detected

2.2 Material samples

As can be seen in table 7, fecal microorganisms did not occur in any samples of sterilised meat-and-bone pulp during the research period. In pressed meat-and-bone pulp after degreasing, an inconsiderable number of fecal streptococci occurred twice, and E.coli - once. It should be stressed that Salmonella spp. did not occur in any of the examined semi-finished products' or meat-and-bone meal samples.

Table 7. The number of *E.Coli*, group *D streptococci* and *Salmonella* spp (cfu/g) in the samole taken from the production cycle of meat-and-bone meal

Kind of product	Specification	Period of investigations				
		July August	September October	November December	January February	March April
Sterillized meat-bone pulp	E	n.d.	n.d.	n.d.	n.d.	n.d.
	D	n.d.	n.d.	n.d.	n.d.	n.d.
	S	n.d.	n.d.	n.d.	n.d.	n.d.
Pressed meat-bone pulp	E	n.d.	n.d.	n.d.	0.4×10^{-1}	n.d.
	D	n.d.	3.0×10^1	n.d.	9.5×10^0	n.d.

	S	n.d.	n.d.	n.d.	n.d.	n.d.
Meat-bone meal	E	n.d.	n.d.	n.d.	n.d.	n.d.
	D	n.d.	n.d.	n.d.	n.d.	n.d.
	S	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. – not detected, E – *E. Coli*, D – group *D streptococci*, S –*Salmonella* spp.

DISCUSSION

The results of one's own research indicate a high fecal bacteria contamination of rooms and machines in the septic part of the plant. The bacteria occurred in a high quantity in the dissection room and on the unloading platform. Dirty walls and floors could be observed mainly near the shredder and in the unloading room. They were covered with a grease layer and the material residue. It was a result of their direct contact with the processed material. The utilisation material contamination can trend at 1010 - 1015 level of microorganisms in 1g [15]. On animal skin and hair one can find most frequently proteolitic and psychrofilnic bacteria as well as bacteria generating resting spores [3]. Among the isolated microflora of cattle and swine inner organs, positive Gram bacteria amount to 60 - 70%. The dominating kinds are: Micrococcus, Corynobacterium, Staphylococcus and Bacillus [11,12]. Among the negative Gram bacteria occurring in cattle liver, heart and kidneys, and in swine liver, Escherichia coli was most frequently isolated. Even though the high contamination is often unpreventable due to the material storage, still it is usually a result of basic negligencies on the part of workers.

E.coli, fecal streptococci and salmonella spp. could also be observed on load-carrying bodies and tyres of cars transporting carcass. As it can be seen from the conducted research washing aaand decontamination was not always effective. Tielman and Willinger [15] show that surface disinfection, when using a proper medium, causes bacteria reduction to about 70%. In the examined rooms they proved the presence of numerous coli and fecal streptococci. Moreover, they isolated, among others, Pseudomonas spp., Proteus vulgaris and anaerobic resting spore bacilluses.

The regulations concerning keeping the proper hygienic conditions in the utilisation plant are focused mainly on the septic part of the plant, where the input utilisation material is to be found. On the basis of observations conducted in the plant of investigation it can be stated that separating the clean and dirty parts was in practice insufficient to prevent bacteria propagation in the internal environment of the plant. The contact between both parts was possible not only through sanitary locks. These rooms often lacked disinfection mats or pools filled with a disinfection medium. Besides, there was no proper protection of the clean part rooms against insects and rodents. Inappropriate separation of both parts may be one of the main reasons for contamination of feed meal microorganisms [8]. The hygienic condition of the final product depends considerably on the cleanness degree of the non-septic part [1,2,14]. The research conducted in the clean part of the utilisation plant shows insignificant coli contamination (tab.4). From 1cm^2, maximally 2.5 x 10^2 of Escherichia coli bacteria were isolated. Similar results were obtained by Tielman and Willinger [15] and Sskovgaard [13], who report that coli bacteria occur sporadically in the non-septic part. On the other hand, in the mill room, where the degreased meat-and-bone pulp was stored, one could observe Escherichia coli in autumn - winter period in the number of 0.9 x 10^0 (September - October) and 2.5 x 10^2 of microorganisms on cm^2 (November - December). It should be highlighted

that degreased and hot pomace were placed on the floor, and not on the required ground beam 6cm above the floor, which could influence the bacteriological contamination of the semi-finished product. The bacteria also occurred in the engine room. On 1cm^2 of surface there were from 1.5 x 10^0 to 2.5 x 10^0 of examined microorganisms. It should be stressed that meat-and-bone pulp obtained after utilisation material sterilisation was free from Escherichia coli bacteria. Polish norms concerning the sterilisation process are regarded as the strictest in Europe. Despite keeping and following the sterilisation rigours one sometimes happens to produce a product contaminated with microorganisms. On the basis of data presented by Kwietniak [5] one can see that out of 111 samples 76 were completely sterile, and in 26% of the samples oxygen bacteria occurred in the number of 50 to 4000 in 1 gram. There were no pathogenic microorganisms in any of the analysed samples.

In one's own research, an insignificant contamination of meat-and-bone pulp after the degreasing process could be observed (from 0.4 x 10-1 to 3.0 x 101 of microorganisms in 1g). Similar remarks were made by Wollmann [16], who, while analysing pressed meat-and-bone pulp, proved the presence of Enterobacteriaceae family bacteria in the number of 780 in 1 gram of sample. The semi-finished product contamination is likely to take place in the later production phase as a result of secondary infections [1]. They can occur due to dust molecules, where the microorganisms are present [14].

As can be observed from the research the insignificant contamination of bacteriological samples of degreased meat-and-bone pulp and the room contamination did not influence negatively the hygienic condition of the final product, i.e. the meat-and-bone meal. However, the lack of these bacteria in semi-finished products does not exclude the possibility of their identification in feed mixtures. For this reason, it is necessary to increase the frequency of conducted disinfections, especially in the clean part of the plant, and place particular stress on tight separation of non-septic and septic parts. Intensified detailed sanitary controls would certainly enable to obtain a radical improvement in the functioning conditions of these, important for epizootic reasons, plants.

CONCLUSIONS

1. The research results indicate a high fecal bacteria contamination of rooms and machines in the septic part of the plant.
2. Low efficiency of conducted disinfection processes in the septic part of the plant was proved.
3. Insufficient room separation of the clean and dirty parts of the plant was the reason for bacteria propagating in the internal environment of the plant.
4. Bacteriological contamination of the semi-finished product was an effect of secondary infections occurring in a later production phase of meat-and-bone meal. 5. It is necessary to increase the frequency of conducted disinfections, especially in the clean part of the plant.6. Intensified, detailed sanitary controls are indispensable for gaining a radical improvement of hygienic conditions of utilisation plants.

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Submited: 21.07.1998

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