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## Consistent implementation of the residue monitoring plan for consumer health protection

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Case report

### Summary

*In order to protect consumer health and to create conditions for the trade of live animals and animal products with the European Union, and with third countries wishing to trade these kind of products with the European Union, there is legal obligation to monitor and control plans. Residues are pharmacologically active substances, which may remain in live animals and animal products, and which may be suspected or proven to be harmful to human health. The implementation of the monitoring plan for residues ensures a systematic method for revealing the possible presence of illegal drugs or certain substances above permissible limits in live animals and animal products. Once residues are detected in samples submitted for laboratory testing, the competent inspection authorities are required to implement all the necessary follow-up measures in order to determine the causes and origins of illegal drugs, or the reasons that led to exceeding the maximum allowable amount in foods of animal origin intended for human consumption. This paper describes a follow-up procedure conducted after the detection of coccidiostats in table eggs from farm hens.*

**Keywords:** Residues, plan, follow-up, coccidiostats

### Introduction

Today's lifestyle and intensive animal production have resulted in human exposure to a variety of adverse effects, such as residues of veterinary drugs, hormones, contaminants and other toxic substances. People are able, as individuals, to reduce these effects to a certain extent, though in most cases, it is necessary to provide an integral system that combines multi-disciplinary science, to ensure protection of the individual. This study gives a practical example describing a systematic approach to protecting the consumer health, i.e. protecting the entire human population from the harmful effects of residues of veterinary medicines (coccidiostats). These may remain in live animals and animal products and enter the human body through the food chain, thus jeopardizing health. This example outlines an effective way in which the profession acted to overcome the shortcomings of individual entities. It represents years of applying this systematic approach to passing legislation, its implementation, proper training, technical equipment and professional training of laboratory staff, and the permanent commitment of enthusiastic individuals. This example will show how the smallest error in the manufacturing process can result in serious consequences for human health and can incur serious financial costs. It also shows that the right implementation plan for residue monitoring can prevent such consequences or at least reduced them to an acceptable level. In order to protect the personal and business interests of the companies involved, the personal data of individuals, details of food business operators, or year of events will not be divulged.

### Residue Monitoring Plan

In addition to the primary objective of consumer protection, the systemic control of substances is intended to ensure the conditions for free trade in live animals and animal products. To this end, the European Union passed Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products, which clearly lays down the necessary measures to be implemented for monitoring substances and groups of residues listed by importance of this segment for the protection of human health (EC, 1996). This includes monitoring the production process of animals and primary products of animal origin, with the aim of detecting the presence of residues of certain substances in live animals, their excrement and body fluids, tissues, animal products, animal feed and drinking water. The Directive also regulates the coordination of work of inspectors implementing the regulation on the ground within their national territory and the central competent authority. In the case the maximum allowable quantity of residues of a certain substance is exceeded, the competent authority is required to conduct an investigation on the farm of origin, and to determine the cause of exceeding the allowable limit. If the presence of prohibited substances or products is detected by unauthorized persons, the detected substance or products is placed under the control of authorized persons until the competent authority implements the adequate measures. This Directive also stipulates the measures to be fulfilled when imported from third countries, and the manner of sampling. All third countries wishing to trade in live animals and animal products with EU Member States are obliged to

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#### "Improving the safety of the food chain through risk prevention in plant and animal production" Bruxelles, 28. studenog 2014.

Znanstveno vijeće Belgijske federalne agencije za sigurnost prehrambenog lanca (Belgian Federal Agency for the Safety of the Food Chain, FASFC) organizira 10. godišnji internacionalni simpozij "Improving the safety of the food chain through risk prevention in plant and animal production".



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submit the results of the analysis of the previous and the sampling plan for the coming year, by 31 March of the current year.

The Residue Monitoring Plan (RMP) was passed pursuant to the Decision on the monitoring of residues of certain substances in live animals and animal products (SG BiH, 2004). It contains species of animals and their products to be controlled, the number of samples to be analyzed during the year, the list of substances to be controlled in different species of animals and their products, methods for their analysis, standards for interpreting the results obtained and the maximum permitted residues of authorized substances. The RMP is adopted for each calendar year.

### Implementation of the Residue Monitoring Plan

All substances to be tested within the RMP were divided into two groups (A and B) and their subgroups (Table 1). For substances of group A, prohibited substances, residue control is carried out in order to detect the illegal administration of a prohibited substance or abuse of approved substances. In group B, approved substances for use, residue control is carried out to verify compliance with the maximum permitted quantity of residues of veterinary drugs and other substances in accordance with the legislation in force. Animal species and animal products/animal with target tissues sampled as part of the RMP for the detection of coccidiostat residues are shown in Table 2.

### Anticoccidials

Anticoccidials belong to group B (B2), for which residue control is intended to control compliance with the maximum allowable quantities of active substances in accordance with the Regulations on the maximum level of pharmacologically active substances in products of animal origin (SG BiH, 2011). Anticoccidials are substances intended to kill or retard the growth of protozoan species that cause disease in animals. Due to their chemical properties, anticoccidials are lagging behind in the pipe wall, high chair and storage for feed. Consequently, in order to avoid feed contamination, it is necessary to perform thorough cleaning of the equipment after the application of coccidiostats and before the withdrawal period. Also, when using coccidiostats containing ionophores, its use with other veterinary medicinal products is contraindicated (MP, 2011). Most coccidiostats are approved for use as additives in animal feed for target species (chickens, turkeys) in accordance with the authorisation conditions. Regardless, the production of animal feed containing certain coccidiostats may result in cross-contamination of animal feed for animals not of the target group. This cross-contamination can lead to exposure of animals that are not the target group (hens) and the potential risk to the health of these animals, and the possibility of residues remaining in food products obtained from these animals. To reduce the possibility of cross-contamination to a minimum, feed business operators are required to abide by the Good Manufacturing Practice princi-

ples (EFSA, 2008).

### Detection of coccidiostats (maduramicin and salinomycin) in table eggs

During the regular implementation of the RMP for coccidiostats (maduramicin), the results of laboratory analysis performed on eggs of farm hens showed non-compliant results. In the case of suspicion or non-compliant results, the laboratory is required to inform the client and the central competent authority thereof as quickly as possible, and to subsequently submit the analysis results. As there is no prescribed maximum permitted level, in this case, the limit of detection was set as the level for taking action. The laboratory test report showed that the amount of maduramicin found was 5 µg/kg, and the level of undertaking measures (level of action) was 2 µg/kg. The Ordinance on the maximum level of coccidiostats or histomonostats in food resulting from the unavoidable contamination of feed with these substances for those species for which their inclusion in food is not intended (SG BiH, 2013) is aligned with Commission Regulation (EC) No 124/2009 of 10 February 2009 setting maximum levels for the presence of coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed (EC, 2009) and Commission Regulation (EU) No 610/2012 of 9 July 2012 amending Regulation (EC) No 124/2009 of 10 of February 2009 setting maximum levels for the presence of coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed (EC, 2012). At the time of the follow-up the mentioned Ordinance was not yet in force. The method used in the quantification of coccidiostats (maduramicin) was liquid chromatography tandem mass spectrometry (LC-MS/MS), which was previously validated and accredited in accordance with Commission Decision 2002/657/EC (EC, 2002).

The RMP is implemented in such a way that samples are taken in the field, properly stored (kept at 4°C or frozen at -18°C if the samples are not submitted immediately to the laboratory) and then shipped to the laboratory for analytical testing. During regular sampling, the period from sampling to analysis should not be longer than 30 days, which is considered acceptable. In the present case, this time limit was abided by, as the sample was taken on 11 November by the competent veterinary inspector, delivered to the laboratory on 25 November, and testing was completed on 30 November.

### Follow Up - taking action in the case of violations

Immediately upon receipt of notification of a positive result, on 1 December, who was officially notified by the competent authority with instructions on taking measures in the case of a violation. In the further procedure, the competent authority found the subject to be a mini hen farm (600 animals), with a daily production of 500 eggs, and that hens were fed with complete mixtures for hens in the original 25 kg packaging. It was also found that the animal feed was imported, and the labelling of

feed included the composition (though no information was given for the included coccidiostatic), instructions for use, expiration date, and the production date. On 2 December, the competent authority performed additional sampling of eggs, one sample of animal feed was taken from the feeders and one sample of animal feed from the original packaging, and an interim ban on animals and animal products from the farm was issued, pending the results of laboratory analyzes (Figure 1).

As is the case with suspicious samples, the delivery of samples to the laboratory and performance of laboratory results was carried out by the accelerated procedure. The test results were submitted on 13 December, 11 days after re-sampling was performed. The analysis results showed increased levels of coccidiostats (maduramicin) in the egg samples in the amount of 8 µg/kg, while the sample taken from the animal feed feeders showed no increased concentration of coccidiostats (maduramicin). However, a high concentration of coccidiostats (salinomycin) of 5 µg/kg was determined, while the sample of feed from the package showed high amounts of coccidiostats (maduramicin) in the concentration of 25 µg/kg.

Immediately upon receipt of the laboratory results, the central competent authority notified the competent veterinary inspector, and suggested action according to Articles 21 and 22 of the Decision on the monitoring of residues.

On that same day, 13 December, the authorized veterinary inspector visited the mini-farm, ordered the safe disposal of 4,560 table eggs produced in the period from 2–13 December, and extended the ban on live animals and products until a negative result was obtained. The owner was ordered to stop administration of the food in question due to the elevated concentrations of coccidiostats (maduramicin and salinomycin) found, and suggested the use of animal feed from another manufacturer (Figure 2). On 15 December, eggs were re-sampled from a small farm, and as part of regular monitoring, eggs were sampled from neighbouring farms which did not use the same animal feed supplier. According to the results of laboratory analysis, on 20 December, the concentrations of coccidiostats (maduramicin and salinomycin) in both samples were found to be within the acceptable limit, <2 µg/kg, which indicates that the eggs are hygienically safe for human consumption, and the earlier enacted ban on live animals and animal products was lifted (Figure 2).

Due to the presence of residues of coccidiostats (maduramicin and salinomycin) in animal feed samples, collected at the mini farm on December 2, and acting on the principle of traceability of food on 13 December, the Court examined the dynamics of imports of animal feed in the premises of the importer in the period between 20 September and 16 December, and ordered a ban on the distribution of animal feed pending the results of laboratory analysis. After reviewing the documentation and information on imports in that time period, on 22 December, two samples of feed from the original package were taken from the warehouse, and eggs were re-sampled on a small farm and delivered to the laboratory for analytical testing. On 27 December, laboratory reports showed the presence of coccidiostats (maduramicin) in

the egg sample to be within the prescribed maximum allowable amount, while both animal feed samples were found to be inconsistent with elevated concentrations of coccidiostats, with maduramicin and salinomycin found in concentrations of 14 µg/kg and 7 µg/kg, respectively, in one sample, while the second sample contained salinomycin concentration in the amount of 56 µg/kg. Upon the receipt of laboratory results, on the same day the Central Competent Authority informed the competent veterinary inspector who had banned the circulation of animal food produced between 2–14 December (Figure 3). On December 27, the order was made for the official veterinarian to ensure compliance with the applicable legislation in the next 10 consignments of feed declared to contain coccidiostats from the producers of the exporting country. These consignments were sampled at the border and an order was issued prohibiting trade to obtain the same results matched analysis. The director of the Veterinary Administration of the exporting country received a detailed report on the follow-up results, with a request that they take all measures to determine the manner in which the coccidiostats was found in the packages of food for hens, but was not specified in the label.

On 16 January, the director of the Veterinary Administration of the exporting country, within competence is feed mixing facility, forwarded a memo informing us that their investigations confirmed our findings. They determined that the cause for the findings of coccidiostats in animal feed originated from suppliers of a premix whose declaration stated that it or not contain coccidiostats.

### Follow-up results

i. The most important result of this follow-up is the fact that we continued to prevent the possibility of contamination of final consumers of products of animal origin containing coccidiostats residues (maduramicin and salinomycin).

ii. We undoubtedly identified the origins and causes for the presence of coccidiostat residues (maduramicin and salinomycin) in table eggs and animal feed.

iii. We stopped the circulation of feed containing illicit substances in their country.

iv. We proved that such veterinary services operate efficiently and that we implemented all the prescribed measures to protect consumer health.

v. We proved the justification of budgetary funds used for the annual implementation of the RMP.

### Discussion

The consequences of the production and use of feed containing coccidiostats (maduramicin and salinomycin) that is not clearly listed on the label can be considered from several aspects.

The most important is that the end consumers consumed eggs containing coccidiostat residues (maduramicin and salinomycin), and were exposed to the possible harmful effects that these residues may cause for at least 32 days (Figure 1). Figure 2 shows that economic damages caused by the use of feed containing illegal substances was significant for the owner of the mini hen

farm, considering the trade ban on live animals and products which lasted 19 days and resulted in the disposal of 4,560 pieces of table eggs.

Also, we concluded that the animal feed importer suffered economic damage in terms of the limited or total trade bans for animal feed (Figure 3), and the additional costs incurred due to sampling at border crossings and the trade bans on goods pending the results of the laboratory analyzes.

Economic damage was also incurred for the feed mixing factory in another country during the period of investigation and implementation of corrective actions. The factory was brought under enhanced inspection, which included a ban on its products on the domestic and export markets, and additional laboratory testing was performed on its finished products and premixes, until it could be determined that the cause of every manufacturer and supplier of premix that is not produced and declared their products in accordance with the applicable legislation of that State.

All food business subjects for which certain incompatibles according to the current legislation were ascertained in the course of the follow-up, will be subjected to targeted sampling for coccidiostats residues (maduramicin and salinomycin) in addition to regular audits in the implementation of the RMP in the coming years.

### Conclusions

During the implementation of the RMP, past practice has shown that laboratory results of analyzed samples almost always show compliance with the prescribed standards. For this reason, some have questioned the justification of spending financial resources for such programs if the results are still unsatisfactory. The example outlined here shows that such programs are justified and that they should continue to be implemented and improved, both in providing targeted education for veterinary inspectors, laboratory personnel and food business operators, as well as for developing new laboratory methods and information systems, for the purpose of protecting consumers and creating conditions for free trade in live animals and animal products. The starting point in this case was the negligence of the premix manufacturers, who disregarded the principles of Good Manufacturing Practice

and did not clearly declare the presence of coccidiostats on their product. Damage in terms of endangerment of human health and the economic damages that it caused to safely assumed larger proportions of the RMP is not spent on a professional and highly responsible manner.

We hope that this example will assist other colleagues dealing with these or similar situations, by sharing the lessons we have learned. The complete procedure of regular sampling carried out in legally defined terms was that the period from sampling to its delivery to the laboratory must be short, which since then has been applied in practice. We are confident that any future follow-up in the same or a similar situation should be carried out as in Figure 4, where the period of consumer exposure to harmful impacts of certain residues and the resulting financial costs caused by the follow-up is significantly reduced.

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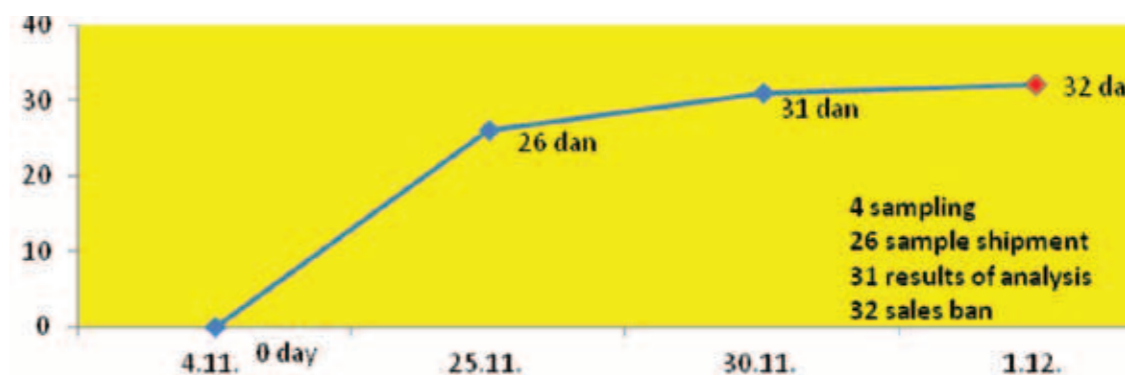
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**Table 1.** Classification of substances to be tested as part of the RMP.

Group A – Substances which have an anabolic effect and unapproved substances	
(1)	Stilbenes, stilbene derivatives, their salts and esters ( <i>diethylstilbestrol /DES/, hexestrol, dienestrol</i> )
(2)	Antithyroid substances ( <i>2-thiouracyl</i> )
(3)	Steroids ( <i>estradiol-17β, testosterone, progesterone, trenbolone acetate, melengestrol acetate</i> )
(4)	Resorcylic acid lactones and zeranol ( <i>zeranol</i> )
(5)	Beta agonists ( <i>clenbuterol, salbutamol</i> )
(6)	Prohibited substances ( <i>aristolochia spp., chloramphenicol, chloroform, chlorpazomazine, colchicine, dapsone, dimetridazole, metronidazole, nitrofurans (including furazolidon), ronidazole</i> )
Group B – Veterinary drugs and contaminants	
(1)	Antibacterial substances, including sulfonamides and quinolones
α)	Antibiotics ( <i>amoxicilin, ampicillin, benzylpenicillin, clavulanic acid, cloxacillin, dicloxacillin, oxacillin, cefazolin, cefquinome, spiramycin, tilmicosin, tylosin, eritromycin, linkomycin, josamycin, thiamphenicol, chlortetracycline, tetracycline, oxytetracycline, doxycycline, aminosidina, apramycin, streptomycin, dihydrostreptomycin, gentamycin, neomycin, spectinomycin, kanamycin</i> )
β)	Derivatives of diamino pyrimidine ( <i>trimethoprim</i> )
χ)	Sulfonamides ( <i>sulfamethazine, sulfathiazol, sulfadimetoxine, sulfamethoxazole, sulfadiazine, sulfaquinoxaline, sulfadimidine, sulfamerazine, sulfaguanidine, other compounds from the group of sulfonamides</i> )
δ)	Quinolones ( <i>danofloxacin, difloxacin, enrofloxacin, sarafloxacin, norfloxacin, flumequin, decoquinat, ciprofloxacin oxcolinic acid, marbofloxacin</i> )
(2)	Other veterinary drugs
α)	Anthelmintics ( <i>closantel, levamisole, albendazole, febantel, fenbendazole, flubendazole, oxfendazole, oxiabendazole, thiabendazole, triclabendazole, ivermectin</i> )
β)	Anticoccidials including nitroimidazoles ( <i>toltrazuril, amprolium, robenidin, nicarbazin, narazin, lasalocid, monensin, slinomycin</i> )
χ)	Carbamates and pyrethroids ( <i>amitraz, flumethrin, permethrin, cyfluthrin, deltamethrin</i> )
δ)	Sedatives/ including a tranquilizer and beta-blockers ( <i>azaperone, carazolol</i> )
ε)	Non-steroidal anti-inflammatory drugs ( <i>caprofen</i> )
φ)	Other pharmacologically active substances ( <i>dexamethasone, methylprednisolone</i> )
(3)	Other substances and environmental contaminants
α)	Organochlorine pesticides and PCBs ( <i>aldrin, benzene, chlordane, dieldrin, DDT and metabolites, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene, hexachloride (α,β,δ), lindane, metoxychlor, PCBs</i> )
β)	Organophosphorus pesticides ( <i>diazinon, azametifos, cumafos, dichlorvos, trichlorfon (neguvon), dioxantion, methyl-parathion</i> )
χ)	Chemical elements ( <i>arsenic, cadmium, lead, mercury</i> )
δ)	Mycotoxins ( <i>aflatoxin, ergotamine, ochratoxin, zearalenone</i> )
ε)	Dye ( <i>malachite green leucomalachit green</i> )
φ)	Other

**Table 2.** Types of animals and animal products from various tissues.

Species / product	Target tissue
Cattle	Liver
Sheep and goat	Liver
Pigs	Liver
Poultry	Liver/feed
Egg	Egg



**Figure 1.** Trade ban on the farm.

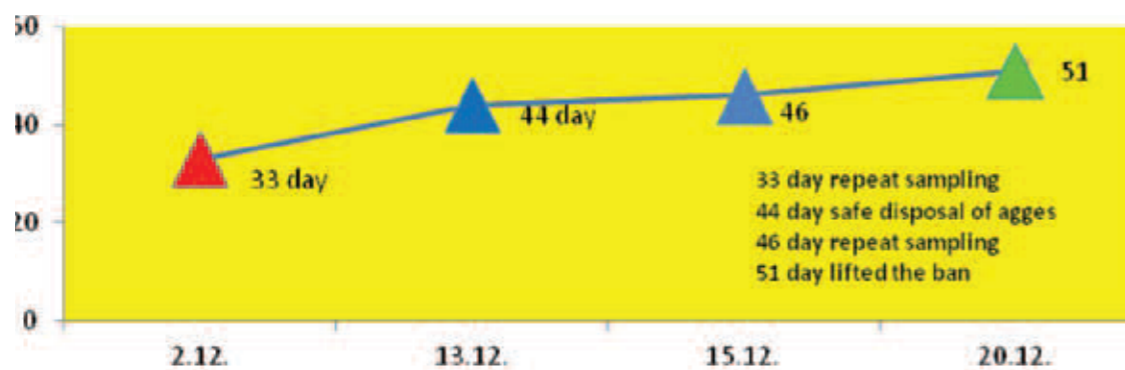


Figure 2. Destruction of eggs, re-sampling and lifting the trade ban on the farm.

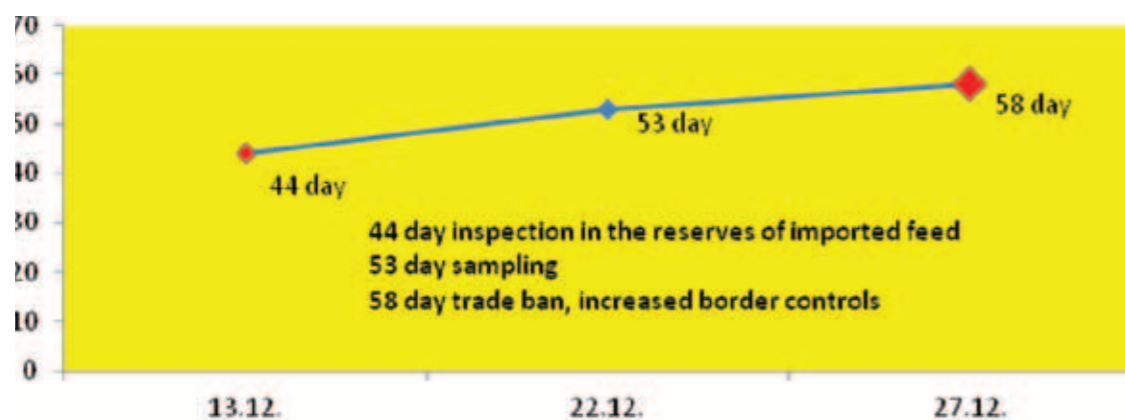


Figure 3. Sampling of feed and sales ban.



Figure 4. Chronological comparison of survey conducted and a future follow-up procedure.

U broju 4/14 za rad pod nazivom „Izolacija zrakom prenosive bakterije *Listeria spp* u mesno-prerađivačkoj industriji“ objavljena je kategorizacija rada kao Pregledni rad umjesto Znanstveni rad.

## Izolacija zrakom prenosive bakterije *Listeria spp* u mesno-prerađivačkoj industriji

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Znanstveni rad

### Sažetak

*Listeria monocytogenes* i druge bakterijske vrste roda *Listeria* su koje ukoliko se nalaze u mesu i mesnim proizvodima mogu izazvati štetno djelovati na zdravlje potrošača. Zanimala nas je mogućnost aerogene kontaminacije mesa i mesnih proizvoda ovim mikroorganizmima koji čine bioaerosol. Ovaj rad proučava prisutnost bakterije *Listeria monocytogenes* i *Listeria spp* u bioaerosolu u zraku mesno-prerađivačkih objekata te važnost izbora između impakcijske ili ciklonske metode uzorkovanja zraka. Ciklonska metoda pokazuje veću osjetljivost za otkrivanje bakterije *L. monocytogenes* i drugih vrsta *Listeria spp* u uzorcima bioaerosola u zraku mesnih industrija. Ciklonskim načinom uzorkovanja bioaerosola pronašli smo prisutnost *Listeria spp* u 41% uzorka od čega je u 24% potvrđena prisutnost *L. monocytogenes*. Rezultati ukazuju na značajnu mogućnost aerogene kontaminacije mesa i mesnih proizvoda bakterijom *L. monocytogenes* putem bioaerosola u mesnoj industriji. Ciklonska metoda uzorkovanja zraka za otkrivanje prisutnosti *L. monocytogenes* u bioaerosolima pokazala se pouzdanijom od impakcijske metode.

**KLjučne riječi:** *Listeria monocytogenes*, impakcijska i ciklonska metoda uzorkovanja zraka, proizvodnja mesa

## Isolation of airborne *Listeria spp* in meat processing industry

Scientific paper

### Summary

*Listeria monocytogenes* and other *Listeria* species are microorganisms which can significantly affect the health of consumers transferring by meat and meat products. Special interest was emphasized due to the possibility of aerogene contamination of meat and products with microorganisms forming bioaerosol. In this paper the presence of *Listeria monocytogenes* and *Listeria spp* and in bioaerosol in air of meat processing plants and the importance of selecting the methods of air sampling as impaction or cyclone method were studied. In experiment the cyclone method shows higher sensitivity for the detection of sampling *L. monocytogenes* and other *Listeria spp* in bioaerosols of the air in meat industry. With cyclone bioaerosol sampling method, we found the presence of *Listeria spp* in 41% of the sample, of which 24% of the sample confirmed on the presence of *L. monocytogenes*. The results show a significant potential for aerogene contamination of meat and meat products with *L. monocytogenes* via bioaerosol in the meat industry. Cyclonic method indicated more reliable air sampling in detecting the presence of *L. monocytogenes* in bioaerosols compared with the impact method.

**Key words:** *Listeria monocytogenes*, impaction and cyclonic air sampling methods, meat production