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BACTERIAL RISKS AND DETERMINATION OF CRITICAL CONTROL POINTS AT THE INDUSTRIAL PRODUCTION OF CHICKEN EDIBLE EGG

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The aim: *study the bacterial risks and to determine the control critical points at the industrial production of chicken edible egg.*

Materials and methods. *The first stage of our research was to study the degree of risk of contamination by opportunistic and pathogenic microflora at all stages of production of chicken edible eggs according to ISO 22000:2007. In order to achieve the goal, bacteriological research was conducted on pathological material from day-old chickens and adult birds, the content of marketable eggs from birds of different age groups; as well as washed from the equipment of poultry farms, the repair young poultry department, the industrial herd department, egg sorting and certification workshops, from the surface of the egg, from the working surfaces of special vehicles. Bacterial contamination of air, droppings, bedding, complete feed was studied. Bacteriological studies were carried out according to generally accepted schemes, using accumulative, selective and differential diagnostic media (heptadecyl sulfate agar, endo, xylose-lysine agar, differentiated agar with diamond green, Muller-Hinton). The sampling was carried out using universal sterile applicators "Voles".*

The next stage was to analyze the results of bacteriological studies and determine the basic list of criteria for creating critical control points (CCPs) for the further development of a risk management scheme for the bacterial biosafety of edible eggs according to HACCP principles.

Results. *Isolation of S. aureus from the heart and lungs, E. coli, P. aeruginosa, Enterobacter spp, Enterococcus spp - from the intestines of day-old chickens and litter – indicate violations of veterinary and sanitary standards in the hatchery and the low quality of disinfection before the placement of day-old young birds, as well as non-observance of veterinary and sanitary norms for the transportation regime of day-old young birds. Isolation of E. coli, Enterobacter spp, Klebsiella spp.Str. zymogenes from compound feed, and E. coli, Enterobacter spp, Shigella spp. in washings from the equipment of the feed mill is evidence of insufficient veterinary and sanitary control of incoming raw materials, compound feed, as well as poorly carried out disinfection of equipment and specialized vehicles.When examining objects from the production line, E. coli, Enterobacter spp., P. aeruginosa, and S. epiderrmidis were most often isolated. From the pathological material during the bacteriological examination, microflora of the genus E. coli, Streptococcus spp., S. aureus (most often pathogenic serotypes) prevailed. As a result of the analysis of the conducted studies, we determined a basic list of criteria for creating a CCP and developing a risk management scheme for the bacterial biosafety of edible eggs according to the principles of HACCP.*

Conclusions. *As a result of the bacteriological monitoring of objects of the technological cycle of chicken edible egg production, we established a list of bacteriological risks at all stages of production. As a result of the conducted research, it was determined, that the spectrum of bacterial contamination was mainly represented by opportunistic microflora. In the majority, the following types of microorganisms were detected: E. coli, P. aeruginosa, Enterobacter spp, Enterococcus spp. But C. jejuni, Streptococcus spp., S. aureus. Salmonella spp, which are the most dangerous for the consumer and poultry, were not isolated. We studied the bacterial risks at all the main stages of the industrial production of chicken edible eggs and identified the main critical control points of production according to the principles of the HACCP system*

Keywords: *bacterial risks, control critical points, HACCP system, chicken edible egg, E. coli, P. aeruginosa, S. aureus, C. jejuni, Streptococcus spp., Salmonella spp*

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1. Introduction

The most important sign of the quality of poultry products is their safety indicators, that is, the absence of substances harmful to the health of the consumer, as well as pathogens of infectious diseases [1, 2].

The unlimited use of a wide range of veterinary drugs and feed additives, due to the modern level of poultry farming technology and the increase in the industrial load on the environment with toxic substances due to intensive economic activity, requires increased control

over the safety of poultry products. The threat is created by the use of poultry products, contaminated with residual amounts of antibiotics, which can cause allergic diseases in humans and the development of antibiotic-resistant microorganisms that cause toxic infections and toxicosis, as well as the formation of new forms of bacteria and the reduction or even complete loss of the effectiveness of previously active therapeutic agents. The experience of countries with developed economies shows that effective quality control of poultry products is possible only with the application of a set of measures. These measures should include: control over the breeding of healthy poultry, timely microbiological monitoring during incubation, breeding of poultry and obtaining poultry products; control over the use of environmentally safe disinfectants, veterinary drugs and feed additives. All this indicates the need to organize enhanced control over poultry farming and the microbiological and sanitary quality of poultry products to be sold [3]. Products of animal origin, contaminated with such microorganisms as *E. coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, *Campylobacter fetus*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Salmonella genus*, etc., pose a special biological danger to the health of the consumer.

The presence of bacterial and infectious diseases in the farm negatively affects the epizootic situation. If viral diseases are prevented by vaccination of poultry, then bacterial infections, which cause great economic damage to poultry farming, require constant monitoring by veterinary medicine specialists [4].

There is a need to determine the factors affecting the sanitary quality and safety of poultry products; improvement of the veterinary and sanitary control system to obtain high-quality and safe poultry products; development of a set of measures to improve the sanitary quality and safety of poultry products based on the system of critical points of veterinary and sanitary control; studying the sources of bacterial insemination of poultry products.

High-quality and safe poultry products can be obtained only from healthy birds. Therefore, health control during the period of cultivation and breeding is an important stage of state veterinary control, influencing the indicators of quality and safety of products. Conducting regular microbiological research allows timely detection of hidden bacterial carriers among productive livestock and will contribute to the timely implementation of veterinary-sanitary and medical-prophylactic measures. These measures will contribute to the reduction of the general level of bacterial pathogens, caused by opportunistic microorganisms, and will be able to prevent new outbreaks of the disease. In such cases, it is necessary to consider control systems of poultry products.

The main principle of improving the HACCP system is that the number of HCPs in production should be necessary and sufficient for product safety. **The aim of the research** was to study the bacterial risks and to determine the control critical points during the industrial production of chicken edible eggs.

2. Materials and methods

The research was carried during 2018–2020 out on the basis of the laboratories "Innovative technologies and safety and quality of livestock products" and "Veterinary pharmacy" of the department of microbiology, veterinary and sanitary examination, animal hygiene and quality and safety of livestock products of the Faculty of Veterinary Medicine of Sumy National Agrarian University, Sumy Regional State Laboratory of the State Production and Consumer Service of Ukraine (Sumy), as well as in the conditions of poultry farms, egg processing enterprises of Sumy and Poltava regions.

The first stage of our research was to study the degree of risk of contamination by opportunistic and pathogenic microflora at all stages of production of chicken edible eggs according to ISO 22000:2007. In order to achieve the goal, bacteriological research was conducted on pathological material from day-old chickens and adult birds, the content of marketable eggs from birds of different age groups; as well as washed from the equipment of poultry farms, the repair young poultry department, the industrial herd department, egg sorting and certification workshops, from the surface of the egg, from the working surfaces of special vehicles. Bacterial contamination of air, droppings, bedding, complete feed was studied. Bacteriological studies were carried out according to generally accepted schemes, using accumulative, selective and differential diagnostic media (heptadecyl sulfate agar, Endo, xylose-lysine agar, differentiated agar with diamond green, Muller-Hinton). The sampling was carried out using universal sterile applicators "Voles".

The next stage was to analyze the results of bacteriological studies and determine the basic list of criteria for creating critical control points (CCPs) for the further development of a risk management scheme for the bacterial biosafety of edible eggs according to HACCP principles.

3. Research results

The first stage of our work was to study the risks of contamination by opportunistic and pathogenic microflora at all stages of the production of chicken edible eggs according to ISO 22000:2007.

First, we studied the risks of contamination with bacterial microflora when placement day-old young. For this, bacteriological studies of pathological material from day-old chickens and litter from transport boxes were carried out. Batches of day-old young, obtained from domestic hatcheries and from foreign suppliers, were studied.

The quality of disinfection of poultry houses and pathological material from day-old chickens, litter from transport boxes (meconium) were bacteriologically investigated. The results are presented in Table 1.

Table 1

Isolated microflora during disinfection quality control and from the repair young poultry department

Material for research (washings)	Isolated microflora								
	E. coli	*Enterobacter spp*	*P. aeruginosa*	*S. aureus*	*Salmonella spp.*	*Shigella spp.*	*C. jejuni*	*Enterococcus spp.*	*Streptococcus. spp.*
Cages	_	–	_	_	–	–	_	–	–
Drinkers	–	+	–	–	–	–	–	–	–
Litter removal line	–	–	–	–	–	–	–	–	–
Feeders	+	+	–	–	–	+	–	–	–
Bunkers	+	+	–	–	–	+	–	–	–
domestic incubators									
Heart	–	–	–	+	–	–	–	–	–
Lungs	–	–	–	+	–	–	–	–	–
Intestine	+	_	+	_	_	_	_	+	–
Litter	+	+	+	–	–	–	–	–	–
foreign incubators									
Heart	–	–	–	+	–	–	–	+	–
Lungs	_	_	_	+	_	_	_	_	+
Intestine	+	_	+	_	_	_	_	+	_
Litter	+	+	+	–	–	–	+	+	+

The results of the research, presented in Table 1 – isolation of *S. aureus* from the heart and lungs, *E. coli, P. aeruginosa, Enterobacter spp, Enterococcus spp* – from the intestines of day-old chickens and litter – indicate violations of veterinary and sanitary standards in the hatchery and the low quality of disinfection before placement day-old young birds, as well as non-observance of veterinary and sanitary norms for the transportation regime of day-old young birds. We also pay attention to pronounced bacterial contamination: the selection of *C. Jejuni, Streptococcus spp.* in addition to the above-described types of bacteria from batches of day-old young birds from foreign suppliers.

At the next stage, we conducted bacteriological studies in fodder production shops.

Bacterial microflora was isolated from washings from technological equipment, from raw materials, finished products, and special transport for the transportation of feed in the repair departments of young birds and the production department. Table 2 presents the results of research from the line of the feed production shop. Most often, we detected bacteria of the genus E. coli and Enterobacter spp., which may be a consequence of the introduction of meat-bone or fish meal into the compound feed and the low efficiency of the disinfectants used.

Table 2

Isolated microflora of the fodder production shop

Type of material		Isolated microflora						
		E. salt	*Enterobacter spp*	*Klebsiella spp.*	*Serratia spp.*	*Salmonella spp.*	*Str. zymogenes*	*Shigella spp.*
Fodder raw materials and compound feed	Corn	–	–	–	+	–	–	–
	Soybean meal	–	–	–	+	–	–	+
	Sunflower cake	–	–	–	–	–	–	–
	Final product (combined feed)	+	+	+	–	–	+	–
Wash off	Bunkers	–	+	–	–	–	–	–
	Transporters	–	+	–	–	–	–	+
	Tanks for raw materials	+	+	–	–	–	–	–
	Special transport (body)	+	+	–	–	–	–	–

Isolation *of E. coli, Enterobacter spp, Klebsiella spp., Str. zymogenes* from the final product - compound feed, as well as *E. coli, Enterobacter spp, Shigella spp.* in washings from the equipment of the feed mill, is evidence of insufficient veterinary and sanitary control of incoming raw materials, finished products, as well as poorly carried out disinfection of equipment and specialized vehicles.

The next stage of our research was to study the contamination of edible eggs with opportunistic and pathogenic microflora directly on the egg production line. Bacteriological studies of pathological material from industrial herds, washings from poultry equipment, egg sorting shops, belt conveyors, containers, egg surfaces were carried out. The general bacterial air pollution of poultry premises was investigated using the sedimentation method. As a result of the study, it was found, that the average indicator of bacterial air pollution was 1,122,200 m.c./m^3 in poultry houses, which exceeds the normative indicator (500,000 m.c./m3) by 2.2 times. It was noted, that the poultry stocking density was exceeded in the studied farms.

Table 3

Isolated microflora from the egg production line and pathological material of industrial livestock

Material for research		Microflora from the egg production line								
		E. coli	*Enterobacter spp*	*Enterococcus spp.*	*Streptococcus sp p.*	*Klebsiella spp*	*P. aeruginosa*	*Salmonella spp.*	*S. aureus*	*S. epidermidis*
Wash off	Cages	–	–	–	–	–	_	–	_	-
	Belt conveyor	–	–	–	–	–	–	–	–	-
	Sorting tables	+		–	–	–	–	–	–	-
	Personnel	+	+	+	+	–	–	–	+	+
	Packaging (gaskets)		+	–	–	–	+	–	_	–
	Egg surface	+	+	–	+	–	+	–	–	+
	Internal transport	–	+	–	–	–	–	–	–	–
Egg content		+	+	–	–	–	–	–	+	+
Microflora from pathological material of industrial livestock										
Path material (industriallivestock)	Heart	–	–	–	–	–	–	–	–	–
	Liver	+	–	_	+	–	–	_	_	–
	Spleen	+	–	_	+	–	–	_	_	–
	The bone marrow	+	–	–	+	–	–	–	+	–
	Cecum	+	+	+	–	–	–	–	–	–
	Follicles	+	+	+	+	–	–	–	+	–
	Lungs	–	–	–	–	–	–	–	+	–
	Trachea	–	–	–	+	–	–	–	+	–

When examining objects from the production line, *E. coli, Enterobacter spp., P. aeruginosa,* and *S. epiderrmidis* were most often isolated. From the pathological material during the bacteriological examination, microflora of the genus *E. coli, Streptococcus spp., S. aureus* (most often pathogenic serotypes) prevailed.

The analysis of the data in Table 3 confirms the fact that an excess of stocking density has a negative impact on the sanitary condition of the premises in an industrial flock of laying hens. Bacterial air pollution in poultry houses has a negative effect on the health of birds, the risk of infectious diseases of bacterial etiology increases. During the pathological examination, we found signs of cloacitis, salpingoperitonitis, hepatosis, nephrosis, visceral form of gout.

Based on the established criteria, we defined a list of CCPs for managing the risks of bacteriological biosafety of edible eggs according to HACCP principles:

CCP 1. Intake of day old young birds (control at the hatchery, entrance control at the enterprise, quarantine measures, laboratory control, veterinary supervision. Preparation of premises for the placement of young birds: disinfection, sanitation, technological rest of poultry houses).

CCP 2. The period of rearing in the young repair department, transfer to the industrial department, the beginning of egg laying. Feed production workshop: control of incoming raw materials, manufactured compound feeds, laboratory control, veterinary and sanitary supervision. Disinfecting and sanitary preparation of premises for poultry placement.

CCP 3. Egg production at the industrial unit: veterinary and sanitary control of production (monitoring of diseases of infectious and non-infectious etiology, control of microclimate parameters, personal hygiene of personnel, functioning of sanitary passes, control of the sanitary condition of poultry premises and devices of the egg collection system – elevators, belt conveyors). Automatic sorting and packaging of eggs. Veterinary – zootechnical quality control of sorting directly at the production site. Removal and disposal of non-compliant products.

CCP 4. Egg certification workshop. Packaging materials: incoming control of packaging materials, storage, delivery. Egg marking: incoming control of marking paint, storage of marking paint, refueling of the marking machine (control of marking quality and conformity). Egg storage: control of the microclimate of warehouses, veterinary and sanitary control (ovoscopy, measurement of the air chamber).

CCP 5. Output control of finished products. Traceability. Feedback and withdrawal, receiving complaints.

4. Discussion

As a result of the bacteriological monitoring in poultry farms, it was established, that the spectrum of bacterial

contamination is mainly represented by opportunistic microflora. Mainly the following types of microorganisms were found: *E. coli, P. aeruginosa, Enterobacter spp, Enterococcus spp.* Bacteria *C. jejuni, Streptococcus spp., S. aureus, Salmonella spp.,* which are the most dangerous for humans and poultry, were not isolated. Our results and the data of other researchers regarding the isolation of bacterial flora from poultry farms differed somewhat. Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly and other authors report that a review of risk factors for *Salmonella* in laying hens revealed that overall evidence points to a lower occurrence in non-cage compared to cage systems. [5–7].

We established that from the pathological material during the bacteriological examination, microflora of the genus *E. coli, Streptococcus spp., S. aureus* (most often pathogenic serotypes) prevailed. Similar results were shown by researchers Karunarathna, R., Ahmed, K. A., Goonewardene, K., Gunawardana, T., Kurukulasuriya, Moreau, T., Gautron, J., Hincke and other [8–10].

Advantages and disadvantages of the obtained research results. The advantage of the obtained research results is that the obtained data of bacteriological monitoring of poultry farms became the basis for establishing control critical points according to the HACCP system. The disadvantage of the results of the conducted research is that not all private enterprises have free access to conduct bacteriological research.

Limitations of the study. Limitation of the study is that most poultry farms in Ukraine are private, so there may be difficulties in access for such studies.

Prospects for further research. Our further research is aimed at a detailed study of each proposed control critical point in order to develop proposals for production.

5. Conclusions

As a result of the bacteriological monitoring of objects of the technological cycle of chicken edible egg production, we established a list of bacteriological risks at all stages of production. As a result of the conducted research, it was determined, that the spectrum of bacterial contamination was mainly represented by opportunistic microflora. In the majority, the following types of microorganisms were detected: *E. coli, P. aeruginosa, Enterobacter spp, Enterococcus spp.*

C. jejuni, Streptococcus spp., S. aureus, Salmonella spp., which are the most dangerous for the consumer and poultry, were not isolated. We confirm this with our own research results and research, conducted within the framework of the State program for the control of poultry salmonellosis (laying hens) in poultry farms of Ukraine.

We studied the bacterial risks at all the main stages of the industrial production of chicken edible eggs and identified the main critical control points of production according to the principles of the HACCP system.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results, presented in this article.

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Data availability

Manuscript has no associated data.

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