

MONITORING OF THE ISOLATION OF PATHOGENS OF BACTERIAL INFECTIONS OF POULTRY

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In modern commercial poultry farming, the main problem remains the fight against infectious diseases, which cause significant economic losses due to reduced productivity and costs of eradication measures. Broilers are particularly vulnerable to bacterial diseases due to their intensive growth rate and high productivity. The article is devoted to the definition and analysis of the spread of bacterial infections in poultry farms in the northeastern part of Ukraine. The main attention is paid to determining the etiological factors of infectious diseases in broiler chickens of different age groups. It was established that bacterial pathogens were isolated mainly in associations and were represented by microorganisms of the Enterobacteriaceae. Most often isolated pathogens Salmonella spp., E. coli, Proteus spp. and other opportunistic microorganisms. The study carried out a microbiological analysis of 385 samples of pathological material taken from broilers of different age periods (1–7, 10–30 and 30–42 days). The results revealed a significant role of Salmonella spp. as an etiological factor of diseases in early age period, E. coli in middle age groups and Proteus spp. with Pseudomonas spp. in the older age group. Salmonella spp., E. coli and Proteus spp. were most often isolated in 89.7% of the investigated samples of biomaterial from chickens 1–7 days, the association of bacterial isolates of Salmonella spp., P. mirabilis represented the largest part – 36.9%. From samples of biomaterial from broiler chickens 10–30 days, isolates of E. coli and Salmonella spp. make up the dominant share – 41.8%, E. coli and Staphylococcus spp. – 22.1%, Salmonella spp. and C. perfringens – 14.9%, E. coli and Enterococcus spp. – 10.2%, Pseudomonas spp. and Staphylococcus spp. – 6.3%, Bacillus spp., Proteus spp. – 4.7%. Campylobacter spp., Proteus spp., Pseudomonas spp., Clostridium spp. were the largest share of isolates from the examined samples of chicken carcasses 30–42 days – 25.8%, which indicates their predominant role in the development of primary and secondary infections in poultry and the potential risks of spreading foodborne toxic infections in humans when consuming insufficiently thermally processed poultry meat. Studies confirm the influence of the microbiological background on the development of primary and secondary infections associated with the conditions of housing, feeding and biosecurity in farms.

Key words: bacterial infections, bacteria, isolates, broilers, microorganisms.

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Introduction. Poultry breeding is an important direction of agricultural production, which provides the population with high-quality food products and plays a significant role in the development of the agricultural sector of Ukraine (Muzyka et al., 2023; Golovko et al., 2024). At the same time, the successful functioning of this industry faces numerous challenges, in particular infectious diseases of poultry. Bacteriosis has a significant impact on poultry productivity, which leads to economic losses for farms and a decrease in product quality. The epizootic situation regarding infectious diseases of poultry is complicated by a number of factors, in particular, the intensity of production and the level of biosecurity. These conditions contribute to the active spread of bacterial infections, such as escherichia, pasteurellosis, salmonellosis, etc., which requires in-depth study to develop effective preventive measures (Darbandi et al., 2022; Christensen et al., 2021; Wang et al., 2021; Zhang et al., 2024).

The purpose of the study – determination of the distribution of pathogens of bacterial infections among

poultry of different age groups in poultry farms specializing in broiler breeding.

Research materials and methods. The research work was carried out in the period 2022–2024 in accordance with the thematic plan of scientific research of the Department of Epizootology and Parasitology of the Sumy National University «Optimization of the set of measures to prevent the occurrence and spread of infectious animal diseases in the farms of the northeastern region of Ukraine», 0122U001254, 2022–2027.

Research results. We conducted a study of pathological material, which was selected from the carcasses of broiler chickens of different age groups in poultry farms in the northeastern part of Ukraine. Biological material was examined (poultry carcasses, heart, bone marrow). Research on the level of isolation of pathogens of bacterial infections was carried out from dead poultry of different age groups, namely 1–7 days, 10–30 days and 30–42 days. A total of 385 samples were investigated.

According to the research results of 165 samples of pathological material from chickens 1–7 days, the largest

share was made up of isolates of *Salmonella spp.* and *P. mirabilis* – 36.9%, the share of isolation of pathogens *E. coli*, *Proteus spp.*, *Staphylococcus spp.* – 24.8%, the share of isolation of microorganisms of the genera *Proteus spp.*, *Klebsiella spp.*, *Citrobacter spp.*, *Enterobacter spp.*, *Yersinia spp.*, *Campylobacter spp.*, *Clostridium spp.* were 18.2%, the share of the isolation of pathogens *Salmonella spp.*, *Campylobacter spp.* and *Enterobacter spp.* – 17.6%, the share of isolation of microorganisms of the genus *Staphylococcus spp.*, *Proteus spp.* and *Neisseria spp.* are not significant and is 2.4%.

The results of microbiological studies of samples from dead chickens 1–7 days are presented in the tab. 1

So, opportunistic pathogens of the Enterobacteriaceae family were isolated in various associations in 89.7% of the samples taken from chickens 1–7 days. According to the results of the study of samples of pathological material, it was established that the most common isolates are *Salmonella spp.*, *E. coli* and *Proteus spp.* The obtained data indicate the key role of isolated isolates in the formation of the infectious process in poultry. *Salmonella spp.* is one of the main causative agents of intestinal infections, and *P. mirabilis* is an opportunistic microorganism that can complicate the course of infections under the influence of negative factors. The specific gravity of isolated microorganisms from carcasses of poultry 1–7 days is presented in Fig. 1

Table 1

Data on the isolation of microorganisms from dead poultry 1–7 days, n=156

Results of microbiological research		
Type of microorganisms	the number of positive samples	positive samples,%
<i>Salmonella spp.</i> , <i>P. mirabilis</i>	61	36.9
<i>E. coli</i> , <i>Proteus spp.</i> , <i>Staphylococcus spp.</i>	41	24.8
<i>Proteus spp.</i> , <i>Klebsiella spp.</i> , <i>Citrobacter spp.</i> , <i>Enterobacter spp.</i> , <i>Yersinia spp.</i> , <i>Campylobacter spp.</i> , <i>Clostridium spp.</i>	30	18.2
<i>Salmonella spp.</i> , <i>Campylobacter spp.</i> , <i>Enterobacter spp.</i>	29	17.6
<i>Staphylococcus spp.</i> , <i>Proteus spp.</i> , <i>Neisseria spp.</i>	4	2.4
total	165	100

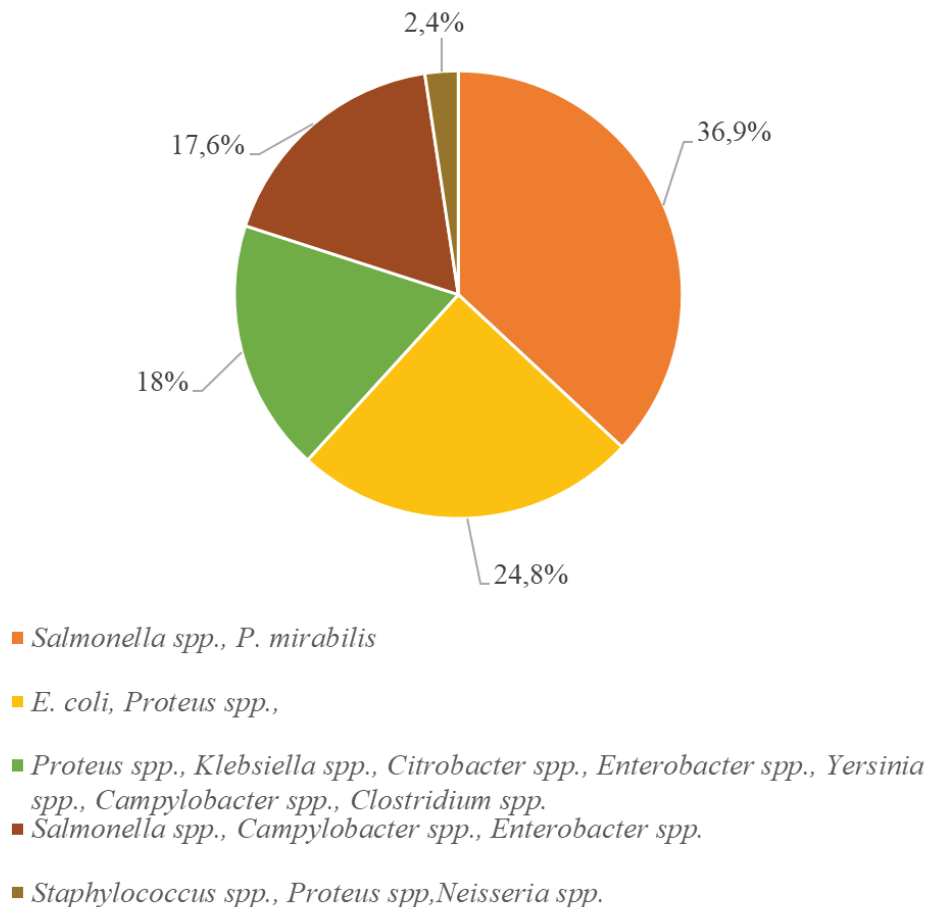


Fig. 1. The levels of isolation of microorganisms from pathological material samples, (1–7 days)

Isolation of pathogens *E. coli*, *Proteus spp.* and *Staphylococcus spp.* (24.8%) demonstrates the role of these microorganisms in the development of mixed infections. Isolation of a group of microorganisms, which includes *Proteus spp.*, *Klebsiella spp.*, *Citrobacter spp.*, *Enterobacter spp.*, *Yersinia spp.*, *Campylobacter spp.* and *Clostridium spp.* (18.2%) indicates their role in the development of secondary infections, which occur as complications associated with a violation of the intestinal microbial balance. The obtained results support the monitoring of the state of advanced pathogens such as *Salmonella spp.* and *P. mirabilis*, to reduce the risk of infections and to develop effective veterinary and sanitary measures to reduce the risk of spread or to minimize the registration of bacterial infections.

At the second stage of the research, the level of isolation of bacterial microflora from dead poultry 10–30 days was determined. A total of 127 samples were examined (heart, bone marrow, intestinal contents). The results of microbiological studies of samples from dead chickens 10–30 days are presented in the tab. 2.

It was established that among the isolated pathogens, *E. coli* and *Salmonella spp.* make up the dominant share – 41.8%, *E. coli* and *Staphylococcus spp.* – 22.1%, *Salmonella spp.* and *C. perfringens* – 14.9%, *E. coli* and *Enterococcus spp.* – 10.2%, *Pseudomonas spp.* and *Staphylococcus spp.* – 6.3%, *Bacillus spp.*, *Proteus spp.* – 4.7% (Fig. 2).

The presented results regarding the levels of isolation of bacterial microflora from poultry to 30 days of age are associated with a decrease in the adaptive characteristics of the body of poultry due to an incompletely formed immune system. This aspect makes this age category of poultry sensitive to the effects of stress, negative environmental factors, hygienic violations of housing conditions, feeding and watering, which in turn creates conditions for the development of pathogenic and opportunistic microflora.

We also conducted microbiological studies to determine the levels of isolation of bacterial microflora from dead poultry of slaughter condition, namely 30–42 days. The results of microbiological studies of the samples taken from the dead bird are presented in the tab. 3.

Table 2

Data on the isolation of microorganisms from dead poultry of 10–30 days, n=127

Results of microbiological research		
Type of microorganisms	the number of positive samples	positive samples, %
<i>E. coli</i> , <i>Salmonella spp.</i>	53	41.8
<i>E. coli</i> , <i>Staphylococcus spp.</i>	28	22.1
<i>Salmonella spp.</i> , <i>C. perfringens</i>	19	14.9
<i>E. coli</i> , <i>Enterococcus spp.</i>	13	10.2
<i>Staphylococcus spp.</i> , <i>Pseudomonas spp.</i>	8	6.3
<i>Bacillus spp.</i> , <i>Proteus spp.</i>	6	4.7
total	127	100

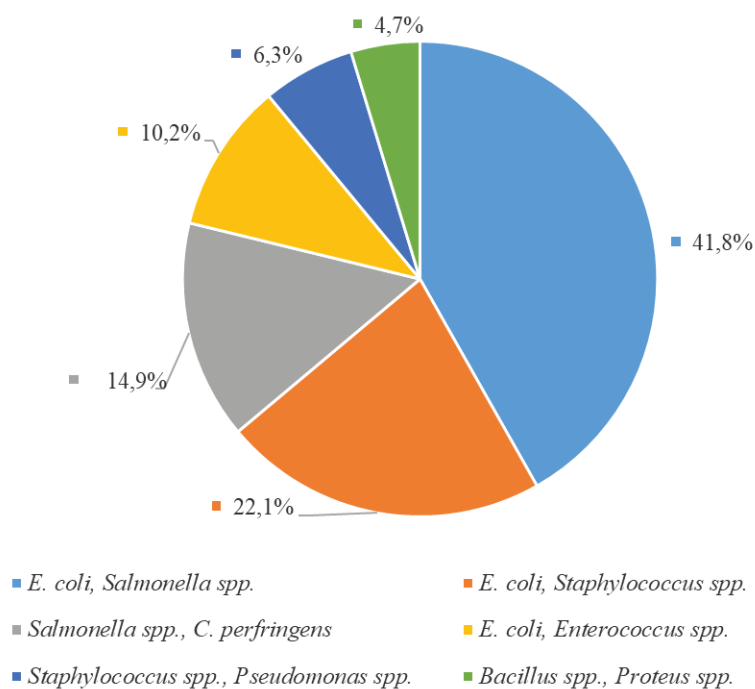


Fig. 2. The levels of isolation of microorganisms from pathological material samples, (10–30 days)

At this age, the bird is in an active phase of growth, demonstrates high metabolic activity of processes and an increase in productivity indicators, which in turn causes a decrease in natural resistance to infections. Under such conditions, there are risks of colonization of tissues and organs by intestinal microflora. The majority of intestinal microorganisms are normally commensals and opportunistic microorganisms, which, under conditions of disruption of homeostasis in the bird's body, exhibit pathogenic properties. According to the data in the tab. 3 recorded the simultaneous isolation of bacterial pathogens of several genera of the *Enterobacteriaceae* family from the studied samples (Fig. 3).

Campylobacter spp., *Proteus spp.*, *Pseudomonas spp.*, *Clostridium spp.* were isolated with the highest

specific gravity from among the studied samples, which was 25.8%, which indicates their role in the development of the infectious process in this age period of broilers. The high frequency of isolates of a combination of *E. coli* and *S. aureus* isolates (24.7%) indicates their potential to cause both local and systemic infections. A significant proportion of isolates of *E. coli* and *Salmonella spp.* (18.3%) emphasizes the importance of monitoring these pathogens, as they are often the cause of food poisoning associated with the consumption of insufficiently thermally processed poultry meat. Therefore, the results obtained by us regarding the levels of isolation of opportunistic and pathogenic microflora from pathological material allow us to identify potential dangers that can potentially be an etiological factor of both concomitant infections and the cause of bird death.

Table 3

Data on the isolation of microorganisms from dead poultry of 30–42 days, (n=93)

Results of microbiological research		
Type of microorganisms	the number of positive samples	positive samples, %
<i>E. coli</i> , <i>S. aureus</i>	23	24.7
<i>Campylobacter spp.</i> , <i>Proteus spp.</i> , <i>Pseudomonas spp.</i> , <i>Clostridium spp.</i>	24	25.8
<i>E. coli</i> , <i>Salmonella spp.</i>	17	18.3
<i>C. perfringens</i> , <i>E. coli</i>	13	13.9
<i>E. coli</i> , <i>Enterococcus spp.</i>	11	11.9
<i>Staphylococcus spp.</i> , <i>Klebsiella spp.</i>	5	5.4
total	93	100

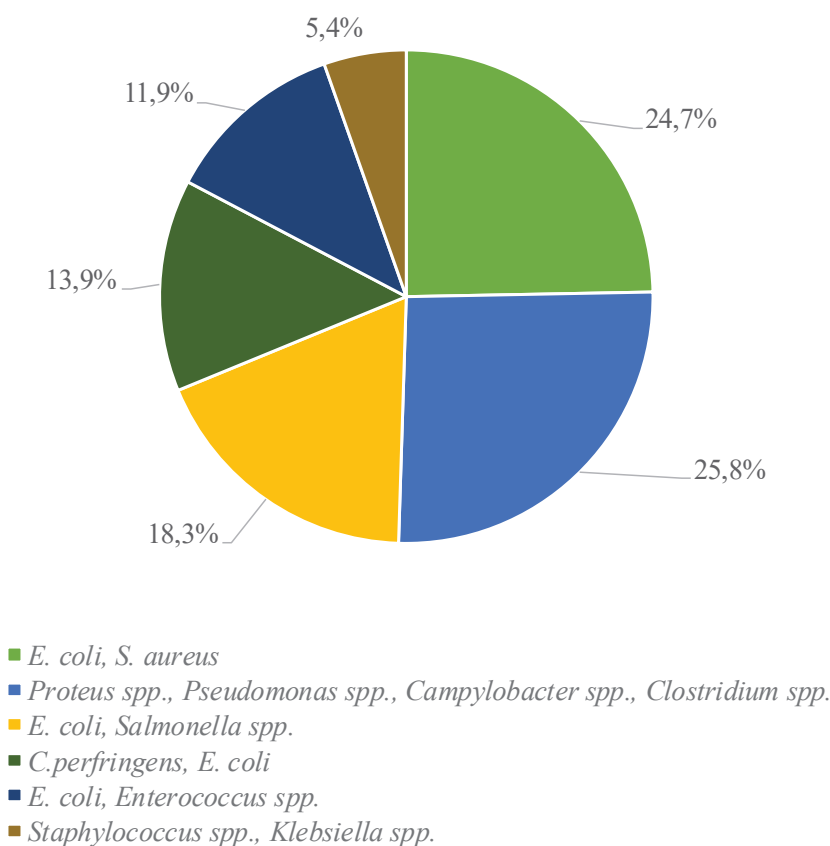


Fig. 3. The levels of isolation of microorganisms from pathological material samples, (30–42 days)

Discussion. Our research results revealed a high level of spread of opportunistic and pathogenic microorganisms among broilers of different age groups in poultry farms of northeastern Ukraine. The analysis of the obtained data shows that representatives of the genera *Salmonella spp.*, *E. coli*, *Proteus spp.*, which are the leading etiological factors of the infectious process, were most often found among the pathogens.

It was established that *Salmonella spp.* dominated in the age group (1–7 days), and *P. mirabilis*, which may be due to the imperfection of the immune system and stress factors in the early period. In poultry aged 10–30 days, combinations of *E. coli* and *Salmonella spp.* were significantly spread, which confirms their role as primary pathogens and factors of mixed infections. In the older age group (30–42 days), a high frequency of isolation of *Proteus spp.*, *Pseudomonas spp.* were observed, and *Clostridium spp.*, which indicates possible secondary complications associated with violation of housing and feeding conditions. The obtained results emphasize the need for the implementation of comprehensive monitoring of bacterial pathogens in poultry farms for the purpose of timely diagnosis and prevention of infections. A special emphasis should be placed on improving biosecurity, increasing the level of vaccination, and optimizing the conditions for keeping poultry. This study also demonstrates the importance of developing individual strategies for each age group, taking into account the specificity of their immune response and physiological state. Further research should be aimed at studying the resistance of microorganisms to antibacterial drugs, which will help to improve methods of fighting infections and reduce economic losses in poultry farming. The results of the study indicate a high frequency of isolation of opportunistic and pathogenic microorganisms in broilers of different age groups. *Salmonella spp.*, *E. coli*, *Proteus spp.*, *Pseudomonas spp.* and other bacteria, which confirms the global trend in the spread of these pathogens in poultry. Studies by foreign authors confirm the revealed patterns. In particular, the works of Y. Shang (Shang, 2018, p. 254–255) emphasize the key role of *Salmonella spp.* in the development of intestinal infections in poultry, which leads to significant economic losses due to reduced productivity and increased mortality. They also indicate the high adaptability of these bacteria to various environmental conditions.

In researches (Fancheret et al., 2020; Kathayat et al., 2021) emphasized the role of *E. coli* as one of the main causative agents of mixed infections. The authors note that the high pathogenicity of this microorganism is due

to its ability to produce toxins and form biofilms, which complicates the treatment of infections. In addition, they indicate the increasing resistance of *E. coli* to antibacterial drugs, which makes it difficult to fight infections (Wu et al., 2021; Fancher et al., 2020). Special attention should be paid to the role of *Proteus spp.* and *Pseudomonas spp.* in secondary infections. According to research (Gieryńska et al., 2022; Shang et al., 2018; Zhang et al., 2024), these microorganisms often cause complications, in particular with weakened immunity in poultry. Their allocation in older age groups, as it was recorded in our study, may indicate imperfect conditions of maintenance and hygiene in poultry farms. The obtained results emphasize the importance of introducing systematic monitoring of the state of microbial flora in poultry farms. In addition, foreign experience suggests that the successful fight against bacteriosis requires a comprehensive approach, which includes improving biosecurity, using effective vaccination programs, and increasing the awareness of workers in the industry about hygienic requirements (Shiet et al., 2022; Gieryńska et al., 2022). Thus, the combination of the data we received with the results of global research can become the basis for improving strategies for the prevention and treatment of bacterial infections in poultry farming.

Conclusions. It was established that several opportunistic microorganisms of the *Enterobacteriaceae* family were isolated simultaneously from samples of biological material from the carcasses of broiler chickens of different age groups. From samples of pathological material from chickens 1–7 days, *Salmonella spp.* and *P. mirabilis* were most often isolated – 36.9%, *E. coli* and *Salmonella spp.* were isolated simultaneously from chickens 10–30 days in 41.8%, *Campylobacter spp.* were isolated simultaneously from carcasses of chickens 30–42 days in 25.8%, *Proteus spp.*, *Pseudomonas spp.*, *Clostridium spp.* The analysis of these levels of isolation of opportunistic and pathogenic microflora from pathological material identifies potential dangers that are the etiological factor of both concomitant infections and the cause of bird death.

Studies emphasize the importance of monitoring the spread of bacterial infections, improving veterinary and sanitary measures and developing adapted preventive programs for each age group of poultry. The proposed results are of practical importance for reducing economic losses in poultry farming and improving the effectiveness of measures to combat bacterial infections.

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Моніторинг ізоляції збудників бактеріальних інфекцій птиці

У сучасному промисловому птахівництві основною проблемою залишається боротьба з інфекційними захворюваннями, які викликають значні економічні збитки через зниження продуктивності та витрати на заходи ліквідації. Бройлери особливо вразливі до бактеріозів через інтенсивний темп росту та високу продуктивність. Стаття присвячена визначенню та аналізу поширення бактеріальних інфекцій у птахогосподарствах північно-східної частини України. Основну увагу приділено визначенню етіологічних чинників інфекційних захворювань у курчат-бройлерів різних вікових груп. Встановлено, що бактеріальні патогени ізолювалися переважно в асоціаціях і були представлені мікроорганізмами родини *Enterobacteriaceae*. Найчастіше ізолювали патогени *Salmonella* spp., *E. coli*, *Proteus* spp. та інших умовно-патогенних мікроорганізмами. У дослідженні проведено мікробіологічний аналіз 385 зразків патологічного матеріалу, відібраного від бройлерів різних вікових періодів (1–7, 10–30 та 30–42 днів). Результати виявили значну роль *Salmonella* spp. як етіологічного чинника захворювань у молодняку раннього вікового періоду, *E. coli* у середніх вікових групах та *Proteus* spp. разом із *Pseudomonas* spp. у старшій віковій групі. В 89,7% досліджених проб біоматеріалу від курчат 1–7 добового віку найчастіше ізолювали *Salmonella* spp., *E. coli* та *Proteus* spp., найбільшу питому вагу представляли асоціація бактеріальних ізолятів *Salmonella* spp., *P. mirabilis* – 36,9%. З проб біоматеріалу від курчат-бройлерів 10–30 добового віку домінуючу частку складають ізоляти *E. coli* та *Salmonella* spp. – 41,8%, *E. coli* та *Staphylococcus* spp. – 22,1%, *Salmonella* spp. та *C. perfringens* – 14,9%, *E. coli* та *Enterococcus* spp. – 10,2%, *Pseudomonas* spp. та *Staphylococcus* spp. – 6,3%, *Vacillus* spp., *Proteus* spp. – 4,7%. Найбільшу частку ізолятів від досліджених проб трупів курчат 30–42 добового віку склали *Campylobacter* spp., *Proteus* spp., *Pseudomonas* spp., *Clostridium* spp. – 25,8%, що вказує на переважну їх роль в розвитку первинних і секундарних інфекцій у птиці та потенціальних ризиків поширення харчових токсикоінфекцій у людей при вживанні недостатньо термічно обробленого м'яса птиці. Дослідження підтверджують вплив мікробіологічного фону на розвиток первинних і вторинних інфекцій, пов'язаних із умовами утримання, годівлі та біобезпеки в господарствах.

Ключові слова: бактеріальні інфекції, бактерії, ізоляти, бройлери, мікроорганізми.