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# AVIAN SALMONELLOSIS. EPIDEMIOLOGICAL PARTICULARITIES, DIAGNOSIS AND PROPHYLAXIS MEASURES

**431.03. Microbiology, virology, epizootiology, mycology, and veterinary immunology**

The abstract of the doctoral thesis in veterinary medical sciences

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**CONTENTS**

## CONCEPTUAL FRAMEWORKS IN RESEARCH

**Relevance of the topic.** Birds, both domestic and wild, can be carriers and shedders of *Salmonella* and may exhibit clinical infections of varying severity, often being the source of foodborne illnesses in humans. The sources of contamination are highly variable, including contaminated feed, environmental objects, caregivers, transportation, rodents, etc. The primary source of contamination for humans is contaminated poultry products, such as poultry meat and eggs [48, 82, 107, 129].

Numerous microbiological investigations conducted by various researchers confirm that, despite a 'healthy' appearance, poultry meat is frequently contaminated with pathogenic microorganisms harmful to humans. Currently, *Salmonella spp.* and *Campylobacter spp.* are the most well-known hazards associated with the consumption of poultry meat. The incidence of diseases caused by these bacterial infections is steadily increasing in all countries where these diseases are monitored. Although *Salmonella* is recognized as the most important pathogen associated with poultry meat, the exact incidence of human illnesses due to poultry consumption is still unknown. However, based on published global statistics, it is estimated that illnesses caused by *Salmonella* following poultry consumption account for approximately 20-25% of all human salmonellosis cases [5, 81, 99, 152].

**Description of the situation in the research field and identification of research problems.** Salmonellosis is the second most frequently reported gastrointestinal infection in the European Union and a significant cause of foodborne outbreaks in humans. According to the data from the European Centre for Disease Prevention and Control (Annual Epidemiological Report, 2021), in 2021, there were 61 236 confirmed cases of salmonellosis, of which 73 were fatal. The EU/EEA notification rate for salmonellosis was 16,6 cases per 100,000 inhabitants. These data confirm that, despite the systematically implemented veterinary sanitary measures in poultry units, avian salmonellosis remains a current problem for the veterinary service, contributing to the strengthening and diversification of diagnostic and prophylactic methods [1, 22, 48, 77].

Considering the increasing role of domestic and wild birds, as well as their products (meat and eggs) in human nutrition and the numerous cases of illnesses and foodborne infections in humans, we aim to investigate, on one hand, the aetiology and prevalence (frequency) of *Salmonella* infections in birds, and on the other hand, the presence of *Salmonella* spp. serotypes in poultry products intended for public consumption (eggs and meat).

**The aim and objectives of the research**

**The aim** of the study was to establish the epidemiological situation and the impact factors of avian salmonellosis on poultry populations and public health by analysing the main disease transmission vectors.

**Research objectives:** (1) Monitoring the diversity of pathogenic microflora and the presence of bacteria from the genus *Salmonella* spp. in some poultry units for current egg production and broiler chick rearing; (2) Analysis of the presence and diversity of *Salmonella spp.* serotypes in faecal matter, pathological material collected from poultry enterprises for current egg production, broiler chick rearing, from samples taken from incubators, and from poultry markets for bird commercialization; (3) Analysis of the presence and diversity of *Salmonella spp.* serotypes in poultry carcasses and eggs, produced under the conditions of the Republic of Moldova, as well as those imported and marketed at the Central Agricultural Market

in Chisinau; (4) Serotyping of *Salmonella spp*. bacteria isolated and determining their sensitivity to some groups of antibiotics commonly used in poultry enterprises; (5) Developing scientific-practical recommendations regarding the methods of prophylaxis and control of avian salmonellosis and *Salmonella spp.* induced food poisoning in humans.

**Research hypothesis.** The presence and systematic spread of salmonella infections in bird populations and the increased incidence of foodborne illnesses in humans due to the consumption of poultry products demonstrate the impact of this disease and the need for comprehensive studies, as well as the improvement of monitoring methods and strengthening of veterinary sanitary measures aimed at the prevention of avian salmonellosis.

**Scientific research methodologies.** It is based on the scientific research strategy of classical and modern epidemiological and microbiological methods. Epidemiological research was conducted on avian salmonellosis at some hatcheries in the republic, poultry units for broiler chick rearing and egg production for current consumption, as well as at the Poultry Market for the commercialization of birds of various species and age categories in Chisinau, including some units for poultry slaughter in the republic. Some units for the commercialization of poultry products (eggs and poultry meat) were also under surveillance. Samples were collected from these units for research purposes (substrate and equipment washes, faecal matter, pathological material from carcasses). Bacteriological, bacterioscopic, serological, and serotyping research was conducted on bacteria from the genus *Salmonella spp.,* against the background of the existing microbiome in the monitored units. Both classical research methods and modern methods were used, including conventional, enrichment, differential, selective, and special culture media, monoreceptor sera for serotyping bacteria from the genus *Salmonella spp*., as well as methods for determining antimicrobial resistance of *Salmonella* isolates identified in poultry units and their products. The obtained results were systematized and statistically processed.

**The scientific novelty and originality.** For the first time, epidemiological monitoring of avian salmonellosis was conducted in poultry enterprises for broiler chick rearing and egg production to assess the main disease transmission vectors and evaluate the risk factors for the poultry sector and consumers of poultry products. The diversity of circulating bacterial microflora in bird populations, meat, and eggs was established, with isolation, serotyping, and determination of the incidence of *Salmonella spp.* types. The antibiotic resistance of *Salmonella* isolates to some antibacterial agents used in poultry farming was assessed, as well as the effectiveness of certain vaccine strains used in the immunoprophylaxis of avian salmonellosis.

**The important scientific issue addressed in this field.** Avian salmonellosis remains the most widespread foodborne infection in poultry, posing the greatest risk of foodborne illnesses in humans. Assessing the incidence of bacteria from the genus *Salmonella spp.* in poultry units and poultry products enables the implementation of sanitary measures to reduce the risk of salmonellosis spreading in bird populations and consequently reduces the risk of *Salmonella* foodborne illnesses in meat and egg consumers. Scientific-practical recommendations allow for the elimination of risk factors for *Salmonella* transmission in the poultry farming chain and the production of safe poultry products for consumers.

**The practical value of research.** The incidence of bacteria from the genus *Salmonella spp.* in poultry units and poultry products has been assessed, with critical contamination points highlighted and proposed for monitoring. The level of sensitivity of salmonellas to commonly used antibacterials in poultry farming has been assessed, allowing for the refinement of treatment

protocols for certain bacterial diseases in birds, as well as the post-vaccination effect of some vaccines used in the prevention of avian salmonellosis.

**The main scientific results presented for defense.** New findings regarding: the epidemiological situation concerning avian salmonellosis within poultry enterprises for meat and egg production in the Republic of Moldova; the incidence of bacteria from the genus *Salmonella spp.* within poultry enterprises, hatcheries, poultry markets, as well as in pathological material collected from birds; the incidence of bacteria from the genus *Salmonella spp.* in poultry products (meat and eggs), from the commercial network of the Agricultural Market in Chisinau and from some slaughterhouses in the republic; data regarding the immunological efficiency of certain vaccines used in preventing salmonellosis in laying hen populations; the results of serotyping bacteria from the genus *Salmonella spp.* from poultry units, poultry products, and the resistance of *Salmonella* isolates to some antimicrobials used in poultry farming.

**Approval of the thesis at national and international scientific forums.** The materials of the thesis were presented at various national and international scientific forums: Scientific Conference with International Participation "Management of the genetic fund of animals – problems, solutions, outlooks", ISPBZMV, Chisinau, Republic of Moldova (2023); International Conference on Farm Animal Infectious Diseases, Faculty of Veterinary Medicine, Timișoara, Romania (2021); Scientific Conference with International Participation "Innovations in animal husbandry and safety of animal products - achievements and outlooks", ISPBZMV, Chisinau, Republic of Moldova (2021); International Congress "Life Sciences a Challenge for the Future", Faculty of Veterinary Medicine, Iasi, Romania (2020); Scientific Conference with International Participation "45 years of High Veterinary Medicine Health Education in the Republic of Moldova", Faculty of Veterinary Medicine, Chisinau, Republic of Moldova (2019); Scientific Congress with International Participation "Forward to Global Health", Faculty of Veterinary Medicine, Iasi, Romania (2019); Scientific Congress with International Participation "Forward to Global Health", Faculty of Veterinary Medicine, Iasi, Romania (2018); Scientific Congress with International Participation "Forward to Global Health", Faculty of Veterinary Medicine, Iasi, Romania (2017); International Scientific Symposium "Zootechnical Science - an important factor for European-type agriculture" (2016).

**Publications on the thesis topic**. Seventeen scientific papers have been published on the subject of the thesis, including sixteen articles, out of which one in SCOPUS-indexed international journals, the "One Health and Risk Management" journal, eight articles in peer-reviewed international journals, one article in journals listed in the National Register of Profile Journals, category B, "Agricultural Sciences", and six articles in the proceedings of national and international scientific conferences and congresses (two single-author articles) and a thesis. Additionally, a scientific-practical recommendation has been published.

**Volume and structure of the thesis.** The thesis consists of an introduction, 5 chapters, general conclusions and recommendations, bibliography with 233 titles, 13 annexes, 118 pages of main text, 36 figures, and 19 tables. The obtained results are published in 17 scientific papers, and scientific-practical recommendations have been developed.

**Keywords:** chicks, laying hens, carcasses, eggs, faecal matter, cadavers, microorganisms, *Salmonella* spp., serotypes, culture media.

## THESIS CONTENTS

In the Introduction, the relevance and motivation for the necessity of studying the epidemiological situation of avian salmonellosis and its impact on poultry flocks are argued, as well as the risks of consuming poultry products (meat and eggs) for public health at both global and national levels. The factors contributing to the spread of avian salmonellosis in poultry flocks are emphasized, along with the approach to disease prevention measures.

### 1.EPIDEMIOLOGICAL SITUATION, DIAGNOSIS, PREVENTIVE AND CONTROL MEASURES IN AVIAN SALMONELLOSIS.

In Chapter 1, data from the synthesis of scientific literature regarding the geographical distribution and socio-economic importance of avian salmonellosis are presented, arguing the importance of the disease for public health. The characteristics and antigenic properties of the serotypes of the genus *Salmonella spp.* are described, specifying the serotypes that primarily affect flocks of chicks and adult birds, as well as the serotypes that are epidemiologically dangerous to humans. The main focus was on methods of comprehensive diagnosis, considering epidemiological data, clinical and pathological findings, and confirmation of the diagnosis through bacteriological, bacterioscopic, and serotyping investigations of *Salmonella spp.* isolates. Concurrently, general and specific sanitary-veterinary measures for the control and eradication of avian salmonellosis are presented, as well as the importance of prophylactic vaccinations with polyvalent vaccines for controlling salmonellosis in laying hens and minimizing the risk of transmitting pathogenic *Salmonella* serotypes to humans through poultry products.

### 2. MATERIALS AND METHODS IN RESEARCH.

Chapter 2 contains a description of the methods used for the research, the number, and types of samples collected for study. The research materials included faecal samples, washes from rooms, equipment, surfaces, transport, and poultry products such as bird carcasses and eggs. To achieve the proposed objectives, both classical and modern research methods were used, including epidemiological, clinical-pathological methods, and laboratory investigations: microbiological, microscopic, serological, biochemical, and statistical methods. Statistical data processing was performed by calculating the arithmetic mean, standard deviation, and confidence interval for a mean using MO Excel.

The research was conducted at the Department of Food Safety and Public Health of the Faculty of Veterinary Medicine at the Technical University of Moldova, at the Republican Centre for Veterinary Diagnosis (the food product testing laboratory and the Animal Health Diagnosis laboratory), as well as in the microbiology laboratory of the Scientific-Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine. Some research was also conducted within the State Project No. 20.8009.5107.12 (2020-2023) 'Strengthening the “food – animal – production” chain by using new feed resources, innovative sanitation methods, and schemes,' carried out by the Scientific-Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine.

*As research material served:*

-samples (swabs) from the equipment of poultry units for egg production and for raising broiler chicks, faecal matter and swabs from equipment at broiler chick rearing enterprises, faecal matter from poultry units, swabs and faecal matter from units transporting birds to poultry markets (Poultry Market in the city of Chisinau), swabs from bird transport crates, current

consumption eggs from poultry enterprises in the republic, samples from frozen and refrigerated bird carcasses raised within the republic, marketed in the network within the Central Agricultural Market in Chisinau where poultry products from all poultry producers in the republic are delivered for sale, swabs from supports in poultry product marketing halls, eggshells after chick hatching, and swabs from some incubators in the republic.

For the proposed research, during the period of 2017-2021, a total of 853 samples were collected, including:

216 - from laying hens (faecal matter, dust, swabs);

62 - from deceased laying hens;

254 - from broiler chicks (faecal matter, bedding, swabs);

46 - from deceased broiler chicks;

135 - poultry carcass samples;

140 - egg samples.

Out of the serotypes isolated from the samples investigated, 14 *Salmonella* spp. isolates were tested for their susceptibility to commonly used antibiotics in poultry farming.

## 3. EPIDEMIOLOGICAL SITUATION AND THE SANITARY-EPIDEMIOLOGICAL AND SOCIO-ECONOMIC IMPORTANCE OF AVIAN SALMONELLOSIS INCIDENCE IN THE REPUBLIC OF MOLDOVA.

Chapter 3 describes the epidemiological situation and the incidence of salmonellosis in poultry populations in the country. The presented results reflect the prevalence of bacteria from the genus *Salmonella* spp. against the background of bacterial types that prevail in laying hen flocks and broiler chick rearing units. The epidemiological situation regarding foodborne infections in humans caused by pathogenic serotypes of *Salmonella* spp. is also described.

### 3.1. Incidence of avian salmonellosis in poultry populations in the country.

In the process of analysing the epidemiological situation, the study was conducted at poultry units over a period of 5 years (2017-2021). Some data regarding the incidence of salmonellosis cases against other microbial pathogens at poultry units are presented in Figure 3.1.

This study was conducted by sampling (faecal matter) from poultry units using a random sampling method. Analysing the data presented in Figure 3.1, it can be noted that bacteriological research on the parameters of conditionally pathogenic and pathogenic bacterial flora composition in broiler chick units was represented by a complexity of different bacterial genera, with a percentage ratio as follows: bacteria from the genus *Salmonella* spp. - 6%, *E. coli* (coliform bacteria) - 37%, *Mycoplasma* - 11%, *Streptococci* - 22%, *Staphylococci* - 15%, *Proteus* - 6%, Fungal - 3%. Colonies of bacteria from the genus *Salmonella spp.* were identified and serotyped.

According to the serotyping results, out of 254 samples tested, 22 samples were confirmed with the *Salmonella pullorum gallinarum* serotype, which respectively constituted 8.6% of the total number of samples examined.

The obtained results indicate the circulation of pathogenic serotypes of *Salmonella spp.* in broiler chick rearing units, posing a potential risk of contamination to the chicks and subsequent spread to poultry products, thereby prompting analysis of intrinsic and extrinsic factors in the spread of avian salmonellosis.

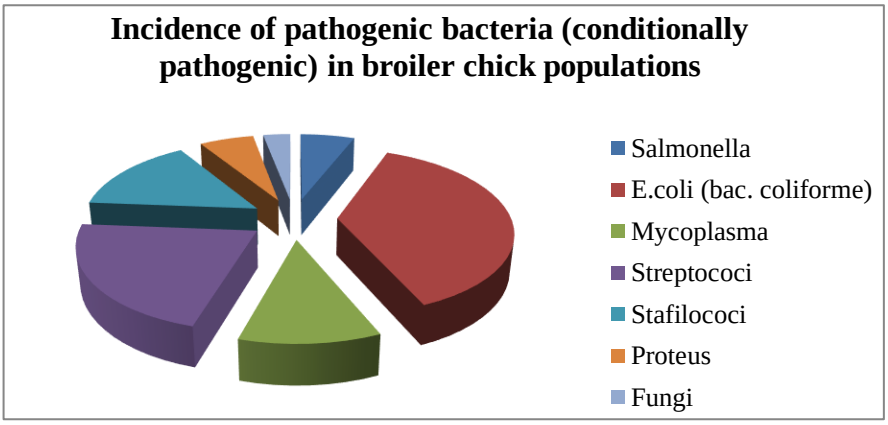


**Fig. 3.1. Composition of bacterial flora in broiler chick populations.**

The results of monitoring the epidemiological situation in poultry units for current consumption egg production are presented in Figure 3.2.

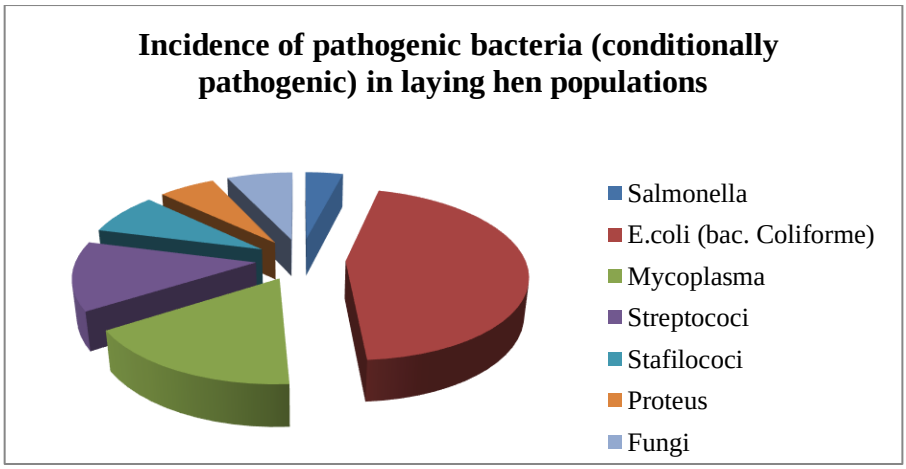


**Fig. 3.2. Composition of bacterial flora in laying hen populations.**

Bacteriological research was conducted on faecal samples randomly collected from the premises housing the laying hens. The results of this study are presented in Figure 3.2. The data presented indicate that the parameters of conditionally pathogenic and pathogenic microflora composition in laying hen units were represented by bacteria from the following genera: *Salmonella* spp. - 4%, *E. coli* (coliform bacteria) - 45%, *Mycoplasma* - 17%, *Streptococci* - 13%, *Staphylococci* - 8%, *Proteus* - 66%, Fungal - 7%. A total of 216 faecal samples were investigated. Colonies of bacteria from the genus *Salmonella* spp. were subjected to serotyping. As a result of serotyping the bacteria from the genus *Salmonella spp.*, it was established that out of 216 samples examined, 9 samples were confirmed to have the presence of the *Salmonella pullorum gallinarum* and *Salmonella typhimurium* serotypes, which constituted 4,2% of the total number of samples examined.

This fact indicates the presence of the risk of transmission of bacteria from the *Salmonella* spp. genus through poultry inventory, as well as through eggs to consumers.

### 3.2.The sanitary-epidemiological situation regarding *Salmonella* spp. foodborne infections in humans.

The epidemiological analysis of the situation regarding salmonellosis in Chisinau and the comparison of the obtained results were conducted based on official data from Statistical Reports F-2, regarding infectious and parasitic diseases, Registers of infectious diseases F.060, sheets of epidemiological evaluation F.362/e, the sheet of epidemiological investigation of the infectious disease outbreak, and the results of laboratory investigations. Over several years, the morbidity of salmonellosis in the republic and especially in Chisinau shows a lingering trend with annual fluctuations in incidence ranging between 23,85 and 49,23 cases per 100 000 populations (National Public Health Agency annual report, 2020). The morbidity of salmonellosis is more pronounced for *Salmonella enteritidis*. The etiological analysis of salmonellosis outbreaks indicates that *S. enteritidis* - 23% is followed by *S. typhimurium* - 31%, then by other *Salmonella* - 8%.

## 4. MONITORING OF EPIDEMIOLOGICAL SITUATION REGARDING *SALMONELLA SPP.* WITHIN POULTRY ENTERPRISES IN THE REPUBLIC OF MOLDOVA

Chapter 4 describes the results of microbiological research at some broiler chicken farms and poultry units for the production of consumer eggs in the republic, reflecting the incidence of bacteria from the *Salmonella* spp. genus against the background of bacterial types predominant in poultry units, as well as the selective serotyping of *Salmonella spp.* serotypes of epidemiological importance for bird stocks and with negative impact on public health.

### 4.1. Microbiological investigations at broiler chicken farms.

Research regarding the monitoring of the circulation of *Salmonella* spp. serotypes was conducted at 8 poultry farms for broiler chicken breeding located in different regions of the republic (Centre, North, South), from which samples were periodically and randomly collected for research. In total, 254 material samples were collected for research purposes over the years 2017-2021. Additionally, a total of 46 samples were randomly collected from carcasses during this period.

**Table 4.1. Microbiological Composition of Faecal Matters Collected from Broiler Chickens (n=5), log CFU/g**

| Type of bacteria | Sampling period for examination | | |
|---|---|---|---|
| | At the age of hall population (1-2 days) | At the age of 20 days | At the age of 40 days |
| ***Salmonella* spp.** | 2,09±0,18 | 2,49±0,19 | 3,52±0,20** |
| ***E.coli* (coliform bacteria)** | 6,65±0,35 | 8,33±0,21* | 8,74±0,04** |
| ***Mycoplasma*** | 2,01±0,20 | 2,07±0,10 | 3,68±0,34* |
| ***Streptococci*** | 3,63±0,14 | 3,89±0,27 | 4,40±0,14* |
| ***Staphylococci*** | 2,02±0,24 | 2,29±0,05 | 2,47±0,07 |
| ***Proteus*** | 1,91±0,25 | 2,11±0,16 | 2,44±0,18 |
| ***Fungal*** | 1,22±0,21 | 1,52±0,10 | 2,24±0,11* |

*** B= 0,95 (p>0,05); ** B=0,99 (p>0,01)**

The composition of the bacterial flora, the prevalence of various bacterial genera, as well as the prevalence of *Salmonella spp.* bacterial forms, were studied.

The results of bacteriological research on the composition and prevalence of bacterial forms in faecal matter, collected from broiler chicken stocks, are presented in Table 4.1.

Analysing the data from Table 4.1, we observe that the index of *Salmonella spp.* bacteria at the age of 1-2 days was 2,09±0,18, increasing subsequently at the age of 20 days to 2,49±0,19, and reaching 3,52±0,20** at the age of 40 days. The highest index of faecal matter microflora with 6,65±0,35 log CFU/g is attributed to coliform bacteria, which at 20 days of age amounted to 8,33±0,21 log CFU/g (p>0,05), and at 40 days of age, this index increased to 8,74±0,04 log CFU/g (p>0,01). Relatively high indices of microorganisms were also found in *Streptococcus* bacteria with values of 3,63±0,14 log CFU/g at 1-2 days of age, 3,89±0,27 log CFU/g at 20 days of age, and 4,40±0,14 log CFU/g (p>0.05) at 40 days of age. The lowest index, with values of 1,22±0,21 log CFU/g (at 2 days of age), was observed in fungal, with an increase in the number of microorganisms up to 2,24±0,11 log CFU/g (p>0.05) at 40 days of age. Figures 4.1 and 4.2 present images of the specific pathomorphological picture for salmonellosis (pullorum disease in chicks), the liver showing infarcts (necrosis) of various sizes, as well as massive accumulations of urates in the ureters, prominently highlighted in a sandy colour (Fig. 4.2).

**Fig. 4.1.** Salmonellosis – Pullorum disease in broiler chickens (miliary hepatic infarcts)

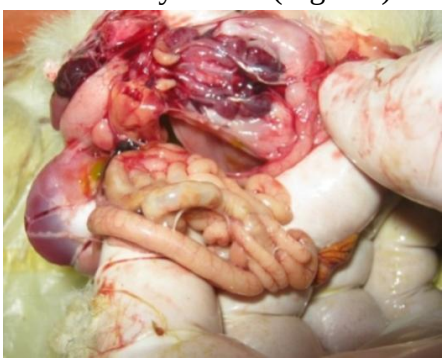

**Fig. 4.2.** Salmonellosis – Pullorum disease in broiler chickens (massive accumulations of urates in ureters)

The results of bacteriological research from the deceased chicks are in Table 4.2.

**Table 4.2. Microbiological composition of the research material sampled from the deceased broiler chicken, (n=5), log CFU/g.**

| **Type of bacteria** | **Sampling period for examination** | | |
|---|---|---|---|
| | **At the age of hall population (1-2 days)** | **At the age of 20 days** | **At the age of 40 days** |
| ***Salmonella* spp.** | 2,67±0,11 | 3,63±0,32* | 4,28±0,15*** |
| ***E.coli* (coliform bacteria)** | 8,12±0,38 | 9,27±0,43 | 9,04±0,24 |
| ***Mycoplasma*** | 1,47±0,04 | 3,11±0,15*** | 4,14±0,35** |
| ***Streptococci*** | 4,23±0,21 | 3,95±0,37 | 4,96±0,75 |
| ***Staphylococci*** | 3,00±0,27 | 3,08±0,18 | 3,31±0,06 |
| ***Proteus*** | 3,01±0,42 | 3,06±0,11 | 3,75±0,08 |
| ***Fungal*** | 2,43±0,38 | 2,42±0,28 | 3,20±0,13 |

*** B= 0,95 (p>0,05); ** B=0,99 (p>0,01); *** B=0,990 (p>0,001);**

Bacteriological research reported that the highest index of bacterial flora in the deceased chicks was constituted by coliform bacteria with indices of 8,12±0,38 log CFU/g at the age of 1-2 days, increasing to 9,27±0,43 log CFU/g at 20 days of age, and respectively to 9,04±0,24 log CFU/g at 40 days of age.

Regarding the number of *Salmonella* spp. bacteria at the age of 1-2 days, it was 2,67±0,11 log CFU/g, with variations of 3,63±0,32 log CFU/g (p>0,05) at 20 days of age, and with an increase to 4,28±0,15 log CFU/g (p>0,001) at 40 days of age.

Relatively increased indices were found in *Streptococcus* bacteria, which at the age of 1-2 days varied from 4,23±0,21 log CFU/g, with a slight decrease to 3,95±0,37 log CFU/g at 20 days of age, and an increase to 4,96±0,75 log CFU/g at 40 days of age. Other bacterial forms such as *Staphylococci* at the age of 1-2 days had values of 3,00±0,27 log CFU/g, which at 20 days of age amounted to 3,08±0,18 log CFU/g, and at 40 days of age, this index increased to 3,31±0,06 log CFU/g.

*Proteus* bacteria at the age of 1-2 days had values of 1,22±0,21 log CFU/g, with an increase to 2,24±0,11 log CFU/g (p>0.05) at 40 days of age.

The presence of fungal flora was relatively high, constituting 2,43±,38 log CFU/g at the age of 1-2 days, with an increase of indices up to 2,42±0,28 log CFU/g at 20 days of age, and an index of 3,20±0,13 log CFU/g at 40 days of age.

The index of *Proteus* bacteria presence varied from 3,01±0,42 log CFU/g at the age of 1-2 days, with a dynamic increase to 3,06±0,11 log CFU/g at 20 days of age, and an index of 3,75±0,08 log CFU/g at 40 days of age.

The results presented indicate that the incidence of *Salmonella spp.* bacteria in deceased broiler chicks is over 2 log units higher compared to the index in live chicks.

### 4.2. Microbiological investigations at some poultry farms for the production of consumer eggs.

For the respective study during the years 2017-2021, periodically, samples of faecal matter were collected from bird stocks according to the applicable standards. In total, 216 samples were collected. Concurrently, from the carcasses of laying hens, faecal matter was collected from ceca and samples from the oviducts. In total, 62 samples were collected from carcasses.

**Table 4.3. Microbiological composition of faecal matter collected from laying hens (n=5), log CFU/g**

| Type of bacteria | Sampling terms during the egg-laying period (days) | | |
|---|---|---|---|
| | 145-165 (beginning of laying) | 290-310 (middle of the laying period) | 450-470 (end of the laying period) |
| ***Salmonella* spp.** | 0,65±0,16 | 0,71±0,08 | 1,66±0,24* |
| ***E.coli* (coliform bacteria)** | 8,85±0,29 | 8,37±0,74 | 8,79±0,61 |
| ***Mycoplasma*** | 0,68±0,21 | 0,68±0,13 | 1,45±0,18* |
| ***Streptococci*** | 2,52±0,09 | 2,16±0,22 | 3,14±0,20* |
| ***Staphylococci*** | 1,15±0,37 | 1,22±0,25 | 2,57±0,24* |
| ***Proteus*** | 0,70±0,09 | 0,57±0,11 | 3,06±0,20*** |
| ***Fungal*** | 0,69±0,25 | 0,37±0,11 | 3,18±0,22** |

*** B= 0,95 (p>0,05); ** B=0,99 (p>0,01); *** B=0,990 (p>0,001);**

At laying hens, the index of coliform bacteria during the beginning of laying (145-165 days) was 8,85±0,29 log CFU/g, with virtually constant variations of 8,37±0,74 log CFU/g in the middle of the laying period (290-310 days) and 8,79±0,61 log CFU/g at the end of the laying period (290-310 days).

It is worth noting that the index of *Salmonella spp.* bacteria showed relatively minor variations, such as (0,65±0,16 log CFU/g at 145-165 days of age), but with a twofold increase to 1,66±0,24 log CFU/g (p>0,05) at the end of the laying period (450-470 days). A heightened index of bacterial forms was constituted by *Streptococci*, which had values of 2,52±0,09 log CFU/g at 145-165 days of age, with an increase to 3,14±0,20 log CFU/g (p>0,05) at the end of the laying period (450-470 days). The index of *Staphylococci* varied from 1,15±0,37 log CFU/g at the beginning of the laying period to an increase of up to 2,57±0,24 log CFU/g (p>0,05) at the end of the laying period (450-470 days). *Proteus* bacteria at the beginning of the laying period had variations of 0,70±0,09 log CFU/g with a significant increase at the end of the laying period to up to three times, with values of up to 3,06±0,20 log CFU/g (p>0,001) at the end of the laying period (450-470 days). The number of fungi also increased considerably, ranging from 0,69±0,25 log CFU/g at the beginning of the laying period to 3,18±0,22 log CFU/g (p>0,01) at the end of the laying period. Regarding *Mycoplasma* bacteria, it is mentioned that at the beginning of the laying period, it had values of 0,68±0,21 log CFU/g, while at the end of the laying period, this index doubled, reaching 1,45±0,18 log CFU/g (p>0.05).

The obtained data indicate that throughout the laying period, all bacteriological indices double or triple, indicating increased risks of avian salmonellosis occurrence.

Figures 4.5 and 4.6 present images depicting dead laying hens with typical signs of vent pecking. Figure 4.3 depicts coagulated masses of broken eggs in the abdominal cavity, as well as numerous affected eggs, dark brown in colour, while Figure 4.4 shows exudate containing broken eggs, atrophied and mummified eggs of small dimensions in the lumbar regions.

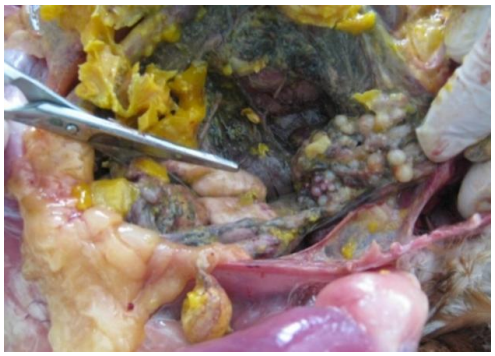

**Fig. 4.3.** Egg peritonitis, with ruptured, deformed eggs.

**Fig. 4.4.** Egg peritonitis with atrophied, mummified eggs.

Sixty-two samples were collected, comprising intestinal contents, peritoneal contents, and oviduct contents. The results of this study are presented in Table 4.4. In laying hen cadavers, indicators of intestinal and oviduct microflora showed significantly elevated levels of bacteria across all genera.

During the specified periods, the coliform bacteria index in hen cadavers ranged from 10,43±0,12 log CFU/g at the onset of the laying period (145-165 days), fluctuating with variations of 10,27±0,21 log CFU/g in hen cadavers aged 450-470 days (end of the laying

period). Meanwhile, the index of *Salmonella* genus bacteria increased from 2,61±0,15 log CFU/g in laying hen cadavers (beginning of laying 145-165 days) to 3,38±0,26 log CFU/g in hen cadavers aged 450-470 days.

**Table 4.4. Microbiological composition of the material for research collected from laying hen cadavers (n=5), log CFU/g.**

| Type of bacteria | Sampling terms during the egg-laying period (days) | | |
|---|---|---|---|
| | 145-165 (beginning of laying) | 290-310 (middle of the laying period) | 450-470 (end of the laying period) |
| ***Salmonella* spp.** | 2,61±0,15 | 2,77±0,08 | 3,38±0,26 |
| ***E.coli* (coliform bacteria)** | 10,43±0,12 | 9,43±0,68 | 10,27±0,21 |
| ***Mycoplasma*** | 2,04±0,20 | 2,65±0,11 | 2,77±0,24 |
| ***Streptococci*** | 3,33±0,25 | 3,14±0,33 | 3,84±0,19 |
| ***Staphylococci*** | 2,30±0,30 | 2,05±0,42 | 3,84±0,12** |
| ***Proteus*** | 2,08±0,28 | 2,25±0,11 | 3,93±0,41* |
| ***Fungal*** | 2,02±0,25 | 1,73±0,20 | 3,88±0,17** |

*** B= 0,95 (p>0,05); ** B=0,99 (p>0,01);**

The *Streptococcus* bacteria index showed variations from 3,33±0,25 log CFU/g in hen cadavers aged 145-165 days, reaching values of up to 3,84±0,19 log CFU/g in hen cadavers towards the end of the laying period (450-470 days).

The numerical indices of *Staphylococcus* bacteria varied from 2,30±0,30 log CFU/g at the beginning of the laying period, reaching values of 3,84±0,12 log CFU/g (p>0,01) in laying hen cadavers towards the end of the laying period. The numerical indices of fungi were also relatively high, ranging from 2,02±0,25 log CFU/g at the beginning of the laying period with a significant increase to 3,88±0,17 log CFU/g (p>0,01) in hen cadavers at the end of the laying period. Towards the end of the laying period, high variations were also observed in the indices of bacteria such as *Proteus* and *Mycoplasma*, with values up to 3,93±0,41 log CFU/g (p>0,05) and 2,77±0,24 log CFU/g, respectively.

The results obtained demonstrate that towards the end of the laying period, the number of conditionally pathogenic bacteria increases, and at the same time, the number of *Salmonella spp.* bacteria significantly increases, which could lead to the onset of salmonellosis.

### 4.3. Microbiological research on some incubators.

In order to establish the incidence of *Salmonella spp.* bacteria in some incubators in the republic, 45 samples were randomly collected from 5 incubators that provide day-old chicks to owners in rural areas of the republic as well as some poultry entrepreneurs.

The research material consisted of washes taken from inside the incubators and from room surfaces, equipment, and at the end of the incubation period, eggshell samples from hatched chicks were used as research material.

The results of this study are presented in Table 4.5. Analysing the data presented in Table 4.5, we observe that the highest indices were observed in the genus of bacteria represented by *Streptococci*, with values of 1,86±0,21 log CFU/g up to the incubation process, nearly doubling after the incubation period, constituting 2,78±0,09 log CFU/g (p>0,01).

**Table 4.5. Microbiological composition of the research material collected from some incubators. (n=5), LOG/g.**

| Type of bacteria | The sampling period for research | |
|---|---|---|
| | At the beginning of the incubation period | After the incubation period |
| ***Salmonella* spp.** | 0,22±0,04 | 1,01±0,17** |
| ***E.coli* (coliform bacteria)** | 1,30±0,11 | 3,69±0,13*** |
| ***Mycoplasma*** | 0,26±0,05 | 1,05±0,17** |
| ***Streptococci*** | 1,86±0,21 | 2,78±0,09** |
| ***Staphylococci*** | 0,84±0,22 | 2,42±0,17*** |
| ***Proteus*** | 0,22±0,06 | 0,31±0,05 |
| ***Fungal*** | 0,29±0,07 | 1,58±0,14*** |

*** B= 0,95 (p>0,05); ** B=0,99 (p>0,01); *** B=0,990 (p>0,001);**

The next index after the microbial load level was represented by coliform bacteria, with an index of 1,30±0,11 log CFU/g until the beginning of the incubation process, reaching variations of up to 3,69±0,13 log CFU/g (p>0,001) after the completion of the incubation process.

The index of *Salmonella spp.* bacteria had the highest growth variations from the beginning of the incubation period – 0,22±,.04 log CFU/g, with variations of 1,01±0,17 log CFU/g (p>0,01) after the incubation period.

Intense growth was also recorded in the case of fungi, which ranged from 0,29±0,07 log CFU/g at the beginning of the incubation period to an increase in fungi practically five times – 1,58±0,14 log CFU/g (p>0,001) at the end of the incubation period. A negligible increase was recorded in the case of *Proteus* bacteria, with values of 0,22±0,06 log CFU/g before incubation and a small increase of 0,31±0,05 log CFU/g at the end of the incubation period.

The indices of *Staphylococcus* bacteria were also negligible, constituting 0,84±0,22 log CFU/g before the incubation period and 2,42±0,17 log CFU/g (p>0,001) after the incubation process. *Mycoplasma* bacteria before incubation constituted 0,26±0,05 log CFU/g, increasing fourfold to 1,05±0,17 log CFU/g (p>0,01) at the end of the incubation period.

This study has demonstrated that the incubation process increases the number of bacteria by 3-5 times, with the highest indices being observed in *Salmonella* spp. and *Streptococcus* bacteria.

### 4.4. Monitoring the presence and diversity of *Salmonella* spp. bacteria in some poultry markets.

The research material consisted of washes collected from within the Agricultural Market of Chisinau, where day-old chicks are provided, as well as adult birds of various species from most regions of the republic.

The results of the bacteriological study are presented in Table 4.6. The data in the table indicate that the largest proportion of bacteria in samples collected from poultry maintenance cells intended for the sale of birds consisted of *E. coli* bacteria (coliform bacteria) with an index of 6,76±0,37 log CFU/g, followed by *Streptococci* with 4,60±0,19 log CFU/g, and *Salmonella spp.* bacteria, with an index of 3,05±0,12 log CFU/g.

Lower intensities were recorded in the case of bacterial forms such as fungi 2,96±0,18 log CFU/g, *Staphylococci* – 2,35±0,08 log CFU/g, and a further reduced intensity was recorded in the case of *Proteus* bacterial forms with 0,80±0,23 log CFU/g.

Variations in the microflora composition on the interior surfaces of the hall were significantly lower compared to those on poultry maintenance cells.

The highest index was represented by coliform bacteria, with variations of 5,22±0,57 log CFU/g (p>0.05), followed by *Streptococcal* flora with variations of 3,40±0,29 log CFU/g (p>0,01). *Salmonella* spp. bacteria constituted 2,18±0,2 log CFU/g (p>0,01).

The smallest variations were recorded in fungal forms, with variations of 1,78±0,13 log CFU/g (p>0,001), followed by *Staphylococcus* bacteria with variations of 1,65±0,18 log CFU/g (p>0,01), and *Proteus* bacterial forms with an index of 0,69±0,16 log CFU/g.

The research conducted has demonstrated that the highest incidence of bacteria was found in those collected from within the vehicles used for poultry transportation.

**Table 4.6. Microbiological research results of samples collected from the poultry market (washes) (n=5), log CFU/g**

| **Type of bacteria** | **Type of samples collected (washes)** | | |
|---|---|---|---|
| | **From the surface of cells, trays** | **From the surfaces of the hall** | **From inside the transport** |
| ***Salmonella* spp.** | 3,05±0,12 | 2,18±0,23** | 3,27±0,22 |
| **E.coli (coliform bacteria)** | 6,76±0,37 | 5,22±0,57* | 6,83±0,40 |
| ***Mycoplasma*** | 2,66±0,14 | 1,75±0,10*** | 3,05±0,17 |
| ***Streptococci*** | 4,60±0,19 | 3,40±0,29** | 4,20±0,23 |
| ***Staphylococci*** | 2,35±0,08 | 1,65±0,18** | 3,10±0,25* |
| ***Proteus*** | 0,80±0,23 | 0,69±0,16 | 1,63±0,04** |
| ***Fungal*** | 2,96±0,18 | 1,78±0,13*** | 3,34±0,16 |

The results of the research on faecal matter from birds for sale at the Poultry Market in the city of Chisinau are presented in Table 4.7.

**Table 4.7. Microbiological research results of samples collected from the poultry market (faecal matter) (n=5), log CFU/g**

| **Type of bacteria** | **The type of samples collected (faecal matter)** | | |
|---|---|---|---|
| | **From chicks aged 0-2 days** | **From chicks aged 50-60 days** | **From adult birds** |
| ***Salmonella* spp.** | **0,58±0,13** | **1,48±0,09*** ** | **3,42±0,19*** ** |
| ***E.coli* (coliform bacteria)** | **3,46±0,05** | **4,36±0,24** ** | **7,21±0,22*** ** |
| ***Mycoplasma*** | **0,82±0,22** | **1,59±0,31** | **2,39±0,10*** ** |
| ***Streptococci*** | **1,38±0,36** | **3,19±0,10** ** | **4,83±0,17*** ** |
| ***Staphylococci*** | **0,51±0,13** | **2,44±0,09*** ** | **3,50±0,19*** ** |
| ***Proteus*** | **0,38±0,08** | **1,24±0,17** ** | **2,04±0,14*** ** |
| ***Fungal*** | **0,44±0,07** | **2,70±0,09*** ** | **3,14±0,12*** ** |

*** B= 0,95 (p>0,05); ** B=0,99 (p>0,01); *** B=0,990 (p>0,001);**

The data from the table indicate that the highest number of bacteria was found in coliform bacteria, with values of 3,46±0,05 log CFU/g in chicks aged 0-2 days, increasing to 4,36±0,24 log CFU/g (p>0,01) in chicks aged 50-60 days. Meanwhile, in adult birds within the poultry market, this index was practically double compared to young chicks, constituting 7,21±0,22 log CFU/g (p>0,001). *Salmonella spp.* bacteria constituted 0,58±0,13 log CFU/g in chicks aged 0-2 days, increasing to 1,48±0,09 log CFU/g (p>0,001) at 50-60 days of age, while in adult birds, this index was 3,42±0,19 log CFU/g (p>0,001), showing a difference five times higher compared to young chicks.

An increased incidence was also recorded in *Mycoplasma* bacteria. In chicks aged 0-2 days, the bacterial count index was 0,82±0,22 log CFU/g, reaching variations of up to 1,59±0,31 log CFU/g in chicks aged 50-60 days, while in adult birds, this index was 2,39±0,10 log CFU/g (p>0,001), showing a difference three times higher compared to young chicks.

Fungal flora had moderate relative variations, with values of 0,44±0,07 log CFU/g in chicks aged 0-2 days, with an increase in the index to 2,70±0,09 log CFU/g (p>0,001) in chicks aged 50-60 days, while in adult birds, there were variations of 3,14±0,12 log CFU/g (p>0,01). *Proteus* bacterial forms had smaller variations compared to other bacterial forms, constituting 0,38±0,08 log CFU/g in young chicks in the first days of life, with the index increasing to 1,24±0,17 log CFU/g (p>0,01), and a much larger difference in adult birds, constituting 2,04±0,14 log CFU/g (p>0,001), practically four times higher compared to young chicks.

The results obtained demonstrate that the bacteriological indices are more than 3-5 times higher in adult birds compared to young chicks, which constitutes a major risk of direct contamination with *Salmonella* spp. bacteria during their temporary presence in the poultry market.

## 5.MONITORING THE PRESENCE AND DIVERSITY OF BACTERIA OF THE SALMONELLA SPP. GENUS IN POULTRY MEAT AND EGGS.

Chapter 5 presents the findings of microbiological research conducted on refrigerated and frozen poultry carcasses, focusing on the incidence of *Salmonella* spp. bacteria. It also includes bacteriological research results on eggs from the retail network, with a particular emphasis on the incidence of *Salmonella* spp. bacteria and the antimicrobial resistance of isolated serotypes. Additionally, the chapter provides data on the effectiveness of using vaccine strains at facilities producing eggs for consumption.

### 5.1. Microbiological study of refrigerated poultry carcasses.

The research concerning the impact of *Salmonella spp.* bacterial contamination on refrigerated poultry carcasses was carried out by sampling carcasses from the poultry product distribution network in Chisinau (Central Agricultural Market).

A total of 87 samples were collected for research purposes. Microbiological research focused on studying the presence and incidence of *Salmonella spp*. bacteria at the time of placement on the market for sale and after 48 hours. The results of this study are presented in Table 5.1.

The data from the table indicate that in samples taken at the time of placement of poultry carcasses for sale, *Salmonella spp.* bacteria had values of 85,0±10,4 colonies, which doubled to 161,4±19,8 colonies after 48 hours (p>0,001). Additionally, the number of *E. coli* bacterial colonies varied from 198,2±11,4 at the time of placement for sale to 210,8±19,0 after 48 hours. A significant increase was observed in the number of fungal colonies, which doubled from

52,8±8,5 at the time of placement of refrigerated carcasses for sale to 105,6±11,1 (p>0,001) after 48 hours. *Streptococcus* bacteria also showed a noticeable increase in colony numbers, from 186,8±21,8 colonies at the beginning of the sales period to 278,6±9,1 (p>0,001) after 48 hours in the distribution network. Similarly, colonies of *Staphylococcus* bacteria doubled in significance, from 116,6±10,7 colonies at the beginning of the sales period to 242,8±9,7 (p>0,001) colonies after a 48-hour interval designated for sale.

The obtained data have demonstrated that even under refrigeration conditions of +4 to +6 degrees Celsius, bacterial microflora develops very intensively. The number of *Salmonella spp.* colonies doubled in just 48 hours.

**Table 5.1. Number of colonies of isolated microorganisms on refrigerated poultry carcasses (n=5), colony count.**

| **Type of bacteria** | **The sampling period for research (number of colonies)** | |
|---|---|---|
| | **At the time of placement for sale** | **After 48 hours** |
| ***Salmonella* spp.** | 85,0±10,4 | 161,4±19,8** |
| **E.coli (coliform bacteria)** | 198,2±11,4 | 210,8±19,0 |
| ***Streptococci*** | 186,8±21,8 | 278,6±9,1** |
| ***Staphylococci*** | 116,6±10,7 | 242,8±9,7*** |
| ***Fungal*** | 52,8±8,5 | 105,6±11,1** |

****B=0,99 (p>0,01) ***B=0,999 (p>0,001)**

**5.2. Microbiological study of frozen poultry carcasses.**

The presence and incidence of bacteria of the *Salmonella spp.* genus, as well as other bacterial forms, were analysed at the time of placement on the market for sale and after 48 hours. A total of 52 samples were collected for research purposes. The results of this study are presented in Table 5.2.

**Table 5.2. Number of colonies of isolated microorganisms on frozen poultry carcasses (n=5), colony count.**

| **Type of bacteria** | **The sampling period for research (number of colonies)** | |
|---|---|---|
| | **At the time of placement for sale** | **After 48 hours** |
| ***Salmonella* spp.** | 19,4±1,6 | 31,6±10,1* |
| ***E.coli* (coliform bacteria)** | 50,0±9,6 | 69,2±5,7 |
| ***Streptococci*** | 62,0±10,7 | 72,2±7,2 |
| ***Staphylococci*** | 41,6±6,2 | 57,4±7,1 |
| ***Fungal*** | 24,4±7,5 | 37,2±8,8 |

*** B= 0,95 (p>0,05)**

In the case of bacteriological research on frozen poultry carcasses, at the time of placement for commercialization, the index of *Salmonella spp.* bacteria had values of 19,4±1,6 colonies, and after 48 hours, this index increased by values of 31,6±10,1 colonies (p>0,05), constituting the highest increase in bacterial index compared to other bacterial forms. *E. coli*

bacteria had values of 50,0±9,6 at the time of placement for commercialization with an increase in the index to 69,2±5,7 colonies within the 48-hour interval. *Streptococcus* bacteria were represented by 62,0±10,7 colonies at the time of placement for commercialization, with a non-significant increase to 72,2±7,2 colonies within the 48-hour interval. A non-significant increase was also recorded in the colonies of *Staphylococcus* bacteria with indices from 41,6±6,2 colonies initially to 57,4±7,1 colonies after 48 hours of the start of commercialization, while the number of fungi varied from 24,4±7,5 colonies initially to a slow increase to 37,2±8,8 colonies over the 48-hour interval.

The study presented demonstrated that despite the fact that frozen carcasses are kept at temperatures below 0°C, microorganisms of the *Salmonella spp.* genus and other bacterial forms are present on poultry carcasses, and moments of even temporary handling in the commercialization process (removal and placement in the freezer) contribute to the slow development of the microflora, which has been shown to multiply with a difference of up to 50% over a 48-hour period.

**5.3. Study of the presence and diversity of bacteria of the *Salmonella* spp. genus at some poultry slaughtering enterprises in the republic.**

The research activity was conducted within a collaborative program between the National Public Health Agency and the National Food Safety Agency, aimed at monitoring the circulation (incidence) of *Salmonella spp.* serotypes at poultry slaughtering units, in abattoirs (2018). For this study, 65 samples were collected and examined from various districts of the republic, including: Anenii Noi district - 15 samples, Orhei district - 5 samples, Floresti district - 5 samples, Calarasi district - 4 samples, Ungheni district - 4 samples, Chisinau - 6 samples, Donduseni district - 4 samples, Falesti district - 4 samples, Briceni district - 3 samples, Criuleni district - 5 samples, Ialoveni district - 5 samples. Additionally, 16 samples were collected from poultry carcasses from import batches from Ukraine, Poland, Brazil, and Romania.

**Table 5.3. The type of samples collected from poultry slaughterhouses for bacteriological research.**

| **Nr.** | **Samples for research** | **Total researched samples** | **Including positives for** | |
|---|---|---|---|---|
| | | | *Salmonella* | |
| **1** | **Chicken carcass** | **17** | **3 (17.6%)** | **-** |
| **2** | **Chicken meat (thighs, drumsticks, quarters, back)** | **12** | **1 (8.3%)** | **-** |
| **3** | **Chicken neck (with skin/without skin)** | **10** | **1 (10%)** | **-** |
| **4** | **Edible chicken organs (heart, gizzard, liver)** | **28** | **-** | **-** |
| **5** | **Chicken cecum with contents** | **4** | **1 (25%)** | **-** |
| **6** | **Turkey meat (thighs, breast)** | **4** | | **-** |
| | **TOTAL** | **75** | **6 (8%)** | **-** |

The results of the respective study (Table 5.3) showed that out of 75 samples subjected to serotyping, 6 samples, or 8%, tested positive for certain serotypes of bacteria from the *Salmonella* spp. genus. The highest number of *Salmonella spp.* serotypes (17,3%) was found in samples collected from whole broiler chicken carcasses.

The results from Table 5.4 indicate that from the refrigerated poultry carcasses, the serovars detected were *S. othmarschen, S. lagos,* and *S. senftenberg,* while from the frozen carcasses, the serovar isolated was *S. isangi.*

**Table 5.4. Data on serotyping of bacteria from the *Salmonella spp.* genus in poultry carcasses.**

| Type of sample examined | Number of positive samples (n) | Isolated serovars |
|---|---|---|
| **Refrigerated chicken carcass** | 1 | **S. othmarschen** (6,7,14:g,m,[t]:-) |
| **Refrigerated chicken carcass** | 1 | **S. lagos** (1,4,[5],12: i:1,5) |
| **Frozen chicken carcass** | 1 | **S. isangi** (6,7,14:d:1,5) |
| **Refrigerated chicken thighs** | 1 | **S. senftenberg** (1,3,19:g,[s],t:-) |
| **Refrigerated chicken neck with skin** | 1 | **S. senftenberg** (1,3,19:g,[s],t:-) |
| **Chicken cecum with contents** | 1 | **S. pasing** (4,12:z35:1,5) |
| **TOTAL** | **6** | |

**5.4. Microbiological investigation of eggs for current consumption from food trade networks.**

Egg samples were collected from units selling eggs for current consumption from poultry farms in various regions of the country, sold at the Agricultural Market in Chisinau, and from egg sorting stations of poultry farms. The number of egg samples taken for bacteriological investigations amounted to 140. The results of this study are presented in Table 5.5.

**Table 5.5. Microbiological research results of egg samples collected (n=5), colony count.**

| Type of bacteria | The sampling period for research (number of colonies) | |
|---|---|---|
| | From the surface of the shell (1cm$^2$) | From the egg packaging |
| ***Salmonella spp.*** | 47,6±7,0 | 111,2±6,9*** |
| ***E.coli* (coliform bacteria)** | 124,8±19,7 | 213,0±16,0** |
| ***Streptococci*** | 77,0±12,4 | 183,6±6,1*** |
| ***Staphylococci*** | 54,2±8,3 | 95,2±7,0** |
| ***Fungal*** | 19,8±4,1 | 116,6±9,4*** |

*** B=0,95 (p>0,05)**B=0,99 (p>0,01)***B=0,999 (p>0,001)**

The results obtained indicate that eggs obtained from laying hens contain colonies of *Salmonella spp.* with indices of 47,6±7,0 colonies, while the data from the egg packaging showed indices of 111,2±6,9 colonies (p>0,001), thus exceeding the number of colonies on the eggshell by more than 2 times. Coliform bacteria presented indices of 124,8±19,7 colonies on the eggshell and 213,0±16,0 colonies (p>0,01) on the egg packaging. The number of *Streptococcus* colonies on the eggshell was 77,0±12,4 colonies, while on the egg packaging it was 183,6±6,1

colonies (p>0,001). *Staphylococcus* colonies on the eggshell had indices of 54,2±8,3 colonies, while on the packaging they were 95,2±7,0 (p>0,01).

The largest difference in colony numbers between the eggshell and packaging was found in fungal colonies. On the eggshell, the number of colonies was 19,8±4,1, whereas on the egg packaging, it was 116,6±9,4 colonies (p>0,001). Thus, the number of fungal colonies on the packaging was 5,9 times higher compared to their presence on the eggshell.

From the colonies of different bacterial forms isolated on various culture media, smears were prepared and stained using the Gram staining method. This study aimed to investigate the bacteriological properties of various bacterial forms, as well as their belonging to their respective bacterial genera (as studied in bacteriological research).

For the isolation and identification of *Salmonella spp.* colonies, special media for the *Salmonella spp.* genus were used, such as Bismuth Sulphite Agar, *Salmonella Shigella* Agar, and Brilliance *Salmonella* Agar. Figures 5.5-5.7 represent the shape, colour, and arrangement of *Salmonella spp*. colonies on the Petri dish. On each culture medium, *Salmonella spp.* colony forms are represented differently. In Figure 5.5 (SS Agar medium, swabs from room surfaces), *Salmonella spp.* colonies are represented with oval or spherical shapes, dark brown in colour, arranged in chains, evenly distributed over the entire surface of the plate. In Figure 5.6 (swabs and samples from the surface and depth of carcasses), *Salmonella* spp. colonies are brown in colour, with oval shapes, clustered, with a higher intensity in samples taken from the surface of the carcasses. In Figure 5.7 (samples taken from poultry cells in the poultry market), *Salmonella spp.* colonies are isolated on Brilliance *Salmonella* Agar medium, they are violet in colour, spherical in shape, with a massive intensity over the entire surface of the Petri dish.

**Results of bacteriological and microscopic examinations. Types of colonies of microorganisms isolated from poultry breeding units and poultry products.**

**Fig. 5.5.** Colonies of *Salmonella spp.* on SSA medium.

**Fig. 5.6.** Colonies of *Salmonella spp.* on Bismuth SA medium.

**Fig. 5.7.** Colonies of *Salmonella spp*. on Briliance SA medium.

In the results of bacteriological investigations conducted in poultry units and poultry products, in association with bacteria of the genus *Salmonella* spp., it was found that in the majority of the samples examined, a microflora was isolated, associated with the predominance of colonies of E. *coli, Streptococci, Staphylococci* and microscopic fungi. Some of the bacterial forms isolated from the examined samples are presented in Figures 5.8-5.10. Figure 5.8 shows the forms of *Salmonella* coliform bacteria with rod-shaped gram-negative forms, placed with

uniform intensity throughout the microscopic field. Figure 5.9 represents an associated flora of microscopic fungi and *E. coli*, while Figure 5.10 depicts a flora of *Staphylococcus spp.* bacteria, which have a round or oval shape, blue in colour, placed separately or in clusters, spread throughout the entire microscopic field, predominantly in the central area.

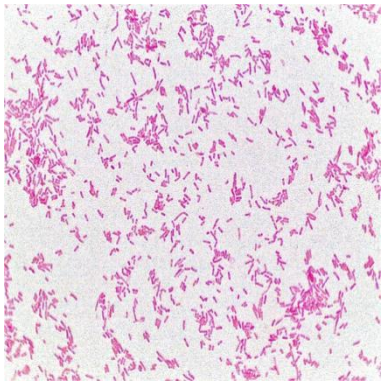

**Fig. 5.8.** *Salmonella spp.* bacteria (Gram-negative rods).

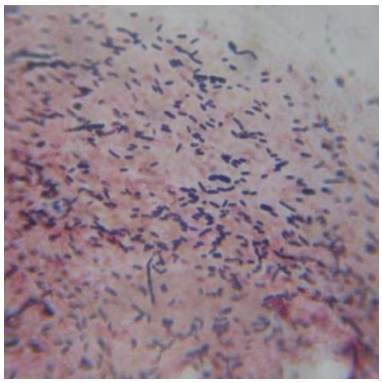

**Fig. 5.9.** Associated flora with *E. coli* and fungi.

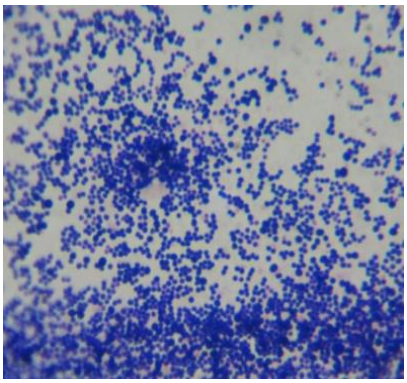

**Fig. 5.10.** Bacteria of the genus *Staphylococcus spp.*

**Data referring to the serotyping of bacteria of the genus *Salmonella* spp. isolated from birds and poultry products**.

The procedure for serotyping bacteria of the genus *Salmonella spp.* was carried out in the bacteriology department of the Republican Centre for Veterinary Diagnosis. From the research samples where typical colonies of *Salmonella spp.* developed, repeated passages (seedings) were subsequently performed, selecting the most evident colonies for serotyping.

From the number of egg samples examined, pathogenic serotypes of *Salmonella spp.* such as *Salmonella gallinarum*, *Salmonella dublin*, and *Salmonella typhimurium* were identified in 6%, while bacteriological research conducted on poultry carcasses showed that out of the total number of samples examined, 4% of samples tested positive for *Salmonella spp.*, with a predominance of serotypes *Salmonella gallinarum*, *Salmonella enteritidis*, and *Salmonella infantins.*

Monitoring the incidence of serotypes of *Salmonella spp.* is also carried out according to the strategic plan of anti-epidemic measures, which is coordinated at the National Agency for Food Safety level. Some of the serotypes of epidemiological importance for the health of bird populations and public health are presented in Table 5.6. According to official control data conducted throughout the 2018-2022 period by the Republican Centre for Veterinary Diagnosis, a total of 75 positive samples were confirmed with the *Salmonella enteritidis* serotype, with the most positive serotypes being identified in bird faeces (40 samples), followed by poultry meat (18 samples), and 7 positive samples each isolated from droppings taken from poultry premises and 3 samples from dust and manure samples examined. The *Salmonella typhimurium* serotype was confirmed in 17 samples, of which 8 positive samples were identified in faeces, 4 in poultry meat samples, and 2 in meat products.

The epidemiological importance was also represented by the *Salmonella Infantis* serotype. Throughout this period, 93 positive samples with this serotype were identified, of which 74 were found in samples taken from poultry carcasses, and 17 in poultry meat products.

**Table 5.6. Information regarding the detection and serotyping of bacteria from the *Salmonella* spp. genus during the years 2018-2022 (data from the National Agency for Food Safety).**

| No | Researched material | *S. enteritidis* | *S.typhimurium* | *S.kentucky* | *S. infantis* | *S. farsta* | *S.winneba* | *S.newport* |
|---|---|---|---|---|---|---|---|---|
| 1 | **Droppings** | 7 | 3 | 4 | 2 | - | - | - |
| 2 | **Faecal matter** | 40 | 8 | 1 | - | - | - | - |
| 3 | **Meconium** | 1 | - | - | - | - | - | - |
| 4 | **Dust samples** | 2 | - | - | - | - | - | - |
| 5 | **Poultry meat** | 18 | 4 | 9 | 74 | 2 | 1 | 5 |
| 6 | **Meat products** | 7 | 2 | - | 17 | - | - | - |
| TOTAL | | 75 | 17 | 14 | 93 | 3 | 1 | 5 |

Concurrently, other serotypes of *Salmonella* spp. were isolated, such as: *S. Kentucky* - 14 positive samples, *S. Farsta* - 3 positive samples, *S. Winneba* - 1 positive sample, and *S. Newport* - 5 positive samples. However, these serotypes do not pose a significant risk to poultry flocks or public health.

**5.5. Investigations on the antimicrobial resistance of *Salmonella spp.* serotypes isolated from poultry meat and eggs.**

Research on the susceptibility of serotypes isolated from samples collected from poultry units, poultry carcasses, and eggs was conducted to test sensitivity to antibiotics commonly used in poultry farming to combat contamination or for the prophylaxis of bacterial diseases.

The Kirby Bauer disk diffusion method was used for antimicrobial sensitivity testing, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) from HI Media, India. In total, 14 isolates of *Salmonella spp.* were tested.

The criteria used to assess the antibiogram results were: R-resistant, I-intermediate, and S-sensitive. The results of this study are presented in Table 5.7.

In the study, inhibition disks with antimicrobial solutions of 9 commercial preparations, available in veterinary pharmacies with antimicrobial action, were used, including: Colistin 5mg, Florfenicol 30mg, Tetracycline 30mg, Gentamycin 10mg, Enrofloxacin 10mg, Doxycycline 30mg, Ciprofloxacin 5mg, Ofloxacin 5mg, and Amoxicillin 30mg.

Analysing the obtained data, it can be stated that the highest resistance of the isolated bacterial flora was found for Amoxicillin at 72,8%, followed by Colistin at 66%, and Doxycycline at 64%, which also showed the lowest sensitivity at 12%, 18%, and 15%, respectively.

At the same time, the highest sensitivity was recorded for active substances such as Ofloxacin at 82%, Genta Plus at 73%, and Enrofloxacin at 56%. An average sensitivity with variations from 31 to 49% was established for active substances such as Tetracycline and Florfenicol.

**Table 5.7. Antibiogram of the sensitivity of the microflora isolated from poultry carcasses and table eggs (n=14).**

| Or. nr. | Name of the antimicrobial agent | | | Type of strains as a result of the antibiogram | | |
|---|---|---|---|---|---|---|
| | **Name of the antibiotic** | **Symbol** | **Concentration in micrograms of the antibiotic in the disc** | **R (%)** | **I (%)** | **S (%)** |
| 1 | Colistin | ES | 5mg | **66** | 16 | 18 |
| 2 | Florfenicol | F | 30mg | 32,4 | 18,6 | 49 |
| 3 | Tetracycline | TE | 30mg | 47,2 | 21,8 | 31 |
| 4 | Genta Plus | GEP | 10mg | 21 | 6 | **73** |
| 5 | Enrofloxacin | EF | 10mg | 34,5 | 9,5 | **56** |
| 6 | Doxycycline | DO | 30mg | **64** | 21 | 15 |
| 7 | Ciprofloxacin | CF | 5mg | 37 | 17 | 46 |
| 8 | Ofloxacin | OF | 5mg | 12 | 6 | **82** |
| 9 | Amoxicillin | AMX | 30mg | **72, 8** | 15,2 | 12 |

A comparative study to establish the correlation of sensitivity of *Salmonella* spp. strains in humans to some antibacterial agents used in the treatment of infectious bacterial diseases in humans (The National Public Health Agency Report, 2022). The study was conducted within The National Public Health Agency and included: Public Health Centre Chisinau, Public Health Centre Ungheni, Public Health Centre Edinet, Public Health Centre Cahul, Public Health Centre Comrat, Public Health Centre Hancesti, Public Health Centre Soroca.

The results of this study confirm that out of the 187 serotypes of *Salmonella* spp., 31 active substances were tested. The antibiogram results showed diverse sensitivity to these substances. A practical resistance of almost 100% was recorded for Cefuroxime and Tigecycline preparations, while a 100% sensitivity was established for preparations such as Ampicillin-sulbactam, Imipenem, and Co-trimoxazole.

The results obtained confirm that out of the total number of *Salmonella* spp. serotypes under investigation, there was an average resistance of 7,76% and an average sensitivity of 92,24%.

### 5.6. Data regarding the use of vaccine strains in the immunoprophylaxis of avian salmonellosis

In the Republic of Moldova, avian salmonellosis is monitored through the prism of the national strategic program, which involves sampling from poultry flocks represented by faeces or dust from poultry maintenance facilities, and for food safety, samples are taken from carcasses or table eggs.

Despite the security measures taken at poultry facilities, as well as monitoring measures, the risk of contamination of poultry flocks persists. Evidence of this is the sporadic cases of salmonellosis incidence in some poultry units.

The primary measure that would contribute to the control and prophylaxis of contamination of poultry flocks with pathogenic serotypes, as well as those of public health importance (*Salmonella enteritidis* and *Salmonella typhimurium*), is immunoprophylaxis. In this

context, poultry breeders can use vaccines approved in the Republic of Moldova with notification to the representatives of the Territorial Directorates for Food Safety, as well as making available to the reference laboratory (Republican Centre for Veterinary Diagnostics) the methods for detecting post-vaccination specific antibodies.

The results of this study are presented in Tables 5.8 and 5.9.

**Table 5.8. Data regarding the unvaccinated laying hen flocks against salmonellosis during the year 2020**

| Or. nr. | Name of the poultry enterprise | Locality | Number of birds | Total number of dead birds | Number of cadavers with egg peritonitis | Percentage of lethality with egg peritonitis | Percentage of non-standard eggs, % |
|---|---|---|---|---|---|---|---|
| 1 | LLC ,, Pasarea Argintie,, | Chisinau | 71300 | 758 | 61 | 8,1 | 2,6 |
| 2 | LLC ” Artex Group” | Ciorescu commune | 42000 | 447 | 41 | 9,1 | 2,8 |
| 3. | PLC ,,Avicola-Nord,, | Dubasari town | 54500 | 689 | 52 | 7,5 | 1,8 |
| 4 | LLC ”Aviton Agro” | Falesti town | 62400 | 505 | 39 | 7,7 | 3,1 |
| 5 | LLC „Speranta” | Taraclia district, V-Perjii village | 44700 | 474 | 35 | 7,3 | 2,4 |
| **Total** | | | **274900** | **2873** | **228** | **7,94** | **2,54** |

In table 5.8 are presented data representing the actual number of poultry units where laying hens in the republic were not subjected to prophylactic vaccinations against avian salmonellosis, with monitored cases of mortality, including the cause of mortality, with a focus on egg peritonitis. Laying birds were vaccinated against Infectious Bronchitis, Egg Drop Syndrome, and Avian Pseudopestis, which effectively protect the hens throughout the productive period. This argues that egg peritonitis is usually the cause of the circulation of pathogenic serotypes of *Salmonella spp.* in laying hen populations. Analysing the data from table 5.9, we observe that the laying hen populations from 5 poultry farms, totalling 274 900 laying hens, were under monitoring. The monitoring period lasted 6 months (starting from the beginning of laying).

Typically, deceased birds were subjected to necropsy examinations and selective (random) microbiological examinations. Special attention was paid to cases of egg peritonitis during necropsy. Out of the total number of bird carcasses (2979), the number of carcasses with egg peritonitis was 228 birds, or 7,94%. The percentage of carcasses with egg peritonitis varied from 7,3 to 9,1%, averaging 7,94%. At the same time, the percentage of non-standard eggs (shell-less, cracked, irregularly pigmented) varied from 2,4 to 3,1%, with an average of 2,54%. Simultaneously with the aforementioned, the peak of egg productivity lasted on average 2 weeks, with maximum egg productivity variations of 84-86%.

During the same period, another 5 poultry farms specialized in producing eggs for consumption, where bird populations were immunized with associated vaccines against avian salmonellosis, were under monitoring. The results of this study are presented in table 5.9.

**Table 5.9. Data regarding the vaccination of birds against salmonellosis in 2020**

| Or. nr. | The poultry farm name | Location | Vaccine type | Number of vaccinated birds | Number of dead birds | Number of dead birds (egg peritonitis) | Percentage of egg peritonitis lethality | Percentage of non-standard eggs |
|---|---|---|---|---|---|---|---|---|
| 1. | LLC ,,Acustic Tehnologie” | Anenii Noi district | Inactivated Vaccine for *S. ent.* PT 4, *S. typh.* DT104 | 66200 | 455 | 17 | 3,7 | 1,2 |
| 2. | LLC,,Intervetcom” | Cimislia district | Inactivated vaccine "Salmin plus", for *S. typh., S.ent., S.inf.* | 71000 | 563 | 22 | 3,9 | 1,1 |
| 3. | LLC SC,,Rom-Cris” | Donduseni district | Inactivated vaccine "Nobilis SG3R", for *S. Gall.* 9R. | 86500 | 629 | 31 | 4,9 | 0,7 |
| 4. | LLC I.M. ”PB Nord” | Edinet district | Inactivated vaccine "Nobilis salenvac T", for *S. Enter., S.Typh.* | 53200 | 466 | 19 | 4,0 | 0,9 |
| 5. | LLC ’’Dant-Agro’’ | Ungheni district | Inactivated vaccine "Nobilis salenvac T", for *S. Enter., S.Typh.* | 93600 | 712 | 23 | 3,2 | 0,6 |
| | **Total** | | | **299500** | **2825** | **112** | **3,93** | **0,9** |

Comparing the effectiveness of vaccinations against avian salmonellosis, it can be mentioned that the introduction of vaccination in some poultry units in the republic has contributed to a reduction in bird mortality by 3,97%, and the incidence of egg peritonitis by 4,1%. At the same time, the quantity of non-standard eggs has decreased by 1,64%.

## GENERAL CONCLUSIONS

1.Despite the implementation of a comprehensive set of sanitary veterinary and curative prophylactic measures within poultry enterprises, avian salmonellosis remains a significant issue for the health of bird populations, causing considerable economic losses as well as major risks to public health.

2.Research on the circulation of pathogenic serotypes of bacteria from the *Salmonella spp.* genus has demonstrated that the veterinary measures currently undertaken are not sufficient to prevent the incidence of poultry product contamination with these bacteria and to minimize the risks of contamination.

3.Epidemiological and bacteriological research on broiler chicken populations has shown that the incidence of circulation of bacteria from the *Salmonella spp.* genus is 8,6%, with the

predominance of *Salmonella pullorum gallinarum* serotypes, constituting a major impact on the health of broiler chicken populations.

4.The study of the incidence of bacteria from the *Salmonella spp.* genus in laying hen populations reports a presence percentage of up to 4,2%, with the predominance of *Salmonella pullorum gallinarum* and *Salmonella typhimurium* serotypes.

5.The most significant contamination risks with bacteria from the *Salmonella* spp. genus are considered to be: egg and meat packaging, transport units intended for poultry and products, and poultry product marketing places (poultry markets).

6.The results of monitoring pathogenic *Salmonella* spp. bacteria in poultry units over the past 5 years indicate that the highest contamination with *Salmonella spp.* is found in poultry meat, followed by faecal matter and consequently meat products. The highest percentage was observed with *S. infantis* serotypes - 36%, followed by *S. enteritidis* – 29,3%, and *S. typhimurium* – 6,7%.

7.Bacteriological research on eggs for consumption has reported the presence of pathogenic *Salmonella* spp. serotypes such as *Salmonella gallinarum*, *Salmonella dublin*, and *Salmonella typhimurium* in 6,25% of the examined samples.

8.The use of vaccines against certain serotypes of *Salmonella* spp. has contributed to a 2% reduction in non-standard eggs and a 4% reduction in egg peritonitis in laying hens, demonstrating the effectiveness of preventing salmonellosis in birds through specific immunization.

## PRACTICAL RECOMMENDATIONS

The practical recommendations "Measures for the Prevention and Control of Avian Salmonellosis" (2022 Edition), 32p, were developed and approved by the Zooveterinary Commission within The Ministry of Agriculture and Food Industry.

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**2. Articles in scientific collections**

**2.1. In the proceedings of international scientific conferences (Republic of Moldova)**

11. JUNCU, O., STARCIUC, N., OSADCI, N., MALANCEA, N., SPATARU, T., MANCIU, A., CIUCLEA, A. Microflora ouălor de consum şi riscurile de contaminare cu Salmonella spp. In: *Lucrări ştiinţifice*, Univ. Agrară de Stat din Moldova. 2018, vol. 49: Medicină Veterinară: materialele Simpozionului Ştiinţific Internaţional "*Probleme actuale şi tendinţe ale sectorului de creştere a animalelor şi medicinei veterinare",* dedicat aniversării a 85 de ani de la fondarea Universităţii Agrare de Stat din Moldova, Chişinău, 2018, pp. 117-121. ISSN 978-9975-64-298-9.
12. JUNCU, O., STARCIUC, N., OSADCI, N., ANTOHII, T., MANCIU, A., MALANCEA, N., CIUCLEA, A., MOSCALIC, R. Monitorizarea bacteriilor din genul Salmonella spp. în cadrul unor incubatoare. In: *Lucrări ştiinţifice*, Univ. Agrară de Stat din Moldova. 2019, vol. 54: Medicină Veterinară: *Materialele simpozionului ştiinţific internaţional "45 ani de învăţământ superior medical veterinar din Republica Moldova*. Chişinău, 2019, pp. 219-224. ISBN 978-9975-64-310-8.
13. ANTOHII, T, STARCIUC, N., OSADCI, N., JUNCU, O. Monitorizarea unor boli zoonotice transmisibile caracteristice animalelor domestice şi impactul acestora pentru sănătatea publică. *Lucrări ştiinţifice,* Univ. Agrară de Stat din Moldova. 2019, vol. 54: Medicină Veterinară: *Materialele simpozionului ştiinţific internaţional "45 ani de învăţământ superior medical veterinar din Republica Moldova*. Chişinău 2019, pp. 224-229. ISSN 978-9975-64-310-8.

**2.2. In proceedings of national scientific conferences with international participation**

14. JUNCU, O., STARCIUC, N., OSADCI, N., MOSCALIC, R. Indicii microbiologici și impactul prezenței bacteriilor din genul Salmonella spp. în unele unități avicole din republică. In: Inovații în zootehnie și siguranța produselor animaliere – realizări și perspective: conferința științifico-practică cu participare internațională, dedicată celei de-a 65-a aniversări de la fondarea Institutului Ştiinţifico-Practic de Biotehnologii în Zootehnie şi Medicină Veterinară, 30 sept.-01 oct. 2021, Maximovca: Culegere de lucrări științifice. Maximovca, 2021, pp. 652-660. ISBN 978-9975-56-911-8.
15. JUNCU, O. Incidența bacteriilor din genul Salmonella spp. la unele unități de creștere a puilor broiler și a găinilor ouătoare. In: *Conferința științifico-practică cu participare internațională "Gestionarea fondului genetic animalier – probleme, soluții, perspective*", 28-

30 sept., 2023: Culegere de lucrări științifice. Maximovca: Print-Caro, 2023, pp. 407-415. ISBN 978-9975-175-38-8.

16. JUNCU, O., STARCIUC, N., OSADCI, N., BORDOS, X., PANFILII, A. Unii indici microbiologici ai carcaselor de pasăre în perioada de comercializare. In*: Stiința zootehnică – factor important pentru o agricultură de tip european:* Culegere de lucrări a Simpozionului științific cu participare internațională dedicat aniversării a 60-a de la fondarea IȘPBZMV, 29 sept-01 oct. 2016. Maximovca, 2016, pp.166-170. ISBN 978-9975-56-367-3.

**3. Theses in scientific collections**

**3.1. In the proceedings of international scientific conferences (abroad)**

17. STARCIUC, N., JUNCU, O., NATALIA, O. The indices of microflora diversity of chickens saled in the poultry market from Chisinău. In: *Scientific Congress with International Participation. 56-th Anual Meeting of Veterinary Sciences „Towards a Global Health*: program and abstract booklet, 19-20 October. Iași, 2017, p. 54.

## ADNOTARE

JUNCU Olga**:" Salmoneloza aviară. Particularități epidemiologice, diagnosticul și măsuri de profilaxie"**, teză de doctor în științe medical-veterinare, Chișinău, 2024.

**Structura tezei**: introducere, 5 capitole, concluzii generale și recomandări, bibliografie cu 233 titluri, 13 anexe, 118 pagini text de bază, 36 figuri, 19 tabele. Rezultatele obținute sunt publicate în 17 lucrări și sunt elaborate Recomandări științifico-practice.

**Cuvinte-cheie:** pui, găini ouătoare, carcase, ouă, materii fecale, cadavre, microorganisme, salmonella, serotype, medii de cultură.

**Scopul lucrării:** stabili situația epidemiologice si a factorilor de impact al salmonelozei aviare asupra păsărilor si a sănătății publice prin analiza vectorilor principali de transmitere a bolii.

**Obiectivele cercetării:** Monitorizarea diversității microflorei patogene și prezenței bacteriilor din genul *Salmonella spp*. în unele unități avicole de producere a ouălor și de creștere a puilor broiler; Analiza prezenței și diversității serotipurilor de *Salmonella spp.* în carcasele depasăre și ouă, în materiile fecale, materialul patologic, probele de la incubatoare, din piețele avicole; Analiza prezenței și diversitatea serotipurilor de *Salmonella spp.,* serotipizarea și stabilirea sensibilității la unele antibiotice; Elaborarea recomandărilor științifico-practice în profilaxia și combaterea salmonelozei aviare.

**Ipoteza de cercetare.** Prezența și răspândirea sistematică a infecțiilor salmonelice la păsări și incidența crescută a cazurilor de toxiinfecții alimentare la om demonstrează impactul acestei boli și necesitatea studiilor și perfecționarea metodelor de control și de profilaxiei.

**Noutatea şi originalitatea ştiinţifică**: În premieră a fost efectuată monitorizarea situației epidemiologice față de salmoneloza aviară la întreprinderile avicole pentru creșterea puilor broiler și de producere a ouălor de consum în vederea evaluării vectorilor principali de transmitere a bolii și evaluarea factorilor de risc pentru sectorul avicol și consumatori de produse avicole. A fost stabilită diversitatea microflorei bacteriene circulante în efectivelor de păsări, în carnea și ouăle de pasăre cu izolarea, serotipizarea și stabilirea incidenței tipurilor de *Salmonella spp*. A fost apreciata antibiorezistenței izolatelor de *Salmonella* față de unele antibacteriene folosite în în avicultură, precum și eficiența unor tulpini vaccinale utilizare în imunoprofilaxia salmonelozei aviare.

**Rezultatul obținut, care contribuie la soluționarea unei probleme științifice importante:** Salmoneloza aviară rămâne ce-a mai răspândită toxiinfecție la păsări și cu cel mai important risc de toxiinfecții alimentare la om. Aprecierea incidenței bacteriilor din genul *Salmonella spp*. în unitățile avicole și în produsele avicole permit întreprinderea unor măsuri sanitare pentru reducere riscului de răspândire a salmonelozei în efectivele de păsări și respectiv reduce riscul toxiinfecțiilor cu *Salmonella* la consumatorii de carne și ouă. Recomandările științifico-practice permit excluderea factorilor de risc de transmitere a sărmănelelor în lanțul creșterii păsărilor și obținerii produselor avicole sigure pentru consumatori.

**Semnificația teoretică:** Datele obținute prezintă interes teoretic pentru domeniul creșterii păsărilor precum și servește ca material pentru dezvoltarea studiul de perspectivă în monitorizarea și prevenirea salmonelozei aviare.

**Valoarea aplicativă**: A fost apreciată incidența bacteriilor din genul *Salmonella spp.* în cadrul unităților avicole și în produsele avicole, fiind evidențiate și propuse spre monitorizare punctele critice de contaminare cu bacterii din genul *Salmonella spp.* S-a apreciat nivelul de sensibilitatea a salmonelelor față de antibacterienele frecvent utilizate în avicultură, precum și efectul postvaccinal al unor vaccinuri folosite în prevenirea salmonelozei aviare.

**Implementarea rezultatelor științifice:** rezultatele obținute vor fi utilizate ca material teoretic și practic pentru crescătorii de păsări și medicii veterinari din republică în scopul controlului salmonelozei aviare, precum și folosirea materialului în procesul de instruire a studenților anilor 4 și 5 de la facultatea de Medicină Veterinară a Universității Tehnice din Moldova

## АННОТАЦИЯ

ЖУНКУ Ольга: ”**Сальмонеллёз птиц. Эпидемиологические особенности, диагностика и меры профилактики”,** диссертация по соисканию учёной степени доктора ветеринарных наук, Кишинев, 2024.

**Структура диссертации:** введение, 5 главы, общие выводы и рекомендации, библиография 233 источников, 13 приложений, 118 страниц основного текста, 36 фигур, 19 таблиц. Полученные результаты опубликованы в 17 научных работах есть научно-практические рекомендации.

**Ключевые слова**: цыплята, куры-несушки, тушки, яйца, фекалии, тушки, микроорганизмы, сальмонеллы, серотипы, питательные среды.

**Цель работы:** установить эпидемиологическую ситуацию и факторы риска сальмонеллеза на здоровье птиц и людей путем анализа основных векторов передачи заболевания.

**Задачи исследования**: Мониторинг разнообразия патогенной миктофлоры и привалентность бактерий рода Сальмонелла на птицеводческих предприятиях кур несушек и цыплят-бройлеров, в мясо птицы и яйцахб в фекальных массах и патматериале, в инкубаторах и птицеводческих рынков; серотипирования и установление чувствительности к антибиотиков; разработка научно-практических рекомендаций.

**Гипотеза исследовании.** Наличие и систематическое распространение сальмонеллезной инфекции у птиц и рост случаев пищевых отравлений у людей свидетельствуют о важности заболевания и необходимости совершенствования методов профилактики.

**Научная новизна и оригинальность**: Впервые проведен мониторинг эпидемиологической ситуации по сальмонеллезу птиц с целью оценки основных векторов передачи заболевания и оценки факторов риска для отрасли птицеводства и для потребителей продукции птицеводства. С помощью выделения, серо-типирования и установления заболеваемости бактериями рода Сальмонелла установлено разнообразие бактериальной микрофлоры циркулирующей в птицеводческих предприятии, в тушах и яйца птицы. Оценена антибиорезистентность изолятов сальмонелл к некоторым антибактериальным препаратам применяемым в птицеводстве, а также эффективность некоторых вакцинн в иммунопрофилактике сальмонеллеза птиц.

**Полученный результат, способствующий решению важной научной проблемы**: Сальмонеллез птиц остается наиболее распространенной токсической инфекцией среди птиц и наиболее важным риском пищевых отравлений у людей. Оценка заболеваемости бактериями рода сальмонелла в птицеводческих хозяйствах и в птицеводческой продукции позволяет проводить санитарные мероприятия по снижению риска распространения сальмонеллеза в птицеводческих хозяйств и соответственно снижает риск зараженности сальмонеллами мясо и яйца для потребителей. Научно-практические рекомендации позволяют исключить факторы риска передачи сальмонелл в птицеводческой цепочке и получить безопасную для потребителя продукцию.

**Теоретическая значимость**: Полученные данные представляют теоретический интерес для отрасли птицеводства, а также служат материалом для разработки перспективных исследований по мониторингу и профилактике сальмонеллёза птиц.

**Прикладное значение**: выявлены и предложены для мониторинга критические точки заражении бактериями рода Сальмонелла. Установлен уровень чувствительности сальмонелл к используемым антибактериальным препаратам в птицеводстве а также оценивался поствакцинальный эффект применяемых вакцин.

**Внедрение научных результатов**: Полученные результаты использованы в качестве теоретического и практического материала для птицеводов, ветеринарных врачей республики с целью борьбы с сальмонеллезом птиц, а также использованы в процессе обучения студентов 4 и 5 курсов факультета Ветеринарной Медицины, Технического Университета Молдовы.

## ANNOTATION

JUNCU Olga**:** ”Avian salmonellosis. Epidemiological particularities, diagnosis and prophylaxis measures”, doctoral thesis in medical-veterinary sciences, Chisinau, 2024.

**Thesis structure:** introduction, 5 chapters, general conclusions and recommendations, bibliography with 233 titles, 13 annexes, 118 pages of basic text, 36 figures, 19 tables. The obtained results are published in 17 scientific articles, are elaborated scientifical-practical recommendations.

**Key words:** chickens, laying hens, carcasses, eggs, faces, microorganisms, salmonella, serotypes, nutrient media.

**The purpose of the work:** to establish the epidemiological situation and the impact factors of avian salmonellosis on birds and public health by analyzing the main transmission vectors.

**Objectives of the research:** Monitoring of the diversity of pathogenic mycroflora and the presence of bacteria of the genus *Salmonella spp.* in poultry units producing eggs and for growth the broiler chicks; Analysis of the presence and diversity of *Salmonella spp.* serotypes in carcasses, eggs, faces, pathological material, incubators and poultry markets; Serotyping of isolated *Salmonella spp.* and establishing the sensitivity to some antibiotics; Development of scientific-practical recommendations for prevention and control of avian salmonellosis.

**Research hypothesis.** The presence and systematic spread of salmonella infections in birds and the increased incidence of cases of food poisoning in humans demonstrate the impact of this disease and the need for studies and improvement of control and prevention methods.

**Scientific novelty and originality:** For the first time, was performed the monitoring of the epidemiological situation with regard to avian salmonellosis in poultry enterprises for growth broiler chickens and laying hens in order to evaluate the main vectors of salmonellosis transmission and the evaluation of risk factors for poultry sector and consumers of poultry products. Was established the diversity of the bacterial microflora circulating in poultry flocks, on carcasses and poultry eggs with the isolation, serotyping and establishment of the incidence of *Salmonella* serotypes. Was appreciated the antibiotic resistance of *Salmonella* isolates to some antibiotics and efficiency of some ant salmonella vaccines.

**The result which contributes to solving an important scientific problem:** The assessment of the incidence of bacteria of the genus *Salmonella spp*. in poultry units and in poultry products allows the undertaking of sanitary measures to reduce the risk of spreading salmonellosis in poultry flocks and reduces the risk of toxic infections with *Salmonella* on consumers. Scientific-practical recommendations allow the exclusion of risk factors for the transmission of *salmonella* in the poultry breeding chain and obtaining safe poultry products for consumers.

**Theoretical significance:** The obtained data have the theoretical interest for the field of poultry breeding as well as serving as a material for the development of the perspective study for monitoring and prevention of avian salmonellosis.

**Applicative value of the work.** Was assessed the incidence of bacteria of the genus *Salmonella spp.* in poultry units and in poultry products, being highlighted and proposed for monitoring the critical points of contamination with bacteria of the genus *Salmonella spp*., and the level of sensitivity of *salmonella* to the antibacterial commonly used in poultry farming.

**Implementation of scientific results.** The obtained results will be used as theoretical and practical material for poultry breeders and veterinarians for controlling avian salmonellosis, as well as use of the material in the training process of students of the 4-th and 5-th years of the faculty of Veterinary Medicine of Technical University of Moldova.

**JUNCU OLGA**

# AVIAN SALMONELLOSIS. EPIDEMIOLOGICAL PARTICULARITIES, DIAGNOSIS AND PROPHYLAXIS MEASURES

## 431.03. MICROBIOLOGY, VIROLOGY, EPIZOOTIOLOGY, MYCOLOGY, AND VETERINARY IMMUNOLOGY

The abstract of the doctoral thesis in veterinary medical sciences

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