

## SUMMARY

### COMPARATIVE RESEARCHES REGARDING MONITORING AND CONTROL OF DIFFERENT TYPES OF *SALMONELLA* IN BROILER AND LAYING HENS FARMS IN ROMANIA

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The PhD thesis with a total of 224 pages, divided into 12 chapters, is structured according to the requirements in force in two parts: "**Bibliographic study**" with a total of 54 pages and "**Personal research**", which spans in 170 pages. The paper presents a total of 37 tables and 156 images, most of them being inserted in the personal part (28 tables and 138 photos).

**PART I "BIBLIOGRAPHIC STUDY"** contains 6 chapters, in which are presented the main bibliographic aspects regarding salmonellosis, related to etiology, sources and ways of infection, pathogenesis of animal salmonellosis, symptomatology and lesion picture. Data on diagnostic techniques, with emphasis on classical and modern laboratory methods, used in official control, are specified. The last chapter of the first part briefly presents information on epidemiological dynamics in humans and animals, both in the international context and the evolution at national level. All of this information is based on consulting 150 bibliographical titles, including two articles of the PhD student, with preliminary data obtained during the researchers conducted.

**CHAPTER I**, entitled "**Data on enterobacteriaceae family bacteria**", presents historical data, aspects related to the morphobiology of bacteria belonging to the Enterobacteriaceae family, as well as current data on morphobiology, cultivation and biochemical properties of bacteria of the *Salmonella* genus.

**CHAPTER II "Salmonella etiology and taxonomy"** presents a series of references to taxonomy of *Salmonella* genus. In fact, the World Health Organization (WHO), in collaboration with the Salmonella Reference and Research Center (Pasteur Institute, Paris), proposes that the *Salmonella* genus be divided into two species: *S. enterica* and *S. bongori* (former subspecies V). Salmonellosis is produced by members of the *Salmonella* genus which according to the last nomenclature comprises two species, namely *Salmonella enterica* and *Salmonella bongori*. After the 2000s, a third presumptive species, namely *Salmonella subterranea*, was proposed based on the isolation from the environment of a single and unusual strain. *Salmonella enterica* is divided into 6 subspecies that differentiate through certain biochemical features: Subspecies I= Subspecies *enterica* with 1.531 serovars; Subspecies II= Subspecies *salamae* with 505 serovars;

Subspecies IIIa = Subspecies *arizonae* with 99 serovars; Subspecies IIIb= Subspecies *diarisonae* with 336 serovars; Subspecies IV = Subspecies *houtenae* with 73 serovars; Subspecies V= Subspecies *indica* with 13 serovars. *Salmonella bongori* contains less than 22 serovars, with the mention that they are extremely rare. *Salmonella* strains are classified as serovars based on the large diversity of "O" lipopolysaccharide antigens (LPS) and "H" flagella antigens proteins in accordance with the Kauffmann-White scheme that is constantly updated.

There are also issues related to antigenic structure, serotyping, phagotyping or biotyping, as well as pathogenicity of *Salmonella*.

**CHAPTER III “Sources, transmissions and pathogenesis of animal salmonellosis”** treats the main reservoirs, the *Salmonella* circuit in nature and pathogenesis. All *Salmonella* serovars may be pathogenic, but the main condition is that the infectious dose of viable *Salmonella* is significantly high (10<sup>7</sup>-10<sup>9</sup> germs), the invasive power (virulence) of the ingested *Salmonella* strain being another condition for triggering the infection.

**CHAPTER IV " Clinical and morphopatological aspects of salmonellosis"** briefly presents aspects related to the symptomatology and anatomopathological picture of salmonellosis in birds, mammals and humans.

**CHAPTER V "Laboratory Investigations"** deals with the main laboratory investigations used in the diagnosis of salmonellosis, with particular reference to the methods used in the official control of the disease: serological investigations, isolation and identification of *Salmonella*, serotyping, genome identification by molecular biology techniques. Laboratory diagnosis remains a major step in identifying the presence of the *Salmonella* zoonotic agent.

**CHAPTER VI "Epidemiological dynamics"** mentions the evolution of *Salmonella* infections (salmonellosis) in humans and animals, both at international and national levels.

The actual incidence of salmonellosis in humans is difficult to assess because many countries do not have an epidemiological surveillance system and even if there is one, sporadic cases are usually not reported. In countries where the declaration is mandatory, it has been observed that the number of cases has increased considerably in recent years, but this observation is also partly real due to an improvement in case reporting. In 2016, the 28 EU Member States reported 96.039 cases of salmonellosis in humans, of which 94,530 cases were confirmed, resulting an EU notification rate of 20,4 cases per 100.000. In 2014-2016, the serotypes involved in human cases detected from foods (egg and egg products, poultry meat and meat products) as reported in the EFSA database. At EU level, Member States are required to implement *Salmonella* national control programs, in line with Community legislation (Regulation (EC) No 2160/2003), the target serotypes for surveillance being *S. Enteritidis* and *S. Typhimurium*, including the monophasic strains.

The prevalence of *Salmonella* varies from one country to another, depending on the type of poultry production, but also on the detection methods used in the laboratory. Thus, it has been observed that eggs and poultry meat are the most important source of human infection, *S. Enteritidis* and *S. Typhimurium* being the most commonly reported serotypes.

**PART II "PERSONAL RESEARCH"** contains 6 major chapters, each with several subchapters, namely the purpose of the paper, a chapter devoted to the material and methods used during the research, and then general and specific aspects regarding the national control programs of zoonotic *Salmonella*, the interpreted results of the investigations, the sanitary veterinary measures applied in case of a positive result for *Salmonella* Typhimurium and Enteritidis in broilers and laying hens flocks, the prevalence of *Salmonella* serovars in Romania during the period 2013-2016 and the final part of the conclusions and a few recommendations.

**The aim of the paper** is to investigate the prevalence of circulating *Salmonella* serotypes in different animal species in order to determine the prevalence of *Salmonella* serovars that circulate in different animal species in Romania, as well as to achieve a spatial distribution of isolates of *Salmonella* at the county level between 2013 and 2016.

Morphopathological lesions were evaluated, correlating with the histopathological ones and following the way in which the cellular tropism of the presence of bacterial injury influences the global morphopathological picture. A flow chart was made on the diagnosis of the disease, both with classical bacteriological methods and with the modern tools of bacterial genomic detection, given that the current requirements of official control demand accreditation in accordance with the international standards in force. The ultrastructural aspects of *S. Enteritidis* flagella structure have been studied, compared to *S. Typhimurium*'s helical flagella protostructures, an important aspect to be highlighted, especially in the studies conducted in the production of vaccines obtained from mobile strains.

**CHAPTER VII "Materials and methods"** shows the types of test samples / matrices taken for testing, as well as the methods of anatomoclinical and laboratory investigation for these samples taken. The investigations were conducted within the National Reference Laboratory for *Salmonella* in Animals coordinated by the Bacteriology Service, the Morphopathology Service and the Service of Molecular Biology from the Bucharest Institute of Diagnostic and Animal Health, in accordance with the capability for diagnostic methods within the official control. Bacteriological investigations were carried out in a total of 1.229 samples, of which 967 were from laying hens and 262 from broilers, as follows: 409 dead bodies from laying hens (339) and broiler (70 cases), 756 faeces samples derived from the two categories (564 from laying hens and 192 from broilers) and 64 composite samples (caeca + oviducts) from laying hens.

The necropsy examinations that allowed the detection of morphopathological lesions were performed on 824 dead bodies (627 from laying hens and 197 from broilers). A series of staining methods specific to histopathology (HE, HEA, Martius Scarlet Blue, Brown and Brenn) were applied to tissue / organ sections subjected to microtomy technique and paraffin inclusion from 409 samples (339 laying hens dead bodies and 70 from broilers). Also, molecular biology tests were performed on a number of 44 isolates from laying hens diagnosed with *Salmonella*, as well as electron microscopic investigations on 13 isolates from laying hens with *Salmonella*.

**CHAPTER VIII "General and Specific Aspects of Zoonotic *Salmonella* National Control Programs"** puts into question information on the European and national legislation

applicable in this field, the Zoonotic *Salmonella* National Control Programs for different categories of poultry, programs approved by the EU for Romania through Decisions Grant, as well as their co-financing, issues related to the sampling, packaging, labeling and samples transport to laboratory as well as the main objects of the zoonotic *Salmonella* national control programs in broiler and laying hens flocks.

**CHAPTER IX "Results and discussion obtained from the application of laboratory methods for the detection of zoonotic *Salmonella* agents"** are presented in a detailed manner on the results of the investigations carried out on the samples taken.

A flow chart for the isolation and identification of *Salmonella* has been applied, following which the cultural, biochemical and morpho-tinctorial features specific to several strains of *Salmonella* diagnosed in both laying hens and broilers have been observed. The serological identification consisted in the determination of the somatic antigenic structure with mono- and polyvalent anti-*Salmonella* serum and the classification of *Salmonella* strains in serovars was performed according to ISO/TR 6579-3: 2014 Food Microbiology - Horizontal Method for the detection, counting and serotyping of *Salmonella* - Part 3 of the Guide for Serotyping *Salmonella* spp. " and in accordance with the Kauffmann-White-Le Minor scheme based on the identification of " O "lipopolysaccharide antigens," H "flagella protein antigens and "Vi" coating antigens". Detection of the specific genome of the different *Salmonella* serovars was performed using 2 tests: the multiplex PCR test and the sequencing test on a number of 44 isolates from laying hens diagnosed with *Salmonella*, strains that were initially identified phenotypically on the basis of cultural, morpho-tinctorial, biochemical and serologically typing by specific bacteriology techniques. From the analysis of the results obtained from the electron microscopy we have found that, in the structure of the *S. Enteridis* flagella, the protoflagellin networks show an elongated, parallel and filamentous aspect, compared to *S. typhimurium* spiral flagella protostructures. The true image of the result of the presence of bacterial injury in the tissues and organs was highlighted by the classic histopathological techniques, and in all cases examined there was an increased incidence and frequency of proliferative inflammation of lymphoid and / or macrophage type having topohistopathological expression in outbreaks and / or diffuse, more frequently identified in cases where *S. Gallinarum* and *S. Enteritidis* infections occurred compared with infections produced by *S. Pullorum*, where the lesion constantly identified is lymphohistiocytar myocarditis, the diffuse form.

**CHAPTER X "Sanitary veterinary measures applied after identification of a positive result of *Salmonella* Typhimurium and Entertidis, in broiler and laying hens flocks"** sets out very important aspects relating to the declaration of a positive flock with *Salmonella* and the restrictive measures concerning the flock (s) concerned, separately on the two main categories studied: laying hens and broilers.

**CHAPTER XI "The prevalence of *Salmonella* serovars in Romania during the period 2013-2016"** presents the investigation of the prevalence of *Salmonella* serotypes circulating in different animal species in Romania and the achievement of a spatial distribution of *Salmonella* isolates at county level between 2013 and 2016. The data generated from the

collection of information on the 5067 samples from the analyzed period were entered into a Microsoft Excel 2007 Software. The frequency of each isolated *Salmonella* serotype, from different matrices and county, was determined. In the official activities on *Salmonella* control programs, namely the implementation of programs for the control of salmonellosis in animals, 1323 samples were tested for typing in 2013, 1199 in 2014, in 2015 were analyzed a number of 1357 samples, and in 2016, 1188 samples. In the period 2013-2016, the study was conducted on samples from 20 domestic and wildlife species across the country, farms, backyards or zoos.

**CHAPTER XII "Conclusions and Recommendations"** sets out 34 well-grounded conclusions drawn from the research and 2 recommendations.