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**UNIVERSITY OF AGRICULTURAL SCIENCES AND  
VETERINARY MEDICINE „ION IONESCU DE LA BRAD” IAȘI  
FACULTY OF VETERINARY MEDICINE  
SPECIALIZATION MICROBIOLOGY- IMUNOLOGY**

**Drd. OBADĂ MIHAI DORU**

**PhD THESIS ABSTRACT**

**„RESEARCHES REGARDING THE USE OF A PROGRAM FOR SCREENING AND  
CONTROL OF FOOD POISONING WITH SALMONELLA SPECIES AND  
CAMPYLOBACTER SPECIES BY PHENOTYPICAL AND MOLECULAR INVESTIGATION”**

***Keywords: collective consumption, serotyping, biological materials  
food, typing M.L.S.T.***

In public health problems, food poisoning continues to occupy an important place since they are present all over the globe. The OMS reports indicate that all over the world, millions of people suffer from diseases caused by contaminated food.

Food poisoning is a much bigger problem for governments and for the food industry today, compared to the last decades.

Food poisoning, they must always and necessarily adapted to individual situations encountered locally or nationwide even if there are general guidelines.

Implementing a screening and control program requires consideration and specific details of a territory or country of culinary education, religious orientation, geographical conditions and professional ethics implementation and control device such programs.

Outbreaks of food poisoning requires profound implications in different fields as internal medicine, epidemiology, laboratory medicine, microbiology and food chemistry, food and health control of the quality management.

The novelty of the thesis is that the M.L.S.T. technique in Romania has not hitherto been used, and the two protocols work *Salmonella spp.* and *Campylobacter spp.* not have been tested and optimized in Romania. This study brings new information both nationally and internationally on the genetic structure of *Salmonella spp.* and *Campylobacter spp.* strains isolated from food



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and biological materials in Romania and provides the interested researcher, typing protocol and a study to identify the factors virulence of *Salmonella spp* and *Campylobacter spp*.

Thesis entitled "***Research on the use of a screening program and control of toxic infections with Salmonella spp and Campylobacter spp by optimal models phenotypic and molecular investigation***" includes 269 pages and is written in chapters IX and is structured according to the criteria in force in two parts .

The first part (chapters I, II, and III) is 20,44 % and summarizes the main bibliographic database of literature regarding the characterization of bacterial genera *Salmonella spp* and *Campylobacter spp* brief description of the types of food poisoning caused by *Salmonella spp* and *Campylobacter spp* ., and describe techniques mentioned gender typing of isolates, representing "***Current state of knowledge***" and Part 2 (chapters V, VI, VII, VIII, IX) refers to personal research and is 79,56 %. Each chapter in Part 2 has included work material and methods, results, and discussing their partial conclusions.

The paper is illustrated with a number of 69 figures, 40 tables and 205 is based on evidence literature.

In **chapter IV**, entitled "***Objectives of the thesis***" research objectives which are pursued: performing microbiological and molecular biology investigation and control of outbreaks of food poisoning caused by bacterial genus *Salmonella spp* and *Campylobacter spp*.

The proposed research was to contribute to the elaboration of a program of screening and control of food poisoning caused by *Salmonella spp* and *Campylobacter spp*.

The research was based on the following objectives:

- isolation of *Salmonella spp* strains from different ecological niches *Campylobacter spp*: food animal faeces and identification based on cultural characteristics, biochemical, and serological typing;
- identification P.C.R. genes that synthesize virulence factors for *Salmonella spp*. and *Campylobacter spp*;
- identify the types of nucleotide sequence specific strains of *Salmonella spp*. and *Campylobacter spp*. isolated from biological material (feces, urine, etc.) and food (eggs, cheese, meat, etc);
- characterization of isolates from food and biological materials and their comparison;



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- ancestral genotype identification and phylogenetic relationships between different types of sequence;
- monitoring and investigating outbreaks of foodborne *Salmonella spp.* and *Campylobacter spp.* in Iasi county.
- measures to control foodborne *Salmonella spp.* and *Campylobacter spp.*
- establish a procedure for screening and control of foodborne *Salmonella spp.* and *Campylobacter spp.*

In **chapter V**, entitled "**Materials and Methods**" describes the traditional methods of isolation and identification of *Salmonella spp* and *Campylobacter spp* gender and PCR and M.L.S.T. of these bacterial genera.

In **chapter VI**, entitled "**Prevalence and risk factors associated with dissemination of *Salmonella spp***" were identified serotypes of *Salmonella spp* using the slide agglutination method. The antigens represented by isolated strains and polyvalent and flogging monovalent antisera were classified into seven serovars with the following implications: *Salmonella enteritidis* (50,88%), *Salmonella typhimurium* (33,34%), *Salmonella kottbus* (5,28%), *Salmonella litchfield* (3,50%), *Salmonella virchow* (3,50), *Salmonella umbilo* (1,75%), *Salmonella rissen* (1,75%). From the 57 strains studied, 54 (94,73%) were isolated from patients with acute diarrhea and 3 strains (5,267%) in people with food poisoning.

The different reactions P.C.R. were investigated 30 strains of *Salmonella spp* to identify specific virulence factors.

Importance of determining the characteristics of isolates from food and biological materials is highlighted by the study of virulence factors that allowed the identification of isolates with epidemic - *sopE* gene carriers and those whose virulence plasmid is transmitted - *pefA* gene carriers.

Through different reactions 30 strains were investigated to identify specific virulence factors. In our study, all isolates of *Salmonella spp* showed fragments of human genes and *sopE*, *pefA* so human isolates showed a strong transmission of epidemic and virulence plasmid transmission has been made through plasmid.

Allelic profiles of these strains were determined by MLST technique and the type of frequency of these strains was assigned by comparison with those held in the MLST database. MLST technique was performed on a total of 12 *Salmonella* strains isolated from various human



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pathological materials and food products. Through MLST technique there were identified 5 types of sequence: ST-11, ST-19, ST-212, ST-582 and ST-142.

Sequences types ST 11(3 isolates) and ST (3 isolates) were identified for isolates of human origin. ST sequence type 11 was identified for *Salmonella heidelberg* isolates and ST 19 was identified for isolates of *Salmonella enteritidis* and *Salmonella typhimurium*. *Salmonella bovismorbificans* ST 142 was identified for strains of human origin isolated from a pool of human faeces collection in England.

In an outbreak associated with *Salmonella kottbus* it was demonstrated that isolates were epidemic, but the transmission of virulent factors was not made through the plasmids. The current study suggests that *Salmonella kottbus* ST 212, ST identified in 3 strains and 582, identified in one strain has emerged as a major public health problem in various food products in Romania. *Salmonella enterica* ST 212 has not been previously reported from eggs, poultry and sheep's cheese and it was identified a source(chicken neck skin) from which sequence type ST 582 can be isolated. In addition we suggest a distinction between ST 212 and ST 582. *Salmonella kottbus* ST 212 isolates analyzed have epidemic character (have *sopE* gene) while *Salmonella kottbus* ST 582 isolates don't.

The Phylogenetic tree analysis based on gene fragment sequences using the *Molecular Evolutionary Genetic Analysis* program through Neighbour Joining method showed phylogenetic similarity between ST 582 and ST 142 and ST 19 and ST 212. Phylogenetic tree analysis based on fragment sequences of *dnaN* showed the relatedness between ST 11 and ST 582 and ST 142 and ST 212. Phylogenetic tree analysis based on *sucA* gene fragment sequences showed phylogenetic relatedness between ST 212 and ST 11 both emerging from ST 19 and based on *thrA* gene sequence fragment showed phylogenetic relatedness between ST 19 and ST 212, both emerging from ST 142 and ST 582. Based on pure gene fragment sequences phylogenetic relatedness was shown between ST 142 and ST 11, ST 19 and ST 582 are similar and based on phylogenetic gene fragment sequences revealed *hemD* phylogenetic relatedness between ST 142 and ST 212 and ST 11 and ST 582.

In **chapter VII**, entitled "**Prevalence and risk factors associated with dissemination of bacteria of the genus *Campylobacter***" were identified serotypes of *Campylobacter* spp and genetic characteristics of *Campylobacter jejuni* strains, we characterized 10 isolates from England with two molecular techniques: PCR and M.L.S.T.



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If the 10 isolates of *Campylobacter spp* was identified genes *groEL* and *hypO* presence. In other words, the identification of these genes has been demonstrated that these isolates are *Campylobacter spp* and *Campylobacter jejuni* exact species. Sequence type ST-21 (4 isolates) were identified for isolates of human origin. He is reported as the most common clonal complex isolated from humans. Sequence type ST-45 (6 isolates) were identified for isolates of animal origin (poultry). He is reported as the most common clonal complex isolated from the environment.

In the period 2008-2011 were officially registered 322 cases of food poisoning by bacteria of the genus *Salmonella*, and official reports on Romania, *Campylobacter ssp* infections occur in 2008 (2 cases confirmed) and 2009 (254 confirmed cases).

In **chapter VIII**, entitled "*Planning and preparing a program of screening and control of foodborne Salmonella spp and Campylobacter spp*" describes a range of strategies for surveillance and monitoring of food poisoning caused by bacterial genera mentioned above.

Food poisoning caused by bacteria of the genus *Salmonella spp* and *Campylobacter spp* have always maintained an epidemiological alert system in Europe, they are a high risk to human health. It requires an ongoing screening program, especially in our country where source control is very hard to do because most of the foods of animal origin are not subject to veterinary control, holding them with the origin of non- professional.

In **chapter IX** are summarized in the 22 final conclusions, key issues drawn from the investigations and some recommendations regarding P.C.R. techniques and M.L.S.T. in Romania.

This study provides a high degree of novelty, in that Romania, although it is a country where there are surveillance and control programs, genetic progress in the study of local strains of *Salmonella* and *Campylobacter spp* spp.sa done in a slow pace where techniques P.C.R. and M.L.S.T. just beginning to be applied, with remarkable results.