

REVIEW

by Assoc. Prof. Violeta Valcheva Ruseva, PhD

The Stephan Angeloff Institute of microbiology, Bulgarian Academy of Sciences, Department of Infectious microbiology, head of the Laboratory of molecular biology of mycobacteria

Concerning: Review about the PhD dissertation, selected as a member of the Scientific Jury by order № I-115/2.10.2023 of the Director of the Stephan Angeloff Institute of Microbiology, BAS and the reviewer of the Scientific Jury by order № 1 /05.10.2023 of the Director of the Stephan Angeloff Institute of Microbiology, BAS

for obtaining the educational and scientific degree "Doctor" in the professional field 4.3. Biological Sciences, scientific specialty "Microbiology"

PhD candidate: Maya Angelovska

Dissertation topic: Prevalence and characteristics of enteropathogenic *Y. enterocolitica* strains isolated from pigs

Scientific supervisor: Prof. Hristo Najdenski, DSc, Corresponding Member of BAS

1. General information of the received materials

The author of the dissertation is Maya Angelovska - PhD student at the Laboratory of Bacterial Virulence, Resistance and New Antimicrobial Agents of the Department of "Infectious Myrobiology", the Stephan Angeloff Institute of Microbiology, BAS with scientific supervisor: Prof. Hristo Najdenski, DSc, Corresponding Member of BAS. The set of materials presented for review, both in printed and electronic form, complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulation for the Implementation of LDASRB, and the Regulation of the Bulgarian Academy of Sciences (BAS) for the implementation of LDASRB. It also meets the criteria of the Regulation on the conditions and procedure for acquiring academic degrees and academic positions at the Stephan Angeloff Institute of Microbiology, BAS for obtaining the educational and scientific degree of "Doctor."

2. Brief biographical data

The doctoral student Maya Angelovska was born on May 2, 1982 and graduated secondary education in Bitola, North Macedonia. In 2004 graduated Sofia University "St. Kliment Ohridski", Faculty of Biology with a bachelor's degree in molecular biology and in 2006 in the same faculty a master's degree in virology. During the period 2006-2007 is a

virologist specialist at the Stephan Angeloff Institute of Microbiology, BAS, and since 2012 until now she is a specialist microbiologist at the same institute. In 2019 started a doctoral dissertation in the professional field of Biological Sciences, scientific specialty: Microbiology 01.06.12 in the Department of Infectious Microbiology at the Stephan Angeloff Institute of Microbiology, BAS.

3. Relevance of the topic

Yersinia enterocolitica is a zoonotic pathogen that causes yersiniosis in humans and animals. This disease is third in importance among the most reported foodborne zoonoses in the countries of the European Union. Yersiniosis has been proven and described in various animal species, including wild animals and birds. The two enteropathogenic bacterial species are ubiquitous in the environment and easily adapt to different conditions in it, which is an important prerequisite for infecting a wide range of hosts and contaminating water, soil, feed, vegetables, etc. In light of the growing importance and public health concern of human pathogenic biotypes and serotypes of *Yersinia*, as well as the role of pigs meat as a major reservoir for the spread of *Y. enterocolitica*, this dissertation addresses a current health issue. The relevance of the dissertation is also due to the choice of new modern molecular-biological methods and epidemiological data on the distribution of *Y. enterocolitica*, isolated from slaughter pigs originating from different regions of the Republic of Bulgaria.

4. General characteristics and structure of the dissertation work

The dissertation corresponds in content and structure to the requirements for formulating a dissertation for the acquisition of the educational and scientific degree "doctor". The scientific work is written on 157 standard pages and illustrated with 16 tables and 26 figures. The structural distribution includes an introduction of 2 pages, a literature review of 45 pages, aim and objectives - 1 page, materials and methods - 14 pages. The results of the own studies are presented in 42 pages, divided into points, each of which has in several sub-points. The discussion of the results is presented on 16 pages, and the conclusions are on 1 page. The contributions are on 1 page, 3 of them with original nature and 1 with a recommendation for practice. The literature is on 20 pages including 294 sources.

5. Literature review

The literature review is written extremely comprehensively, arranged in a good structure and chronological sequence and clearly shows that the PhD student demonstrates an excellent knowledge of the key studies concerning the dynamics of the epidemiological process in yersiniosis caused by *Y. enterocolitica* as well as the studies of other groups in the field. Virulence factors in *Y. enterocolitica*, which can be both chromosomally encoded and

plasmid encoded, are very well described. Data on *Yop effector* proteins of *Y. enterocolitica* and their function during infection as well as the presence of enteropathogenic strains of *Y. enterocolitica* in different slaughter pigs samples are presented. The virulence genes are also described in details.

A comprehensive review of conventional and molecular biological methods for the detection of *Y. enterocolitica* has been done. The student's knowledge of the material is also evident from the presentation of the application of the various amplification methods, that are more efficient approaches for gene proofing. The LAMP method for proving pathogenic *Yersinia enterocolitica* is well studied and characterized by high sensitivity and specificity, which is a prerequisite for its inclusion in the doctoral student's own research. At the end of the literature review, attention is also paid to the analysis of unsolved problems on the topic affecting the genetic diversity of yersiniosis strains of pathogenic bioserotypes from different geographical regions of the country and most often isolated in Europe by monitoring both the spread of these strains among pigs and their entry into the food chain and their subsequent spread to humans.

Based on the literature review, the goals are logically formulated and 8 research objectives have been given.

6. Research methodology

The methodology used in the development of the dissertation is impressive – various microbiological, biochemical and molecular biological tests were used for isolation and identification of *Y. enterocolitica*. This demonstrates a in depth knowledge of the methodology. The geographical specificity of the areas from where the samples were taken, the techniques used to collect the biological materials and the examinations through a series of tests are described in detail.

7. Results and Discussion

In two consecutive chapters "Results" and "Discussion of the Results", the obtained data are presented and illustrated with tables, graphics and photos. After each result, the PhD student presents an in depth analysis and discussion from an epidemiological point of view. Maya Angelovska used a large number of samples - 790 , 601 from tonsils and 189 from faeces collected for period of five years (January 2016 - December 2021). The pigs were randomly selected. All slaughtered pigs were originated from seven pig farms or fattening pig farms, distributed in 4 different geographical regions and 5 different cities of Bulgaria. The implementation of the activities on the other tasks was done on isolated 920 colonies, which according to their macromorphological characteristics correspond to the representatives of the

genus *Yersinia*. 136 isolates were phenotypically and biochemically characterized as *Y. enterocolitica*, 106 of which were isolated from tonsils, and the remaining 30 originated from pig feces. 43 strains isolated from tonsils and faeces were identified as *Y. enterocolitica* by 16S rRNA gene PCR. She also makes a comparative analysis regarding the frequency of distribution of the isolated *Y. enterocolitica* strains in different regions in Bulgaria, proving that the geographical region of origin of the slaughter pigs does not influence the presence of *Y. enterocolitica*. The analysis and comparison between individual farms and the importance of the season is impressive. The sensitivity of the strains to antibiotics was also demonstrated in detail and a bank of strains and their DNA was created. The virulence profile of the isolated *Y. enterocolitica* strains and their serological group affiliation were also determined. All strains were shown the same serogroup characteristics, and all of them belongs to the serotype O:3. Regarding the virulence determinants of *Y. enterocolitica*, the presence of the *ail* and *ystA* genes were found in all 43 isolated strains and the presence of the plasmid-encoded *yadA* gene only in 41.

Regarding the genetic diversity of the 43 isolates after macrorestriction analysis of genomic DNA, the strains were grouped into five pulsotypes (I, II, III, IV and V). The pulsotypes were characterized, and pulsotype I was widespread (38 strains). Based on the obtained information, a dendogram was built and the strains were divided into two clusters S01 and S02, and three single pulsotypes: SP1, SP2 SP3. Clusters S01 and S02 had a high percentage of genetic similarity sharing 97.4% similarity. The SP3 single pulsotype is genetically the most distant from the clusters and pulso types. The loop-mediated isothermal reaction LAMP is a method with high possibilities for usage in diagnostics in laboratories with limited resources. The PhD dissertation highlighted the advantage of the optimized LAMP protocol for direct detection of the *phoP* gene of pathogenic *Y. enterocolitica*, directly from pig tonsil and faeces tissues. The sensitivity of the method is higher than that of the conventional PCR for the detection of pathogens of the genus *Yersinia*. This validates its use as a rapid and reliable method for proving pathogens in food or pig samples in field conditions as well as in laboratories with limited resources.

The interpretation of the obtained results is logical and in the conclusion of the dissertation 9 conclusions are formulated. The author's original contributions, especially those with recommendations for practice, also deserves a high evaluation.

8. Evaluation of publications

PhD student Maya Angelovksa presents 2 publications in connection with the dissertation work. The first publication is in “Antibiotics” an MDPI journal, indexed in

Scopus and Web of Science with quartile Q1 and the second publication is in the “Acta Microbiologica Bulgarica” journal with quartile Q4. Maya Angelovksa participated in 9 international and 3 national scientific conferences, which proves her significant contribution to the scientific work of the PhD dissertation. She also participates as a member of scientific teams of different projects financed by the National Science Fund of the Ministry of Education and Science.

9. Summary of the dissertation

The summary of the Maya Angelovksa's dissertation work reflects the main results achieved in the dissertation and complies with all the generally accepted requirements in the Regulations for the Application of the Law on the Development of the Academic Staff in the Republic of Bulgaria (RASASRB) for its preparation.

10. Conclusion

The dissertation work contains scientific and scientifically applied results that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (RASASRB), the Regulations for the Application of RASASRB, and the Regulations for the Application of RASASRB at BAS. The presented dissertation results fully comply with the specific requirements of the Rules of the IMikB for the application of ZRASRB.

The dissertation work demonstrates that Maya Angelovska possesses in-depth theoretical knowledge and professional skills and show qualities and skills for independent scientific research. Based on the above, I confidently give my positive assessment of the conducted research, and recommend to the esteemed jury to award Maya Angelovska the educational and scientific degree "Doctor" in the field of Biological Sciences, scientific specialty: Microbiology 01.06.12.

October 30, 2023

Reviewer:

/Assoc. Prof. Violeta Valcheva Ruseva, PhD/