SCIENTIFIC OPINION

from **Prof. Maria Bogomilova Angelova, DSc**, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
on a dissertation presented to a Scientific Jury, formed by order No. № I-115/2.10.2023 of the Director of the Stefan Angeloff Institute of Microbiology at BAS for obtaining the educational and scientific degree "Doctor" in Professional field: 4.3. Biological Sciences (Microbiology)
Doctoral candidate: **Maya Angelovska**
Dissertation topic: Prevalence and characteristics of enteropathogenic *Y. enterocolitica* strains isolated from pigs
Scientific supervisors: **Prof. Hristo Najdenski, DSc, Corresponding Member of BAS**

Relevance and significance of the Ph.D. thesis subject

Yersinia enterocolitica is one of the most important enteropathogens and yersiniosis is one of the leading zoonoses in Europe. Data published by the ESFA for 2020 demonstrate the global spread of this disease. This pathogen is responsible for human infections, especially in young children and patients with co-morbidities, which is a prerequisite for the development of systemic diseases. Despite the serious problems, data on the prevalence of *Y. enterocolitica* in humans and animals is insufficient.

Pigs are the most important reservoir for *Y. enterocolitica*. It is known that at slaughter the bacterium can pass from the tonsils to all organs and so contaminate the carcass. As a result, there is a risk of transmission of the pathogen to humans. Due to its psychrophilic nature, *Y. enterocolitica* grows and multiplies at low temperatures during food storage. This is the reason why refrigerated and frozen foods can be potential reservoirs and sources of infection as well as factors responsible for the transmission of pathogenic strains. Therefore, assessing the microbiological risk of the presence of *Y. enterocolitica* in slaughtered pigs is of particular importance. The methods used to prove the bacterium are laborious and time-consuming. There is a need to develop sufficiently efficient methods combining molecular biological and conventional approaches for rapid identification of *Y. enterocolitica* in samples from pigs destined for slaughter. All this highlights the relevance and prospectively of the chosen topic. I would like to note that the interest of scientists in the problem is constantly growing, as evidenced by the huge number of publications in recent years. Many of them are devoted to the characterization of this pathogenic disease on a national scale for various European countries. With this dissertation, Bulgaria joins the common effort. Moreover, in our country, there are still no effective methods implemented in veterinary practice.

Knowledge of the subject

The dissertation is constructed in a traditional form with relevant sections. It is written in 158 computer pages and illustrated with 16 tables and 26 figures. I would like to note the excellent

quality of the photographs. The literature review is purposefully and specifically prepared. It contains 294 publications, corresponding to the tasks set. All aspects of the problem of Yersiniosis are covered here in detail, including studies from recent years but without neglecting the historical reference. At the end, a sub-section highlights the unresolved issues and argues the need for the study. In my opinion, this is a very good idea which, together with the 2 figures and 5 tables included in the review, makes it easier for the reader to appreciate the relevance of the thesis.

The purpose of this thesis is derived from the need to use scientific approaches to develop a reliable method for determining *Y. enterocolitica* and to transfer it to laboratory practice. It is clear, well formulated, and integrates all areas of experimental work. To realize this goal, 8 specific, interrelated tasks have been formulated, which include all the mandatory criteria for the establishment of a new method. Already here one can see the serious amount of work set before the PhD student.

Methodology of the study

The section Materials and Methods demonstrates an extremely wide range of methods. They are tailored to the specific requirements of the experiment. Routine microbiological methods are included, as well as state-of-the-art molecular biological, biochemical, etc. such as PCR and RT PCR, LAMP, etc. It should be noted that all methods are described in great detail, which allows their correct reproduction.

Characterization and evaluation of the dissertation and contributions

The section Results shows the scale of the development. The very first lines impress with the number of 790 samples processed and collected from farms in different regions of the country and in different seasons. On this broad basis, 920 colonies were isolated, which correspond in their macromorphological characteristics to the representatives of the genus *Yersinia*. Angelovska performed very detailed biochemical tests on all of them and found that 136 (21%) of them probably belong to the species *Y. enterocolitica*. The identification of the isolates was confirmed by molecular genetic methods. The statistically processed data for each strain were analyzed in great detail. The next steps in the study concern the characterization of the strains in terms of biotype, serotype, and phagotype, and their sensitivity to antibiotics. Of interest are experiments on the identification of genes responsible for pathogenic potential. Data were obtained on the presence of the *ail*, *ystA*, and *yadA* genes in 43 of the isolated strains, and a detailed interpretation of their presence or absence is presented. The figures in this subsection once again highlight the considerable volume of research. Because of the work carried out, a collection of strains and their DNA material has been established, and this may form the basis for future studies. The analysis made by Angelovska on the regional distribution of infected pigs, the comparison between different farms, and the importance of the season should also be highlighted.

In accordance with the set tasks, experiments have been carried out to develop new molecular methods to detect the presence of *Y. enterocolitica*. As a result of a very detailed study, data were

obtained to optimize the LAMP protocol based on the demonstration of the *phoP* gene in pathogenic strains. The study included logical following of modern methodological approaches for the determination of DNA concentration, primer's selectivity, the sensitivity of the LAMP reaction, and its real-time readout (qPCR). Thus, the optimized protocol showed 100% efficiency in artificially contaminated porcine fecal samples. The same result on diagnostic specificity, sensitivity, and accuracy of the LAMP reaction was achieved for strains isolated using the ISO standard and those isolated directly from the tonsil and fecal tissues of slaughtered pigs. The data obtained demonstrate that the optimized LAMP protocol for the detection of pathogenic *Y. enterocolitica* directly from tonsils and feces is more sensitive than the conventional PCR method.

The presentation of the results is combined with a skillful discussion of all stages of the study based on relevant literature sources. This section also demonstrates the doctoral student's serious experimental work and her in-depth knowledge of the field. Her approach of discussing each stage of the study in great detail, evaluating the sensitivity of the method, suggesting better conditions to avoid false positives and sample contamination, and comparing the results with data of other authors is impressive. At the end of the discussion, Maya Angelovska underlines the advantages of the new protocol and justifies the need for its implementation in laboratories and the field.

I would like to emphasize with satisfaction the very good layout of the thesis, the concise scientific style in which it is written, the correct reflection of the results in tables and figures, and their professional presentation.

In my opinion, the conclusions are a logical consequence of the experimental data and provide the necessary information about the value of the research conducted. It should be pointed out that the stated aim has been achieved. I accept the formulation of the contributions and would like to emphasize their significance in theoretical, scientific, and applied aspects. I also appreciate the recommendation made for practice.

Assessment of publications and personal contribution of the PhD student

Data from the dissertation have been included in 2 scientific articles and 12 participations in scientific forums. The articles have been published in the journal Antibiotics with quartile Q1 and Acta Microbiologica Bulgarica with quartile Q4. The article in Antibiotics has now 2 citations although it was published in 2023. The reports and posters have been presented at 9 International and 3 National forums. This gives me reason to believe that Maya Angelovska's results have become available to our and the international scientific community. In all the papers and 9 reports in scientific forums the PhD student is ranked first, which proves her significant contribution to the development of the thesis.

Personal impressions

I know Maya Angelovska from her work at the Institute of Microbiology. For me, she is a promising young scientist with the ability to overcome obstacles and cope with challenges. I am

convinced that Angelovska will continue to work in the field of microbiology with the same desire and enthusiasm.

Conclusion

In conclusion, I would like to emphasize that the PhD student has fulfilled the requirements ZRASRB and the additional criteria of the IMicB for obtaining the educational and scientific degree "Doctor". The material presented by Maya Angelovska is dissertable, the topic is relevant and offers a contemporary level of an important issue for theory and practice. The conducted experiments are methodologically correct, the obtained results are reliable and are a solid basis for further scientific and applied developments. A great deal of experimental work has been carried out, and the problem posed has been studied in a multifaceted and detailed way at a contemporary level, significant contributions have been made, and a proposal for application in practice has been formulated. To this characteristic of the dissertation I would like to add that, in my opinion, Maya Angelovska came out of the Ph.D. as a well-trained specialist in the field of Infectious Microbiology and Molecular Biology, she has mastered a large number of modern methods, she has gained experience in interpreting scientific data. All this gives her the qualification of a young scientist, a worthy competitor to colleagues from foreign laboratories.

Because of the analysis made and the proven growth of the PhD student, I propose to the distinguished members of the scientific jury formed by Order No. I-115/2.10.2023 of the Director of the Stefan Angeloff Institute of Microbiology at BAS, to award **Maya Angelovska** the Educational and Scientific degree "**Doctor**" in the scientific field 4.3 Biological Sciences (Microbiology).

27. 10. 2023
Sofia

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/Prof. M. Angelova, DSc/