

Opinion about Maya Angelovska's dissertation „Distribution and Characterization of Enteropathogenic Strains of *Yersinia enterocolitica* isolated from pigs“

from
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The chosen topic of the dissertation is on a current problem of veterinary microbiology, affecting an issue related to human health - the food zoonosis yersiniosis. The dissertation begins with an "Introduction" (2 pages), representing a summary of the thesis. A "Literature review" (45 pages) follows, which comprehensively examines the state of the *Yersinia*, *Yersinia enterocolitica* problem, incl. taxonomy and classification, bio-/sero-/genotypes, virulence factors, epidemiology, incl. presence of the pathogen in pigs and humans, disease pathogenesis and clinic. Next, a thorough review of microbiological proof methodology with particular attention to biological-molecular methods. My assessment of the review is positive - it shows excellent knowledge and systematization of data from the literature. In general, the overview is centered on the problem of research in the dissertation, and it should be emphasized that the dissertation student has marked the unresolved problems on the topic. From here follows the derivation of the purpose and tasks set for research in the dissertation.

Since an important part of the thesis is the methodology for isolation and typing of *Y. enterocolitica*, the author examines this issue in the most detail - sample collection, microbiological and biochemical tests for isolation and proof of *Y. enterocolitica*, with a detailed description of the molecular-biological methods used tests - isolation of DNA from pig tonsils and faeces, amplification of the 16S rRNA gene region, of virulence genes, gel electrophoresis to prove the PCR and LAMP products, microrestriction analysis with endonucleases, disc diffusion method to determine antibiotic sensitivity . The focus is on the description of the LAMP (loop-mediated isothermal amplification) protocol.

Own research (43 pages) is richly illustrated with 24 figures and 8 tables. The "Discussion" section impresses with the precise analysis of the obtained experimental data and the comparison with data from the literature.

Nine conclusions have been formulated, which contain the achievements of the dissertation in a concentrated form. It was established that 6.7% of slaughter pigs from different regions of Bulgaria carry pathogenic strains of *Y. enterocolitica*. *Yersinia* are isolated only in the autumn-winter season - October-March. For the entire 5-year period of the study, one predominant genotype was demonstrated. The isolated strains were of biotype 4/serotype 0:3, demonstrating high carriage of the virulence determinants *ail*, *ystA* and *yadA*. Three resistance profiles against the most commonly used antibiotics in practice have been identified. Multiresistant strains have also been demonstrated.

A high achievement of the thesis is the optimization of the LAMP protocol for the detection of the *phoP* gene in enteropathogenic *Y. enterocolitica* in tonsils and faeces of slaughter pigs. This optimized method for the detection of enteropathogenic *Yersinia* is equal in

efficiency to that of qPCR. This method allows application both in field conditions and in laboratories with limited resources, with more modest equipment.

The research carried out in the dissertation is the first of its kind in our country to establish intraspecies genetic similarity between strains of *Y. enterocolitica* isolated over a 5-year period from different farms. For the first time in Bulgaria, multi-resistant strains of *Y. enterocolitica*, resistant to more than four groups of antibiotics, were isolated from slaughter pigs, which is a definite risk to human health after the spread of these strains in the food chain.

The literature list of the dissertation includes 326 sources (all in Latin), of which 119, i.e. about 1/3 of the publications are after 2015. This shows that the dissertation is based on a state-of-the-art literature foundation.

The author has 2 articles in journals with an impact factor and an international editorship. She is a co-author of 12 reports at scientific forums, 4 of which are international.

Critical notes

The author should publish another part of her research in international journals.

Conclusion

It is a very well -selected topic on an up -to -date problem of veterinary microbiology, closely related to human nutrition. The author shows an excellent knowledge of the issues related to *Yersinia Enterocolitica*. The studies have achieved the purpose of the dissertation and the experimental tasks have been developed and solved. It makes an impression of the excellent knowledge and application of state-of-the-art molecular-biological methods. It is worth pointing out that a precise method has been developed, and in field conditions and in laboratories with modest equipment.

Based on the research done and in accordance with what I have stated so far and the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, I appeal to the Scientific Council of the Stefan Angelov Institute of Microbiology to award Maya Angelovska the educational and scientific degree "Doctor".

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Reviewer:

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