

RESPONSE

of the official opponent on the PhD thesis Zhike Liu on the topic: «Construction of multiplex PCR assay based on the *citE2* gene to identify *Salmonella Pullorum* and its effector *SteE* in pathogenicity and immunity», submitted for the degree of Doctor of Philosophy in the field of knowledge 21 «Veterinary Medicine» in the specialty 211 «Veterinary medicine»

Topic relevance. *Salmonella Pullorum*, as causative agent of Pullorum disease in poultry, is among the most important pathogens which have resulted in substantial economic losses to the poultry industry worldwide. Pullorum disease causes severe septicemia, mainly in young birds, with clinical symptoms such as loss of weight, decreased laying, diarrhea, lesions and disorder of the reproductive system. Today, scientists from all over the world testify that *S. Pullorum* is still very common in many regions, especially in developing countries, although it has been the target of disease eradication schemes in most industrialized countries.

The disease can be asymptomatic and transmitted to offspring vertically through the ovary. *S. pullorum* has been largely eradicated in developed countries, but it is still widespread in most developing countries, causing serious economic losses to poultry farms. Current effective measures of both prevention and control of *S. Pullorum* which are mainly aimed at the elimination of sick chickens suspected of *S. Pullorum*, surveillance of healthy chickens and termination of transmission routes. However, there is still a lack of related epidemiological data on *S. pullorum* which is the first step in formulating effective control strategies. Thus, the development of rapid and accurate methods for the identification of *S. Pullorum* will improve prevention and control measures.

The topic of the dissertation is relevant, as the work is devoted to the development and scientific justification of the prevention and accurate detection of *S. Pullorum*, and clarify the mechanism of persistent *S. Pullorum* infection in chickens.

Connection of the work with scientific programs, plans, topics. Work carried out in accordance with the main directions of scientific research of the National Natural Science Foundation of China-Henan Joint Fund (Grant Number U1904117), Key Science and Technology Program of Henan Province (Grant Number: 21210210100 and 212102110009), Sun Yat-sen University and Henan Institute of Science and Technology within the framework of scientific programs of research work. The materials of the dissertation work are part of comprehensive scientific research of the Department of Veterinary Expertise, Microbiology, Zoohygiene and Safety and Quality of Livestock Products of the Sun Yat-sen University according to the following thematic plans of research works: "System of monitoring methods of control and veterinary and sanitary measures, regarding the quality and safety of livestock products in diseases of infectious etiology" (state registration No. 0114U005551, 2014-2019); "Forecasting the risks of cross-border introduction and spread of particularly dangerous animal diseases and the development of scientifically based disinfection

systems based on innovative import-substitutable highly effective means" (state registration No. 0115U001342, 2018-2023).

Purpose, reliability and novelty of the scientific provisions, practical significance, conclusions and recommendations formulated in the work.

The purpose of the dissertation work is that development and scientific justification of the prevention and accurate detection of *S. Pullorum*, and clarify the mechanism of persistent *SSP Pullorum* infection in chickens.

The author achieved her purpose by confirmation of the causative agent of sick chickens with suspicion of *S. Pullorum* and conducting a test for its sensitivity to drugs; establishment of a multiplex PCR method by targeting the *citE2* region of difference and the intergenic sequence for accurate and rapid detection of *B. Phoktamum* than analysis of the association of the *steE* gene with *B. Phoktamum* virulence determining the role of *steE* in inflammation caused by *B. Phoktamum* and studying the molecular mechanism of persistent *S. Pullorum* infection in chickens.

The PhD thesis was completed using sufficient number of laboratory animals and practical material with the involvement of modern methods, namely: the microbiological method (isolation and purification, biochemical identification), drug susceptibility test, PCR, analysis of clinical symptoms and autopsy, λ -Red recombination system, prokaryotic expression vector method, transfection, flow cytometry, histopathology method, immunofluorescence staining, immunohistochemistry, qRT-PCR, western blotting, statistical analysis.

The conclusions and proposals for the production of the dissertation work are fully substantiated and correspond to the obtained results of own research.

Scientific novelty of the obtained results is that, the proposed method is the first multiplex PCR method based on the *citE2* gene and the intergenic sequence of *SPS4_00301-SPS4_00311* was established for the accurate detection of *S. Pullorum* in clinical and food samples. Confirmed the pathogenicity associated with *steE* in *Salmonella Pullorum*. These results dissecting the molecular mechanism of *steE* in regulated the balance of Th1/Th2 cytokines during *S. Pullorum* infection for the first time. This thesis established the theoretical basis of prevention and treatment of *S. Pullorum* in chickens.

The practical significance of the obtained results is that, based on the results of theoretical and experimental studies provide a scientific reference for the prevention and accurate detection of *S. Pullorum* in chicken fanners. The developed multiplex PCR system had high sensitivity and specificity, and could be a valuable tool for the clinical diagnosis of *S. Pullorum*, which is an important pathogen in chickens. In addition, *steE* was associated with the persistent infection of *S. Pullorum*, which modulated Th1/Th2-related immune responses by *STAT3/STAT1/SOCS3* and *NF- κ B* axis and could be a unique drug candidate for salmonellosis.

The importance for science and national economy of the results obtained by the author of the dissertation, recommendations for their use.

The main provisions of the PhD thesis were included in the guidelines according to prevention and detection of *Salmonella* and the mechanism via which

steE inhibits the host inflammatory response by STAT3/SOCS3 and NF- κ B axis, approved by the Academic Council of SNAU (Protocol № 5, dated 29.12.2019).

The dissertation materials are included in the syllabus, work program of courses "Veterinary microbiology", "Veterinary medicine" for masters of the Faculty of Veterinary Medicine of Sumy National Agrarian University, and are used in distance learning of students based on the platform «Moodle».

And for the courses "Veterinary microbiology" and "Veterinary medicine" for masters of the Henan Institute of Science and Technology (HIST).

Zhike Liu's dissertation on the topic: «Construction of multiplex PCR assay based on the *citE2* gene to identify *Salmonella Pullorum* and its effector SteE in pathogenicity and immunity» is the completed scientific work, completed according to the set goal and objectives. The obtained results are important and reliable, since the work was carried out on a sufficient number of animals with the involvement of modern effective methods of research.

The dissertation is presented on 133 pages of computer text, illustrated with 7 tables and 45 figures and consists of an annotation, introduction, 4 chapters, conclusions, a list of sources used and 2 annexes. The list of references includes the name of 294 sources.

Approbation of research results. The main provisions and results of the research were reported and received general scientific approval at BTRP Ukraine 2021 International BioThreat Reduction Symposium, (29 June - 2 July, 2021, Kyiv, Ukraine); Chinese Association of Animal Science and Veterinary Medicine, 2022 Academic Forum (14-16 August, 2022. Foshan, China); The III Scientific and Practical International Distance Conference «Microbiological and Immunological Research in Modern Medicine», (March 24, 2023, Kharkiv, Ukraine); The XI International Scientific and Practical Conference «Problems of the development of science and the view of society», (March 21-24, 2023, Graz, Austria); Chinese Association of Animal Science and Veterinary Medicine, 2023 Academic Forum (12-15 May, 2023. Yangzhou, China).

Based on the materials of the dissertation work, 14 scientific papers were published, including: 3 articles in professional editions of Ukraine, 2 articles in Web of Science or Scopus scientific metric publication and 9 abstracts for reports at scientific and practical international conferences.

Personal contribution to solving a scientific problem or solving a specific scientific task. The author took part in the implementation of scientific programs based on PhD thesis, and conceived and designed experiments. Setting objectives, discussing the results, forming conclusions were conducted together with tutors. PhD student analyzed the data as well as interpretation of the data on the topic of the dissertation, and conducted experimental research using modern methods and softwares with co-authors of scientific papers. The applicant wrote dissertation and published articles in which the main material of the PhD thesis. The author thanks all participants in the manuscript for kindly help.

The materials of Zhike Liu's dissertation are practically important and relevant for bacteriologists, veterinarians, infectious disease specialists and

epizootologists who study the problem of bacterial safety of feed and food products.

The results of Zhike Liu's work confirmed that SteE as anti-inflammatory effector contributed to the persistent infection of *S. Pullorum* by regulating the host's innate immune response though modulate the STAT3/SOCS3 and NF- κ B axis, which provided a novel therapeutic strategy for salmonellosis. A rapid and accurate method Multiplex PCR system was established for the detection of *S. Pullorum*.

Scientific propositions, conclusions and proposals are formulated accordingly in the dissertation. They are justified and are a logical continuation of the obtained results of experimental research.

General assessment of the essence of the dissertation, its value and shortcomings in terms of content and design.

Zhike Liu's PhD thesis on the topic: «Construction of multiplex PCR assay based on the *citE2* gene to identify *Salmonella Pullorum* and its effector SteE in pathogenicity and immunity» corresponds to specialty 211 - «Veterinary Medicine». The work was performed at the appropriate level and meets the requirements for the design of theses and the Procedure for awarding the degree of Doctor of Philosophy approved by the Resolution of the Cabinet of Ministers of Ukraine dated 12.01.2022 No. 44, which cancels the previous orders of the Ministry of Education and Culture of Ukraine dated January 12, 2017 No. 40 and Ministry of Education and Culture of Ukraine dated May 31, 2019 No. 759 with changes and additions.

Evaluating positively Zhike Liu's PhD thesis on the topic: «Construction of multiplex PCR assay based on the *citE2* gene to identify *Salmonella Pullorum* and its effector SteE in pathogenicity and immunity», I would like to make certain shortcomings and get answers to some questions:

1. Not all terms in Latin in the text of the dissertation are in italics.
2. In our opinion, in the content of the dissertation in Chapter 2. "Materials and methods" it is not necessary to allocate a list of all types of research into subparagraphs.
3. Are there any features of using your proposed diagnostic method? Are there cases or conditions under which a false result is possible?
4. Do you have perspective plan to develop attenuated *S. Pullorum* vaccine?

The above comments do not reduce the positive assessment of the PhD thesis, because they do not relate the essence of the dissertation work and do not reflect on the conclusions and proposals for production, and the questions posed are clarifying and debatable.

CONCLUSION

Zhike Liu's PhD thesis on the topic: «Construction of multiplex PCR assay based on the *citE2* gene to identify *Salmonella Pullorum* and its effector SteE in pathogenicity and immunity» was performed at the appropriate level, is completed

scientific research work, according to relevance, scientific novelty, theoretical and practical significance of the obtained results, it meets the requirements for the design of theses and the Procedure for awarding the degree of Doctor of Philosophy approved by the Resolution of the Cabinet of Ministers of Ukraine dated 12.01.2022 No. 44, which cancels the previous orders of the Ministry of Education and Culture of Ukraine dated January 12, 2017 No. 40 and Ministry of Education and Culture of Ukraine dated May 31, 2019 No. 759 with changes and additions, and its author deserves to be awarded the scientific degree of Doctor of Philosophy in the field of knowledge 21 «Veterinary Medicine» in the specialty 211 «Veterinary Medicine».

Official opponent,
Doctor of Veterinary Sciences,
Head of Department
of veterinary medicine and pharmacy
National University of Pharmacy
Ministry of Health of Ukraine



Yevheniia VASHCHYK

Signature of Yevheniia Vashchuk certifies:
Head of the personnel department
National University of Pharmacy
Ministry of Health of Ukraine

