

## REVIEW

official reviewer Dolbanosova R.V. Doctor of Veterinary Medicine, associate professor for Zhike Liu's dissertation work «Construction of multiplex PCR assay based on the citE2 gene to identify Salmonella pullorum and its effector SteE in pathogenicity and immunity», which was submitted for obtaining the scientific degree of Doctor of Philosophy at the one-time special council at Sumy National Agrarian University, branch of knowledge 21 – «Veterinary medicine», specialty 211 – «Veterinary medicine».

### **1. The degree of relevance of the dissertation topic and its connection with scientific programs, topics, plans**

Bacteria of the genus Salmonella are one of the causes of acute and chronic infectious diseases in poultry. In addition to being a reservoir for Salmonella, infected poultry can transmit the pathogen through the food chain to humans. Based on the reported detection of Salmonella in food, it can be concluded that it is more often isolated from poultry from its products than from any other animal species.

This fact indicates the high prevalence of infectious diseases caused by salmonella in poultry, in particular among chickens and turkeys raised for sale. Therefore, nationwide programs to identify infected flocks of birds are of great importance.

The current effective measures to the prevention and control of S. Pullorum that mainly to eliminate the diseased chickens with suspected S. Pullorum, monitor the healthy chickens, and cut off the route of transmission. However, there is still a lack of related epidemiologic data of S. Pullorum, which is the first step of formulating effective control strategies. Therefore, a rapid and accurate method is urgently needed to identify S. Pullorum and help us make corresponding prevention and control measures.

Due to long-term unjustified use of antibiotics, bacterial resistance has increased, and antibiotic treatment disrupts the normal homeostasis of the body and intestinal flora. The problems caused by long-term, large-scale use of antibiotics are becoming more and more serious, and the development of effective and safe substitutes for antibiotics is inevitable and urgent. Therefore, there is a need to urgently search for an alternative to antibiotics.

The thesis is devoted to providing development and scientific justification of the prevention and accurate detection of *S. Pullorum*, and clarifies the mechanism of persistent *S. Pullorum* infection in chickens. *S. Pullorum* is a host-specific pathogen, causes severe economic losses to the chicken farms, and also can induce an anti-inflammatory response in chickens. A rapid and accurate method can help us make corresponding prevention and control measures in clinical and food samples. Additionally, *S. Pullorum* effectors with anti-inflammatory function can be a unique drug candidate for targeting salmonellosis.

Work carried out in according 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), Sumy National Agrarian 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 Sumy National Agrarian 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).

## ***2. The degree of validity and reliability of scientific statements, conclusions and recommendations formulated in the dissertation***

Evaluating the main results of the dissertation work submitted for defense, there is a need to emphasize their theoretical validity and the applicant's orientation to a clear formulation of his own position.

Confirmation of the scientific argumentation and validity of the provisions, conclusions and recommendations of the dissertation work is a logical sequence in the setting and execution of tasks according to the purpose of the research. The dissertation contains the necessary theoretical, methodological, methodical and analytical studies, properly covered in the relevant sections.

The scientific validity and reliability of the results presented in the dissertation work is ensured by the creative use of scientific approaches, systematic study and generalization of the results of scientific developments by scientists.

During the study following methods were used: the microbiological method (isolation and purification, biochemical identification), drug susceptibility test, PCR, analysis of clinical symptoms and autopsy,  $\lambda$ -Red recombination system, prokaryotic expression vector method, transfection, flow cytometry, histopathology method, immunofluorescence staining, immunohistochemistry, qRT-PCR, western blotting, statistical analysis.

## ***3. Scientific novelty of scientific provisions, conclusions and recommendations formulated in the dissertation***

Acquaintance with the content of the graduate student's dissertation and his main scientific works made it possible to determine the most important main points of provisions, conclusions and recommendations that prove scientific novelty. The results of the work are formulated by the recipient independently and fully reflect his scientific contribution.

This thesis established the theoretical basis of prevention and treatment of *S. Pullorum* in chickens. This 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.

#### **4. Scientific significance and practical value of the obtained research results.**

The research results presented by the dissertation have theoretical and practical significance.

Based on the results of theoretical and experimental studies provide a scientific reference for the prevention and accurate detection of *S. Pullorum* in chicken farmers. 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/SOCS3 and NF- $\kappa$ B axis and could be a unique drug candidate for targeting salmonellosis.

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- $\kappa$ 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).

**5. *The completeness of the presentation of scientific provisions, materials, conclusions and recommendations of the dissertation in published scientific works, counted according to the topic of the dissertation***

Zhike Liu 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 software's 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 main provisions of the dissertation were reported and discussed and approved at a meeting of: 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).

According to the results of research, 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 of reports at scientific and practical international conferences.

**6. *Academic integrity***

Violations of academic integrity (academic plagiarism, self- plagiarism , fabrication, falsification) in the dissertation work on scientific papers, which

highlight the main scientific results of the applicant's dissertation research, were not detected.

### **7. Discussion questions and comments to the dissertation work.**

Along with the positive evaluation of Zhike Liu's dissertation, it can be noted that some statements are debatable and need to be revised:

1. After tab. 2.2. it is possible to describe not only the conditions of the research, but also to describe their result.
2. In section 3.1. after the figures and tables, you start new research subsections. You need to describe your results. This will help us to understand the material presented more quickly.
3. The conclusions in chapter 3.1 need to be expanded. You have more results than what you described
4. Fig 3.16 Anatomical symptoms of chickens infected with WT and  $\Delta steE$  strains needs to be described in more detail.
5. 3.7.8 Conclusions in chapter 3.8 - indicate the correct chapter. expand your conclusions to the chapter.

The stated debatable provisions and certain shortcomings do not significantly affect the overall positive assessment of the dissertation work.

### **8. Dissertation design**

The design of the dissertation in terms of structure, language and presentation style meets the requirements for the design of dissertation works, which are approved by the order of the Ministry of Education and Culture of Ukraine dated 12.01.2017 No. 40 with changes and additions introduced by the order of the Ministry of Education and Culture of Ukraine dated 31.05.2019 No. 759. The materials of the dissertation clearly highlight the scientific and practical results obtained during the research.

### **9. General conclusion**

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», which is presented for obtaining a doctor of philosophy, is the final independent study containing scientifically based results in the field of veterinary medicine.

In terms of content and formal features, the dissertation meets the requirements for the design of dissertations and the Procedure for awarding the degree of Doctor of Philosophy approved by the Resolution of the CMU 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.

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