FEEDBACK
from the official opponent
Doctor of Veterinary Sciences, Senior Research Fellow,
Head of the laboratory of animal bacterial diseases of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences
Ayshpur Olena for Liu Mingcheng's Thesis «Experimental research on pathogenesis of Streptococcus suis infection», submitted for the degree of Doctor of Philosophy from the field of knowledge 21 «Veterinary» in the specialty 211 «Veterinary medicine»

1. Relevance of the topic of the dissertation.

Pig farming is one of the modern areas of animal husbandry and a competitive type of agribusiness. Infectious diseases cause significant economic losses from reduced productivity and death of pigs. Among the causative agents of bacterial diseases in pigs, the role of pathogenic and opportunistic microorganisms is increasing dramatically. Streptococcus suis is a zoonotic pathogen that causes diseases such as meningitis, pneumonia, endocarditis, polyserositis, arthritis, septicemia and abortion in pigs. A significant number of pigs in China's pig farms suffer from streptococcal infection, the infection is also registered in Ukraine and other countries of the world. In recent years, the incidence rate of streptococcal meningitis has shown a significant upward trend. Due to its widespread spread, the causative agent of swine streptococcosis, Streptococcus suis, is becoming increasingly resistant to antibiotics. The danger of introducing pathogenic strains into the farms calls for constant monitoring of these diseases and effective therapeutic measures.

This pathogen is also dangerous for humans. In China, two outbreaks of streptococcal infection among people were registered, the etiological factor of which was the bacterial pathogen S. suis. The course was characterized by a high level of morbidity and mortality. Antibacterial drugs used to treat the infection are unable to penetrate the blood-brain barrier to reach the therapeutic target site, which is key in controlling bacterial meningitis. Therefore, strengthening the prevention and control of streptococcal infection in pigs has become an urgent task. The solution to this scientific problem is based on the study of the pathogenesis of streptococcal infection. The prerequisite for the induction of meningitis is the penetration of S. suis through the blood-brain barrier and subsequent damage to the central nervous system. Endothelial cells of microvessels of the brain are the main component of the blood-brain barrier, the dysfunction of which is associated with various neurological diseases. Therefore, the study of the interaction between S. suis (serotype 2) and microvascular endothelial cells of the brain is of great importance in the study of the pathogenesis of meningitis.

Therefore, the experimental study of the pathogenesis of streptococcal infection in pigs is an important step in the development of a strategy to control streptococcal infection in pigs.

2. Connection of work with scientific programs, topics, plans.

The materials of the dissertation research are part of complex scientific of studies of the department of epizootology and parasitology of the Faculty of Veterinary Medicine of the Sumy National Agrarian University according to the thematic plan of the scientific research work «Optimization of the set of measures to prevent the occurrence and spread of infectious diseases of animals in the farms of the northeastern region of Ukraine», state registration number 0122U001254 (2022–2027 years). Experimental research on the topic of the dissertation have conducted in the period from 2019 to 2023 years. Department of Epizootology and Parasitology of Sumy National Agrarian University. The dissertation is a fragment of the research programs of the National Natural Science Foundation of China «Project for the Development of Young Talents of Henan Province».

3. Scientific novelty of the obtained results.

The following new scientific results were obtained in the Thesis: it was theoretically and experimentally established for the first time that pyroptosis is involved in the pathogenetic process of developing meningitis due to streptococcal infection of pigs caused by S. suis.

The use of the culture of endothelial cells of brain microvessels (bEnd.3) for infection with S. suis serotype 2 was experimentally substantiated in in vitro experiments. A study was conducted to determine the effectiveness of various schemes for infecting cell culture (Bend3) with the zoonotic bacterial pathogen Streptococcus suis of type 2. The optimal multiplicity of infection (MOI) has screened and the time and dose of infection of the endothelial cells of the microvessels of the brain with the pathogen S. suis 2 has determined experimentally.

For the first time, he conducted a study of genes related to pyroptosis, based on the extraction of total RNA from an infected culture of cells of endothelial microvessels of the brain of white mice and subsequent transcription into complementary DNA (cDNA). The content of proteins in the supernatant of infected cells was determined experimentally. Molecular genetic methods for detecting cytokines released by infected cells have developed experimentally.

For the first time, he has investigated the stages of pyroptosis development in endothelial cells of brain microvessels infected with S. suis 2 (bEnd.3).

With the help of an electron microscope, morphological changes characteristic of pyroptosis were established for the first time in the S. suis 2 infected culture of endothelial cells of brain microvessels (bEnd.3).

4. Scientific and practical significance.

The pathogenesis of meningitis due to streptococcal infection caused by the zoonotic pathogen S. suis serotype 2 was theoretically and experimentally substantiated. On the basis of in vitro studies, a stepwise process of pathogenetic processes of the development of pyroptosis in the culture of endothelial cells of microvessels of the brain of mice (Bend3) infected with S. suis 2 was established.

The obtained research results can be used in the development of vaccines for streptococcal infection of pigs caused by S. suis serotype 2 and targeted paratherapeutic agents in veterinary humane medicine and means of preventing pathological changes in the brain by similar processes.

The main results of the dissertation work are presented in the scientific and practical recommendations «Streptococcus suis infection (Etiology, Epidemiology, Laboratory diagnosis, Prevention and Treatment)», protocol № 18, dated 29.05. 2023, authors: Mingcheng Liu, Oksana Kasianenko.

The obtained research results and conclusions were implemented in the educational process during the teaching of the disciplines: «Veterinary technologies for the prevention of infectious diseases of animals», «Epizootology and infectious diseases», «Anti-epizootic measures in animal husbandry» at the Department of Epizootology and Parasitology in the training of specialists for the degree of higher education «Master» from the field of knowledge 21 «Veterinary» in the specialty 211 «Veterinary Medicine» at the Sumy National Agrarian University.

The obtained research results are recommended for implementation in the educational process during the study of the educational component «Veterinary Microbology» at the Henan Institute of Science and Technology, China.

5. Completeness of presentation of the dissertation material in scientific publications.

Based on the materials of the dissertation, 13 scientific works were published, including 5 articles in specialized scientific publications of Ukraine, 7 theses of reports in collections of conference materials, 1 scientific and practical recommendations.

6. The degree of validity of scientific statements.

Liu Mingcheng's Thesis is a completed scientific work on which he worked during 2019–2023 years, it fully meets the set goal and objectives of the research. The results of his own research obtained by the doctoral student are properly substantiated and logically compared with the data of other researchers. The conclusions are formulated on the basis of the obtained practical data, which prove the author's awareness of the problem under study.

The results presented in the dissertation determine the modern ideas about the pathogenetic processes of the development of meningitis due to streptococcal infection caused by S. suis.

7. The structure and content of the dissertation, its completeness and compliance with the established requirements for design.

The dissertation consists of sections: an Introduction, Literature review on the topic and choice of research directions, Objects and methods, Results of own research and Summary and analysis of results, Conclusions, Production proposals, Liturature reveur (222 names), Applications. The work is laid out on 129 sheets of computer text, contains 5 tables, 30 figures.

In the introduction, the author substantiated in detail the relevance of the conducted research, taking into account modern knowledge on the topic to which the dissertation is devoted; clearly defines the object and subject of research; lists the research methods used by her; objectively highlights the scientific novelty and practical significance of the obtained data; determines the personal contribution, indicates the place and period of approval of scientific research and the number of publications prepared on the topic of the work.

Chapter 1. «Literature review on the topic and choice of research directions» is informative and analytical, corresponds to the purpose of the research, written at a high scientific and methodical level with the use of a sufficient number of primary sources. The list of references is presented in order of citation in accordance with the current standard. In general, this section of the dissertation is quite voluminous, written competently and reflects the author's ability to critically analyze literature data and draw objective conclusions from them. Such an analysis of the literature, made after processing a significant mass of foreign literature, allowed him to clearly define the problem and correctly formulate the purpose of the work and the tasks for its solution.

Chapter 2. «Objects and methods». The author substantiated the objects and methods of research, stages and conditions of conducting experiments. In this section, he presented specific research methods, namely: molecular genetic (polymerase chain reaction in real time), microbiological (microscopy, electron microscopy), analytical (Western blot method for detecting specific proteins in cells), immunological (ELISA method to detect the expression of individual genes in cell supernatants), and statistical (processing of research results).

Thus, to perform the work, the dissertation student used modern methods of experimental research, which allowed him to realize the tasks set before him.

Chapter 3. «Results of own research». The results of experimental studies conducted by the acquirer are presented here. The materials of this part of the dissertation work are presented in the form of 10 subsections.

In section 3.1. «Bacterial count» PhD student has presented research on determining the level of adsorptioncell cultureof different breeds Streptococcus suis under the influence of waves of different lengths: 450 nm, 570 nm, 600 nm, 650 nm. In section 3.2. «The preparation, identification and culture of brain microvascular endothelial cells» the applicant has presented the results of research on preparation for infection and immunofluorescent identification of the culture of endothelial cells of brain microvessels. In subsection 3.3. «Screening of optimal multiplicity of infection» the results of the study are presented relative expression of cytokines from the culture of cells during different times after infection S. suis. Experiments have established the optimal conditions for infection of brain endothelial cell culture. In section 3.4. «Cellular total RNA extraction and reverse transcription» the author presented data on the development of a method of RNA extraction using the Trizol method from endothelial cells of brain microvessels infected with S. suis (serotype 2) isolates. A study on the assessment of the quality of extracted nucleic acids is given. The obtained data provide an experimental basis for further work. In section 3.5. «QPCR detection» PhD student presented

research, the results of which confirm the presence of pyroptosis in the investigated infected cell culture (bEnd.3) based on the application of the method fluorescent quantitative PCR. In section 3.6. «Western blot detection» the author has presented the data of experimental studies regarding detection of protein expression of cytokines that are associated with pyroptosis, such as IL-18, IL-1β, caspase-1, GSDMD and GSDME. These studies have conducted using Western experimental methodblot. In section 3.7. «ELISA detection» the author has described experimental studies on determining the expression of genes and protein concentrations in the supernatant of the cells of the experimental groups and the control. In section 3.8. «Lactate dehydrogenase. Detection» PhD student has presented experimental studies, the results of which are confirmed cellular pyroptosis of infected cells. He has established the release of lactate dehydrogenase from infected cells, which is an indicator of cytotoxicity. The obtained results indicate a violation of the integrity of the cell membrane. In the section 3.9. «Electron microscopy observation» the applicant has presented the results of research on morphological changes of infected S. suis 2 endothelial cells of brain microvessels (bEnd.3), which are characteristic of pyroptosis. He compared the obtained data with the control.

In the section 3.10. «Conclusions tochapter3» the dissertation presented a logically structured, deep and comprehensive analysis and discussion of the obtained results. In this section, he has summarized the obtained data, compared them with the available data of the literature, and emphasized the fact that the author brought something new to the solution of this scientific problem.

The conclusions consist of 6 points, contain concise information about the results of the research, are structured according to the tasks set in the dissertation, stem from the results of own research and are based on the experimental data presented in the dissertation. Proposals for production are proposed by the dissertation student, properly designed and documented.

So, in general, this section is presented professionally, competently and characterizes the dissertation as a comprehensively prepared and erudite scientist.

8. Discussion clauses and remarks to the dissertation.

Giving an overall assessment of Mingcheng Liu's dissertation, it is necessary to point out some identified shortcomings, inconsistencies, and also ask some debatable questions that need clarification:

1. What are the precautions during cell culture?
2. What are the methods for counting bacteria?
3. What are the precautions for cell RNA extraction?
4. What is the significance of fluorescence quantitative CT values?
5. What factors can affect the results of Western-blot?
6. What's the difference between LDH method and CCK-8 method?
7. What's the difference between scanning electron microscope and transmission electron microscope?
8. Why are the mice cells being tested instead of pig cells?
9. Epidemiology of Streptococcus suis
10. Public health impact of Streptococcus suis.

Grammatical errors caused by inattention to computer typing, some incorrect expressions, etc. should be included among other shortcomings in the dissertation. On page 8 are indicated virulence factors of S. suis 2 such as CPS, SLY, MRP, EF, SAO, Srt, FBPS, SadP, and Eno, but not all abbreviations are listed in the list of abbreviations. The work is not without stylistic flaws. However, the above-mentioned remarks are not fundamental and do not reduce the overall positive assessment and scientific significance of the dissertation work Liu Mingcheng.

9. General conclusion

Liu Mingcheng's Thesis «Experimentalresearch on pathogenesis of Streptococcus suis infection», which was submitted for defense to the specialized scientific council for obtaining the degree of Doctor of Philosophy in the field 21 «Veterinary» with the specialty 211 «Veterinary medicine» according to its relevance, scientific and theoretical level, main results of validity, main provisions and the results published in professional publications, the novelty of the proposal and its practical significance meet the requirements of the Ministry of Education and Culture of Ukraine Order № 40 of January 12, 2017 «On approval of requirements for the preparation of a dissertation» and Resolution of the Cabinet of Ministers of Ukraine of January 12, 2022 № 44 «On approval of the Award Procedure degree of Doctor of Philosophy and annulment of the decision of the one-time specialized academic council of the institution of higher education, scientific institution on awarding the degree of Doctor of Philosophy» with changes introduced in accordance with Resolution of the Cabinet of Ministers № 341 dated 03.21.2022.

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ВІДДІЛ КАДРІВ

The signature of O. Ayshpur is certified
The Head of HR Department Tetiana Sydorenko