



REVIEW

By assoc. prof. Vladimir Svetoslavov Petrov, PhD - Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University - Stara Zagora

Regarding: PhD thesis "Antimicrobial action and biological effects of electrochemically activated water solutions", presented by Toshka Evgenieva Petrova for awarding of the educational and scientific degree "Doctor of Philosophy" in the scientific specialty "Epizootology, infectious diseases and prevention of infectious diseases in animals", in the field of higher education 6.0 "Agricultural science and veterinary medicine", professional area 6.4. Veterinary Medicine.

The review is presented in implementation of decisions of a scientific council, appointed order ZPS-370/05.07.2023 of the Rector of University of Forestry, taken at the first meeting (Protocol No. 10/14.07.2023).

Toshka Evgenieva Petrova has been enrolled in PhD studies, regular form of study by order No. ZSD-21/24.01.2020 of the Rector of University of Forestry. Prof. Dr. Teodora Petrova Popova was appointed as scientific supervisor. The topic of the doctorate was extended by order ZSD-57/17.02.2023. After completing the training activities, successfully passing exams according to the individual study plan and preparing a dissertation project, she was awarded with the right of defense by order No. ZSD-128/24.04.2023 of the Rector of the University of Forestry. By decision of the extended council of the "Infectious Pathology, Hygiene and Control of Foods of Animal Origin" department of the Faculty of Veterinary Medicine at LTU (Protocol No. 192/22.06.2023), the PhD thesis is directed for defense before a scientific council.

I. Relevance of the problem.

Progressively increasing resistance among bacteria to antimicrobial agents and chemical substances used for disinfection is one of the biggest problems in modern medicine. At the same time, a significant part of them are emitted by the treated animals or are of low biodegradability, which favors their entry and retention in the environment. All this necessitated the search for alternative approaches to traditional antimicrobial agents, which are not dangerous for patients and nature, without developing resistance to them. Studies on the efficacy of biologically active plant extracts, essential oils, oligosaccharides, saturated fatty

acids with medium carbon chain length (caprylic, octanoic and decanoic acids) are being deepened. In this regard, I find the presented dissertation work up-to-date and with potential for application in the practice of the presented research models. Electro- or electrochemically activated aqueous solutions (catholytes and anolytes) are non-toxic to higher organisms, do not pollute the environment and are an accessible means to which microorganisms do not develop resistance. These advantages make them suitable for use in medicine for the treatment of various bacterial and viral diseases, as well as for the disinfection of various surfaces.

2. Structure

The PhD thesis "Antimicrobial action and biological effects of electrochemically activated water solutions" is written on 209 pages, illustrated with 38 tables and 58 figures. In writing it, the structure for a dissertation and the balance between the individual parts have been respected. The following sections are distinguished - Contents (3 pp.), Abbreviations used (1 pp.), Introduction (2 pp.), Literature review (47 pp.), Own research with purpose, tasks, materials and methods (19 pp.), Results (65 pp.), Discussion (23 pp.), Conclusions (2 pp.), Recommendations for practice (1 pp.), Contributions (2 pp.), Publications in connection with the dissertation work (1 pp.), References (37 pp.). Added Acknowledgments and Summary in Bulgarian and English.

3. Literary awareness of the PhD student

When writing the dissertation, 348 literary sources were used, of which 29 were by Bulgarian authors. In the last 10 years, 157 (45%) scientific works have been published. This statistic convincingly testifies to the level of literary awareness of the PhD student. In my opinion, it is not correct to indicate only links (references № № 118-132, 228) in the References, especially since some of them are to articles in periodicals, company websites, product prospectuses, YouTube videos, etc. similar.

4. Literature review

The first major part of this section is devoted to the evolution in concepts of electrolysed aqueous solutions. It is focused on the construction of the first devices, the attempts to apply the obtained solutions in different spheres of life. Following is a presentation of the basic device of an electrolyzer and the different types of ionizers. The chemical and physical prerequisites for the activity of the solutions obtained during electrolysis are described in detail. A classification is presented according to the ionic composition, due to the impact of the electric current, according to the composition of the salts in the initial salt solution in the

electrochemically activated waters, and the physical parameters - based on the available ions in the obtained water electrolytes. The main data obtained from the research on the characteristics of the solutions in the anode zone (anolyte, or popularly called "dead water") and those in the cathode part (catholyte, or popularly called "living water") are presented in detail. An economic analysis for the replacement of conventional disinfectants and methods with electrolyzed water solutions is also presented.

I find the section to be well structured, concise and shows the author's good literary awareness, which lays the necessary foundation for presenting one's own research.

5. Own research

The aim is short, clear and concretely formulated. Five scientific tasks have been set for the implementation.

In the "Materials and methods" section, the types of electrolyzed aqueous solutions, the bacterial species and strains on which the antimicrobial effect of the investigated anolytes and catholytes was tested are presented sequentially. The nutrient media for cultivating the bacteria in the experiments and the equipment used are described. In my opinion, HiDetect rapid identification discs are not reliable enough for identification of microbial species in scientific research. The section describes the surfaces on which the disinfection effect was studied, as well as the commercial preparations used for controls. Information is presented on the experimental animals used, clinical patients and obtaining samples from them for subsequent research. All experimental setups for in vitro and in vivo tests, experiments and studies are described in great detail. A statistical analysis of the obtained results has been declared, using appropriate methods.

Due to the small number of animals involved, the impossibility of randomization and control of conditions, studies in clinical patients have the character of pilot trials rather than in-depth scientific studies or, as the doctoral student formulated in task 5, rather of case reports.

6. Results

The obtained results are illustrated with 52 figures, of which 47 are photographs of test tubes, petri dishes, experimental animals and patients, often combined 5 or more in one figure. Not all of the author's original photos are of good quality. Thirty-seven tables are also attached. For the most part, the section follows the sequence of tasks.

Some technical errors have been made in the numbering of the figures. On page 98 the figure should be numbered 12, not 10; on p. 127 respectively 42, not 38. The legend of Table 16 (p. 105) is after the title, but should be below the table itself.

Notes:

In the dogs with conjunctivitis, only patient 3 can correctly discuss the effect of the anolyte used. In patient 1, the lack of microbiological examination before treatment does not allow to categorically claim an antibacterial effect. In patient 2, removal of the foreign body alone was usually sufficient for clinical improvement. For this animal, it is difficult to draw unequivocal conclusions about the antibacterial effect of the anolyte.

When studying the clinical effect of catholytes and anolytes, for greater reliability of the results, it is correct to compare treatments of one ear with a conventional approach (suitable antimicrobial agents, after sensitivity testing) and with tested alternative preparations of the other.

In patients 7, 11-14, with infected wounds caused by a foreign body and ectoparasites, again the effect of the tested anolytes cannot be unambiguously determined, because the removal of the cause of the inflammation is often a sufficient condition for clinical recovery.

7. Discussion

In this section, a thorough, professional analysis of the obtained results is made. In it, the author successfully interprets both his data and those presented by other researchers. Skillfully, scientifically based, he manages to defend his own positions, which allows me to strengthen my opinion about the good literary awareness of the doctoral student. In this discussion, her deep knowledge of the field is even more clearly outlined.

8. Conclusions, Contributions and Recommendations for practice

Based on the obtained results, 9 conclusions are presented. In my opinion, conclusions 7 and 8 are too boldly formulated based on the small number of patients followed.

I accept the proposed 12 original contributions. Like the conclusions, I find some too strongly worded based on the small number of patients followed.

In my opinion, some of the recommendations for practice are too generally formulated.

9. Evaluation of the personal participation and publication activity of the doctoral student.

The doctoral student personally participated in the implementation of the set scientific tasks, confirmation of which are the presented 4 publications, three of which he is the lead

author of. Therefore, the experiments performed, the processing of the results, the presented conclusions and contributions are the personal work of the doctoral student.

No data on participation in scientific forums is presented.

10. Abstract

The abstract of the dissertation was prepared according to the requirements of the Regulations for the Development of the Academic Staff at the University of Forestry. It is written in 51 pages, and over 1800 characters per page. The abstract correctly reflects the purpose, the tasks, the methods used, the results obtained, the formulated conclusions, contributions and recommendations for practice. In my opinion, it is advisable for the abstract to include part of the discussion.

CONCLUSION

The peer-reviewed PhD thesis, with author Toshka Evgenieva Petrova, submitted for the acquisition of the educational and scientific degree "Doctor of Philosophy" in the scientific specialty "Epizootology, infectious diseases and prevention of infectious diseases in animals", is up-to-date, sufficient in volume and structure, which meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the regulations for its implementation and the Regulations for the Development of the Academic Staff at the University of Forestry. I accept the results obtained from the conducted laboratory studies as reliable and personal work of the author. I assess the formulated conclusions, contributions and recommendations for practice as adequate and up-to-date. In the text, single mistakes were made in the spelling of some words. The remarks I have made are more technical or guidelines for further research in pets, so they do not change my overall positive attitude towards the scientific work under review.

I propose to the respected members of the scientific council to evaluate the merits of the discussed dissertation work and to vote positively for awarding Toshka Evgenieva Petrova the educational and scientific degree "Doctor of Philosophy" in the scientific specialty "Epizootology, infectious diseases and prevention of infectious diseases in animals", in the field of higher education 6.0 "Agricultural science and veterinary medicine", professional area 6.4. Veterinary Medicine.

14.08.2023
Stara Zagora

Reviewer:
(Assoc. prof. V. Petrov, PhD)