Opinion

by Prof. Rayko Dimitrov Peshev, Ph.D., head. Of Department "Epizootology and Infectious Animal Diseases" in NDNIVMI, Sofia, on a dissertation on the topic: Antimicrobial action and biological effects of electrochemically activated aqueous solutions for the award of a scientific and educational degree "Doctor" in the field of higher education: 6. Agricultural sciences and veterinary medicine, Professional direction: 6.4. Veterinary medicine, scientific specialty: "Epizootology, infectious diseases and prevention of infectious diseases in animals", presented by Toshka Evgenieva Petrova, from the department: "Infectious pathology, hygiene, technology and control of food of animal origin" from the Faculty of Veterinary Medicine at Forestry University in Sofia.

The dissertation work presented to me for drafting an opinion is written on 209 pages and includes: Introduction -2 pages, Literature review - 45 pages, Own research, Aim and tasks -1 page, Materials and methods - 17 pages, Results - 65 p., Discussion -23 p., Conclusions - 2 p.., Recommendations for practice - 1 p., Scientific applied contributions - 2 p., Publications in connection with the dissertation work - 1 p., Acknowledgments - 1 p., Summary in Bulgarian and English language - 2 pages, bibliography - 36 pages. The same is illustrated with 58 figures and 38 tables.

In recent years, due to the indiscriminate use of antibiotics and chemotherapeutics, bacterial strains have acquired resistance and poly resistance, which in turn leads to great difficulties in the treatment of diseases. In addition to resistance, the widespread use of antimicrobial agents leads to the appearance of a number of allergic conditions in the body. Gram-negative bacteria are characterized by a higher resistance to chemical effects, which makes it difficult to take measures aimed at effective control. Particularly important are *Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa,* and more recently *Campylobacter jejuni and Campylobacter coli*. Pollution of the environment with chemical means used to fight microorganisms leads to undesirable changes in the ecological balance and biodiversity in nature. All this necessitates the search for new, efficient antimicrobial agents, which are not dangerous for humans, animals and the environment, and which do not lead to the development of resistance to them. One of the promising modern approaches in this aspect is the treatment of water with an electric current, in which electroactivated water solutions (catholytes and anolytes) with specific physicochemical properties are obtained by changing the electrochemical characteristics of water. These activated waters are an efficient alternative to antibiotics, since, in addition to being non-toxic to higher organisms, they are also an environmentally friendly and affordable means of disinfection to which (due to their modes of action) microorganisms do not develop resistance.

In the literature review section, the candidate provides historical facts about electrolyzed water solutions (EWS), the principle of the devices for obtaining electrolyzed waters called electrolyzers, the anolytes and catholytes obtained by the devices and their impact on microorganisms and economic aspects of the application of electrolyzed water solutions. Scientific research shows that EWS is a promising tool with a variety of positive effects, applied alone or in combination with other tools. They show not only ecological purity, but are also economically advantageous, easily available and from a chemical point of view, completely safe to work with. The data in the world scientific literature up to this point refer to studies in

relation to the substance, the mechanisms of action, the ways of application, as well as the biological effects in humans. In veterinary medicine, there are few scientific studies of EWS, especially on their impact on clinical (field) pathogenic bacterial strains, as well as in the therapy of infectious processes in animals. The biological effects after oral administration in mammals and birds are also poorly studied.

From the literature review presented in this way, it can be seen that the doctoral student is familiar with the latest research in the field of electrolysis, the mechanisms of EWS's impact on pathogenic bacteria in animals, freely handles the literature on the problem, which is a condition for conducting studies that have not been carried out previously.

In the section own research, aim and tasks, the author aims to study the antimicrobial action and some biological effects of electro - and electrochemically activated aqueous solutions. For the realization of this goal, 5 tasks have been set for implementation. I think that the goal and tasks are correctly set.

In the Material and methods section, the doctoral student describes the use of 17 types of electrolyzed aqueous solutions (EWS), of which 4 are electroactivated (anolytes and catholytes) and 13 are electrochemically activated: 5 types of catholytes and 8 types of anolytes. The antimicrobial effect of different EWS was monitored against 4 types of bacteria and 7 different strains (reference and field, isolated from animals, patients in the FVM at LTU and the period of preservation of the antimicrobial effect of the studied anolytes and catholytes (after their storage in the dark at room temperature for 7, 21, 28 and 29 days). A comparative test of the action of EWS with 3 broad-spectrum, pharmaceutical and commercially approved disinfectants was also carried out. 66 animals were used for the in vivo studies, of which 20 were ducks; 21 dogs of different breeds and ages for paw antiseptics with anolyte and 25 animals with clinical infections. The bacterial strains that were studied, the nutrient media for carrying out the bacteriological studies, the surfaces from which they were obtained and the instruments with which they were studied are described. As controls in these studies 3 commercial preparations were used - vircon, chlorhexidine and derma intensive. 20 ducks and 25 ducks were used in the research. clinical patients – dogs, cats, guinea pigs, gerbils. The feed and blood samples obtained from the animals are described. The devices with which the electrolysis was carried and all other devices with which the studies were performed are described. The methods and the experimental set-up for the in vitro study of the antimicrobial activity of anolytes and catholytes towards different bacterial strains, after their preparation and after different periods of storage, are successively described. The experimental set-up for studying the antimicrobial activity of anolyte in the treatment of mixed sludge samples from an urban sewage treatment plant is given. The experimental set-up for studying the disinfectant and antiseptic effect of anolyte applied to laboratory and skin surfaces, the experimental set-up and sampling for studying the biostimulating effect of catholyte in White Peking ducks and the methods of sampling, isolation, identification and determination of antibiotic sensitivity of bacterial and dermatomycosis pathogens in patients are described. Statistical processing of the data was also performed.

In the results section, the PhD student chronologically gives data from the studies of the antibacterial effect of electro-activated and electrochemically activated aqueous solutions - anolytes and catholytes in vitro against bacteria of different pathogenicity and tracking the preservation of the ECAS effect after different periods of storage at room temperature in the

dark. The antimicrobial activity of an anolyte in the treatment of a sample of mixed sludge from an urban sewage treatment plant was investigated and the efficacy of the application of the anolyte to the sludge was determined. The antimicrobial effect of anolyte when used as a disinfectant and antiseptic agent was studied on different surfaces and materials and the results were compared with the use of a commercial disinfectant. Experiments were conducted and the biostimulating effect of catholyte applied per os to improve the growth indicators of ducklings of the White Peking duck breed was established. The results of the effect of anolytes and catholytes in the treatment of some bacterial and mycotic infectious diseases in patients (in vivo) are reflected, and the good effect of their application has been established.

In the discussion section, the research results are discussed, comparing them with the data of other researchers and hypothesizing the mechanisms of action of ECAS. The results of freshly prepared and aged ECAS are interpreted and it is concluded that both types are active and act on the investigated microorganisms. The data on the impact of ECAS on bacteria found in waste water from the city's treatment plant were also discussed. The results of the oral administration of catholyte in White Peking duck and the action of anolytes and catholytes in the treatment of some bacterial and mycotic infectious diseases in patients are interpreted. From the discussion, it is clear that the doctoral candidate knows the literature well, can correctly interpret the obtained results and express her own opinion on the problem being developed.

Nine conclusions are given that fully correspond to the research results, there are 5 recommendations for practice and 12 original contributions arising from the dissertation work. I believe that the colleague took into account the notes of the preliminary reviewers and the other colleagues present at the previous meeting. In connection with the dissertation work, 4 scientific reports were printed in 3 of which the doctoral student was in first place. With this, it fulfills the requirements of the LDASRB and the regulations of the VMF of LTU.

I also have the following notes on the dissertation work: On page 97, figure 11 precedes figure 10, which is on the next page. It should be fig.12 as mentioned in the text. A technical error was made in it reflection. On page 132 there is fig.38, then on page 135 there is a repetition of fig. 38, and this figure should be 42. I believe that a technical error was made. Figure 10 demonstrating different dilutions of the microorganisms on Muller Hinton agar and Chapman Stone agar the results are clear and for the other two agars not, because the petri dishes are inverted. On page 129 there is a figure 38, and there exist a figure with such a number on page 126, probably a technical error was made in the writing and placement, and because the first concerns a burn, and the second a fungal disease. On page 132 the explanation is for figure 48, not as written below figure 44. The notes I have indicated are not substantive, but technical in nature and do not detract from the value of the development.

Conclusion: The dissertation work presented to me for opinion on the topic: Antimicrobial action and biological effects of electrochemically activated aqueous solutions for the award of the scientific and educational degree "Doctor" by Toshka Evgenieva Petrova, is an up-to-date work of high importance for veterinary medicine. ECAS are antimicrobial agents that are not dangerous for patients and the environment, and do not lead to the development of resistance to them. Their cost compared to classically used antimicrobials and disinfectants is negligible, and their efficacy is high, which is confirmed by the current studies. The set goals and tasks are correctly set and fulfilled, and the results and conclusions drawn are credible. I believe these data will be of benefit to veterinarians and other professionals dealing with

bacterial and fungal infections. I believe that the dissertation work fulfills the criteria of the LDASRB and the regulations of the VMF at LTU and I give a positive opinion.

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