STATEMENT

by Prof. Vasil Kostadinov Manov, PhD,

professor in Department "Internal noninfectious diseases, pathology and pharmacology", Faculty of Veterinary Medicine, UF-Sofia, appointed as a member of Scientific jury by order of the Rector of UF-Sofia No. ZPS-567/14.11.2023.

Subject: Dissertation for the award of the educational and scientific degree "PhD" in the field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.4. Veterinary medicine, scientific specialty "Parasitology and invasive diseases of animals and humans".

Topic: "Studies on cardiac dirofilariasis in dogs in Bulgaria"

Author: Master of Veterinary Medicine Radoslav Mitkov Rafailov

Research supervisor: Assoc. Prof. Kostadin Pavlov Kanchev, PhD

The dissertation of PhD student Radoslav Rafailov is particularly relevant, both in a scientific and purely practical aspect. It is associated with cardiac heartworm disease, a common disease in dogs. The reason for the wide spread of this disease is climate change and the ban on the use of some insecticides, which leads to an increase in the populations of mosquitoes that carry *Dirofilaria immitis*. The obtained results from this study will undoubtedly contribute to updating the information on the prevalence of cardiac dirofilariasis, the changes that occur in the animal organism, as well as to determine the risk periods for the spread of the disease in the country.

The dissertation is presented in 187 pages and is properly structured. The author has included the required sections, according to generally accepted requirements such as: introduction, literature review, aim and tasks, materials and methods, results, discussion, conclusions, contributions, recommendations for practice and references. The cited literature includes 393 titles, of which 62 are in Cyrillic and 331 are in Latin.

The literature review is well structured and thorough, showing a good awareness of the author. It presents the main thematic questions that are related to the aim and tasks of research. A thorough review of the data in the scientific literature has been made, and the information is logically and consistently arranged.

The aim and tasks are precisely formulated, indicating the stages of the research, the specific approaches and methods for their implementation. The six tasks set are well defined and realistic.

The selection of the experimental material corresponds to the aim of the study. The significant number of dogs (2626) used in the screening study is impressive. Classical and modern methods are used in the experiments, which are well described. The statistical processing of the obtained data is done by means of a multivariate dispersion analysis (multiple ANOVA), followed by a Tukey test. The various methods applied (rapid antigenic tests, Knott's method for detecting microfilariae in blood, conventional PCR test, Real time PCR, pathoanatomical methods, etc.) show that the PhD student has mastered a number of research techniques.

In the course of the study, a large number of results are obtained and successfully analyzed. They are very well illustrated with 13 tables and 105 figures, which are sufficiently informative and make possible a real assessment of the established findings. This section shows the PhD student's literary awareness, the ability to analyze and process scientific information, as well as to interpret the obtained results. The data from the conducted researches are compared with results obtained by other researchers at home and abroad.

The obtained results are summarized in 14 conclusions. The presented scientific contributions are objective and properly defined. Divided into four original and two confirmatory. The most important of them are: A complex study of the spread of cardiac heartworm disease by region in Bulgaria was carried out, and the types of mosquito vectors and the dependence between their spread and temperature and seasonal characteristics were established. Through Real time PCR, *D. immitis* microfilariae were proven in the following mosquito species: *Anopheles albopictus*, *Culex pipiens*, *Anopheles cinereus*, *Anopheles vexans* and *Anopheles maculipennis* complex. The period of effective infection of mosquitoes during the summer months in nine cities of the country was established. The screening test for antigen from the parasite in dogs confirmed the presence of the species *D. immitis* in the designated six regions of the country. Mosquitoes of the genera *Culex*, *Anopheles* and *Aedes* were confirmed as intermediate hosts and vectors of *D. immitis*.

I accept the contributions of the dissertation as correctly reflected, precisely defined and justified. They can be defined as supplementing and enriching a scientific field of the presented dissertation.

The PhD student offers seven recommendations for practice related to the diagnosis and intime prevention of the disease.

Two scientific articles are published on the topic of the dissertation - one is independent, and the other is the first author. Three citations are noted, which is indicative of their scholarly value. The PhD student presented part of the obtained results at two scientific conferences, one of which is awarded first place.

The personal contribution of the PhD student in the preparation of the scientific work is undeniable.

In the final version of the dissertation, many of the observed inaccuracies, technical and typographical errors during the preliminary discussion of the project-dissertation work are corrected.

Conclusion

The dissertation on the topic "Studies on cardiac dirofilariasis in dogs in Bulgaria" shows that the PhD student has in-depth theoretical knowledge and professional skills, demonstrating qualities for independent conduct of scientific research. The set tasks have been completed, as a result of which the goal has been achieved. Original scientific results with real application in veterinary practice have been obtained. I accept the scientific contributions and consider that they correspond to the professional field of the developed topic. The scientific metrics fully cover the criteria for acquiring the "PhD" degree according to the Law on the Development of Academic Staff of the Republic of Bulgaria and the regulations for its application.

All this gives me reason for a positive assessment of the qualities of the dissertation work of Radoslav Mitkov Rafailov and to recommend to the members of the honorable Scientific Jury to vote "**POSSITIVE**" awarding the educational and scientific degree **"PhD"** in the Higher Education District 6. Agrarian sciences and veterinary medicine, Professional field 6.4. Veterinary medicine, Scientific specialty "Parasitology and invasive diseases of animals and humans".

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Sofia

Prepared the opinion:
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