



UNIVERSITY OF FORESTRY - SOFIA
FACULTY OF VETERINARY MEDICINE



STATEMENT

of a dissertation for the awarding the educational and scientific degree „Doctor“, field of higher education 6.0 Agricultural sciences and veterinary medicine, professional field 6.4 Veterinary medicine, in the scientific specialty „Parasitology and invasive diseases in animals and humans“

Author of the PhD thesis: Radoslav Mitkov Rafailov, PhD student at the department of „Infectious Pathology, Hygiene, Technology and Control of Foods from Animal Origin“, Faculty of Veterinary Medicine, University of Forestry, Sofia.

Title of the PhD thesis: „Studies on the heartworm disease in dogs in Bulgaria“.

Member of the scientific jury: Assoc. Prof. Roman Pepovich, DVM, PhD, from the department of „Infectious Pathology, Hygiene, Technology and Control of Foods of Animal Origin“, Faculty of Veterinary Medicine, University of Forestry, Sofia, according to Order № 567 / 14.11.2023 of the Rector of University of Forestry, Sofia.

1. General presentation of the PhD student

Radoslav Mitkov Rafailov was born in 07/02/1990. He completed his secondary education in the city of Lovech, at the Science and Mathematics High School profile "Biology and health education with the study of English" in 2009. In 2015 he graduated as a master veterinarian in University of Forestry - Sofia. After that, he worked as a veterinarian at "Koopdoverie Lesidren" OOD, and subsequently at "St. George" Veterinary Clinic Estrella Vet OOD. He began his teaching career in 2016 as an assistant in the department of "Infectious Pathology, Hygiene, Technology and Control of Foods from Animal Origin", Faculty of Veterinary Medicine of the University of Forestry, Sofia. By order № ZSD-28 / 27.01.2020 of the Rector of University of Forestry, he was enrolled in a doctoral course of independent training in the scientific specialty "Parasitology and invasive diseases in animals and humans". On 14.01.2023, by order № ZSD-65 / 22.02.2023 of the Rector of University of Forestry, he was dismissed with the right of defense.

2. Structure of the dissertation

The presented dissertation work is written on 187 standard pages and contains the sections required for the preparation of this type of scientific work: introduction - literature review - purpose and tasks - material and methods - results - discussion - conclusion -



contribution - recommendations for practice - publications in connection with the dissertation - bibliography.

3. Actuality of the topic

Dirofilariasis caused by the nematode *Dirofilaria immitis* is a widespread vector-borne disease leading to the death of dogs in endemic areas. The main factors leading to the expansion of the range of distribution are related to natural ecological and climatic changes in the direction of a global increase in temperatures, the resistance and adaptation of mosquito intermediate hosts to lower temperatures and the emergence of new species, as well as the free transport of humans and animals that become mechanical carriers of infectious and parasitic diseases in new territories. Most of the dogs with established disease do not show a clinical picture, and the infestation in these animals is often incidentally detected after routine screening. In animals with a very high number of heartworms, a clinical picture characteristic of the disease is observed - disturbances in the function of the pulmonary arteries, the right side of the heart and the vena cava. The outcome of the disease depends on the degree and severity of the course: good with adequate treatment for I and II degrees to questionable with a poor prognosis despite treatment for III and IV degrees. All this necessitates the search for modern diagnostic methods for the early detection of the disease. Based on this, I consider the current study to be actual and significant.

4. Literary awareness

The literature review contains a large volume of well-arranged literature information based on the vast number of 393 literature sources. This shows that the PhD student is well acquainted with the research and scientific publications on the issue, as well as his skills to analyze the available information with a focus on clarifying problematic issues having a practical orientation. Data on the prevalence of the disease in Europe and Bulgaria are presented in detail. The general characteristics of the etiological agent and the clinical manifestation of the disease in dogs are reviewed. The possibilities for diagnosis are outlined, paying attention to the most important methods for establishing cardiac dirofilariasis in animals (PCR, ELISA, rapid tests, the modified Knott method, autopsy and immunochromatography), as well as some auxiliary methods (radiography, echocardiography and electrocardiography). A general characterization of the vectors has also been made in detail (species composition of mosquitoes in Europe and Bulgaria; important climate-geographic indicators and data on the development of mosquitoes). In this way, the literature review leads logically to the research objective set by the PhD student.



5. Aim, tasks and research methods

The purpose of the present work is to study the prevalence of heart heartworm disease in dogs in different regions of Bulgaria, as well as to specify the possibilities for diagnosis of the disease, to deepen the knowledge about the pathological changes in the final hosts, about the species composition of the intermediate hosts and the seasonal dynamics of mosquitoes in different areas of the country and to make a correlation between their number and their carriage of *D. immitis*. To achieve this purpose, 6 tasks have been formulated. Although in-depth studies on the spread of the disease in dogs have been carried out in our country, many questions remain unclear and their answer lies in the purpose and tasks.

The study was conducted in the period 2017-2021, and 2626 dogs were examined, distributed by region, habitat, sex, age and breed. 3180 female mosquitoes were caught from different cities in Bulgaria and some meteorological indicators were tracked. The diagnostic methods used, their sensitivity and specificity are considered with the purpose of the study.

6. Results

The section contains a sufficient amount of factual material (66 pages), which is presented consistently and accurately, based on the individual tasks. The results of the studies are reflected in 9 tables and illustrated with 97 figures. The results data showed a prevalence of 11.12% in dogs, with the highest prevalence of *D. immitis* positive dogs being found in the Central South region of our country. Stray dogs between the ages of 2 and 7, mostly larger breeds, suffer from this disease to the greatest extent. Global warming and climate change, accompanied by an increase in average daily temperatures, as well as the extension of the "warm" annual periods, are favorable predisposing conditions for the maintenance of the invasion and its spread on the territory of our country. The main mosquito vectors of the disease on the territory of our country are the species: *Culex pipiens*, *Aedes albopictus*, *Aedes cinereus*, *Aedes vexans*, *Aedes caspius*, *Anopheles maculipennis complex* и *Culiseta sp.*, as it is most often established *Culex pipiens*. The complex studies conducted show that to achieve an objective result in the diagnosis of cardiac dirofilariasis, the combined application of clinical serological tests (SNAP® 4Dx® Plus) and modern laboratory methods (conventional PCR) is required.

7. Discussion

The discussion is divided into parts, following the individual tasks of the dissertation work. A thorough and competent analysis of the obtained own results with the available data from the literature has been made, which gives the section the necessary character of a scientific discussion and emphasizes the skills of the PhD student in handling scientific facts.



8. Conclusions, contributions and recommendations for practice

Based on the results obtained from the conducted research, 14 conclusions were formulated. I accept contributions that are grouped as original (4 pcs.) and confirmatory (2 pcs.). Seven recommendations were made for the practice, but some of them (№ 3, 4, 5 and 6) need to be specified because they are of a conclusion nature.

9. Evaluation of dissertation publications

There are 2 publications in English on the subject of the dissertation in refereed scientific publications, in which the PhD student is the first author, which is proof of the personal participation of the PhD student in the conducted research, which are part of the dissertation work being developed. Some of the results were also presented at 2 national scientific conferences with international participation. Three citations of the PhD student's publications were also found.

10. Dissertation abstract

The submitted dissertation abstract is 39 pages, prepared according to the requirements and reflects the main results achieved in the dissertation.

11. Critical notes and questions

With the notes and recommendations made by me, as a member of the extended departmental council when discussing the dissertation project, the PhD student complied and corrected.

CONCLUSION

The presented dissertation on the topic: „Studies on the heartworm disease in dogs in Bulgaria“ contains sufficient original and confirmatory scientific contributions which enrich our knowledge of vector-borne infections in the dog. Taking into account the large volume of scientific research carried out, the obtained original results and their promotion in refereed scientific publications, meeting all the requirements of Law of development the academic staff of Republic of Bulgaria its Rules, as well as the relevant Rules of University of Forestry, give me reason to evaluate it **POSITIVE**.

I propose to the honorable Scientific Jury to also vote positively and award Radoslav Mitkov Rafailov the educational and scientific degree „Doctor“ in the scientific specialty „Parasitology and invasive diseases in animals and humans“.

14/02/2024

Sofia

Statement prepared by:

(Assoc. Prof. Roman Pepovich, DVM, PhD)