

REVIEW

by Prof. DVM Emil Ivanov Sapundzhiev, Ph.D., DSc
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Concerning: The dissertation presented by DVM Iliyan Stefanov Georgiv on the topic: "MORPHOLOGICAL STUDIES OF THE SPLEEN OF THE DOG", with scientific supervisor Assoc. Prof. DVM Georgi I. Georgiev, Ph.D., from Department: "Anatomy, physiology and Animal Husbandry", of Faculty of Veterinary Medicine (FVM) of University of Forestry (UF), for awarding the educational and scientific degree "DOCTOR", in the field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.4. Veterinary medicine, in the scientific specialty "Morphology".

By order of the Rector of University of Forestry № ЗПС 303/from 09.06.2023 I was appointed a member of the Scientific Jury on the procedure and at its first meeting on 06.07.2023 I was elected as reviewer of the procedure for this dissertation defense.

1. Compliance with the procedure.

The set of materials provided to me on paper and electronic arrangement is in accordance with the list of National documental requirements for the defense of a dissertation, according to the Law and the Regulations for its application at LTU. It contains a total of 13 documents - an application from the postdoctoral student, the printed dissertation work and the author's abstract for it, a report on the fulfillment of the minimum national requirements for scientific and publication activity, in the form of an opinion from the competent commission of the UF, a report on the scientific contributions related to the dissertation work, a bibliography of the published scientific articles in connection of the dissertation, a Protocol of the preliminary discussion (approbation) of the extended CS, a CV according to the European model, a diploma for higher education for "master degree", Certificate of passed exams for the doctoral minimum, Declaration of originality according to Art. 27, para. 2 of Educational Law, Order for enrollment in doctoral studies, Order for deduction with the right of defense of Dissertation, Information card according to the model from NACID, in Bulgarian and English. In the available documentation, there are no negative signals and objections regarding the terms of the procedural steps, the topic of the dissertation, or any reason for its development, which is why, and in connection with the above, the opinion is convincingly imposed that the documents correspond to all legal, moral and ethical norms for the implementation of the procedure of defense.

2. Brief biographical data about postdoctoral student.

The veterinarian Iliyan Stefanov Georgiev studied at a specialized technical school, after which he studied "veterinary medicine" and completed his higher education at the Faculty of Veterinary Medicine of UF, Sofia in 2018 with good result, certified by the attached copy of the diploma with No. 19332/10.04.2018. For about a year and a half, he worked in the UF system, as support staff for the learning process. Since the beginning of the new teaching year 2019/2020, after winning a competition, he has been appointed and works as an assistant in "Anatomy of animals" in the department of "Anatomy, Physiology and Animal Breeding Sciences" of the FVM at UF, where he still works. He was enrolled as a postdoctoral student in an independent form of study by Decree №250/21.07.2022. During this period of formulating the dissertation work, he also passed the necessary exams in the specialty and was directed for defense in due course, by Decree № 160/02.06.2023. He is fluent in and uses English and computer programs in his work. In the FVM of UF, he is involved in the organizational activities of the Scientific Annual Conference of the FVM, of its section "Morphology and Animal Breeding Sciences".

3. Relevance of the dissertation topic and expediency of the set goals and tasks.

The dissertation is thematically related to the issue of blood supply to the dog's spleen. These studies are associated with the state of the body under normal conditions, anesthesia, hematological studies and pathological conditions as a result of acute trauma or immunological response of the body. Although in practice there are many modern and diverse approaches to clarifying the general process of blood supply and blood stream in the spleen, there are still some unclarified statements on this issue, and even more precise and up-to-date scientific evidence is required. In this regard, the choice of the topic is relevant and gives theoretical-applied value not only to the morphological specialty of science, but also to the clinical practice of veterinary medicine.

4. General presentation of the dissertation, knowledge and mastery of the issue.

The text of the dissertation, including 69 figures and 1 table, is presented on 168 computer-formed pages and includes contents, introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusions, contributions, recommendations, list of cited literature, list of publications in connection of the dissertation work, acknowledgments and summaries in Bulgarian and English languages. At the examination, the postdoctoral student showed fluency in the issues being developed and handled the matter in an accessible and

understandable scientific language, with data for evaluation at the post-master's level.

The postdoctoral student is elaborated very well the literature review and it is balanced on relation of the topic of the dissertation from a total of 142 sources, of which 23 are in Bulgarian. They are systematized according to the developed tasks, the obtained results and adequately to the discussion. The review is focused on the anatomotopographic location of the spleen, its main and microcirculatory blood supply, as well as on the use of some practical traditional and new approaches to the diagnosis of the vascular bed, from the combination of which a more complete picture of the anatomical arrangement and topography of the vessels is obtained. The review finishes with a logical conclusion that explains the need for a conventional morphological study of splenic angioarchitecture in the dog with a variety of scientific methods used in the morphological specialty and clinical practice.

5. Research methodology.

The goal of the elaborated dissertation is purposefully formulated, although stylistically the text should have been reshaped according to my preliminary notes, since in the current form two goals are understood, and the main goal is really to conduct the morphological study in several directions - macroscopic, microscopic and clinical-diagnostic. Five main tasks are logically planed, which are also performed consistently and with the necessary scientific ambition, for the additional illumination of the investigated problem. The selection of the 70 dogs used in the research is appropriately made and their distribution by number and gender according to the tasks was consistent with the possibilities for statistical processing and reliability of the data. Although almost double the number of female dogs predominated, no sex-specific variability is reported. The classic anatomical methods used are adequate for the performance of the tasks on the subject, including macroscopic dissection, preparation of corrosion anatomical preparations, x-ray and histological slides - according to three main methods, as well as modern diagnostic clinical methods, including image diagnostics for computed tomography angiography, echography - conventional, contrast-enhanced and color Doppler ultrasonography, and generally correspond to the purpose of the study. The description of the methods and approaches used is correct and consistent and gives the impression that the postdoctoral student has mastered them and can work independently as well as arrange the data obtained from them.

6. Evaluation of dissertation results.

The presentation of the own results in the dissertation is made clear and understandable, as they represent the opportunity for the doctoral student to

conduct independent scientific research, to which both the scientific consultant and the traditions in the department, passed down through the generations of teachers and research morphologists, undoubtedly make a significant contribution. The discussion is conducted simultaneously with the presentation of the own results, following the sequence of the tasks and the data are comparable with those of the different referred authors. Their discussion is correctly interpreted in both confirmatory and original aspects.

Following the results of the first task, it is established that the most common position of the dorsal end of the spleen is in the transverse planes between the thirteenth thoracic and second lumbar vertebra, and the ventral end is respectively between the second and fourth lumbar vertebra. The general position of the organ largely depends on the filling of the stomach, in which it varies and may be tucked into the chest cavity on a full stomach, or in contact with the diaphragm and the left costal wall on a completely empty stomach. The ventral edge of the spleen touches the abdominal wall between the cartilago xyphoidea and the navel in a moderately full stomach, and passes to the right of the median plane, reaching the regio pubica in front of the pecten ossis pubis in a very full stomach. It resembles the boot-like shape of the organ and the S-shaped curve of the cranial rim. One dog (French bulldog) was found to have an accessory spleen, which is a sporadic case and unknown etiology, but is not a precedent for the scientific community.

According to the second task of specifying the anatomotopographical location of the main arterial and venous vessels and their branches of the spleen in the dog the incoming and outgoing blood vessels were traced, and some variations of the separation of the abdominal artery and the number of separated rami lienales from the dorsal and ventral branches of the splenic artery were also confirmed, as well as two similar branches from a. gastroepiploica sinstra. Aa. trabeculares, aa. pulpae albae, sinus venosus (lienalis; venularis), vv. pulpae rubrae and vv. trabecularis are visualized. The impermeability of the efferent sphincter of the sinus venosus (lienalis; venularis) was also tested, by Biodur auxiliary for plastination.

The results of the third task present a visualization of the circulatory system and terminal vascular bed of the spleen in the dog at the microscopic level, considering its closed type of blood supply and the reservoir type of the organ, using a combination and different type of anesthesia protocols. Through the combination of various histological methods on light microscopic and illuminated preparations, all arterial and venous blood vessels of the microcirculatory bed of the spleen of the dog, as well as some structures of the organ's stroma and parenchyma, were identified. During practical work with histological slides is recommended the separate injection

of ink-gelatin solution only in the arteries, or only in the veins, with subsequent staining with hematoxylin-eosin. The proportion of the histological structure of the spleen is changed between red and white pulp when a combination of the anesthetics Acepromazine and Diazepam are used, as well as a normal micromorphology of the spleen with no change of the ratio of the pulps is established, when a combination of Propofol and Acepromazine is used. The greatest change is observed in the color, size and histostructure of the ventral end of the spleen. The observed morphological structures - sinus venosus (syn. venularis; lienalis), are filled with erythrocyte formalin precipitates and accumulated hemosiderin. In the accessory spleen of a dog (French bulldog), synchronous with the main spleen histostructural change was also found, using the same combinations with anesthetics.

According to the fourth task, CT-scans, conventional and Doppler ultrasonograms in real time are used to establish the blood vessels in the parenchyma of the spleen and outside it. Through the CT slides made, the splenic artery and vein, their branches along the hilus, the trabecular arteries and veins were visualized, and it is recommended that in future studies, for better visualization of the vessels, the injection of the contrast agent should be made through v. jejunalis, but through v. cephalica and a. brachialis, should be made as a reserve approach. The "zebra" effect was confirmed, representing the appearance of hyperdense rounded and tubular areas at the end of the arterial and the beginning of the venous phase in the parenchyma of the spleen, emphasizing the observation of the sinus venosus (lienalis, venularis) and vv. pulpaе rubrae also. The silhouette of the spleen in the dog was observed in the transverse planes between the thirteenth thoracic and third lumbar vertebrae on CT-scans. Using ultrasonography, it was established that the homogenous hyperechoic echotexture of the spleen does not change before and during xylazine-ketamine anesthesia. On ultrasonograms, the trabecular veins, which are often highlighted, are confirmed and vv. pulpaе rubrae are identified, which have a hyperechoic wall due to the fatty tissue surrounding them. Aa. trabeculares and arterial rami lienales are visualized, and the splenic artery is demonstrated with a regular oval and tubular structure with a hyperechoic wall, when transversally and longitudinally the transducer is oriented, while the vein of the same name is elliptical in shape.

According to the fifth task, by using of contrast-enhanced ultrasonography, all the phases of contrasting the spleen parenchyma in the dog were observed, namely the arterial phase (wash in), the venous phase (wash out), the phase of powerful pumping to the portal system and the phase of powerful evacuation of the contrast from the organ. Confirmed sequentially, the arterial phase observed between 16-30

sec, with the appearance of a "zebra" effect around 30 sec, then the parenchymal or venous phase between 35-120 sec, followed by the contrast pumping phase through the portal vessels, and between 2 and 3 min complete contrast evacuation as the organ recovers its initial echotexture. Through contrast-enhanced ultrasonography, the sinus venosus (lienalis, venularis) and v. pulpae rubrae and the subtle mechanism of contrast migration between them has been traced, explaining the appearance of the "zebra" effect on ultrasonograms and CT-scans. Retention of contrast after the third minute in the spleen was not observed in the animals studied, therefore it is recommended that studies of the canine spleen should be performed during this time interval, when contrast-enhanced ultrasonography is done. From the results using color doppler, a clear picture of blood flow mapping is obtained, with the different coloring providing information on both the direction and speed of blood flow. The main vessels of the canine spleen are clearly visualized, and the color shades obtained in connection with the strong pressure and eddying of the blood against the transducer, or the coincidence of its direction with that of the outflow of blood, are explained. No pathological findings were reported on the venous vessels, which is theoretically the main goal of this research method and also aiming the prevention of thrombosis and its complications.

In this part, I should note that the prepared figures in the dissertation are made very qualitatively, which I have the impression is the personal work of the post-doctoral student. This also presents him in a good light, as a diligent and immersed in his work scientist. The figures are detailed and clearly marked, which gives a high educational and scientific level to the dissertation work, and can even be used in teaching students.

7. Discussion of the conclusions, contributions and publications to the dissertation.

I accept in substance the formulated conclusions, as they are made on the basis of the fulfillment of the five tasks of the dissertation and they include the general aspects of the results of the dissertation. Their quantitative expression - 16, is an impressive number, but this is the right of the author and is a consequence of the advice of the scientific supervisor of the dissertation, which is tradition it self.

The contributions derived from the dissertation are also of proven scientific and applied value. Of the 6 original contributions mentioned, I think 4 should be accepted, since the presence of an additional spleen (contribution 3) and the establishment of the location of the spleen (contribution 4) are of a confirmatory nature. I perceive the remaining 8 affirmative contributions as proof of thorough, purposeful and successfully completed work.

The post-doctoral student also formulated 15 recommendations that can be used for future research and routine anatomical studies aimed at visualizing the blood supply of the spleen and similar experimental work. They have theoretical and scientific value and are the result of acquired experience and practical work.

In connection with the developed dissertation, a list of three publications is presented, one of which is in press process and I formally accept it. All of them are related to the topic of the dissertation and demonstrate results of the scientific elaboration on the topic. The first two articles are printed in our specialized scientific journal - TMVM, which is widely referenced with a database, and the third is also in the process of being printed in it. Generally, the 3 publications related to the dissertation so far meet the criteria of the Regulations for the Application of the law, regulations and the requirements of the NACID to the Ministry of Education and Science and have fulfilled the indicator of publication activity. I take the liberty of recommending to look for opportunities to publish in journals with an impact factor as well, which is a necessity for future development.

8. Critical notes.

At the approbation, I made a recommendation that the reader should be introduced to the controversy about the use of the terms spleen, which are two in the Bulgarian language, as well as the use of the two terms for the organ in medicine, lien and spleen. I attribute this omission to the technical category, as the same applies to the spelling mistakes made and the formatting of some of the inscriptions on the figures. When presenting the results and discussing the microcirculation of vessels, one must be very careful with the terms arteries and veins, and give precise formulation when it comes to arterioles and venules blood vessels. I also expected to be attached a list of participations in scientific forums during the time of developing the dissertation, which has been discussed so far, for which indicator I have indirect positive data. These judgments do not detract from the merits and qualities of the present dissertation as a whole, and in this case remarks and also trivial comments are unavoidable.

9. Personal impressions.

I know an assistant DVM Iliyan Stefanov Georgiev from the period when he was a first-year student at the FVM of UF - Sofia, in 2012. Even then he demonstrated the experience gained from his studies at the Veterinary Medicine College in Kostinbrod and his attitude to the morphological subjects and in particular to anatomy was expressive, which opinion is shared by the leading teachers of these disciplines. He demonstrates very good working skills and creative abilities in the preparation of anatomical teaching models, as well as ethical collegial feelings and the ability to work

in a team. He knows the necessary rhetoric when work with students and knows how to attract their attention, through which he shows the ability to involve them in the learning process. I dare to recommend him to improve his knowledge of English, which is an urgent necessity on the steep career path as a teacher and scientist.

10. Abstract.

With the necessary procedural assessment of the abstract prepared by the doctoral student, which has the same topic as the dissertation work, I declare that it corresponds in content and results to the dissertation and presents its qualities and merits in full, and can be perceived as an individual publication.

11. Conclusion:

The dissertation submitted to me for review on the topic "Morphological studies of the spleen in the dog", presented by an assistant and post-doctoral student in a free form of study DVM Iliyan Stefanov Georgiev, from the Department of "Anatomy, Physiology and Animal Breeding" at the FVM of UF, with a scientific supervisor Assoc. prof., DVM Georgi Ivanov Georgiev, PhD, is an up-to-date, complex, scientific study with specifically formulated and fulfilled goals and tasks, and obtained results with conclusions and recommendations from a purposeful individual work, and corresponds in full compliance on the criteria of the Law and the Regulations for its application. It contains undoubted scientific, educational and applied contributions to the investigation of the location, blood supply and vascular bed in normal and anesthetized conditions of the spleen in the dog, combining a variety of research morphological and imaging diagnostic paraclinical methods.

In connection with all these mentioned qualities, and also based on my individual impressions of the post-doctoral work, I personally give a positive opinion on the awarding of assistant, DVM Iliyan Stefanov Georgiev, the educational and scientific degree "DOCTOR (PhD)", in the field of higher education 6. Agricultural sciences and veterinary medicine, professional direction 6.4. Veterinary medicine, in the scientific specialty "Morphology".

Reviewer's signature:

(Prof. DVM Emil Sapundzhiev, PhD, DSc)

The review was submitted on: 28 July 2023