REVIEW

by Prof. Dr. Angel Petrov Vodenicharov, Ph.D., Dsc, Faculty of Veterinary Medicine, Trakia University - Stara Zagora, currently retired, member of the Scientific Jury appointed by ZPS 365/03. 07. 2023 of the Rector of University of Forestry

REGARDING: Dissertation work "Morphological studies of the spleen in the dog", presented by master - veterinarian Ilian Stefanov Georgiev for the acquisition of the educational and scientific degree "Doctor" in the specialty (scientific specialty) "Morphology" (01.06.26), in professional direction 6.4. "Veterinary medicine", field of higher education 6. Agricultural sciences and veterinary medicine

The work of assistant professor Georgiev submitted for review for the acquisition of the educational and scientific degree "Doctor" is on a number of aspects of the morphology of the dog's spleen - an interesting organ from both a medicobiological and a clinical point of view.

The dissertation is written on 168 pages and contains 69 figures and one table. The bibliographic reference contains 142 sources, of which 30 are in Cyrillic and 112 are in Latin.

The dissertation is well structured and laid out according to the usual scheme for this type of work: Title page, Table of Contents - 2 pages, Introduction - 2 pages, Literature review - 37 pages, Aim and tasks - 2 pages, Material and methods - 13 pages, of which Material – 2 pages and Methods – 11 pages, Results and Discussion – 77 pages, Conclusions – 3 pages, Contributions – 2 pages, Recommendations – 2 pages, Literature – 16 pages. Publications on the dissertation work - 1 page, - 3 publications are listed, 2 printed and 1 under print - all in the faculty magazine "Tradition and modernity in veterinary medicine". They are collective developments, the PhD student is the lead author and the scientific consultant is in the collective of all three publications. Acknowledgments - 2 pages, Summary in Bulgarian and English languages - 2 pages.

In a spirit of criticism towards the "Literature" section, one can point out the non-uniform way (lack of the so-called uniformity) of writing the text of the literary sources - e.g. in those in Cyrillic, the year of publication is at the end, while in the rest it is after the last author, although here also in some of the publications (Nos. 48, 50, 52, etc.), the year is also at the end.

The **literature review** is well structured, with four subsections, in which the general structure and reasons for the different topographic position of the spleen in the dog, the blood vessels, the histological features and angioarchitectonics, and the imaging-diagnostic methods for visualizing the blood vessels are discussed.

The anatomotopographical location of the spleen is described in detail, with particular attention being paid to changes in its location depending on the filling of the stomach, with which it is in close syntopical relationships, as well as on the degree of blood saturation. Data important for clinical practice are given that with moderate blood loss, the spleen gives off a volume equal to 35% of the lost blood - 2.5 times more than the liver and 5 times more than that of the intestines.

The blood vessels involved in the blood supply of the dog's spleen are examined in detail, and data from publications printed in recent years are presented along with what is established in modern textbooks. A place is also devoted to the peculiarities of the organ's blood supply in other mammals, as well as in humans.

Useful, although somewhat common knowledge, are the data on the microscopic structure of the spleen. The identification of individual subsections 2.3.2.1. and 2.3.2.2. respectively "Spleen Histology" and "Spleen Microcirculation" is, in my opinion, not entirely appropriate, since it is clear from the exposition that both places contain data on both the structure and the organ's blood vessels. In addition, the numbering of said subsections should be 2.3.1.1. and 2.3.1.2. It is better to add the text "Angioarchitectonics of the spleen" for the dog to the main subsection.

Quite logically, in accordance with the research undertaken, special attention was also paid to the application of imaging-diagnostic methods for the visualization of the abdominal organs and especially their blood vessels. The significance of these studies is well outlined in the conclusion of the literature review, where it is clearly stated that the limited number of studies on splenic blood vessels provide motivation for further studies. Also, another thing that is essential for clinical practice is specially noted - the changes in the organ that occur under the influence of various anesthetics.

In this form, the structure of the literature review is in accordance with the scientific problem, it is saturated richly and thoroughly with data presented especially well in the last 10 years - in the literature index 67 sources (41.18%) are from the specified period,

in this number even publications from 2022. The overview ends with a "Conclusion", in which the dissertation's good knowledge, orientation and creative use of the data known so far is evident. The correct orientation and creative interpretation of the literary data allowed the author to convincingly and clearly formulate the goal and the resulting tasks of his dissertation work.

The **goal** is well formulated and is consistent with the title of the dissertation, and the 5 tasks set for its implementation are clearly and specifically described. It is necessary to note that in the final design of the dissertation, the doctoral student complied with the recommendations regarding the tasks and their number was reduced to five, without affecting the scope of the research.

The number of animals used is 70 – 23 males and 47 females, which are sufficient for the performance of the tasks. In addition to the considerable number of animals, the author also applied a wide methodological set including: native preparation, injection of the vessels with two types of plastic, post mortem radiography (also with air) and live, staining with hematoxylin-eosin, incl. and after injection of the vessels with ink-gelatin, enlightened preparations also after Indian ink-gelatin filling, computer tomographic angiography, and three types of ultrasonography – conventional, contrast and Doppler.

The description of the methodical procedures in its predominant part is clear and precise. It can be summarized that with the selected methodological approaches, the necessary prerequisites for obtaining objective scientific data have been created.

It is specifically stated that all statutory requirements for the protection and humane treatment of experimental animals, according to the existing documents, have been complied with.

Own results and comments on them are presented in the summary section "**Results and Discussion**". This approach is relatively less used in this kind of scientific development, but the data presented in an appropriate way and their professional discussion with the known in the literature, gives reason to assume that the merger is successful and can be accepted.

The observed findings are clearly documented, their interpretation is professional, which is the result of the good literary awareness of the author on the one hand and the excellent mastery of the methodologies.

In the studies on the position of the spleen, a significant conclusion was made that the most frequently observed cases are those characteristic of its position with a moderately full stomach. This, according to the author, is most likely due to the enlarged stomach as a result of feeding the dogs with pellets and the advanced age of the animals studied. An important fact not only for the breed anatomy of the dog, but also for clinical practice is the establishment of an additional spleen during dissection of a dog of the French bulldog breed, located in the *lig. gastrolienale*.

In the course of research on the blood supply of the spleen, certain differences have been established compared to what is known in the specialized literature. This was achieved primarily by applying two or more techniques to visualize the blood supply sources of the spleen and the organ angioarchitectonics. As an example in this regard, it can be stated that *a. gastroepiploica sinistra* gives two branches to the splenic hilus, which divide into *a. trabecularis*. The latter continue as the laterally directed *aa. pulpae albae* demonstrated on both corrosion preparations and radiographs.

Also of interest are the established *sinus venosus /venularis, lienalis/* on contrast X-ray images, macroscopic dissection and corrosion preparations after filling with Biodur. The data gave reason to confirm that the blood supply to the spleen in the dog is of a closed type.

Regarding the microscopic structure of the spleen in the dog, the data on the normal structure are mostly confirmatory. However, one cannot fail to point out the interesting and original results obtained for the accessory spleen (see above), as well as the established features of an opposite nature in the white/red pulp ratio when using Acepromazine and Diazepam and Acepromazine and Propofol.

An important part of the dissertation is the data from the computed tomography and ultrasonography studies. Through these non-invasive methods, data have been obtained that have a certain significance for clinical practice. Among them, the following could stand out:

- Intraorgan vessels, as well as those outside the spleen, are demonstrated by applying contrast-enhanced computed tomography angiography

- When applying contrast computerized angiography and enhanced contrast ultrasonography, the "zebra" effect at the end of the arterial phase by filling the *sinus venosus* was established.

- No changes were found in the echotexture, size and shape of the spleen in the dog before and during Xylazine-Ketamine anesthesia.

- When following the phases of the passage of echographic contrast, the parenchyma of the spleen shows differences during the arterial and venous phases, as well as during the last phase - of contrast pumping. It was found that the recovery of the normal echotexture of the spleen (homogeneous hyperechoic) was about 3 min after the introduction of the contrast agent.

- In the Doppler ultrasonography, *sinus venosus /lienis*, *venularis/* in the red range and *v. pulpae rubrae* in the blue or black range.

The attached photographic documentation, with few exceptions, is of high quality and convincingly supports the described results on the one hand, as well as the appropriate choice and appropriate combination of morphological and imaging methods. The absence of a scale line in the figures can be pointed out as a gap in the visualization of the observed finds, which undoubtedly leads to difficulty in their interpretation.

The results and their discussion are summarized in 15 conclusions. In principle, such a number is large for such a dissertation development, but considering the considerable methodological set, it can be considered realistic.

The dissertation contains 6 originals and 8 corroborative contributions that faithfully present the achievements of the development. To them I would add a third group - methodical contributions, which would include the combination of morphological and imaging methods for complex studies, both in norm and in experiment.

The development is also of an applied nature, which is contributed by the considerable number of recommendations – 15, some of which have a clear practical orientation.

My impressions of the presentation and discussion of the dissertation work at the extended departmental council, as well as the opinion of the scientific consultant expressed during the development of the dissertation, are enough to consider that this work is the personal work of the doctoral student.

The relevance and significance of the problem treated in the dissertation, the well-formulated research objectives, the good literary awareness, the high methodical level of the development and the achieved original contributions are indisputable.

The indicated critical remarks are mainly of a technical nature and do not reflect the overall positive assessment of the dissertation work.

The abstract is written and formatted in accordance with the requirements.

CONCLUSION

The dissertation "Morphological studies of the spleen in the dog" is relevant and relevant, both for species anatomy and clinical practice. The purpose of the research is formulated on the basis of a good knowledge of the literature on the problem. The methods used are selected correctly, and their combination is successful. As a result of the conducted research, data were obtained, enriching the knowledge of the topographical anatomy, vascular system and morphology of the spleen in the dog. The conclusions are well founded. It can definitely be assumed that the doctoral studies were conducted correctly, as a result of which the doctoral student acquired sufficient skills for further independent planning, organization and conduct of morphological research.

The dissertation contains scientific, scientific-applied and applied results, which represent contributions to science and meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the mentioned law and the relevant Regulations for the Development of the Academic Staff at the Forestry University.

Bearing in mind the above, I give my positive assessment of the presented dissertation work, and this gives me reason to confidently propose to the honorable Scientific Jury to award the educational and scientific degree "DOCTOR" to Master Ilian Stefanov Georgiev in the specialty (scientific specialty) "Morphology", in a professional direction 6.4. Veterinary medicine and field of higher education 6. Agricultural sciences and veterinary medicine.

28. 07. 2023.
Stara Zagora

Reviewer:
(Prof. Angel Vodenicharov)