REVIEW

from Prof. Nasko Yovchev Vasilev, DVM, PhD

Department of "Obstetrics, reproduction and reproductive disorders", faculty of Veterinary medicine, Trakia University, Stara Zagora, Bulgaria, appointed as a member of the scientific jury according to order № ЗПС-640/01.12.2022 г. of the Rector of UoF, Sofia..

of the materials presented for the defense 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 "Obstetrics and gynecology of animals and diseases of newborn animals" by Aleksandar Stoimenov - PhD student with scientific consultant Assoc.Prof. PhD Kalin Hristov.

Biographical data

Aleksandar Stoimenov was born on 16.02.1993. He graduated as a Master of Veterinary Medicine in 2018 at the University of Forestry , Faculty of Veterinary Medicine, Sofia. In the period 2018-2019, he worked at the Veterinary Center "St. Antim". At the beginning of 2019, he started as an assistant at the Faculty of Veterinary Medicine of UoF, Sofia. Enrolled in the doctoral program of self-study with order No. ЗПС - 164 of 28.06.2021. Dissertation topic „Research on the prevalence, aetiology and natural defence mechanisms of the mammary gland in sheep with subclinical mastitis“. Participant in scientific conferences and symposiums in the country. He uses English language and computer programs in his teaching and research work.

The dissertation is properly structured and meets the generally accepted requirements.

Actuality of the problem

Sheep breeding is a promising sub-sector of animal breeding, having an important economic importance for many countries in the world, including Bulgaria. The biological characteristics of sheep allow them to adapt to climatic conditions, terrain, feeding and breeding.

The products obtained from the sheep have unique properties, which makes them highly valued in the market. In terms of chemical composition, sheep's milk is significantly superior to cow's milk. It has much larger amounts of fat, calcium, protein, cobalt and phosphorus. Vitamins of group "B" are also in larger quantities. Sheep's milk is extremely rich in replaceable and irreplaceable fatty acids and is very well absorbed by the organism. It contains large amounts of vitamin A and D.

The presented dissertation examines the issues related to the prevalence and etiology of subclinical mastitis in sheep breed for milk, the changes in the cytological composition, the physico-chemical parameters of the milk and some of the protective mechanisms of the mammary gland.

The existing data from the conducted studies are for periods distant in time, while at the same time there have been changes in the breed composition and breeding technologies. New research with a modern approach is needed to highlight the relevance of the topic.

The last paragraph of the introduction is very definitive, but the subsequent sections, with the exception of the literature review, do not deal with the issues regarding the diagnosis of subclinical mastitis in dairy sheep, as well as the optimization of early diagnosis, therapy and prevention. No research, results or discussion was subsequently conducted.

Degree of knowledge of the state of the problem and interpretation of literature data

A literature review is prepared on the state of the problem, including data on the anatomy and physiology of the mammary gland. The issues related to the protective mechanisms of the mammary gland, the prevalence and etiology of mastitis in sheep were examined. Data from targeted studies on the main microorganisms causing mastitis in sheep in different countries and breeds are cited. Predisposing factors for the occurrence and development of inflammation of the mammary gland in sheep are described. The applicable methods for diagnosis, treatment and prevention of mastitis are summarized. 254 literary sources are included, of which 8 in Cyrillic and 246 in Latin.

It would be good to include a short conclusion based on the presented extensive literature review. Point six Treatment and prevention of mastitis remains unexamined in the following sections.

Aim and tasks.

The stated aim corresponds to the subsequent tasks and gives the opportunity to confirm the relevance and need for conducting research in sheep farms in the country. The PhD student sets five tasks with the resolution of which to update the results on the prevalence and etiology of subclinical mammary gland inflammation in dairy sheep.

Material and methods

Animals of four breeds from five sheep farms were included in the studies. The research was carried out on a flocks with 3,800 sheep over a two-year period, aged from 2 to 6 years, with different methods of breeding and milking in compliance with the requirements of the regulatory documents for animal welfare. But actually 78 sheep or 156 halves, representing 2% of the sheep on the five farms, were tested. Presented as an episiotological study… The prevalence of subclinical mastitis is studied, and methods for establishing clinical signs of inflammation, such as the coagulation test, are described.

Methods were used to assess the clinical condition of the animals, microbiological, cytological and physico-chemical studies, as well as statistical processing of the obtained results.

Presentation of the results

The obtained results are presented in an appropriate manner through twenty-seven tables and twelve figures.

Current data on the prevalence of subclinical mastitis in the six sheep farms are presented, showing a high relative proportion ranging from 29.4% for farm B to 53.3% in farm D, an average of 41.3% (isolated microorganism and somatic cells over 500,000 in ml) with greater frequency in both milk halves. Regarding the main microorganisms that were isolated from the obtained milk samples, it was found that S. aureus ssp. aureus in 40%, Enterococcus sp. - 25%, S. epidermidis - 25%, S. saprophyticus ssp. saprophyticus -5% and S. lentus -5% for farm A. For farm B, the isolated microorganisms are of different genera and species - L. lactis ssp. lactis - 44%, S. xylosus -31%, S. epidermidis - 10%, S. haemolyticus -6%, S. cohnii ssp. urealyticum -6% and Micrococcus sp. 3%. Particularly informative in my opinion is table 11 with the summary results for the most frequently isolated microorganisms from the milk samples. The studies carried out on sensitivity and resistance are particularly relevant, both for animal and human health. Sensitivity of individual isolates is still good to individual antibiotics, but resistance to Colistin, Kanamycin, Amikacin, Gentamicin Novobiocin, Amoxicillin and Penicillin in individual farms is of concern. The following question arises: *How to explain the results obtained in terms of sensitivity and resistance to different antibiotics in individual farms? Quote from the Discussion section* „This is most likely due to the excessive and inappropriate use of antibiotic preparations on farms and failure to comply with the prescribed therapeutic courses". It deserves attention and consideration when implementing the drug policy in our country regarding the regime of use of antibiotic preparations in farms.

No number of milk halves, milk samples or isolates is indicated in any figure or table from point 1 of the Results section. At the same time, the relative share, minimum, maximum and average values are presented. Yes, animals are insufficient, but that doesn't mean we shouldn't point them out. Thus, one does not get an idea of how the presented results were obtained and how significant they are. As the results obtained from the biochemical blood test are presented - table 20.

For the good overview of the obtained results, it would be good to clarify the terms used - infected, healthy, affected and unaffected …. Characteristics such as "most sensitive microorganism" or "most resistant", can an equal sign be put. *The use of expressions such as - microbicidal activity of milk could have been avoided - ? Microbicidal oxygen-dependent activity of milk?*

The established changes in the cytological composition and physicochemical parameters of milk in lactating sheep with subclinical mastitis confirm that significant changes occur only in individual

parameters, such as % fat and % protein. An increase in serum total protein concentration by 5.95% was registered in animals with bilateral subclinical mastitis ($P<0.05$) with a decrease in albumins (18.11%) and an increase in γ-Globulins by 5.3%. But these results cannot be used as diagnostic criteria.

The conducted studies of the humoral and cellular factors of natural resistance contribute to complementing the knowledge on the mechanisms of development and ongoing processes in subclinical mastitis in sheep. The determined changes in the content of immunoglobulins in the blood serum show a significant decrease in IgG in animals with subclinical mastitis compared to healthy ones. A similar trend was observed for IgM. At the same time, when examining the same indicators in milk, the exact opposite trend of increasing the values of IgG, IgM and IgA was registered. These results can be included in the survey of sheep for the prevalence of subclinical mastitis and also with prognostic value in applied treatment after future studies are carried out.

Discussion of results and references

A discussion of the obtained results was carried out, comparing them with studies carried out in different countries. A parallel comparison of the established changes in the studied indicators is made, both between individual studies and for different breeds of sheep. A successful attempt has been made to compare and logically explain the recorded dynamics of the studied indicators regarding the prevalance, etiology and natural protective mechanisms of the mammary gland in sheep with subclinical mastitis. There are also some inaccuracies, such as page 112 our results show a higher prevalence of *mastitis* (*clinical or subclinical*) in certain farms at the present time“ and the subsequent explanation "This is most likely due to the increasing resistance of pathogenic microorganisms to antibiotics and non-compliance with zoohygiene requirements" in all or individual isolates, farms and studies. A very strong generalization, but according to the results there are differences between individual farms. As indicated, the milk samples for the individual farms in the present study were obtained in different seasons. This fact should be taken into account when discussing the obtained results. On page 125, a comparison is made between the results of the BMT examination of the milk halves and the microbiological examination. *It does not make a good impression* to add uncommented results in the previous section and insert them in the Discussion section.

Conclusions

I accept the presented conclusions except № 5 *„Thermography is not suitable as a stand-alone method for diagnosing subclinical mastitis in sheep. It could be used as an additional screening method“*. In which cases and how will its use contribute?

Scientific and scientific-applied contributions

Three original and four confirmatory contributions are presented in the dissertation.

Recommendations for practice

Recommendation needs editing „*For the diagnosis of subclinical mastitis in sheep based on somatic cell count, a reference value of 500,000 cell elements in 1 milliliter of milk should be used*". The presence of subclinical mastitis cannot be assumed on the basis of an increased number alone.

Evaluation of the degree of personal involvement of the PhD student

The research was planned in advance and carried out in good faith, therefore I consider that the PhD student has enriched his theoretical knowledge and increased his practical skills.

Published articles

The results of the developed tasks are included in two scientific works published in the scientific journal of the FVM. I allow myself to recommend the PhD student to publish the results of his studies in journals with impact factor (Web of Science) and impact rank (SCOPUS).

The presented dissertation abstract reflects the structure and content of a dissertation work.

Conclusion

On the basis of the different research methods learned and applied by the doctoral student, the correctly conducted experiments, the generalizations and conclusions made, I consider that the presented dissertation meets the requirements of the Law of development the academic staff of Republic of Bulgaria its Rules, which gives me the reason to evaluate it Positively.

I suggest to the Honorable members of the Scientific Jury to vote positively for awarding the educational and scientific degree "Doctor" to Aleksandar Stoimenov in the scientific specialty "Obstetrics and gynecology of animals and diseases of newborn animals", field of higher education 6.0 "Agricultural sciences and veterinary medicine", professional field 6.4 "Veterinary medicine".

06.01.2023

Signature:.....

(Prof. Nasko Yovchev Vasilev, DVM, PhD)