

SHORT TERM SCIENTIFIC MISSION (STSM) - SCIENTIFIC REPORT -

Action number: CA15224

STSM title:

Assesment of individual keel bone damage using different diagnostic methods - Phase II

STSM start and end date: 20/01/2021 to 12/02/2021

Grantee name: **BECSKEI Zsolt**

PURPOSE OF THE STSM

The intensive scientific and practical work on studying the Keel Bone Damage for last few years resulted in different methods available for detection of the KBD.

The aim of this STSM was to continue the collaboration between the Faculty of Veterinary Medicine in Belgrade and the Faculty of the Veterinary Medicine in Stara Zagora in the field of analysing and practice of the available diagnostic methods for detecting and assessing the KBD. This collaboration and research focus on KBD started in 2020, by approval of my first STSM. Then, I had the opportunity to practice and study the standard palpation method, ultrasonography and radiography, the visual and palpatational exploration at necropsy. As it is well known that the KBD includes not only a deviations but in many cases fractures also, for better understanding the pathogenesis and the overall nature of KBD it is crucial to have an insight into the deeper structures of the affected keel bone. The analysis of the histopathological sections of the deviated or fractured keel bone could have application value in the diagnosis, patophysiology, pathomorphology of the KBD. In my second STSM, we will focus on this aspect of analysing the KB.

All the mentioned available diagnostic methodologies have different practical, diagnostical and scientific value, but all together can help the better understanding of the KBD in laying hens.

Using all those facts in mind, the main purpose of my second STSM was to further gain my skills and knowledge in the field of different diagnostic methods for detecting and defining the KBD in laying hens, with special attention on histopathological aspect.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Just like for the first time, the Host institution of my STSM work was the Faculty of veterinary medicine University of Trakia, Stara Zagora, Bulgaria. During my stay at the Department for internal medicine and therapeutics, under the supervision and coordination of Assist. Prof. Lazarov Lazarin, I spent a three week course using variety of diagnostic tools for assesement of the KBD in laying hens, but the main focus was analysing the histological sections of keel bones from KBD affected laying hens. The very friendly and helpful team of the Department of Pathology of the Faculty of Stara Zagora, especially dr Ismet Kalkanov, a pathologist, ensured all the technical and professional support needed. He also helped a lot in commenting the histological findings.

After collecting the literature data, dealing with pathological and pathohistological aspects of the KBD we realized that there is a limited publications available in this field. There are some scientific teams working on the topic, but publications are still missing and this fact make us more enthusiastic in our small research work.

The Pathology department of the faculty collaborates with a local egg producing farm, where the laying hens arrived from, all as corps. The farm has the LSB line, in total 6000 laying hens. The rareing management at the farm is cage free, the hens are on the floor with wood-shaving bedding, without special enrichment tools. The nests are situated at 75cm heigh. The birds have metal perches situated at 40-120cm height distanced from each other 2 meters.

After individual labelling of all the corps, the body condition was evidenced, signs of cannibalism or other damagin behaviour were noticed. The palpation method was applied on all the corps and the detected deviations of the keel bone, fractures of the keel bone were evidenced, and the localization and severity of tese changes wese assingned. The corps were assessed radiographycally, using both the dorso-ventral and lateral position for exposition. After that, the the keel bone region were analyzed using ultrasonography. Finally, necropsy was done on all the tested hens, while the keel bone was detailly analysed macroscopically and evidence of the kell bone deformities and fractures were evidenced and assessed. Samples of the keel bone with detected fracture and deviation were taken for histopathological analysis.

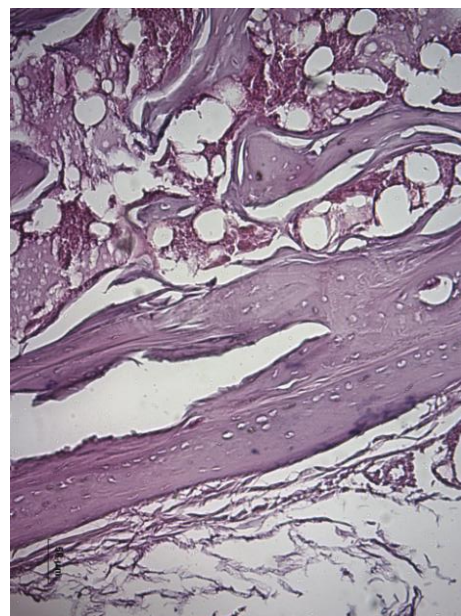
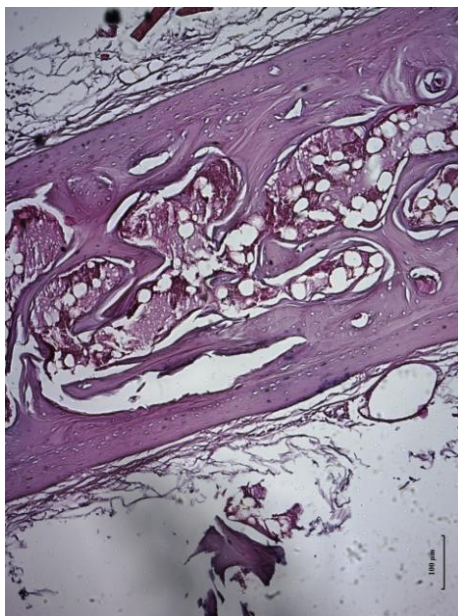
The keel bone samples were decalcified applying the 5% nitric acid method. After the decalcification the standard procedure of paraffine embedding was done. Appr. 4 micrometers thin histological sections were made using a mechanical microtome and for staining the samples the Hematoxiline and Eosine method was applied. For some sections Toluidine Blue staining was additionally carried out, as a special staining method for better visualisation of the connective tissue. The photodocumentation of the histological sections of the analysed keel bone samples was done using "Optica Microscope - Italy" microscope. In accordance with the standard pathohistological procedures, the measure barcode was assigned to the images, indicating the size of the visible elements at the sections.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

As it was expected, different diagnostic methods gave different results, regarding the prevalence of KBD. Just like in my first STSM, the palpation method was the less sensitive and showed that near 50% of the tested birds had KBD. As usually, the detected fractures were positioned at the distal part of the KB. Also, C and S type deviations with variable severity were evidenced by palpation in 38% of the cases. Similarly to the results of my first STSM, using the ultrasonography and the radiography method showed more sensitivity than the palpation. 58% of the ultrasonographically tested hens and 64% of the radiographs showed an evidence of KBD. The localisation of the fractures were mainly in the mid part and at the caudal part of the KB. Macroscopic analysis of the dissected keel bones at necropsy revealed a presence of variety degree and stage of KBD in 60% of the tested KB.

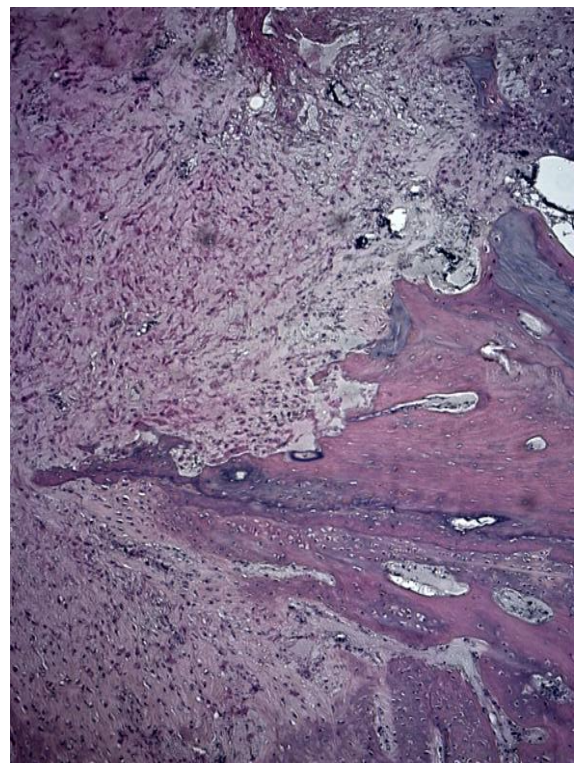
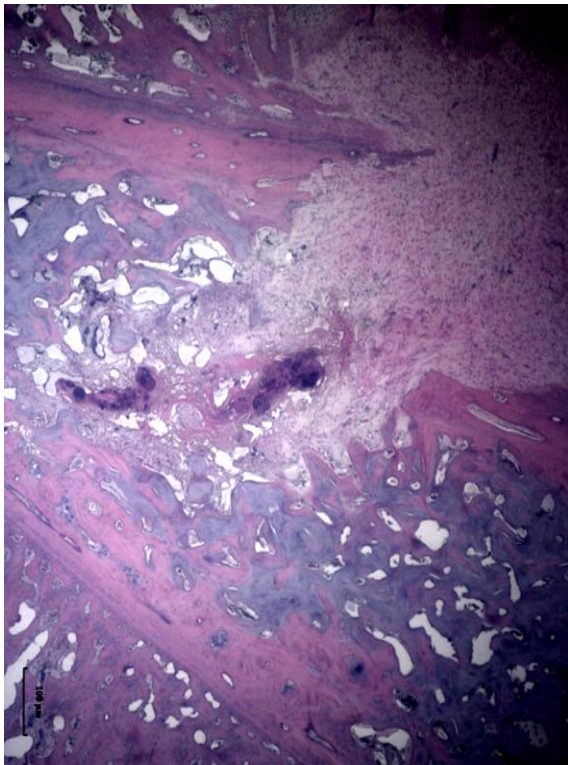
Analysing the histological sections of intact keel bones and the selected ones with detected fractures and deviations, it was evidenced that the keel bone tissue of the affected birds shows a wide range of pathological changes.

The intact keel bone has a specific structure. The compacta of the bone is covering the spongiosa. The thickness of the compacta is different between the tested hens and between the localization of the samples of the same keel bone. Outside the compacta, the thin layer of the periosteum is covering the bone (Fig 1 and 2).



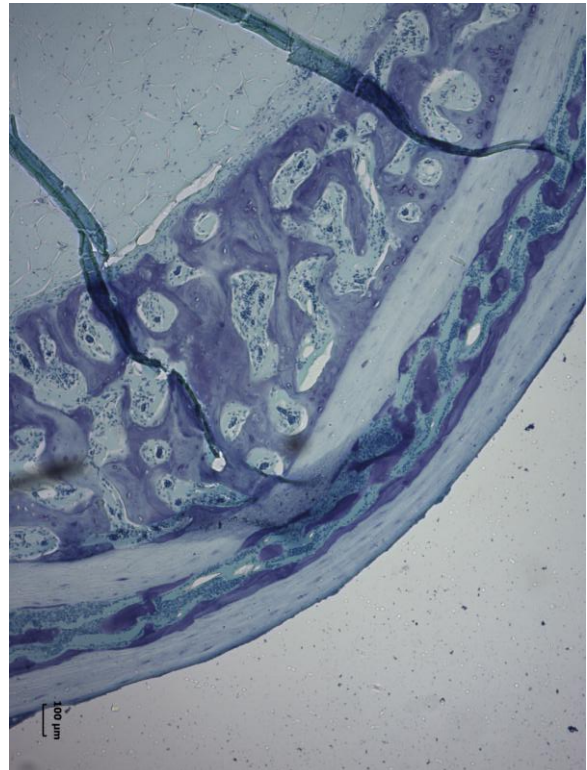
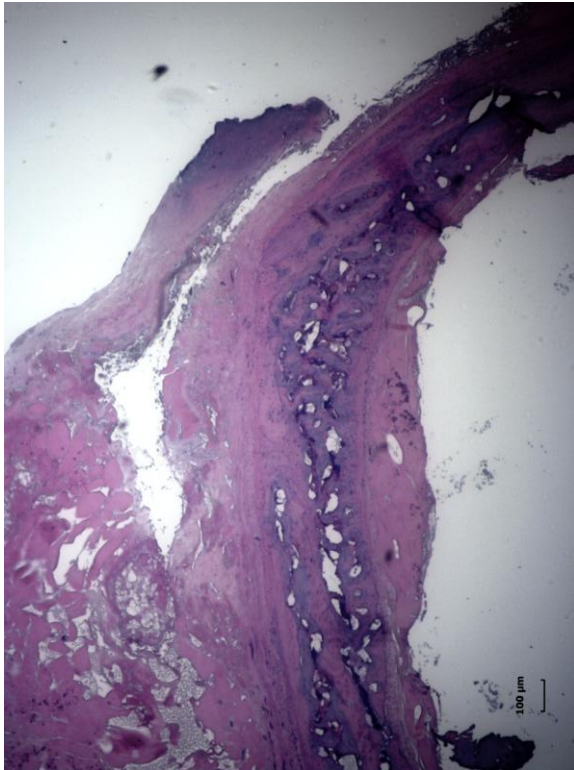
Analysing the KB with fractures, we evidenced a discontinuity of the compacta, mostly on one side of the KB (Fig. 3), but there were evidenced cases where both sides of the KB showed the evidence of the fracture. In the literature data, there is an indication about the presence of hemorrhage and inflammatory infiltration of the fractured site and the surrounding tissue. Our observation didn't find evidence of hemorrhage, nor inflammatory cells at the sites of fractures. What we found is a presence of a variety amount of connective tissue, at the direct site of the fracture and in surrounding site (Fig. 4). The connective tissue was the main component of the callus in the majority of the samples. The presence of a connective tissue tells us about the fact

that the pathological change we have is not a fresh one (not an acute, fresh fracture). It needs time for making a connective tissue agglomeration at the site of the fracture. According to the literature data, the time elapsed is variable and depends on a numerous factors (is there a complication and infection of the fracture site, the species, the age of the birds, the overall health status, the stress status, etc.). In some cases, the outer line of the callus material consisted of islands of cartilaginous tissue (Fig. 4), what is a physiological process of bone wound healing. The amount of the callus tissue was variable between the tested KBs. No sample was found with a totally calcified callus material. This might be because the birds got injured not too long time before the analysis, but the fracture had enough time to resolve the hemorrhage and the local inflammation. It might be that if they lived further, in the next phase of wound healing the cartilaginous phase will occur, and after that via the remodeling, the aged bone callus will appear and strengthen the injured KB. There is a discussion about the pain, which occurs at fracture sites. We did not have the opportunity to measure it, but analysing the fracture sites, it is evident that the continuity of the compacta is interrupted, with damaging the periosteal layer, which is known to be well innervated. Taking those simple facts in mind, it could be concluded that pain is a following symptom of KBF. It might be that the size and the localization of the fracture can affect the level of the pain, but those are all our speculations. Further investigations are needed to define those questions, and ensure better understanding of welfare of the KBF affected birds.



Interesting was the finding that the KBs which showed an evidence of deviation also showed changes in the bone and surrounding structure. Histological sections of some of the C and S type keel bone deviations showed additional ossification sites. Actually, what we found is that outside

the compacta of the deviated site of the keel bone, but below the periosteum, a new cartilaginous and fibrous tissue occurred (Fig. 5), reminds a new growth site of the bone. It could be explained with a theory that as some forces acting on the bone resulted in change its shape (C and S shaped curves), but as an adaptation process to that forces, besides that the bone changed its shape and got deformed, it is trying to strengthen its structure and trying to make those sites thicker and stronger with newly synthesized tissue, outside the compacta.



In conclusion we can say that these and lot of other questions that we are not able to answer at the moment, needs further investigation with the aim of better understanding the nature of the KBD. Real time monitoring of the live birds and analysing their corpses could surely give us more essential information we need. Using histological analysis of the KBD sites we can explain some details about the nature of the accidents which causes the KBD and about the robustness of the birds to cope with that insult.

At the end, we can underline that even though different diagnostic methods have different applicability in practice, all of them have their value, not only in assessment of the KBD, but in better understanding of the nature of laying hens in general, their behaviour, physiology and pathology, in one word "their short lives".

FUTURE COLLABORATIONS

This fruitful STSM, supported by the COST Action 15224, resulted in a new and continuing collaboration between the Faculty of veterinary medicine in Belgrade, Serbia and the Faculty of veterinary medicine in Stara Zagora, Bulgaria.

After the succesful first STSM in 2020, we continued the research on KBD what resulted in a approved application for a second STSM. The results and experiences from these two STSMs will be published in a form of common article and soon, as the epidemiological circumstances allow, will be presented in some of the upcoming International Symposias with an acknowledgement note to the Cost Action 15224. The two faculies are working on extension of research collaboration, including students and lecturers. Also, through the COST action, we are actively working on further opportunities for collaboration in the area of precision livestock farming and defining a bilateral project.

For the second time, I would like to express my grateful thanks to the Action leaders of the CA15224, and my supervisor Prof. dr Lazarin Lazarov, for their support and the oppurtunity to gain my skills in the field of KBD. Also, many thanks to Prof. dr Ismail Kalkanov form the Pathology department, who shared his time and thoughts focusing on histopathological assessement of the KBD lesion.

2021. February 15.

STSM Applicant

BECSKEI Zsolt

Some of the new and old photos from the STSMs - gallery:

