Report for Short Term Scientific Mission

CA15224

Applicant: Christin Habig
Home Institution: Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics
Host/Host Institution: The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh
Start and End of STSM: 13.02.2017 – 24.02.2017

1. Purpose of the STSM:

At the Friedrich-Loeffler-Institut I am working on a project comparing relatively unselected with current commercial lines of hen to understand how selection has played a part in bone stability, including keel bone development and its susceptibility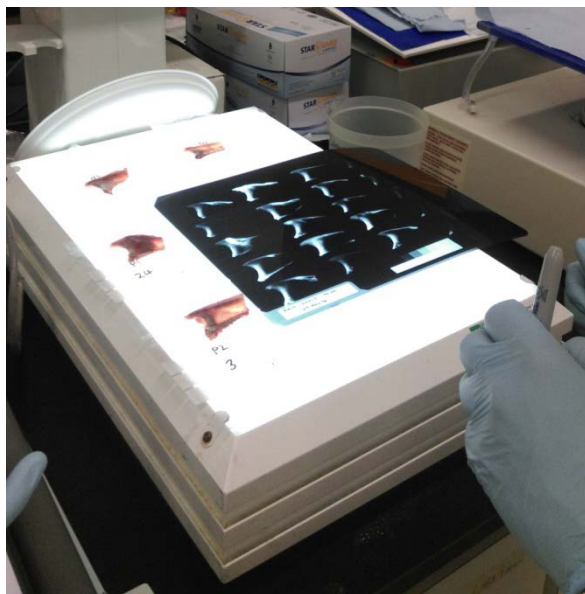 to damage. Based on my previous and current activities I received a lot of experience in keel bone palpation. As this method mainly allows evaluation of the carina sterni, I am very interested in new techniques, which enable the assessment of the whole keel bone and more thorough analyses. Therefore, the purpose of the STSM was to learn and train in other methods to assess bone quality in laying hens.

Figure 1. Pieces of the keel bone were scored for deformities and fractures by palpation and X-ray imaging.

2. Description of the work carried out during the STSM:

During my first week at the Roslin Institute I primarily worked with Heather McCormack. We took X-rays of dissected *tibiae* from hens at the age of ten and 18 weeks. I was involved in X-ray imaging as well as developing the images from the film and digitisation using an X-ray scanner. As the structure of bones in laying hens is strongly influenced by their reproductive state, it is very important to adjust the voltage of the X-ray machine according to the hens' age to ensure adequate penetration of the bone by the X-ray.

Afterwards, Pete Wilson, one of the researchers of the working group, and I analysed these and other already existing images of the keel bone, humerus and tibia independently of each other using ImageJ software. Each X-ray image was displayed in false colour and calibrated in terms of millimetres of aluminium equivalence on a 16 step aluminium step-wedge, which was part of the radiograph. Then the boundary edge of each bone was delineated and the mean radiographic density was calculated for pixels contained within the boundary. Moreover, I was shown how to measure the bone length and thickness, as well as the thickness of the cortex. After we completed the analyses, an inter-observer comparison based on regression analyses was made.

Within the first week of the STSM we also did a method comparison of keel bone palpation and X-ray imaging. Again, Pete and I collected data independently of each other. Using a four-scale scoring system we palpated 4 cm long pieces of the keel with regard to deformities and fractures (0 = no deformity/fracture to 3 = severe deformity and/or fracture). Furthermore, X-rays of these bones were scored separately using the same scoring system. Results were used for inter-observer comparison as well as for comparison of the two methods.
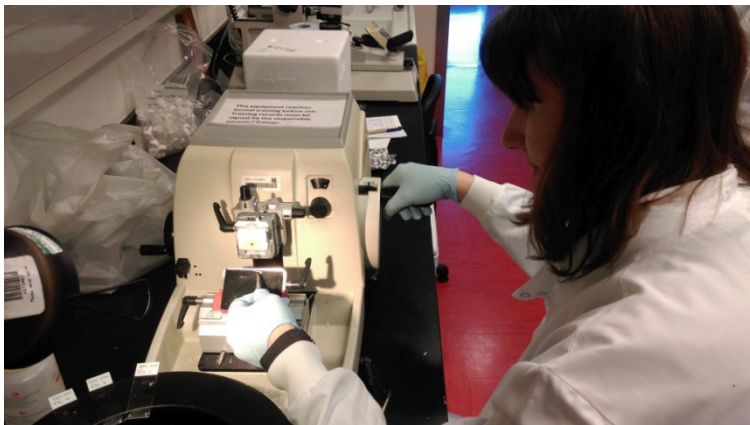

Figure 2. Microtome for the preparation of histological tissue sections.

I also made a presentation, to give an overview of my project and our activities regarding bone stability in laying hens to an extended laboratory group.

In the second week at the Institute I mainly worked with Bob Fleming in the bio-imaging facility. I learned how to prepare bones, in particular long bones, for histomorphometric analyses and cut a lot of slices (6 µm) of *tibiae* dissected from the ten- and 18-week-old hens mentioned above. I examined haematoxylin and eosin stained sections under a fluorescence microscope coupled to a camera. A total of twelve sections out of three slices per bird were photographed and bone content was calculated subsequently using ImageJ software. Bob showed me a lot of histological images and gave an explanation of the different types of bone.

3. Description of the main results obtained:

During the two weeks at the Roslin Institute I gained a lot of knowledge in bone morphology and bone quality assessment. In particular, X-ray imaging might be a good method to be established in our Institute. It enables a detailed depiction of the bone structure and bone damage, including keel bone fractures in vivo at different weeks of age as well as post mortem. Nevertheless, results of our method comparison showed, that keel bone deformities couldn't be determined with certainty by X-ray analysis, while fractures of the keel were not always detected by palpation in cases where they occurred. Therefore, a combination of palpation and X-ray imaging seems to be necessary to investigate the full extent of keel bone damage. The freely accessible software ImageJ opens up vast possibilities to analyse a high number of radiographs and histology images more or less standardised and certainly will be more applied in my home institution. The discussions during the STSM were substantive and fruitful and showed me other approaches for the consideration of bone stability. Overall, the STSM was a great experience, given the possibility for exchange of experience and knowledge. I really appreciate to get to know other researchers working in the field of bone stability in poultry for many years.

4. Future collaboration with the host institution (if applicable):

At the last day of the STSM we had a debriefing and talked about future cooperation, but at present, there is no concrete plan for joint activities but we hope to work towards joint proposals.

5. Foreseen publications/articles resulting from the STSM (if applicable):
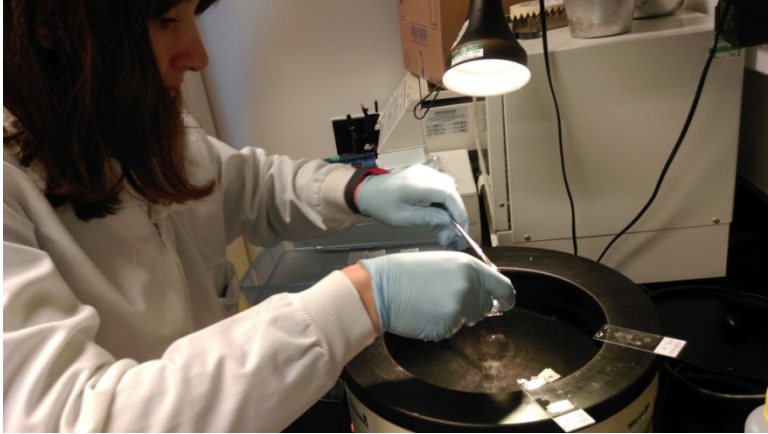There are no foreseen publications resulting from the STSM.

Figure 3.Transfer of tissue sections on slides.