Application for the Short Term Scientific Mission (STSM) COST action FA1308

Work plan

The purpose of this work plan is to frame the most important aspects of our research, in order to expand the projects initiated and to outline new avenues for future results.

The emergence of new collaborations give us the opportunity to expand our projects. These chances allow us to share not only human resources (Italian and Slovenian) and knowledge but also animal resources, which are the basis of our research and our true target.

We will look for genes involved in production and reproduction, by comparing the Sarda breed sheep (dairy production) with 3 native Slovenian sheep breeds with different attitudes: Istrska Pramenka (dairy), Jezersko Solcavskva Ovka (dairy and meat), Bovska Ovka (dairy and meat).

Previous studies we conducted at the Section of Endocrinology and Animal Welfare of the Department of Veterinary Medicine, of the Sassari University, were:

- Investigation of polymorphisms in KISS-1 and GPR54 genes in dairy and meat sheep.
- SREBP-1 gene polymorphisms in dairy and meat sheep

Our research is aimed at identifying genes with favourable or neutral effect on productivity, reproduction and immunity. We will expand the screening of genes involved in the sexual maturation (KISS-1, kisspeptine and GPR54, G protein-coupled receptor 54) in different composition and quality of milk (SREBP-1, Sterol regulatory element-binding transcription factor 1) and in resistance to diseases (IFNG [interferon-gamma], IL8 [interleukine-8]). A promising approach is the use of genetic resistance of the host to disease; the last genes will be structurally and functionally characterized by PCR and SSCP analysis combined to sequencing technologies and bioinformatics to understand the possible association between genetic variant and the immune function.

500 blood samples from Sarda sheep recorded in the Herd Book will be analyzed. In Slovenia, for each listed sheep breed (Istrska Pramenka- Jezersko Solcavskva Ovka- Bovska Ovka) at least 75 individual blood samples will be taken. All animal data about age, health, feeding, production (yield and quality) and reproduction (reproductive efficiency - fertility and pregnancy rate - seasonal anoestrus, litter size), will be recorded.

We will use academic and work resources from Sassari and Ljubljana University. I will work at Sassari University with my tutor Professor Vincenzo Carcangiu. He since 2005 serves as an associate professor at the Department of Veterinary Medicine, teaching Physiology of the domestic animals, Ethology and Animal Welfare. I will work at the University of Ljubljana with the professor Staric Jože (Assist. Prof. DVM, MSc, PhD Erasmus & CEEPUS coordinator) which is head clinic for ruminants with ambulatory clinic at the Veterinary Faculty of Ljubljana.

During the first part of the project I, Dr. Federico Farci, will work at the University of Ljubljana from 15th January, 2016 with prof. Staric Jože. We will use part of the resources of the Sassari University and part of the Faculty of Ljubljana. My research project will begin collecting blood samples from Slovenian sheep breeds. Sampling days will be decided and organized under the supervision of the Prof. Staric Jože. Samples will be taken from the jugular vein, using tubes with EDTA as an anticoagulant. All animal data about age, health, feeding, productions (yield and
quality) and reproduction (reproductive efficiency -fertility and pregnancy rate- seasonal anoestrus, litter size) will be recorded. Samples will be analysed at the institute of Microbiology and Parasitology of the Ljubljana University. 4 aliquots of blood samples from each animal, each will be sampled for convenience in storage and in order to take and use them for future analysis. It is expected to take approximately 250 samples, using about 1,000, 1 ml microfuge tubes. One of these aliquots will be used for DNA extraction. The others will be stored at -20°C. Genomic DNA will be extracted using commercial kit (NucleoSpin Blood 250 prep, Machery Nagel). At this stage we will use other 500 tubes, 1 ml. After opening, some kit components require storage at -20°C (proteinase k). Nucleic acid extraction requires the use of a thermostatic bath to maintain T of BE (elution buffer) around 70°C and to break cells. During all this process, we will use micropipettes from 10µl, 100 µL, 1000 µL. 500 tips dedicated to all kinds of micropipettes will be used. The concentration and quality of the extracted DNA will be assessed using spectrophotometer reading. Finally, DNA will be stored at -20°C or even better at -80°C.

This period in Slovenia will last for three months and will end on 15th April, 2016.

The second part of the project will be made at the Department of Veterinary Medicine, of the Sassari University. The team of the Prof. Carcangiu will begin collecting blood samples from Sarda sheep breeds. Samples will be taken from the jugular vein, using tubes with EDTA as an anticoagulant. All animal data about age, health, feeding, productions (yield and quality) and reproduction (reproductive efficiency -fertility and pregnancy rate- seasonal anoestrus, litter size), will be recorded. Genomic DNA will be extracted using commercial kit (NucleoSpin Blood 250 prep, Machery Nagel).

After this, I will be involved by performing amplification of the target genes (KISS-1, GPR54, SREBP-1, IFNG and IL8) and then, SSCP migration in order to check possible polymorphisms in their nucleotide sequence. The samples showing different migration pattern will be sequenced in both directions.

For complete sequencing samples will be purified and sent to a commercial sequencing service. An appropriate Statistical Analysis will be performed in order to determine if polymorphisms of genes sequences could be associated with the health status and production performances in different sheep breed.

The person in charge of this project is prof. Carcangiu for the University of Sassari and the prof. Staric for the University of Ljubljana.

Yours faithfully,

[Signature]

(Federico Faro)

DIPARTIMENTO MEDICINA VETERINARIA

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