**Ain’t no mountain high enough**

**Many miles were travelled by Greece’s HERITAGESHEEP project team’s – sometimes across snow covered mountains in the winter – in order to achieve its semen collection target. And even when the team did manage to reach the farms, things were not always straightforward. But despite the great distances and other obstacles, the team succeeded.**

Travel – and lots of if – was on the cards for the team of semen collectors charged with fulfilling the remit of the Greek HERITAGESHEEP project team. Semen had to be collected, on farm, from rams based on farms all over the country and, in some instances, an overnight stay was required. “So we didn’t run into problems, so much as just difficulties with distance, compounded by some mountainous regions that had to be crossed,” says team leader Christina Ligda, who is also a researcher at the Animal Genetics and Breeding Centre for Genetic Resources, at Thessaloniki in Greece.

That said, things were not always so straightforward once a farm was reached. Collecting semen from live rams can be fraught with difficulties, not least that some breeds are more ‘shy’ than others, as Christina and the team discovered.

The Greek team’s goal was to collect semen from three breeds – the Boutsiko, the Katsika and the Frisarta. The first two are local mountain breeds and well adapted to the mountains of Epirus, while the Frisarta is a plain breed with high performance in the area of Arta,” explains Christina. “Katsika is a small population breed, which is under an in-situ conservation programme, and the Boutsiko is a breed with a decreasing population numbers.”

Heritage sheep breeds (HSBs) are defined as genetically distinct, geographically concentrated and adapted to their environments. Typically, these sheep breeds are ‘local’ breeds, traditionally farmed for commercial use, and play an important role in the culture and rural economy of the regions in which they are managed.

What is HERITAGESHEEP?

The aim of the HERITAGESHEEP EU project is to establish a European-wide conservation programme of HSB genetic resources for the diversification of production in livestock agriculture and for their central importance in the long-term sustainability of medium- to low-input farming systems. More information can be found on www.heritagesheep.eu.

And it's for this reason that semen from some of the best rams of each breed are being collected and stored for future generations – should they ever be needed. A threat facing all HSBs is the risk of disease entering the region in which the breed is geographically concentrated. Bluetongue and foot-and-mouth disease are just two that are fresh in farmers' minds. The impact of these diseases and from procedures such as culling, taken to prevent disease spread, can be catastrophic. This risk was highlighted during the foot-and-mouth disease epidemic in the UK in 2001, when regional breeds located in the disease centres suffered disproportionate losses to their gene pools.

## FIRST CONTACT

Before collection could begin, it was vital to get the sheep breed societies and farmers on side. "And we made our first contact with them through a questionnaire, which offered an assessment of the threats and values to the heritage sheep genetic resources. This made the farmers more aware of the objectives of the project and, as a result, they agreed to participate in semen collection," explains Christina.

"They were more than happy to cooperate and initial contact with them was made through the Animal Genetic Improvement Centre, based in Ioannina, and the key people at the centre have very good contacts with the breeders," she added.

## COLLECTION PROTOCOL

Semen was collected using a ewe in oestrus. Once the ram mounts the ewe, the procedure is repeated, as many times as possible, until the ram allows semen to be collected into an artificial vagina.

"It is then prepared for collection by the introduction of warm water (between 40 and 42°C) and air between the outer casing and soft inner sleeve, lubrication with gel in the end where intromission of the penis

occurs, and attachment of a graduated collecting glass tube at the opposite end," explains Christina.

The semen was diluted with a soybean lecithin-based extender (commercial medium). For laparoscopic insemination, $100x10^6$ spermatozoa are required. And after dilution the semen is stored in a cold cabinet for a two-hour cooling period and cooled at a rate of 0.5°C per minute – quickly and steadily.

The semen is then packages in 0.5ml straws and these are labelled with the breed name, the code of the farm where it comes from, as well as the ear-tag number of the ram. Date of collection and freezing is also recorded.

"And we also collected and stored other information about the ram, such as scrotal circumference measurements, wither height and a photo of the ram," adds Christina.

Straws are cooled slowly, at a rate of 5°C per minute to -25 °C. Below -25 °C, the cooling rate is higher at 50°C per minute until -170°C is reached. The straws are then plunged into liquid nitrogen and stored in a tank.

## ORGANISING FARM VISITS

"This protocol was easy to follow once we were on farm," says Christina. "The hardest thing was organising the visits to the farms, as the team had to move from Thessaloniki to Ioannina and Arta, and therefore the whole process had to be organised in two-day trips on a weekly base.

"And in the collection locations we had to organise some basic laboratory facilities in order to have a first evaluation of the quality of the semen. For the Boutsiko breed we worked on the experimental farm of the NAGREF Research Station of Ioannina, and for the Katsika and Frisarta breeds we did this on specific farms."

Boutsiko sheep

Katsika sheep.


Frizarta sheep.

She says that selection rams and ‘training’ them also took a lot of effort and time. “We didn’t expect this and we found that the different breeds had different attitudes towards the ewe and the artificial vagina”

“For example, the rams of the mountain-based Katsika breed needed more time to get to know the personnel working closely with them – they seemed a little shy. And for this reason the whole process needed more time than we originally expected.”

## SUCCSSFULL COLLECTION

But despite the additional times required, Christina and the team – comprising vets Aristotelis Lymberopoulos and Tarek Khalifa, and technician Angelos Kokotas – have met and continue to meet their collection targets for the three breeds. They collected a total of 1,000 doses from five Boutsiko rams – 1000 doses and collections for the other two breeds are continuing.

“We’re very pleased with our results so far – not bad when you consider the mileage involved and the logistics of getting a team and all the collection and storage equipment on farm,” says Christina. “We’ve proved that we can do it both efficiently and effectively and that’s what

we’ll continue to do until we’ve collected enough semen from all three breeds.”

Rachael Porter - Journalist