

Annex 10: Work Package 4 report on semen collection

Heritage Sheep Breeds

Title page:

- Title
 - HeritageSheep Project: “Collection and storage of semen of heritage sheep breeds in five European countries” (WP4)
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- Abstract of 2 sentences
 - In total, semen of 152 rams of 15 breeds in 5 European countries is collected. Each of the five partners have chosen at least two heritage breeds in their countries from which to collect germplasm. Semen from these rams is now safely stored and is available to restore a breed or a particular genetic characteristic, in case it would get lost in the event of a disaster.
- Key words
 - cryopreservation, heritage sheep, semen collection

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Foreword

This report reflects the results of Work package 4 of the HERITAGESHEEP project. The project started on 1 April 2007. The end-date was 30 September 2009. An extension of 6 months was provided given the difficulties encountered in germplasm collection during the Blue Tongue virus epidemic of 2008. The partners were specialists from five Member States – France, Greece, Slovenia, the Netherlands and the United Kingdom. Heritage Sheep was awarded with funding from the European Commission (EU). The co-financing for the Netherlands was provided by the Centre of Genetic Resources, the Netherlands (CGN) and the Dutch Ministry of Agriculture, Nature and Food Quality.

Summary

The aim of Work package 4 (WP4) of the EU project HERITAGESHEEP is the *ex situ* conservation of heritage sheep breeds (HSB), by undertaking the practical work to cryopreserve semen of HSBs in Europe. Firstly, each of the five partners in this project (i.e. The Netherlands, United Kingdom, France, Greece, and Slovenia) selected at least two breeds for *ex situ* conservation based on criteria developed from WP1 and discussions held at the 2nd meeting of the partners in Amsterdam in 2008. Secondly, the optimal *ex situ* conservation method of HSBs was constructed for each country. Thirdly, the semen of the prioritised HSBs was actually collected, frozen and stored in genebanks.

Breeds were prioritised and selected based on several issues, of which the most important ones were the geographical isolation, the effective population size, the existing resources, and the unique traits associated with the breed and participation. The only restriction was that the breed had to participate in Work Package 1 of this HeritageSheep Project. The United Kingdom had chosen two breeds (Shetland and Lonk), Greece had chosen three breeds (Frisarta, Boutsiko and Katsika), France had chosen four breeds (Causse du Lot, Rava, Limousine, and Bizet), The Netherlands had chosen three breeds (Kempen Heath, Milkshoop and Black Blazed), and Slovenia had chosen three breeds (Istrian Pramenka, Bovec and Bela Krajina Pramenka).

The most optimal *ex situ* conservation strategy differs between countries, as well as the procedures to freeze semen, without clear preference for a particular freezing medium or freezing procedure.

In total, semen of 152 rams of 15 breeds was collected and now safely stored in genebanks. This semen can be used to restore a breed or a particular genetic characteristic, in the event of a disaster (e.g. disease outbreak). For the maintenance of the genetic diversity of heritage sheep breeds this is an incredible contribution.

1. Introduction

There are many different breeds of sheep currently existing in the world. Out of a total number of some 1200 breeds, more than a third of them – 417, are native to the European Union. Unfortunately, the native sheep breeds of Europe are now losing numbers at such a rate that the breeds are at risk of becoming lost forever. These sheep breeds are a resource for future generations, and should be preserved.

From the breed society estimates 19 out of the 46 breeds across the partner countries (The Netherlands, United Kingdom, France, Greece, and Slovenia) are thought to have 95% or more of breed numbers actively farmed in the geographical region associated with the breed. This geographical concentration – endemism – presents a major risk to the genetic resources of Heritage Sheep Breeds. Disease outbreaks (like Foot and Mouth Disease) and epidemics are also ever-present threats, with risks made much worse when each heritage breed is concentrated within a single region.

To be able to restore a breed or a particular genetic characteristic that could be lost in the event of a disaster, germplasm is collected. The germplasm can be semen from rams or very young embryos flushed from ewes after artificial insemination. The stores are an archive of sheep breeds at threat of extinction, and the genetic material is kept stored frozen in genebanks.

Work package 4 (WP4) of the EU project HERITAGESHEEP concerns the *ex situ* conservation of heritage sheep breeds (HSB), by undertaking the practical work to cryopreserve semen of HSBs in Europe. Each of the five partners in this project selected at least two heritage sheep breeds in their countries from which to collect germplasm, and to store the semen from these rams safely. This represents the beginnings of a European-wide genebank of Heritage Sheep Breeds. The objectives of WP4 are:

1. Identification of breeds for *ex situ* conservation
2. Construction of the optimal *ex situ* conservation method of HSBs for each country
3. Collection, freezing and storage of semen (or other genetic material) from the prioritised HSBs.

2. Identification of breeds

Each of the five partners in this project has selected at least two breeds in its country for the collection of semen in WP4, based on the current situation and aided by the information contained within the results presented in the WP1-survey. Important issues for the determination of breeds to be included, are:

- Existing resources *Ex situ* conservation resources (see WP3)
- Effective population size Number of males in population
 Number of females used for pure-bred breeding
- Geographical isolation
- Existing conservation schemes and effect on population, if there are no conservation schemes in place for a decreasing population of sheep then these should be a priority
- Co operation of the breed society in coordinating animals for collection of semen
- Disease crisis situations
- Breeds which participated in WP1
- Unique traits associated with the breed
- Scientifically documented phenotypic traits
- Consideration of values and promotional activities determined by the dataset of WP1

Following the assessment each country sought to make an initial selection of two breeds for the collection of semen in WP4. Where, for any reason, it was not possible to access sufficient rams for collection, or where other factors prevented collection of sufficient semen, additional breeds were added to the list for collection. The initial list of proposed breeds, by country, was as follows:

United Kingdom:

1. **Shetland.** The endemism survey conducted by The Sheep Trust identified 77 Shetland flocks on the Shetland Islands with a total of 16,238 sheep, 15,630 females and 608 males. The number of sheep producing coloured wool is declining and has reached a critical level. Coloured wool is important for knitting wool for which the breed is internationally famous. The breed society The Shetland Flockbook Trust was approached to suggest semen collection from coloured rams to protect this resource and to enhance the National semen archive in the UK, in the event that it becomes a national archive for genetic resources. At present, it only contains semen from rams selected on scrapie genotype. The breed society proved very keen to participate. Currently, there are no other conservation schemes for the breed.

In addition because the breed is indigenous to the Isles of Shetland and Orkney it is geographically isolated. Greater than 95% (15,545 sheep) of the breed numbers are within a radius of 53 Km of the breed mean centre. The breed maintains a high health status and promotional efforts on the part of the breed society are extensive. However, the breed has great difficulty marketing meat due to the geographical position of the island and high transport costs.

2. **Lonk.** The endemism survey conducted by The Sheep Trust identified 32 flocks of sheep from 36 breed society members. A total of 15,925 were counted, 15,551 females and 374 males. Mapping showed that 77% (12,275 sheep) of the numbers located within a radius of 62 Km and > 95% (15,355 sheep) within a 71 Km radius in the Pennine districts of Yorkshire and Lancashire in the North West of England. The breed has semen from only 1 ram currently in the National Semen archive (currently under construction) and semen collection would enhance the semen archive. The

breed has the smallest membership of all the UK breeds surveyed in WP1. The breed is disadvantaged because promotional activities for agricultural products in the region where the Lonk is farmed do not include the Lonk.

Greece:

1. **Frisarta.** This breed is geographically concentrated, with a stable population, and not endangered. Frisarta is a breed with high milk production and is under a milk recording scheme. No semen has been collected for cryopreservation.
2. **Boutsiko.** The breed used to be very common in the mountain regions of Epirus. However, the total population now holds 10,000 sheep and is decreasing. For this reason and due to the adaptation of the breed in the region, it was suggested to collect semen from a number of rams of the breed, according to the possibilities.
3. **Katsika.** This is a small population, and was not included in the beginning of the project. According to the population size, the breed is considered as endangered. However, the partners concluded that since the farmers are making efforts for the development of the breed it was considered a good opportunity to collect semen from a number of rams from this breed also.

France:

1. **Causse du Lot.** This breed has a large population (100,000 ewes but only half are pure-bred), but geographically they are very isolated and they are threatened by FCO-disease. They have no semen cryopreserved. The association is very interested in the project and there is a ram centre.
2. The other breeds are (1) **Limousine** and (2) **Rava** (none of them have any frozen semen yet). All breeds are managed by the same organisation, and semen of 10 rams each was collected.
3. Two other possible breeds are (1) **Bizet** and (2) **Grivette**. No semen is collected from these breeds so far, and when possible, it will be tried to collect semen from those breeds as well.

Netherlands:

1. Above all, it was decided to not collect semen from the three synthetic Dutch breeds (i.e. North Holland, Flevoland, Swifter). No semen from the Drenthe Heath and Schoonebeek will be collected either, since that was already done very recently in 2008. The chosen breeds are: (1) **Kempen Heath**, (2) **Milksheep** (Friesian & Zeeland), and (3) **Black Blazed**.
2. The **Kempen Heath** is a small population with 2,000 ewes in 10 flocks in only the southern province of The Netherlands, called Zuid-Limburg. Semen has been cryopreserved before, but not recently.
3. No semen has ever been cryopreserved from the **Milksheep**, neither Friesian nor Zeeland Milksheep. Both populations exist of approximately 3,000 ewes and 50-80 rams. The Milksheep are either kept for professional farming or for hobby farming. Originally, the sheep are located along the coast (South-West, West and North-West of the Netherlands). Currently, they appear more nationwide.

4. Not much semen of **Black Blazed** rams has been cryopreserved until now; only 170 doses of 7 rams. The population is approximately 4,000 ewes. The sheep are located nationwide. Therefore, this breed is of interest for this project.

Slovenia:

The effective population size, geographical isolation, and unique traits were the main factors used to consider which breeds to select. In addition emphasis was placed on breed vulnerability due to the outbreak of Blue Tongue disease in the neighbouring Croatia, Italy and Austria. The two most endangered breeds of sheep located most closely to these neighbouring countries. Predation by the brown bear, wolf and lynx is a serious issue. Three breeds were selected including (1) **Istrian Pramenka**, (2) **Bovec** and (3) **Bela Krajina Pramenka**.

Short summary

To summarise, the UK selected 2 breeds (Shetland and Lonk), Greece selected 3 breeds (Frisarta, Boutsika and Katsika), France selected 3 breeds for sure (Causse du Lot, Rava and Limousine), but would like to collect semen from one or two other breeds as well, if possible (Bizet and Grivette), The Netherlands selected 3 breeds (Kempen Heath, Milksheep and Black Blazed), and Slovenia selected 3 breeds (Istrian Pramenka, Bovec and Bela Krajina Pramenka).

3. Construction optimal conservation method

There are two ways of collecting semen; ejaculated semen or epididymal semen. Which of these two is the most optimal *ex situ* conservation strategy differs between countries. For example, on farm collection of ejaculated semen and collection of epididymal semen is prohibited in France, as it does not comply with EU regulations. Or as another example, The Netherlands do not have EU certified sheep AI stations. Therefore, the Netherlands have predominantly used epididymal semen from testes obtained from slaughterhouses, or ejaculated semen collected in the field.

Ejaculated semen is collected from selected rams approximately twice a week. Epididymal semen is obtained from the epididymis of selected rams. The rams are not culled for the semen collection specifically, but were already intended for culling. Testes are transported from the slaughterhouse in coolers with freezer packs around them to keep them at the right temperature. It is important not to wait too long before obtaining the semen from these testes. Semen is obtained on the day of slaughter with a procedure from the caudae of the epididymides.

Semen freezing procedures varies between the countries, with no clear preference for a particular freezing medium or freezing procedure. Freezing media contain egg yolk and glycerol as cryoprotectants.

Laparoscopic inseminations generally result in pregnancies over 60%. However, laparoscopic inseminations are prohibited in the Netherlands for animal welfare reasons. Cervical inseminations may result in 30% pregnancies. Epididymal semen seems to have at least as good as, or better fertility than ejaculated semen.

The rams of which semen was collected were all serologically tested for a number of diseases, but which diseases varied per country. See also an example of a form for the ram information in Annex 1

3.1 A comparison of two media for freezing ram semen: “Red Ovine” versus “Tris”

A comparison is made between a commercial medium from IMV (“Red Ovine” extender) and ‘home made’ Tris-Egg yolk freezing medium.

Tris-egg yolk freezing medium

One litre of complete medium contains:

- 0.200 mol (24.22 g) Tris(hydroxymethyl)-aminomethane,
- 0.0644 mol (13,44 g) of citric acid.1H₂O,
- 0.0555 mol (10.0 g) of D- fructose,
- 0.05 g of Tylan,
- 0.25 g of Gentamycin sulphate,
- 0.676 g of ‘Lincospectin 100’ (lincomycin/spectinomycin),
- 200 ml of pasteurized egg yolk (Eiproma, Wormerveer, The Netherlands), and
- 0.766 mol (70.56 g) of glycerol = 5.6 % (v/v).

The medium is not clarified by centrifugation or filtration. Tris-citric acid extender generally ensures that egg yolk is well emulsified, rendering only very little sediment. The medium is stored frozen and thawed shortly before use. The freezing and thawing further enhances the emulsification of the yolk.

Red ovine

This extender is obtained from IMV-Technologies (www.imv-technologies.com). This extender already contains glycerol but not egg yolk. Four volumes of the extender are mixed with 1 volume of egg yolk (from fresh eggs). The medium is not clarified by centrifugation or filtration. It wasn't frozen and thawed before use as it was advised to be used 'fresh'. Just like Tris, the Red Ovine extender was able to emulsify the egg yolk quite well, and the complete Red Ovine-egg yolk did not develop much sediment.

For the six ejaculates frozen in July 2009 the media were prepared as described above. That means that the source of egg yolk for the Tris-egg yolk and the red ovine – egg yolk was different, as the Tris-egg yolk was already complete. In August 2009, five more ejaculates were frozen. In this case, we made new Tris-egg yolk and fresh Red ovine-egg yolk, using the same source of egg yolk (from fresh eggs).

Table 1. *Characteristics of media*

	pH	mOsm/kg of water
Tris – egg yolk –July	6.8	1,217
Red ovine – egg yolk –July	6.9	1,171
Tris – egg yolk – August	6.5	1,241
Red ovine – egg yolk – August	6.7	1,179

Semen collection

Rams of the Dutch Milk Sheep breed (Nederlands Melkschaap) were used. In total, nine rams were used. From two rams, two subsequent ejaculates from the same day were processed as separate ejaculates. So in total, the comparison of the media was made with 11 ejaculates.

The two media were placed in a 30 °C waterbath. Semen was collected on farm, using an artificial vagina. The collection tubes were immediately placed in a 30 °C waterbath. A small aliquant was used to determine the sperm concentration. The remaining ejaculate volume was divided evenly over two tubes, and these aliquots were diluted with 3 volumes of the respective media. The semen tubes were cooled slowly by placing them enwrapped in kleenex tissue in a 5 °C coolbox. At three hours after semen collection the semen was then diluted to a final concentration of 200 x 10⁶ sperm/ml. In some ejaculates only a little semen could be used for the experiment, so in these cases the final concentration was lower in order to obtain the required volume to fill at least two 0.5-ml straws per medium.

Freezing

Straws are filled, sealed, and placed on racks (30-40 straws/rack) and placed in a nitrogen vapour freezer with forced ventilated nitrogen vapour at -80 °C during 10 minutes (average effective cooling rate inside the straws over the range +5 °C to -60 °C is approximately 30 °C/min. Maximum cooling rate after dissipation of heat of fusion = approximately 60 °C/min), then plunged in LN₂ and transferred to storage tanks.

Thawing and semen assessment

Straws were thawed by holding them in a stirred water bath of 37 °C for 30 seconds. The straws were wiped dry and the contents were brought into small tubes in the 37 °C Dry Block. Percentage motile sperm is estimated by an experienced technician on five different spots of one glass slide wet Mount. The five values were then averaged for that semen sample.

Membrane integrity was assessed by fluorescence microscopy, using 4',6-diamidino-2-phenylindole (DAPI), as a fluorescent dye-exclusion dye) 20 µl of the semen was mixed with 20 µl of 10 µM of DAPI in Na₃citrate solution (28.5 g/l). After 2 minutes, 80 µl of 2 % glutaraldehyde in Na₃citrate solution (28.5 g/l) was added. 100 cells were assessed in a glass slide wet mount. The percent 'intact' (not fluorescent) cells is shown in Table 2.

Results

No differences were found between the media in pre-freeze and post-thaw % motile sperm and % sperm with an intact membrane (DAPI fluorescent dye exclusion).

Overall data for 11 ejaculates:

Table 2. *The percent 'intact' (not fluorescent) cells*

	Pre-freeze		Post-thaw	
	% motile	% motile	% motile	% intact
Tris	80.0 ± 2.5	37.1 ± 4.6	37.1 ± 4.6	41.9 ± 3.0
Red ovine	80.5 ± 2.5	35.5 ± 4.2	35.5 ± 4.2	39.8 ± 2.6

If the data from ejaculates frozen in July (two media with different source of egg yolk) and August (two media with the same source of egg yolk) were analyzed separately, the same picture is seen: No differences between the two media.

Conclusion

As described in the report of WP3, different media and protocols are being used by France, Netherlands, UK, and Greece, with adequate results. The comparison with methods and media used in France could have been interesting as these deviated more strongly from those used in the UK and in NL. For practical reasons it was not feasible now to compare freezing methods. Also, unfortunately, we could not include the French media for freezing ram semen.

The present comparison made between the commercial medium (Red ovine) and the 'home made' Tris-egg yolk showed that there is no difference, albeit the post-thaw values seem to be slightly better using the home-made medium. The physical characteristics of the two media (egg yolk dispersion ability, pH and osmolality) suggest these media are quite similar anyway.

The decision to use either medium can thus be made largely on practical considerations.

4. Collecting, freezing, storing

Collecting, freezing and storing semen began early in 2009 for this project. Annex 2 shows a summary for each country with the non-scientific information you have think about when organising the collection of semen. Like, what to do when you have to get the rams from the Shetland Islands to the main land and the weather is very bad? Or how to collect semen of rams in the middle of the countryside without lights and/or electricity? Or when the rams turn out to be too shy at the start? Each story tells the unexpected situations you can end up in when getting the rams together, and learns you where to think about when starting collecting semen.

Final breeds for *ex situ* conservation are identified in each country: Lonk and Shetland for UK, Causse du Lot, Bizet, Limousine and Rava sheep for France, Kempen Heath, Milksheep and Black Blazed for The Netherlands, Bovec, Istrian Pramenka and Bela Krajina Pramenka for Slovenia and Frisarta, Boutsika and Katsika for Greece.

In the Netherlands, semen from 30 Milksheep rams, 15 Kempen Heath rams and 4 Black Blazed rams was collected. In total 1,750, 2,500 and 300 doses, respectively (Table 3). Each dose in The Netherlands contained 0.5 ml with 100-200 millions of sperm cells.

In UK, semen of in total 21 Lonk rams and 15 Shetland rams was collected with 5,028 and 3,497 straws respectively (Table 3). Each dose in the United Kingdom contained 0.25 ml of sperm with average 125 million total sperm, 25-30 million progressively motile.

Table 3. *Number of doses of semen cryopreserved per breed*

Country	Breed	No. of rams	No. of doses
United Kingdom	Shetland	15	3,497
	Lonk	21	5,028
Greece	Frisarta	4	790
	Boutsiko	5	1,255
	Katsika	3	550
France	Causse du Lot	22	2,827
	Rava	5	967
	Limousine	10	1,934
	Bizet	3	525
The Netherlands	Kempen Heath	15	2,500
	Milksheep	30	1,750
	Black Blazed	4	300
Slovenia	Istrian Pramenka	6	225
	Bovec	4	442
	Bela Krajina Pramenka	5	314

In France, semen of Causse du Lot, Rava, Bizet and Limousine was collected; 22, 5, 3 and 10 rams per breed, respectively. In total, 2,827, 967, 525 and 1,934 straws are now conserved for each breed, respectively (Table 3). Each dose in France contained 0.25 ml with 100 million sperm cells.

In Greece, semen from Frisarta, Boutsiko and Katsika (4, 5, and 3 rams, respectively) was collected. In total, 2595 doses are now stored (Table 3). Each dose in Greece contained 0.5 ml with 800 million sperm cells, when ejaculated semen was collected. However, for the Katsika breed epididymal semen was collected and each dose contained 0.5 ml with 200 million sperm cells.

In Slovenia, semen has been collected from 4 Bovec sheep, 6 Istrian Pramenka and 5 Bela Krajina Pramenka, with in total 442, 225 and 314 straws per breed, respectively (Table 3). Each dose contains different number of sperm cells. However, an average number is between 200 – 300 millions of cells.

4.1 Storage and Usage

The Netherlands

The semen is stored by the Centre of Genetic Resources, The Netherlands (CGN). They are owner of the semen as well. When epididymal semen was collected, CGN bought the rams from the breeders and were therefore direct owner of the animals. When ejaculate semen was collected, an agreement was set up with the breeder. In this agreement it was stated that CGN will be the owner of the semen, but they can only distribute the semen after consulting the breed organisations. This embargo usually holds for a fixed term. For ejaculate semen, the breeders do not get a reimbursement; the breeders are all participating voluntarily. It is of interest for the breed organisations to secure their breed, and to collaborate in maintaining the genetic resources and unique alleles for the future.

United Kingdom

The semen is stored by The Sheep Trust and is held in tanks by Innovis (East Lothian). The Sheep Trust produced an agreement with each breeder, which includes guidance notes and conditions for transfer of ownership of semen. In short it is stated that 40% of the semen collected from a single animal is available for use by the owner of the tup, 10% by the breeder of the ram (these may be the same person) and 50% for use at the discretion of the Sheep Trust. A proportion of the Sheep Trust Quota may be available for purchase, subject to an application submitted to and approved by the Sheep Trust. The owner/breeder has access to material for 3 years from the date of collection, after which the usage will revert to the Sheep Trust. Costs of long term storage for 3 years will be covered for the owner/breeder by the Sheep Trust.

France

The semen are stored in the National Cryobank. The owner of the semen is the breed organisation, but the doses are not accessible directly for the farmers. To be authorised to take doses from the National Cryobank we need the agreement of the National Cryobank itself and the owner of the semen (the breed society in this case).

Greece

The collected semen is stored in the Cryo Bank of the Veterinary Research Institute of the National Agricultural Research Foundation in Thessaloniki. The owner of the semen are the breeders. The partners agreed that the selected material in the frame of the project will be transferred to the National Cryo Bank of Greece, when this will be established. In the meantime the use of the collected material will be decided with the agreement of the research team of the project and representative of the Ministry of Rural Development and Food.

Slovenia

The semen is stored by the University of Ljubljana, Veterinarian Faculty in Ljubljana. Owner of the semen is national gene bank. Artificial insemination is not in use in Slovenia and therefore semen is not in use by farmers.

5. Conclusions

In total, semen of 152 rams of 15 breeds is collected. Each of the five partners (The Netherlands, United Kingdom, France, Greece, and Slovenia) have chosen at least two heritage breeds in their countries from which to collect germplasm. Semen from these rams is now safely stored and represents the beginnings of a European – wide genebank of Heritage Sheep Breeds. The stores can be an archive and are available to restore a breed or a particular genetic characteristic, in case it would get lost in the event of a disaster (e.g. disease outbreak). For the maintenance of the genetic diversity of heritage sheep breeds this is an incredible contribution. It is essential to realise that heritage sheep breeds contribute to and shape their ecosystem – without the breeds, the environments will be lost forever and farms will disappear. Given the threats to their existence in the challenging regions where they are kept, support is urgently required for conservation of their genetic resources for future generations.

Annex 1: Ram information

Flock Number / Farm id	
Animal id / Ear Tag Number	
Name	
Sire id	
Dam id	
Species	
Breed	
Date of Birth	
Sex	
Breeding association	
Latitude and Longitude of farm (if available)	
Photo	
Other comment/remarks of animal	

Scrapie Information

EID (NSP) if applicable	
Scrapie Genotype	
Private Scrapie Certificate Number	

Bluetongue Vaccination Status

Has the Ram had Bluetongue vaccination Yes/No	
Date of BTV vaccination	
Manufacturer	
Batch Number	
If not vaccinated do you agree to your ram receiving Bluetongue Vaccination at the collection centre	YES NO (please delete)

Status other diseases

Different per country; e.g.	
Maedi Visna Virus (MVV), or Caprine Arthritis/Encephalitis (CAE)	
Brucella Ovis	
...	

Annex 2: Interviews with ‘soft information’

The interviews in this Annex are done by Rachael Porter, agricultural journalist in UK.

1. The Netherlands

Ad-hoc race against the clock

A determined and persistent approach is key to the HERITAGESHEEP Dutch project team’s success. And despite many obstacles and set backs, it’s well on its way to achieving its semen collection target

A logistical nightmare and a race against time describe the conditions and circumstances surrounding the programme of semen collection, recently undertaken by the HERITAGESHEEP project team in the Netherlands. But grit and determination sees it well on its way to completing the task with considerable success, despite the odd hiccup along the way.

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.

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 Netherlands in 2001, when regional breeds located in the disease centres suffered disproportionate losses to their gene pools.

SEMEN COLLECTION

With this in mind, the somewhat unconventional if not slightly bizarre practice of collecting ram semen has a somewhat sober and incredibly serious side. And with the threat of Bluetongue looming over all European flocks, none more so than those in The Netherlands, it is also being carried out with a degree of urgency.

But, as is always the case when working with animals, nothing is ever straightforward. But for the Dutch team the first ‘stumbling block’ was people! The team – which includes Yvette de Haas and Lucia Kaal, who are both based at the Animal Breeding and Genomics Centre at Wageningen University in Lelystad – first had to convince the breed organisations to participate and explain what the project was trying to do and why. And then began the wait –

for the societies to come back to them with names, addresses and contact details of farmers with rams who were willing to donate semen.

Semen is being collected from three different breeds in the Netherlands – Kempen Heath, Milk Sheep and Black Blazed – and from rams all over the country.

KEMPEN HEATH

“The Kempen Heath society was relatively keen to cooperate, once we explained that we wanted to collect and freeze semen from several rams – between 10 and 20 – to help maintain and protect biodiversity both now and in the future,” says Lucia.

“But convincing them was just the first step – we then had to track down and contact individual farmers who owned rams and then explain what we wanted to do all over again. This was very time consuming and the Bluetongue disease threat meant that the clock was ticking.”

Kempen Heath is a breed that is usually kept in small flocks in The Netherlands’ southern areas and rams usually go from one flock to another to ‘spread’ their genes in a wide breeding circle.

“The breed organisation has been very successful in creating a special market for Kempen Heath meat and demand is such that rams are often also slaughtered for their meat. So there aren’t many rams available,” explains Yvette.

“But luckily the breeding organisation and the farmers also saw how valuable our project was for the future security and success of the breed and they sold us 12 rams.”

The project team had to buy the rams as semen was collected ‘epididymally’ – or post mortem. “To put it bluntly, we had to go to the slaughter house on the day the rams were slaughtered and collect their balls!” says Yvette. Samples of each rams’ blood were also taken so that they could be tested for disease.

To date, 2,500 straws of semen has been collected from 15 rams Kempen Heath rams – that’s between 125 and 250 straws per ram.

BLACK BLAZED

People certainly proved to be more problematic than the sheep as far as the Black Blazed breed was concerned. “We found the owners of this breed of sheep were not only hard to convince to take part in the scheme, but also difficult to get on board,” says Yvette.

She explains that they simply weren’t very willing to participate and those who did help wanted only the very best rams added to the gene bank – and for a high price. “But when a breed is already classed as ‘rare’, you can’t be too fussy about the semen you collect. As far as we were concerned, it was all good and certainly much better than none at all.”

After a lot of negotiation, the team finally ‘banked’ a few Black Blazed rams, all the more satisfied with their achievement after such a tough struggle.

A total of 300 straws of Black Blazed semen has been collected from four rams.

MILK SHEEP

Milk sheep are kept by Dutch farmers for both commercial and pedigree ‘hobby’ reasons. The rams that the team collected semen from were predominantly from commercial farms and had to be ‘sampled’ while still alive. “We were collecting ejaculate semen because the rams were all still in use,” explains Lucia.

This presented its own set of problems, not least being that The Netherlands, unlike the other countries involved in the project, has no central holding centre where rams can go to be ‘sampled’. This meant that semen collectors had to go out and visit individual farms.

And to add to their problems, most of sheep farms they visited were organic and, therefore, were not allowed to use hormones to synchronise the ewes and get the rams to ‘perform’.

“At first we thought that we could draw up a schedule so we could visit several farms in one day, but we soon realised that this simply wasn’t going to be possible. The chance of ewes being in heat on several farms on the same day was very slim indeed if left to Mother Nature,” says Yvette.

“So we had to visit the farms on an ‘ad hoc’ basis, when there was a ewe on heat on the farm where the rams were so we could collect the ejaculate.

“If we got the call to say that a ewe was in heat we’d have to literally drop everything and head off to that farm immediately – there was no time to lose if we were going to make the most of the window of opportunity.”

‘LIVE’ COLLECTION

With this in mind, it’s hardly surprising that the Dutch project team preferred the post-mortem semen collection method compared to the ‘live’. “The former not only proved to be less time consuming and considerably easier but it was also a lower cost exercise, despite the fact that we had to buy some of the rams before they were slaughtered.”

Henk Sulkers and Kees Zuidberg, two of the project’s semen collectors, would often get the ‘call of nature’ and dash off to the farm, which could be at the other end of the country, only to find that the ewe wasn’t in heat after all and the ram or rams wouldn’t perform. “Very frustrating for us and embarrassing for the farmer,” says Henk.

“There would be three or more people there, all poised and ready with the semen collection equipment – and nothing would happen. So, a lot of time and money was spent and there was no semen to show for it!”

To date 1,750 straws of Milk Sheep semen has been collected from 30 rams.

MISCOMMUNICATION

Miscommunication also caused confusion and embarrassment. Yvette recalls one instance where the team was expecting to collect semen from 25 rams at one farm, only to arrive and find that there were just five. Again, a disproportionate amount of labour, equipment, fuss and fanfare resulted in a few red faces.

And the job was made even more difficult on one unit when they arrived to find no electricity and no hot water. “Some farms were pretty remote also and proved difficult to find. We did get lost quiet a few times – that was embarrassing for us,” says Henk.

Successful collections comprise around 16 straws of semen per ram. Semen has to be transported back to the lab at a constant temperature of 10°C and it's then frozen.

“As you can imagine, collection days were long days – whether we were on farm or at the slaughter house,” adds Henk.

EARLY STARTS

Slaughter house collections required a 5.30am start and we were rarely finished before 7.00pm. We have to be there from start to finish to ensure that the testicles didn't get muddled up!”

As of March 2009, the Dutch team was about half way through its collection programme, with semen from 20 rams. Semen from a further 20 rams, predominantly Milk Sheep that are based in the south of the country, is still awaiting collection.

Henk is well built and can handle the more ‘wild’ sheep. “But when we began collecting semen, some of the sheep we were handling were not at all used to humans – far from ideal when collecting semen from live rams. And it took the best part of four weeks to make a successful collection,” he says.

“Rounding up some the rams was a real eye opener. Some of them were so wild that they jumped two metres in the air to get away from us when we tried to corral them – I've never seen anything like it.”

ENTHUSIASM

All in all, the farmers and breeders of the heritage sheep breeds in the Netherlands have been and continue to be eager to help the project succeed. And why wouldn't they? “They're passionate and enthusiastic about their sheep breeds and, ultimately, want to ensure that these breeds survive,” says Yvette.

Logistical difficulties caused by the 2007 and 2008 outbreaks of Bluetongue have hindered the project, but have also served to heighten awareness about what the team are trying to do and just how vital its work is.

“Semen collected from rams that have or are recovering from the disease is also less fertile,” she adds. “So it really is a race against time – and disease – to collect as much quality, fertile semen as possible and store it away securely to safeguard the future of these sheep breeds.”

2. United Kingdom

Managing movement restrictions and high mileage

Thorough planning and problem solving were both key to the HERITAGESHEEP UK project team's successful semen collection programme. And despite many obstacles and set backs, it has banked important and unique sheep genes for future generations

Movement restrictions, many miles of travel – over land and sea – and heavy snow were just some of the obstacles that the UK's HERITAGESHEEP project team managed to overcome during a vital programme of semen collection from Shetland and Lonk rams.

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COLLECTION CENTRE

The UK programme set out to collect semen from Shetland and Lonk rams. And collecting semen in the UK had its own unique problems, namely that sheep had to be transported to the collection centre in Edinburgh. "Unlike the other project teams in Europe, our semen collectors did not go 'on farm' to do their job. And all the collections were from live sheep too," explains The Sheep Trust's Amanda Carson, who heads up the HERITAGESHEEP project team in the UK.

All sounds relatively simple, and it would be if the many sheep that needed to be transported came from just one or two farms on mainland Britain, but the reality was that many of the sheep had to be transported from several different farms dotted across the Shetland Islands, which is situated more than 200 miles north of Aberdeen. "But the first obstacle was to convince sheep farmers to take part in the programme," explains Amanda, although she admits that this task was made slightly easier due to her role within The Sheep Trust and her contacts within the sheep breed societies.

"Fortunately both breed societies were willing to co-operate – that's a huge help and got us off the starting blocks as far as getting farmers to volunteer their rams for collection semen was concerned."

THE SHETLAND

Shetland sheep produce a fleece that is very fine and supports the tradition of knitting on the island – the wool is famous across the world. Shetland sheep also have the widest ranges of colours of any breed.

There are 11 main colours as well as 30 markings, many still bearing their Shetland dialect names including light grey, grey, white, emsket (a dusky bluish grey), musket (light greyish brown), shaela (a dark steel grey), black, fawn, and moorit (shades from fawn to dark reddish brown).

“The sheep’s grazing habits can influence wool colour,” explains Amanda. “And the programmes to control scrapie led to a massive reduction in the number of coloured rams so I suggested that we select coloured rams to participate in the semen collection programme.”

THE LONK

Lonk sheep were badly affected by the UK’s foot-and-mouth disease epidemic in 2001 and The Sheep Trust collected semen from this breed in an attempt to conserve genetic resources that were at risk.

“More recently the National Scrapie Plan in the UK set up a semen archive to ensure that genetic material was not lost as a result of the removal of animals with the VRQ genotype.

“The Lonk breeders only submitted one ram to this project and so, of all the native breeds in the UK, the Lonk is the most under represented breed in the semen archive. Through our longstanding association the breeders were willing to participate with us for this project,” adds Amanda.

SHETLAND COLLECTION PROGRAMME

Shetland sheep are scattered across the Shetland Islands, so the first task was to get them all transported to the island’s main port – Lerwick. Here they were inspected by a vet before setting sail, over night, to Aberdeen.”

Since the rams had to be in tip-top condition, in order for semen collection to be successful, they were housed in luxury accommodation – by sheep standards anyway – both on the ferry and in Aberdeen. “The ferry livestock accommodation had recently been re-vamped, so that was some good news for us,” says Amanda, “particularly since Shetland rams are prone to fighting.”

She says that Shetland rams are very naughty and bully each other. “They can’t be transported together and the new accommodation allowed rams to be housed individually.”

After a brief stay in Aberdeen, the rams were then collected by a representative from Innovis (a specialist company providing technical support for UK-based breeding organisations) and transported by road to its collection centre in Edinburgh – another 175 miles south.

“So it was a long journey with a difficult start. Getting all the rams to Lerwick at the same time to catch the ferry was the toughest part. And the whole operation was made a whole lot tougher by the weather. We had some of the worst snow in the UK for 20 years while we were running this operation.

“It’s not something we expected and it did make things a lot harder. But we managed to get there in the end.

“Getting the sheep home proved most difficult as that was when the snow really hit us. It was difficult to organise the ferry crossing, since there’s only one sailing a day. And the snow meant that this phase was very much a case of ‘stop-start stop start’. But we got all the rams home eventually.”

LONK LOGISTICS

Snow also hampered efforts to collect semen from the second of the two UK sheep breeds – the Lonk.

There were two opportunities to collect semen, due to the seasonal nature of sheep breeding. And when the team made their first attempt, the six-day stand still for sheep was in place to help prevent the spread of disease from flock to flock.

“We couldn’t collect them by travelling from farm to farm and similarly they couldn’t be returned in this fashion either.

“So we organised for them to be both collected by us and returned to their owners at Clitheroe Market in Lancashire and both times we made provisions similar to those made on a sale day.

“And after collection and return these rams and all the sheep on their home farms were subject to the same six-day stand still rule as all other sheep,” explains Amanda.

Again it all sounds relatively simple, although it did again require a considerable amount of co-operation on the part of the farmers involved. And once again the weather served to complicate matters.

“Returning these sheep also proved extremely difficult due to heavy snow. Three times we arranged a date for the rams to be returned via Clitheroe and twice the snow intervened and we had to postpone. But we got them all home in the end.”

OBSTACLES AND CONCERNS

Bluetongue also caused a few headaches. Collection was scheduled to take place in October – the typical breeding season ‘window’, but the disease made an appearance in late 2008 and the team had to wait until November.

“All in all it was a logistical nightmare – complicated by geography, the weather and disease.

“And we were also really worried that once we got the sheep to the collection centre that the rams wouldn’t ‘perform’. We used artificial vaginas at the Edinburgh centre, for example, and we couldn’t be 100% sure that that would work as Shetland rams can be a little shy!

But all the TLC on route to the centre meant that the rams were ‘raring to go’. So Amanda and the team had worried unnecessarily. “And they’d all put on a bit of weight, according to their owners, during their ‘holiday’ – don’t we all!”

PROGRAMME SUCCESS

So semen from a total of 24 Lonk and 17 Shetland rams was successfully collected. “We were aiming to collect from 24 Shetland rams, but seven didn’t make it to the ferry. They’re

seasonal breeders and we missed the window of opportunity with these rams,” explains Amanda.

“But that said, we’re really pleased with what we achieved and how well we overcame the difficulties that we were expecting – as well as those we were not.

“I think we did well and we’ve certainly done what we set out to do, which is to preserve and safeguard the genetics of these two HSBs for future generations.”

3. Greece

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 it – 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 in the region of Epirus, where the breeds were raised and in the majority of the cases, 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 a researcher of the National Agricultural Research Foundation, 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.”

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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, 100x106 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 packaged 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.”

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.”

SUCCESSFUL 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 – 1,000 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.”

4. France

Collaboration eased semen collection

Willing farmer participants and help from AI organisations helped the French HERITAGESHEEP project team to achieve its semen collection target. But considerable organisation and planning was still required on its part.

French sheep farmers were keen to collaborate with France's HERITAGESHEEP project team. They seem to understand the urgency and the vital nature of what the team was trying to do. This attitude went some way towards helping the French team collect a huge amount of semen from four 'meat' breeds – there's already some semen 'banked' from the country's native and 'local' dairy sheep breeds. But the process still involved a lot of planning, patience and hard work, according to leader Delphine Duclos, from the Institut de l'Élevage.

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CO-OPERATIVE COLLECTION

The French programme involved collecting semen from four different breeds – Rava, Limousine, Bizet and Causses du Lot. Semen was taken from live rams. Therefore, rams from these breeds of flock and situated in two different parts of France, had to be transported to two collection centres.

This was made considerably easier due to the exceptionally good co-operation of the sheep farmers involved. "Nothing exists in cryoconservation for their breeds of sheep and they were all too aware that this left them in a vulnerable position should, for example, a serious disease wipe out their flocks. They didn't have any way of preserving their breed's genes, but we were offering them a solution – a 'back up' plan, if you like," explains Delphine.

FOUR 'LOCAL' BREEDS

The Rava is an ancient Celtic breed that has acquired plenty of strength and hardiness. The rams are able to live on a meagre diet for part of the year and the fleece protects the breed in severe weather.

The Rava's characteristics are distinct black, or sometimes russet, patches on their heads and they have no horns. Their legs are covered with fleece.

The Bizet has an ability to breed out of season and this means that a ewe can lamb up to three times in two years. Around 50% of ewes are pure-bred and also have good milk production, making the breed highly valued for crosses with rams for meat production. The sheep are biscuit and white coloured, with black heads and legs, white nose blazes and white hooves. The male has rolled horns, the female is polled.

The Causses du Lot is well adapted to its environment and is well suited to free-range or 'sheepfold' management systems. The ewe matures early and breeds well out of season. In large flocks there can be two, three or even four lambing times spread throughout the year. Ewes achieve a prolificacy rate of 1.59. The sheep are white with black eye patches. Neither the male nor female is horned.

The Limousine is noted for its early sexual maturity, with some ability for out of season breeding, and for its fertility and good milk quality. The breed is white with horns on both males and females.

All four are 'local' breeds – they come from a specific region of France and were, therefore, also collected from here to be taken to the semen collection centre. The Rava, Bizet and Limousine breeds all come from Auvergne and were collected by, among others, Jerome Gueux. The Causse du Lot is, not surprisingly, from the Lot department, in the Midi Pyrénées region and one of the people collecting these rams for delivery to the collection centre, as well as their semen, was Hubert Issaly.

FREEZING SEMEN

In France, we chose the breeds among the meat breeds because semen is already stored for the dairy breeds. Furthermore, we have to choose organisations able to collect and freeze semen.

“We worked closely with the Races de France – Fédération des Organismes de Selection, which already has good contacts with two of the breed organisations we contacted and this certainly helped a lot with some of the logistics of rams and semen collection,” says Delphine.

She explains that ram and semen collection was carried out by two organisations. “The first collected rams and semen from three breeds – Rava, Bizet and Limousine – because all these breeds are situated in the same area and managed by the same structure.”

This AI centre has Limousine and Rava rams that visit on a regular basis, so these made up the majority of our 'donor' rams. Bizet rams visited the centre only for the purpose of this project. All rams stayed at the centre for several months – for quarantine, training and collection – which took several weeks.

But the results were well worth the wait. Five Rava rams produced 893 doses of semen, 10 Limousine rams gave 1,783 doses, and three Bizet rams produced 317 doses of semen.

A second centre dealt with the Causses du Lot rams. A few are selected each year for AI, but only for fresh semen collection – it isn't frozen. “The season for fresh semen begins in the

middle of March, so we made sure that the collection for frozen semen happened just before that, at the end of February,” says Delphine.

“We selected 22 rams and the centre collected and ‘trained’ them for several weeks. But to freeze the semen, that particular AI centre had to collaborate with another organisation that was specialised and equipped to freeze sheep semen.

“And that’s why the collection was carried out in just four days!” she adds.

RESULTS

A record time – with record results to match. A total of 2,771 doses of semen were collected from 22 rams. “The most productive ram gave 150 doses in three collections,” says Delphine. “And the least productive have just 27 does in one collection.”

Individual performances aside, the French team achieved what it set out to do and now, thanks to its hard work and dedication, the gene bank for these four French ‘meat’ breeds is well and truly in the black. Their semen has joined the national collection in France’s cryobank. And producers, breeders and sheep enthusiasts can rest assured that their breeds are now safe for future generations.

5. Slovenia

Plenty of muscle and the power of persuasion

Brawn – as well as brains – has been key to the success of the semen collection programme carried out by the Slovenian HERITAGESHEEP project team. And despite many obstacles and set backs – including heavy snow and wolves – it has collected an impressive amount of semen so far.

A battle against the elements, and native predators, has done little to dampen the enthusiasm of the Slovenia's HERITAGESHEEP project team, which is charged with collecting semen from some of the country's heritage breeds. In some instances brawn – not brains – won the day and despite some bad luck the team is well on its way to completing the task with considerable success.

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PARTICIPATION PERSUASION

Persuading sheep breeders to take part – or rather to offer their rams for semen collection – was relatively straight forward, according to Drago Kompan, who headed up the Slovenian team. He's assistant professor at the University of Ljubljana's zootechnical department.

“Because the national selection centre in Slovenia is part of the institution that is also taking part in this project, we have permanent contacts with the breeders' organisations. All the breeding work is carried out in partnership with the breeders and their organisations,” he explains.

“Our co-workers and extension personal have attended their meetings, on both a national and local level, regularly during the past three years.”

The HSB project and its aims were explained to them all during their annual meetings and they were all more than willing to assist in the project. They understood its importance in protecting the future prosperity of their respective sheep breeds, according to Drago.

WHICH BREEDS?

Deciding which breed to choose for semen collection was also relatively straight forward. The project team looked at the effective population size, geographical isolation, and other unique traits. And a huge emphasis was given to each breed's vulnerability in the case of possible disease outbreak.

“Due to the outbreak of bluetongue disease in neighbouring Croatia, Italy and Austria we decided to collect semen from the two most endangered breeds of sheep. Both are located close to the bluetongue-infected neighbouring countries,” says Drago.

Predators, such as the brown bear, wolf, and lynx, also ‘make trouble’ and threaten the breeds, particularly the Istrian Pramenka and Bela Krajina Pramenka breeds. “So these were the breeds that we selected.”

BREED DESCRIPTIONS

The Istrian Pramenka milking breed has quite a large frame, owing to the highly placed torso and long strong legs. Its udders are a good shaped, fastened high, with large teats. The sheep's multicoloured wool does not cover the animals' entire body – their legs and stomach are naked and other body parts are only covered with shaggy fleece.

As with other breeds, Istrian sheep are not all alike, and this characteristic has given way for the animals to be named after the location rather than their appearance (since the sheep look different from one breeder to the next). The breeders breed, for example, white, black, spotted, patchy sheep, short-eared sheep, horned sheep, and horn-less sheep.

Istrian sheep have a couple of traits in common: their tenaciousness and adaptability to relatively rough surroundings. And their milk contains, on average, 7.2% fat and 5.9 % protein. Some ewes will produce milk with more than 8% fat and 6% protein.

Slovenia's Bela Krajina Pramenka sheep have long fringed wool, which acts as a good protection against the cold and rain but it is not appropriate for manufacturing because all woollen products are extremely rough. The most common coat colour is white, although it is almost impossible to find an animal that would be purely white because black dots or spots on the head and legs are characteristic of the breed.

The sheep's tails are unusually long, and reach down to just a few centimetres above the ground. Rams have extremely large horns, which are curled several times as the animals get older. Sheep may have horns too but they are short.

TRAVEL ARRANGEMENTS

Despite their willing participation, not so straight forward was getting breeders to take their rams to the semen collection centre in Ljubljana. And sometimes team members had to go to individual farms to collect rams themselves.

“One of the team travelled to one farm with a jeep and livestock trailer to pick up some rams. But, due to the unexpected weather, he got stuck in the snow just 500 metres from the farm.

“So he had to lead the rams to the trailer, through the snow, on a leash. He even had to carry some of the rams,” explains Drago. “When you consider the weight of the rams that was far from easy to do – particularly in those weather conditions. That’s dedication for you!”

With another Bela Krajina Pramenka breeder, the team agreed to collect semen from one particular ram. However, in the meantime, the breeder’s flock had an unexpected ‘visitor’. “A wolf attacked the flock and killed the chosen ram. How unfortunate was that?” says Drago.

HAPPY ENDING

But despite these set backs – however unusual and unlucky – the team continued to work hard. Semen was taken by the veterinary service in Ljubljana, in the department of reproduction, using the electro ejaculation technique. And, up to now, the team has collected semen from a total of 15 rams – 5 per breed.