



BushaLive (PO No. 299432)

Determination of different types and strains of Busha Cattle in the Balkans, Sustainable use of Busha Cattle: Comprehensive overview in the field, development of an overall-crossborder conservation model

Final Report March 2015



Introduction

The BushaLive project, funded under the Funding Strategy for the Implementation of the Global Plan of Action for Animal Genetic Resources, targets the autochthonous Busha cattle breed of the Balkans, which survives in small, highly endangered, populations. The breed is hardy and well-suited to extensive farming, but has relatively low production yields. It is an important part of the local identity, but will be lost if conservation measures are not put in place to protect it. Stakeholders across the various nationalities and religions present in the Balkans share a common willingness to collaborate in conserving the breed.

Background

The BushaLive Project was implemented 2013-2015. Previously, SAVE Foundation spent many years gathering information about Busha and other shorthorn cattle as well as networking stakeholders in the Balkan region. The International Workshop on Shorthorn Cattle of the Balkans, held at Pogradec, Albania (11-13 September 2008), brought together the partners for a cross-border regional co-operation for indigenous livestock breeds of the Balkans region particularly on Shorthorn cattle (Busha, Illyrian, Brachyceros Cattle), which occur in all countries of the Balkans. Statements and reports about the current state of the Shorthorn Cattle in each country were given as short reports, published on the internet before the workshop, and presentations held during the workshop. The participants exchanged information on the special problems of breeding in the different countries in three working groups. The presentations and reports from this meeting can be found here: <http://agrobiodiversity.net/balkan/Pogradec/workshop.asp>

The BushaLive project was based the following needs and actions that were identified in Pogradec in 2008:

Needs

Securing the Breeds

- Securing and documenting the traditional Agro-Eco-Systems
- Identification of the different cattle types / local phenotypes
- Identification of the individual animals
- Define breed standards
- Build nucleus groups and nucleus of farmers
- Include from beginning on all pure animals to minimise in-breeding
- Organise farmers in breeder associations in each country
- Strive for a breeding population of at least 200 purebred animals and make security doubles by dividing in groups
- Breed with a minimum of 10 unrelated males
- Organise an efficient bull keeping system
- Ask for subsidies under Art. 11 of Convention on Biodiversity
- Storing of semen as additional security measure
- Gene bank with somatic cells central for Shorthorn Cattle

Balkan Network

- Continuation of identification and evaluation of shorthorn cattle
- Molecular genetic characterisation and distance of the different types of Shorthorn Cattle in the Balkan Countries
- Database of Shorthorn Cattle
- Integrated information system for the exchange of data, animals and products
- Continuation of meetings with all three pillars (government, Science, breeders), but main focus on the Farmers: strengthening crossborder cooperation
- Umbrella organisation

Network for in situ conservation

- Evaluation of the current state in each country
- Stakeholder listing on the SAVE Webpage (open listing)
- Webpage for Shorthorn Cattle of the Balkans
- Creation of a Forum for in-situ conservation (possibly with "top-farms")
- A printed catalogue of the different types and breeds, contacts etc. for spreading best practice
- Busha breeder associations necessary in each country

Establishment of in situ conservation

- Establishment of breed standards
- clear guidelines for breeding
- Exchange of breeding material within similar types
- Evaluation of good bulls
- "contest of best farmers" to stimulate an accurate breeding

Herd book

- establishment of (comparable) herd books
- to optimize mating and for crossborder exchange the same herd book programmes should be used, which should be cheap and easy to handle
- minimal herd book standards have been defined

Promotion

- Transfer of knowledge to the breeders and to the public
- Collection of the knowledge of the breeders
- Listing of project activities in the different countries
- Promoting quality Food and making use of labels
- Making best use of low-input-agriculture to become profitable
- Promotion of special services (e.g. grazing)
- Promoting as cultural heritage and for tourists
- exhibition on national level and if possible on international level

The most important actions for the nearer future were stated as follows

- Annual meetings shall go on
- continuation of the exchange of data and information
- promotion of rare breeds of the Balkans especially of Short-horn breeds (Busha; Illyric Cattle, Brachyceros Cattle) by printed brochures or catalogues and by Internet



The BushaLive Project took some of the key ideas from this list in order to perform genotyping, to collect data at both animal and farm level and to further promote networking and information exchange between stakeholders.

The Steering Committee of the BushaLive project met in Sarajevo on the 18-19th April 2013. A summary of the information available and work already done was presented. The situation of Busha in each country in the Balkan region was discussed along with a systematic approach to monitoring, characterising and

collecting samples for DNA analysis. A map of the area was marked with all known populations – black to show known populations, red for places that need to be verified.

A field visit was made to a near-by small-holding so that the survey that had been prepared could be tested. It was concluded that only minor changes were needed. It was clear that the farm level data is a very important factor in this data collection. As many animals as possible should be recorded. The draft survey was completed and sent to the members of the Steering Committee for use in the individual regions where Busha occur. The field information was then entered into an excel file in order to provide an overview of the information available.

Participants in Sarajevo were also given the equipment required for the blood sampling and were shown how to use it. This was followed by a lengthy discussion of the best way to send the samples to the laboratory. Although the formalities for importing blood samples had already been discussed and approved by the German authorities, it was concluded that sending blood samples to the laboratory in Germany would be problematic as many countries do not allow the export of samples. After discussion it was concluded that, in some cases, DNA extraction should be undertaken within the national borders. Those who could send blood without problem sent them by airmail to the Ludwig-Maximilians-University. In other countries it was important that cattle were sampled before they went to their summer pastures. In some countries this was delayed and samples could then only be taken in autumn. This led to a delay in the schedule as no tests were carried out in the laboratory until all of the samples had been received in order to keep costs within the budget.



The aim was that about twenty representative animals should be sampled in each country. Sampled animals should be possible candidates for a crossborder programme therefore they should be:

- Young/fertile
- Purebred
- Breeding animal (i.e. not male going for slaughter)
- From as many different bloodlines as possible
- If possible from different breeders
- Phenotypically a wide range – it is not necessary to choose “perfect” animals as long as they meet the above requirements

The survey was also completed as a part of the field work. For each animal sampled, a range of phenotypical characteristics was also recorded. Details about each farm were also recorded, so that the use of Busha can also be assessed. Due to the slow pace of the sampling process, the surveys were also not returned to schedule, which led to a delay in the data analysis.

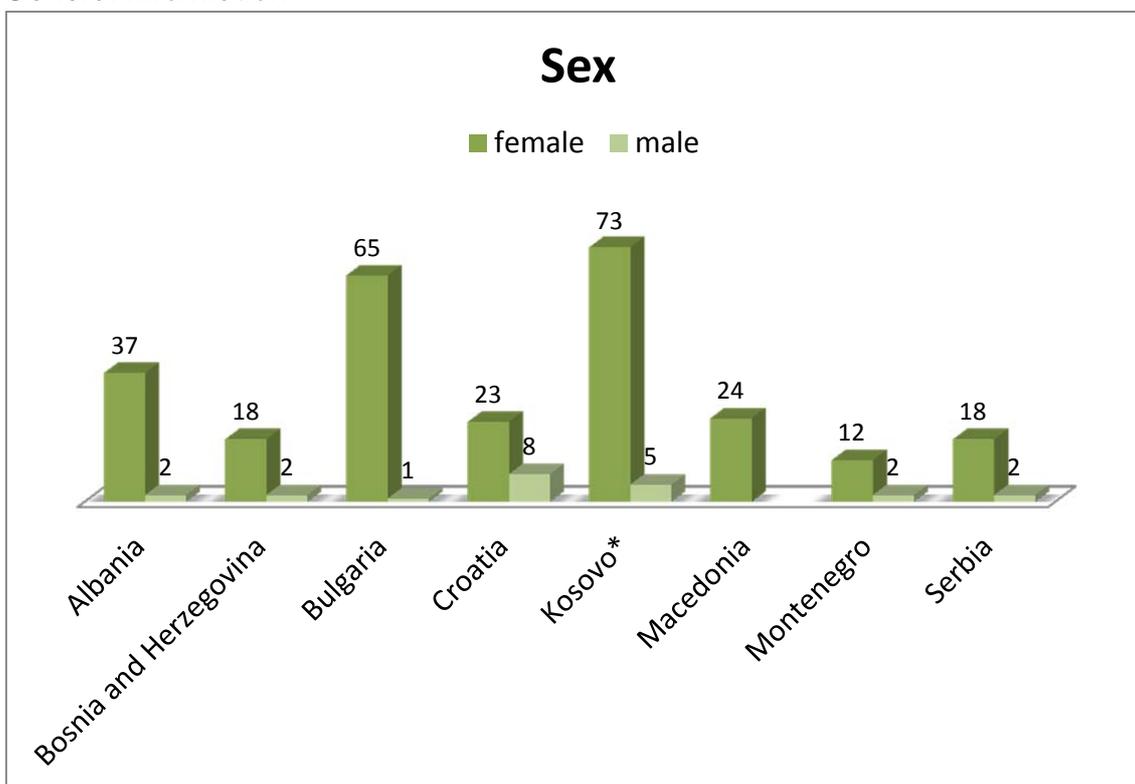
Due to the delays, the planned roundtable with stakeholders with a study visit to an example of best practice to come to a consensus about a suitable recording system and to exchange experience of promoting local, quality food products and selling "cultural heritage" and "tradition" was been delayed and therefore adapted. An online conference was held on 18th December 2014 to discuss the issues and share the state of knowledge at that moment. The exchange of experience of promoting local, quality food products and selling "cultural heritage" and "tradition" was incorporated into the final workshop that took place in Dubrovnik in March 2015. Nearby is an interesting Busha farm that was visited as an example of best practice, see: <http://www.busa-dubrovnik.com/>

Survey Data – Summary of the presentation at the Final Workshop, Dubrovnik, March 2015

When presenting the results, it became clear that some of data provided on the survey must be incorrect. Therefore, this data needs to be corrected before all the results are presented. Those results that are distorted by mistakes in the data are not presented here.

224 surveys were received from 7 countries; there were 203 females / 21 males

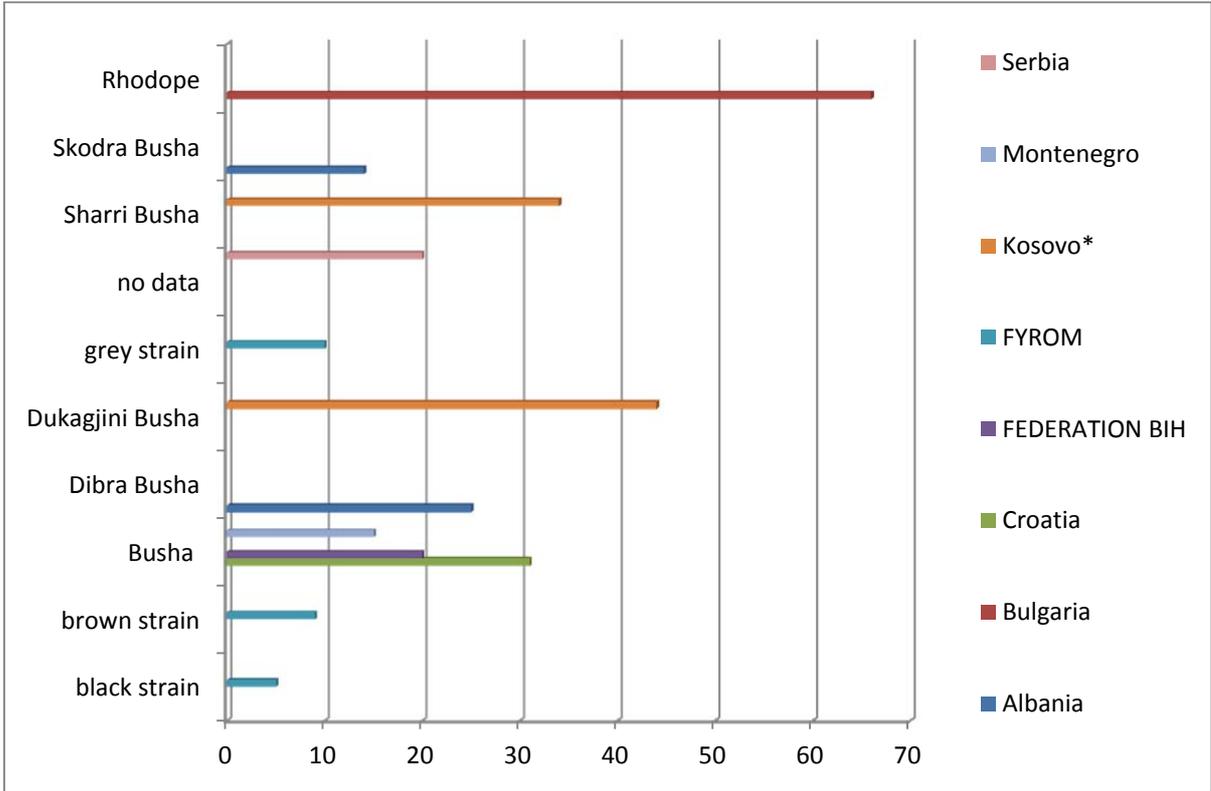
General Information



Population trends

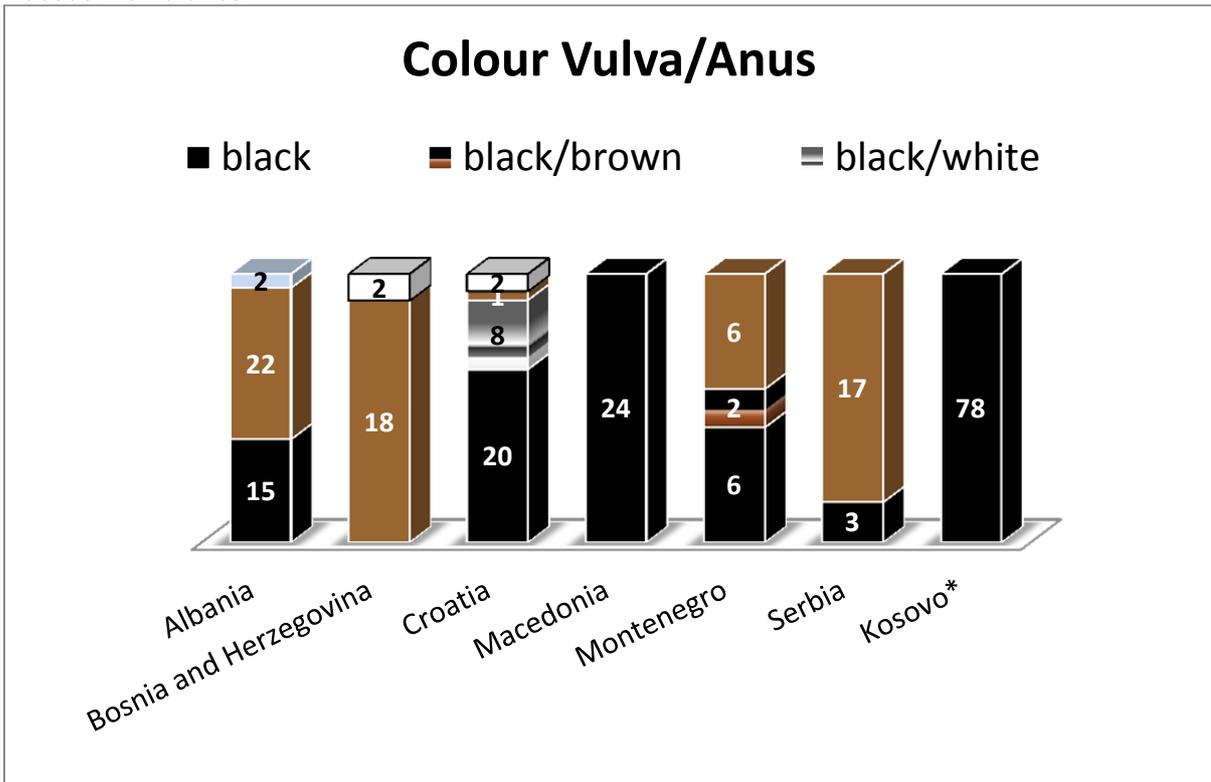
Country	Decreasing	Increasing	No data	Stable	Total
Albania			39		39
Croatia		16	15		31
FEDERATION BIH	20				20
FYROM	3			21	24
Kosovo*	33	36		9	78
Montenegro	9			6	15
Serbia	20				20
Total	85	52	54	36	227

Strains per country



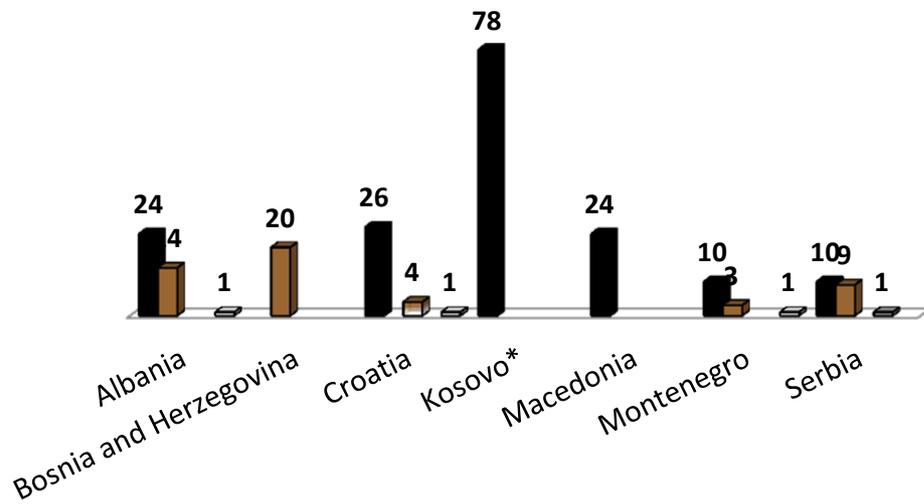
Physical Characteristics

The occurrence of black or dark mucous membranes has been used as a phenotypical indicator of the Busha breed. However, animals with "tiger" markings or in the same blood lines tend to have light mucous membranes.



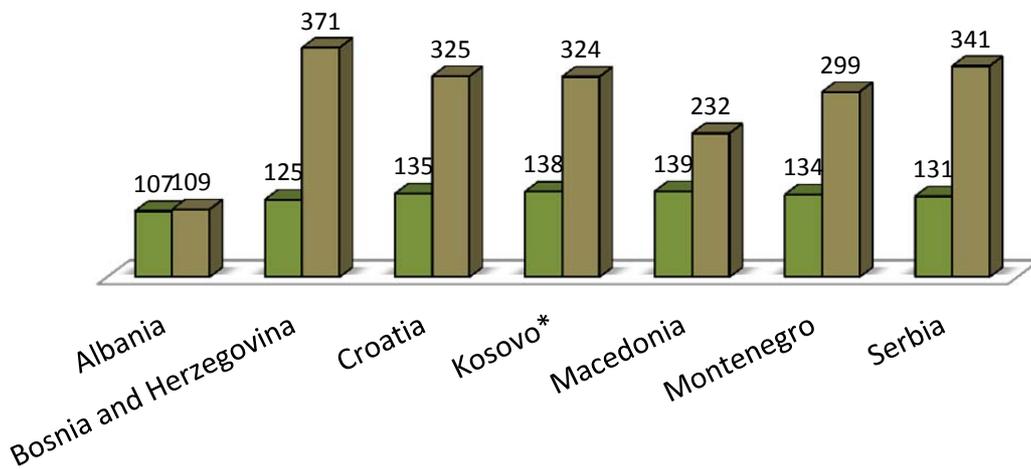
Hoof colour

black
 brown
 brown/white
 grey
 white

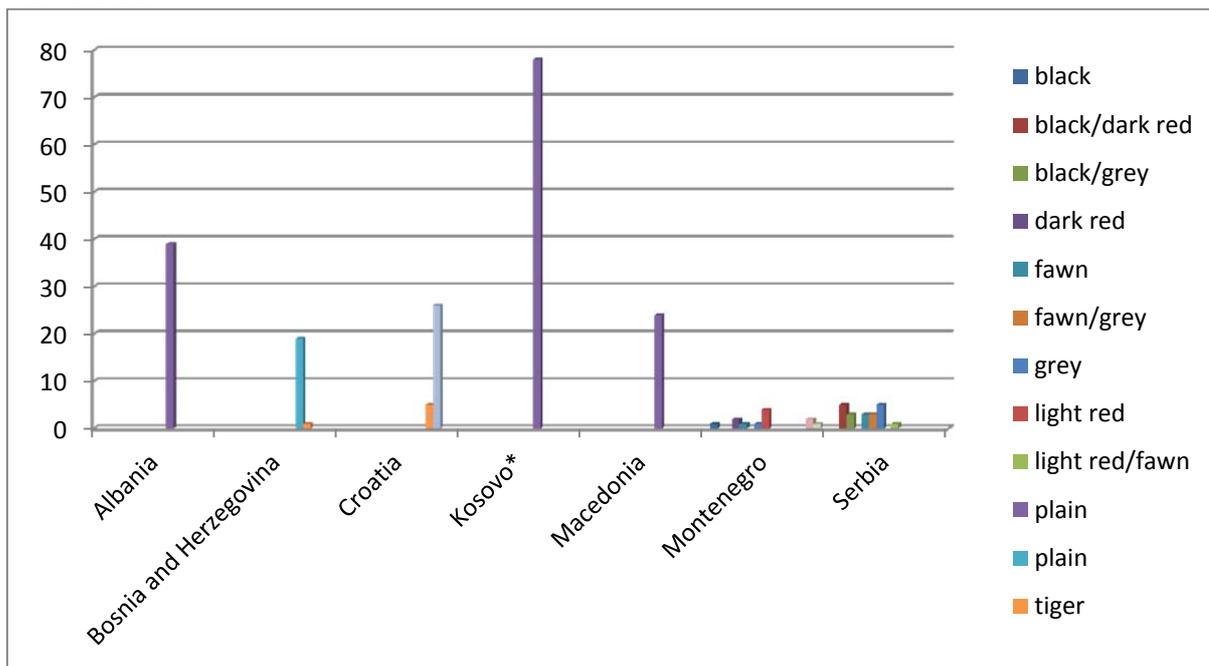


Body sizes

average body length (cm)
 average body weight (kg)



Hair colour



The hair colour of Busha is always unicolour and not patched or in different colours. The so called “tiger” Busha are usually tigated (brindled) over the whole body.

The occurrence of an “eelstripe” has previously been used as a phenotypical indicator of Busha. However, this is not present in all countries:

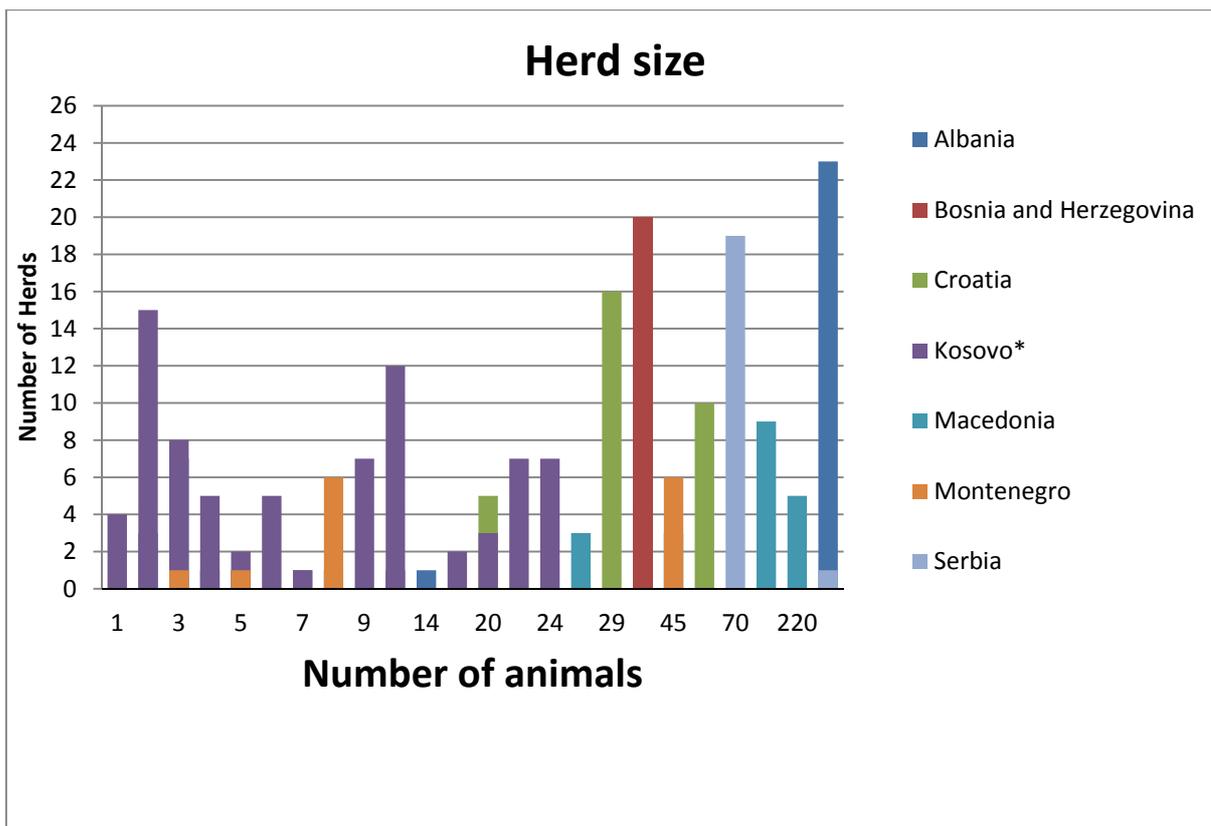
Country	Eelstripe	Country	Eelstripe
Albania	No	Kosovo*	No / no data
BiH	No	FYROM	no
Croatia	Partwise farm 1 Šestanovac / Imotsk	Montenegro	no
Serbia	yes		

Farm level data

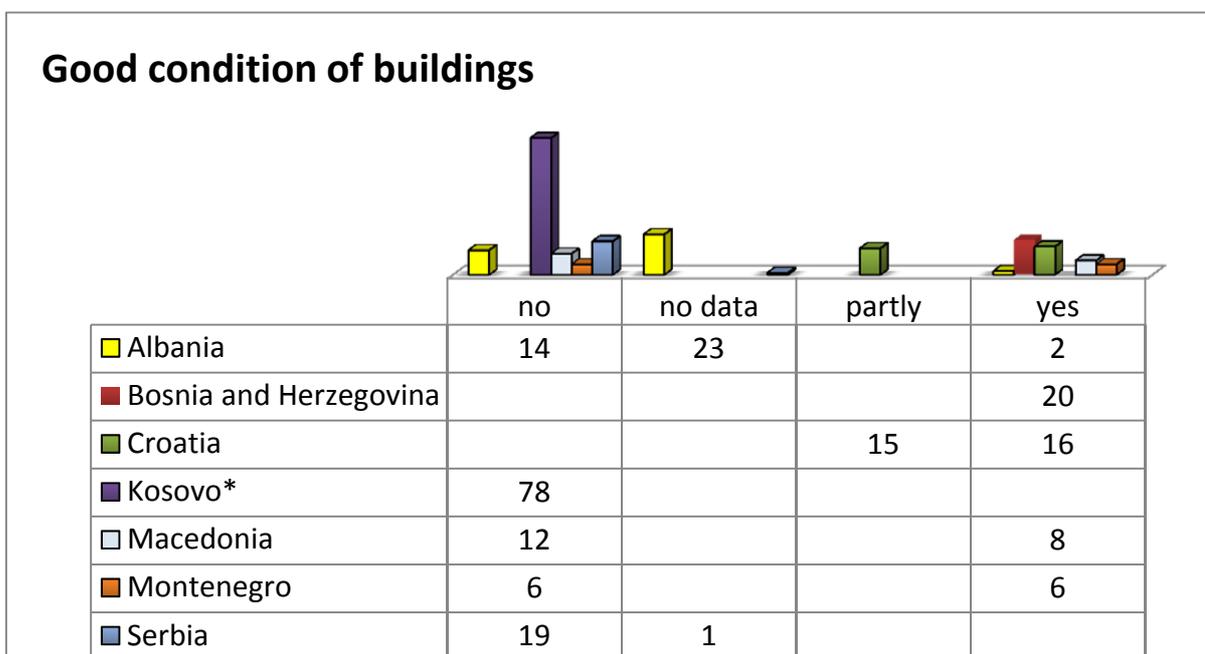
The “Farm Level Data” should give an expression of how well secured the animals on the respective farm are. All investigated farms are peasant and extensive.

Mating practice

- Multiple sires: Albania, FYROM, BiH
- 1 sire per herd: Kosovo*, Croatia, Montenegro (ca. 3 bulls)
- No artificial insemination etc.

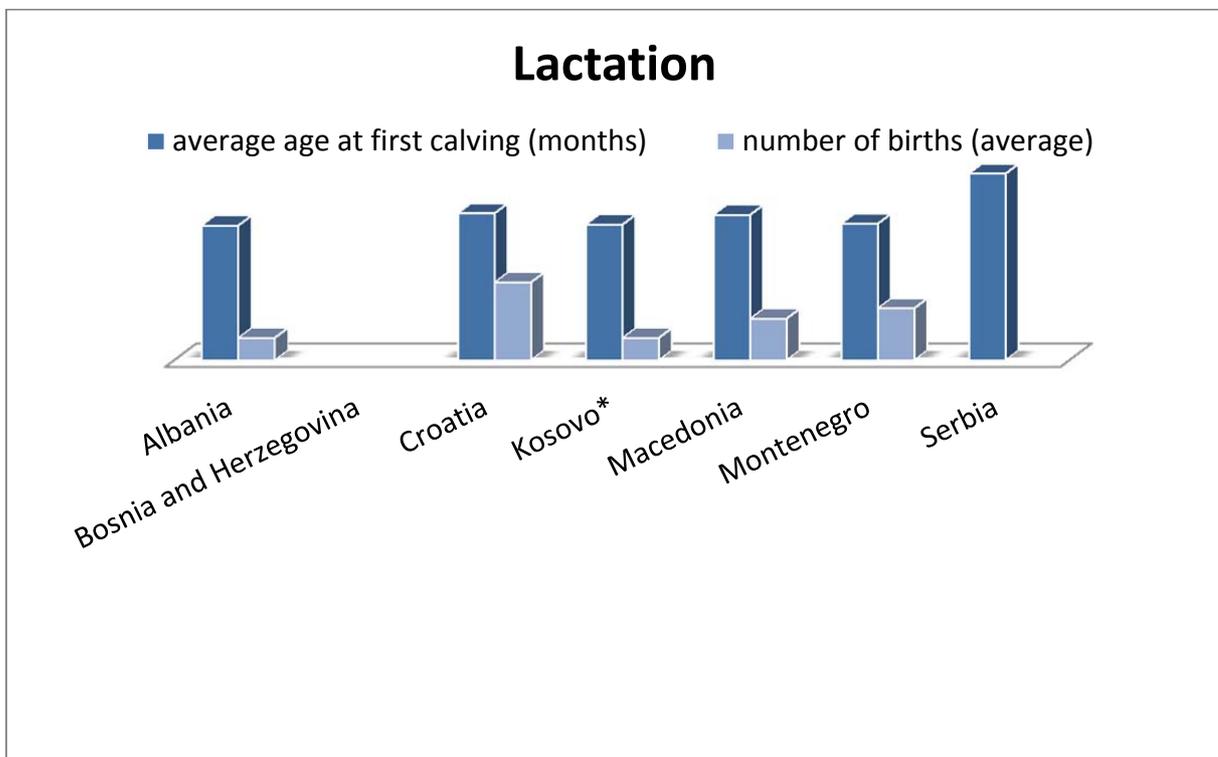
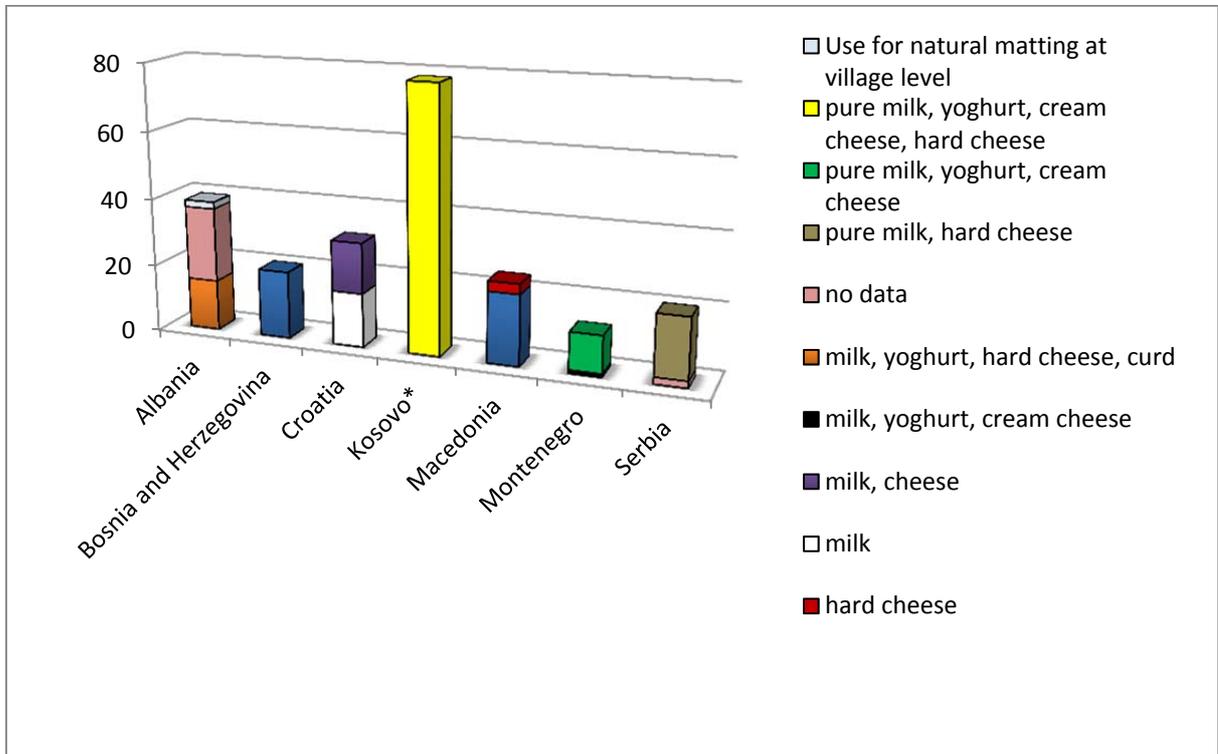


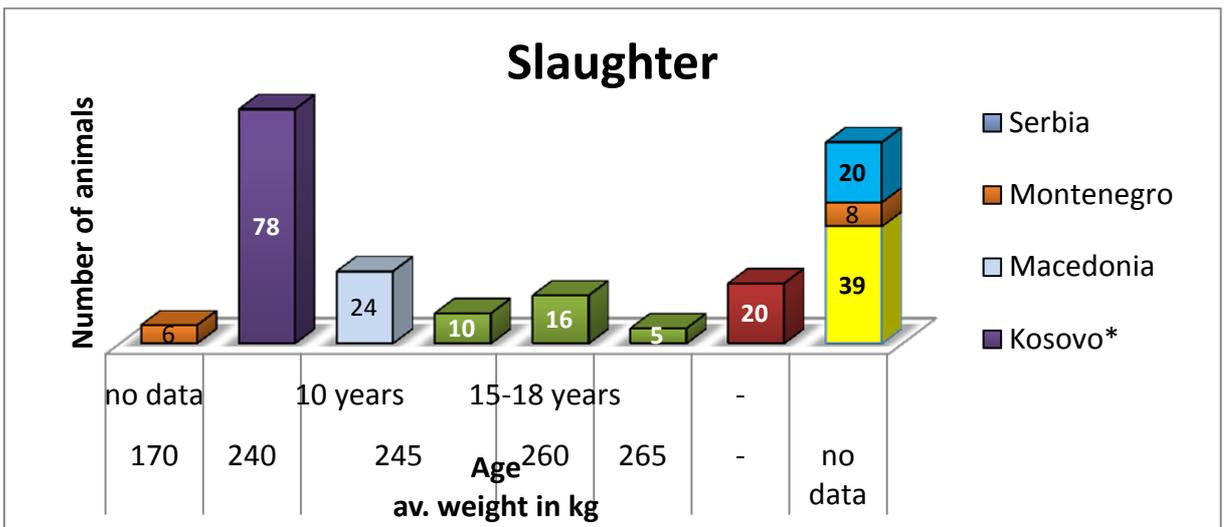
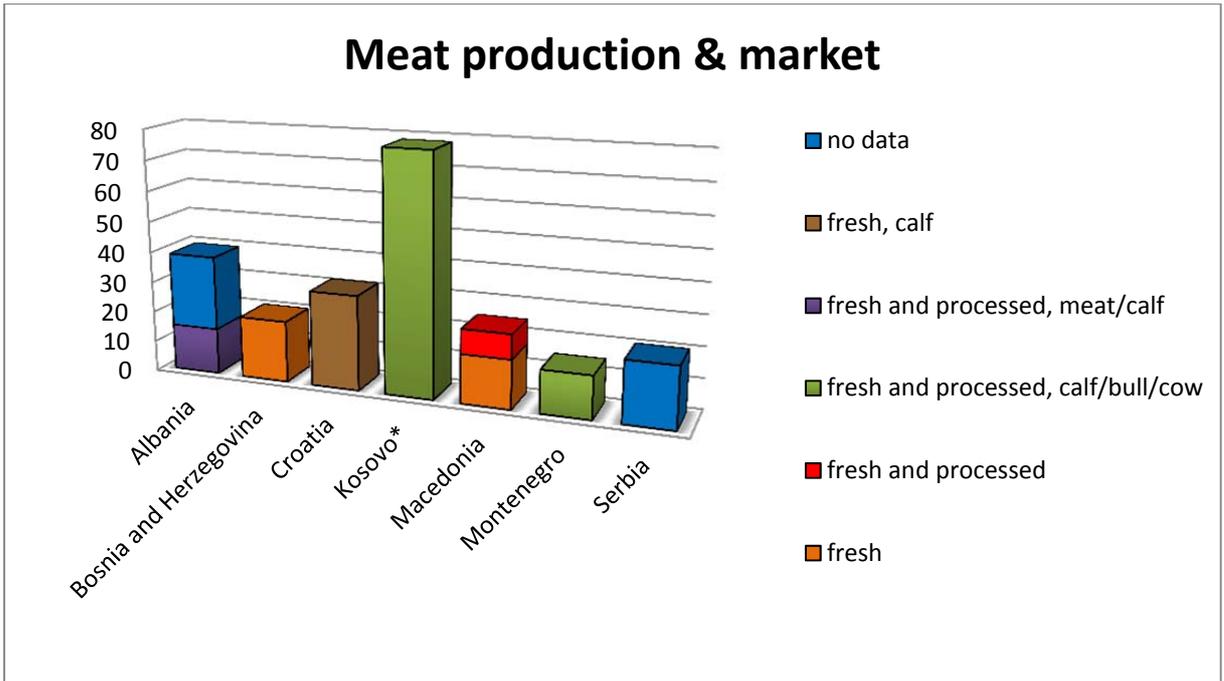
The ownership is not secured in Serbia, BiH, Montenegro and in some cases in Albania. The conditions of buildings can be a sign of the economic situation of the farm:



Products and Marketing

The production process is in all countries traditional. In some cases, calves are produced for meat, but mostly milk is an important product:





Survey Conclusion

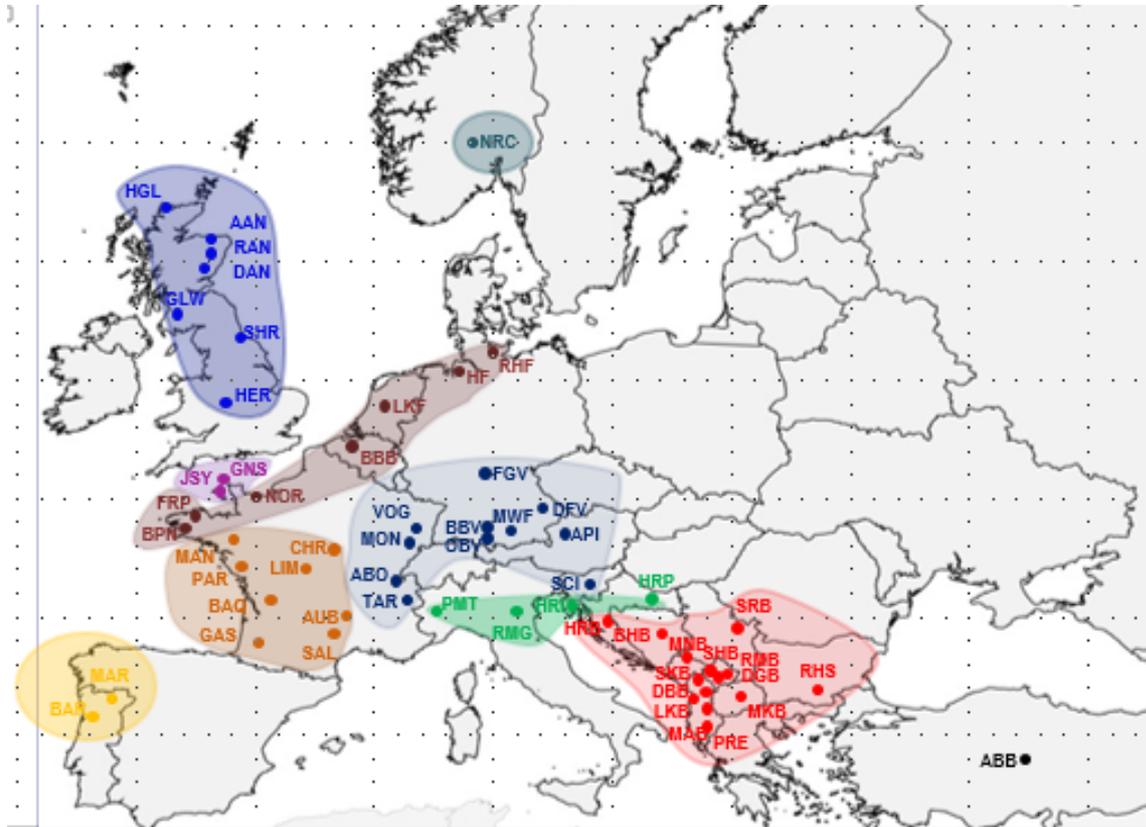
The survey data shows the difficulty of comparing phenotypical data collected by different people as much of it is a question of interpretation. Some data needs to be checked or updated so that the most correct version is available for the project. Only the data that is objective (withers height etc) can really be used together with the genotyping to create a profile of the animals. In the case of the “tiger” (brindled) Busha, the recorded colour will be helpful in determining why this marking occurs.

Genetic Data – summary of the presentation at the Final Workshop, Dubrovnik, March 2015

Material

The samples used in the data analysis were from 58 breeds

- 2 outliers (ZEB $N=36$, NDA $N=37$)
- 14 Busha breeds, collected through BushaLive and also samples previously collected.
- 42 breeds from Europe breeds from a large geographical area divided into clusters.

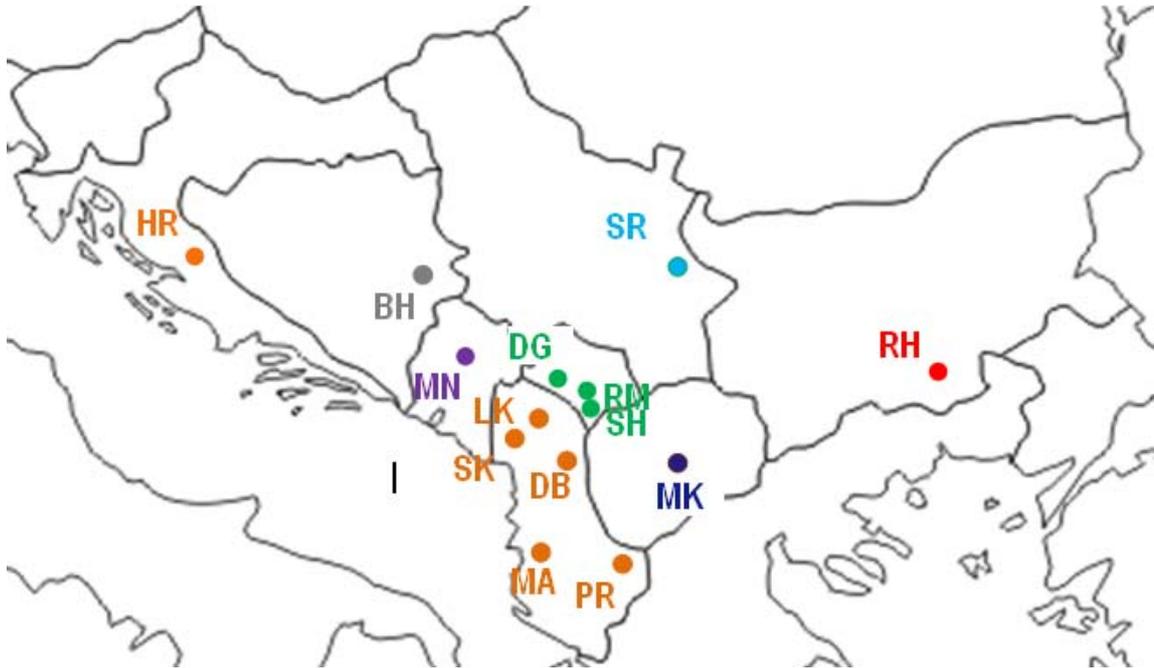


The

Busha samples were taken from the following places:

Country	Breed name	Abr. Breed	Sampling area	Country	Breed name	Abr. Breed	Sampling area
Albania	Lekbian Busha	LKB	(Lekbibaj)	Kosovo	Dukagjini Busha	DGB	Deqan
Albania	Skodra Busha	SKB	Back Rrjoll, Rrjoll	Kosovo	Red Metohan B.	RMB	(Blag, Kuklibeg)
Albania	Dibra Busha	DBB	Dibra Lashkize & Dardhe	Kosovo	Schaari Busha	SHB	Dragash
Albania	Middle Albania B.	MAB	(Divjake)	Monte Negro	Motenegrian B.	MNB	Andrijevica, Plav-Gusinje, Ulcinj, Berane, Plav, Herceg Novi, Niksic
Albania	Prespa Cattle	PRE	(Prespa)	Serbia	Serbian Busha	SRB	Stara planina
Macedonia	Macedonian B.	MKB	Strumica, Ohrid-Kicevo, Kvadraci, Trpejca	Bosnia and Herzegovnina	B&H Busha	BHB	Buhovo
Bulgaria	Rhodope Shorthorn	RHS	Kardzali, Haskovo, Smolyan	Croatia	Croatian Busha	HRB	Sestanovac, Gospic

An exact location of the sampling places on a map plus a detailed breed description from each country has been requested for the publications. However, an approximate location of the sampled herds is as follows:



Genotyping costs

Some costs, for shorthorn animals not found within the region covered by the BushaLive project, were covered by SAVE Foundation; costs for historical samples were covered by other sources external to the BushaLive project. The cost for the BushaLive project of the SNP chips worked out at about 80 Euros per animal. However, this cost does not include the costs of sampling, ca. 85 Euros per animal and, also the costs of an analysis and writing up of results for publication, in this case covered by a post-doctoral post at Ludwig-Maximilians-University Munich.

Therefore, it can be concluded that sampling and genotyping of a geographically wide-spread population, usually found in marginal landscapes, costs in the region of US \$180 per animal, not included the costs of fully analysing the data. This high cost is still prohibitive for many breeds and populations, even though it is urgently needed for conservation, as the results below indicate.

Genetic diversity - within 14 Busha strains

Results showing the diversity of Busha were explained so that everyone could understand the significance of what was being presented. The Busha results showed high genetic diversity in the population. The Busha were then clustered or grouped into the following:

B01 = Macedonia (MKB) + Kosovo (RMB+SHB) + Bulgaria (RHS) + Serbia (SRB) # **PRE** = Albania (PRE)

B02 = Albania (MAB+LKB+DBB+SKB)

B03 = Kosovo (DGB) + M. Negro (MNB) + Bosnia & Herzegovina (BHB) + Croatia (HRB)

The groups were then more closely analysed so that the relationships between the strains could be more clearly understood.

Global genetic diversity - between cattle breeds and IBD analyses within and among Busha strains and other breeds

The relationships between the individual animals samples were studied using Intity by Descent (IBD) Probability to provide a Genome-wide realised relationship based on genetic marker.

This way the expected inbreeding could be assessed to show the state of the sampled cluster population and compared to the global diversity. In this way the introgression from other breeds can also be judged so the status of “purebred Busha” can be given as it occurs. Also, the relationships between the strains sampled in the projects can be shown. This was explained in detail for each

cluster group. Through this animals can be excluded from the conservation programme and others can be chosen for genetic exchange with other groups. The introgression cannot always be seen in an external inspection but the genetic testing shows clearly which animals should be excluded from conservation programmes. This is the reason why so many European breeds were included as references in the analysis. Tyrolean Grey will be included in a future analysis, it has already been arranged that 50 samples of unrelated animals can be used. When possible the Danish Red could be included as its influence is indicated in some of the results. Including some older samples in the project and comparing with the 2014 samples shows that both inbreeding and introgression are slowly increasing.

Summary of the results presented at the Final Workshop

PopID	Inbreeding		IBD(W)		Minimal IBD	Maximal IBD
	mean	SD	mean	SD	(Pop)	(Pop)
MKB	1,102	0,077	0,004	0,183	SHR	RHS
PRE	1,032	0,026	0,002	0,087	SHR	RHS
MAB	1,044	0,059	0,001	0,057	SHR	JSY
LKB	1,035	0,042	0,003	0,097	SHR	RHS
DBB	1,062	0,066	0,002	0,200	AAN	JSY
SKB	1,070	0,085	0,005	0,123	SHR	JSY
RMB	1,035	0,028	0,002	0,056	SHR	MKB
SHB	1,066	0,070	0,005	0,132	SHR	MKB
DGB	1,053	0,069	0,006	0,181	AAN	RMB
RHS	1,080	0,053	0,003	0,105	SHR	MKB
SRB	1,101	0,086	0,001	0,118	SHR	MKB
MNB	1,049	0,042	0,004	0,090	SHR	MKB
BHB	1,120	0,081	0,019	0,348	SHR	MKB
HRB	1,031	0,031	0,002	0,042	SHR	RHS

The complete data will be made available to members of the steering committee for the future conservation work. The results will be published in full in the near future.

Conclusion

The results of the genotyping need to be taken into the practical work in the field. The results will help decision making for conservation practice; this has previously been based on phenotypical information, now decisions can be more accurate. Exchange over borders can also be facilitated now that the IBD can be used to show how populations are related. The influence of the Tyrolean Grey needs to be included as it is historically documented that the influence in all the populations is present.

The SNP genotypes will be made available in each country to be used in practical work and further research. A common publication will be made of all the results. The data can then be used for combined publications and for next steps in the research.

The Busha is a part of the cultural value of the area and this project has connected it to the most modern technologies, which is a very important for the future conservation. This project BushaLive shows that SNPs are very important for the future of conservation projects of animals in small populations as inbreeding and introgression can be minimised.

A post-doctoral post at Ludwig-Maximilians-University Munich has been funded to do a full analysis of the data and to prepare publications about the results even after the BushaLive project has been completed.

Consensus finding

The above summaries of the sampling and the survey data were followed by discussions in two working groups:

Working group 1 / Sustainable use of Busha Cattle

General overview of the situation

- Many small farms, many small herds, few young breeders.
- Direct marketing from the farm.
- Small, old fashioned structures.

How can the number of cows be increased?

- Encouragement to keep them via subsidies
- Modernise the structures, however many modern structures aren't suited to Busha
- Cooperation between farmers

How can young people be encouraged to keep the animals?

- Quality of life
- Infrastructure
- Family/women leave the villages
- Making money available through loans/grants

What can the Busha offer the market?

- Grazing
- Nature/habitat protection
- Products such as cheese or meat - are easy to sell, often there is no special marketing needed. Regional customers will buy the products, if they are good quality.

Main barriers

- Breeding – wolves, administration, inspections, vet services, labour costs, extensive pastures required, access to water, private forestry, lack of education/literacy
- Production – small pastures, cheap imported meat, too little pasture, no special ecological supports for the breeding, small populations.

Good practice

- Prespa – ecotourism (increasing Prespa cattle stocks)
- Croatia - Selling veal for a higher price rather than selling beef that does not compare well to commercial breeds
- Macedonia – gene bank (cry conservation of semen, blood, tissue, hair and from 2015 embryos)
- Montenegro – keeping small breeding groups of Busha cattle in monasteries

Conclusions

- Busha cannot survive without governmental support – this support does not just need to be financial (e.g. structure, legislation, tourism programme, access to pastures)
- Governments do not pay anything or do not pay enough and this probably will not change.
- Keeping breeding groups in publicly owned areas
- Keepers of Busha should not have to pay to graze on publicly owned land (e.g. nature parks) where a service is being provided.
- Cultural identity – heritage value is important
- Promotion of new farms with Busha cattle is very important
- Combined production systems – meat/milk/grazing
- There should be research into the special qualities of the products.
- Organic farming can increase the value of the products (perhaps even in the subsidies)
- Products – if production is increased then health and hygiene standards will make the increased production more complex and expensive.
- It's easier to fight the wolves than the government!

Working Group 2 / Model for a cross border data management

Recording system

- important for track data and exchange of animals
- must be unique for easiest exchange of animals

- ISO code of each state
- establish who has responsibility for keeping the recording system
- who is the responsible person for communication
- problems related to recording system:
 - lack of collaboration (between breeders, institutions and organizations, county level), ...
 - existing data is incomplete
 - existing breeding program for Busha is not implemented in practice (e.g. Serbia)

Country	Recording System	Ear tag	Recording system	Association of Busha breeders
Albania	-	+	-	-
Montenegro	+	+	1. Veterinary directory (Animal Identifying and Registration) 2. Biotechnical Faculty Livestock Selection Service (Animal recording)	-
Macedonia	+	+	Faculty of agricultural Sciences and Food Skopje	NGO Busha Skopje (2011)
Kosovo				
Serbia	+	+	Institute for animal husbandry Faculty of Agriculture Novi Sad	Local and regional level
Bosnia and Herzegovina	-	+	Agency for identification in Banja Luka (no check-out ear tags)	-
Croatia	+	+	Croatian Agriculture Agency	Association of Busha breeders (2003)

Exchange of animals between Busha metapopulations

- collaboration with others countries and exchange of experiences
- responsible person (organisation) in each country
 - Information about breeder?
- controlled exchange of semen, embryos, ...
- refreshing blood method (mating with most similar breeds)
 - must kept at a low level
 - introducing the most similar strains of Busha
 - short-term bull exchange
- respect veterinarian laws of each country

Committee

- talking about problems, things need to be changed, exchange some positive experiences
- Meeting in certain period of time?
 - expensive in this time
 - visiting and meeting at one breeder
 - e-conference
 - sending annual report

Results

Roundtable Discussion: Do we need a common Breed Standard?

It is difficult to define a common standard as there is a large range. A standard can also be damaging to conservation as it restricts the diversity found within the population to one type of Busha and some valuable traits may be lost. The diversity and the range are important for conservation. It is better to speak of guidelines for common characteristics to conserve the crossborder breed in a meta-population with many strains.

Roundtable Discussion: A strategy for ongoing breeding management

Within each country it is important that the breeders are in contact with each other and that the recording of the strains takes place. This recording should also include the ISO codes to support the ongoing exchange of animals or material with a unique ID of the animals. The exchange, guided by the results of the genotyping, can be used to solve the problems of inbreeding and introgression within population groups

Conclusion

The results of the blood samples and the survey show that Busha cattle are a crossborder metapopulation with different strains in each country. The project results can be used as a tool for a crossborder exchange of animals. With the results, breeders and universities are able to decide an adapted strategy to maintain the different strains in the respective countries. Over decades, an exchange of animals between the neighbouring countries has taken place. The influence of foreign breeds has been small up to now. There is a need to keep the practice of exchange with neighbouring populations for blood refreshing but it is important to not to take foreign breeds for breeding with at least the nucleus populations of Busha cattle. Crossbreeding for e.g. a higher milk yield is only a possibility for animals which are not used for any nucleus population. It was agreed to keep the different identified strains in each country. An exchange of breeding material should only take place for blood refreshing and within neighbouring populations based on the results of the DNA sampling.

Busha cattle are well adapted to the common extensive farming practice in the Balkan countries. The use of additional, concentrated feed is unnecessary. Under these conditions the performance of Busha cattle is much better than that of commercial breeds, which need a lot of input and are not able to graze in the dinaric-karst fields. Mainstream breeds usually have about three to four lactations. Busha cattle often have eight or more lactations. The output of milk within the whole lifetime of a Busha cow is therefore much higher than the milk yield of a mainstream breed under the same conditions. The use of the animals is the best way to conserve them and to increase the stock numbers.

Not only products but also services need to be assessed and promoted. Busha keeping is one of the best models for eco-conservation in protected areas – for example in grassland management to support diversity in plants and also in insects and worms for the food chain. Organic farming is a way of adding value to products and gaining more subsidies and recognition for the ecological value of the Busha cattle which is kept up to today under extensive conditions. This causes a big impact into the ecological balance of an area: The lessened use of Anthelmintics (Wormers) and Antiparasitic treatments in the practice of keeping Busha cattle causes a bigger occurrence of worms and beetles. Habitats that are grazed by Busha in this extensive way can therefore additionally be colonised by many of today's endangered species of insect and bird.

These facts are often not seen by the governmental authorities and agricultural science. Therefore the BushaLive project was a good opportunity – among others - to draw the attention on the advantages of Busha cattle in their region of origin. New and, up to now, less recognised arguments will help to improve the attention to this unique breed.

The most important objective to keep the aims of the BushaLive project alive is an ongoing communication between the countries and stakeholders. A regular exchange and reporting shall assure the conservation of the Busha cattle. This exchange shall be realized through a short reporting template, which can be published on the BushaLive website on www.agrobiodiversity.net in a regular cycle. A photo gallery of made up of the photos provided for the project plus a “breed description” for each country will also be made available on Agrobiodiversity.net in the near future.

The project strengthened the willingness of collaboration between the stakeholders across the different nationalities, religions, and other interests on the Balkans. Not only the conservation of an important part of the agrobiodiversity of the Balkans could be strengthened through BushaLive project, but also the personal responsibility of the actors and the dialogue across the borders was encouraged.

First Publication

Broxham ET, Kugler W and Medugorac I (2015) A case study on strains of Buša cattle structured into a metapopulation to show the potential for use of single-nucleotide polymorphism genotyping in the management of small, cross-border populations of livestock breeds and varieties. *Front. Genet.* 6:73. doi: 10.3389/fgene.2015.00073
<http://journal.frontiersin.org/article/10.3389/fgene.2015.00073/full>

** This designation is without prejudice to positions on status, and is in line with UNSCR 1244 and ICJ Advisory opinion on the Kosovo declaration of independence*