

IN-SITU AND EX-SITU GENE CONSERVATION PROGRAMS OF AUTOCHTHONOUS BREEDS IN CROATIA

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Summary

Protection of the autochthonous breeds in Croatia is performing more than one decade. National protection program is based on government subvention, and negative trend in endangered breeds was stopped, thanks to them. During the years of protection programs conduction none of autochthonous breeds were lost. Protection programs of autochthonous breeds are conducted primary through *in situ* models. Some new opinion concerning protection of autochthonous breeds point out their economic utilization, integration in protected areas, collecting genetic materials and storage in national "Genebank". Croatia has lot of protected areas, fullness of pasture land in which autochthonous breeds can be easily integrated. It is necessary to establish national 'Genebank', and new knowledge and instructions about storage genetic materials open the possibility of its quickly foundation and occupancy. Collecting somatic cells of autochthonous, primary endangered breed, is solution, mainly because of its simplicity of collecting and storage procedure, and lowest investment assets at the beginning. Along with collecting tissue cells, collecting of semen, embryos and oocytes is necessary. Information about tissue samples stored in national gene bank will be integrated in national animal information system.

Introduction

Active care about genetic resources in Croatia began at the end eightieth years of twenty century, after it was realized that some specific breeds disappear very quickly from their environment. First activities were directed to introducing professional and general public with problem of loss certain breeds and necessity of their preservation. Despite the transition in agricultural production and war events in nineteen years of twenty century, government took care about animal genetic resources, primarily through acceptance subvention assets intended for breeders of the autochthonous domestic animals. Growth of public interests for autochthonous breeds, prerequisites for activation conservation programs are created, primary through *in situ* models. At the same time, first steps *ex situ in vivo* and *ex situ in vitro* were done, but lately weren't systematically developed. Awareness the needs of more active integration *ex situ* models in actual *in situ* models of protection autochthonous breeds, establishment of programs of unique genetic bank and storage of tissue samples (semen, embryos, oocytes, tissue) are noticed on national level. It is well known that economical, political and other reasons (diseases) can lead to quick changes of interests in breeding autochthonous breeds, and contribute to doubtful survival if they are protected mainly through *in vivo* programs. In stimulant environment, which is obtaining in Croatia, enormous interests for breeding autochthonous, especially endangered breeds are present, mainly because of high stimulation assets. Violation stimulant environment like this, can autochthonous breeds (whose protections are based on *in situ* programs) again lead on the edge of survival. Establishment *ex situ* programs of endangered autochthonous breeds is necessary for several reasons. One of the most important reason to establish *ex situ* models (Cryogenic conservation) is opportunity of reconstruction of autochthonous breed in cases it's biological disappearance. New experience with appearance certain diseases in swine populations (swine fever, brucellosis) and sheep (blue tongue) dedicate on vulnerability *in situ* models in preserving autochthonous breeds, with respect to fast establish 'National Genebank'. Except above mentioned, deposited genetic material in 'Genebank', allows more concrete selection procedures in population, creating new or activating older lines/ genus's. Deposited material in genetic base is continuously support to already established *in situ* and *ex situ – in vivo* protection models, in cases of looses genetic (allelic) diversity, lines disappearance, increasing the inbreeding level and genetic 'drift'.

Actual state of Animal Genetic Resources in Croatia

On the list of autochthonous and protected breeds in Croatia are three cattle breeds (Istrian Cattle, Busha, Slavonian Syrmian Podolian Cattle), three horse breeds (Croatian Posavac, Croatian Coldblood, Murinsulaner horse), three ass breeds (Istrian donkey, North-Adriatic donkey, Littoral-Dinaric donkey), six sheep breeds (Ruda Sheep, Pag Island Sheep, Cres Island Sheep, Istrian Milk Sheep, Lika Sheep, Tsigai), two goat breeds (Croatian Spotted and Croatian White Goat), two pig breeds (Black Slavonian Pig, Turopolje Pig), two poultry breeds (Zagorje Turkey, Hrvatica Hen). Except above mentioned proprietary breeds, in Croatia also habit several breeds which are not on the list of autochthonous and protected breeds, because they are still in phase of morphometric, production and genetic defining, what is main criterion to get the breed status (Gray Dalmatian Cattle, Rab Island Sheep, Dalmatian Pramenka, Krk Island Sheep, Dravska Goose). Number and status indicator in autochthonous and protected breeds in Croatia are given in table 1.

Table 1. Status of autochthonous and protected breeds of domestic animals in Croatia

Species	Breed	Breed autochthonism confirmed	Number adult animals			Estimation of trend in population	Estimation <i>in situ</i> programs	
			Total (<i>estimate</i>)	Under control				
				Males	Females	(%)		
Horse	Lipizzaner	Yes	454	157	297	100	*↑	+++
	Croatian Coldblood	Yes	2120	127	2093	100	**↑	++
	Croatian Posavac	Yes	1618	117	1501	100	*↑	++
	Murinsulaner horse	Yes	25	3	22	100	*↓	-
Ass	Istrian donkey	Yes	150*	30	80	73	*↑	+
	Littoral-Dinaric donkey	Yes	2 500*	250	660	43	**↓	+
	North Adriatic donkey	Yes	80*	17	45	77	*↓	+
Cattle	Busha	Yes	111	11	100	100	**↑	++
	Istrian cattle	Yes	361	18	343	100	*↑	+++
	Slavonian Syrmian Podolian Cattle	Yes	75	1	74	100	≈	+
	Dalmatian Grey Cattle	No	5 000*	-	-	-	*↓	+
Sheep	Pag Island Sheep	Yes	30 000*	54	2 389	8,14	≈	+++
	Krk Island Sheep	No	15 000*	-	-	-	≈	++
	Lika Sheep	Yes	30 000*	105	4 877	16,61	≈	+++
	Ruda Sheep	Yes	307	17	290	100	*↑	+
	Rab Island Sheep	No	6 500*	21	775	12,25	≈	++
	Dalmatian pramenka	No	200 000*	75	2 761	1,42	*↑	+++
	Istrian Milk Sheep	Yes	2 500*	57	2 204	90,05	*↑	+++
	Cres Island Sheep	Yes	15 000*	19	728	4,98	*↑	++
Tsigai	Yes	3 000*	64	2 498	85,4	≈	+	
Goat	Croatian White Goat	Yes	≈ 1 000*	-	-	-	≈	+
	Croatian Spotted Goat	Yes	≈ 5 000*	7	210	5	≈	+
Pig	Black Slavonian Pig	Yes	650	46	604	100	**↑	+++
	Turopolje Pig	Yes	150	13	137	100	≈	+
Poultry	Dravska Goose	No	800*	-	-	-	≈	+
	Croatian Hen	Yes	1 000*	-	22	2,20	*↑	+
	Zagorje Turkey	Yes	1 816	-	1816	100	≈	++

↑ positive trend, ↓ negative trend, ≈ in equilibrium, * trend pronouncedly;

Noticed positive trends, in larger amount, are result of stimulant subvention politics. Breeders realize rights for annual stimulating assets per one herdbook animal and offspring. If the effective population size are smaller than one regulate by the government ($N_e \approx 100$), breeders will get 50% larger stimulation assets.

Table 2. Annual amounts of stimulating assets for autochthonous and protected breeds in Croatia

Species	EURO/Year/Head	EURO/Year/Head (+50%; $N_e < \text{critical values}$)	EURO/Year/Head (+50%; <i>adult head + offspring</i>)
Cattle	411	616	1233
Horse	274	411	822
Donkey	137	205	411
Pig	96	144	288
Sheep	48	72	144
Goat	48	72	144
Turkey	21	31	62
Hen	8	12	25

The oversight of the status of autochthonous and protected breeds (table 1) we noticed different level of being endangered for some breeds. According to that, it is necessary to deposit genetic material through adequately *ex situ* programs, for preservation genotype originality.

Actual protection program of autochthonous breeds in Croatia

In horse population *in situ* protection programs effectively held population of Croatian Posavac and Croatian Cooldblood in necessary level. Further breeding consolidation is done. At this moment, these breeds are in the relatively convenient situation, because their survival is connected with marginal grassland, unsuitable for more intensive exploitation in agriculture (pasture on awash area near Sava river). The state of Murinsulaner horse is not satisfied, the reason is partly in non existence of grasslands (environment) for breeding Murinsulaner horse. During years, population amount is 30-40 individuals, thanks to minor number horse enthusiast from Međimurje. Breeding approach like this, leads to nonreturn loss of genetic identification of Murinsulaner horse. Because of that, this breed takes high place on priority list of *ex situ* programs, depositing genetic materials in 'Genebank', and later serves in reconstruction of breed. In revitalization of Murinsulaner horse, more active collaboration with related breeds from neighboring state will be helpful.

Autochthonous donkey breeds are protected within *in situ* conservation programs, prior by subvention assets. Although the overall number of donkey under breeding consideration is quite large ($\approx 1\ 000$ individuals), small number of reproductively active population is concerning, meaning small number of annual registered offspring (20-70 individuals/year). It is noticeable that existing *in situ* donkey preservation model is not efficient enough, and it is necessary to make review of actual model and establish additional programs which will insure preservation of genetic variability. Adequate *ex situ* programs will create and protect one part of genetic reserve which will be activated, if necessary. Although interests for donkey breeding in Croatia exist, breeding organizations that will represent the breeder's interests still are not established. Earlier experience in conservation of autochthonous breeds show that establishment of breeding organizations have positive effect on conduction of breeding program, and also have initiative in preparing *ex situ* programs. We expect that breeding organizations after settings will complete existing *in situ* programs, by introducing adequate *ex situ* conservation programs.

Autochthonous and protected cattle breeds are, in the last few years, in phase of recovery and breeding stabilization. Positive trend is expressed primary in Istrian Cattle breed whose *in situ* protection program was started at first. During the first year of protection Istrian Cattle, semen was collected from several qualitative bulls, and first steps in embryo collecting have been made. Today 'Center for Animal Reproduction of Croatia' disposes with embryo from Istrian Cattle, but more important, it has few thousand doses of semen from Istrian bulls, which are used in insemination of istrian cow population. Activity in collecting semen and embryos from Istrian Cattle population is the first steps in establishing 'Genebank'. Unfortunately, it was stopped after first promising results, although with that action we confirmed possessing the equipment and experts who can establish 'Genebank'. Slavonian Sarmian Podolian Cattle population is under monitoring more than decade, but its retention for a long time at one area has stopped growth of population. Last few years, new herds of Slavonian Sarmian Podolian Cattle were created. For the example, we can mention inhabit one part of population in Park of nature 'Lonjsko polje', where cattle maintain biotope and keep the environment. Interests for breeding Slavonian Sarmian Podolian Cattle increase, what can be seen by increased advantage of breeders for breeding animals. Concerning actual population vulnerability, Slavonian Sarmian Podolian Cattle is also on the first place in establishing *ex situ* programs (depositing genetic material in 'Genebank'). Busha as autochthonous breed was included in conservation programs three years ago. Although the number of Busha population increasing, it's still too small, so deposit genetic material in 'Genebank' make reasonable grounds. Till now collecting and saving semen from several 'Busa' bulls have been done. That is important contribution to sustainability of this small and vulnerable population.

Two autochthonous pig breeds are protected exclusively through *in situ* programs. Certain events in last few years have shown population vulnerability if only this type of protection is applied. Turopolje pig protection program is based on using this breed in traditional way (free range on pastures, feeding by acorn). However, it was realized that free ranging, especially during winter months, disastrously effects on reproduction efficiency (low rate of piglets survival). Also, stopping prevention vaccination against swine fever makes this breed, located in Turopolje lug, even more vulnerable. Newer thinking on need for complete breed's economical affirmation has initiated implementation of several nucleuses that should have certain economical benefit. Regarding population vulnerability, it is necessary to urgently collect tissue samples and conserve them in "Gene Bank". Black Slavonian pig population is at a moment in much better position that resulted from breed activating in economical exploitation

programs (production of 'Kulen'). Population size and breeders interest for this breed gives Black Slavonian pig sustainability good chance, but because forward mentioned reasons (diseases) there is need for conservation part of genetic material in 'Genebank'.

Autochthonous sheep breeds in Croatia are mostly in better position regarding breeds sustainability. Most sheep breeds (Pag Island Sheep, Istrian Milk Sheep, Cres Island Sheep, Dalmatian pramenka, Krk Island Sheep or Rab Island Sheep) are in economical function (milk, cheese, meat), and existing breeding supervision and selection work are giving breeds sustainability very good chance. Exception is Ruda Sheep because individual number puts this breed into critical endangered group. This breed is economically less interested while breeds breeding area (Dubrovnik) is economically oriented towards tourism. Therefore there is relatively small number, especially younger breeders, on this area that are interested in breeding Ruda Sheep. Blue tongue disease on this area had put breed in even more difficult state. Encountering all these problems (disease, decreasing interest for breeding) emphasizes need for implementation *ex situ* preservation programs.

Two autochthonous goat breeds in Croatia are economical active. Croatian Spotted Goat is under systematical selection supervision, while Croatian White Goat is in typesetting phase and is not under systematical selection supervision. Croatian Spotted Goat population number (350 heads) makes this breed critically endangered. Regarding population status, with redefinition *in situ* preservation programs, it is needed implementation of adequate *ex situ* program, primarily through conservation genetic material samples in 'Genebank'.

Two autochthonous poultry breeds in Croatia are protected. Croatian Hen population is more stabile, and programs for breed's economical exploitation are implemented. Achievements of breed's economical activation are partial, and major problem appeared with avuim flue disease, that is, forbidding poultry free ranging, making it traditional recognizable breeding way that produced meat (Zagorje Turkey) impossible.

Implementation of *ex situ* autochthonous breed protection models in Croatia

Status of autochthonous and protected domestic animal breeds in Croatia points out necessity for appropriate models and *ex situ* protection programs implementation in order to prevent irretrievable loss of genetic resources. Natural parks and other protected aeries give possibility for returning autochthonous breeds in theirs earlier habitat, benefiting to human as well as nature it self. Mentioned examples of embryos and sperm collecting (Istrian Cattle), point out technical and human potentials for 'Genebank' implementation.

Implementation of *ex situ-in vivo* endangered autochthonous breed protection models in Croatia is in initial phase. Existing pasture resources, especially in national parks and protected aeries are offering large opportunities for autochthonous breeds reintegration in theirs earlier habitat. In that way autochthonous breeds are getting place for their survival, taking care on total biodiversity, preventing pasture and other bio-systems devastation, becoming recognizable habitats and becoming more attractive for tourists- visitors. Newer example like including Slavonian Syrmian Podolian Cattle in life cycle of Park of the nature Lonjsko filed affirms justification of these actions. ZOO parks are also one of possible *ex situ-in vivo* model, but ZOO's absorption possibility is minor in comparison to protected aeries.

Implementation of *ex situ- in vitro* models i.e. 'Genebank' is far more important for preservation of total genetic resources in Croatia. There are all presumptions for implementation of centre in which genetic material would be collected. It is important to mention washing out and storing more embryos of Istrian Cattle fourteen years ago in 'Centre for Animal Reproduction in Croatia'. Now days it is important to make decision about foundation of 'Genebank', choose most appropriate location, according to financial means start collecting most appropriate genetic material.

Decision about type and quantity of needed genetic material (semen, oocytes, embryos, tissue cells) must to be balanced in order to insure most effective functioning. Actual collecting and storing of tissue cells is most inexpensive and the fastest although activating genetic material from tissue cells is at moment most difficult. It is thought that technological advances in further decades will simplify process of activating genetic information from tissue cells. Thus it seems completely reasonable at moment to start fast and simple collecting sufficient number of tissue cell samples from specific, especially critical endangered breeds, and reactivating genetic material will soon become much more suitable (more secure and inexpensive).

Semen collecting now days in relatively simple procedure needed in function of breed preservation. Stored semen is valuable genetic material, but it is known that from semen itself it is not possible to reconstruct the breed (absence of cytoplasmic DNA information). Collecting and conservation of oocytes and embryos is more demanding, and more expensive at start of *ex situ-in vitro* program being programs disadvantage.

Most experts think that in breed protection from disappearing most efficient is collecting combined tissue material (tissue cells, semen, oocytes, embryos) in which case 'Genebank' can be continuous support to *in situ* programs (sperm, embryos). Thus in 'Genebank' foundation in Croatia will be used FAO recommendations and earlier experiences. In 'Genebank' will be stored earlier collected genetic material and in one place will be noted information on stored genetic material. Actions that are needed to be taken during foundation of 'Genebank' can be put in several points:

- make inventarisation of autochthonous and protected breeds in Croatia and according to breed status make priority list for genetic material collecting (in present situation as priority breeds we suggest Slavonian Syrmian Podolian Cattle, Busha, Murinsulaner horse, Ruda sheep, Turopolje Pig);
- depending on capacity, urgency and investment, make decision about tissue type that will be conserved in 'Genebank' (we suggest this order: tissue cells, semen, oocytes, embryos);
- depending on technical and human potentials make decision about place of 'Genebank' foundation;
- visit terrain and collect sufficient number of tissue samples and store them in 'Genebank';
- information on individuals, tissue type, place of conservation need to be stored into appropriate information system;
- after collecting and conservation, tissue samples of those breeds from a top of priority list, complete 'Genebank' with sufficient number and structure of genetic material collected from all other domestic animal breeds, in first place autochthonous and than all others.

Table 3. Integration of *ex situ* into active *in situ* protection models of autochthonous breed in Croatia

Species	Breed	Active <i>in situ</i> program	<i>Ex situ</i> – Cryogenic conservation program			
			Necessity of <i>ex situ</i> program*	<i>Ex situ</i> priority (1 - 5)	Conserved tissue (<i>till now</i>)	Priority tissue in 'Genebank'
Horse	Lipizzaner	Yes	-	5	-	-
	Croatian Coldblood	Yes	+	5	-	Tissue cells
	Posavina horse	Yes	+	4	-	Tissue cells
	Murinsulaner horse	Yes	+++	1	-	Tissue cells, semen
Ass	Istrian donkey	Yes	++	3	-	Tissue cells,
	Littoral-Dinaric donkey	Yes	+	4	-	Tissue cells,
	North Adriatic donkey	Yes	+++	2	-	Tissue cells,
Cattle	Busha	Yes	++	1	semen	Tissue cells, semen, embryos
	Istrian cattle	Yes	++	2	semen, embryos	Tissue cells, semen, embryos
	Slavonian Syrmian Podolian Cattle	Yes	+++	1	semen	Tissue cells, semen, embryos
	Dalmatian Grey Cattle	No	+	5	-	Tissue cells,
Sheep	Pag Island Sheep	Yes	-	5	-	Tissue cells,
	Krk Island Sheep	No	+	3	-	Tissue cells,
	Lika Sheep	Yes	-	4	-	Tissue cells,
	Ruda Sheep	Yes	+++	1	-	Tissue cells, oocyte, semen
	Rab Island Sheep	Yes	+	3	-	Tissue cells,
	Dalmatian pramenka	Yes	-	5	-	Tissue cells,
	Istrian Milk Sheep	Yes	+	3	-	Tissue cells,
	Cres Island Sheep	Yes	++	3	-	Tissue cells,
Tsigai	Yes	++	2	-	Tissue cells,	
Goat	Croatian White Goat	No	++	2	-	Tissue cells,
	Croatian Spotted Goat	Yes	+	3	-	Tissue cells,
Pig	Black Slavonian Pig	Yes	+	3	-	Tissue cells, semen, oocyte
	Turopolje Pig	Yes	+++	1	-	Tissue cells, semen, oocyte
Poultry	Dravska Goose	No	+++	2	-	Tissue cells
	Croatian Hen	Yes	+++	2	-	Tissue cells
	Zagorje Turkey	Yes	++	3	-	Tissue cells

*(estimate from 1 to 3)

Conclusions

Revision of autochthonous and protected breed status in Croatia points out some positive results, but also need for implementation additional programs that should diminish risks of genetic inheritance loses. Experiences in previous functioning of autochthonous and protected breeds' program protection can be summarized in several notations:

- programs based exclusively on governmental subvention do not give assurance in long-term sustainability of endangered autochthonous breeds (decrease in level of financial subvention results decreasing of interest for breeding same breeds),

- autochthonous breeds, especially endangered one, need to be economically active through special food production programs (ecological bonus), tourist attraction and other;
- governmental subside politic should extra stimulate herd nucleus, test stations and reproduction centres foundation (in order to ensure qualitative reproduction, changes of pure lines and genus's, ...);
- it is necessarily to disperse protected breed throughout breeding area (establish more nucleus herds), in order to decrease risk of disease;
- coordinate application of preventive measures with population status because of risk losing genetic resources (vaccination against pig plaque);
- it is necessary to utilize geographical advantages (natural parks, protected areas, ZOO parks) for activating *ex situ – in vivo* programs;
- it is necessary to implement national *ex situ – in vitro* program (cryogenic conservation program) and establish 'Genebank' for systematic conservation of all tissue samples from autochthonous, especially endangered breeds.