

Institutional and legal framework for ex-situ conservation in the Republic of Croatia

Ramljak, J.¹, Ivanković, A.¹, Dražić, M.², Dadić, M.³, Jeremić, J.⁴, Šimpraga, M.⁵, Karadžole, M.⁵, Pejaković, J.⁵, Čačić, M.², Cerjanec, D.³

Historical overview

In January 2012 final version of Operational Programme for the establishment of the domestic animals gene bank in the Republic of Croatia was done. It was provided by the National Programme for the protection of autochthonous – native and protected breeds of domestic animals in the Republic of Croatia which was adopted earlier, at the beginning of 2010 year. The Operational Programme for the development of the Gene Bank is a well-designed, systematic, coherent and focused set of procedures and measures aimed at collecting, processing and storing tissue specimens of genetic material of interesting specimens with the function of their preservation.

Objective(s) of national cryopreservation programme/policy

Collection goals

The main task of the Operational Programme for the establishment of the domestic animals gene bank in the Republic of Croatia is to point out and enumerate the main activities (measures) required for the establishment of the gene bank, which will be implemented from 2012 to 2016.

This multi-annual operational programme encompasses 26 autochthonous and protected breeds of domestic animals (9 sheep breeds, 4 horses breeds, 3 breeds of donkeys and cattle, 2 breeds of pigs, goats and poultry, and one breed of bees, Annex 1). Furthermore, the multi-annual operational programme predicts the collection and management of genetically interesting material of the conventional breeds of domestic animals.

Gene Bank is consist of systematically stored tissue and reproductive cells at selected locations and a traceable documentation and information system monitoring the storage of genetic material in Gene Bank Collection, its manipulation within the Collection and the accompanying operating procedures for specific situations.

Collection categories

The genetic material stored in the Gene Bank Collection is categorized according to:

- the level of storage (Core Collection, Working Collection),
- type of domestic animals (cattle, sheep, goats, pigs, horses, donkeys, poultry and other),
- type of tissue (semen/sperm, ova, embryos, tissue cells).

Whole collection of gene bank genetic material is divided in two categories:

1. Core collection of genetic material (which takes 65% of gene bank material)
 - genetic material permanently store in the Gene Bank. It is not generally used in the implementation of breeding programmes and may be used only in crisis situation (natural disasters, disease) which directly threatens the biological survival of the breed.
2. Working collection of genetic material (which takes 35 % of gene bank material)
 - material which serves as support to the *in situ* conservation programme of a breed, to research, needs of breeding organizations and use in genetic construction of other breeds.

Organizational structure of the Gene Bank of the Republic of Croatia

The institutional framework of the Gene Bank is consist of: state administration bodies and public institutions (Ministry; CAA and Croatian Horse Breeding Centre – Đakovo and Lipik National Stables), scientific and educational institutions; partner institutions with the required references in the area of agronomy and veterinarian medicine; breeding organizations (central breeders' unions,

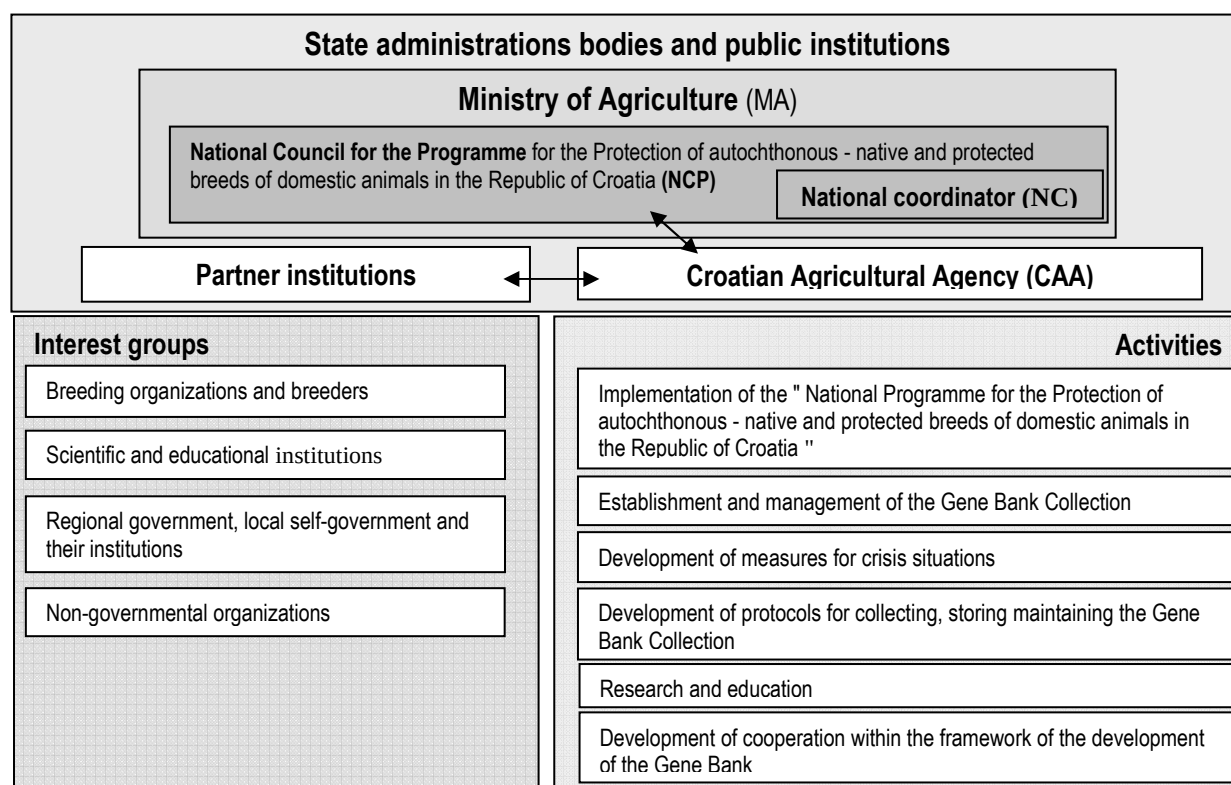
¹Faculty of Agriculture University of Zagreb, Svetosimunska 25, 10 000 Zagreb, Croatia; ²Croatian Agriculture Agency, Ilica 101, 10 000 Zagreb, Croatia; ³Ministry of Agriculture, Ulica Grada Vukovara 78, 10 000 Zagreb, Croatia; ⁴State Institute for Nature Protection, Trg Mažuranića 5, 10 000 Zagreb, Croatia; ⁵Faculty of Veterinary Medicine, Heinzelova 55, 10 000 Zagreb, Croatia.

breeders' unions, breeders' associations); non-governmental associations; companies from the related area (artificial insemination centres).

The genetic material stored in the gene bank is the property of the Republic of Croatia. Management of stored genetic material in the name of the Croatian Ministry of Agriculture is carried out by the Croatian Agricultural Agency (hereinafter: CAA), the central authority responsible for managing the Gene Bank Collection.

Although transboundary breeds exist, among Croatia and Slovenia, Austria, or other country contract or agreement on subject of exchange of genetic material is not yet established.

Scheme 1. Overview of the organizational structure of the Republic of Croatia's Gene Bank



Decision making process (selection and quantity of the genetic material)

The genetic material stored in the Gene Bank Collection is chosen in a systematic and balanced way. The Gene Bank Collection primarily stores the genetic material of critically endangered breeds, followed by highly endangered breeds and potentially endangered breeds and, finally, non-endangered breeds (Annex 1). The type of genetic material collected and stored in the Gene Bank Collection is stored semen of male breeding animals (sperm), ova (oocytes), embryos, body tissues (somatic cells, blood, etc.), and isolated DNA. Two main principles on which the breeds and types of tissues stored in the Gene Bank Collection are selected are:

- the level of a breed's endangerment of extinction (i.e. the real and effective population size, proportion of population under the scope of breeding, degree of inbreeding, the level of risk exposure (disease, natural disasters, etc.), demographic structure of breeders of autochthonous species (i.e. age, motivation, education level).
- the conservation value of a breed (i.e. genetic uniqueness of the breed, genetic variability within the breed, possession of unique and economically important traits, genetic adaptability of the breed to a unique environment, cultural and historic value of the breed).

The necessary quantity of genetic material per breed is as follows:

- semen of male breeding animals (sperm): minimally 50 male breeding animals/breed; 300 doses/breeding animal; minimally 15 000 doses/breed

¹Faculty of Agriculture University of Zagreb, Svetosimunska 25, 10 000 Zagreb, Croatia; ²Croatian Agriculture Agency, Ilica 101, 10 000 Zagreb, Croatia; ³Ministry of Agriculture, Ulica Grada Vukovara 78, 10 000 Zagreb, Croatia; ⁴State Institute for Nature Protection, Trg Mažuranića 5, 10 000 Zagreb, Croatia; ⁵Faculty of Veterinary Medicine, Heinzelova 55, 10 000 Zagreb, Croatia.

- of this number, 200 doses per breeding animal (total 10 000 doses/breed) should be placed in permanent storage and named as Category I at two locations
 - the remaining 100 doses of each breeding animal (total 5 000 doses/breed) would be in the function of supporting the *in situ* programme (breeding programme), named Category R.
- b) ova (oocytes): minimally 50 female breeding animals/breed; 4 to 12 ova/female animal; 200 to 600 ova/breed
- c) embryos: minimally 100 embryos/breed
- d) body tissues (blood, somatic cells): minimally 200 animals/breed
- e) semen of allochthonous breeds interesting for breeding purposes (old bulls lines): 200 doses

For breeds with a small census number (autochthonous or rare breeds, critically endangered breeds, $N_e < 50$), the genetic material of every available (and healthy) specimen is collected regardless of the degree of kinship. Selection of specimens within a breed should be conducted on the basis of pedigree (if any) in such a way that kinship between specimens is minimized, which contributes to the preservation of greatest genetic diversity.

Depending on the available source, genetic material is collected based on an annual plan made by the CAA together with its partner institutions, and which must be adopted by the NP Council. The genetic, reproductive and zoohygienic aspects are taken into account while drafting the annual action plan for storing genetic material in the Gene Bank Collection.

Storage and documentation of genetic material

Storage of samples will be medium size with all necessary equipment and facilitates. Measures of precautions should be always taken due to possibility of contamination of the genetic material stored in containers (like conservation of previously contaminated material, storing of samples of doubtful sanitary status, for example taken outside of planned collection location or animals not recoded by protocol, by persons unauthorized for collecting, etc.).

All the genetic material stored in the Gene Bank Collection must be properly labelled and labelling system depends on the type of genetic material that is stored. Packaging in which the genetic material stored is stamped with text (country's code, animal's unique number, tissue type code, and date of processing of the genetic material).

Additional data entered into the CRYO-IS HR database which accompanies the collection, processing, storage and activation of the stored genetic material. The information stored in CRYO-IS HR is selectively available to all stakeholders in accordance with their powers and rights.

Sanitary arrangements (measures) / regulations

There are three important factors that shall be observed in order to reduce the risk of transferral of some diseases. They are: setting up quarantine, observing the procedures of collecting of genetic materials, and correct procedures with the genetic material after collection. Use of sanitary protocols and disabling of samples contamination will be consequent from personnel (use protective equipment defined by regulations: rubber boots or protective feet covers, protective aprons or overalls, rubber gloves etc.) and also applicable in using material like portable refrigerators, collectors, medical equipment, etc.

The genetic material of animals which are tested positive for diseases and are contagious, is not taken for storing. Since quarantined animals sometime don't show visible symptoms of diseases, sampling of blood or other tissue is recommended (for example nose wipes) for complete tests for presence of infectious agents.

Legal framework related to genetic material and data

Existing legal frame (>21 laws, orders and ect.) and legal regulations that will be made shall govern the relationships between all the participants storing the genetic material into the gene bank,

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including the scope of genetic reserves, manner and collection procedures, storing and use and managing of records on biological material.

There are several approaches to the exchange of material between the gene bank and the animal owner, and the choice of most suitable one is on the team of professionals. Approaches are: the gene bank can buy an animal from the owner, the animal owner can donate the genetic material of the animal to the Gene Bank, providing a waiver of all the rights of the donated material, the owner of the donor animal can store the genetic material in the Gene Bank for certain time period (embargo) and after the embargo expires, the ownership over of the genetic material belongs to the Gene Bank. Documentation related to the ownership of genetic material is in phase of preparation.

Below stated elements should be taken into consideration: proprietary rights (proprietorship over cryoconserved genetic material should be clearly specified), costs of collecting, processing and freezing, rights of intellectual property (if the Gene Bank is founded as public property of a state, research results of use of materials in the Gene Bank should be publicly available, without the requirement for intellectual property), veterinary / sanitary issues (contract on ownership of the genetic material should contain the list of diseases the animal has been tested for), and data protection (the gene bank decides which information on stored genetic material shall be available to the public).

International transfer of the genetic material stored in the Gene Bank Collection

Secondary role of national Gene Bank is evident in the possibility of the international exchange of the genetic material where attention should be put to the regulations which govern the issue of health aspect. In this case main regulations are defined by the World Organisation for Animal Health (Office International des Epizooties, <http://www.oie.int/>). Protocols for transfer of genetic material between countries are used by the signatory countries of the Sanitary and Phytosanitary measures (SPS, http://www.wto.org/english/tratop_e/sps_e/sps_e.htm).

Conclusions and future plans

Founding, management and sustainable use of the Gene Bank is a complex project with interinstitutional cooperation as well as support of state institutions. Based on current knowledge, we present concise recommendations:

- project should be seen as the occurrence of permanent value which holds growth potential throughout the years,
- legislation should be regulated and harmonized with the relevant European legislation,
- proprietary rights should be regulated (samples),
- intellectual rights of property should be regulated,
- quality management system should be introduced,
- communication strategy and transparency should be defined.

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Annex 1. Overview of the status of endangerment of native and protected breeds in the Republic of Croatia

Species	Breed	Number of animals				Effective size of population (Ne)	Categorisation of breed endangerment status
		Total (*estimate)	of				
			Male	Female	Young		
Horses	Lipicanac horse	1550	177	462	909	511.89	Potentially endangered
	Croatian cold-blood horse	6300	265	2849	3181	969.79	Potentially endangered
	Croatian Posavac horse	4900	124	2072	2713	467.99	Potentially endangered
	Medimurje horse	57	5	21	31	16.15	Critically endangered
Donkeys	Istrian donkey	220*	20*	150*	50*	70.58	Critically endangered
	Littoral Dinaric donkey	2000*	200*	1300*	500*	693.33	Highly endangered
	North Adriatic donkey	150*	20*	100*	30*	66.66	Critically endangered
Cattle	Buša	563	33	308	222	119.23	Highly endangered
	Istrian cattle	1041	36	627	378	136.18	Highly endangered
	Dalmatian Gray	2000*	200*	1300*	500*	693.33	Highly endangered
	Slavonian Symirian podolac	257	9	145	103	33.90	Critically endangered
Sheep	Pag island sheep	30000*	218	4143	680	828.41	Not endangered
	Krk island sheep	18000*	7	111	41	26.34	Potentially endangered
	Lika sheep	30000*	159	6553	1032	620.93	Potentially endangered
	Ruda sheep	712	38	564	110	142.41	Highly endangered
	Rab island sheep	6500*	18	443	75	69.19	Potentially endangered
	Dalmatian "pramenka	200000*	293	8184	838	1131.49	Not endangered
	Istrian sheep	2314	96	1769	449	364.23	Potentially endangered
	Cres island sheep	15000*	32	737	143	122.67	Potentially endangered
	Tzigai sheep	3000*	26	1135	255	101.67	Potentially endangered
Goats	Croatian white goat	5000*	4	58	13	14.97	Potentially endangered
	Croatian spotted goat	35000*	30	394	97	111.51	Potentially endangered
Pigs	Black Slavonian pig	5000*	109	896	297	388.71	Potentially endangered
	Turopolje pig	400*	129	30	75	97.36	Critically endangered
Poultry	Hen "Hrvatica"	1500*	103	949		371.66	Potentially end
	Zagorje turkey	3000*	428	2255		1438.90	Not endangered
Bees	Grey bee	320000*+		6500			Not endangered

* estimate; source: annual report CAA, * bee hives