

SANCO/10244/2014

Programmes for the eradication, control and monitoring of certain animal diseases and zoonoses

Survey programme for Avian Influenza

Croatia

Approved* for 2014 by Commission Decision 2013/722/EU

* in accordance with Council Decision 2009/470/EC

version: 2.22







PROGRAMME for ERADICATION : ANNEX IV

Member States seeking a financial contribution from the Community for national programmes for the control and monitoring of avian influenza in poultry and wild birds shall submit applications containing at least the information set out in this form.

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Friday, August 23, 2013 14:39:30

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Identification of the programme

version: 2.22

1.	identification of the programme
	Member state: HRVATSKA

Disease: avian influenza in poultry and wild birds

Request of Union co-financing for the period :

FROM

2014

TO

2014

1.1 Contact

Name: Ankica Labrovic

Phone: +385 6443 535

Fax.: +385 6443 899

Email: labrovic@mps.hr

- 2. Description and implementation of the surveillance programme in poultry
- 2.1.1 Designation of the central authority in charge of supervising and coordinating the departments responsible for implementing the programme

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(max. 32000 chars):

Veterinary Directorate of the Ministry of Agriculture is the national competent authority responsible for supervision and coordination of implementation of the Program.

Animal Health Sector of the Veterinary Directorate (VD) is responsible for developing the Program in coordination with a AI diagnostic laboratory of the Croatian Veterinary Institute (CVI).

The Program is implemented by authorised veterinarians who provide for sampling and submission of samples to the AI diagnostic laboratory.

Supervision of implementation of the Program on the field is carried out by veterinary inspectors (officials of the VD).

2.1.2 System in place for the registration of holdings

(max. 32000 chars):

According to the provisions of Article 38, paragraph 1 of the Veterinary Act (Official Gazette 41/2007, 55/11), farms of ungulates and equidae exceeding 20 conditional animal units, poultry and rabbit farms exceeding 10 conditional animal units, hatcheries, wild game breeding farms, establishments for farming of fish and molluscs and other facilities of aquaculture must comply with the stipulated veterinary-health and biosecurity conditions.

Pursuant to the provisions of Article 38, paragraph 3 of the Veterinary Act, all farms are to be registered in the Farms Register, which is an integral part of the Central Register of Domestic Animals (CRDA), the responsibility for which lies with the MA's Veterinary Directorate. The Directorate has entered into contract with the Croatian Agricultural Agency (CAA) delegating to the CAA maintenance of CRDA. All holdings keeping gallinaceous birds (chickens, turkeys, pheasants, partridges and quails) and ratites (ostriches) are registered. Also holdings keeping domestic waterfowl (e.g. ducks, geese and mallards for restocking supplies of game) are registered in the CDRA. For every holding CRDA contain information on unique holding number, name of the keeper, address, geographical coordinates, animal species and number of animals.

2.1.3 Design (risk based or surveillance based on representative sampling)

(max. 32000 chars):

Al surveillance based on representative sampling scheme will be implemented in 2014.

Number of poultry holdings to be sampled is calculated based on Tables 1 and 2 of the Annex I of the Commission Decision2010/367/EU and according to the poultry species present on the poultry holding. Holdings to be subject of sampling will be randomly selected form the list of all holdings of a specific poultry species and production categories keeping more than 100 animals. This will assure

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representativeness so generalization of findings to all holdings of a specific poultry species and production categories keeping more than 100 animals.

Backyard poultry in settlements where poultry is kept in proximity to large surface waters and migratory birds resting or nesting areas as well as backyard poultry in holdings where more than 50 animals of different poultry species (e.g. gallinaceous and domestic waterfowl, mixed) are kept together will also be included in the Program.

Target population (poultry production categories) to be included in the surveillance program, are:

- 1. laying hens
- 2. free range laying hens
- 3. chicken breeders
- 4. turkey breeders
- 5. fattening turkey
- 6 farmed game birds (pheasants, partridges and quails)
- 7. backyard flocks

Number of holdings from each of the production categories mentioned above, to be subject of sampling, will assure identification of at least one infected poultry holding if the prevalence of infected holdings of this category is 5% or more, with 95% confidence interval (CI).

As regards backyard poultry, those keeping more than 50 of animals of different poultry species (e.g. gallinaceous and domestic waterfowl, mixed) are considered as an independent sampling frame and number of holdings to be samples will ensure identification of at least one infected poultry holding if the prevalence of infected holdings in that group of backyard holdings is 5% or more, with 95% Cl. In the sampling frame of backyard holdings, some holdings will be included based on the prior information on risk, collected within the frame of Al surveillance program (Tables providing an information of the results of Al surveillance in domestic poultry are attached to this document).

Number of birds to be sampled, per holding included in the Program, will ensure detection of at least one AI seropositive bird if the prevalence of seropositive birds on a holding is 30 % or more, with 95% confidence. Blood samples for serological examination will be collected from all poultry production categories and species and 10 birds will be sampled per one epidemiological unit on the holding (e.g on laying hens holdings in average there are 2 epidemiological units, while on chicken breeders in average there are 1,5 epidemiological unit).

Samples will be taken at farm level. Whenever possible samples will be taken at the slaughterhouse.

The following domestic waterfowl categories will be included in the program:

- 1. ducks breeders
- 2. geese breeders
- 3. fattening ducks
- 4. fattening geese
- 5. farmed game birds (waterfowl).

Number of duck, goose and mallard holdings to be sampled will ensure identification of at least one infected holding if the prevalence of infected poultry holdings of a specific poultry production category is 5% or more with 99% CI.

Number of ducks, geese and mallards to be sampled, per holding included in the program, will ensure detection of at least one AI seropositive bird if the prevalence of seropositive birds on a holding is 30 % or more, with 95% confidence.

Blood samples for serological examination will be collected form all poultry production categories and species and 20 birds will be sampled per one holding.

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Samples will be taken at farm level. Whenever possible samples will be taken at the slaughterhouse.

In case of detection AI seropositive birds sampling for virological testing for AI will follow.

In case of change of epidemiological situation the Program will appropriately be amended.

Responsibilities

- 1. To ensure the implementation of the Programme is the responsibility of the VD of the Ministry of Agriculture.
- 2. Bird samples shall be taken and submitted to the laboratory by authorised veterinarians.
- 2.1 The authorised veterinary organizations shall:
- once a month submit reports to the VD on the sampling of poultry in accordance with this Programme.
- submit, together with reports referred in the previous paragraph, a copy of the completed Sample Submission Forms as specified in the Annex to this Programme.
- 3. The laboratory testing of samples shall be carried out by Poultry Centre of the Croatian Veterinary Institute, Heinzelova 55, Zagreb, which is the National reference laboratory for AI. The national reference laboratory shall:
- report all positive results to the Veterinary Directorate as soon as possible;
- report all negative results to the Veterinary Directorate once a month.
- 4. Competent veterinary inspectors shall supervise the implementation of the Programme on the spot.

2.1.3.1 Short description of predominant poultry population and types of poultry production

(max. 32000 chars):

Poultry production in Croatia may be described as follows: 92% of poultry production refers to hens, 2% to turkeys, 2% to ducks and 3% to geese.

Other types of poultry, such as guinea fowls, quails, pheasants are insignificant within poultry population. Fattening of broilers, as well as of turkeys assures significant meat production, while breeding of ducks and geese is not as represented, being just semi-intensive for local market supply. Croatia has enough supplies of poultry meat for its own market. Around 70% of fattened poultry originates from intensive systems, other 30% refers to traditional, semi-intensive production for individual consumption. In Croatia, there is a yearly production of 600 000 breeding broiler layers, 40 million broilers and around 1.2 million turkeys. There are also 22 000 breeding commercial layers and around 1.7 million layers for egg production. The existing poultry production capacities are greater, however they are not fully exploited. Egg production is intensive in large companies and on family farms. There is also extensive and semi-intensive seasonal production on family infields. Around 30% of egg production is realized within semi-intensive systems and 70% within intensive production systems.

Source: Kralik G. at al, Current condition and development perspectives of poultry production in Croatia. http://www.cabi.org/animalscience/Uploads/File/AnimalScience/additionalFiles/

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2.1.3.2 Criteria and risk factors for risk based surveillance(1)
(max. 32000 chars):
n/a
(1) Including maps showing target sampling sites identified as being particularly at risk for the introduction of avian influenza virus, taking into account criteria set out in point 4 of Annex I to Commission Decision 2010/367/EC.
2.2 Target populations (2)
(max. 32000 chars) :
n/a
(2) including MS specific exceptional circumstances as described in Annex I point 3 of Commission Decision 2010/367/EU)

2.2.1 POULTRY HOLDINGS ^(a) (except ducks, geese and farmed game birds (waterfowl e.g. mallards) to be sampled

Serological investigation according to Annex I to Commission Decision 2010/367/EU

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

Category: laying hens

delete this category

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	138	53	20	1 060	Haemagglutination-inhibition-test (HI)	X
Total	138	53	20	1 060		

Add a new row

Holdings or herds or flocks or establishments as appropriate.

Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested

Total number of holdings of one category of poultry in concerned NUTS 2 region.

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In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

Category: free range laying hens

delete this category

	NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	a	18	18	10	180	Haemagglutination-inhibition-test (HI)	X
	Total	18	18	10	180		

Add a new row

Holdings or herds or flocks or establishments as appropriate.

Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested

Total number of holdings of one category of poultry in concerned NUTS 2 region.

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

Category: chicken breeders

delete this category

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	NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis		
Hr	vatska	60	42	15	630	Haemagglutination-inhibition-test (HI)	X	
	Total	60	42	15	630			
						Add a now your		

Add a new row

- (a) Holdings or herds or flocks or establishments as appropriate.
- Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested:

 Total number of holdings of one category of poultry in concerned NUTS 2 region.

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

Category: fattening turkeys

delete this category

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	76	42	10	420	Haemagglutination-inhibition-test (HI)	X
Total	76	42	10	420		
					Add a new row	

- Holdings or herds or flocks or establishments as appropriate.
- Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested
- (c) Total number of holdings of one category of poultry in concerned NUTS 2 region.

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

Category: turkey breeders

delete this category

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	15	15	10	150	Haemagglutination-inhibition-test (HI)	X
Total	15	15	10	150		

Add a new row

- $Holdings\ or\ herds\ or\ flocks\ or\ establishments\ as\ appropriate.$
- (b) Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested
 - Total number of holdings of one category of poultry in concerned NUTS 2 region.

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

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Category: farmed game birds (gallinaceous)

delete this category

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	11	11	10	110	Haemagglutination-inhibition-test (HI)	X
Total	11	11	10	110		
						[[

Add a new row

n) Holdings or herds or flocks or establishments as appropriate.

Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested Total number of holdings of one category of poultry in concerned NUTS 2 region.

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

Category: backyard flocks

delete this category

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	314	60	10	600	Haemagglutination-inhibition-test (HI)	X

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	Total 314 60 10	600
		Add a new row
(a) (b) (c)	Holdings or herds or flocks or establishments as appropriate. Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be u Total number of holdings of one category of poultry in concerned NUTS 2 region.	sed, region as defined in the programme by the Member States is requested

Add a category

Total Poultry	632	241	85	3 150	

DUCKS, GEESE AND FARMED GAME BIRDS (WATERFOWL e.g. MALLARD) HOLDINGS (a) to be sampled. 2.2.2

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Serological investigation according to Annex I to Commission Decision 2010/367/EU

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

Category: duck breeders

delete this category

NUTS (2) (b)	Total number of duck and geese holdings	Total number of duck and geese holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	29	29	20	580	Haemagglutination-inhibition-test (HI)	x
Total	29	29	20	580		

Add a new row

Holdings or herds or flocks or establishments as appropriate.

Refers to the location of the holding of origin. In case NUTS (2) code can not be used, region as defined in the programme by the Member State is requested

Category: geese breeders

delete this category

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(a) (b)

NUTS (2) (b)	Total number of duck and geese holdings	Total number of duck and geese holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	15	15	20	300	Haemagglutination-inhibition-test (HI)	x
Total	15	15	20	300		

Add a new row

Holdings or herds or flocks or establishments as appropriate.

Refers to the location of the holding of origin. In case NUTS (2) code can not be used, region as defined in the programme by the Member State is requested

Category: fattening ducks

delete this category

NUTS (2) (b)	Total number of duck and geese holdings	Total number of duck and geese holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	53	47	20	940	Haemagglutination-inhibition-test (HI)	X
Tota	53	47	20	940		

Add a new row

(a) Holdings or herds or flocks or establishments as appropriate.

Refers to the location of the holding of origin. In case NUTS (2) code can not be used, region as defined in the programme by the Member State is requested

Category: fattening geese

delete this category

NUTS (2) (b)	Total number of duck and geese holdings	Total number of duck and geese holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
Hrvatska	17	17	20	340	Haemagglutination-inhibition-test (HI)	x
Total	17	17	20	340		

Add a new row

Holdings or herds or flocks or establishments as appropriate.

Refers to the location of the holding of origin. In case NUTS (2) code can not be used, region as defined in the programme by the Member State is requested

Add a category

Total Ducks and geese	114	108	80	2 160	

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Grand Total Poultry + Ducks/Gueese	746	349	165	5 310	
Grand Total ELISA Poultry + Ducks/Gueese				0	
Grand Total agar Poultry + Ducks/Gueese				0	

2.3 Sampling procedures, sampling periods and frequency of testing

(max. 32000 chars):

Blood samples will be taken at farm level. Whenever possible samples will be taken at the slaughterhouse.

Sampling on poultry holdings included in the Program will be carried out once a year, during the period from 1 January to 31 December, 2014. Bird samples shall be taken and submitted to the laboratory by authorised veterinarians.

Samples shall be taken from poultry during their production age or, in the case of fattening poultry, during the final fattening phase.

Samples may be taken during the vaccination of poultry against Newcastle disease (in accordance with the Annual Order).

Blood samples taken to check immunity against Newcastle disease may also be used for the surveillance of antibodies to AI virus subtypes H5 and H7.

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At least 2 ml of blood (without anticoagulants) shall be taken.

During storage and transportation, the samples must be kept at a temperature not exceeding $4\text{Å}^{\circ}\text{C}$ and they must be submitted to the national laboratory for avian influenza within 48 hours. If a sample cannot be transported to the national laboratory within this period, the blood serum must be separated, frozen, and sent to the laboratory in the frozen state.

Blood samples shall be sent to the following national laboratory:

Croatian Veterinary Institute

Poultry Centre

Heinzelova 55, Zagreb 10000

Contact telephones: 01/2441 392; 2441 394; 2440 211.

Fax: 01/2441 396.

2.4. Laboratory testing: description of the laboratory tests used and follow up investigations

Description of the used serological tests: (max 32000 chars)

The laboratory testing of samples shall be carried out by Poultry Centre of the Croatian Veterinary Institute, Heinzelova 55, Zagreb, which is the National reference laboratory for Al.

Standard haemagglutination-inhibition test for detection of AI antibodies of H5 and H7 subtypes is used according to the Council Directive 2005/94/EC, OIE Terrestrial Manual 2009 (Chapter 2.3.4.) and Diagnostic Manual for Avian Influenza (Official Gazette 99/2008).

For H5 subtype for initial testing teal/England/7894/06 (H5N3) antigen is used. All positives are to be tested with chicken/Scotland/59 (H5N1) antigen to eliminate N3 cross reactive antibodies; for H7 subtype for initial testing turkey/England/647/77 (H7N7) antigen is used. All positives are to be tested with African starling/983/79 (H7N1) antigen to eliminate N7 cross reactive antibodies. I

If standard antigens are not supplied by the EU AI reference laboratory, following antigen will be used instead: for H5 primary antigen is Av-R5371/Croatia/2007 (H5N1) and secondary antigen is Mallard/Croatia/1/2006 (H5N3), for H7 primary antigen is Av-R7152/Croatia/2007 (H7N2) and secondary antigen is Turkey/Italy/3560/1999 (H7N1).

The national reference laboratory shall:

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- report all positive results to the Veterinary Directorate as soon as possible;
- report all negative results to the Veterinary Directorate once a month.
- 3. Description and implementation of the surveillance programme in wild birds
- 3.1.1 Designation of the central authority in charge of supervising and coordinating the departments responsible for implementing the programme and relevant collaborating partners (e.g. epidemiologists, ornithologists, nature bird observation and hunter organisations).

(max. 32000 chars):

VD of the Ministry of Agriculture is the national competent authority responsible for supervision and coordination of implementation of the Program. Animal Health Sector of the Veterinary Directorate is responsible for developing the Program in coordination with AI laboratory expert as well as ornithologist from Croatian Veterinary Institute and Institute of Ornithology of the Croatian Academy of Sciences and Arts. Ornithologists take a major part in sampling and identification of a species of wild bird.

The implementation of the 2014 Programme for AI in Wild Birds will be the responsibility of the VD of the Ministry of Agriculture. The following institutions also participate in the implementation of the Programme:

- Croatian Veterinary Institute â€" Poultry Centre;
- Institute of Ornithology of the Croatian Academy of Sciences and Arts.
- 3.1.2 Description and delimitation of the geographical and administrative areas in which the programme is to be applied

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max. 32000 chars):

The spatial distribution of the locations at which the survey for influenza virus in wild birds will be conducted in 2014 is shown on the Map provided in the the attachment of the last year Program. Locations identified on the Map are areas in the country where abundance and diversity of birds, particularly water birds is significantly higher than on other areas in the country. Also on these locations a large number of different species of migratory birds (i.e. the sites are an important crossing of migration routes or a mixing sites of passage migrants from various areas) are present. It is expected that on such sites expected mortality or morbidity of wild birds is higher. Active searching and monitoring of dead or morbiund wild birds in particular those of target species on such locations will allow for timely detection of HPAI of subtypes H5N1 in wild birds.

The following locations are of the main interest for active searching of dead or moribund wild birds:

Fish ponds

- 1. «Draganic », Jastrebarsko
- 2. «Fish pond», Oriovac/Jelas Polje, Slavonski Brod
- 3. «Fish pond», Donji Miholjac
- 4. «Fish pond» KonÄŤanica, Daruvar
- 5. «Fish pond Grudnjak», 33515 Grudnjak
- 6. «Fish pond 1905» NaĹ ¡iÄŤka Breznica, NaĹ ice
- 7. «Fish pond Poljana», Poljana / «Riba GareĹ nica», GareĹ nica
- 8. «Fish pond ÄŚesmi» (Narta, SišÄŤani, Vukšinec, Blatnica) Dubrava, Kostanj bb / Narta
- 9. «Fish pond» Lipovljani, Novska

Artificial water reservoirs

- 10. Varazdin Drava
- 11. Donja Dubrava Drava
- 12. Ĺ oderica Koprivnica

Major wetland areas (nature parks)

- 13. Lonjsko Polje Nature Park
- 14. Kopacki Rit Nature Park
- 15. Vransko Lake
- 16. Delta of the Neretva river

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Waste disposal area

- 17. Prudinec (Jakusevac, Zagreb)
- 18. Waste disposal area Kosambra, Porec.

Also taking into consideration experience during the occurrence of HPAI of subtype H5N1 in wild birds in Croatia in 2005 and 2006, the majority of dead birds submitted to the laboratory for testing were originating from these locations. On two locations mentioned above HPAI of subtype H5N1 was confirmed in wild birds in 2005 and 2006.

3.1.3 Estimation of the local and/or migratory wildlife population

(max. 32000 chars):

Estimation of size of wild birds populations in the country was done by ornithologist from Croatian Veterinary Institute and Institute of Ornithology of Croatian Academy of Sciences and Arts and it is summarized in the Table provided within the frame of the last year Program.

3.2 Design, criteria, risk factors and target population(3)

(max. 32000 chars):

Passive surveillance scheme will be implemented in order to provide for laboratory investigation of moribund or wild birds found dead so to allow for timely detection of HPAI of subtypes H5N1 in wild birds. Active searching and monitoring of dead or moribund wild birds of target species will in particular be focused on locations where increased incidence of morbidity and/or mortality wild birds is expected to be observed easier than on other areas of the

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country (e.g. areas close to lakes and waterways, wetlands, ponds, sea, waste disposal area).

Target population are mainly migratory water birds that have been shown to be at a higher risk of becoming infected with and transmitting the HPAI H5N1 virus. Wild bird species to be targeted for sampling and testing will be those as listed in Part 2 of the Commission Decision 2010/367/EU, that are present in the country.

The working group (made up of ornithologists, virologists and epidemiologists) continuously monitor and provide for analysis of results of the Programme. The group also provide for instructions on sampling and submission of samples to the laboratory as well as information dissemination about water bird species that are the main target for passive surveillance for HPAI.

(3) Areas at risk (wetlands in particular where links with high density poultry populations), previous positive findings as referred to in point 2 of Part 1 of Annex II to Commission Decision 2010/367/EC should be taken into account and if possible complemented by a map.

3.2.1 WILD BIRDS focussed on target species

Investigations according to the surveillance programme set out in Part 2 of Annex II to Decision 2010/367/EC

In case of multiannual programme, please provide targets on annual basis.

If your targets differ between different implementation years please provide separate tables per year in attachment.

NUTS (2) code/region (a)	Wild birds to be sampled (b)	Total number of birds to be sampled	Estimated total number of samples to be taken for active surveillance (c)	Estimated total number of samples to be taken for passive surveillance	
Hrvatska	Target species	720	0	1 440	X

version · 2 22

	Total	0	720	0	1 440
				Add a n	ew row
(a) Membe	Refers to the place of collection of birds/samples. In case No r State is requested. Please fill-in these values directly in the f		rial Units for Statistics) can i	not be used, region as define	d in the programme by the
(b)	General description of the wild birds are intended to be sar. Voluntary, to be included for information purposes, not elic	· ·	active and passive surveillar	nce.	

3.3 Sampling procedures and sampling periods

max 32000 chars:

Sampling will be carried out in accordance with the Diagnostic Manual.

Samples from wild birds will be taken by skilled persons (mainly ornithologists) who are equipped for collection and transport of samples. The ornithologist shall determine the species and, if possible, the age and sex of each wild bird caught.

At least 5 sick/dead wild birds (or all if number of sick or dead wild birds is lower than 5) found on a location must be adequately sampled for virological examination (molecular detection /RT-PCR/ followed by virus isolation in case of positives).

In case adequate sampling can not be assured on the location, sick or dead wild birds must be collected as a whole and submitted to the Al laboratory. Cloacal and tracheal/oropharyngeal swabs and/or organs (brain, heart, lung, trachea, kidney and intestines) from wild birds found dead or moribund, must be taken.

An appropriately taken cloacal swab must be covered with a visible quantity of faeces.

If available, swabs may be placed in an antibiotic or specific virus transport medium so that they are fully immersed. Regardless of whether the swabs are submitted in a transport medium or not, they must be chilled.

If a transport medium is not available, the swab must be placed in a protective casing and submitted dry to the laboratory.

Samples must not be frozen unless absolutely necessary. If rapid transport (in transport medium at 4oC) is not guaranteed, the samples must be immediately frozen and then transported on dry ice to the laboratory.

Samples must be submitted to Croatian Veterinary Institute; Poultry Centre; Heinzelova 55, Zagreb 10000 (Contact telephones: 01/2441 392; 2441 394;

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2440 211) as soon as possible.

It is expected that majority of samples from dead or moribund wild birds will be taken during spring and in particular autumn migrations when birds are grouping but also during winter. During these periods of the year ornithologist are more frequently present on locations of interest.

3.4 Laboratory testing: description of the laboratory tests used

max 32000 chars:

Detection of Al viruses is carried out fully in accordance with the Council Directive 2005/94/EC and Diagnostic Manual for Avian Influenza (Official Gazette 99/2008).

Both, classical and molecular pathways are used, and combined if needed.

For molecular pathway, EU AI reference laboratory recommended Real Time PCR methods for detection of influenza A matrix gene, Eurasian H5 avian influenza, Eurasian H7 avian influenza and N1 AIV gene detection are used.

In addition, EU AI reference laboratory recommended One Step RT PCR for detection of H5 & H7 avian influenza & cleavage site sequencing protocols are used.

Pathotyping of H5 and H7 positive samples is based on sequencing of HA-cleavage site and putative amino acid motif.

4. Description of the epidemiological situation of the disease in poultry during the last five years

max 32000 chars :

No evidence of HPAI viruses was observed in poultry during the last five years.

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5. Description of the epidemiological situation of the disease in wild birds during the last five years

(max. 32000 chars):

No evidence of HPAI viruses was observed in wild birds during the last five years.

The last occurrence of HPAI of H5N1 subtype was detected in wild birds in March 2006. Description of introduction and spread of avian influenza A (H5N1) subtype in Croatia including seventeen isolates identified during the period from October 2005 to March 2006, is provided in the paper (Savic at al, 2009)

6. Measures in place as regards the notification of the disease

(max. 32000 chars):

According to the Veterinary Act (Official Gazette No 41/2007, 55/11), Articles 13 to 16, owner of animals and any veterinarian is obliged to notify an infectious disease suspicion or a case. Disease notification procedures and forms are prescribed in details in the Ordinance on the notification of animal diseases (Official Gazette No 62/11, 114/11).

In case of notification of AI suspicion measures are applied in accordance with Ordinance on measures to control and eradicate avian influenza (Official Gazette No 131/06). This Ordinance is fully aligned with Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC; and the Ordinance on the diagnostic manual for avian influenza (Official Gazette No 99/08). This Croatian ordinance is fully aligned with Commission Decision 2006/437/EC of 4 August 2006 approving a Diagnostic manual for avian influenza as provided in Council Directive 2005/94/EC).

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7. Costs

7.1 Detailed analysis of the costs

7.1.1 Poultry

(max. 32000 chars):

Serological test: HI test for H5 and H7

- Number of samples per holding are calculated based on epidemiological unit.
- 10 samples are taken per epidemiological unit. Average number of epidemiological units per Laying hens is 2 and Chicken breeders holdings is 1,5 so sample size is increased by 2 and 1,5 times, respectively, on such holdings. According to the design and taking into account the number of epidemiological units it is expected that 5310 samples will be serologically tested within the frame of this Program.
- It is assumed that up to 1% of samples might need to be further investigated due to positive serological test results. In such cases additional sampling for the purpose of serological and virological investigation on a holding concerned will be performed. For that reason additional 400 serological tests is included in the costs.
- So overall expected number of serological tests to be carried out within the frame of this Program is 5710.

Virological tests

- RT PCR test: it is assumed that up to 1% of samples might need to be further investigated due to positive serological test results. In such cases additional sampling for the purpose of serological and virological investigation on a holding concerned will be performed.

For that reason 200 RT PCR tests, 30 virus isolation tests are included in the costs.

When calculating costs only costs eligible to be reimbursed and those applied in the Decision 2012/761 (for MS programmes in 2013) are considered.

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7.1.2 Wild birds

(max. 32000 chars):

Virological testing

- Search for dead or moribund wild birds of target species will be carried out on 18 locations (specified under point 3.1.2).

It is assumed that on each location 40 dead or moribund wild birds will be found and submitted for laboratory investigation during the 2014. This will result with 720 wild birds to be sampled and 1440 samples (cloacal sample and tracheal or organ sample) to be virological tested.

- In addition 50 virus isolation tests are included in the costs. When calculating costs only costs eligible to be reimbursed and those applied in the Decision 2012/761 (for MS programmes in 2013) are considered.

version: 2.22

7.2 Summary of the annual costs: 2014

7.2.1 Poultry surveillance

Detailed analysis of the cost of the programme - poultry

In case of multiannual programme, please provide estimations on annual basis.

If your cost estimations differ between different implementation years please provide separate tables per year in attachment.

Laboratory testing			
Methods of laboratory analysis	Number of tests	Unitary test cost (per method) in €	Total cost (€)
ELISA test	0	0	0
agar gel immune diffusion test	0	0	0
Haemagglutination-inhibition-test (HI) for H5 (specify number of tests for H5)	5 310	10	53100
Haemagglutination-inhibition-test (HI) for H7 (specify number of tests for H7)	5 310	10	53100
Virus isolation test	30	27	810
PCR test	200	34	6800

Other please specify here	0	0	0	
			Add a new row	
Sampling				
	Number of samples	Unitary cost in €	Total cost (€)	
Samples	5 710	1.9	10849	
Other measures				
	Number of samples	Unitary cost in €	Total cost (€)	
Other please specify here	0	0	0	X
			Add a new row	
Total poultry Testing + Sampling + Other measures	16 560		124 659,00 €	

Wild bird surveillance 7.2.2

Detail analysis of the cost of the programme - wild birds

Laboratory testing			
Methods of laboratory analysis	Number of tests	Unitary test cost (per method) in €	Total cost (€)
Virus isolation test	50	27	1350
PCR test	1 440	34	48960
Other please specify here	0	0	0
			Add a new row
Sampling			
	Number of samples	Unitary cost in €	Total cost (€)
Samples	720	5	3600
Other measures			

	Number	Unitary cost in €	Total cost (€)	
Other please specify here	0	0	0	X
			Add a new row	
Total wild birds Testing + Sampling + Other measures	2210		53 910,00 €	
Grand Total Poultry + Wild birds	18770		178 569,00 €	

Attachments

IMPORTANT:

- 1) The more files you attach, the longer it takes to upload them .

- 2) This attachment files should have one of the format listed here: <u>jpg, jpeg, tiff, tif, xls, doc, bmp, pna, pdf.</u>
 3) The total file size of the attached files should not exceed 2 500Kb (+- 2.5 Mb). You will receive a message while attaching when you try to load too much.
 4) IT CAN TAKE <u>SEVERAL MINUTES TO UPLOAD</u> ALL THE ATTACHED FILES. Don't interrupt the uploading by closing the pdf and wait until you have received a Submission Number!



REPUBLIC OF CROATIA

Ministry of Agriculture, Fischeries and Rural Development Veterinary Directorate

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Table 1

birds Species (Lat) Phalacrocorax aristotelis Ardea cinerea Cygnus olor Anser anser Anas penelope Anas strepera Anas crecca Anas plathyrhynchos Anas acuta Anas querquedula Anas clypeata	ds found	birds with observed clinical	clinical signs	wild birds	No of live wild birds with clinical signs	birds withouth	Total No of wild birds tested in	Total No of wild birds tested in		Total No of	Total No of
Phalacrocorax aristotelis Ardea cinerea Cygnus olor Anser anser Anas penelope Anas strepera Anas crecca Anas plathyrhynchos Anas acuta Anas querquedula Anas clypeata	6 8				tested	_	1.	active surveilance			wild birds POSITIVE
Anser anser Anas penelope Anas strepera Anas crecca Anas plathyrhynchos Anas acuta Anas querquedula Anas clypeata	8					5		5	5	5	
Anas querquedula Anas clypeata							6		6	6	
Anas penelope Anas strepera Anas crecca Anas plathyrhynchos Anas acuta Anas querquedula Anas clypeata	1					18	8	18	26	26	
Anas strepera Anas crecca Anas plathyrhynchos Anas acuta Anas querquedula Anas clypeata			2				1	2	3	3	
Anas crecca Anas plathyrhynchos Anas acuta Anas querquedula Anas clypeata			71					71	71	71	
Anas plathyrhynchos Anas acuta Anas querquedula Anas clypeata			17					17	17	17	
Anas acuta Anas querquedula Anas clypeata			1152					1152	1152	1152	
Anas acuta Anas querquedula Anas clypeata	2		926				2	926	928	928	
Anas clypeata			9					9	9	9	
			39					39	39		
Audhus fasisa			20					20	20	20	
Aythya ferina			101					101	101	101	
Aythya nyroca			30					30	30	30	
Aythya fuligula			7					7	7	7	
Porzana porzana			1					1	1	1	
Fulica atra			262					262			
Larus michahellis	2					383	2	383			
Larus ridibundus	1					941	1	941	942		
Sterna hirundo						59		59	59		
Chlidonias hybridus						421		421	421		
Columba livia domestica	2						2		2		
Larus canus						27		27	27		
Larus cachinnans						20		20	20		
Larus argentatus						3		3	3	3	
Tringa erythropus			1					1	1	1	
Netta rufina			1					1	1	1	
Aquila chrysaetos	1						1		1	1	
Vanellus vanellus			3		<u> </u>			3	3	3	1
Larus fuscus									·		



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Table 2 No of wild birds found positive on PCR test / virus isolation / serology in 2012

All birds were tested either by PCR or virus isolation. None of the tested wild birds was found positive for H5 and/or H7 Al virus. None of the birds was tested using serology methods.



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Table 1: Report of surveillance for AI in poultry in Croatia in 2012

					•														1
	Poultry category																		
	Chicken breeders			Laying hens			Broilers			Backyard poultry			Ducks			Turkeys			
			Number			Number			N			Number						Number	
	Number	Number of positive	of blood samples	Number	Number of positive	of blood samples	Number	Number of positive	Number of blood samples	Number	Number of positive	of blood samples	Number	Number of positive	Number of blood samples	Number	Number of positive	of blood samples	
	of farms	farms	tested	of farms	farms	tested	of farms	farms	tested	of holdings	holdings	tested	of farms	farms	tested	of farms	farms	tested	
First half	10	0	472	34	0	730	0	0	0	864	3	8280	0	0	0	6	0	186	Total No. of
Second half	24	0	879	85	0	1543	1	0	10	1526	2	13074	1	0	100	3	0	99	samples tested
Samples/year																			25373

One serum sample out of 10 from a backyard in Draž was tested positive for H7 Al antibodies in April 2012. One serum sample out of 10 from a backyard in Bokšić Lug was tested positive for H7 Al antibodies in May 2012. At the same time and the same village in another backayrd, 4 samples out of 10 were found positive for H7 Al antibodies. In the same backyard in September 2012, one out of 10 samples was found positive for H7 Al antibodies. All three backyards are in Osječko-baranjska county.

One serum sample out of 10 from a backyard in Zokov Gaj was tested positive for H5 Al antibodies in September 2012. The backyard is in Virovitičko-podravska county.

Subsequently, poultry from the backyard with H7 Al antobody positive results in May and September in Osječko-baranjska county was sampled for virological testing. Also, poultry from the backyard with positive result for H5 Al antibodies in Virovitičko-podravska county was also sampled for virological testing. Resuts of virological testing for all samples were negative for Al virus.

Virological testing for AI in villages where H5/H7 seropositive backyard poultry were found												
Village(s)	Sampling time	Number of poultry sampled	oultry cloacal		Total number of samples	Al positive (M gene rRT-PCR)	(H5 gana	H7 positive (H7 gene rRT-PCR)				
Bokšić Lug	18.10.2012.	10	10	10	20	0	0	0				
Zokov Gaj	18.10.2012.	17	17	17	34	0	0	0				
Total		27	27	27	54	0	0	0				

Map 1: Spatial distribution of locations/sites at which the 2014 passive surveillance for avian influenza in wild birds will be conducted (the same locations are included in AI surveillance from the year 2006)

