



EUROPEAN COMMISSION
HEALTH & CONSUMERS DIRECTORATE-GENERAL
Unit 04 - Veterinary Control Programmes

SANCO/3859/2008

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

**Survey programme for Avian Influenza in
poultry and wild birds**

Approved* for 2009 by Commission Decision 2008/897/EC

Bulgaria

* in accordance with Commission Decision 90/424/EEC



REPUBLIC OF BULGARIA
MINISTRY OF AGRICULTURE AND FOODS

PROGRAMME
FOR
SURVEILLANCE OF AVIAN INFLUENZA IN
DOMESTIC POULTRY AND WILD BIRDS IN THE REPUBLIC OF BULGARIA FOR
THE YEAR OF 2009

1. IDENTIFICATION OF THE PROGRAMME:

Member State: Republic of Bulgaria

Disease: Avian Influenza

Year of implementation: 2009

Reference to this document:

- **Law on veterinary activity** (SG 87/1 November 2005 in force since 1 May 2006)

“Art. 123. (1) The NVS shall draft national programs for epizootic surveillance of some specific contagious diseases.

(2) The Minister of Agriculture and Forestry shall approve the programs under paragraph 1 upon a proposal of the Director General of the NVS.”

- **Ordinance № SG-103** of 21 August 2006 on the measures for prevention, control and eradication of Avian Influenza (H5N1) disease; issued by the Ministry of Agriculture and Forestry and entered into force from 01.07.2007, SG No 83 of 13 October 2006 (harmonized with Council Directive 2005/94 EEC).
- **Commission Decision 2007/268** on the implementation of surveillance programmes for avian influenza in poultry and wild birds to be carried out in the Member States and amending Decision 2004/450/EC

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2. DESCRIPTION OF THE SURVEILLANCE PROGRAMME IN POULTRY:

2.1 Objectives, general requirements and criteria

A. Objectives:

The serological surveillance of highly pathogenic Avian Influenza subtypes H5 and H7 has the following objectives:

- To detect clinical and subclinical infections and to undertake early protection measures for control the spread of the disease and possible mutation of the virus.
- To detect poultry populations infected with H5 and H7, which are susceptible to the disease and are reared in areas where the risk of disease introduction is considered to be higher.
- To prove that a region is free from notifiable avian influenza in the frame of intercommunity trade from 01.01.2008 and in the frame of international trade according to the rules of World Organisation for Animal Health (OIE), Paris.

B. General requirements and criteria:

1. The sampling shall not extend beyond 31 December 2009 and shall cover a production period.
2. Testing of samples shall be carried out at National Reference Laboratory for Avian Influenza under the National Diagnostic and Research Veterinary Institute /NDRVI/, Sofia National Reference Laboratory for Diagnostics of Avian Influenza and Newcastle Disease in Varna.
3. All results, both serological and virological, shall be sent to the Community Reference Laboratory for Avian Influenza. All isolates positive for Avian Influenza will be submitted to the Community Reference Laboratory.

The samples should be addressed to:
Avian Virology, VLA Weybridge, New Haw, Addlestone, and Surrey KT15 3NB, United Kingdom
Community Reference Laboratory contacts

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2.2 DESIGN AND IMPLEMENTATION:

Serological and virological examination of domestic poultry

Serological EXAMINATION for Avian influenza of the following species and birds category:

- Domestic hens, kept in non-commercial holdings or in industrial holdings;
- Domestic birds of the gallinaceous species /breeding stocks, laying hens, turkeys, of ratites/;
- Domestic waterfowls /ducks and geese/;
- Birds, reared and used for hunting (pheasants, partridge, quails, half-savage birds);

SAMPLE SELECTION, COLLECTION AND SENDING:

For serological analyses are sent serum samples of clinically healthy birds.

- **Blood** obtained by a single-use closed blood sampling system. The blood is collected of the internal part of the wing from v. cutanea ulnaris and v. Brachialis, using vacuum container type butterfly.

The samples are chilled immediately on ice and submitted to the laboratory as quickly as possible. If rapid transportation can not be guaranteed the samples can be stored for 48 to 72 hours at 0 - 4°C and for a longer period – at -70° C. The specimens for virological sampling should not be transported on dry ice, since the CO₂ inactivate immediately the avian influenza virus.

The plan for laboratory surveillance for 2009 is based on a regional principle as regards to the samples which have to be taken from different bird species and sent for analyses. The programme includes examination of live poultry. It is preferable the samples taken from domestic poultry from gallinaceous species and waterfowls to be sent with a separate cover letters. The samples should be taken within the migratory period of the wild birds and can include considerable number of slaughtered domestic poultry.

In case of taking samples from one settlement the latter must be collected from at least three different backyards/ flocks.

Table 2.2.1 Poultry holdings (except ducks and geese):

NUTS (2)code	Total number of holdings	of	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of Laboratory analysis
<u>BG311</u>	1- hens	laying	1	20	19- ELISA; 1 - AGID	ELISA; PCR; AGID HI;
<u>BG312</u>	1- hens	laying	1	20	19- ELISA; 1 - AGID	ELISA; PCR; AGID HI;
<u>BG313</u>	2- hens	laying	2	20	37- ELISA; 3 - AGID	ELISA; PCR; AGID HI;
<u>BG314</u>	3- hens	laying	3	20	55- ELISA; 5 - AGID	ELISA; PCR; AGID HI;
<u>BG315</u>	2- hens	laying	2	20	37- ELISA; 3 - AGID	ELISA; PCR; AGID HI;
<u>BG321</u>	2- hens	laying	2	20	37- ELISA; 3 - AGID	ELISA; PCR; AGID HI;
<u>BG322</u>	1- hens	laying	1	20	19- ELISA; 1 - AGID	ELISA; PCR; AGID HI;
<u>BG323</u>	3- hens	laying	3	20	55- ELISA; 5 - AGID	ELISA; PCR; AGID HI;
<u>BG324</u>	1- hens	laying	1	20	19- ELISA; 1 - AGID	ELISA; PCR; AGID HI;

<u>BG325</u>	2- hens	laying	2	20	37- ELISA; 3 - AGID	ELISA; PCR; AGID	HI;
<u>BG331</u>	4- hens	laying	4	20	74- ELISA; 6 - AGID	ELISA; PCR; AGID	HI;
<u>BG332</u>	1- hens	laying	1	20	19- ELISA; 1 - AGID	ELISA; PCR; AGID	HI;
<u>BG334</u>	3- hens	laying	3	20	55- ELISA; 5 - AGID	ELISA; PCR; AGID	HI;
<u>BG341</u>	2- hens	laying	2	20	37- ELISA; 3 - AGID	ELISA; PCR; AGID	HI;
<u>BG342</u>	1- hens	laying	1	20	19- ELISA; 1 - AGID	ELISA; PCR; AGID	HI;
<u>BG343</u>	2- hens	laying	2	20	37- ELISA; 3 - AGID	ELISA; PCR; AGID	HI;
<u>BG344</u>	3- hens	laying	3	20	55- ELISA; 5 - AGID	ELISA; PCR; AGID	HI;
<u>BG411</u>	2- hens	laying	2	20	37- ELISA; 3 - AGID	ELISA; PCR; AGID	HI;
<u>BG412</u>	4- hens	laying	4	20	74- ELISA; 6 -AGID	ELISA; PCR; AGID	HI;
<u>BG413</u>	1- hens	laying	1	20	19- ELISA; 1 -AGID	ELISA; PCR; AGID	HI;
<u>BG414</u>	2- hens	laying	2	20	37- ELISA; 3 -AGID	ELISA; PCR; AGID	HI;

<u>BG415</u>	1- laying hens	1	20	19- ELISA; 1 -AGID	ELISA; PCR; AGID	HI;
<u>BG421</u>	4 -laying hens	4	20	74- ELISA; 6 -AGID	ELISA; PCR; AGID	HI;
<u>BG422</u>	2 -laying hens	2	20	37- ELISA; 3 -AGID	ELISA; PCR; AGID	HI;
<u>BG423</u>	1-laying hens	1	20	19- ELISA; 1 -AGID	ELISA; PCR; AGID	HI;
<u>BG424</u>	1-laying hens	1	20	19- ELISA; 1 -AGID	ELISA; PCR; AGID	HI;
<u>BG425</u>	1- laying hens	1	20	19- ELISA; 1 -AGID	ELISA; PCR; AGID	HI;
Total		53	20	984- ELISA 76- AGID		

Table 2.2.2 Pheasants holdings to be sampled:

NUTS (2)code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of Laboratory analysis
<u>BG314</u>	1- pheasants	1	20	20-HI	ELISA; PCR; AGID HI;
<u>BG343</u>	2- pheasants	2	20	40- ELISA	ELISA; PCR; AGID HI;
<u>BG344</u>	1- pheasants	1	20	20- ELISA	ELISA; PCR; HI;

				1- HI	AGID
Total		4		80-	ELISA

Table 2.2.3 Farmed game holdings to be sampled:

NUTS (2)code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of Laboratory analysis
<u>BG342</u>	1- farm game birds	1	20	20 HI	ELISA; PCR; HI; AGID
Total	1	1		20-	HI

Table 2.2.4 Turkey holdings to be sampled:

NUTS (2)code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of Laboratory analysis
<u>BG331</u>	2- turkey	2	20	40- III	ELISA; PCR; HI; AGID
<u>BG342</u>	2- turkey	2	20	40 HI	ELISA; PCR; HI; AGID
<u>BG342</u>	2- turkey	2	20	40- HI	ELISA; PCR; HI; AGID
Total		6	20	120-	HI

Table 2.2.5 Duck and geese holdings to be sampled:

NUTS code	Total number of ducks and geese holdings	Total number of ducks and geese holdings to be sampled	Number of samples per holding	Total number of samples to be performed per method	Methods of laboratory analysis
<u>BG315</u>	3- ducks mallards	3	40	120- HI	HI ; AGID
<u>BG324</u>	2- ducks mallards	2	40	80- HI	HI ; AGID
<u>BG325</u>	1- ducks mallards	1	40	40- HI	HI ; AGID
<u>BG331</u>	5- ducks mallards	5	40	200- HI	HI ; AGID
<u>BG332</u>	4- fattening ducks	4	40	160- HI	HI ; AGID
<u>BG342</u>	5- ducks mallards	5	40	200- HI	HI ; AGID
<u>BG344</u>	13- ducks mallards	13	40	520- HI	HI ; AGID
<u>BG422</u>	17- ducks mallards	17	40	680- HI	HI ; AGID
<u>BG423</u>	9 fattening ducks	9	40	360- HI	HI ; AGID
Total		59	40	2360- HI	

Table 2.2.6 Backyard flocks:

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
<u>BG413</u>	Byalo Pole village	gallinaceous species –625	81 backyards	53	ELISA; PCR; HI; AGID
		Water birds – 30	5 backyards	25	
	Cherniche village	gallinaceous species - 460 Waterfowls 10	58 1	42 5	ELISA; PCR; HI; AGID
	Toplonitza village	gallinaceous species 260	22	22	ELISA; PCR; HI; AGID
	Total:			147	
<u>BG341</u>	Cherni Vrah village	gallinaceous species - 3000	215	53	ELISA; PCR; HI; AGID
		waterfowls - 70	9	45	
	Dolno Ezerovo village	gallinaceous species - 3200	207	60	ELISA; PCR; HI; AGID
	Kraimorie village	gallinaceous species - 318 waterfowls - 12	22 2	22 10	ELISA; PCR; HI; AGID
	Dimchevo village	gallinaceous species - 545 waterfowls 55	55 6	42 30	ELISA; PCR; HI; AGID
	Total:			262	

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
BG331	Kazashko village	gallinaceous species - 250 waterfowls - 55	52 9	42 20	ELISA; PCR; HI; AGID
	Strashimirovo village	gallinaceous species - 1175 waterfowls - 18	105 3	53 15	ELISA; PCR; HI; AGID
	Ezerovo village	gallinaceous species - 661 waterfowls - 23	60 3	42 15	ELISA; PCR; HI; AGID
	Total:			187	
BG321	Town Svishtov	Gallinaceous species - 1532	48	35	ELISA; PCR; HI; AGID
	Vardam village	Gallinaceous species - 396	21	21	ELISA; PCR; HI; AGID
	Total:			56	
BG311	Vrav village	gallinaceous species - 2100 waterfowls - 86	50 9	35 30	ELISA; PCR; HI; AGID
	Koshava village	gallinaceous species - 3100 waterfowls - 65	50 8	35 20	ELISA; PCR; HI; AGID

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
	Archar village	gallinaceous species - 4128 waterfowls - 87	70 11	42 20	ELISA; PCR; HI; AGID
	Total:			182	
BG313	town Oryahovo	gallinaceous species - 5000 waterfowls - 2000	50 55	35 70	ELISA; PCR; HI; AGID
	Ostrov village	gallinaceous species - 15 250 waterfowls - 800	1109 60	60 70	
	Town. Kozlodui	gallinaceous species - 48 000 waterfowls - 1200	600 86	60 90	ELISA; PCR; HI; AGID
	Total:			385	
	BG332	Shabla village	gallinaceous species - 5 900 waterfowls - 920	610 92	60 100
Ezerets village		gallinaceous species - 1512 waterfowls - 110	51 11	42 20	ELISA; PCR; HI; AGID

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
	Durankulak village	gallinaceous species - 3200 waterfowls - 900	184 815	153 300	ELISA; PCR; HI; AGID
	Vaklino village	gallinaceous species - 1532 waterfowls - 612	135 89	53 100	ELISA; PCR; HI; AGID
	Total:			828	
BG425	Ostrivitca village	gallinaceous species - 120 waterfowls - 12	12 3	12 10	ELISA; PCR; HI; AGID
	Shiroko Pole village	gallinaceous species - 600 waterfowls - 8	59 2	42 4	ELISA; PCR; HI; AGID
	Gluhar village	gallinaceous species - 886 waterfowls 28	68 5	42 15	ELISA; PCR; HI; AGID
	Padartci village	gallinaceous species - 350	19	19	ELISA; PCR; HI; AGID
	Kokoshane village	gallinaceous species - 66	7	7	ELISA; PCR; HI; AGID
	Total:			151	
BG312	Slivata Village	hens - 800 waterfowls - 90	80 6	53 15	ELISA; PCR; HI; AGID
	Dolno Ligavo village	hens - 1 729 waterfowls - 79	88 7	53 15	ELISA; PCR; HI; AGID

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
	Dolni Teibar village	hens - 240 waterfowls - 89	29 4	29 10	ELISA; PCR; HI; AGID
	Gorno Tserovene village	hens -2047 waterfowls - 35	186 3	53 10	
	Total:			238	
<u>BG423</u>	Poibrene village	gallinaceous species – 1 216 waterfowls - 315	56 26	42 40	ELISA; PCR; HI; AGID
	Total:			82	
	<u>BG314</u>	Zagrajden village	gallinaceous species- 4 340 waterfowls – 4100	55 67	
	Dolni Vit Village	gallinaceous species- 4 680 waterfowls – 5120	115 86	53 100	
	Total:			265	
<u>BG421</u>	Belovitca village	gallinaceous species – 3 000 waterfowls - 68	240 9	53 20	ELISA; PCR; HI; AGID

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
	Malo Krushovo village	gallinaceous species - 1210 waterfowls - 52	80 6	42 18	ELISA; PCR; HI; AGID
	Trilistni village	gallinaceous species - 5 500	4	4	ELISA; PCR; HI; AGID
	Total:			137	
BG324	Kroyach village	gallinaceous species - 820 waterfowls - 315	41 32	35 50	ELISA; PCR; HI; AGID
	Total:			85	
BG323	Krivina village	gallinaceous - 9125 waterfowls - 315	96 8	53 20	ELISA; PCR; HI; AGID
	Batin village	gallinaceous - 4250	77	53	ELISA; PCR; HI; AGID
	Ryahovo Village	gallinaceous - 5556 waterfowls - 213	72 14	53 30	ELISA; PCR; HI; AGID
	Total:			209	
BG325	Srcharna village	gallinaceous species - 7000	42	35	ELISA; PCR; HI; AGID
		waterfowls - 120	8	30	

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
	Vetren village	gallinaceous species -5842 waterfowls - 152	51 9	42 40	ELISA; PCR; III; AGID
	Popina village	gallinaceous species - 4125 waterfowls - 70	46 5	35 10	ELISA; PCR; III; AGID
	Total:			192	
BG342	Banya village	gallinaceous species - 2252 waterfowls - 131	125 35	53 35	ELISA; PCR; III; AGID
	Total:			88	
BG334	Lomtei village	gallinaceous species – 3 200 waterfowls – 526	55 30	42 50	ELISA; PCR; HI; AGID
	Total:			92	
BG422	Galabete village	gallinaceous species - 490 waterfowls – 21	47 3	35 10	ELISA; PCR; III; AGID
	Nikolovo village	gallinaceous species -190 waterfowls – 45	22 6	22 20	ELISA; PCR; III; AGID
	Total:			87	

NUTS code	Settlement:	Bird species and number of samples:	Number of backyards:	Total number of samples:	Methods of laboratory analysis
BG333	Kiolinen village	gallinaceous species - 112 waterfowls - 94	5 6	5 20	ELISA; PCR; HI; AGID
	village Malomir	gallinaceous species - 412 waterfowls - 80	25 7	25 20	ELISA; PCR; HI; AGID
	Tushovitca village	gallinaceous species - 641 waterfowls - 512	48 32	35 40	ELISA; PCR; HI; AGID
	Sushina village	gallinaceous species - 191 waterfowls - 45	19 4	19 10	ELISA; PCR; HI; AGID
	Kochovo village	gallinaceous species - 1680 waterfowls - 1320	105 60	53 70	ELISA; PCR; HI; AGID
	Han Krum village	gallinaceous species - 675 waterfowls - 758	78 42	53 50	ELISA; PCR; HI; AGID
	Total:			400	
Total for the country:				4073	

Total number of sample per method:

Gallinaceous species: ELISA- 2094; AGID- 158;

Waterfowls: HI- 1821

2.3 LABORATORY TESTING: DESCRIPTION OF THE LABORATORY TESTS:

1. Laboratory tests shall be carried out in accordance with the avian influenza diagnostic manual (Commission Decision 2006/437/EC) laying down the procedures for the confirmation and differential diagnosis of avian influenza (including examination of sera from ducks and geese by haemagglutination-inhibition (HI) test).
2. All positive serological findings shall be confirmed by the National Laboratories for avian influenza by a haemagglutination-inhibition test, using designated strains supplied by the Community Reference Laboratory for Avian Influenza:
 - H5 (a) initial test using Ostrich/Denmark/72420/96 (H5N2);
 - (b) test all positives with Duck/Denmark/64650/03 (H5N7) to eliminate N2 cross reactive antibody.
 - H7 (a) initial test using Turkey/England/647/77 (H7N7);
 - (b) test all positives with African Starling/983/79 (H7N1) to eliminate N7 cross reactive antibody.

3. DESCRIPTION OF THE SURVEILLANCE PROGRAMME IN WILD BIRDS:

3.1 Objectives, general requirements and criteria:

- **A. Objectives:**

Virological surveillance for avian influenza in wild birds aim to identify the risk of introduction of AI viruses (LPAI and HPAI) to domestic poultry by:

— to ensure early detection of HPAI H5N1 by investigating increased incidence of morbidity and mortality in wild birds, in particular in selected 'higher risk' species.

— in the event that HPAI H5N1 is detected in wild birds, then surveillance of live and dead wild birds shall be enhanced to determine whether wild birds of other species can act as asymptomatic carriers or 'bridge species' (see table below).

— to continue a 'baseline' surveillance of different species of free living migratory birds as part of continuous monitoring of LPAI viruses. Anseriformes (water fowl) and Charadriiformes (shorebirds and gulls) shall be the main sampling targets to assess if they carry LPAI viruses of H5 and H7 subtypes (which would in any case also detect HPAI H5N1 and other HPAI, if present). 'Higher risk species' must be targeted in particular.

- **B. General requirements and criteria:**

1. Sampling shall not extend beyond 31 December 2008.
2. Testing of samples shall be carried out at National Reference Laboratory for Avian Influenza under the National Diagnostic and Research Veterinary Institute /NDRVI/, Sofia National Reference Laboratory for Diagnostics of Avian Influenza and Newcastle Disease in Varna.

3. All results, both serological and virological, shall be sent to the Community Reference Laboratory for Avian Influenza. All isolates positive for Avian Influenza will be submitted to the Community Reference Laboratory.

The samples should be addressed to:

Avian Virology, VLA Weybridge, New Haw, Addlestone, and Surrey KT15 3NB, United Kingdom

Community Reference Laboratory contacts

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The list of wild bird species presenting a higher risk of susceptibility in relation to avian influenza.

List of wild bird species presenting a higher risk in relation to avian influenza *

Species	LATIN NAME
Bewick's Swan	<i>Cygnus columbianus</i>
Whooper Swan	<i>Cygnus cygnus</i>
Mute Swan	<i>Cygnus olor</i>
Geese	
Pink-footed Goose	<i>Anser brachyrhynchus</i>
Bean Goose	<i>Anser fabalis</i>
Greater White-fronted Goose (European race)	<i>Anser albifrons</i>
Lesser White-fronted Goose	<i>Anser erythropus</i>
Greylag Goose	<i>Anser anser</i>
Barnacle Goose	<i>Branta leucopsis</i>
Brent Goose	<i>Branta bernicla</i>
Red-breasted Goose	<i>Branta ruficollis</i>
Canada Goose	<i>Branta canadensis</i>
Ducks	
Eurasian Wigeon	<i>Anas penelope</i>
Common Teal	<i>Anas crecca</i>
Mallard	<i>Anas platyrhynchos</i>
Northern Pintail	<i>Anas acuta</i>

Garganey	<i>Anas querquedula</i>
Northern Shoveler	<i>Anas clypeata</i>
Marbled Teal	<i>Marmaronetta angustirostris</i>
Red-crested Pochard	<i>Netta rufina</i>
Common Pochard	<i>Aythya ferina</i>
Tufted Duck	<i>Aythya fuligula</i>
Waders	
Northern Lapwing	<i>Vanellus vanellus</i>
Eurasian Golden Plover	<i>Pluvialis apricaria</i>
Black-tailed Godwit	<i>Limosa limosa</i>
Ruff	<i>Philomachus pugnax</i>
Gulls	
Black-headed Gull	<i>Larus ridibundus</i>
Common Gull	<i>Larus canus</i>

LIST OF BIRDS LIVING IN PROXIMITY TO DOMESTIC POULTRY

Species	Latin name	EURING code
Domestic Goose	<i>Anser anser domesticus</i>	01610
Domestic mallard	<i>Anas platyrhynchos</i>	01860
Domestic Muscovy Duck	<i>Cairina moschata</i>	01750
Feral Pigeon	<i>Columba livia</i>	06650
House Sparrow	<i>Passer domesticus</i>	15910

3.2 DESIGN AND IMPLEMENTATION:

1. It is necessary the participation of ornithological institutions and organizations responsible for the ringing of birds. Where necessary this must be done under the supervision of these organizations or by the hunters.
2. The active surveillance in living or hunted birds shall be targeted on:
 - a) populations from different wild birds species presenting a "higher risk" identified on the basis of:

- origin and the migratory flyways;
- the number of the wild birds in the Community;
- the probability of contact with domestic poultry;

b) identification of areas of a higher risk based on:

- mixed sites frequented by high number of different species migratory birds but mostly those in annex I;
- in proximity to domestic poultry farms;
- location of the migratory flyway;

3. The passive surveillance in wild birds, found dead, should be focused on the presence of increased mortality or outbreaks of acute infectious disease:

/a/ in wild birds listed in p.3.1 and other wild birds in contact with them;

/b/ in areas described p. 2 */b/*

Increased incidences of mortality of different bird species concentrated in one place is an additional factor that should be taken into consideration.

A detailed description of the number of samples per villages and the sampling method (virological).

The frequency of the surveillance in the critical points should be identified individually depending on each of the specificity of the critical points and on the type of the disease (infectious agent, sources of the infection, way of transmission, driving forces of the infectious process, incubation period, natural reservoirs, environmental factors, season, agricultural activities and etc.).

The surveillance frequency could be with decreased intensity in regions considered of lower risk.

The National Veterinary Service has prepared model cover letters, according to the European Commission requirements, for submission of the samples to the National Reference Laboratories "Newcastle disease and Avian Influenza A".

Sampling procedures:

1. Oropharyngeal and cloacal swabs for virological examination shall be taken from apparently healthy free living birds. If for any reason it is impractical to take cloacal swabs from live birds carefully collected fresh faeces samples may serve as an alternative. However, traceability in case of mixed sites frequented by different bird species must be ensured.

2. Cloacal and tracheal/oropharyngeal swabs and/or tissues (namely the brain, heart, lung, trachea, kidney and intestines) from wild birds found dead or shot shall be sampled for virus isolation and molecular detection (PCR).

3. Specific care has to be taken for the storage and transport of samples. Swabs must be chilled immediately on ice or with frozen gel packs and submitted to the laboratory as quickly as possible. Samples must not be frozen unless absolutely necessary. If available, swabs must be placed in antibiotic or specific virus transport medium so that they are fully immersed. Placing samples in medium for transportation must be done in addition to chilling and not as an alternative to chilling. In the absence of such medium, swabs must be returned to their casing and submitted dry. If rapid transport within 48 hours to the laboratory (in transport medium at 4° Celsius) is not guaranteed, samples shall be immediately frozen, stored and then transported on dry ice. Storage and transport of samples may be affected by a variety of factors so the method selected must be fit for purpose.

4. Sampling procedures shall be carried out in accordance with the avian influenza diagnostic manual laying down the procedures for the confirmation and differential diagnostic of avian influenza.

Table 3.2.1

Wild birds:

NUTS code	Wild birds to be sampled	Total number to be taken for active surveillance	Total number to be taken for passive surveillance
<u>BG413</u>	40	35	5
<u>BG341</u>	120	100	20
<u>BG331</u>	90	80	10
<u>BG321</u>	20	20	-
<u>BG311</u>	100	90	10
<u>BG313</u>	30	25	5
<u>BG322</u>	40	35	5
<u>BG332</u>	80	70	10
<u>BG415</u>	110	100	10
<u>BG425</u>	60	55	5
<u>BG315</u>	40	35	5
<u>BG312</u>	80	70	10
<u>BG423</u>	170	140	30
<u>BG414</u>	40	35	5
<u>BG314</u>	40	35	5
<u>BG421</u>	120	100	20
<u>BG324</u>	40	35	5
<u>BG323</u>	60	55	5
<u>BG325</u>	50	45	5
<u>BG342</u>	80	70	10
<u>BG324</u>	40	35	5
<u>BG412</u>	20	20	-
<u>BG344</u>	80	70	10

<u>BG334</u>	60	55	5
<u>BG422</u>	170	140	30
<u>BG333</u>	60	55	5
<u>BG343</u>	60	55	5
Total	1900	1660	240

3.3 LABORATORY TESTING: DESCRIPTION OF THE LABORATORY TESTS:

The diagnostics is carried as per the following methods:

1. Virus isolation in chick embryos as per the routine procedures for testing of samples from faeces or oro-tracheal swabs.
2. Detection of the nucleic acids of the viruses of Avian Influenza and Newcastle disease through real-time reverse transcriptase Polymerase Chain Reaction assay.
3. Identification of the isolated virus through agar gel precipitation test for avian influenza virus and haemagglutination inhibition test for paramyxovirus.
4. Characterization of the Newcastle disease isolated viruses for identification the type of the strains - velogenic, mesogenic or lentogenic through biological tests: determining the mean death time of 10 days-old chick embryos, determining of intracerebral pathogenicity index in day-old chickens and in case of necessity sending of material for genetic analyses to an international reference laboratory sending of material for genetic analyses to an international reference laboratory.

4. DESCRIPTION OF THE EPIDEMIOLOGICAL SITUATION OF THE DISEASE IN POULTRY DURING THE LAST FIVE YEARS:

4. 1. Each year since 2000, the NVS has been implementing an annual AI Surveillance Programme in poultry.

Measures included in the programme for poultry surveillance:

- ✦ Observation of health status of the poultry kept in the poultry farms of intensive mode of keeping /the large poultry holdings/;
- ✦ Observation of health status of the poultry kept in backyards;
- ✦ Testing of samples taken from the birds kept in all regions of the country considered to be of higher risk with regards to AI;
- ✦ Testing of poultry carcasses collected in case of mortality rates higher than the normal poultry ones;
- ✦ Strict control on the movements of poultry and poultry products;
- ✦ Thorough disinfection of transport vehicles entering into the country from third countries;
- ✦ Control on the implementation of bio-security measures.

Epidemiological situation in birds throughout the last 5 years:

During the last 5 years not a single case of highly pathogenic Avian influenza (HPAI) has ever been identified in poultry populations in Republic of Bulgaria.

4.1.1. The NVS of Bulgaria is the national competent authority responsible for the implementation of the AI Surveillance Programme.

This Programme is directly performed by registered veterinarians exercising private practice /registered private practitioners/ under the control of the official veterinarians directly responsible for all the country municipalities located within the 28 administrative districts (= 28 veterinary regions) of Bulgaria.

The outcomes of implementation of the AI Surveillance Programme are reported in writing on monthly basis by the 28 "Animal Health" Department Chiefs (with the 28 Regional Veterinary Services) to the "Animal Health" Directorate at the NVS Central Administration.

Each week the National reference Laboratory send information, regarding the samples from the Surveillance Programme to the Animal Health Directorate in the National Veterinary Service.

4.1.2 All poultry holdings (including backyard poultry) are registered and have an identification number according to the Law of veterinary activity.

4.1.3 The prophylactic vaccination against Avian Influenza is prohibited. In Republic of Bulgaria the vaccination against the disease was never carried on.

5. DESCRIPTION OF THE EPIDEMIOLOGICAL SITUATION OF THE DISEASE IN WILD BIRDS DURING THE LAST FIVE YEARS:

5.1. Each year since 2000, the NVS has been implementing an annual AI Surveillance Programme in wild birds.

Measures included in the programme for wild birds surveillance:

- ↓ Monitoring of the wild birds migration;
- ↓ monitoring of the mortality in wild birds;
- ↓ capture and taking samples from wild bird;
- ↓ survey and laboratory testing of samples from wild birds;

Epidemiological situation in wild birds throughout the last 5 years:

AI in poultry has never been found out up to 31.01.2006:

On 31.01.2006 in river Danube near the town of Vidin a sick swan was found. AI virus, strain H5 was isolated from the swan at the National Reference Laboratory on AI in Sofia. The isolate was sent to Central Reference Laboratory of the European Community in Waybridge, Great Britain and on 10.2.2006 the isolate was confirmed as influenza A H5N1;

On 09.02.2006 in samples taken from dead swans found in the lake of Durankulak, region of Dobrich, a virus was isolated determined as influenza of the type A-H5;

On 09.02.2006 virus H5N1 was isolated from a dead swan found out in the dam Tzonevo, region of Varna.

On 11.02.2006 a virus of influenza H5 was confirmed in dead swan found out on the beach of Karimoric residential quarter, town of Burgas.

Regions of Bulgaria where influenza in swans (*Cygnus olor*) was found out:



All data for surveillance of wild birds for 2007 were on-line submitted to DG SANCO;

On 08.02.2008 a Low pathogenic avian influenza virus H7N7 was confirmed in a mallard duck shot near to the village of Han Krum, municipality of Veliki Preslav, administrative district of Shoumen.

- 5.1.1 The NVS is the national competent authority responsible for the implementation of the AI Surveillance Programme. The Programme is been performed under the assistance rendered by the local associations of ornithologists and by the local units of the national Union of Hunters and Anglers of Bulgaria.
- 5.1.2 The Programme is been implemented over the territory of the whole country, major share of the total number of samples foreseen therein being taken from all those 10 administrative districts /veterinary regions/ identified as such of higher risk with regards to AI.

5.1.3 Throughout the whole season of intensive wild bird migration, the “Animal Health” Directorate at the NVS Central Administration would receive the daily information about the numbers and the health status of the wild birds observed.

6. MEASURES IN PLACE AS REGARDS THE NOTIFICATION OF THE DISEASE:

The Law on Veterinary Activities, Art.124 and Ordinance No.23 / 14.12.2005 on the order and the way of notification and registration of infectious diseases in animals, which is harmonized with Council Directive 82/894/EEC

7. COSTS:

7.1 Detailed analysis of the costs:

7.1.1 Poultry:

1. Costs related to taking and transportation of samples for testing to diagnostics laboratories
2. Costs for the purchase of the required diagnostics kits
3. Costs for compensations to the owners of compulsively killed or slaughtered birds
4. Costs for remuneration of the labor of the executers of the program

7.1.2. Wild birds:

- 1.Costs related to capture of wild birds
- 2.Costs related to taking and transportation of samples for testing to diagnostics laboratories
- 3.Costs for the purchase of the required diagnostics kits
- 4.Costs for remuneration of the labor of the executers of the program

7.2 Summary of the costs:

7.2.1 Poultry surveillance

Methods of laboratory analysis	Measures eligible for co- financing surveillance in poultry		
	Numbers of tests perform per method	Unitary test cost (per method)	Total cost
Serological pre-screening:			
ELISA	3158	1	3158
AGID	234	1.2	281
HI for H5/H7	4321	12	51 852
Virus isolation test	500	30	15 000
PCR test	1000	15	15 000
Other measures to	Specify activities		

be covered			
Sampling	9 213	1	9 213
Others /transport	9 213	1	9213
Total			103 717

7.2.2 Wild bird surveillance

Measures eligible for co- financing surveillance in wild birds			
Methods of laboratory analysis	Numbers of tests perform per method	Unitary test cost (per method)	Total cost
Virus isolation test	1000	30	30 000
PCR test	900	15	13 500
Other measures to be covered	Specify activities		
Sampling	1900	3	5 700
Others /transport	1900	1	1900
Total			43 500

TOTAL AMOUNT UNDER THE AVIAN INFLUENZA SURVEILLANCE PROGRAMME OF R. OF BULGARIA FOR 2009 – 147 217 €

