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*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 2010 by Commission Decision 2009/883/EC**

**Denmark**

\* in accordance with Council Decision 2009/470/EC

# Ministry of Food, Agriculture and Fisheries

Danish Veterinary and Food Administration



European Commission  
DI - Animal Health and Standing Committees  
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**Plan for implementation of surveillance programmes for avian influenza in poultry, game birds for restocking and wild birds to be carried out in Denmark in 2010 and application for financial contribution**

## 1. Identification of the programme

Member State: Denmark.

Disease: Avian influenza.

Year of implementation: 2010.

Reference of this document: File no. 2009-20-321-00026/BRB

Format used: Annex IV in Commission Decision 2008/425/EC

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## **2. Description of the surveillance programme in poultry**

### **2.1 Objectives, general requirements and criteria**

The objectives for the surveillance programme for avian influenza (AI) in poultry and game birds for restocking in 2010 are:

- Detecting sub-clinical infections with LPAI of subtypes H5 and H7 thereby complementing early detection systems and subsequently preventing possible mutations of these viruses to HPAI.
- Detecting infections of LPAI H5 and H7 subtypes in specifically targeted poultry populations at specific risk of infection due to their husbandry system or the susceptibility of specific species.
- Contributing to the demonstration of a free status in the frame of international trade according to OIE rules.

The programme will be implemented for the entire Denmark.

The National Veterinary Institute, Hangevej 2, DK-8200 Aarhus N, Denmark is the National Reference Laboratory (NRL) for the diagnosis of AI. The NRL performs all virological diagnostic analyses for AI in Denmark. Serological tests are performed at the National Veterinary Institute, Bülowsvej 27, DK-1790 Copenhagen V. Serological and virological tests will be performed according to methods required by the Diagnostic Manual (Decision 2006/437/EC).

Requirements and criteria stated in Commission Decision of 13 April 2007 (2007/268/EC) on the implementation of surveillance programmes for avian influenza in poultry and wild birds carried out in the Member States and amending Decision 2004/450/EC will be complied with.

All laboratory results from the survey in poultry and game birds for restocking will be transferred to a poultry database at the Danish Meat Association, where the results are recorded. The DVFA has on line access to the database. Positive results are also send by e-mail directly from the National Veterinary Institute to the DVFA.

### **2.2 Design and implementation**

Since 2006, the Danish surveillance programmes in poultry and game birds for restocking have been more extended and more intensified than previous programmes in relation to number of holdings included and test frequency.

Instead of testing only a part of poultry and game birds holdings in Denmark, all commercial holdings with more than 100 animals in target group are included and tested once or more

frequently. Breeder flocks are tested once a year, pullets before release to egg production, outdoors layers four times a year and outdoor slaughter poultry before slaughter. In addition, turkeys are tested before slaughter. In risk areas, breeder flocks and indoor layers are tested twice a year. Risk areas are defined as areas 3 km from the coastal line and around large lakes.

Herds of game birds for restocking are tested four times a year during the season from February to August. The first test is on blood samples from breeding animals and the three other tests are PCR test on offspring. The inclusion of game birds for restocking in the programme and including PCR-test of offspring, is considered to be very important, due to the fact that especially mallards are known to be asymptomatic carriers of avian influenza virus. The strategy with multiple samplings of game bird holdings has been justified as Denmark during the summer 2006 detected LP H5 (H5N2 and H5N3) on three locations in the last of the samplings from offspring.

Further, when poultry and game birds are traded, they had to be accompanied by a certificate stating that the herd had been tested within the preceding three months for poultry and two months for game birds.

The Danish AI surveillance strategy is planned to continue in 2010. However the DVFA are at the moment considering to amend the existing programme at some points. The considerations are to put emphasis on larger commercial holdings with fattening ducks and geese kept indoors but with a poor biosecurity and to reduce the sampling activity in a few other poultry categories, but still fulfil the criteria in Commission Decision 2007/268/EC. Also the use of PCR-tests instead in holdings with ducks and geese is under consideration.

Because the considerations at this moment not are finalised, this plan and application for poultry and game birds for restocking in 2010 contains the design from the present programme.

The application for financial contribution for the programme in poultry and game birds for restocking only includes the estimated total expenditure on eligible measures. That means that only the number of holdings to be sampled according to table 1 and table 2 in Commission Decision 2007/268/EC are stated in tables 2.2.1 and 2.2.2, rather than the total number of holdings, under here PCR-tests of samples from game birds offspring, to be tested according to the Danish AI surveillance programme.

However, we kindly ask the European Commission to reconsider the Communities co-financing policy on surveillance programmes for avian influenza in poultry and game for restocking to ensure co-financing on routine PCR tests also.

**Present routine surveillance in poultry and game birds for restocking:**

**Hens**

Risk areas are defined as: Land areas 3 kilometres from all coastal areas and inlets, around larger named lakes and along larger rivers in Denmark.

**Breeding flocks:**

In risk areas: 10 blood samples twice a year from each flock.

Outside risk areas: 10 blood samples once a year from each flock.

**Central-rearing and pullet rearing flocks:**

10 blood samples from each flock.

**Laying hens:**

Indoors flocks in risk areas: 10 blood samples twice a year from each flock.

Free range flocks: 10 blood samples four times a year from each flock.

**Free ranging broilers:**

10 blood samples four times a year from each holding.

**Turkeys**

**Breeding flocks:**

In risk areas: 10 blood samples twice a year from each flock.

Outside risk areas: 10 blood samples once a year from each flock.

At the moment there are no such flocks in Denmark.

**Fattening turkeys:**

10 blood samples from each flock.

**Ducks and geese**

**Breeding flocks:**

In risk areas: 45 blood samples twice a year from each flock.

Outside risk areas: 45 blood samples once a year from each flock.

**Free ranging fattening geese and ducks including mallards:**

45 blood samples four times a year from each holding.

### **Game birds for restocking**

#### **Mallards for restocking:**

Holdings are tested four times during the breeding season:

First test: 45 blood samples from breeding animals before egg-laying.

Second test: 10 killed, 2 weeks old ducklings from the first hatch.

Third test: 10 killed, 2 weeks old ducklings from the middle hatch.

Fourth test: 10 killed, 2 weeks old ducklings from the last hatch.

#### **Pheasants and partridges:**

Holdings are tested four times during the breeding season:

First test: 10 blood samples from breeding animals before egg-laying.

Second test: 10 killed, 2 weeks old chickens from the first hatch.

Third test: 10 killed, 2 weeks old chickens from the middle hatch.

Fourth test: 10 killed, 2 weeks old chickens from the last hatch.

### **Holdings with trade of poultry and game birds**

If the holding has not been involved in a regular quarterly sampling scheme, the holding has to be tested before sale.

From holdings with more than 100 animals at the time of trade:

1) From hens, turkeys, pheasants and partridges: 10 blood samples.

2) From geese and ducks including mallards: 45 blood samples.

Test results of these holdings are valid up to three months for hens, turkeys, ducks and geese and two months for pheasants, partridges and mallards.

**Table 1 - Poultry and game birds to be sampled and estimated expenditure according to the present routine surveillance programme**

<b>Poultry and game birds</b>	<b>Number of flocks/herds</b>	<b>Frequency</b>	<b>Number of samples</b>	<b>Number of samples in total</b>	<b>Laboratory examinations</b>	<b>Price of analysis DKK</b>
Breeders (flocks)						
- Chicken	489	1 / year	10	4.890	Serological	528.120
- Geese and ducks	23	1 / year	45	1.035	Serological	111.780
Free range laying hens (flocks)	121	4 / year	10	4.840	Serological	522.72
Free ranging fattening (herds)						
- Broilers	7	4 / year	10	280	Serological	30.240
- Geese and ducks	54	4 / year	45	9.720	Serological	1.049.760
Fattening turkeys	46	3 / year	10	1.380	Serological	149.040
Risk areas*						
- Laying hens	58	2 / year	10	1.160	Serological	125.280
- Breeding, chicken	49	2 / year	10	980	Serological	105.840
- Breeding, geese and ducks	2	2 / year	45	90	Serological	9.720
Game birds (hers)						
- Mallards	35	1 / year 3 / year	45 10	1.575 1.050x2 = 2.100 (210 pools)	Serological PCR	170.100 90.720
- Pheasants and partridges	213	1 / year 3 / year	10 10	2.130 6.390x2 = 12.780 (1.278 pools)	Serological PCR	230.040 552.096
<b>Total expenditure serology and PCR</b>					Serology: PCR:	3.032.640 DDK 642.816 DDK <b>3.675.456 DDK</b> <b>493.389,53 Euro</b>

\* It is assumed that risk areas covers 10% of Denmark and therefore 10% of the national population of the poultry production type will be concerned.

Holdings with trade of poultry and game birds for restocking are not included in the estimation of expenditure. That category will be added up in the individual categories in the final report for 2010.

Serological test: HI test for H5 and H7.

Virological test: Real-time-RT-PCR or RT-PCR-test.

Price for one H5 test: 54 DKK = 7,25 Euro

Price for one H7 test: 54 DKK = 7,25 Euro

10 blood samples (H5 and H7) in total for 10 birds: 1080 DDK = 144,98 Euro

45 blood samples (H5 and H7) in total for 45 birds: 4860 DDK = 652,40 Euro

10 chicken/duckling (PCR) in total for 10 birds:

2 x preparation of samples: 390 DDK

2 x PCR: 374 DDK

Handling of samples, tubes: 100 DDK

Total: 864 DDK = 115,98 Euro (price pr. pool: 432 DDK = 59,99 Euro)

Exchange rate 23 April 2009: 7,4494 Reference:

<http://www.ecb.int/stats/exchange/eurofxref/html/index.en.html>



**Table 2.2.1 POULTRY HOLDINGS<sup>(a)</sup> (except ducks and geese) TO BE SAMPLED**

**Serological investigation according to point B of Annex I to Commission Decision 2007/268/EC<sup>1</sup> on holdings of free ranging broilers**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

NUTS (2) code <sup>(b)</sup>	Total number of holdings <sup>(c)</sup>	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.
Denmark	7	7	10	70 HS - 70 HF - 140	H
Total	7	7	10	140	

(a) 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) 2 code can not be used, coordinates (long-lat) are requested

(c) Total number of holdings of one category of poultry in concerned NUTS 2 region

\* Data (no. of holdings) recorded in the poultry database.

**Table 2.2.1 POULTRY HOLDINGS<sup>(a)</sup> (except ducks and geese) TO BE SAMPLED**

**Serological investigation, according to point B of Annex I to Commission Decision 2007/268/EC<sup>1</sup> on holdings of fattening turkeys**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

NEUTS (2) code <sup>(b)</sup>	Total number of holdings <sup>(c)</sup>	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.
Denmark	46	46	10	460 H5 + 460 H7 + 920 H11	
Total	46	46	10	920	

(a) 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) 2 code can not be used, coordinates (long/lat) are requested.

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

• Data (no. of flocks) recorded in the poultry database.

**Table 2.2.1 POULTRY HOLDINGS<sup>(a)</sup> (except ducks and geese) TO BE SAMPLED**

**Serological investigation according to point B of Annex I to Commission Decision 2007/268/EC<sup>1</sup> on holdings of chicken breeders**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

NUTS (2) code <sup>(b)</sup>	Total number of holdings <sup>(c)</sup>	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.
Denmark	489	60	10	600 H5 + 600 H7 = 1200	H1
Total	489	60	10	1200	

(a) 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) 2 code can not be used, coordinates (long/lat) are requested.

(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.  
\* Data (no. of flocks) recorded in the poultry database.

**Table 2.2.1 POULTRY HOLDINGS <sup>(a)</sup> (except ducks and geese) TO BE SAMPLED**

**Serological investigation according to point B of Annex I to Commission Decision 2007/268/EC<sup>1</sup> on holdings of laying hens in risk areas**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

NUTS (2) code <sup>(b)</sup>	Total number of holdings <sup>(c)</sup>	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.
Denmark	58	42	10	420 H5 + 420 H7 = 840	HI
Total	58	42	10	840	

(a) 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) 2 code can not be used, coordinates (long/lat) are requested.

(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.  
Data (no. of flocks) recorded in the poultry database.

**Table 2.2.1 POULTRY HOLDINGS<sup>(a)</sup> (except ducks and geese) TO BE SAMPLED**

Serological investigation according to point B of Annex I to Commission Decision 2007/268/EC<sup>1</sup> on holdings of free range laying hens

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

NUTS (2) code <sup>(b)</sup>	Total number of holdings <sup>(c)</sup>	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.
Denmark	121	53	10	530 H5 · 530 H7 · 1060 H1	H1
Total	121	53	10	1060	

(a) 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) 2 code can not be used, coordinates (long/lat) are requested.

(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.  
 \* Data (no. of flocks) recorded in the poultry database.

**Table 2.2.1 POULTRY HOLDINGS <sup>(a)</sup> (except ducks and geese) TO BE SAMPLED**

**Serological investigation according to point B of Annex I to Commission Decision 2007/268**

**/EC<sup>1</sup> on holdings of farmed feathered game (pheasants, partridges, rock partridges, red-legged partridges)**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

NUTS (2) code <sup>(b)</sup>	Total number of holdings <sup>(c)</sup>	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.
Denmark	213	53	10	530 H5 - 530 H7 - 1060	HI
Total	213	53	10	1060	

(a) 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) 2 code can not be used, coordinates (long/lat) are requested.

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

\* Data (no. of holdings) recorded in the poultry database.

**Table 2.2.2 DUCK (incl. mallards) AND GEESE HOLDINGS TO BE SAMPLED<sup>(b)</sup> according to point C of Annex I to Decision 2007/268/EC**

<u>Serological investigation</u>						
NUTS (2) code <sup>(b)</sup>	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 to be performed per method	Methods of laboratory analysis.	
Denmark						
	Mallards: * 33	35	45	1575 H5 + 1575 H7 = 3150	HI	
	Ducks and geese - brooders**; 21	23	45	1035 H5 + 1035 H7 = 2070	HI	
	Free ranging fattening ducks *	43	45	1935 H5 + 1935 H7 = 3870	HI	
	Free ranging fattening geese*	11	45	495 H5 + 495 H7 = 990	HI	
Total		112	180	10080		

(a) 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) 2 code can not be used, coordinates (long./lat) are requested.

\* Data (no. of holdings) recorded in the poultry database.

\*\* Data (no. of flocks) recorded in the poultry database.

### **2.3 Laboratory testing; description of the laboratory tests used**

#### **Serological methods and examination**

Serological tests will be carried out using haemagglutination inhibition test (HI test) in accordance with the avian influenza diagnostic manual (Commission Decision 2006/437/EC). The antigens and control sera will be received from the Community Reference Laboratory. Using four HA units of antigen in the tests, sera with titres equal to or above 16 ( $4 \log_2$ ) will be considered positive.

The virus strains provided by the Community Reference Laboratory will be used as antigen in the initial test. Samples that are positive in tests with the initial antigen will be subjected to a further confirmatory test with the recommended strain for the specific H-subtype. A serum sample will be considered positive only if HI titres were equal to or above 16 with both antigens of the same subtype.

#### **Virological methods and examination**

The primary diagnostic procedures will be based on real-time RT-PCR or RT-PCR methods, but will also include virus isolation by inoculation in SPF embryonated eggs. The methods conform to the methods required by the avian influenza diagnostic manual (Commission Decision 2006/437/EC).

The specific RT-PCR analysis for general influenza A applied primers are specific to the viral matrix (M) gene. The H5 and H7 specific analyses apply primers, which only detects the viral haemagglutinin (HA) gene of the H5 and H7 subtypes, respectively.

#### **Surveillance of game birds (offspring):**

Analyses for general influenza virus (M-gene) RRT/ RT-PCR detection are performed on all samples received for influenza diagnosis.

- 1) If a sample is tested positive in general supplementary H5 and H7 analysis are performed.
- 2) If a sample is detected positive for H5 or H7 the RT-PCR product is sequenced for confirmation of the H5 or H7 subtype and for characterisation of the virus in terms of pathogenicity.
- 3) If a sample is positive by the M-gene RT-PCR the sample is inoculated in SPF embryonated eggs.

#### **Samples from poultry, suspected infected with subtype H5 or H7 on the basis of serological test results are tested with:**

- 1) M specific PCR in combination with H5 or H7 specific PCR.
- 2) If a sample is detected positive for H5 or H7 the RT-PCR product is sequenced for confirmation of the H5 or H7 subtype and for characterisation of the virus in terms of pathogenicity.



Virus cultivation utilise 8-10 days old embryonated SPF eggs, which are inoculated by the allantoic route. The eggs are incubated for one week and the harvest of allantoic fluid is tested for presence of haemagglutinating viruses. Agglutinating viruses are H-typed by HI test. In addition, identification RT-PCR and sequencing is carried out in accordance with the above description.

A final characterisation of a virus isolate is done by conventional neuraminidase test (N-typing). In addition, a N-1 specific RT-PCR method may be applied to samples collected either directly from sick or dead birds or harvested from inoculated SPF embryonated eggs.

### **3. Description of the surveillance programme in wild birds**

#### **3.1 Objectives, general requirements and criteria**

- A) Surveillance of HP AIV H5N1 in wild birds in Denmark.
- B) Surveillance of the presence of LP and HPAI AIV in host species with 'higher risk'.
- C) Surveillance of the presence of LP and HP AIV in host species living in proximity to domestic poultry.
- D) Examination of hunted game birds.

Tracheal/oropharyngeal swabs and/or tissues from wild birds found dead (objective A) will be sampled for virus isolation and molecular detection (PCR).

Oropharyngeal and cloacal swabs for virological examination will be taken from apparently healthy free living birds (objective B and C) and from hunted game birds (objective D).

Testing will be carried out at the National Reference Laboratory, Aarhus.

#### **3.2 Design and implementation**

##### **Objective A Surveillance of HP AIV H5N1 in wild birds**

The aim is to perform laboratory investigation of AI on birds, which have died of natural causes in Denmark. The examinations are aimed at populations, where an unusually high mortality occurs. The focus will especially be on water related species and game birds in areas near the coast, with the presence of unusual deaths (number, species, special circumstances). A cooperation have been established with the Danish Forest and Nature Agency, the National Environmental Research Institute, the Danish Hunters Association, the Danish Fishing Association, the Danish Ornithological Society and the regional veterinary and food administration centres in order to enhance the alertness among the general public.

##### **Objective B Surveillance of host species with 'higher risk'**

In relation to the autumn flyways, Denmark is the first important rallying ground on the migratory route for water birds breeding in the neighbouring countries. The collection will embrace species breeding in Scandinavia including Denmark, Russia and The Baltic countries in September to December in the Danish part of the Wadden Sea.

Ornithological experts will collect the samples.

Besides, samples from hunted ducks, collected in collaboration with Danish Hunters Association will be included in this category.

**Objective C Surveillance of host species living in proximity to domestic poultry**

These samples will be collected in relation to the normal ringing procedure of, among others, migratory birds at the ringing stations. The collection of samples will be coordinated by Zoological Museum at the University of Copenhagen. The focus will be on birds, which have a high probability of contact with domestic poultry. The samples will be collected over the year.

**Objective D Examination of killed game birds**

Every year a large number of mallards and pheasants are bred for and released in nature and subsequently used for hunting. Samples will be collected from game birds in the period 1 September to 20 December at an authorised game slaughterhouse. The focus will be on game birds from large manor huntings, which are expected to comprise mainly mallards bred for release in nature.

The surveillance programme can be adjusted, if the epidemiological situation changes.

**Table 3.2.1 WILD BIRDS - investigation according to the surveillance programme for avian influenza in wild birds set out in Annex II to Decision 2007/268/EC**

NUTS (2) code/region <sup>(a)</sup>	Wild birds to be sampled <sup>(b)</sup>	Total number of birds to be sampled	Total number of samples to be taken for active surveillance	Total number of samples to be taken for passive surveillance
DK	Objective A, wild birds died naturally	200		200
DK	Objective B, live birds from bird sanctuaries	1.500		
DK	Objective C, live birds living in proximity to domestic poultry	500	2300x2= 4600	
DK	Objective D, hunted game birds	300		
Total		2.500	4600	200

(a) Refers to the place of collection of birds/samples. In case Nuts 2 code can not be used, region as defined in the programme by the Member State

(b) General description of the wild birds are intended to be sampled in the framework of the active and passive surveillance.

### **3.3 Laboratory testing: description of the laboratory tests used**

The primary diagnostic procedures will be based on real-time-RT-PCR or RT-PCR methods, but will also include virus isolation by inoculation in SPF embryonated eggs. The methods conform to the methods required by with the avian influenza diagnostic manual (Commission Decision 2006/437/EC).

The specific RT-PCR analysis for general influenza A applied primers are specific to the viral matrix (M) gene. The H5 and H7 specific analyses apply primers, which only detects the viral haemagglutinin (HA) gene of the H5 and H7 subtypes, respectively.

#### **Samples from healthy wild birds:**

- 1) Samples from healthy wild birds are tested by M specific PCR.
- 2) Any positive samples are tested by H5- and H7 specific PCR.
- 3) Samples positive by M will be inoculated in SPF embryos.
- 4) If a sample is detected positive for H5 or H7 the RT-PCR product is sequenced for confirmation of the H5 or H7 subtype and for characterisation of the virus in terms of pathogenicity.
- 5) Samples giving doubtful results will be inoculated in SPF embryos.

#### **Samples from dead wild birds:**

Analyses for general influenza virus (M-gene) and specific H5 subtype RT-PCR detection are performed on all samples received for influenza diagnosis.

- 1) If a sample is tested positive in general and negative for H5, a supplementary H7 analysis is performed.
- 2) If a sample is detected positive for H5 or H7 the RT-PCR product is sequenced for confirmation of the H5 or H7 subtype and for characterisation of the virus in terms of pathogenicity.
- 3) If a sample is positive by the M-gene RT-PCR the sample is inoculated in SPF embryonated eggs.

Virus cultivation utilise 8-10 days old embryonated SPF eggs, which are inoculated by the allantoic route. The eggs are incubated for one week and the harvest of allantoic fluid is tested for presence of haemagglutinating viruses. Agglutinating viruses are H-typed by HI test. In addition, identification of RT-PCR and sequencing is carried out in accordance with the above description.

A final characterisation of a virus isolate is done by conventional neuraminidase test (N-typing). In addition, a N-1 specific RT-PCR method may be applied to samples collected either directly from sick or dead birds or harvested from inoculated SPF embryonated eggs.

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

A comprehensive screening programme for AI in poultry was established in 2003. The screening programme included samples from the major poultry types in Denmark, i.e. fattening turkey, chicken breeders, broilers, layers and ducks. The screening programme in poultry was only slightly revised in 2004, where the two changes were, that only free ranging broilers were sampled, and ducks were examined serologically. All test results from the screening programme for AI virus in poultry were negative.

In addition to the results from the serological surveillance programme in 2003, the presence of LPAI was detected on 4 September 2003 in a holding with mallard ducks, which was examined due to elevated mortality. The presence of LPAI subtype H5N7 was detected by virus isolation during the laboratory diagnostic examination of the holding. There were 11,000 mallard ducks on the holding, which were raised for restocking for hunting purpose. The DVFA decided for precautionary reasons to cull and destroy the affected flock. The epidemiological investigations supported the assumption that the clinical signs found in the flock of mallards was not caused by the LPAI virus strain. Isolation of the H5N7 strain was considered to be a coincidental event.

The screening programme in poultry was only slightly revised in 2005, when the main change was that all holdings with free range laying hens were examined. All test results from the screening programme for AI virus in poultry were negative.

In 2006, the surveillance of AI in poultry was extended and more intensified than previous programmes. Besides the surveillance programme for poultry, the programmes included game birds for restocking and holdings (with more than 100 animals) trading poultry or game birds. Holdings situated in appointed risk areas were tested more frequently than holdings outside risk areas.

In 2006, one outbreak of HPAI H5N1 was reported in a back-yard flock with clinically diseased and dead birds. The outbreak of HPAI was the first outbreak in poultry ever recorded in Denmark. The virus was of the same type as the one that had previously been detected in wild birds in the area. It was concluded, that the source of infection most likely was wild birds, possibly transmitted by direct contact. However, no direct evidence exists for this hypothesis. This outbreak did not cause any secondary outbreaks, and reoccurrence of HPAI H5N1 in poultry has not been observed since then.

Due to the extended and more intensified surveillance programme, detection of LPAI was done in three game bird holdings (one H5N2 and two H5N3) in 2006. These three holdings with mallards for restocking were all culled due to the detection of LPAI H5. In two of these holdings, the infection was thought to originate from contact with wild birds. In the third holding, the infection most likely originated from indirect contact with one of the other in-

ected holdings. There were no clinical signs in any of these holdings. In 2006, ten flocks or holdings were found to have single or more serologic positive samples. The holdings were managed as holdings under suspicion for avian influenza. However, testing with PCR showed no circulating virus in any of these holdings.

The extended and more intensified surveillance programme, which started in 2006, continued in 2007 and 2008.

In 2007, eight flocks or holdings were found to have single or more serologic positive samples. The holdings were managed as holdings under suspicion for avian influenza. However, testing with PCR showed no circulating virus in any of these holdings.

In 2008, seven holdings were found to have single or more serologic positive samples. The holdings were managed as holdings under suspicion for avian influenza. The holdings were investigated and samples for virological examination were taken. One holding was found positive by virological tests. The holding had breeding geese, ducks and mallards. There had been a serological reaction against H5 in the breeding geese. Following an investigation of tracheal and cloacal swabs low pathogenic H7N1 was detected by PCR followed by sequencing in cloacal swabs from the domestic ducks. The most likely source of infection is introduction by wild birds as low pathogenic avian influenza H7N1, with sequence identical with isolates from the domestic ducks, were detected in wild mallards in a nearby lake.

#### **4.1 Measures included in the programme for surveillance in poultry**

##### **4.1.1 Designation of the central authority charged with supervising and coordination the departments responsible for implementing the programme**

The DVFA carry out the programme assisted by its 3 regional offices<sup>1</sup>. The central coordination activities at the DVFA are placed in the Animal Health Division in collaboration with the 3 regions, The National Veterinary Institute and Danish Meat Association.

##### **4.1.2 System in place for the registration of holdings**

Commercial holdings with poultry like holdings with cattle, pigs, sheep, goats, and commercial holdings with deer, foxes, minks and fish are recorded in a central database, called the Central Husbandry Register (CHR), which is owned by the Ministry of Food, Agriculture and Fisheries. The rules for registration are laid down in Order No. 815 of 14 July 2006 on registration of animal husbandry in the CHR. The CHR stores information on the unique holding code, the address and the geographic coordinates of the holding, data on the farmer, number of animals of all species and veterinary information. Commercial poultry farmers are obliged to register their holding in the CHR. Likewise poultry farmers are obliged to report if the holding is closed down. It is voluntary for owners of backyard flocks to register their holding

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<sup>1</sup> The regional veterinary and food administration centre, Region North, South and East

in CHR. However, if outbreaks of HP AI occur in wild birds or in a poultry holding, then it is also mandatory for owners of backyards flocks in zones to register their holding in CHR.

#### 4.1.3 Data on vaccination

In Denmark it is prohibited to vaccinate against avian influenza except susceptible birds kept in zoos, which can be vaccinated according to Commission Decision 2007/598/EC concerning measures to prevent the spread of highly pathogenic avian influenza to other captive birds kept in zoos and approved bodies, institutes or centres in the Member States.

However no birds in zoological gardens are included in the programme for poultry surveillance. Zoological gardens are regarded as permanent quarantines, where birds are kept isolated from other poultry and captive birds in Denmark.

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

A comprehensive screening programme for AI in wild birds was established in 2003, with a wider covering in both time and space than previous. Bird droppings were sampled in both spring/summer (March to June) and autumn/winter (September to December), and were sampled from 20 locations. The bird droppings were collected from cormorant, lapwing, Bewick's swan, mallard, widgeon, teal and six different geese species. AI virus was detected by RT-PCR in 34 of 579 pools and could be isolated in 15 pools. The AI virus was mainly found in the duck species in the autumn months. Neither H5 nor H7 subtypes of AI virus could be isolated, but LP H5 and H7 subtypes were detected with RT-PCR. The following AI virus subtypes were isolated in the 15 pools: H1N1, H3N2, H3N6, H3N8, H4N6, H6N5, H6N8 and H10N7.

The screening programme for AI in wild birds was slightly revised in 2004, when the bird droppings were sampled in September to December from 16 locations. The bird droppings were only collected from mallard, widgeon, teal and pintail. AI virus was detected by RT-PCR in 131 of 696 pools and 65 individual samples and could be isolated in 14 samples. LPAI virus subtype H5N2 was isolated from two pools, and LPAI virus subtype H5 could be detected with RT-PCR in 13 pools. The H7 subtype was neither isolated nor detected with RT-PCR. The following AI virus subtypes have been isolated in the 15 pools: H2N3, H3N2, H3N8, H5N2 (LP), H6N2, H8N1 and H8N4.

In the screening programme for AI in wild birds in 2005, bird droppings were sampled in August to December from two locations. The bird droppings were collected from mallard, teal, widgeon, goose and pheasant. The samples were pooled in pools of five samples and tested by both virus isolation and RT-PCR. AI virus was detected by RT-PCR in 140 of 558 pools and could be isolated in 16 samples. LPAI virus subtype H5 or H7 was isolated from three pools, and LP AI virus subtype H5 or H7 could be detected with RT-PCR in 27 pools. All the H5 and H7 positive findings were from mallards. The following avian influenza

virus subtypes isolated in the 16 pools were: H1N1, H1N9, H3N8, H4N6, H5Nx (LP), H7N5 (LP), H7N7 (LP), H9N1 and H11N9.

The survey programme for AI in wild birds in 2006 consisted of a passive surveillance for AI in wild birds found dead and an active surveillance in waterfowl reservoirs and along migratory flyways. In January and the beginning of February 2006, only very few dead birds (passive surveillance) were sent in for examination, but after the infection spread among wild birds in Europe from the middle of February, this changed considerably. The number of birds examined was highest in March falling to a lower level in May and June. This was due to both increased awareness towards AI among the general public and the DVFA's call via the media for sending in dead birds. Many birds died during the winter, which was colder and lasted longer than normally. The first finding of HPAI H5 in wild birds in Denmark was confirmed on 14 March 2006. Subsequently, it was shown to be of the type H5N1, which had been found in large parts of Europe. In total, 44 wild birds were found infected with HPAI H5N1 in six counties in March-May of 2006. The last case was confirmed on 29 May. In total, 1190 dead birds were examined in 2006. The dead wild birds were sent in from the whole country but the positive findings were concentrated in the southeastern part of Denmark especially along the Baltic Sea.

In 2006, 5512 samples of bird dropping (active surveillance) were analysed in 1102 pools by PCR. All samples, except one pool with samples from five herring gulls, gave negative test results for HPAI H5N1. Other avian influenza virus subtypes isolated in the active surveillance: Two H2N3 (LP), one H13N6 (LP) and one H14N5 (LP).

The surveillance programme for AI in wild birds in 2007 consisted of a passive surveillance in wild birds found dead and an active surveillance in waterfowl reservoirs and along migratory flyways, birds living in proximity to domestic poultry and surveillance of hunted game birds. In 2007, 212 dead wild birds were tested, and only one of these were found positive for other LPAI than H5 or H7. In the active surveillance, 4054 live wild birds were tested. Of these, 414 birds were living in proximity to domestic poultry and 3640 were birds from "high risk" species. Finally, 758 hunted game birds, ducks and pheasants, were tested. All samples were negative for HP H5N1. The following low pathogenic avian influenza virus subtypes were isolated: H3N6 (one sample), H3N8 (two samples), H4N6 (two samples), H6N1 (one sample), H6N2 (one sample), H10N3 (one sample), H11N2 (two samples), H11N9 (one sample), H14N5 (one sample). By PCR AIV was detected in 48 samples, 8 of which were LP H5 and 2 were LP H7.

The surveillance programme for avian influenza in wild birds in 2008 consisted of a passive surveillance in wild birds found dead or sick and an active surveillance in waterfowl reservoirs and along migratory flyways, birds living in proximity to domestic poultry and surveillance of hunted game birds. In 2008, 70 dead wild birds were tested. All birds were found negative for HPAI H5N1. In total, 629 birds living in proximity to domestic poultry were tested. Nine of these birds were found positive for LPAI. Two was H5 and seven were found positive for other than H5 or H7. In total, 297 were birds from "high risk" species. 19 of these



birds were found positive for LPAI. Two were H5, one was H7N1 and 16 were found positive for other than H5 or H7. Of this 16 one was H11N9, one was H12N5 and one was HxN8.

In total, 280 hunted game birds, mainly mallards, were tested. 38 of these birds were found positive for LPAI. Two was H5. One was H7 and 35 were found positive for other than H5 or H7. Of these 35 one was H1N2.

Additionally, extraordinary surveys were established due to the outbreak of LP H7N1 in a holding with breeding geese, domestic ducks and mallards. In a nearby lake 24 wild mallards were hunted and tested for AI. LP H7N1, with a sequence identical with the isolates from the domestic ducks in the holding, was detected. Further, in 33 localities around the infected holding samples from in total 10 shell ducks and 46 wild mallards were tested. All those 56 birds were negative for AIV.

## **5.1. Measures included in the programme for wild birds surveillance**

### **5.1.1 Designation of the central authority charged with supervising and coordination the departments responsible for implementing the programme**

The DVFA is the central authority responsible for implementing the programme. The Regional Veterinary and Food Administration Centres, the National Veterinary Institute, the National Environmental Research Institute, the Zoological Museum at the University of Copenhagen and the Danish Emergency Management Agency are each responsible for parts of the programme.

### **5.1.2 Description and delimitation of the geographical and administrative areas in which the programme is to be applied**

The programme shall cover the whole country. However, the focus will be on localities near the coast and areas where the concentration of resting water birds is highest.

### 5.1.3 Estimation of the local and/or migratory wildlife population

Table showing the local and migratory wildlife population in Denmark

Species	Total population size
Widgeon	150000
Teal	50000
Mallard	450000
Pintail	60000
Shoveler	40000
Tufted Duck	120000
Goldeneye	100000-130000
Common Pochard	35000
Common Eider	76000
Greater Scaup	31000
Great Cormorant	7500 <sup>a</sup>
Whooper Swan	59000
Mute Swan	250000
Bewick's Swan	20000
Taiga Bean Goose	70000-90000
Pink-footed Goose	42000
Greater White-fronted Goose	1000000
Greylag Goose	500000
Barnacle Goose	420000
Light-bellied Brent Goose	7000
Dark-bellied Brent Goose	200000
Lapwing	5100000-8400000
Golden Plover	140000-210000
Great Black-backed Gull	180000
Herring Gull	1700000-3600000 <sup>b</sup>
Common Black-headed Gull	3700000-4800000
Common Gull	1200000-2250000

<sup>a</sup> Subspecies *sinensis* breeders in Denmark

<sup>b</sup> Subspecies *argentatus* breeding/wintering i NW-Europe

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

All suspicions of AI including poultry showing clinical symptoms of the disease must be reported to the veterinary authorities as laid down in Order No. 693 of 21 June 2007 and Order No. 943 of 14 September 2006 with later amendments. Seropositive holdings will be managed as holdings under suspicion for avian influenza. Holdings will be investigated and samples for examination will be taken. The local Animal Health Unit in the regional veterinary and food administration centres will impose movement restrictions on the farm and on possible contact farms.

Rules for compensation of farmers with animals, that have to be killed due to infection with AI subtype H5 or H7, are laid down in Order No. 239 of 12 April 1991 concerning expenses and compensation related to eradication and prevention of animal diseases as amended by Order No. 812 of 29 October 1999. The animals are compensated at the market value and the DVFA cover 20% of the estimated loss of profits.

In case the general public find dead birds in nature they have to contact the local Animal Health Unit in the regional veterinary and food administration centres. If AI is suspected the birds are under appropriate safety measures collected by personnel from the Danish Emergency management agency and brought to The National Veterinary Institute for virological examination. The wild birds shall be collected on the same day or if notified after 1 pm on the following day.

## **7. Costs**

### **7.1. Detailed analyses of the costs**

#### 7.1.1 Poultry

##### **Survey in poultry and game birds for restocking - estimation of expenditure:**

Serological test: HI test for H5 and H7.

Price for laboratory examinations:

Price for one H5 test: 54 DKK = 7,25 Euro

Price for one H7 test: 54 DKK = 7,25 Euro

10 blood samples (H5 and H7) in total for 10 birds: 1080 DKK = 144,98 Euro

45 blood samples (H5 and H7) in total for 45 birds: 4860 DKK = 652,40 Euro

Exchange rate 23 April 2009: 7,4494 Reference:

<http://www.ecb.int/stats/exchange/eurofxref/html/index.en.html>

#### 7.1.2 Wild birds

##### **Passive surveillance in wild birds**

Tracheal swabs from each bird will be tested.

Estimated number of birds to be tested: 200

Total number of PCR tests: 200

##### **Active surveillance in wild birds**

Cloacal and tracheal swabs from each bird will be tested individually.

Estimated number of birds to be tested from bird sanctuaries: 1500

Estimated number of birds to be tested from surveillance on birds living in proximity to domestic poultry: 500.

Estimated number of hunted game birds from authorised game slaughterhouse to be tested: 300.

The above mentioned number of birds to sampled from the different categories in the active surveillance will finally be laid down in the beginning of 2010 when the contracts between the DVFA, the National Veterinary Institute, the National Environmental Research Institute and the Zoological Museum at the University of Copenhagen are signed.

**Total number of PCR tests**

Total number of birds tested: Up to 2500.

200 birds will be tested once (tracheal swabs) and 2300 birds will be tested twice (cloacal and tracheal swabs). Total PCR tests:  $200+2300+2300 = 4800$ .

**Costs of sampling wild birds**

The costs for sampling cloacal and tracheal swabs from 2000 birds (1500 birds from bird sanctuaries and 500 birds living in proximity to domestic poultry) are estimated to: 871.000 DKK (436 DDK per bird).

The costs for collecting 200 dead or sick wild birds are estimated to: 202.125 DKK (1.011 DDK per bird).

**Total estimation of expenditure passive and active surveillance in wild birds**

The estimated total costs for PCR test and virus isolation: 1.545.536 DKK = 207.471,20 Euro.

The estimated costs for sampling cloacal and tracheal swabs: 871.000 DKK = 116.922,16 Euro.

The estimated costs for collecting dead or sick wild birds: 202.125 DKK = 27.133,06 Euro.

Exchange rate 23 April 2009: 7,4494    Reference:  
<http://www.ecb.int/stats/exchange/eurofxref/html/index.en.html>

## 7.2 Summary of the costs

### 7.2.1 Poultry surveillance

Measures eligible for co-financing surveillance in poultry			
Methods of laboratory analysis	Number of tests to perform per method	Unitary test cost (per method)	Total cost
Serological pre-screening <sup>1</sup>			
Haemagglutination-inhibition-test (HI) for H5/H7 <sup>2</sup>	H5 = 7.650 H7 = 7.650	54 DKK = 7,25 Euro 54 DKK = 7,25 Euro	826.200 DKK = 110.908,26 Euro
Virus isolation test	16	965 DDK = 129,54 Euro	15.440 DDK = 2.072,65 Euro
PCR test	320 (64 pools)*	171,2 DDK = 22,98 Euro	54.784 DDK = 7.354,15 Euro
Other measures to be covered	Specify activities		
Sampling			
Others			
<b>Total</b>			<b>896.424 DKK</b> <b>120.335,06 Euro</b>

<sup>1</sup> Specify the laboratory test to be used

<sup>2</sup> Specify number of tests for H5 and for H7

\* Minimum confirmatory PCR-tests: From a positive serological holding 6 pools from 40 animals will be tested. Estimated 8 positive holdings in 2010, 8 holdings x 8 pools = 64 pools. Neighbour PCR-tests: 64 pools x 5 = 320 PCR tests.

Preparation of samples: 64x195,-

PCR tests (M, H5, H7): 64x3x187,-

Handling of samples, tubes: 64x100,-

Total:

12.480 DDK

35.904 DDK

6.400 DDK

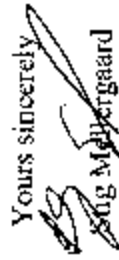
54.784 DDK

7.2.2 Wild bird surveillance

Measures eligible for co-financing surveillance wild birds			
Methods of laboratory analysis	Number tests to perform per method	Unitary test cost (per method)	Total cost
Serological pre-screening			
Haemagglutination-inhibition-test (HI) for H5/H7			
Virus isolation test	70	965 DDK = 129,54 Euro	1.545.536 DDK
PCR test	4800		207.471,20 Euro
<b>Other measures to be covered</b>	<b>Specify activities</b>		
Sampling	Sampling 2000 wild live birds		Active survey: 871.000 DDK 116.922,16 Euro
Others	Sampling 200 dead or sick wild birds		Passive survey: 202.125 DDK 27.133,06 Euro
<b>Total</b>			<b>2.618.661 DDK = 351.526,43 Euro</b>

Exchange rate 23 April 2009: 7,494, Reference: <http://www.ecb.int/stats/exchange/eurofxref/html/index.en.html>

Yours sincerely

  
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