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

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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 2009 by Commission Decision 2008/897/EC

# Denmark

* in accordance with Commission Decision 90/424/EEC

**Ministry of Food, Agriculture and Fisheries**
Danish Veterinary and Food Administration

European Commission
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ANIMAL HEALTH DIVISION

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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 2009 and application for financial contribution**

**1. Identification of the programme**
Member State: **Denmark.**

Disease: **Avian influenza.**

Year of implementation: **2009.**

Reference of this document: **File no. 2008-20-221-02896/BRB.**

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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 in poultry and game birds for restocking in 2009 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, Hangoevej 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.

### 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 the holdings in Denmark and test the targeted holdings only once per year, all commercial holdings in a target group are included and tested frequently.

The surveillance programme in 2009 will, similarly to the programme in 2008, be based on routine samples from hens, turkeys, ducks and geese, game birds for restocking (mallards, pheasants and partridges) from holdings with more than 100 animals, and holdings with more than 100 animals trading poultry or game birds.

Holdings situated in appointed risk areas will be tested more frequently than holdings outside risk areas. A risk area is defined as land areas 3 kilometres from all costal areas and inlets, around larger named lakes and along larger named rivers in Denmark.

The DVFA consider the inclusion of game birds for restocking in the programmes to be very important, due to the fact that especially mallards are known to be asymptomatic carriers of avian influenza virus. Evaluation of the data from the Danish programme in 2006 indicates that game bird holdings, which are tested 4 times during the breeding season, may be tested negative for LPAI subtype H5 or H7 in the first serological test (which is performed on breeding animals) but may test positive in one of the following routine tests in offspring (offspring are tested with PCR). 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 four samplings. All outbreaks were stamped out.

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

All laboratory results from the survey in poultry and game birds 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.

The application for financial contribution for the surveillance programme in poultry and game birds for restocking in 2009 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 to be tested according to the Danish AI surveillance programme.

**The routine surveillance in poultry and game birds for restocking will consist of:**

## Hens

Risk areas are defined as: Land areas 3 kilometres from all costal 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 batch.
Third test: 10 killed, 2 weeks old ducklings from the second batch.
Fourth test: 10 killed, 2 weeks old ducklings from the last batch.

**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 batch.
Third test: 10 killed, 2 weeks old chickens from the second batch.
Fourth test: 10 killed, 2 weeks old chickens from the last batch.

## 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.

**Poultry and game birds to be sampled and estimated expenditure according to the extended Danish routine surveillance**

| Poultry and game birds | Number of flocks/ herds | Frequency | Number of samples | Number of samples in total | Laboratory examinations | Price of analysis DKK |
|---|---|---|---|---|---|---|
| Breeders (flocks) | | | | | | |
| - Chicken | 438 | 1 / year | 10 | 4380 | Serological | 411.720 |
| - Geese and ducks | 19 | 1 / year | 45 | 855 | Serological | 80.370 |
| Free range laying hens (flocks) | 128 | 4 / year | 10 | 5120 | Serological | 481.280 |
| Free ranging fattening (herds) | | | | | | |
| - Broilers | 14 | 4 / year | 10 | 560 | Serological | 52.640 |
| - Geese and ducks | 54 | 4 / year | 45 | 9.720 | Serological | 913.680 |
| Fattening turkeys | 120 | 3 / year | 10 | 3600 | Serological | 33.840 |
| Risk areas* | | | | | | |
| - Laying hens | 58 | 2 / year | 10 | 1160 | Serological | 109.040 |
| - Breeding, chicken | 49 | 2 / year | 10 | 980 | Serological | 9.212 |
| - Breeding, geese and ducks | 2 | 2 / year | 45 | 90 | Serological | 8.460 |
| Game birds (hers) | | | | | | |
| - Mallards | 33 | 1 / year | 45 | 1.785 | Serological | 139.590 |
| | | 3 / year | 10 | 990 (198 pools) | PCR | 92.565 |
| - Pheasants and partridges | 219 | 1 / year | 10 | 2190 | Serological | 205.860 |
| | | 3 / year | 10 | 6.570 (1314pools) | PCR | 614.295 |
| **Expenditure** | | | | | | |
| Serology: 2.445.692 | | | | | | |
| PCR: 706.860 | | | | | | |
| **Total** | | | | | | **3.152.552** |

* 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.

Seroligical test: HI test for H5 and H7.
Virological test: RT-PCR-test.

10 blood samples (H5 and H7) in total for 10 birds: 940 DKK.
45 blood samples (H5 and H7) in total for 45 birds: 4230 DKK.
10 chicken/duckling (PCR) in total for 10 birds: 935 (price pr. pool: 467,50 DKK)

**Table 2.2.1 POULTRY HOLDINGS [a] (except ducks and geese) TO BE SAMPLED**

**Serological investigation according to point B of Annex I* to Commission Decision 2007/268/EC[48] on holdings of free ranging broilers**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

| NUTS (2) code[b] | Total number of holdings[c]* | 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 | 14 | 14 | 10 | 140 H5 + 140 H7 = 280 | HI |
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| | | | | | |
| Total | 14 | 14 | 10 | 280 | |

(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.

[48] O J L 115,3.5.2007, p. 3.

## Table 2.2.1 POULTRY HOLDINGS [a] (except ducks and geese) TO BE SAMPLED

**Serological investigation according to point B of Annex I* to Commission Decision 2007/268/EC[48] on holdings of fattening turkeys**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

| NUTS (2) code[b] | Total number of holdings[c]* | 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 | 120 | 80 | 10 | 800 H5 + 800 H7 = 1600 | HI |
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| | | | | | |
| Total | 120 | 80 | 10 | 1600 | |

(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.

[48] O J L 115,3.5.2007, p. 3.

**Table 2.2.1 POULTRY HOLDINGS [a] (except ducks and geese) TO BE SAMPLED**

**Serological investigation according to point B of Annex I* to Commission Decision 2007/268/EC[48] on holdings of chicken breeders**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

| NUTS (2) code[b] | Total number of holdings[c]* | 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 | 487 | 60 | 10 | 600 H5 + 600 H7 = 1200 | III |
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| | | | | | |
| Total | 487 | 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.

[48] O J L 115,3,5 2007, p. 3

**Table 2.2.1 POULTRY HOLDINGS[a] (except ducks and geese) TO BE SAMPLED**

**Serological investigation according to point B of Annex I* to Commission Decision 2007/268/EC[45] on holdings of laying hens in risk areas**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

| NUTS (2) code[b] | Total number of holdings[c]* | 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 |
| | | | | | |
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| | | | | | |
| | | | | | |
| | | | | | |
| 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.

[45] O J L 115,3.5.2007, p. 3.

## Table 2.2.1 POULTRY HOLDINGS [a] (except ducks and geese) TO BE SAMPLED

**Serological investigation according to point B of Annex I* to Commission Decision 2007/268/EC[48] on holdings of free range laying hens**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

| NUTS (2) code[b] | Total number of holdings[c]* | 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 | 128 | 53 | 10 | 530 H5 + 530 H7 = 1060 | HI |
| | | | | | |
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| | | | | | |
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| | | | | | |
| | | | | | |
| Total | 128 | 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.

[48] O J L. 115,3.5.2007, p. 3.

**Table 2.2.1 POULTRY HOLDINGS [a] (except ducks and geese) TO BE SAMPLED**

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

**/EC[48] on holdings of farmed feathered game (pheasants, partridges, rock partridges, redlegged partridges)**

**PLEASE USE ONE FORM PER POULTRY CATEGORY**

| NUTS (2) code[b] | Total number of holdings[c]* | 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 | 219 | 53 | 10 | 530 H5 + 530 H7 = 1060 | HI |
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| | | | | | |
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| | | | | | |
| Total | 219 | 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.

[48] O J L 115,3 5.2007, p. 3.

## Table 2.2.2 DUCK (incl. mallards) AND GEESE HOLDINGS TO BE SAMPLED*[a] according to point C of Annex I to Decision 2007/268/EC

### Serological investigation

| NUTS (2) code[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 to be performed per method | Methods of laboratory analysis. |
|---|---|---|---|---|---|
| Denmark | | | | | |
| | Mallards: * 33 | 33 | 45 | 1485 H5 + 1485 H7 = 2970 | HI |
| | | | | | |
| | Ducks and geese - breeders**: 21 | 21 | 45 | 945 H5 + 945 H7 = 1890 | 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 | | 108 | 180 | 9720 | |

(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 log2) 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 RT-PCR methods, but will also include virus isolation by inoculation in SPF embryonated eggs. The RT-PCR protocols applied at the Danish Veterinary Institute have been tested as a part of an EU-project (AVIFLU) and they are subsequently recommended by the EU-reference laboratory, Weybridge, UK. The methods conform to the methods required by the avian influenza diagnostic manual (Commimission Decision 2006/437/EC).

The specific RT-PCR analysis for general influenza A applied primers are specific to the viral matrix (M) gene or the nucleoprotein (NP) 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) 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.

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 either H5 or H7 specific PCR, depending on the serological result.
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 subtyped 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 prevalence of LP and HP AIV in host species with 'higher risk'
C) Surveillance of the prevalence 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 for avian influenza in Denmark.

### 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 Asso-

ciation, 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.

**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. Their possible risk of occurrence and outbreaks of AI are controversial and should be investigated closer. With a view to this, samples are 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 with the programme for surveillance of avian influenza in wild birds set out in Annex II to Decision 2007/268/EC**

| NUTS (2) code/region[a] | Wild birds to be sampled [b] | 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 | | | 120 |
| DK | Objective B, live birds from bird sanctuaries | | 1500 | |
| DK | Objective C, live birds living in proximity to domestic poultry | | 500 | |
| DK | Objective D, hunted game birds | | 300 | |
| | | | | |
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| | | | | |
| Total | | | 2300 | 120 |

(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 RT-PCR methods, but will also include virus isolation by inoculation in SPF embryonated eggs. The RT-PCR protocols applied at the Danish Veterinary Institute have been tested as a part of an EU-project (AVIFLU) and they are subsequently recommend by the EU-reference laboratory, Weybridge, UK. The methods conform to the methods required by with the avian influenza diagnoatic manual (Commimission Decision 2006/437/EC).

The specific RT-PCR analysis for general influenza A applied primers are specific to the viral matrix (M) gene or the nucleoprotein (NP) 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 NP 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 subtyped 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 the disease has not been observed since then.

Due to the surveillance programme, detection of LPAI was done in three game bird holdings (H5N2 and 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 infected holdings. There were no clinical signs in any of these holdings. In 2006, ten flocks or herds were found to have single or more serologic positive samples. However, testing with PCR showed no circulating virus in any of these herds.

The extended and more intensified surveillance programme, which started in 2006, continued in 2007. In 2007, eight flocks or herds were found to have single or more serologic positive samples. However, testing with PCR showed no circulating virus in any of these herds.

## 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[1]. 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 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 2005/744/EC laying down the requirements for the prevention of highly pathogenic Avian influenza caused by influenza A virus of subtype H5N1 in susceptible birds kept in zoos 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.

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[1] The regional veterinary and food administrations, Region North, South and East

**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 have been examined in 2006. The dead wild birds were sent in from the whole country but the positive findings were concentrated in the south-eastern 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 total, 4054 live birds were tested in 2007. Of these, 414 birds living in proximity to domestic poultry and 2398 bird droppings from "high risk" species. In total, 42 of these were found positive, mainly mallards and teal. All positive samples were LPAI, five of these were H5 other than N1 and two of these were H7 other than N1. Finally, 758 hunted game birds, ducks and pheasants, were tested, and none of these were positive.

## 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 Veterinary Institute at the National Laboratory, 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 |
|---|---|
| Wigeon | 1500000 |
| Teal | 500000 |
| Mallard | 4500000 |
| Pintail | 60000 |
| Shoveler | 40000 |
| | |
| Tufted Duck | 1200000 |
| Goldeneye | 1000000-1300000 |
| Common Pochard | 350000 |
| Common Eider | 760000 |
| Greater Scaup | 310000 |
| | |
| Great Cormorant | 75000[a] |
| | |
| 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[b] |
| Common Black-headed Gull | 3700000-4800000 |
| Common Gull | 1200000-2250000 |

[a] Subspecies sinensis breeders in Denmark

[b] Subspecies argentatus breeding/wintering i NW-Europe

Waterbird Population Estimates. 2006. Simon Delany and Derek Scott (eds.). Fourth Edition. Wetlands International, Hageningen, The Netherlands.

## 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 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:
1 blood sample for H5: 47 DKK.
1 blood sample for H7: 47 DKK.

10 blood samples (H5 and H7) in total for 10 birds: 940 DKK.
45 blood samples (H5 and H7) in total for 45 birds: 4230 DKK.

#### 7.1.2 Wild birds

**Passive surveillance in wild birds**
Tracheal swabs from each bird will be tested.

Number of birds tested: 120

Total number of PCR tests: 120

**Active surveillance in wild birds**
Cloacal and tracheal swabs from each bird will be tested individually.

Number of birds tested from bird sanctuaries: 1500

Number of birds tested from surveillance on birds living in proximity to domestic poultry: 500.

Number of birds tested from surveillance on hunted game birds: 300.

**Total number of PCR tests**
Total number of birds tested: Up to 2420.
120 birds will be tested once (tracheal swabs) and 2300 birds will be tested twice (cloacal and tracheal swabs). Total PCR tests: 120+2300+2300 = 4720.

According to previous experience the estimated number of positive samples is approx. 10% of 2420. That means that 242 positive samples may have to be tested further.

Total amount of PCR tests: 4720+242 = 4962.

**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:
790.000 DKK (395 DDK per bird)

The costs for collecting dead wild birds are estimated to:
The costs for collecting 120 dead, wild birds are estimated to:
110.000 DKK (917 DDK per bird)

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

The estimated total costs for PCR test and virus isolation: 1.416.000 DKK.

The estimated costs for sampling cloacal and tracheal swabs: 790.000 DKK.

The estimated costs for collecting dead wild birds: 110.000 DKK.

## 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[49] | | | |
| Haemagglutination-inhibition-test (HI) for H5/H7[50] | **H5 = 7880**<br>**H7 = 7880** | **47 DKK**<br>**47 DKK** | **740.720 DKK** |
| Virus isolation test | | | |
| PCR test | **128*** | **445 DDK** | **56.960 DKK** |
| **Other measures to be covered** | **Specify activities** | | |
| Sampling | | | |
| Others | | | |
| **Total** | | | **797.680 DKK** |

[49] Specify the laboratory test to be used

[50] Specify number of tests for H5 and for H7

* Minimum confirmatory PCR-tests: From a positive serological holding 8 pools from 40 animals will be tested tested twice with PCR = 16. Estimated 8 positive holdings in 2009: 8 x 16 = 128 PCR tests

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 | **242** | | |
| PCR test | **4720** | | **1.416.000 DKK** |
| **Other measures to be covered** | **Specify activities** | | |
| Sampling | | | **Active survey: 790.000 DKK**<br>**Passive survey: 110.000 DKK** |
| Others | | | |
| **Total** | | | **2.316.000 DKK** |

Yours sincerely

Birgit Hendriksen
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Deputy Chief Veterinary Officer