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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 2011 by Commission Decision 2010/712/EU

Greece

^{*} in accordance with Council Decision 2009/470/EC

AVIAN INFLUENZA SURVEILLANCE PROGRAMME IN POULTRY AND WILD BIRDS IN GREECE FOR THE YEAR 2011

1. IDENTIFICATION OF THE PROGRAMME

Member state: Greece
Disease: Avian Influenza
Year of implementation: 2011

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2. SITUATION OF AVICULTURE IN GREECE AND DESCRIPTION OF THE SURVEILLANCE PROGRAMME IN POULTRY

Based on recent data, Gallus gallus species represent the majority of farmed poultry, reared in two types, egg and meat production. The annual laying population varies between 6,3 and 6,400,000 layers while around 115-120,000,000 broilers are fattened per year. The breeding of other species of poultry in Greece continues to be very limited and consists of seasonal turkey rearing with a rather small number of farms and a small number of turkeys per farm. Ostrich rearing has started declining and the number of farms has reduced since last year. Quail rearing is represented by only a few farms. Ducks or geese are only kept as backyard poultry.

No significant changes have taken place regarding the location of poultry farms. Gallus gallus farms are mainly concentrated in the Northwest area of the country and especially in Epirus territory (Prefecture of Ioannina and Prefecture of Arta). A relatively large number of broiler type production exists also in the Prefectures of Evia, Attica, Viotia, Arcadia, Pieria, and Thessalonica. Very small number or no Gallus gallus farms exist in the other Prefectures. Reproductive flocks are reared mainly in Epirus and Evia and less in Attica and Viotia Prefectures. Turkey farms are located mainly in Northern and Central Greece, while ostrich farms are more evenly distributed throughout the whole country.

The surveillance programme in poultry to be carried out in Greece in 2011 takes into account the guidelines set in Commission decision 2007/268/EC adjusted to the most recent national data regarding the number and location of poultry holdings, production type and poultry population per category.

In the framework of the survey programme, blood samples from commercial flocks are scheduled to be collected from their sheds and/or from abattoirs during slaughtering.

Effort will be made for the surveillance to be extended to backyard duck or geese flocks kept as backyard in the entire country, to backyard gallus gallus flocks in proximity to high risk areas and to holdings rearing game birds.

2.1 OBJECTIVES, GENERAL REQUIREMENTS AND CRITERIA

The surveillance programme for avian influenza in poultry establishes an extended serological survey for LPAI throughout Greece targeting mainly at H5 and H7 subtypes. It aims at detecting subclinical infections with LPAI of subtypes H5 and H7, thereby complementing early detection systems and subsequently preventing possible mutation of these viruses to HPAI. Moreover, it aims at detecting infections of LPAI H5 and H7 subtypes in specifically targeted poultry populations at specific risk for infection due to their husbandry system or species specific susceptibility.

Sampling shall be applied from 1 of January until 31 of December 2011 and shall cover a period appropriate to production periods for each poultry category as required. All kinds of samples will be packaged and sent by the Veterinary Directorate or institution responsible, to the designated laboratories. Four state veterinary laboratories will be involved:

- i) The Avian Pathology Dept. of the Center of Institutions of Thessalonica (80, 26th October Street, 54627 Thessalonica) which is designated as the Greek National Reference Laboratory for Avian Influenza and has the responsibilities that are foreseen in Council Directive 2005/94/EE will be acting as coordinator for the laboratory tests to be performed and will accept samples from the areas of Thrace and Macedonia.
- ii) The Avian Pathology Dept. of the Center of Institutions of Athens (26, Neapoleos Street, 15310 Agia Paraskevi, Athens) which is designated as the Greek National Laboratory for Newcastle Disease, which will accept samples from Peloponnesus and the islands of Aegean Sea.
- iii) The State Veterinary Laboratory of Chalkis, which will accept samples from Center Greece and Evia island.
- iv) The State Veterinary Laboratory of Ioannina, which will accept samples from the area of Epirus (north-western Greece).

Every Prefecture Veterinary Directorate or every local veterinary office, upon dispatching samples to the relevant laboratory will in written notify the relevant State Veterinary Laboratory and General Veterinary Directorate of Ministry of Rural Development and Food about :

- -number and kind of samples sent
- -species and number of farms/birds sampled-location-owner
- -identification number of holding sampled
- -date and means of dispatch

The laboratories upon obtaining the results from the tests they will perform, will in written inform the NRL and the General Veterinary Directorate of the Ministry of Agriculture, mentioning:

- -protocol number of dispatch-relevant veterinary authority
- -number and kind of samples examined
- -species and number of farms/birds sampled-location-owner
- -laboratory tests used and results
- -identification number of holding tested positive

In order to optimise efficiency and avoid additional unnecessary entry of persons into poultry holdings sampling should whenever possible be combined with sampling for other purposes.

Positive findings will be traced-back to the holding for further investigation, while all AI virus isolates shall be submitted to the CRL in accordance to Community legislation.

All necessary measures shall be applied according to Community legislation whenever laboratory results and epidemiological data set the suspicion or confirmation of either HPAI or LPAI in poultry holdings.

Positive and negative results of surveillance of poultry will be submitted to the Commission of the EU by the General Veterinary Directorate of the Ministry of Rural Development and Food through the Commission on-line system. Four 3-month period reports will be submitted by forwarding them within a period of four weeks following the end of the months covered by the report.

2.2 DESIGN AND IMPLEMENTATION

Sampling and serological testing in poultry holdings shall be carried out in order to detect antibodies to avian influenza as defined in Directive 2005/94/EC. Surveillance will be carried out according to a representative sampling scheme. The numbers of holdings to be sampled are listed below and correspond to those in tables 1 and 2 of point B of Annex I to Commission Decision 2007/268/EC depending on the poultry species.

In particular, for each poultry category (except turkey, duck and goose holdings) the number of holdings to be sampled shall be based on figures shown in <u>Table 1</u>, whereas for turkey, duck and goose holdings same number will be calculated according to <u>Table 2</u>.

<u>Table 1</u>: Number of poultry (except turkey, duck and goose) holdings to be sampled in each category

nothings to be sampled in each energory					
Number of holdings per poultry category	Number of holdings to be sampled				
Up to 34	All				
35 -50	35				
51-80	42				
81-250	53				
>250	60				

Table 2: Number of turkey, duck and goose holdings to be sampled

Number of holdings	Number of holdings to be sampled
Up to 46	All
47 -60	47
61-100	59
101-350	80
>350	90

I) Gallus gallus farming

The implementation of the Programme for Gallus gallus farms will take place in 10 administrative districts (NUT 2 code GR 11, GR 12, GR 14, GR 21, GR 23, GR 24, GR 25, GR 30, GR 42, and GR 43).

The local veterinary authorities will collect at least 10 blood-samples of 10 ml each per holding, and from the different sheds, if more than one shed is present on a holding. In case of several sheds the sample size per holding shall increase to 5 blood samples per shed. Besides their sheds, birds from a holding can be sampled in abattoirs during slaughtering.

For Gallus gallus species the number of farms to be sampled will be according to <u>Table 1</u>.

Almost all categories of farmed poultry will be sampled (reproductive poultry of all farmed species, fattening poultry "at risk", layers upon finishing their productive period and farms rearing chickens for use as backyard). Efforts will be made for the even distribution of sampled holdings throughout the territory of Greece and the 12 month duration of the programme.

a) Chicken breeders

Having in mind that according to most recent data there are 110 farms of chicken breeders in whole Greece, the total minimum number of farms for this species to be sampled is 53.

b) Laying Hens

Taking into account that the estimated number of laying hen farms is 408, 60 farms are scheduled to be sampled.

c) Free range/organic laying hens

Since there are 13 farms of free range/organic laying hens throughout Greece, all of them will be included in the sampling plan of this year's survey programme.

d) Free range/organic broilers

From a total of 49 free range/organic broiler farms in Greece, 35 shall be sampled, given the increased risk for this poultry category.

e) Farms rearing poultry (mainly chicken) for use as backyard

In Greece there are 118 commercial farms producing mainly chicken in order to sell them for use as backyard. A number of 53 farms is planned to be sampled.

Analytical presentation of the sampling plan concerning gallus gallus categories mentioned above is laid down in tables 1, 2, 3, 4, and 5 of Annex I.

II) Turkey farming

The implementation of the Programme for fattening turkeys will take place throughout the Greek territory, at the slaughterhouses where they are slaughtered only at Christmas period (seasonal production). The relevant Veterinary Directorates will be responsible to collect 10 blood samples of 10 ml each per flock slaughtered.

The implementation of the Programme for turkey breeders will take place throughout the Greek territory at their holdings and/or abattoirs during slaughtering. The relevant Veterinary Directorates will be responsible to collect 10 blood samples of 10 ml each per holding, and from the different sheds, if more than one shed is present on a holding. In case of several sheds the sample size per holding shall increase to 5 blood samples per shed.

Number of turkey farms to be sampled is calculated based on figures shown in <u>Table 2</u>.

Taking into account the number of fattening turkey farms (50) and turkey breeder farms (7) in Greece, 47 fattening turkey farms and all 7 turkey breeder farms will be sampled and totally 540 turkey blood samples will be collected throughout Greece.

Sampling scheme is laid down in tables 6 and 7 of Annex I.

III) Ostrich farming

Ostrich sampling will take place during their slaughtering in abattoirs used for bovine/porcine animals. The relevant Veterinary Directorates will be responsible to collect 5 blood samples of 10 ml each per animal slaughtered. Taking into account that the number of ostrich farms in Greece is 52, 42 of them will be sampled in accordance with <u>Table 1</u> and totally 210 ostrich blood samples will be collected throughout Greece.

Sampling pattern is described in table 8 of Annex I.

IV) Other species

Due to the fact that ducks or geese are kept as "back-yard" poultry and their premises cannot be considered as normal "farms", a total of 10 blood samples of 5 ml each will be collected from 2 premises per prefecture. Taking into account that the number of the prefectures is 54, it is estimated that totally 540 ducks/geese blood samples will be collected throughout Greece, as described in table 12 of Annex I

Breeder quails are reared in only 4 farms and 50 blood samples of 5 ml each will be taken from every single farm by the relevant Veterinary Directorate of their location. Totally 200 quail blood samples will be collected in accordance with table 9 of Annex I.

V) Surveillance of backyard flocks in designated sensitive areas

In certain areas of Greece where a number of wetlands is located, poultry kept in backyard flocks in neighboring villages, will be randomly sampled for the purpose of obtaining data on AI.

The following four are the wetlands where villages keeping backyard poultry will be sampled:

Evros delta, Vistonis lake, Koronia lake, Axios delta.

Villages from where poultry will be sampled will be located at a distance of 5 kilometres away from the above wetlands. Per wetland 5 villages will be sampled and per village 20 blood samples shall be collected.

400 samples will be totally collected from poultry kept at the above areas according to table 10 of Annex I

Poultry sampled will belong to different species but Gallus gallus is predominant.

VI) Farmed game birds

In Greece 20 farms for the production of game birds exist, i.e. birds farmed for release and hunting. Bird species is partridges, pheasants and quails. As in previous year 2010, these farms will be included in the AI surveillance programme for 2011 focusing mainly in adult birds. Ten blood samples will be collected from every farm according to table 11 of Annex I and from the different sheds, if more than one shed is present. In case of several sheds the sample size per holding shall increase to 5 blood samples per shed.

VII) Cumulative Poultry to be tested

According to the above data it is estimated that the total number of samples, which will be collected throughout the whole Greek territory, will count as in the following Table (total number of dispatches=408):

Species	Number of farms ¹ /prefectures ² /villages ³	Samples per farm ¹ / prefecture ² /village ³	Total samples
Gallus gallus	214 ¹	10 blood samples ¹	2140
Turkeys	541	10 blood samples ¹	540
Ostriches	42 ¹	5 blood samples ¹	210
Breeder quails	41	50 blood samples ¹	200
Farmed game birds	20^1	10 blood samples ¹	200
Ducks and geese	54 ²	10 blood samples ²	540
Backyard flocks	20^3	20 blood samples ³	400
Total			4230

2.3 LABORATORY TESTING: DESCRIPTION OF THE LABORATORY TESTS USED

According to sampling plan, 4.230 serological tests (Elisa pre-screening) shall be performed by the four designated laboratories, in the framework of the survey programme for avian influenza in poultry.

Laboratory tests shall be carried out in accordance with the avian influenza diagnostic manual (Commission Decision 2006/437/EC) laying down the procedures for the confirmation and differential diagnosis of avian influenza.

All positive serological findings shall be confirmed by the NRL by a haemagglutination-inhibition test, using designated strains supplied by the CRL

Positive serological findings shall be followed up by epidemiological investigation at the holding and further sampling for testing by virological methods in order to determine, if active infection of avian influenza virus is present.

The specific protocols provided by the CRL to accompany samples and diagnostic material to be sent to the CRL shall be used.

3. DESCRIPTION OF THE SURVEILLANCE PROGRAMME IN WILD BIRDS

Every year, when the winter and spring migration takes place, water reservoirs (wetlands, river deltas, lakes and ponds), located mainly in northern Greece, are visited by many species of wild birds. Their majority rest for a short period of time and continue their travel through specific flyways, however a noticeable number remains behind. Both migrating and domestic wild birds are under constant monitoring by ornithologists who are involved in species identification, registration of population sizes and behaviour observation regarding migration flyways, main habitats, gregariousness and degree of mixing during migration. This established national information on wild bird's ecology, behaviour and population sizes along with Greece's characteristic geographical terrain and data on distribution of poultry holdings set the framework where Commission guidelines for surveillance of avian influenza in wild birds are implemented.

Total number of samples, targeted bird species and geographical areas in which the programme is to be applied have undergone through slight modifications compared to last year's scheme. Thus 1050 wild birds, mainly from the Anseriformes and Charadriiformes orders, are scheduled to be sampled principally in northern Greece.

Participation of governmental or non governmental organizations, institutions, authorities and federations besides veterinary services has been ensured in order wild bird's programme to be properly designed and implemented. Their role and competence are thoroughly described below.

3.1 OBJECTIVES, GENERAL REQUIREMENTS AND CRITERIA

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

- Ensuring early detection of HPAI H5N1 by investigating increased incidence of morbidity and mortality in wild birds, in particular in selected "higher risk" species.
- Enhancing surveillance of live and dead wild birds, in the event that HPAI H5N1 is detected in wild birds in order to determine whether wild birds of other species can act as asymptomatic carriers or "bridge species".
- Continuing a "baseline" surveillance of different species of free living migratory birds as part of continuous monitoring of LPAI viruses. Anseriformes (water fowl) and Charadriiformes (shorebirds and gulls) shall be the main sampling targets to assess if they carry LPAI viruses of H5 and H7 subtypes (which would in any case also detect HPAI H5N1 and other HPAI, if present). "Higher risk species" will be targeted in particular.

Sampling shall extend from 1 of January up to 31 of December 2010. All kinds of samples will be packaged and sent by the Veterinary Directorate or institution responsible, to the designated laboratories. Four state veterinary laboratories will be involved (same as in poultry monitoring):

- i) The Avian Pathology Dept. of the Center of Institutions of Thessalonica (80, 26th of October Street, 54627 Thessalonica) which is designated as the Greek National Reference Laboratory for Avian Influenza and has the responsibilities that are foreseen in Council Directive 2005/94/EE will be acting as co-ordinator for the laboratory tests to be performed and will accept samples from the areas of Thrace and Macedonia.
- ii) The Avian Pathology Dept. of the Center of Institutions of Athens (26, Neapoleos Street, 15310 Agia Paraskevi, Athens) which is designated as the Greek National Laboratory for Newcastle Disease, which will accept samples from Peloponese and the islands of Aegean Sea.
- iii) The State Veterinary Laboratory of Chalkis, which will accept samples from Center Greece and Evia island.
- iv) The State Veterinary Laboratory of Ioannina, which will accept samples from the area of Epirus (north-western Greece).

Every Prefectural Veterinary Directorate or every local veterinary office, upon dispatching samples to the relevant laboratory will in written notify the relevant State Veterinary Laboratory and General Veterinary Directorate of Ministry of Rural Development and Food about:

- -number and types of samples sent
- -species and number of wild birds sampled
- -date of sampling in the field/place of bird origin
- -geo of sample
- -status of birds

The laboratories upon obtaining the results from the tests they will perform, will in written inform the NRL and the General Veterinary Directorate of the Ministry of Agriculture, mentioning:

- -protocol number of dispatch-relevant-veterinary authority
- -date of receipt of sample in laboratory
- -species and number of wild birds examined
- -number and types of samples examined
- -geo of sample
- -status of birds
- -laboratory tests used and results

All avian influenza virus isolates of cases in wild birds shall be submitted to the CRL in accordance to Community legislation.

Positive and negative results of surveillance of wild birds will be submitted to the Commission of the EU by the General Veterinary Directorate of the Ministry of Rural Development and Food through the Commission on-line system. Four 3-month period reports will be submitted by forwarding them within a period of four weeks following the end of the months covered by the report.

3.2 DESIGN AND IMPLEMENTATION

The design of the survey is adapted to the national situation as regards selection of species to be sampled according to species predominance and bird population sizes. It takes into account the behaviour and movements of bird species as regards migratory flyways, seasonality of migration patterns, main habitats, gregariousness and degree of mixing during migration and the results obtained from previous surveys during 2003-2010.

About 1050 wild birds are scheduled for sampling during the period between January – December 2011. A list of 28 different species, mainly from the Anseriformes and Charadriiformes orders, is included in the sampling plan to be implemented in various geographical and administrative areas of Greece. Analytical data are laid down in table 1 of Annex II.

Sampling plan will take into account both:

-passive surveillance on sick or dead wild birds and -active surveillance on living or hunted birds.

Passive surveillance as in year's 2010 programme will be based on the notification for sick or dead birds from citizens throughout the whole territory of Greece. Veterinary Authorities shall assess and filter incoming reports by focusing mainly on the "high-risk species", listed in part D of Annex II to Commission Decision 2007/268/EC, which are found in areas close to the sea, lakes and waterways where poultry farms are in high densities. Moreover, all bodies engaged in active surveillance will contribute by sampling sick or dead wild birds within their designated survey area, whenever increased incidence of mortality and morbidity occurs. In areas where H5N1 cases have been detected, passive surveillance will expand to bridge species listed in part E of Annex II to Commission Decision 2007/268/EC and in areas epidemiologically linked to these cases. Number of dead wild birds found is not possible to be calculated but for the purpose of the surveillance it can be expected that 200 dead wild birds is possible to be found and sampled. This number will be possibly higher in case of massive deaths.

Active surveillance will mainly focus, as in year 2010, on samples taken from wild migrating or domestic birds of higher risk species arriving or living in wetlands, river deltas, lakes and ponds all over Greece; however, priority is given to the areas of Thrace, Macedonia and Epirus which delimitate the northern frontiers and include the probable flyways of migrating birds towards Greece, as well as to identified areas for concentration and mixing of high number of migratory birds belonging to different species and in particular when these areas are in vicinity with domestic poultry farms.

For the purpose of the collection of samples a number of organizations will be in cooperation with the veterinary services. Among them the Hunters Confederation of Greece and the Hunters Federation of Macedonia-Thrace will play a major role on the sampling of hunted wild birds in the framework of the active surveillance. The number of the hunted wild birds to be sampled is calculated at 300 birds.

Another organization included in the active surveillance part of the survey is the Greek Ornithologists Society which is mainly focusing its activities on bird-watching and ringing of wild birds. As in year's 2010 programme, this organization, in close co-operation with scientists working at the National Agriculture Research Foundation, will be used for the collection of samples from living wild birds. Number of living wild birds to be sampled by the Greek Ornithologists Society is calculated at 350.

Finally, live ill or injured wild birds will also be sampled by the two Greek private institutions the activities of which are focused on the collection of ill or injured wild birds for medicate treatment and release. Number of ill or injured birds is estimated around 200.

When healthy free living birds are sampled, oropharyngeal and cloacal swabs shall be used for virological examination. If for any reason it is impractical to take cloacal swabs from live birds carefully collected fresh faeces samples may serve as an alternative. In case of wild birds found dead or shot, cloacal and tracheal/oropharyngeal swabs and/or tissues (namely the brain, heart, lung,

kidney and intestines) shall be collected for virus isolation and molecular detection (PCR). Specific care shall be taken for the storage and transport of samples. Swabs should be chilled immediately on ice or with frozen gel packs and submitted to the laboratory as quickly as possible.

According to previous years experience a sampling scheme will be prepared and included in the Ministerial Decision for the implementation of the surveillance programme. This sampling scheme will describe the areas and bird species to be sampled and the responsibilities of the above mentioned organizations in the sampling collection in such a manner so all target areas and target species will be covered throughout the whole migrating periods. In the sampling scheme a procedure on the full characterization of the bird species sampled will be prepared. The sampling scheme will be prepared by the Department of Avian Pathology of the GVD in co-operation with the Hunters Confederation of Greece, the Forestry Services General Directorate of the Ministry of Rural Development and Food and the Greek Ornithologists Society.

A steering committee under the veterinary services with the participation of most of the above mentioned organizations is planned to be established with the purpose to co-ordinate the surveillance programme during the whole period.

3.3 LABORATORY TESTING: DESCRIPTION OF THE LABORATORY TESTS USED

Samples taken from wild birds shall be tested as soon as possible by molecular techniques. All four designated laboratories will perform rRT-PCR by using methods which are recognized by the CRL for avian influenza and have produced acceptable results in comparative ring tests of laboratories involved. Initial screening using M gene rRT-PCR shall be conducted, with rapid testing of positives for H5, H7 and N1. In addition, all M gene positive samples shall be appropriately processed and inoculated in embryonated fowl's eggs.

Pooling of up to five samples taken from the same species collected at the same site and same time is permitted when it is ensured that, in case of a positive finding, the individual samples can be identified and retested.

Serological surveillance shall not be applied for avian influenza investigations.

Laboratory tests shall be carried out in accordance with the avian influenza diagnostic manual (Commission Decision 2006/437/EC) laying down the procedures for the confirmation and differential diagnosis of avian influenza.

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

In compliance with Community legislation, surveillance programmes of avian influenza have been implemented since 2003. During this period until today, flocks from every poultry category were sampled and tested negative for both HPAI and LPAI.

4.1 MEASURES INCLUDED IN THE PROGRAMME FOR POULTRY SURVEILLANCE

4.1.1 Designation of the central authority charged with supervising and coordinating the departments responsible for implementing the programme

The Central Authority for the Programme is the General Veterinary Directorate of the Ministry of Rural Development and Food / Animal Health Directorate. The Department of Avian,

Fish and Bee Pathology of the Animal Health Directorate is responsible for the description of the Programme, the follow up of the necessary procedures and the collection and elaboration of the data.

4.1.2 System in place for the registration of holdings

Holdings are registered by using the NUTS (3) code in conjunction with a unique prefectural number assigned to them by the Competent Veterinary Authority. Thus every holding is easily identified and traceability is secured.

4.1.3 Data on vaccination

Based on present epidemiological status of the disease and arguments for and against vaccines application, vaccination for avian influenza is prohibited not only in poultry population, but in captive birds as well.

In case of significant future developments concerning epidemiology of avian influenza and data on vaccines, the Central Competent Authority may at any time reconsider its current vaccination policy and introduce a new one, in accordance to recent Community Legislation (dir. 2005/94/EC).

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

In compliance with Community legislation, surveillance programmes of avian influenza have been implemented since 2003. The first occurrence of the disease was reported during the implementation of the 2006 programme, where virological tests were carried out in more than 2500 wild birds and 33 of them, all found dead in the framework of passive surveillance, were tested positive for HPAI H5N1 between 30/1/2006 and 4/3/2006. In particular, 30 of these cases were detected in mute swans, 1 case in whooper swan, 1 case in a wild goose and 1 case in a cormorant, while these birds were discovered mainly in the northern part of Greece.

5.1 MEASURES INCLUDED IN THE PROGRAMME FOR WILD BIRDS SURVEILLANCE

5.1.1 Designation of the central authority charged with supervising and coordinating the departments responsible for implementing the programme

The Central Authority for the Programme is the General Veterinary Directorate of the Ministry of Rural Development and Food / Animal Health Directorate. The Department of Avian, Fish and Bee Pathology of the Animal Health Directorate is responsible for the description of the Programme, the follow up of the necessary procedures and the collection and elaboration of the data.

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

Primary objective of the sampling plan drawn for the purposes of this programme is to cover as large areas as possible; however, it is imperative to focus on certain regions by taking in account risk assessment results on the basis of epidemiological data. Special emphasis shall be laid on the deltas of Evros, Evrotas, Sperchios and Axios rivers, on the lakes of Vistonis, Karla, Paralimni, Kerkini, Agras and Pamvotis, on the Gulfs of Amvrakikos and Kaloni, on the Mesologi lagoon and finally on the dam of Anapodaris river. These areas are located in the regions of Thrace, Macedonia, Epirus, Thesalia, Aegean Islands, Sterea Ellada, Crete, and Peloponisos, covering 11 out of 13

administrative districts; nevertheless, their majority is concentrated in the northern part of the country (Thrace, Macedonia, Epirus), where a larger wildlife population is observed.

5.1.3 Estimation of the local and/or migratory wildlife population

No relevant data exist. According to ornighological bodies, numbers vary, depending on severety of winter conditions in Asia and Europe.

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

According to recent Greek legislation which has been into full force since 2005 the immediate notification of any abnormal mortality or sickness of migrating or domestic wild birds has been obligatory.

As regards the notification of the disease, currently in force Presidential Decree 33/2008 secures that the suspected presence and confirmed presence of avian influenza are compulsorily and immediately notified to the competent authority in accordance with Council Directive 2005/94/EE.

7. COSTS

7.1 DETAILED ANALYSIS OF THE COSTS

7.1.1 Poultry

Serological pre-screening of Gallus gallus and turkey holdings will be conducted using indirect ELISA. As presented on Tables 1-7 and Table 10 of Annex I, 288 holdings will be sampled and 3.080 sera will be examined. Estimating a ϵ 2 cost per sample, the total cost for serological prescreening using indirect ELISA will reach ϵ 6.160.

Serological pre-screening will also be conducted on holdings of ratites, breeder quails, farmed game birds and backyard ducks and geese using competitive ELISA. As presented on Tables 8, 9, 11 and 12 of Annex I, 174 holdings will be sampled and 1150 sera will be examined. Estimating a €2 cost per sample, the total cost for serological pre-screening using competitive ELISA will reach €2,300.

Haemagglutination Inhibition (HI) tests for H5/H7 will be used for ELISA positive sera which are estimated to reach 1% of the 4.230 examined samples. Thus, 42 sera will be tested with HI. Estimating a €12 cost per sample the cost for HI tests will reach €504.

7.1.2 Wild birds

Real time PCR (rRT-PCR) will be conducted for all wild birds' samples. As presented on Table 1 of Annex II, 1.050 wild birds will be sampled and 2.100 samples (two per bird) will be collected. Estimating a €20 cost per sample the cost for rRT-PCR tests will be €42.000.

Virus Isolation (VI) test will be used for rRT-PCR positive samples which are estimated to reach 5% of the 2100 examined samples. Thus, 105 samples will be tested using VI with a ϵ 4.200 cost.

Additional expenses due to sampling and dispatching are calculated to an amount of €42.000.

7.2 SUMMARY OF THE COSTS

Two tables summarizing the costs of avian influenza surveillance programme in poultry and wild birds are included in Annex III.

ANNEX I

A) POULTRY HOLDINGS TO BE SAMPLED (except ducks and geese)

<u>Table 1</u>
Serological investigation on holdings⁽¹⁾ of chicken breeders

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 21	71	35	10	350	ELISA prescreening and HI if sample tested positive
GR 30	15	8	10	80	>>
GR 12	6	3	10	30	>>
GR 25	6	3	10	30	>>
GR 24	5	2	10	20	>>
GR 43	4	2	10	20	>>
Total	107 ⁽²⁾	53		530	

⁽¹⁾ Holdings equal establishments

<u>Table 2</u>

<u>Serological investigation on holdings⁽¹⁾ of laying hens</u>

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 30	193	23	10	230	ELISA prescreening and HI if sample tested positive
GR 12	100	20	10	200	>>
GR 25	36	8	10	80	>>
GR 24	21	4	10	40	>>
GR 21	11	1	10	10	>>
GR 14	10	1	10	10	>>
GR 42	10	1	10	10	>>
GR 43	5	1	10	10	>>
GR 11	6	1	10	10	>>
Total	392 ⁽²⁾	60		600	

⁽¹⁾ Holdings equal establishments

⁽²⁾ Refers to total number of holdings in NUTS included in sampling plan

⁽²⁾ Refers to total number of holdings in NUTS included in sampling plan

Table 3

Serological investigation on holdings(1) of free range/organic laying hens

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 30	4	4	10	40	ELISA prescreening and HI if sample tested positive
GR 12	3	3	10	30	>>
GR 24	3	3	10	30	>>
GR 25	2	2	10	20	>>
GR 43	1	1	10	10	>>
Total	13	13		130	

(1) Holdings equal establishments

<u>Table 4</u>
Serological investigation on holdings⁽¹⁾ of free range/organic broilers

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 21	43	32	10	320	ELISA prescreening and HI if sample tested positive
GR 25	5	3	10	30	>>
Total	48 ⁽²⁾	35		350	

(1) Holdings equal flocks

<u>Table 5</u>

<u>Serological investigation on holdings⁽¹⁾ rearing poultry (mainly chickens) for use as backyards (Others)</u>

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 30	61	30	10	300	ELISA prescreening and HI if sample tested positive

⁽²⁾ Refers to total number of holdings in NUTS included in sampling plan

GR 12	18	8	10	80	>>
GR 25	15	7	10	70	>>
GR 43	2	1	10	10	>>
GR 14	4	1	10	10	>>
GR 23	11	5	10	50	>>
GR 11	4	1	10	10	>>
Total	115 ⁽²⁾	53		530	

Table 6 Serological investigation on holdings⁽¹⁾ of turkey breeders

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 12	3	3	10	30	ELISA prescreening and HI if sample tested positive
GR 14	3	3	10	30	>>
GR 23	1	1	10	10	>>
Total	7	7	1000000	70	

⁽¹⁾ Holdings equal establishments

Table 7 Serological investigation on holdings⁽¹⁾ of fattening turkeys

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 14	32	30	10	300	ELISA prescreening and HI if sample tested positive
GR 12	13	12	10	120	>>
GR 25	1	1	10	10	>>
GR 11	1	1	10	10	>>
GR 43	1	1	10	10	>>
GR 30	1	1	10	10	>>
GR 21	1	1	10	10	
Total	50	47		470	

⁽¹⁾ Holdings equal establishments

⁽¹⁾ Holdings equal establishments
(2) Refers to total number of holdings in NUTS included in sampling plan

Table 8 Serological investigation on holdings⁽¹⁾ of ratites

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 12	19	16	5	80	ELISA prescreening and HI if sample tested positive
GR 14	13	10	5	50	>>
GR 21	7	6	5	30	>>
GR 11	6	4	5	20	>>
GR 25	3	2	5	10	>>
GR42	2	2	5	10	
GR 24	2	2	5	10	>>
Total	52 ⁽²⁾	42		210	

Table 9 Serological investigation on holdings⁽¹⁾ of breeder quails (Others)

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 12	2	2	50	100	ELISA prescreening and HI if sample tested positive
GR 21	1	1	50	50	>>
GR 25	1	1	50	50	>>
Total	4	4		200	

⁽¹⁾ Holdings equal establishments

<u>Table 10</u> Virological investigation on holdings⁽¹⁾ of backyard poultry (except ducks and geese)

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 11	29163	10	20	200	ELISA prescreening and HI if sample tested positive
GR 12	33833	10	20	200	>>
Total		20		400	

⁽¹⁾ Holdings equal establishments
(2) Refers to total number of holdings in NUTS included in sampling plan

Table 11

Virological investigation on holdings⁽¹⁾ of farmed game birds

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 24	5	5	10	50	ELISA prescreening and HI if sample tested positive
GR 43	2	2	10	20	>>
GR 12	3	3	10	30	>>
GR 30	1	1	10	10	>>
GR 13	3	3	10	30	>>
GR 21	1	1	10	10	>>
GR 22	. 1	1	10	10	>>
GR 25	1	1	10	10	>>
GR 14	1	1	10	10	>>
GR 23	1	1	10	10	>>
GR 11	1	1	10	10	>>
Total	20	20		200	

(1) Holdings equal establishments

B) DUCK AND GOOSE HOLDINGS TO BE SAMPLED

 $\underline{\textbf{Table 12}}$ $\underline{\textbf{Serological investigation on holdings}^{(1)} \ of \ backyard \ ducks \ and \ geese}$

NUT (2) code	Total number of holdings	Total number of holdings to be sampled	Number of samples per holding	Total number of tests to be performed per method	Methods of laboratory analysis.
GR 24	n/a	10	5	50	ELISA prescreening and HI if sample tested positive
GR 43	n/a	8	5	40	>>
GR 12	n/a	14	5	70	>>
GR 30	n/a	8	5	40	>>
GR 13	n/a	8	5	40	>>
GR 21	n/a	8	5	40	>>
GR 22	n/a	8	5	40	>>
GR 25	n/a	10	5	50	>>
GR 23	n/a	6	5	30	>>
GR 11	n/a	10	5	50	>>
GR 14	n/a	8	5	40	>>
GR 41	n/a	6	5	30	>>
GR 42	n/a	4	5	20	>>
Total		108		540	

(1) Holdings equal backyard flocks

ANNEX II

WILD BIRDS TO BE SAMPLED

Table 1

NUT (2) code	Wild birds to be sampled	Total number of samples to be taken for active survey	Total number of samples to be taken for passive survey (estd.)	Total
GR 11	Anatidae	250	50	300
GR 11	Ralidae, Charadriidae, Scolopacidae	5	3	8
GR 11	Corvidae	7	3	10
GR 11	Laridae	60	20	80
GR 12	Anatidae	80	20	100
GR 12	Ralidae, Charadriidae, Scolopacidae	7	3	10
GR 12	Corvidae	5	2	7
GR 12	Passeriformes	60	10	70
GR 12	Ardeidae	15	5	20
GR 14	Anatidae	15	5	20
GR 14	Ralidae, Charadriidae, Scolopacidae	4	1	5
GR 14	Corvidae	3	2	5
GR 21	Anatidae	50	7	57
GR 21	Ralidae, Charadriidae, Scolopacidae	6	4	10
GR 21	Corvidae	3	2	5
GR 23	Anatidae	20	4	24
GR 23	Ralidae, Charadriidae, Scolopacidae	10	3	13
GR 23	Corvidae	3	2	5
GR 24	Anatidae	25	5	30
GR 24	Ralidae, Charadriidae, Scolopacidae	7	3	10
GR 24	Corvidae	3	2	5
GR 25	Anatidae	14	4	18
GR 25	Ralidae, Charadriidae, Scolopacidae	5	0	5
GR 25	Corvidae	3	3	6
GR 41	Anatidae	15	5	20
GR 41	Laridae	15	5	20
GR 41	Ralidae, Charadriidae, Scolopacidae	5	0	5
GR 41	Corvidae	3	2	5
GR 43	Anatidae	13	2	15
GR 43	Corvidae	2	2	4
GR 30	Passeriformes	60	3	63
GR 30	Random	50	15	65
GR 42	Falconiformes	27	3	30
Total		850	200	1050

ANNEX III

SUMMARY OF THE COSTS FOR AVIAN INFLUENZA SURVEILLANCE PROGRAM IN GREECE FOR THE YEAR 2011

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					
(Indirect ELISA)	3.080	€2	€6.160		
Serological pre-screening (Competitive ELISA)	1.150	€2	€2.300		
Haemagglutination-inhibition test (HI) for H5/H7	42	€12	€504		
Other measures to be covered	Specify activities				
Sampling and dispatching					
Others					
Total			€8.964		

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	-	-	-			
rRT–PCR test	2.100 (2 samples per bird)	€20	€42.000			
Virus isolation test	105	€40	€4.200			
Other measures to be covered	Specify activities					
Sampling and dispatching	2.100 samples	€20	€42.000			
Others						
Total	The state of the s		€88.200			

TOTAL COST OF THE PROGRAMME: € 97.164