



**Republic of Serbia**  
**MINISTRY OF AGRICULTURE,**  
**FORESTRY AND WATER MANAGEMENT**  
**- Veterinary Directorate-**  
**Number: 323-07-03772/2021-05/7**  
**01.06.2021.**  
**Belgrade**

**Surveillance plan of avian influenza disease in the population of poultry and wild birds on the territory of the Republic of Serbia**

This Plan details implementation of surveillance of domestic poultry and wild birds (especially birds in aquatic habitats), for the presence of avian influenza disease, in accordance with the Rulebook on the establishment of the Program of Animal Health Measures Program for 2021 ("Official Gazette of RS", No. 36/2021) (hereinafter: the Program of Measures), as well as the method of sampling of testing material, deadlines for execution of the surveillance plan, participating entities, method of financing and the amount of fee for sampling and laboratory tests.

**Introduction**

Avian influenza is a highly contagious infectious disease, which affects primarily poultry and other species of birds, i.e. wild birds. Particular attention must be paid to the possible presence of highly pathogenic strains of avian influenza virus (HPAI), which can also arise from low pathogenic strains of avian influenza (LPAI), primarily from subtypes H5 and H7. Highly pathogenic strains of the virus can cause enormous economic damage to poultry farms, as mortality rate can be close to 100%. In certain subtypes of highly pathogenic avian influenza viruses, occasional transmission to other species of mammals, as well as to humans (for example, subtype H5N1, etc.) has been observed.

A proven reservoir and vector of avian influenza virus are certain species of wild birds (especially migratory), whose habitat is associated mainly with aquatic habitats (geese, ducks, swans), and as such they can be a direct source of infection for the surrounding backyard poultry and other domestic animals, and, of course, humans. These species of wild birds (but also some other species of wild birds associated with aquatic habitats, and occasionally bird species that are not strictly associated with aquatic habitats) can transmit the avian influenza virus over long distances (even intercontinental) with their usual migrations while often showing no clinical signs of the disease.

**Avian influenza epizootic situation**

The current epizootic situation in the world is unfavorable, because the frequency of avian influenza is high, i.e. the number of registered and reported cases of this disease has increased every few years by several tens and hundreds of times, but there is no year that does not record its presence in large number of countries.

The highest number of individual cases of avian influenza in domestic poultry in 2014, 2015, 2016, as well as 2017 was recorded in virtually all European countries and the United States, despite the fact that these countries implemented biotechnological, health and immunoprophylactic measures in all types of intensive highly productive poultry farming at the highest level.

For poultry farming in the Republic of Serbia, as one of its most dynamic branches of animal husbandry, the frequent cases of avian influenza subtype H5N8 in wild birds, but also in poultry in Europe in the entire period from late 2014 to 2017 was particularly worrying, occurring for the most part in the countries which are world leaders in intensive poultry farming (the Netherlands, Germany, France, Italy, Hungary and Great Britain), from which the Republic of Serbia imports high-value registered breeding stock, i.e. reproductive flocks of poultry.

In addition, in the mentioned period, the presence of avian influenza virus was recorded in many other European countries, including those in the immediate vicinity of our country (Austria, Belgium, Czech Republic, Denmark, Greece, Luxembourg, Poland, Slovakia, Spain, Sweden, Slovenia, Finland, Ireland, Lithuania, Cyprus, Bulgaria, Croatia, Romania, Hungary).

The presence of the avian influenza virus subtype H5N6 was also recorded in that period, but fortunately it was not genetically similar to the subtype that was spreading in the Asian Far East at that time.

In the same period, the presence of the highly pathogenic avian influenza virus subtype H5N8 was recorded in the Republic of Serbia in both wild water birds (swans) and domestic poultry in contact backyards, with the aquatic habitat where diseased water birds (swans) lived. One dead swan was diagnosed with the highly pathogenic avian influenza virus subtype H5N5 (the same subtype was also detected in Croatia).

In 2018, the presence of highly pathogenic avian influenza virus subtype H5N8 was recorded in poultry, in Italy, Germany and the Netherlands, as well as in neighboring Bulgaria. In wild birds, HPAI subtype H5N8 was diagnosed in Sweden, but also subtype H5N6 was diagnosed in Denmark, Finland, the Netherlands, Germany, Great Britain, Ireland and Slovakia.

In 2019, detection of HPAI subtype H5N6 continues in European countries, and in the same year, HPAI was also detected in domestic poultry in neighboring Bulgaria.

Last year (2020), the presence of HPAI subtype H5N8 was detected in poultry in Poland, Slovakia, Czech Republic, Ukraine, Germany, the Netherlands, Great Britain, Sweden, France, Belgium, Denmark, but also in the neighboring countries of the Republic of Serbia, such as Romania, Hungary, Bulgaria and Croatia. The same virus subtype was detected in wild birds in many of the mentioned European countries.

At the beginning of this calendar year (2021), the presence of highly pathogenic avian influenza virus (H5N8) was also registered in the Republic of Serbia, in the West Backa district, which was classified as a district with a high level of risk of avian influenza outbreak based on the analysis conducted during the development of this surveillance program. Highly pathogenic avian influenza virus subtype H5N8 was detected in 6 samples taken from swans (6 positive out of 8 analyzed samples taken from this species of aquatic birds). In the mentioned West Backa district, the nature reserve "Gornje Podunavlje" was reported as the critical point which was the reason for such a high level of risk, and where the virus was detected in tested samples taken from wild birds.

In addition, the latest epizootic research using modern biomolecular techniques for characterization of avian influenza virus, has proven that the main reservoirs and vectors, and therefore the sources of the latest "wave" of outbreak of avian influenza in Europe, are migratory wild aquatic birds, especially those species whose natural habitat is associated with water surfaces (swamps, lakes, ponds, rivers), which are abundant in the Republic of Serbia.



## **Objective of the Plan**

The goal of surveillance is early detection, monitoring and containment of avian influenza, as well as detection of the presence of this virus in natural reservoirs (wild birds) and providing timely risk assessment on the possible transmission and spread of the virus to susceptible animal species - primarily domestic poultry but also pigs, as well as human population.

## **Implementation of the Plan**

Within this surveillance, active and passive supervision is carried out both in the population of wild birds, as well as in certain species and production categories of the domestic poultry population, in extensive and intensive poultry farming.

In intensive poultry farming, given the high level of biosecurity measures, only passive surveillance is carried out. We base this decision on the fact that since 2004 and the spread of HPAI subtype H5N1, there has not been a single case of this disease in Serbia on farms where intensive poultry production takes place.

Active surveillance for the presence of avian influenza virus refers only to extensive poultry farming, i.e. keeping poultry in individual holdings, mostly in backyards, where surveillance shall be carried out for the presence of highly pathogenic and low pathogenic strains of avian influenza virus.

Individual holdings on which active surveillance is carried out are farms located in risk areas, ie in areas where due to the proximity of significant aquatic habitats of certain species of wild birds there is an increased risk of potential transmission of the virus to domestic poultry and farmed birds (targeted surveillance).

The following species of domestic poultry are included in the surveillance: ducks, geese, chickens (all age and production categories), quails (Japanese), all other ornamental poultry and farmed birds (hobby birds and poultry).

This surveillance plan also includes wild bird species that represent significant natural reservoirs of avian influenza virus (aquatic habitat birds, with particular emphasis on birds of the family *Anatidae*: geese, ducks, swans, etc.), which have temporary (resting places, wintering areas) or permanent (nesting) habitats on the territory of the Republic of Serbia.

The priority wild birds species, which will be covered by this surveillance program, are listed in the Appendix to this surveillance plan (Appendix 1), but also all wild birds species with suspected presence of avian influenza virus, based on clinical signs of the disease, increased mortality, or their mass presence in the vicinity of poultry facilities, both in those where extensive (backyard) and intensive poultry production takes place.

In the case of a positive virological finding (RT-PCR), swab samples are submitted to the reference laboratory in order to attempt isolation of the avian influenza virus and its further characterization (sequencing).

On all farms where poultry is kept and places where poultry, ornamental poultry and farmed birds are traded (market, fair, exhibition) where the presence of avian influenza virus is detected, measures are taken to contain and eradicate avian influenza in accordance with the Law on Veterinary Matters and the Rulebook on establishment of measures for early detection, diagnosis, containment, control and eradication of infectious avian influenza disease, as well as the method of their implementation ("Official Gazette of RS", No. 7/10).

#### **A. Active surveillance:**

As part of active supervision, the following tests are performed:

1. Testing for the presence of avian influenza virus in samples of cloacal swabs collected on individual farms from domestic poultry (ducks, geese, chickens, turkeys, etc.).
2. Testing for the presence of avian influenza virus in samples of cloacal swabs of domestic poultry, ornamental poultry and farmed birds, sampled at places where trade and public display are carried out (market, fair, exhibition).
3. Testing for the presence of avian influenza virus in cloacal swab or faecal samples in the population of aquatic habitat wild birds (especially species of birds from the family *Anatidae* such as all species of ducks, geese, swans, as the most common reservoirs and vectors of this virus, but also other aquatic habitat bird species, such as storks, herons, and the like).

#### **B. Passive surveillance:**

Passive surveillance includes mandatory testing of poultry throughout the year, regardless of the type of poultry production (extensive/intensive), which shows clinical signs of disease that may indicate avian influenza, as well as testing for the presence of avian influenza virus in samples of all dead and diseased wild waterfowl, both in areas of increased risk of this disease and throughout the country.

#### **V. Sampling site selection:**

Distribution and selection of sampling sites is carried out by the epizootic department of the competent scientific or specialized veterinary institute, taking into account the listed areas of importance for wild birds in the Republic of Serbia (Table 1), which are located in their epizootic area, based on the assessment of risk exposure to the AI virus, taking into account particularly the following:

- 1) existence of areas (swamps, ponds, lakes, creeks, river surfaces, reservoirs, etc.) suitable for stopping, resting, wintering or nesting of those bird species belonging to the so-called aquatic habitat birds (birds, ducks, geese, swans, etc.)
- 2) existence of farms located in the vicinity of previously identified areas significant and suitable for wild aquatic birds, with present populations of various species of domestic poultry (geese, ducks, chickens), for which there is a possibility of close contact with wild aquatic birds;
- 3) existence of poultry farms where intensive poultry production is carried out in the vicinity;
- 4) existence of places where trade and public display of domestic poultry, ornamental poultry and farmed birds (market, fair, exhibition) takes place in the vicinity.



**Table 1.** Areas important for wild birds in the Republic of Serbia

Name of District	Significant areas for wild birds in the District
City of Belgrade	Confluence of the Sava and the Danube rivers
West Backa District	Gornje Podunavlje (Upper Danube Basin)
South Banat District	Becej fishpond Jegricka Srednje Potamisje (Central Tamis Basin) (Baranda fishpond) Vrsac mountains Deliblato Sands Labudovo okno
South Backa district	Becej fishpond Karadjordjevo Titel hill Kovilj marsh Fruska fora (from Novi Sad to Backa Palanka)
North Banat District	Great Bustard Pastures
North Backa District	Subotica lakes and sands
Central Banat District	Slano Kopovo Okanj and Rusanda Carska bara (bog) Gornje Potamisje (Upper Tamis Basin) Srednje Potamisje (Central Tamis Basin) (Orlovat) Danube loess bluffs (from Slankamen to Banovci)
Srem District	Obedska swamp Bosutske forest
Zlatibor District	Tara Uvac and Milesevk Pester
Kolubara district	Valjevo mountains
Macva District	Zasavica Donje Podrinje (Lower Drina Basin) Cer
Moravica District	Ovcar-Kablar Gorge
Pomoravlje District	Gornje Pomoravlje (Upper Morava Basin) Resava Gorge
Rasina District	Donje Pomoravlje (Lower Morava Basin), Celije
Raska District	Golija Kopaonik
Sumadija District	Gruza Lake
Bor District	Djerdap Mala Vrbica Zlotska Gorge
Branicevo District	Djerdap
Zajecar District	Rtanj
Jablanica District	
Nisava District	Suva planina (mountain) Sicevo Gorge
Pirot District	Stara planina (Balkan Mountains) Jerma
Podunavlje District	Danube, Delta of Velika Morava river
Pcinja District	Pcinja Vlasina
Toplica District	

### G. Sampling plan

Based on the degree / level of risk and the danger of the occurrence of avian influenza, areas / districts were performed, as follows: districts with a negligible level of risk, districts with a lower level of risk, districts with a medium level of risk and districts with a high level of risk shown in Table 2. In accordance with the level of risk, a sampling plan was determined, which is shown in Table 3. Table 4 shows the sampling plan at the places where the trade and public display of domestic poultry, ornamental and farmed birds is performed (market, fair, exhibition).

**Table 2.** Division of areas/districts, according to the level of risk of avian influenza disease outbreak

Districts with negligible risk	Districts with low risk	Districts with medium risk	Districts with high risk
Zajecar	Zlatibor	Sumadija	City of Belgrade
Jablanica	Moravica	Pomoravlje	West Backa
Toplica	Raska	Rasina	South Banat
Pirot	Nisava	North Banat	South Backa
Pcinja			North Backa
Kolubara			Central Banat
			Srem
			Macva
			Bor
			Branicevo
			Podunavlje

**Table 3.** Sampling plan for wild birds and poultry on individual farms according to the level of risk of avian influenza disease outbreak

Districts with negligible risk	Districts with low risk	Districts with medium risk	Districts with high risk
Surveillance is not carried out for avian influenza on the population of wild birds and poultry on individual farms.	Once a month - in the periods of the year in which the surveillance is carried out, 3 samples of swabs (or feces) of wild birds and samples of swabs of poultry on individual farms are taken, namely from 3 farms located near the habitat of an area significant for wild birds (3 swabs per farm, representing one sample - pool).	Once a month - in the periods of the year in which the surveillance is carried out, 5 samples of swabs (or feces) of wild birds and samples of swabs of poultry on individual farms are taken, namely from 5 farms located near the habitat of an area significant for wild birds (3 swabs per backyard farm representing one sample - pool).	Once a month - in the periods of the year in which the surveillance is carried out, 10 samples of swabs (or feces) of wild birds and samples of swabs of poultry on individual farms are taken, namely from 10 farms located near the habitat of an area significant for wild birds (3 swabs per farm representing one sample - pool).



**Table 4.** Sampling plan at locations where trade and public display of domestic poultry, ornamental and farmed birds is carried out (market, fair, exhibition)

No.	Name of District	Number of cloacal swab samples	No.	Name of District	Number of cloacal swab samples
1.	City of Belgrade	10	14.	Rasina	10
2.	West Backa	10	15.	North Banat	10
3.	South Banat	10	16.	Zlatibor	10
4.	South Backa	10	17.	Moravica	10
5.	North Backa	10	18.	Raska	10
6.	North Banat	10	19.	Nisava	10
7.	Srem	10	20.	Zajecar	10
8.	Macva	10	21.	Jablanica	10
9.	Bor	10	22.	Toplica	10
10.	Branicevo	10	23.	Pirot	10
11.	Podunavlje	10	24.	Pcinja	10
12.	Sumadija	10	25.	Kolubara	10
13.	Pomoravlje	10			

Surveillance, according to this Plan, includes sampling and testing of cloacal swabs (or feces) of aquatic wild birds, captured or shot near areas which are significant for wild birds, cloacal swabs of poultry on individual farms located in the immediate vicinity of areas significant for wild birds, as well as cloacal swabs of domestic poultry, ornamental and farmed birds taken at locations where trade and public display is carried out (market, fair, exhibition).

Virological tests shall be performed using accredited molecular methods (real-time RT-PCR or RT-PCR), in the laboratory of the Scientific Institute of Veterinary Medicine "Novi Sad" in Novi Sad and in the reference laboratory for avian influenza at the Specialized Veterinary Institute "Kraljevo" in Kraljevo.

Scientific and specialized veterinary institutes submit samples for testing according to the following territorial organization:

**Table 5.** Territorial organization for submitting samples for testing

SVIM "NOVI SAD"	SVI "KRALJEVO"
SIVM "Novi Sad"	SVI "Kraljevo"
SVI "Subotica"	SVI "Sabac"
SVI "Sombor"	SIVMS "Beograd"
SVI "Pancevo"	SVI "Pozarevac"
SVI "Zrenjanin"	SVI "Zajecar"
	SVI "Jagodina"
	SVI "Nis"

All samples which were tested positive for the presence of avian influenza virus by using these methods, shall be submitted to the reference laboratory for avian influenza at the

Specialized Veterinary Institute "Kraljevo" in Kraljevo, for their further characterization, i.e. determining the degree of pathogenicity (low or high), i.e. for virus sequencing.

### **Sampling is performed as follows**

#### **1. Sampling of "waterfowl" wild birds**

Cloacal swabs samples are taken from aquatic habitat wild birds, shot during the hunting season or caught in special traps, particularly those belonging to migratory species, which are better known as the most common reservoirs of the avian influenza virus (from the family *Anatidae*, such as ducks, geese, swans, etc.).

If there is a possibility to observe and locate places where bird colonies spend the night or feed and rest in cooperation with ornithologists, sampling of fresh feces is performed.

If the corpses of dead wild birds belonging to the group of "susceptible species" are found, they are sampled and submitted for examination.

Samples for virological testing are stored and transported while ensuring the maintenance of a cold chain (on ice or frozen swabs) to the competent laboratory.

Collecting samples, i.e. cloacal swabs is performed once a month, by areas and in the amount specified in Table 3.

#### **2. Sampling of poultry on individual farms**

Sampling of cloacal swabs taken from poultry originating from individual farms is performed on farms located in the immediate vicinity of areas which have previously been precisely identified as areas significant for the presence of aquatic wild birds. Samples are taken from free-range poultry in the immediate vicinity of areas where the presence of aquatic wild bird populations has been identified, and for which there is a high probability of direct contact.

Sampling is carried out once a month, by areas and in the amount specified in Table 3.

#### **3. Sampling of poultry at locations where there trade and public display of domestic poultry, ornamental and farmed birds (market, fair, exhibition)**

In the presence of avian influenza virus, cloacal swabs of domestic poultry, ornamental and farmed birds taken from places where traffic and public display is performed (market, fair, fair, exhibition) are examined.

Sampling at places where traffic and public display is performed (market, fair, exhibition fair) is performed at the time of their holding by taking the required number of swabs (listed in Table 4) from the target species and categories of poultry, with obligatory recording of samples by type of poultry and origin.

The stated number of cloacal swabs refers to individual swabs taken from each individual. As a rule, sampling is performed in the stated periods of the year (September, October, November 2021, and March, April and May 2022), but it can also be performed outside that period according to current events that bring risk.

The choice of sampling site in each district will be determined by the competent epizootiological service of the scientific or specialist veterinary institute.



## **Organization and method of sampling and procedure and measures during the detection of avian influenza virus**

Cloacal swab samples of poultry from individual farms are taken by the epizootic service of the competent veterinary institute, in accordance with this Plan, along with taking basic epizootic and anamnestic data, in cooperation with the competent veterinary stations and veterinary inspection.

The epizootic department of the competent institute performs sampling of wild birds in the areas for which it is responsible and delivers samples to the laboratory in accordance with the territorial distribution.

When submitting samples to the laboratory, the Form for sending materials for laboratory testing must be filled in (Appendix 2).

If the presence of avian influenza virus in wild birds or poultry is confirmed by a positive test result during laboratory testing, the competent veterinary inspector and the Veterinary Directorate shall be notified immediately of this fact. Samples which were tested positive for the presence of AI virus shall be sent immediately to the National Reference Laboratory for Avian Influenza in SVI "Kraljevo", for the purpose of conducting confirmatory tests. If the national reference laboratory obtains a positive test result, it shall immediately notify the competent veterinary inspector and the Veterinary Directorate thereof.

In order to ensure smooth, timely and quality implementation of this avian influenza surveillance program in the Republic of Serbia, and especially the part that requires more technically demanding and complex operations (surveillance of wild birds as reservoirs and vectors of AI virus), it is necessary that all surveillance participants timely plan and prepare all resources (staff, equipment, reagents) and inform the Veterinary Directorate about their preparedness.

It is necessary to provide the following for wild birds surveillance:

1. Approval for capture and sampling of birds (obtained from the Institute for Nature Conservation of Serbia);
2. Participation of qualified ornithologists, i.e. certified bird ringer, for field work and accurate identification of wild bird species, from which a sample is taken;
3. Reported dedicated nets and traps for capturing wild aquatic birds;
4. Dry ice or other refrigerant for storage and transport of samples to the laboratory.

In case a veterinary institute is unable to fulfill some of the listed conditions, i.e. cannot provide the necessary resources for this segment of surveillance, it shall immediately inform the Veterinary Directorate of this fact in writing, which will coordinate the implementation of surveillance activities in the epizootic area of that institute.

### **Duration of surveillance**

Active surveillance for the presence of avian influenza, according to this plan, is performed in the period:

- September, October, November 2021
- March, April, May 2022.

Passive surveillance is carried out throughout the year.

### **Reports and invoicing**

Funds for the implementation of this supervision are provided in the budget of the Republic of Serbia.

Scientific and specialist veterinary institutes, by the 10th of the month for the previous month, submit *Reports on the scope of sampling and test results* (Annexes 3, 4 and 5), with all individual test reports as well as an invoice for work performed, addressed to the Ministry of Agriculture, Forestry and Water Management - Veterinary Directorate, Omladinskih brigada 1, 11070 New Belgrade.

The final report on this monitoring, in the form of a study, along with a detailed analysis of data and results shall be prepared by SVI "Kraljevo", in coordination with SIVM "Novi Sad" and other institutes.

**Fees for performed activities:**

Sampling:

- target wild birds (aquatic "habitat" birds), caught alive and cloacal swab taken: RSD 1500.00
- wild birds, sample of the whole corpse (which belongs to the category of target species): RSD 1000.00
- feces or cloacal swab of shot wild birds (also from the category of target "aquatic" species): RSD 1000.00
- sampling of cloacal swab of poultry (pool sample - three swabs from poultry from one farm) RSD 1000.00
- individual sample taken at locations where trade and public display is carried out (market, fair, exhibition): RSD 1000.00

Laboratory testing:

- real time RT-PCR or RT-PCR: RSD 4000.00
- virus sequencing: RSD 9000,00
- virus isolation: RSD 3300,00

Note: Prices are without VAT.



Acting CVO/Director

*Emina Milakara*  
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