

The OIE Member Countries have the possibility to self-declare their country or a zone within their territory free from certain OIE-listed diseases other than those diseases for which the OIE has put in place a specific procedure for official recognition of disease status such as African horse sickness (AHS), bovine spongiform encephalopathy (BSE), classical swine fever*, contagious bovine pleuropneumonia (CBPP), foot and mouth disease (FMD) and peste des petits ruminants*.

*Resolution N° 29 adopted in May 2013 at the 81st OIE General Session

Self-declaration by Spain of freedom from avian influenza

Self-declaration submitted to the OIE on 29 May 2017 by Dr Valentín Almansa, Delegate of Spain to the OIE and Director General of Agricultural Production Health, of the Ministry of Agriculture and Fishery, Food and the Environment (MAPAMA)

Highly pathogenic avian influenza situation

In February 2017, Spain's annual National Avian Influenza Surveillance Programme, which includes active and passive surveillance in domestic poultry and wildfowl, detected the presence of the H5N8 subtype of the highly pathogenic avian influenza (HPAI) virus on ten duck farms in the Catalonia provinces of Gerona (or Girona) and Barcelona.

On 23 February, an initial outbreak was confirmed on a free-range duck farm with 17,800 birds. Later, on 27 February, an epidemiological survey conducted on epidemiologically related farms in regard to live animal movements resulted in the notification of seven secondary outbreaks, all on duck farms (Table I).

Table I
Affected farms

| Farm identification | Community | Province | Reason for sampling | Number of susceptible animals |
|---------------------|-------------------------|-----------|---------------------|-------------------------------|
| ES171630024858 | Sant Gregori | Girona | clinical signs | 17,800 |
| ES172240015515 | Villalonga de Ter | Girona | contact farm | 500 |
| ES172320036206 | Vilopriu | Girona | contact farm | 177 |
| ES170200036717 | Bescano | Girona | contact farm | 450 |
| ES171830034823 | Sant Aniol de Finestres | Girona | contact farm | 0* |
| ES170700034648 | Fontanilles | Girona | contact farm | 5,603 |
| ES171890016583 | La Cellera de Ter | Girona | contact farm | 190 |
| ES081070036954 | Lliçà d'Amunt | Barcelona | contact farm | 380 |

* There were no birds to sample on this holding as they had already been sent to the slaughterhouse by the time of detection. It was assumed to be infected and subjected to full cleaning and disinfection procedures, as per the other properties.

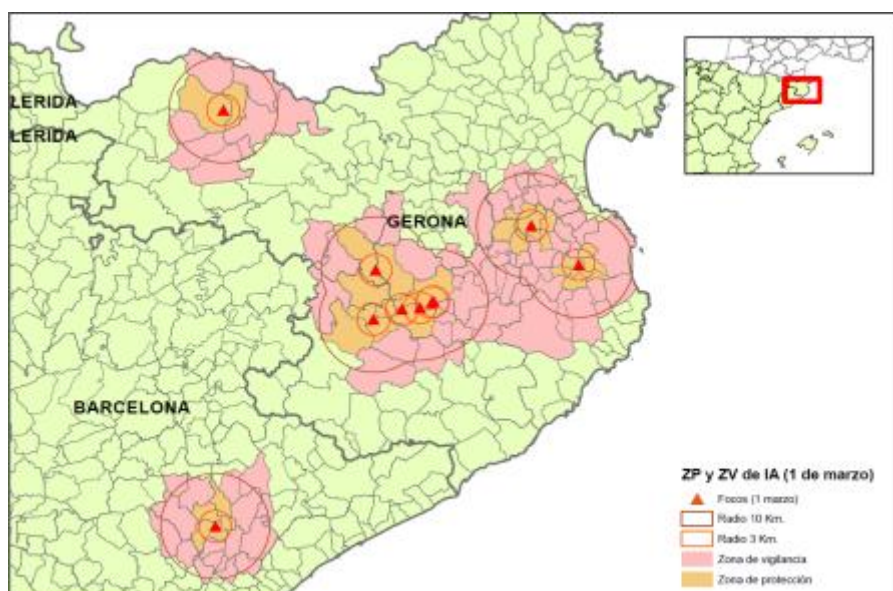
As a result of the surveillance measures operating over a 3-km radius, two additional secondary outbreaks were notified on 1 March in the 3-km protection zone (Table II).

Table II
Secondary outbreaks reported within the protection zone

| Farm identification | Community | Province | Reason for sampling | Number of susceptible animals |
|---------------------|--------------|----------|----------------------|-------------------------------|
| ES171630037061 | Sant Gregori | Girona | within the 3-km zone | 2,450 |
| ES171630007127 | Sant Gregori | Girona | contact farm | 380 |

The map in Figure 1 shows the location of the affected farms and the 3-km and 10-km zones.

Fig. 1
Location of outbreaks



LEGEND (Fig. 1):
 Avian influenza protection and surveillance zones (as of 1 March)
 Outbreaks (1 March)
 10-km radius
 3-km radius
 Surveillance zone
 Protection zone

Positive results were obtained only in the confirmed outbreaks, with the distribution shown in Table III.

Table III

Test results in the confirmed outbreaks

| Farm identification | Municipality | ELISA tested | ELISA + | IHA H5 + | PCR tested | PCR H5+ |
|---------------------|-------------------------|---------------|---------|---------------------------|------------|---------|
| ES171630024858 | Sant Gregori | 40 | 40 | 40 | 76 | 15 |
| ES172240015515 | Villalonga de Ter | 20 | 20 | 12 (8 samples spoiled) | 40 | 40 |
| ES172320036206 | Vilopriu | 20 | 20 | 20 | 40 | 25 |
| ES170200036717 | Bescano | 20 | 13 | 13 | 40 | 40 |
| ES171830034823 | Sant Aniol de Finestres | Empty holding | | | | |
| ES170700034648 | Fontanilles | 20 | 19 | 20 | 40 | 25 |
| ES171890016583 | La Cellera de Ter | 20 | 20 | 17 | 40 | 20 |
| ES081070036954 | Lliçà d'Amunt | 0 | 0 | 20 | 40 | 40 |
| ES171630037061 | Sant Gregori | 20 | 20 | 20 | 40 | 36 |
| ES171630007127 | Sant Gregori | 20 | 19 | 17 | 40 | 38 |

ELISA: enzyme-linked immunosorbent assay

IHA: indirect haemagglutination test

PCR: polymerase chain reaction

Epidemiological study

The epidemiological study produced the following findings.

- No animals were moved from the affected farms to other regions of Spain (apart from the affected region in the map in Figure 1), to the European Union or third countries.
- Initially, seven movements causing risk were identified, from the initially affected farm (the primary outbreak) to final fattening farms in the Gerona and Barcelona provinces. An epidemiological study was conducted on the receiving farms, six of which tested positive, while the seventh had no birds at the time of the outbreak. The total duck population of the seven farms was 7,000.
- Surveillance conducted within the 3-km radius around the primary outbreak revealed two new outbreaks within the same community, also on duck farms. Birds on one of the farms started to display clinical signs, resulting in sampling, and the second had taken delivery of live animals from this newly affected farm. No animal movements took place from these two new farms, apart from those destined for stamping out.

Control and eradication measures

All affected farms were locked down from the moment suspicions were raised, and clinical inspection and sampling were conducted in all the sheds.

The farms were subjected to serological and virological tests, in compliance with the requirements established in Article 10.4.33. of the OIE *Terrestrial Animal Health Code*. The Veterinary Services followed a sampling protocol that specified which samples should be taken, what personal protection equipment to use and the sampling method. On each farm, 20 blood samples, 20 tracheal swabs and 20 cloacal swabs were collected.

Every time a new outbreak was confirmed, the whole population was stamped out and, once completed, this was followed by clean-up and disinfecting operations. Both the (naturally) dead and slaughtered animals were transported from each depopulation site to the Secanim processing plant (Termens, Lerida [Lleida] province), where the carcasses were specially disposed of, to rule out any risk of contamination. Accordingly, leak-proof containers were used to avoid contamination during transport.

The movement of poultry, their products and other materials that could transmit the virus was prohibited in the 3-km zone (the 'protection zone') and the 10-km zone (the 'surveillance zone'). Nonetheless, certain exceptions were permitted, with the application of additional biosecurity measures, in the case of birds intended for slaughter, day-old chicks, ready-to-lay poultry and hatching eggs. These measures remained in force until 1 April 2017, on which date 30 days had elapsed since the end of the clean-up and disinfection operations at the site of the last outbreak, as stipulated in the European Directive 2005/94.

In the protection and surveillance zones, compulsory strengthening of biosecurity measures, as well as other measures, such as a ban on spreading used litter and staging fairs, markets and exhibitions, were enforced as an additional preventative measure. The confinement of free-range poultry was imposed within the 3-km radius.

Epidemiological surveillance

In the protection and surveillance zones, the quarantine and investigation of all poultry farms located in these zones was conducted through clinical surveillance. This included sampling all the farms in the protection zone and, in the event of the display of clinical symptoms or epidemiological links with affected farms, in the surveillance zone. This was in accordance with the heightened surveillance requirements set out in Article 10.4.3.1. of the OIE *Terrestrial Animal Health Code* enabling a country to regain freedom from avian influenza status after an outbreak.

The number of samples taken in the protection zone, and in the other restricted zones, is displayed in Table IV.

Table IV

Number of samples taken in the protection zone and in the other restricted zones

| | Protection zone | Surveillance zone |
|--------------------------------|-----------------|-------------------|
| Number of farms sampled | 55 | 13 |
| Number of samples | 1,375 | 564 |
| Number of ELISA tests | 494 | 370 |
| Number of PCR tests | 881 | 194 |

Negative results from polymerase chain reaction (PCR) and haemagglutination (HI) were obtained in all cases, except in the last two outbreaks detected in the protection zone (farms ES171630037061 and ES171630007127). In regard to enzyme-linked immunosorbent assay (ELISA), seven farms obtained positive results but were confirmed as negative by specific H5 and H7 PCR.

Furthermore, a National Avian Influenza Surveillance Programme has been conducted throughout the country since 2003 to provide early diagnosis of the disease. Its remit includes farmyard poultry, other captive poultry and wildfowl.

Active surveillance is based on the laboratory sampling of farmyard poultry and other captive poultry, to detect the circulation of the H5 and H7 viruses. The sample must be representative of the country's entire poultry population; therefore, a minimum number of samples is set for:

- the various production categories: laying hens, free-range laying hens, breeder hens, breeder turkeys, breeder ducks, breeder geese, fattening turkeys, fattening ducks, fattening geese, gallinaceous game birds (pheasants, partridges and quail), game Anatidae, ratites and others
- each Autonomous Community, to be eligible for consideration as being representative of the whole of Spain.

The sampling strategy chosen in Spain is based on a representative sampling of its entire territory. The sampling period is adapted to the seasonality of production and can also be adapted to other types of timelines identified locally that may potentially involve a higher risk. The number of farms to be sampled (with the exception of duck and goose farms) must ensure detection in the case of an estimated prevalence of 5% with a 95% confidence interval. The number of duck and goose farms to be sampled must ensure the identification of at least one infected farm, involving a minimum prevalence of 5% with a 99% confidence interval. For all categories, the number of samples to be taken on each holding is defined to ensure a 95% probability of identifying at least one seropositive bird, with a considered prevalence of over 30%.

The following table shows changes to the sampling in recent years, which is always in relation to the risk assessment at that time, is detailed in Table V.

Table V
Changes to the sampling in recent years

| Year | 2013 | 2014 | 2015 | 2016 |
|-------------------|-------|-------|-------|-------|
| Farms | 965 | 897 | 833 | 752 |
| Wild birds | 2,772 | 1,435 | 1,201 | 1,148 |

The results of the samples taken in Catalonia from July to December 2016 have been revised. In this campaign, 49 holdings were sampled. All returned negative results by ELISA.

The sampling programme continued through 2017 without detecting new positive cases. The total number of farms to be sampled in 2017 is shown in Table VI, broken down by production category.

Table VI
Total number of farms to be sampled in 2017, broken down by production category

| Production category | Total number of installations | Total number of installations to be sampled | Number of samples per installation | Minimum number of samples to be carried out per method | Laboratory analysis method |
|-------------------------|-------------------------------|---|------------------------------------|--|----------------------------|
| Laying hens | 412 | 60 | 20 | 1,200 | ELISA |
| Breeder hens | 638 | 60 | 20 | 1,200 | ELISA |
| Free-range laying hens | 314 | 60 | 5–10 | 600 | ELISA |
| Ratites | 73 | 42 | 5–10 | 420 | ELISA |
| Gallinaceous game birds | 503 | 60 | 5–10 | 600 | ELISA |
| Fattening turkeys | 629 | 60 | 5–10 | 600 | ELISA |
| Breeder turkeys | 14 | 14 | 5–10 | 140 | ELISA |
| Game Anatidae | 120 | 80 | 20 | 1,600 | ELISA |

| | | | | | |
|-----------------|----|----|----|-----|-------|
| Fattening ducks | 49 | 47 | 20 | 940 | ELISA |
| Fattening geese | 17 | 17 | 20 | 340 | ELISA |
| Breeder ducks | 3 | 3 | 20 | 60 | ELISA |
| Breeder geese | 3 | 3 | 20 | 60 | ELISA |

During the first semester of 2017, 486 samples were taken on 40 holdings in the region of Catalonia, all of them with negative results to the ELISA test.

Moreover, since the first outbreaks of H5N8 HPAI in Northern Europe, the Central Veterinary Services (MAPAMA) have regularly reminded all the sectors involved, and the regional authorities, of the need to boost biosafety measures on poultry farms, primarily those aimed at avoiding contact with wildfowl, and to increase surveillance when faced with an abnormal mortality or compatible clinical symptoms on poultry farms and in wildfowl, as well as to improve passive surveillance and preventative measures.

Description of laboratory tests

The diagnostic method used to detect and identify the virus, the isolation method, and the specific antibodies complied with Chapter 2.3.4. of the OIE 2016 *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* and have been EN ISO 17025-certified by the Spanish National Accreditation Body.

Serum samples were analysed by ELISA (blocking ELISA, ID.VET). Any samples testing positive were confirmed by a haemagglutination inhibition (HI) assay, using designated strains as recommended by the Animal and Plant Health Agency (APHA) European Union Reference Laboratory (APHA–EURL).

The cloacal and tracheal swabs were analysed using the real-time PCR method to detect the influenza A virus.

In the event of a positive result, the method used was real-time PCR to determine the avian influenza virus subtype and sequencing was subsequently carried out to determine its pathogenicity. Lastly, in positive cases, egg embryos were inoculated with the positive samples to attempt to isolate the virus, following the procedure described in Chapter 2.3.4. of the OIE *Manual*.

Phylogenetic results

The region of haemagglutinin H5 of the HPAI virus isolated on farm ES171630024858 was sequenced by the Spanish National Reference Laboratory for avian diseases (*Laboratorio Central de Veterinaria [LCV], Algete*), together with two other HPAI H5N8 isolates obtained in January and February 2017 from wild birds in Palencia province (*Anser anser*) and Gerona province (*Ciconia ciconia*), respectively.

The resulted phylogenetic tree (Fig. 2) shows that the three Spanish HPAI H5N8 isolates are identical in the sequenced region of haemagglutinin H5 and identical to all isolates that appear in that branch of the tree.

Fig. 2

Haemagglutinin phylogenetic analysis of 35 H5 clade 2,3,4,4 nucleotide sequences

Tree scale: 0.001



Sequences were aligned with Muscle (v3.7); 32 from Global Initiative on Sharing All Influenza Data (GISAID) databases and 3 Spanish isolates in green. The region analysed was a 259 base-pair fragment (821–1080 nt) of the H5 haemagglutinin (HA) gene.

The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v 3.0). The HKY85 substitution model was selected assuming an estimated proportion of invariant sites (of 0.001) and 4 gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data (gamma = 0.501). Reliability for internal branch was assessed using the approximate likelihood ratio (aLRT) test (minimum of Shimodaira–Hasegawa [SH]-like).

Conclusions

Bearing in mind that:

- prior to the notification of outbreaks, Spain enjoyed avian influenza-free status,
- stamping-out measures were adopted that included cleaning up and disinfecting all the affected farms,
- three months have elapsed, as stipulated in Point 1 of Article 10.4.3. of the OIE *Terrestrial Animal Health Code*, since the end of the clean-up and disinfection operations,
- surveillance has been performed in accordance with Articles 10.4.27. to 10.4.33. of the OIE *Terrestrial Animal Health Code* during that three-month period,

the official Veterinary Authorities of Spain have decided **to self-declare that Spain's status as being free of notifiable avian influenza has been regained for the whole country.**

The Delegate of Spain to the OIE declares that this country has met the requirements for recognition as a country free from avian influenza as of 2 June 2017, in accordance with Article 10.4.3. of the *Terrestrial Animal Health Code* (2016).