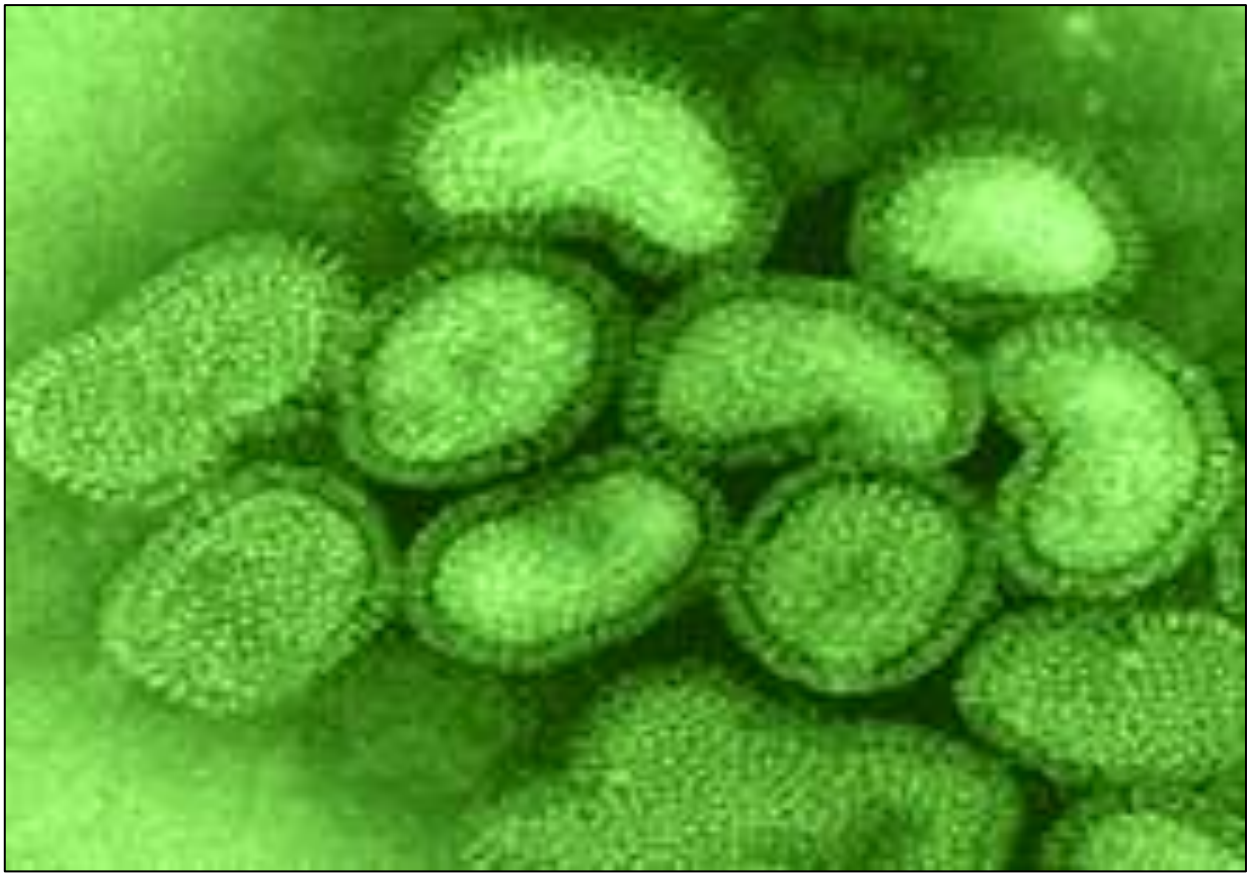
Animal &
Plant Health
Agency

EPIDEMIOLOGY REPORT

Low Pathogenicity Avian Influenza H5N1 (AIV2016/01)
In broiler breeder chickens

Dunfermline, Scotland, January 2016
Situation at 11:00 on Friday 04 March 2016



Electronmicrograph of avian influenza viruses

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DESCRIPTION OF THE PREMISES: The Infected Premises (IP) designated as AIV 2016/01, is a 40,000 **broiler breeder laying unit**, located approx 8km north of the Firth of Forth estuary in the east of Scotland. The birds are owned and managed as a stand-alone unit. It operates as **an intensive (indoor), barn style production system**, and eggs for hatching are collected and delivered to a hatchery in Berwickshire, Scotland.

DESCRIPTION OF THE VIRUS: It has been concluded that this **H5N1 low pathogenicity avian influenza virus** is a **conventional European lineage virus** exhibiting biological properties **consistent with contemporary LPAI viruses that are occasionally detected in the EU** in domestic poultry, and whose closely related progenitors are **maintained in wild waterfowl populations**.

The virus is **clearly distinguishable from viruses associated with the ongoing epidemic in France**, and **distinguishable from the group of HPAI viruses that have caused a global panzootic** in the last 10 years. The genetic analyses suggest that **all eight genes are of avian origin**, **without any specific increased affinity for humans.**

SOURCE AND SPREAD WINDOWS: The most likely time that LPAI infection is estimated to have entered the IP is around **26/12/2015,** with a maximum precautionary source period over which tracings were investigated from 11/12/2015 to 31/12/2015, a day before the precautionary start date for onset of clinical signs. The high risk spread window for LPAI virus opened on **27/12/2015,** with the spread window extending until **09/01/2016** (when restrictions were imposed), with a maximum precautionary spread period over which tracings were investigated extended back to 12/12/2015.

HYPOTHESIS FOR THE SOURCE: The epidemiological investigation has concluded that the most likely source of the outbreak is considered to be indirect contact with wild birds.

EVIDENCE BASE FOR THE SOURCE: This assessment of the source is based on the evidence that: (i) the genetic analyses of the virus indicate a relatively recent introduction from the wild bird population, (ii) there are a number of water bodies in the vicinity where wild fowl have recently been sighted, (iii) there has been a flooded field adjacent to the farm with water fowl sighted in the water, (iv) the IP is in the direct flight pathway between a waste disposal site and Loch Fitty, (v) findings from the official investigation suggest deficiencies in biosecurity on the IP which may have led to introduction of virus, (vi) there is a small risk of fomites (e.g. feathers) being sucked in through the roof of the two houses thought to be the first infected, (vii) there is no evidence suggesting introduction of infection into the houses via direct contact with wild birds, (viii) there have been no other cases of H5N1 identified to date in domestic poultry in the UK, and (ix) there were no poultry or eggs brought onto the IP in the source window and there is also no evidence of contaminated product being brought on.

ASSESSMENT OF POTENTIAL SPREAD: Following extensive investigations, no evidence of avian influenza virus infection has been found on other poultry premises identified as tracings from the IP, or reported on other domestic poultry premises in the United Kingdom.

2. INTRODUCTION / BACKGROUND

This report summarises the epidemiological investigations carried out in order to describe and explain the outbreak of H5N1 Low Pathogenic Avian Influenza (LPAI) infection in broiler breeder chickens on a premises in the administrative territory of Dunfermline, Scotland.

The report will be used to:

(i) Provide evidence to support the UK's position in successfully controlling the outbreak and as a declaration of freedom from H5N1 LPAI to both the EU and OIE and to inform trading partners, in full transparency, with a view to facilitate trade;
(ii) provide source material for the technical annex for UK co-financing claims to the EU;
(iii) record logistics and technicalities of the investigation and disease control to inform future resource planning, contingency plans and training requirements;
(iv) and to highlight gaps in our understanding of notifiable avian influenza and so identify areas for further research or other needs.

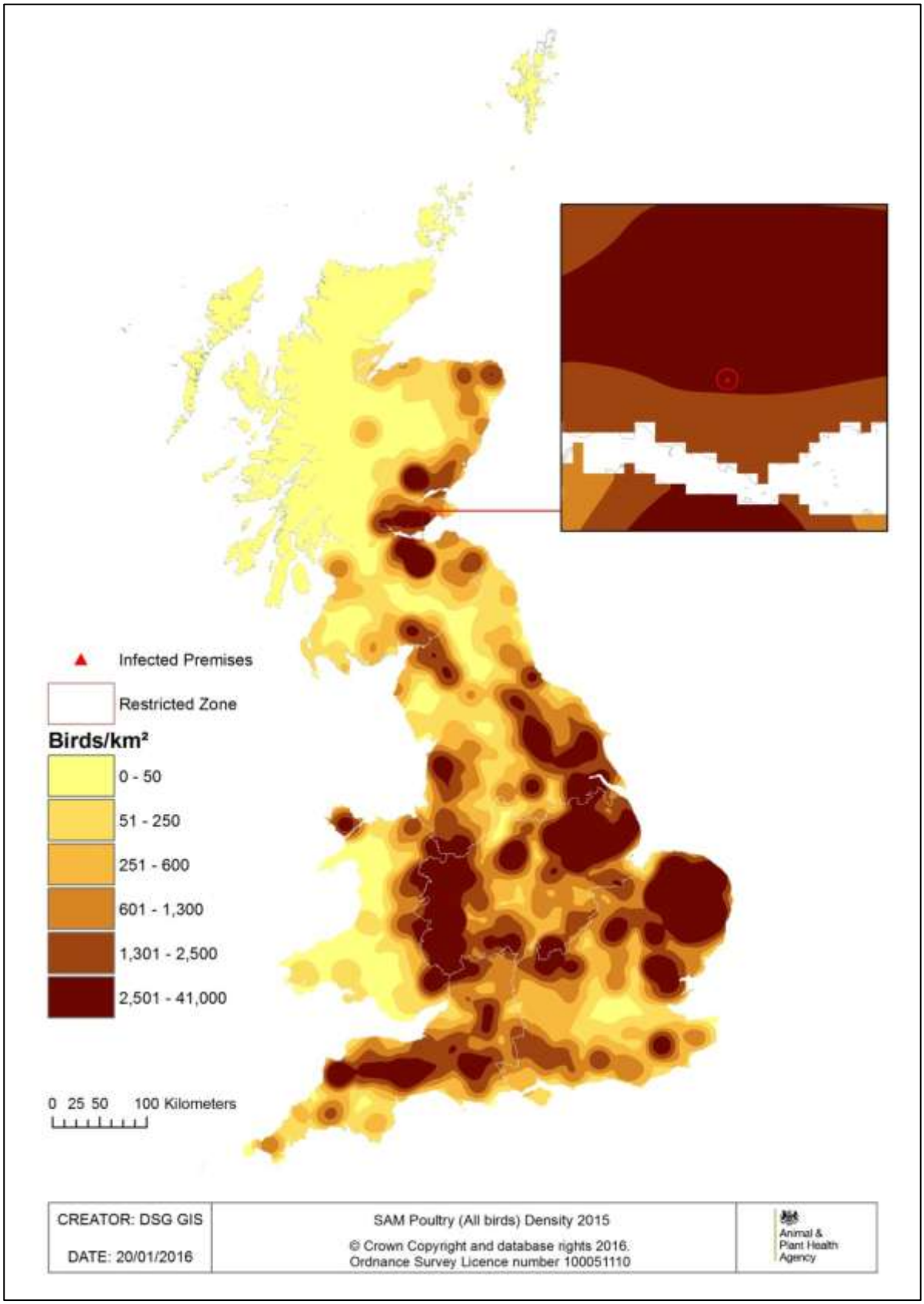
3. DESCRIPTION OF THE INFECTED PREMISES

The Infected Premises (IP) designated as AIV 2016/01, is a 40,000 broiler breeder laying unit, located approx 8km north of the Firth of Forth estuary in the East of Scotland. The birds are owned and managed as a standalone unit. It operates as an intensive (indoor), barn style production system, and eggs for hatching are collected three or four times each week and delivered to a hatchery located in Berwickshire, Scotland.

Within relatively close proximity to the premises there are two major bodies of water (Loch Fitty to the east, Town Loch to the south) and a household refuse landfill site (to the west). The farm manager reports that wild birds, especially seagulls, regularly fly over the poultry farm as it is on their direct daily flight path between Loch Fitty and the landfill site. This is an area with a low density of poultry holdings. There are no other poultry premises within a 1 km radius of the IP, and only two other premises within a 3 km radius. It is important to note that the map in Figure 1 shows the holding to be situated in a high density of poultry area, however, this high density of poultry is entirely attributable to the large number of birds on this one holding.

During December 2015, Great Britain experienced high rainfall, considerably above normal levels, and consequent widespread flooding. In the field directly adjacent to the poultry sheds, within 50 metres, a flooded area has been present for approximately one month prior to the outbreak. The farm manager reported that he has observed wild waterfowl, especially mallards, roosting in this area and making use of this 'temporary pond'.

Figure 1: Map to show location of the IP and density of poultry



Note: The **poultry density** map was created using an extract of APHA's Sam database as at April 2015. Premises with less than 50 birds are likely to be under-represented as poultry registration is only mandatory for premises with 50 or more birds. Premises with less than 50 birds are encouraged to register and so a

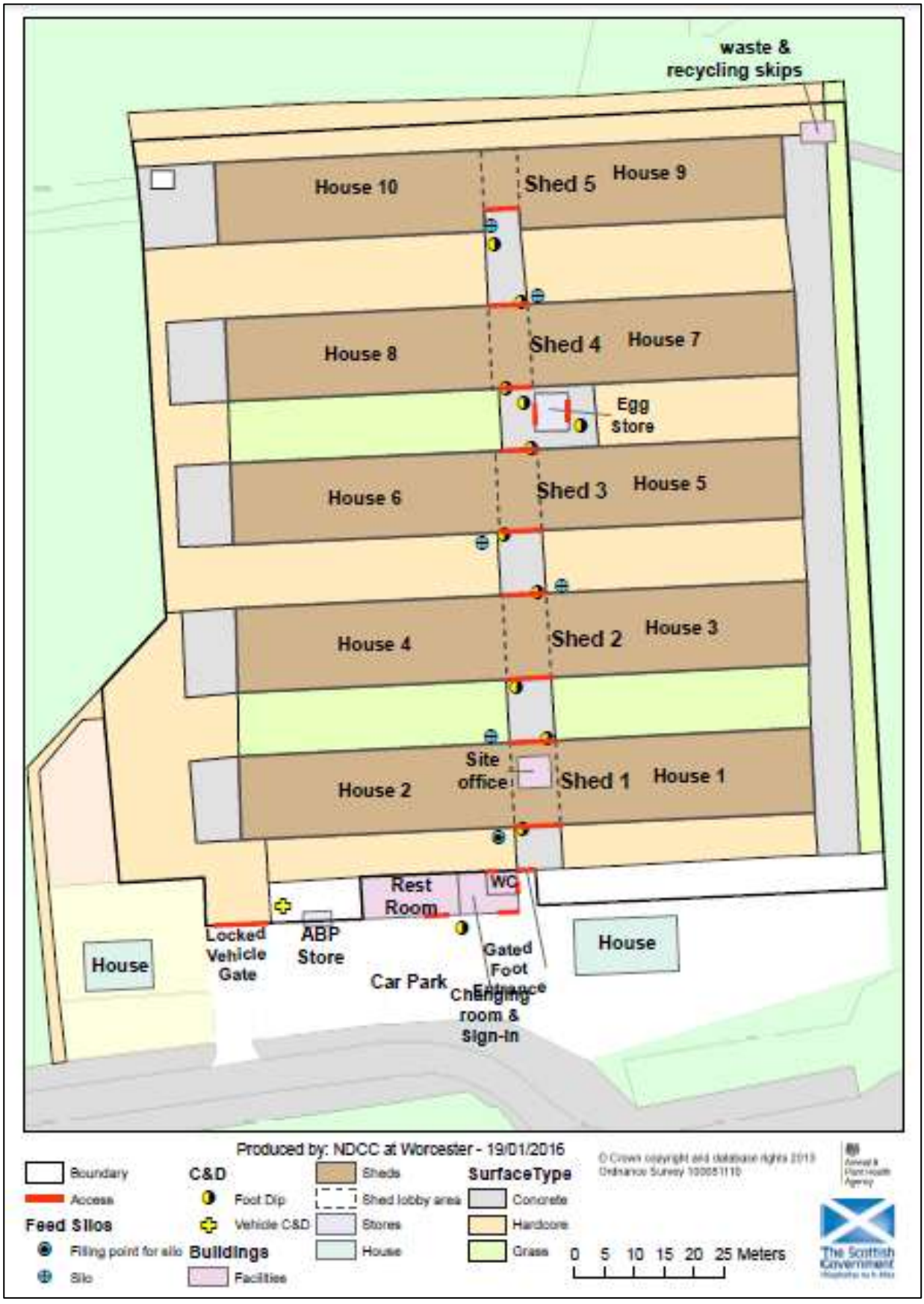
proportion of these premises will be included within the Sam extract. In the event of an outbreak, additional premises may be identified as a result of intensive foot patrols. The density of birds in GB was performed using the kernel density function in ArcGIS using a 20km search radius and output cell size of 1km. The data is classified into six quartiles and the map is suitable only for demonstrating relative density across GB.

Figure 2: Map showing the Infected Premises (IP) and Restriction Zone (1km)

The birds were 57 weeks of age at the time of diagnosis of LPAI, having been placed at 19 weeks of age in April 2015 from a rearing premises in Fife. There is an all-in, all-out policy, with the result that no live birds had moved onto or off the premises since placement of the flock nine months previously.

There are five sheds on the IP, each of which are divided into two poultry houses (numbered 1 to 10) with a central processing lobby. The ten houses were each populated with approximately 4,000 birds. All sheds have fan ventilation, but only houses 9 and 10 have roof inlet ventilation. The birds were fed meal, which is stored securely in sealed feed bins between the sheds, and water from the mains supply.

Figure 3: Diagram detailing IP site



Site processes and biosecurity

Each house has central nest boxes and an egg collection belt which runs into the associated processing lobby. The five lobbies are connected by external concreted walkways, via which the egg collection trollies transfer the eggs to the egg store located near the centre of the site. Site personnel are expected to disinfect their footwear on entry to and exit from each shed. The egg trolley wheels are not disinfected and so present a potential risk pathway for fomite transfer to and from the lobby areas and their external connecting pathways. The floor of the egg store is cleansed and disinfected weekly, but is never fully empty of trolleys.

Dedicated staff work in each of the five sheds (each containing two houses) and are responsible for ‘walking’ the poultry houses to inspect the birds and environment, collecting and cleaning floor eggs, and hand grading all eggs onto trays which are then placed on trolleys. Broken eggs are collected into buckets and disposed of as animal by-product (ABP) waste, along with any poultry carcases. The staff are required to put disposable covers over their footwear on entry to the poultry houses from the lobbies.

There is a power hose for cleansing and disinfecting vehicle wheels at the entry to the site, and delivery drivers are provided with dedicated site wellies and disposable boiler suits at the visitor sign–in room.

A covered ABP store containing plastic barrels crosses the premises boundary. Dead birds are transported in plastic bags and are top-loaded into the barrels from the premises side of the boundary. Broken eggs are placed in a separate barrel. The ABP collector removes the barrels from a side entry door of the ABP store without entering the premises, and replaces them with cleaned and disinfected, empty barrels.

4. TIMELINE OF KEY EVENTS

Table 1: Timeline of key events

Date	Significant event
02/01/16	Clinical signs noted in house 10 at IP
05/01/16	House 10 begins to recover; House 9 onset of first clinical signs: Private Vet. (PVS) requests permission to submit samples for exclusion testing purposes. Blood samples collected by PVS from house 10
06/01/16	Samples collected from houses 9 & 10 to exclude notifiable avian disease
08/01/16	M gene confirmed on exclusion testing sample: Verbal restrictions in place.
09/01/16	Report case: APHA veterinary inquiry commences. Restriction notice served. Official samples submitted from houses 7, 9 & 10. House 7 showing clinical signs
10/01/16	Hatchery visit by APHA. On IP, marked egg drop in House 8. Temporary Control Zone declared.
11/01/16	Hatchery placed under restrictions. Inappetence in houses 5 and 8. Samples collected from houses 5 and 6
12/1/16	Inappetence in house 6
13/1/16	Houses 1 and 3 lethargic. Samples collected from houses 1, 3, 7 and 8
14/1/16	Voluntary cull of eggs at hatchery. On IP, House 2 lethargic. Samples collected from houses 2, 4, 9 and 10.
19/1/16	Cull completed on IP
21/1/16	Preliminary C & D completed on IP

5. INVESTIGATIONS ON THE INFECTED PREMISES

Clinical signs (inappetence and pale eggs) were first noted in house 10 on 02/01/2016. The Private Veterinary Surgeon (PVS) reported that clinical signs were beginning to resolve in house 10 by 05/01/2016, but had developed in house 9.

On 05 & 06/01/2016 the PVS collected samples from houses 9 & 10 for testing to exclude (TTE) the presence of Notifiable Avian Disease. Following reporting of non-negative results on PCR on samples from house 9 (08/01/2016), restrictions were imposed, an official APHA veterinary inquiry visit to the holding was undertaken (09/01/2016), and further official samples were collected from houses 7, 9 and 10.

At the time of the visit on 09/01/2016, clinical signs and a drop in egg production were also observed in house 7. Inappetence was reported on 11/01/2016 in houses 5 & 8, and on 12/01/2016 in house 6. On 13/01/2016 birds from two further houses (1 & 3) and on 14/01/2016 birds from house 2 were reported to be quiet and lethargic.

The results of serological (Haemagglutinin Inhibition Test (HIT) with standard H5N3 antigen) and virological (Nagy PCR) testing of the different houses at various time points is summarised in table 4 in Appendix 1.

An Expert Ornithology Field Assessment was carried out on and around the IP on 13/01/2016. Whilst there were several fly-overs for wild birds across the IP, particularly herring gulls and corvids, there were no roosts observed on the IP itself to suggest that the presence of wild birds was a regular occurrence. In the 1 km restriction zone around the IP, there were several flocks of gulls and starlings, while further afield there were flocks of pink-footed geese on a nearby loch and whooper swans at a nearby reserve, both of which were observed flying over the 1 km zone. The overall likelihood of direct contact with wild birds as the source of LPAI is considered to be low (albeit heightened) and is the same likelihood as for anywhere in the UK at the current time, while the likelihood of spread off the IP to other poultry in the surrounding area is considered to be very low.

Timeline of introduction and progression of infection in the houses on the IP

The analysis of the laboratory results and the production data from the IP, considered alongside the clinical history, suggest the following:

1. **House 10 was the first to be infected on the IP**. There was a marked drop in egg production noted on 02 January and evidence of historical infection at the time that the first samples were collected from the house, i.e. the blood samples collected by the PVS on 05 Jan. This may indicate likely introduction of infection around 26 December.

2. Infection then moved to birds in house 9. There is evidence of acute infection of the birds in that house from the results of samples collected under the testing for exclusion scheme (TTE) on 06 January, with a rise in seroprevalence and decline in PCR positive birds between the report case sampling on 09 January and cull sampling on 14 January. Infection then moved to house 7 (evidence of acute infection on 09 January at report case sampling and increasing levels of seropositivity and no PCR positive birds at cull sampling on 13 January).

3. Infection then moved to house 8 which showed a marked drop in egg production on 10 January: a few birds were seropositive (10%) and PCR (+ve) (20%) at cull sampling on 13 January. Birds in house 5 were probably infected more recently than house 8 (onset of clinical sings on 11 January and evidence of acute infection at pre-cull sampling on 11 January (no seropositives and 55% PCR (+ve)). House 6 was probably infected around the same time as, or after, house 5. Clinical signs in house 6 were observed on 12 January and that house had higher levels of PCR (+ve) (i.e. 70%) than house 5. There were no clinical signs seen in birds from house 2. At pre-cull sampling on 14 Jan there was evidence of early infection (no seropositives and 20% PCR (+ve), so house 2 was likely to be the last house to be infected on the IP. There were no clinical signs reported from houses 1, 3 and 4 and samples at cull taken from those houses on 13th (houses 1 and house 3) and 14th (house 4) January respectively, were negative for serology and PCR. This is indication that birds in these houses were not infected above the design prevalence of 10%.

4. **The available evidence to date supports the introduction of virus into house 10 on, or around, 26/12/2016** after which there was progressive spread within and between houses, up until the point of depopulation by which time seven out of ten houses had become infected.

6. OVERVIEW OF TRACING ACTIVITIES

Evidence based on the clinical picture, laboratory results and expert advice, together with the OIE requirement for a precautionary assumption of a 21 day incubation period prior to clinical signs, gave the following source and spread time windows:

- **Source window:** Most likely date of introduction of infection is around **26/12/2015** with a maximum precautionary source period over which tracings were investigated from 11/12/2015 to 31/12/2015, a day before the precautionary start date for onset of clinical signs.

- **Spread window:** Most likely potential for spread from the premises is between **27/12/2015 – 09/01/2016** (when statutory disease control restrictions were imposed), with a maximum precautionary spread period over which tracings were investigated extended back to 12/12/2015.

There were a large number of personnel and other contacts within the company and with other businesses. All contacts to and from the IP in the whole period from 11/12/2015 to 09/01/2016 were identified and assessed as to their risk of introducing or spreading infection (see Tables 2 and 3, and Figure 4).

As a result, visits were recommended for:

1. seven poultry premises linked to the IP via egg collection and bedding delivery;
2. one ABP plant receiving carcases from the IP; and
3. the single hatchery that had received hatching eggs from the IP.

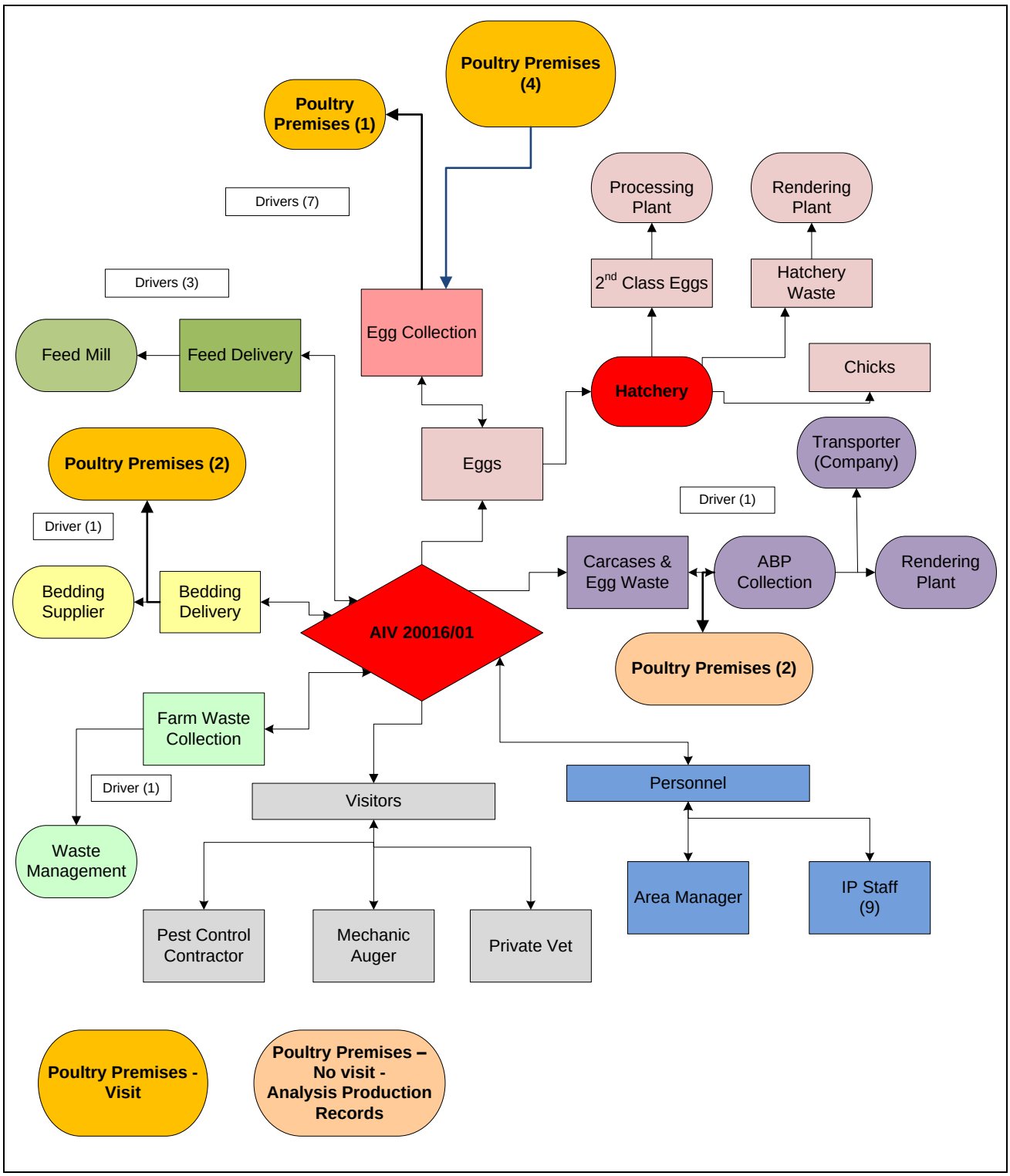
Multiple telephone and email enquiries were generated to confirm information about the different tracing investigations linked to the IP and to inform the risk assessments.

A detailed veterinary risk assessment of the hatchery and the eggs consigned to it during this period was carried out; this was informed by a visit to the hatchery including inspection of records, procedures and biosecurity protocols, in addition to expert virological advice from the Reference Laboratory at Weybridge (see section 13).

A number of potential tracings due to movements of personnel, vehicles and carcases were considered to represent a very low to negligible risk as a result of biosecurity protocols in place for these risk pathways, non-contact with susceptible poultry during the risk period and/or the very low or negligible risk pathways as concluded during the veterinary risk assessments. No further action was considered necessary in respect of these. A breakdown of numbers and types of tracings investigated and assessed is provided at Appendix 2.

The seven poultry premises identified above were considered to be at low risk of onward disease transmission, but were visited in order to establish the degree of contact with the IP and subjected to clinical inspection and record checks with negative findings. One of these premises required further investigation due to increased chick mortality. An overall assessment, including production record checks by staff experienced in analysing poultry production records, concluded that the likelihood of the mortality at this premises being due to spread of LPAI from the IP was very low, no further investigation was required and restrictions were lifted.

Figure 4: Source and spread tracings related to the IP



7. SOURCE INVESTIGATIONS - HYPOTHESES FOR SOURCE

For any outbreak of avian notifiable disease, the source of infection may be related to (i) introduction of live birds from infected flocks, (ii) introduction of infected or contaminated products, (iii) contact with infected wild birds (directly or via fomites) or (iv) contact with contaminated equipment (fomites) including bedding.

A summary of the sources of infection considered is shown in table 2 with definitions of the qualitative risk terms provided in appendix 3.

Table 2: Possible source of infection for the Infected Premises AIV 2016/01, source tracing window 11/12/2015 – 31/12/2015:

Pathway	Comment	Assessment of likelihood of infection via this route
Direct introduction from wild birds	No reports of wild birds in sheds and access unlikely.	Very low likelihood Low uncertainty
Indirect introduction from wild birds	1. Large numbers of waterfowl, wader and gulls present within a few km of the IP within the 14 days prior to restrictions being served, including Loch Fitty and the Firth of Forth. 2. IP in direct flight path of gulls between Loch Fitty and waste disposal site. 3. A fishing lake is situated within 0.5 km of IP. 4. IP is not far from the coast. 5. Flooded field harbouring wild waterfowl adjacent to the IP (for approx. 1 month), approx.50 meters from houses 7, 9 and 10. 6. Small risk of fomite (feathers etc.) being sucked in through roof ventilation system (only houses 9 & 10). 7. The egg trolley wheels are not disinfected and so present a potential risk pathway of fomite transfer to and from the lobby areas and their external connecting pathways into the poultry houses. 8. Cats roaming on site but not believed to enter the sheds.	High likelihood Low uncertainty
Undisclosed infection in the	1. The birds on the IP were placed in	Negligible likelihood Low uncertainty

Pathway	Comment	Assessment of likelihood of infection via this route
UK: Direct introduction by purchased birds	April 2015 at 19 weeks of age. 2. All-in, all-out policy (no live birds moved on or off the premises since placement).	
Undisclosed infection in the UK: Indirect contact with an infected flock (Please note that there is medium uncertainty (see table 10) associated with the likelihood of undisclosed infection due to the clinical signs of LPAI not being readily apparent)	1. Recent analysis of the UK AI poultry survey supports that there has been no undisclosed infection in poultry flocks in the UK.	Very low likelihood Low uncertainty
	2. No clinical signs consistent with the propagating disease profile expected of an LPAI introduction presented at any traced premises visited.	Very low likelihood Medium uncertainty
	3. Personnel & visitors - movements of area manager, staff, PVS, pest control contractor and auger technicians.	Very low-negligible likelihood Medium uncertainty
	4. No poultry kept by staff members at home. Uncertainty regarding other possible contact with susceptible species outside of work e.g. via hobbies, visits to other premises with susceptible species etc. and recent foreign travel. No clear biosecurity strategy for workers on site.	Very low likelihood Medium uncertainty
	5. Feed delivery – Feed delivered every 2-3 days directly from feed mill and straight into silos from delivery lorry via a hose. Potential spread between sheds via hose or driver.	Very low likelihood Medium uncertainty
	6. Water – Mains water. Stored in movable covered storage tanks.	Negligible likelihood Low uncertainty
	7. ABP collection – ABPs collected from locked shed on perimeter of IP. ABP lorries and drivers do not	Very low to negligible likelihood

Pathway	Comment	Assessment of likelihood of infection via this route
	come onto the site. Possibility for introduction of infection via contaminated empty barrels delivered to the IP.	Medium uncertainty
	8. Egg collection – Driver drives around perimeter of sheds to get to the egg store. No cleansing and disinfection (C&D) of lorries or lorry ramp. Lorry wheels have C&D on entry/exit of IP. Driver wears site dedicated wellies and disposable protective clothing but wears multiple-use gloves.	Low to very low likelihood Medium uncertainty
	9. Bedding delivery – Bedding (bales of wood shavings wrapped in plastic) delivered periodically on pallets to outside of sheds and then manually moved into shed lobbies by hand.	Negligible likelihood Medium uncertainty
	10. Other waste - Only one visit was carried out by the waste company (on 15/12/2015) to empty the recycling skip on the IP and leave it on-site empty.	Negligible likelihood Low uncertainty
Infection elsewhere in the world: Direct contact with an infected flock or wildfowl outside GB.	1. No recent trade into the IP of live birds or hatching eggs/day old chicks.	Negligible likelihood Low uncertainty
	2. Wild bird incursion from migratory birds-migration mostly occurs before January. Weather related movements from the south (e.g., France) are unlikely in winter. Large scale weather driven migration unlikely in the high risk source tracing window.	Low likelihood Medium uncertainty
Infection elsewhere in the world: Indirect	Incursion via trade in contaminated poultry products cannot be ruled out. H5 LPAI has caused outbreaks in	Very low likelihood Medium uncertainty

Pathway	Comment	Assessment of likelihood of infection via this route
contact with an infected flock or wildfowl outside GB	Germany, Italy, France and the Netherlands in commercial poultry since January 2015. H5N1 HPAI has been reported from commercial ducks in France. Virus of various H5 and H7 strains (among others) are likely to be circulating in wild birds across the EU. Common progenitor from recent outbreaks in Italy and the Netherlands (probably derived from a similar wild bird ancestral virus).	Low likelihood Medium uncertainty

8. ASSESSMENT OF THE LIKELY SOURCE

The most likely source of the outbreak is considered to be indirect contact with wild birds. This assessment is based on the following key pieces of evidence:

1. The **genetic analyses of the virus** indicate a relatively recent introduction from the wild bird population into poultry.

2. There are **a number of water bodies** in the vicinity of the IP where wild fowl have recently been sighted.

3. There has been **a flooded field** adjacent to the farm (approximately 50 metres from the houses containing the birds that first showed clinical signs) for approximately one month prior to the onset of clinical signs of disease, with water fowl sighted in the water.

4. The **IP is in the direct flight pathway** between a waste disposal site and Loch Fitty.

5. Findings from the investigation suggest some potential **deficiencies of biosecurity on the IP** (e.g. egg trolley wheels not disinfected, mice seen in the poultry houses) which may have led to introduction of virus into the poultry houses.

6. There is a **small risk of fomites (e.g. feathers) being sucked in** through the roof of the two houses in which birds first presented with clinical signs, via the ventilation system.

7. There is **no evidence** suggesting introduction of infection into the houses via **direct contact with wild birds**.

8. There have been **no other cases of H5N1 identified to date in domestic poultry** in the UK despite raised awareness following confirmation of disease and the recent H7N7 HPAI outbreak, tracings investigations undertaken, and the on-going passive surveillance programme (with a legal requirement to report suspicion of avian influenza to APHA). This is supported by the results of the recent UK AI poultry survey.

9. There were **no poultry or eggs brought onto the IP in the source window** and there is also no evidence of contaminated product being brought on).

9. SPREAD INVESTIGATIONS - POTENTIAL AND PROBABILITY OF SPREAD

The 2015 Hampshire LPAI outbreak was used as a model to demonstrate the effectiveness of a risk-based approach to guide and inform a proportionate response to links between the IP and other poultry premises. This ensured that appropriate action was taken, reduced the impact on the industry and saved APHA resources with fewer visits and fewer premises being placed under restrictions. In addition to a veterinary risk assessment initiated on 09/01/2016 for eggs taken to the hatchery from the IP, nine further risk assessments (see table below) were started on 14/01/2016, to assess the probability of risk pathways from other poultry premises being a route for source of infection to the IP, and risk pathways to other premises being a route for spread from the IP. This section discusses the outcomes of these risk assessments regarding spread from the IP.

Spread windows as determined from tracing timelines:

Precautionary (OIE)	12/12/2015 to 18/12/2015
Likely	19/12/2015 to 26/12/2015
High risk	27/12/2015 to 09/01/2016

Hatchery: The overall likelihood of external contamination of eggs from the IP with virus, resulting in the subsequent infection of chicks at hatching, was concluded as being low for the high risk period.

The following: (i) **Feed deliveries**, (ii) a **non animal by-product waste** collection (materials for recycling), (iii) visits by the **company area manager**, (iv) **IP staff,** (v) **private veterinary surgeon**, (vi) **pest control** man, and (vii) **feed auger technician** – were all assessed to pose very low or negligible overall likelihood for the spread of virus to poultry on other premises. No further action was recommended for these.

For **egg collections** and wood shaving **bedding deliveries** a low likelihood level was concluded and visits were recommended for three premises (one in the egg collection round and two linked to the bedding delivery because of uncertainties regarding cleansing and disinfection of a forklift vehicle carried on the lorry). The egg collection linked farm was the subsequent premises visited by the collection lorry following each IP egg collection. The lorry was fully cleansed and disinfected at the hatchery before being used for any further egg collections or chick deliveries. The premises was targeted through early rapid risk assessment as a spread tracing and visited shortly after confirmation of disease.

The risk pathway associated with **animal by-product collections** was assessed as posing a very low level likelihood for spread of virus from the IP. However, because of uncertainties surrounding destination premises specifically the siting of the respective ABP stores (whether on the periphery of the premises or not), and if biosecurity protocols were followed, a recommendation was made for telephone call inquiries to be made for nine premises identified as being within the high risk tracing window, to gather information to further inform the risk assessment. As a result of these inquiries an analysis of production records was carried out for two poultry premises which had been visited by the same ABP collector that visited the IP within the high risk tracing window. These analyses concluded that there were no significant production data changes that would suggest incursion of LPAI onto those two poultry premises during the time period examined.

Table 3: Summary of veterinary risk assessment conclusions for spread and the associated recommendations for action to be taken.

Contact Categories	Outcome of VRA for Spread risk
Egg Collection	Low
Feed Delivery	Very low
ABP Collection	Very low.
Bedding Delivery	Low
Company Area Manager	Very low
Personnel - IP Staff	Very low
Private veterinary surgeon	Negligible
Pest Control man & Auger Technician	Very low
Other Waste Collection	Negligible

A final veterinary risk assessment was carried out to look at the necessity for revisiting any of the spread premises. The time interval between IP contact and date of APHA visits or production record analysis for these spread premises was calculated – see table 4. The veterinary staff who visited the bedding delivery spread premises assessed the transmission pathway as not a credible means of spread. Therefore, the shortest time interval between IP contact to visit (or production record check eligible for consideration) was 13 days for the farm subsequent to the IP in the egg collection round. Expert opinion (Prof Ian Brown, Head of Virology, APHA) was that from observations of virus behaviour on the IP it can be deduced that on a traced premises, spread would occur within a few days and substantially less than 13 days. It was therefore concluded that none of the visits or records analyses were carried out too early to ensure the detection of signs of disease spread. No further action was recommended.

Table 4: Summary of interval between IP contact and spread tracing visit or production record check.

Type of Tracing	**Premise**	**Last date of IP contact**	**Date of visit/production record check**	**Time interval between IP contact and visit/records check**
Egg collection	Farm 1	31/12/2015	13/01/2016	13 days
Bedding delivery	Farm 2	06/01/2016	17/01/2016	11 days
Bedding delivery	Farm 3	06/01/2016	17/01/2016	11 days
ABP collection	Farm 4	04/01/2016 and 06/01/2016	Records: 13/12/2015-21/01/2016	15 days
ABP collection	Farm 5	6/1/2016	Records 18/12/15 - 21/01/2016	15 days

10. SURVEILLANCE IN THE RESTRICTION ZONE

There were no commercial poultry premises within a 1 km radius of the IP, and therefore no further surveillance was required to be conducted.

11. ANALYSIS OF THE VIRUS

Using a range of laboratory tools/analyses we can conclude that this H5N1 low pathogenicity avian influenza virus is a conventional European lineage virus exhibiting biological properties consistent with contemporary LPAI viruses detected in the EU on a regular basis in domestic poultry, whose closely related progenitors are maintained in wild waterfowl populations.

Genetic analyses of haemagglutinin (HA) gene reveals the LPAIV phenotype with a typical European cleavage site motif (PQRETR/GLF). The intravenous pathogenicity index test in chickens confirms this classification. Furthermore phylogenetic analysis of the HA gene of A/chicken/Scotland/532/2016 (H5N1) LPAIV shows that it is closely related, but distinguishable from other contemporary European H5 LPAI viruses from Italy in 2015 (H5N2), Netherlands in 2014 (H5N1) and 2013 (H5N3), Scotland (wild bird – Razorbill) in 2014, Iceland in 2013 (H5N1) and China in 2011 (H5N2).

The A/chicken/Scotland/532/2016 (H5N1) virus is clearly distinguishable from viruses associated with the ongoing epidemic in France. In addition full genome analysis reveals the virus is a ‘classical European low pathogenic avian influenza virus’ that is distinguishable from other contemporary H5 viruses including the group of HPAI viruses that have caused a global panzootic in the last 10 years.

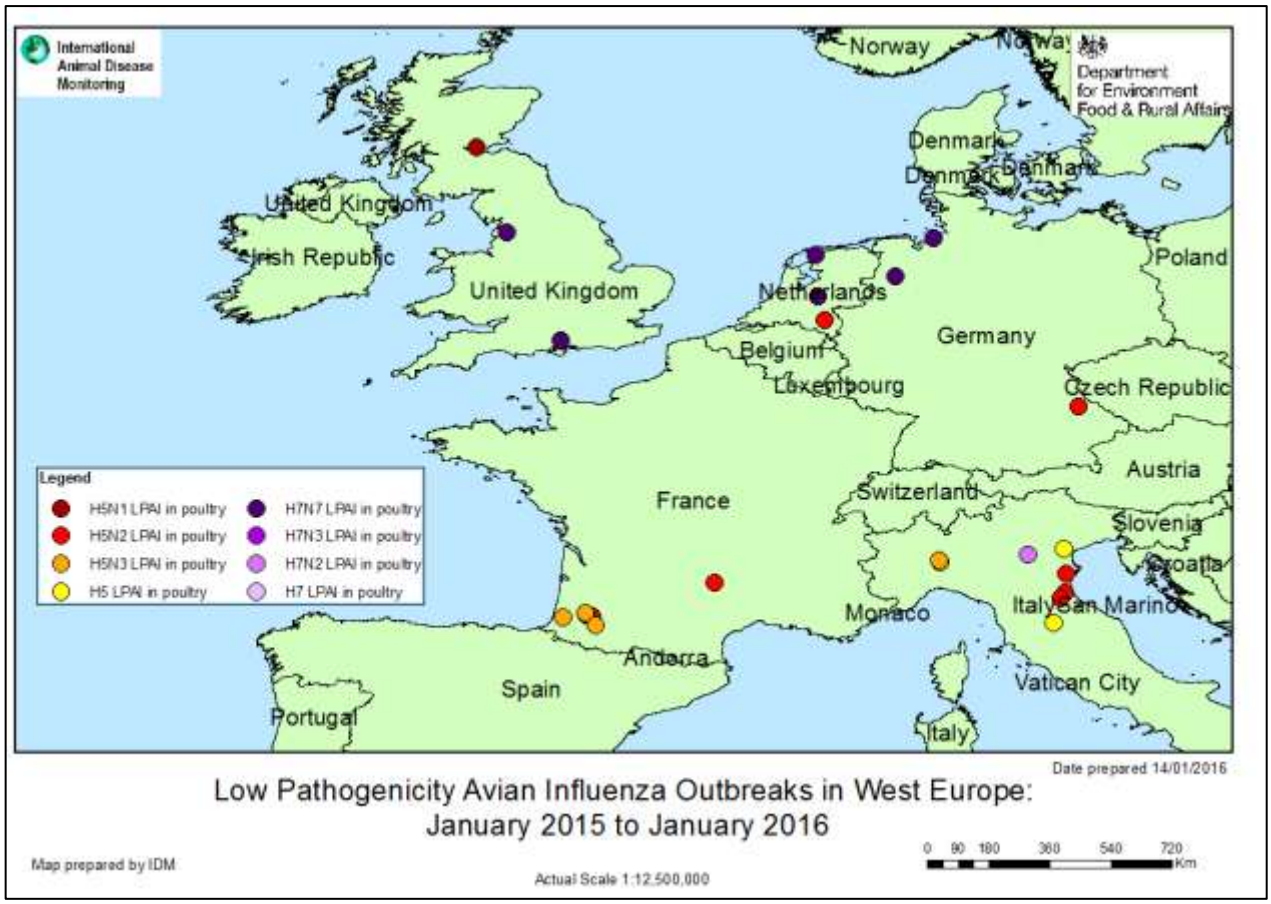
The preliminary genetic analyses conducted by the EU/OIE/FAO Avian Influenza Reference Laboratory (APHA-Weybridge-UK), on the genome of the H5N1 virus to inform estimation of the predicted zoonotic risk, suggests that all eight genes are of avian origin, without any specific increased affinity for humans. The virus does not exhibit any genetic traits suggestive of resistance to antiviral drugs offered to operatives working on the outbreak. None of the major mutations for mammalian adaptation are present including those required for α2-6 receptor binding.

15. INTERNATIONAL CONTEXT

The likelihood of introduction of virus from a poultry source in Europe is considered to be very low; whereas the likelihood of the introduction of virus by direct or indirect contact with wild birds, whether European or UK origin, is considered to be low.

Since January 2015, H5 LPAI has caused outbreaks in Germany, Italy, France and the Netherlands in commercial poultry (see Figure 5: Low pathogenicity avian influenza outbreaks in Western Europe below).

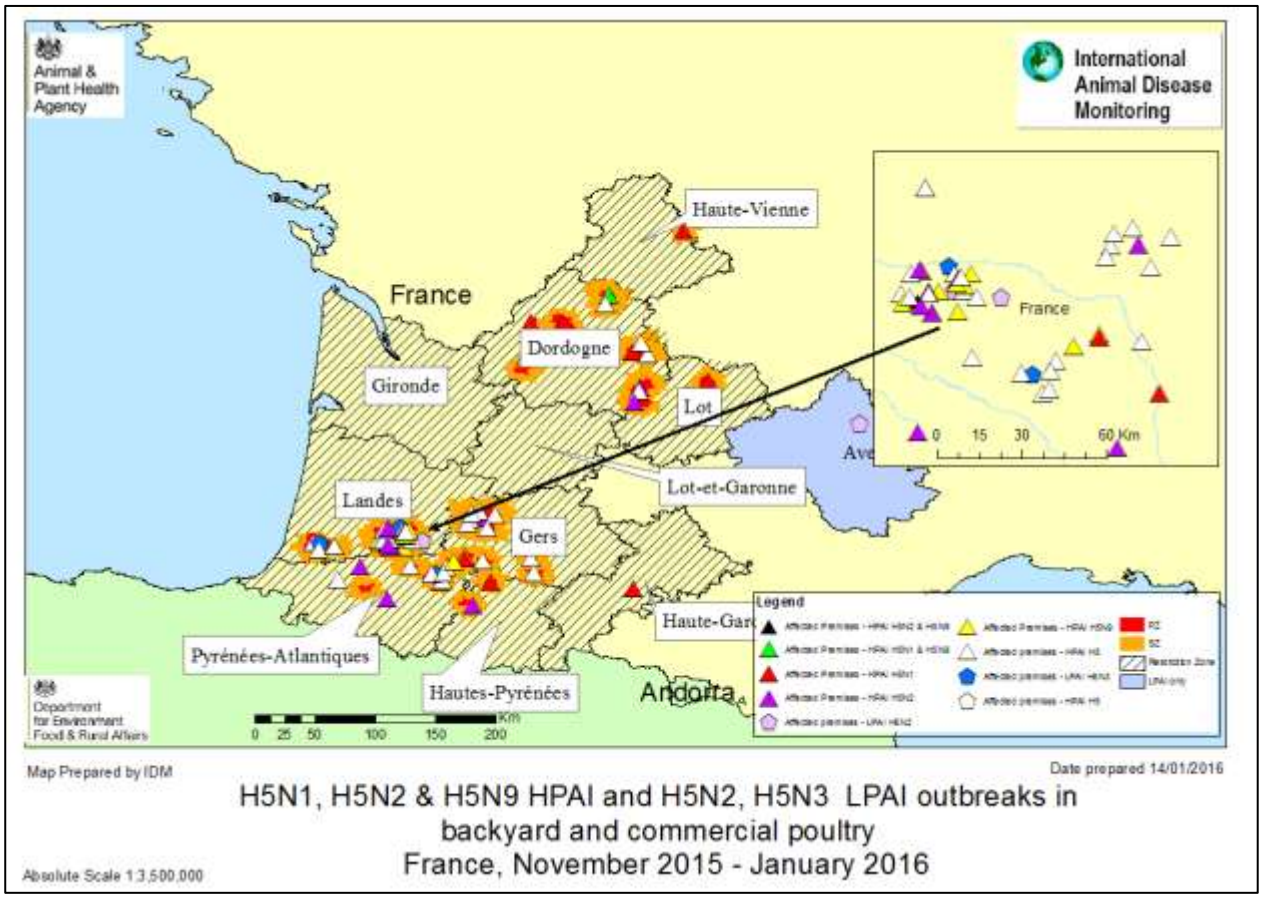
Figure 5: Low pathogenicity avian influenza outbreaks in Western Europe



Low pathogenicity avian influenza viruses of various strains are likely to be circulating in wild birds globally. Therefore, the risk of introduction into domestic poultry will depend on the prevalence and pattern of shedding in wild birds, the level of biosecurity on the holdings and many other factors.

Phylogenetic analyses suggest the viruses isolated from the European outbreaks are very similar (see section on virus analysis/genetic sequencing) and this current H5N1 LPAI virus is likely to be a recent wild bird introduction.

Figure 6: Outbreaks of LPAI in France, November 2015 to January 2016



The level of virus present in the wild bird population is very difficult to assess by routine passive surveillance of wild birds found dead, as this virus causes low pathogenicity in domestic chickens and is not likely to have any significant mortality in wild birds.

According to the investigations on the IP there have been no consignments destined for international trade and no recent imports of live poultry.

The possible source being poultry products from Europe is considered unlikely as disease control measures on commercial poultry premises with notifiable avian influenza infection have generally been rapidly applied and effective.

16. PUBLIC HEALTH IMPACT

Health Protection Scotland (HPS) led an Incident Management Team (IMT) which undertook a continuous risk assessment of the incident following initial suspicion, confirmation and subsequent management of H5N1 LPAI concluding that the risk to the general public was very low – on the basis that there have been no reported cases of human infection with H5N1 LPAI, and the low probability of exposure to infected birds.

The HPS led IMT determined the risk to persons occupationally exposed to H5N1 LPAI (i.e. workers on the IP) to be slightly higher than the general public but still low. HPS provided antiviral prophylaxis and health surveillance to farm staff (and household members of those staff resident on the farm), those directly involved in handling and culling the affected flock and at the identified rendering plant. They provided support to APHA with advice on the need for appropriate Personal Protective Equipment (PPE).

The Food Standards Scotland (FSS) advised that on the basis of current scientific evidence avian influenza does not pose a food safety risk for UK consumers (http://www.foodstandards.gov.scot/news/bird-flu-avian-influenza-advice-food-standards-scotland).

Furthermore, genetic analyses of the virus at APHA Weybridge failed to reveal any mutations known to increase affinity for human infection.

17. REMAINING UNCERTAINTY

1. There is no evidence to suggest that the IP was not the primary case. All available evidence suggests that the IP was the primary case and the level of uncertainty of this is low following completion of the epidemiological inquiry.
2. The source of the LPAI virus and route of introduction into the IP. The most likely hypothesis for introduction of infection remains indirect contact with wild birds.
3. There is a continually present, albeit considered low, risk of further outbreaks of avian influenza (not limited to H5N1 LPAI) as a result of the ongoing presence of AI viruses within the wild bird population throughout Europe, and there is ongoing AI surveillance (both active and passive) in the UK aimed at early detection of such an incursion.

18. CONCLUDING REMARKS

The most likely source of infection is considered to be indirect contact with wild birds. Genetic analysis of the LPAI virus identified on the holding and other epidemiological / laboratory data gathered from all groups on site indicate a relatively recent introduction from the wild bird population.

Investigation of tracings from other premises identified as potential sources have revealed no other premises that could have been the origin of the LPAI infection for the IP. Investigations of similar spread tracings have not revealed any spread of LPAI virus from the IP to other premises.

Although our investigations suggest that the most likely route of introduction of virus onto this infected premises was indirect contact with wild birds, an incursion such as this remains a low likelihood event.

National Emergency Epidemiology Group
04 March 2016

Acknowledgements

The views expressed in this report are those of the National Emergency Epidemiology Group (NEEG). However, we wold like to express our thanks to the members of the Ornithological Expert Panel and the many colleagues who have assisted with this on-going investigation.

The NEEG is comprised of staff from APHA's Veterinary, Operations and Science Directorates.

19. APPENDICES

Appendix 1: Tables summarising selected lab analyses

Table 5: Bird level H5 serology and PCR/shedding results for all samples

House	Date of sampling	Time of sample	Serology				PCR
			No. tested	% Neg	% Inc	% Pos	% Pos
1	13/01/16	Cull	20	100	0	0	0
2	14/01/16	Cull	20	100	0	0	20
3	13/01/16	Cull	16	100	0	0	0
4	14/01/16	Cull	20	100	0	0	0
5	11/01/16	Pre-cull	4	100	0	0	55
6	11/01/16	Pre-cull	2	100	0	0	70
7	09/01/16	Report case	20	100	0	0	90
7	13/01/16	Cull	18	0	0	100	0
8	13/01/16	Cull	20	90	0	10	20
9	06/01/16	TTE*[1]	4*[2]	n/a	n/a	n/a	100
9	09/01/16	Report case	20	0	95	5	20
9	14/01/16	Cull	20	0	0	100	5
10	05/01/16	Pre-TTE	18	5.6	0	94.4	n/a
10	06/01/16	TTE	4*[2]	n/a	n/a	n/a	0
10	09/01/16	Report case	20	30	0	70	0
10	14/01/16	Cull	20	0	0	100	0

*[1] TTE samples = Testing for exclusion
*[2] PCR pools only

Appendix 2: Details of tracings and stock numbers in zones

Details of tracings

The tables below are calculated from data taken from APHA Cardiff Specialist Services Centre (SSC) Tracing Team records on 2nd February 2016. This data describes the pathways and tracing locations investigated by the outbreak tracing team to identify premises from where the LPAI infection may have arrived into the IP (back-tracing for source) and identify premises where there may have been onward spread of infection (forward-tracing for spread) from the IP, thus preventing further spread. In the text, the word "location" is used as catch-all encompassing term that includes poultry premises, premises where there was no susceptible stock or linked-locations that could potentially spread the pathogen

Veterinary risk assessments were carried out to determine the level of risk associated to the different risk pathways either for source and/or spread (Section 7 and 10). These were supported by tracing activities involving data gathering and data verification (record checks, telephone interviews, emails, declarations). The outcome of the VRAs indicated which locations to follow for action: forty one (41) locations were considered for investigation of which only ten (10) required further tracing investigations with a field staff visit. The estimated likelihood of exposure for these locations was nevertheless assessed as Low.

Table 6: Number of locations investigated

Method	
Locations investigated and ruled out by non-exposure to susceptible animals	31
Locations investigated and ruled out by clinical inspection (1)	7
Locations investigated ruled out by production data analysis (2)	2
Locations investigated and ruled out by individual VRA (3)	1
Locations identified as contact premises	0
Total Number of locations	41

(1) "Clinical inspection" – see Section 7 (2) "Production data analysis" – see Section 7 (3) Hatchery

Table 7: Outcome of tracing investigation visits

	Total Number
Locations requiring a tracing visit	10
Locations where restrictions were issued	1
Locations where restrictions were lifted at the conclusion of the tracing investigation	1
Locations containing susceptible animals (1)	7
Locations identified as contact premises	0
Locations negated as contact premises	10
Locations remaining under investigation	0

(1) It does not include the hatchery

Appendix 3: Assessment of wild bird incursion

This assessment covers three specific risk questions:

1. What is the likelihood of introduction into the UK, specifically the infected premises, from the currently affected areas in the EU, through the movement of wild birds?
2. What is the likelihood of spread from the single UK infected premises through the movement of wild birds?
3. What is the likelihood of further outbreaks in poultry in the UK occurring through contact with potentially infected wild birds already present in the UK?

On the risk of introduction: The migration of birds to the UK from Europe may be either seasonal, or prompted by adverse weather. Seasonal migration in the autumn by a wide range of species, originating primarily from Scandinavia and Eastern Europe and mostly occurs before January. Weather-driven migration also occurs, and is largely a response to cold conditions. Many bird species, including waterfowl and gulls will move long distances following a prolonged cold spell, typically below -2^{o}C, which is sufficient to freeze waterbodies and deny access to roosting sites. Most weather related migration to the UK comes from the north and east, as birds move southwest to milder areas. Weather related movements from the south (e.g., France) are unlikely in winter, as birds in these locations would be more likely to move further south to seek milder conditions.

The current epidemiological investigation suggests that the infection may have reached the IP sometime between 18 to 31 December with the most likely date between 26 and 31 December. Examination of the Meteorological Office archive has shown that temperatures in Northern Europe were above freezing between 28 to 31 December, suggesting large-scale, weather driven migration was unlikely during this period.

Based on the current incomplete information, there have not been weather conditions consistent with large scale weather driven bird movements from Europe, although late stage seasonal migration may still have been in progress. We therefore consider that the likelihood of the current outbreak being the consequence of the direct movement of infected wild birds from known infected areas in France as being low. The same risk level applies to other areas of Europe. It should be noted that the uncertainty around the circulation of LPAI in wild birds and the likely lack of clinical signs means that we cannot rule out the possibility of further circulation.

On the risk of spread: The immediate area around the buildings of the IP contained low levels of bird activity and few attractants such as food spillages. There was evidence of small birds, such as robins and magpies, in close association with the buildings, but numbers were low during the period of inspection. Access to the buildings by birds such as robins or starlings was possible, although the frequency of use could not be determined. A small number of starlings were seen on the site perimeter. There was no evidence of birds roosting on the roof of the premise during the period of inspection and no signs of regular bird roosting such as accumulated droppings.

The area within the 1km Restriction Zone contained large flocks of loafing gulls and a flock of around 200 starlings was seen passing through the area. Gulls and corvids made regular overflights of the IP. Overall, gulls, crows and starlings were seen in significant numbers in the 1 km zone although no direct contact with the IP itself was observed. **The likelihood of wild birds contracting infection through direct contact with the IP is**

therefore considered to be low with medium uncertainty, as numbers were small in the immediate vicinity of the buildings and the immediate area of the IP contained few attractions to birds; however, the observations were made at a time when there was considerable human activity on the IP, which may have influenced bird usage patterns.

If wild birds in the area were already carrying the infection, then herring gulls are the most numerous species in the area, and are mobile. We observed significant numbers of gulls (>1000) within the 3 km zone and observed gulls moving from near the IP to roost on the Forth outside the 10 km zone. Swans and geese were also present within the 3 km zone, but their wider movements remain uncertain. Starlings were also present near the IP. Their movements remain unrecorded, but they are a species which may gather in very large numbers to roost communally at night. Swans, geese and starlings are all mobile species in winter and could move significant distances in a short period of time.

If local wild birds were already carrying the infection, then many of the locally abundant species were expected to move distances of over 10 km on a daily basis, as observed for the herring gulls, and significant numbers of birds were using the area around the IP. As a consequence **the likelihood is assessed as medium, but with high uncertainty** as the opportunity exists for infected birds to move beyond the current 10 km zone, but there was no direct evidence of infection in wild birds. This assessment relates to the risk of bird movement. **However, as the number of poultry units in the wider region is also low, then this assessment can be reduced to low likelihood**.

On the likelihood of outbreaks in poultry in the UK from infected wild birds potentially present in the UK: We consider there is a constant low risk of wild birds being infected with LPAI viruses at any time of the year in the UK. Given the current strain is a European one, with evidence of recent incursion into poultry, the report does not change our opinion. EU poultry and wild bird surveillance programmes are not designed to pick up the first incursion of avian influenza, but to look for changes in the strains identified and mass mortality events. The EU wild bird surveillance programme identifies a handful of H5 positive wild birds every year, out of the thousands tested; this is therefore considered a constant low likelihood for source of disease incursion into holdings with less than robustly enforced biosecurity.

Appendix 4: Definitions of qualitative risk terms for likelihood and uncertainty

Table 8: Definitions for the qualitative risk terms based on EFSA (2006) and OIE (2004) with expanded descriptions adapted from NHS (2008), IPCC (2005), and Kahn et al., (1999)

Risk level	Definition	Expanded description
Negligible	Event is so rare, does not merit consideration	The chance of the event occurring is so small it does not merit consideration in practical terms (i.e. < 0.1% probability); it is not expected to happen for years;
Very low	Event is very rare, but cannot be excluded	The event is not expected to occur (very rare) but it is possible (i.e. >0.1-1% probability); it is expected to occur at least annually
Low	Event is rare, but does occur	The event may occur occasionally (rare) (i.e. >1-10% probability); expected to occur at least monthly
Medium	Event occurs regularly	The event occurs regularly (i.e. >10-66% probability); expected to occur at least fortnightly
High	Event occurs very often	The event will happen more often than not (i.e. ≥66-90% probability); expected to occur at least weekly
Very high	Event occurs almost certainly	The event will undoubtedly happen (i.e. >90% probability); expected to occur at least daily

Table 10: Qualitative categories for expressing uncertainty given the available evidence; based on definitions within the literature (EFSA, 2006; ECDC, 2011, Spiegelhalter & Riesch, 2011)

Uncertainty category and definition	Type of information/evidence to support uncertainty category
Low Further research is very unlikely to change our confidence in the assessed risk	• Solid and complete data available (e.g. long term monitoring results) • Peer reviewed published studies where design and analysis reduce bias (e.g. systematic reviews, randomised control trials, outbreak reports using analytical epidemiology) • Complementary evidence provided in multiple references • Expert group risk assessments, specialised expert knowledge, consensus opinion of experts • Established surveillance systems by recognised authoritative institutions • Authors report similar conclusions
Medium Further research is likely to have an important impact on our confidence in the risk estimate	• Some but no complete data available • Non peer-reviewed published studies/reports • Observational studies/surveillance reports/outbreak reports • Individual (expert) opinion • Evidence provided in a small number of references • Authors report conclusions that vary from one another
High Further research is very likely to have an important impact on our confidence in the risk estimate	• Scarce or no data available • No published scientific studies available • Evidence is provided in grey literature (unpublished reports, observations, personal communication) • Individual (non-expert) opinion • Authors report conclusions that vary considerably between them

Appendix 5: Estimated timeline and tracing windows

Source and spread windows indicated by purple and yellow shading respectively; darker shades indicate increased probability of source/spread in this time period)

Table 11: Timeline and tracing window

Source Tracing Window	Spread Tracing Window	Date	
Day 21		11/12/15	Start of precautionary source tracing window, as per OIE guidelines (-21d)
Day 20		12/12/15	Start of precautionary spread tracing window (source +24h)
Day 19		13/12/15	
Day 18		14/12/15	
Day 17		15/12/15	
Day 16		16/12/15	
Day 15		17/12/15	
Day 14		18/12/15	Start of likely source tracing window (-14d)
Day 13	Day 1	19/12/15	Start of likely spread tracing window (source tracing window +24h)
Day 12	Day 2	20/12/15	
Day 11	Day 3	21/12/15	
Day 10	Day 4	22/12/15	
Day 9	Day 5	23/12/15	
Day 8	Day 6	24/12/15	
Day 7	Day 7	25/12/15	
Day 6	Day 8	26/12/15	Start of high risk source tracing window (-6d)
Day 5	Day 9	27/12/15	Start of high risk spread tracing window (source +24h)
Day 4	Day 10	28/12/15	
Day 3	Day 11	29/12/15	
Day 2	Day 12	30/12/15	
Day 1	Day 13	31/12/15	
	Day 14	01/01/16	Precautionary date of onset of clinical signs
	Day 15	02/01/16	Onset of first observed clinical signs. Decline in egg production begins in House 10
	Day 16	03/01/16	
	Day 17	04/01/16	
	Day 18	05/01/16	House 10 largely recovered; House 9 first symptoms: PVS carried out PMs; samples submitted for exclusion testing purposes
	Day 19	06/01/16	Samples submitted for exclusion testing purposes
	Day 20	07/01/16	
	Day 21	08/01/16	**M gene confirmed** on exclusion testing sample: Verbal restrictions in place.

Source Tracing Window	Spread Tracing Window	Date	
		09/01/16	Report case: **EXD1** served. Further samples taken. ETA Weybridge: 9 am 10/01/16
		10/01/16	Hatchery visit at 10:00. EXD40 requested to VENDU. Visit to gather tracings data. **SOS and TCZ declared**
		11/01/16	
	Purple colour reflects source tracing window. Increased intensity of colour reflects increased possibility of introduction on these dates.		
	Yellow colour reflects spread tracing window. Increased intensity of colour reflects increased possibility of spread from the IP on these dates.		

Note: The likely incubation period of AI in birds was agreed to be 2-14 days, with 2-5 days agreed to be a period of higher probability or risk, and with a precautionary window of up to 21 days (in accordance with OIE guidance).

The 02/01/2016 was the reported date of onset of clinical signs on the IP but the 01/01/16 was agreed as a precautionary date of onset of clinical signs.

The 27/12/2015 was the initially agreed date for the start of the high risk source tracing window (i.e. -5d from 01/01/16), and 28/12/15 the initially agreed date for the start of the high risk spread tracing window (i.e. start of high risk source tracing window +1d), but following epidemiological analysis of (i) the laboratory results of all samples collected from the IP (including PVS samples taken on 05/01/2016), (ii) the clinical history and (iii) production records analysis, it was agreed to move the start of the source and spread high risk tracing windows both back by 1 day, i.e. to the 26/12/2015 and 27/12/2015 respectively.

The 31/12/2015 was included in the high risk source tracing window on a precautionary basis as clinical signs were reported to have commenced on the IP on 02/01/2016.