



Animal &
Plant Health
Agency

National Epidemiology Report

Low Pathogenic Avian Influenza H5N3: Suffolk, December 2019

Situation at 08:00 on 14/01/2020

January 2020



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1. Executive Summary

Description of the Premises: The infected premises (IP), designated as AIV 2019/01, is a broiler breeder laying unit and part of a large integrated poultry production company. It operates an intensive indoor barn style production system, supplying hatching eggs to a large associated hatchery. An 'all-in/all-out' flock placement system is operated, with no record of recent movements of poultry onto the farm. The current broiler breeder flock was placed on farm in late May-early June 2019.

Description of the Virus: The haemagglutinin gene of the virus from House 2, named A/chicken/England/032739/19 (H5N3), shares a common progenitor with the virus derived from the H5N1 LPAI chicken case in Scotland in January 2016. It is most closely related to this virus, and as such the data supports indirect/direct introduction from wild birds, rather than undisclosed maintenance in poultry populations during the last three years.

Genetic data at full genome level also supports this interpretation, with all gene segments, matching closely (nucleotide identities of full gene sequences in the range 98-99%) those of contemporary viruses from Eurasian wild birds.

Molecular typing data confirmed that the viruses isolated from houses 2 and 6 were identical.

Source and Spread Windows: The most likely time that LPAI infection is estimated to have entered the IP is on **30/11/2019**, with a maximum precautionary source period over which tracings were investigated from 12/11/2019 to 02/12/2019, a day before the precautionary start date for the onset of clinical signs.

The high risk spread window for the LPAI virus opened on **01/12/2019**, with the spread window extending until **07/12/2019** (when restrictions were imposed), with a maximum precautionary spread period over which tracings were investigated extending back to 13/11/2019.

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 following evidence:

1. The genetic analysis of the viruses isolated from houses 2 and 6 on the IP.
2. There are no relevant, industry-related, international source tracings.
3. There were no poultry or eggs brought onto the IP in the source window, and there is also no evidence of contaminated products being brought on to the IP during this period.
4. There have been no other cases of H5N3 identified to date in domestic poultry in the UK.

There is no evidence suggesting introduction of infection into the houses via direct contact with wild birds; however, some biosecurity deficiencies were identified during the official APHA investigation that may potentially have led to indirect introduction of virus into the poultry housing from a wild bird source (in particular structural damage to the roof of House

2, the first affected house, leading to evidence of ingress of external water and infestation by mice, which are potential mechanic vectors of virus from wild bird faeces).

Assessment of Potential Spread: Following extensive epidemiological investigations and veterinary risk assessments, no indication has been found to suggest that LPAI H5N3 avian influenza is likely to have spread onwards to any other poultry premises investigated in connection with the IP; either by known contacts (source and spread tracings), as a result of proximity (i.e. their location within the Low Pathogenicity Avian Influenza Restricted Zone), or subsequently have been reported on other domestic poultry premises in the United Kingdom to date.

Although the epidemiological investigation concludes that the most likely route of introduction of virus onto the IP was indirect contact with wild birds, an incursion such as this, onto an individual premises, remains a low likelihood event and is largely influenced by the effectiveness of biosecurity measures that have been implemented.

2. Introduction / Background

This report summarises the epidemiological investigations carried out in order to describe and explain the outbreak of H5N3 Low Pathogenic Avian Influenza (LPAI) infection in broiler breeder chickens on a premises in Suffolk, England.

The report will be used to:

1. Provide evidence to support the UK's position in successfully controlling the outbreak and as a declaration to both the EU and OIE of freedom from H5N1 LPAI, and to inform trading partners, with full transparency, in a view to facilitate trade.
2. Provide source material for the technical annex for UK co-financing claims to the EU.
3. Record the logistics and technicalities of the investigation and disease control in order to inform future resource planning, contingency plans and training requirements.
4. 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 2019/01, is a broiler breeder laying unit, and part of a large integrated poultry production company. It operates an intensive indoor barn style production system supplying hatching eggs to a large associated hatchery, which also receives eggs from a further 18 breeding sites. In turn the hatchery then supplies day-old chicks to around 150 rearing farms, most of which are within the same company structure, but some of which are part of different companies.

Hatching eggs are collected from the IP for delivery to the hatchery twice weekly. Whilst stored on the IP prior to collection they are fumigated daily using Virkon TDX at the recommended concentration. Second grade eggs not destined for hatching are also transported to the hatchery at the same time as eggs for hatching, but on separate egg trolleys. These eggs are not fumigated on farm because they are intended for human consumption. On arrival at the hatchery the second grade eggs are stored in a separate chiller outside the main hatchery building, until they are subsequently transported to a human food processing plant, for further processing for human consumption.

An 'all-in/all-out' flock placement system is operated on the IP, with no record of recent movements of poultry onto the farm. The current broiler-breeder flock was placed on farm in late May - early June 2019, and was sourced from three different rearing farms.

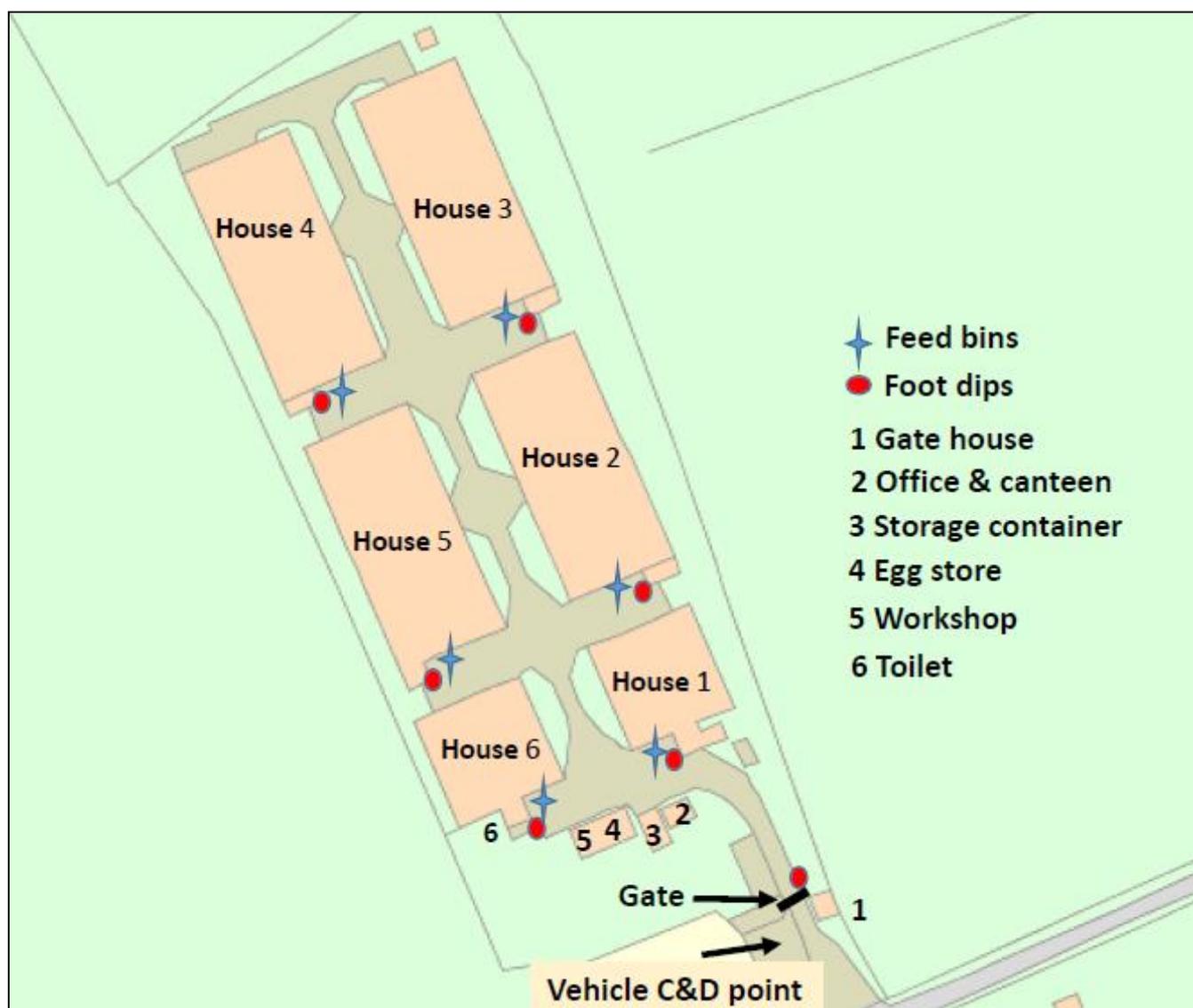
Approximately 28,000 breeding birds were originally placed into six poultry houses on the site (see table 1 and figure 1 illustrating this). The birds were 45 weeks old at the time of the disease investigation and had started laying at 21 weeks, having being transferred onto the premises at 18-19 weeks of age.

Table 1: Details of current flock placement May/June 2019

House No.	Number of birds
House 1	2322 Hens & 129 Cockerels
House 2	5261 Hens & 244 Cockerels
House 3	5550 Hens & 258 Cockerels
House 4	5566 Hens & 253 Cockerels
House 5	5597 Hens & 272 Cockerels
House 6	2447 Hens & 124 Cockerels

At the end of their breeding cycle the birds from this enterprise are normally consigned for slaughter and processing for human consumption.

Figure 1: Plan of the site



Summary Description of the Site:

- 6 separate sheds.
- Conventional poultry housing. Double or triple door access route (outside access/ lobby/egg area/poultry accommodation).
- Mechanical automatic ventilation with covered vents. Very little opportunity for ingress of wild birds.
- Pest control in place, but mice were observed in the poultry houses.
- Boot dips available, and in-use at each entry point (with Defra approved Virocid disinfectant).
- Poultry houses are designed to prevent access of wildlife, fully enclosed accommodation.
- Presence of red mites was confirmed in house 2.

Each house has central nest boxes and two egg belts running on each side into the associated processing lobby. The lobbies from houses 1, 3, 4, 5 and 6 are connected by external concreted walkways, via which the egg collection trollies transfer the eggs from the lobbies to the egg store located towards the entrance of the site. The egg packing station in House 2 is located inside the hygiene barrier, therefore eggs are transferred over the physical barrier (a bench) into the egg trolley. Site personnel are expected to change their footwear on entry to, and exit from, each bird accommodation. The egg trolley wheels are not routinely disinfected, and so present a potential risk pathway for fomite transfer to and from the lobby areas and their external connecting pathways.

Four permanent and two part-time staff attend to birds on the site. Company policy is that staff do not have any contact with other birds or poultry outside of work.

Dedicated staff work in each of the six houses and are responsible for 'walking' through to inspect the birds and environment, collecting and cleaning floor eggs, and hand grading all eggs onto trays which are then placed on trollies. However, there is not a written procedure regarding the flow/order of entering the houses, as that is established at the beginning of the working day, depending on staff availability.

Broken eggs are collected into buckets and disposed of in plastic bags. These are placed into freezers in each house, along with any poultry carcasses, pending weekly collection for disposal by an approved animal by-products (ABP) final user company (egg waste is consigned to rendering and carcasses used to feed maggots produced for use by anglers).

Feed is supplied by a commercial feed mill in the form of dedicated deliveries and delivered directly into the feed bins located near to the entrance of each house.

Litter is supplied as individual plastic wrapped bales which are stacked on additionally outer wrapped pallets and stored outside the poultry houses. The last delivery was on 07/11/19. When required to top up litter in the houses, individual bales are carried inside the houses and across the hygiene barriers. Bales are not allowed to contact the ground during transfer, but are not disinfected before being moved inside.

Pest control is carried out by an external contractor, the last recorded visit being on 15/11/2019.

Waste water is collected into on-farm tanks and is removed by an external contracting company.

At the end of the breeding cycle, and following depopulation of the site, litter is removed from the houses and is stacked and covered with a tarpaulin, before being spread to arable land after harvesting. Litter for the current flock was all still *in situ* in the poultry houses.

4. Description of the Surrounding Area and Other Known Susceptible Livestock in the Locality

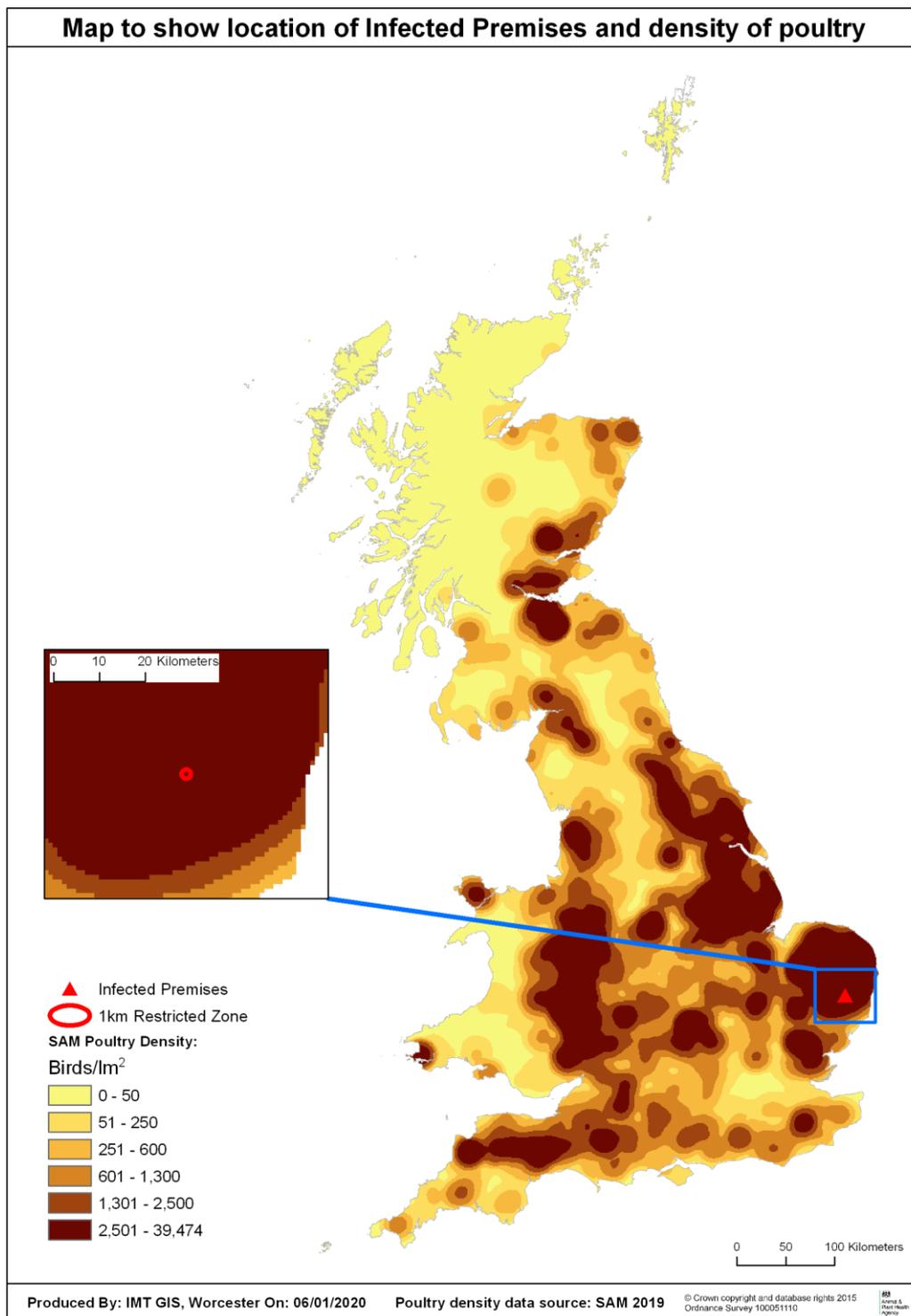
The IP is geographically located within a generally high poultry density area of East Anglia in the east of England (see figure 2).

However, analysis of APHA poultry census data combined with intensive foot patrol activity only identified a total of seven additional poultry premises located within a 1km radius of the IP. These comprised 64 chickens in total, and hence were predominantly very small hobby flocks (<10 birds).

Initial preparatory analysis of centrally held APHA data on registered poultry flocks undertaken at the point of initial suspicion of notifiable avian disease, indicated an additional 12 poultry premises located within a radius of 1 – 3 km from the IP and a further 141 located within a radius of 3-10km.

Surveillance and further action at these premises was subsequently not required, due to confirmation that a LPAI subtype had been confirmed on the IP.

Figure 2: 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 06/01/2020. Premises with less than 50 birds are likely to be under-represented because poultry registration is only mandatory for premises with 50 or more birds. Premises with less than 50 birds are encouraged to register, and therefore 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 estimated 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 demonstrates relative density across GB.

To the east the IP is fenced, and there are public footpaths running along the boundaries on that side. The fence does not continue around the site to the west. The site has ditches on both sides.

There are no ponds on the site itself, but a number of small ponds are present at various locations in the nearby vicinity. During an ornithological study carried out by APHA staff, it was observed that many of the fields and low lying land around the IP were flooded, following a period of prolonged heavy rain.

5. Timeline of Key Events

Table 2: Timeline of key events

Date	Significant event
04/12/2019	<ul style="list-style-type: none"> Drop in egg production noted in House 2 (5200 birds). No other obvious clinical signs other than slight increase in water consumption.
06/12/2019	<ul style="list-style-type: none"> Egg production in House 2 reduced by 10% compared to original levels. Private Veterinary Surgeon (PVS) visited and performed post-mortem examination of 5 birds from House 2 Notifiable avian disease considered unlikely but PVS agrees with APHA to submit swabs (20 oropharyngeal and 20 cloacal) to APHA Weybridge for laboratory 'Testing for Exclusion'. Voluntary restrictions in place pending results.
07/12/2019	<ul style="list-style-type: none"> APHA Weybridge reports four of eight sets of pooled swabs PCR positive for Influenza A virus. Official APHA veterinary inquiry instigated. Official disease restrictions served. Official sampling undertaken in House 2 (paired swabs and blood samples from 20 birds). No significant clinical signs observed, other than some birds with yellowish diarrhoea.
08/12/2019	<ul style="list-style-type: none"> Egg production continued to drop in House 2. Houses 5 and 6 now also affected with a 7 % and 6% egg drop reported respectively. More birds were reported in shed 2 affected with diarrhoea. PCR for M gene (influenza A) was positive. Serology was positive for H5

Date	Significant event
09/12/2019	<ul style="list-style-type: none"> • Update provided only for Houses 2 and 5 which the farm manager attends. Eggs appeared paler. Overall feed consumption normal in all houses, but birds in House 5 taking slightly longer to finish their ration. • Official sampling Houses 5 and 6 (20:20:20)
10/12/2019	<ul style="list-style-type: none"> • • Positive PCR result for H5 reported by APHA Weybridge, cleavage site sequence denotes LPAI H5 detected. • Low pathogenic avian influenza H5 confirmed by CVO (UK) as AIV 2019/01. 1km LPAI restricted zone declared. • Official sampling Houses 1, 3, 4 (60:60:60)
11/12/2019	<ul style="list-style-type: none"> • Epidemiological investigation visits undertaken to IP and associated hatchery.
12/12/2019	<ul style="list-style-type: none"> • Subtype reported as H5N3. • Two premises in LPAI RZ: Clinical inspection and sampling. • Foot patrols in LPAI RZ completed – 5 unregistered hobby flocks detected. • Hatchery placed under restrictions.
13/12/2019	<ul style="list-style-type: none"> • Culling commenced. • Virus isolated from Houses 2 and 6 (identical) confirming LPAI H5N3 in both houses. • Houses 1, 3 and 4 all results negative (PCR and serology). • Negative results reported for the two premises sampled in the LPAI RZ
14/12/2019	<ul style="list-style-type: none"> • Clinical inspections of 5 additional hobby flocks in LPAI RZ completed.
15/12/2019	<ul style="list-style-type: none"> • No epidemiological events reported.
16/12/2019	<ul style="list-style-type: none"> • Culling and disposal completed.
17/12/2019	<ul style="list-style-type: none"> • Preliminary Cleansing and Disinfection completed.
18/12/2019	<ul style="list-style-type: none"> • Preliminary Cleansing and Disinfection officially considered effective.
19 – 20/12/2019	<ul style="list-style-type: none"> • Ornithological assessment of wild bird populations undertaken around IP.
24/12/2019	<ul style="list-style-type: none"> • Restrictions removed from hatchery following supervised cleansing and disinfection.
03 – 07/01/2020	<ul style="list-style-type: none"> • Final clinical inspections +/- sampling completed for total of seven poultry

Date	Significant event
	premises in LPAI RZ.
08/01/2020	<ul style="list-style-type: none"> LPAI RZ lifted.

6. Investigations on the Infected Premises

On 04/12/2019 a drop in egg production was observed in House 2, although no obvious clinical signs of disease were present at the time. Egg production continued to fall and by 06/12/19 was down by 10% compared to original production. An increase in water consumption was noted, although feed consumption remained within normal parameters.

The company's PVS visited the premises on 06/12/19 and carried out a post-mortem examination of five carcasses from House 2 (two dead birds and three culled ones). The findings included one carcass with peritonitis, and another with salpingitis. Nothing else of significance was noted in the other carcasses.

At the time of these visits the PVS noticed a high number of poultry red mites in the affected house.

The PVS considered that suspicion of notifiable avian disease was unlikely, but contacted APHA to request the submission of samples for testing for avian influenza (AI) under the "Testing to Exclude" protocol. Oropharyngeal and cloacal swabs were collected from 20 birds in House 2 on 06/12/19 and submitted for laboratory testing at APHA Weybridge.

Detection of positive PCR results for influenza A viral RNA in four of eight sets of pooled swab samples was reported by the APHA Weybridge laboratory on 07/12/19. Also an APHA veterinary disease inquiry with collection of official samples (paired swabs and blood samples from 20 birds in House 2) was initiated. Official disease restrictions were served on the premises at the time of official sampling.

At the time of the initial APHA investigation, it was reported that egg production in House 2 had reduced by 5.4% over the preceding week, when the expected reduction in production for the stage of lay would have been around 1%. No significant increase in mortality was reported in House 2 (eight birds had died over the previous week, when a baseline level of four would have been expected). No deaths were reported on 07/12/19.

Water consumption in House 2 had peaked at 24% above average on 04/12/19 and remained above average levels, but had reduced to 6.5% above average on 07/12/19.

Mortality in the rest of the houses was as expected, and at a low level. Feed consumption was reported to be as expected in all the other houses.

No respiratory signs, sneezing, abnormal vocalisation, nasal or ocular discharges, or swelling or discolouration of heads and combs were observed in any of the houses and cloacal

temperatures taken from birds were unremarkable (ranging from 40-41°C). However, some yellowish coloured diarrhoea was noted in some birds in House 2.

Over the following days, mortality remained unchanged, as did feed consumption, although birds in House 5 were noted to be taking longer to consume their ration, and a reduction of egg production in Houses 5 and 6 was noted on 08/12/19 (7% and 6% respectively); in addition to a continued drop in production in House 2, with some eggs in houses 2, 5 and 6 becoming paler and misshapen, and a reduction of 3% in production being seen in House 1. Diarrhoea began to be reported in some birds in the remaining houses.

House 2 remained the worst affected house, with birds noted as being slightly lethargic and reluctant to move unless disturbed; however, egg production began to recover.

On 08/12/19 a positive PCR result for M gene (influenza A) and positive serology for H5 were reported by the laboratory.

Additional sampling in the other houses was undertaken on 09/12/19 (houses 5 and 6) and 10/12/19 (houses 1, 3 and 4). On 10/12/19 a positive PCR result for H5 was reported by the laboratory with a cleavage site sequence denoting low pathogenicity H5 avian influenza.

Low pathogenicity avian influenza virus H5 was confirmed by the CVO (UK) and designated as AIV 2019/01 and a 1km LPAI restricted zone was declared. The subtype was subsequently established to be LPAI H5N3.

Virus was also subsequently isolated from the samples from houses 2 and 6, and molecular sequencing showed that the viruses were identical, indicating a single incursion of LPAI H5N3 onto the site; initially into House 2 with subsequent spread to House 6 (based on lack of seropositivity being detected on samples from House 6), supporting a slightly later date of introduction.

Final results were negative on PCR and serology for Houses 1, 3, 4 and 5. However, the spread of virus into House 5 cannot entirely be excluded based on the relatively smaller sampling frame undertaken, even in light of a single sick bird that was euthanased and the carcass submitted for testing with negative results.

A summary of sampling results for all houses is attached as Appendix 1.

Humane culling and disposal of all poultry on the site commenced on 13/12/19 and was completed by 16/12/19. Preliminary cleansing and disinfection was completed on 17/12/19 and considered to be effective on 18/12/19.

Epidemiological investigations on both the IP and the associated hatchery were undertaken on 11/12/19.

Epidemiological investigation on the IP considered that levels of biosecurity were generally good with detailed standard operating procedures in place and likely to be followed. However, a number of potential weaknesses were identified:

1. Leakage of the roof of House 2 above the bird nesting areas, with associated water staining, was noted.
2. A heavy infestation of mice (in House 2 in particular), these are potential vectors of virus from wild bird faeces.
3. Some spillage of feed noted around feed augers, which could potentially attract mice and wild birds (although no particularly notable wild bird activity was reported at the time of the visit).
4. The House 2 egg store is located within the hygiene barrier and eggs are passed across the barrier; however the hand sanitiser was located away from the hygiene barrier posing a potential weakness.
5. Disinfectant foot dips were located at the entrance to each house, but staff entering the bird areas only change footwear, not clothing (although the staff clothing used is dedicated to the site).

An ornithological assessment of wild bird activity in the vicinity of the IP was undertaken by members of the APHA Wildlife Management team on 19-20/12/19 (attached as Appendix 7), although the findings were largely unremarkable.

7. Overview of Tracing Activities

Previous LPAI outbreaks in GB were used as a model to demonstrate the effectiveness of a risk-based approach; to guide and inform a proportionate response to potential links identified between the IP and other poultry premises. This ensured that appropriate action was taken, reduced the impact on the industry, and prioritised APHA resources, with fewer visits required and fewer businesses potentially being placed under restrictions.

A veterinary risk assessment in relation to the hatchery that received hatching eggs from the IP was performed; subsequently licenced moves of day-old chicks hatched from eggs laid and moved into the hatchery prior to the high risk spread tracing window were undertaken. A number of further peer-reviewed veterinary risk assessments (see Appendix 7 below) were undertaken; these were used 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. The following sections discuss the outcomes of these risk assessments regarding the source and spread from the IP.

8. Source Investigations– Hypotheses for the Source

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

Evidence based on the clinical picture, laboratory results and expert advice from the OIE and National Reference Laboratory at APHA Weybridge, 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: The most likely date of introduction of infection is **30/11/2019**, with a maximum precautionary source period over which tracings were investigated from 12/11/2019 to 02/12/2019, a day before the precautionary start date for onset of clinical signs.

Spread window: The most likely potential for spread from the premises is between **01/12/2019 – 07/12/2019** (when statutory disease control restrictions were imposed), with a maximum precautionary spread period over which tracings were investigated extended back to 13/11/2019.

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 isolated from this IP.
2. Findings from the epidemiological investigation suggest some potential breaches of biosecurity on the IP (e.g. egg trolley wheels not disinfected when moving around the site, mice seen within the poultry houses, structural damage and leaking from the roof onto the nesting boxes in House 2 and some feed spillages noted around feed augers, potentially being attractive to wild birds and vermin); all of which are potential transmission pathways for introduction of virus into the poultry houses).
3. There is no evidence suggesting the introduction of infection into the houses via direct contact with wild birds.
4. There were no poultry or eggs brought onto the IP in the source window and there is also no evidence of contaminated products being brought on within the relevant time frame.

Table 3: Possible source of infection for the Infected Premises AIV2019/01

Pathway	Comment	Assessment of likelihood of infection via this route following VRA
Direct introduction from wild birds	<ul style="list-style-type: none"> • No reports of wild birds in sheds and access unlikely. 	<p>Very low likelihood</p> <p>Low uncertainty</p>
Indirect introduction from wild birds	<ul style="list-style-type: none"> • Leak from the roof onto the nesting box area in House 2 observed • The egg trolley wheels are not disinfected and so present a potential risk pathway for fomite 	<p>High likelihood</p> <p>Medium uncertainty</p>

Pathway	Comment	Assessment of likelihood of infection via this route following VRA
	<p>transfer to and from the lobby areas and their external connecting pathways into the poultry houses.</p> <ul style="list-style-type: none"> • Vermin – mice seen in all houses within birds' accommodation, heavy mouse infestation observed in House 2 (these are potential mechanical vectors of virus in wild bird faeces). • Malfunctions of the auger system of the feed bins resulting in feed on the floor, potentially attracting wild birds and rodents. • Increased number of corvids and gulls detected in the area and thought to be secondary to the large amount of flooded land around the IP. During the ornithological survey a small number of direct flights over the IP were observed and these were confined to gulls. 	
<p>Undisclosed infection in the UK:</p> <p>Direct introduction by purchased birds</p>	<ul style="list-style-type: none"> • The birds on the IP were placed in May-June 2019 at 18-19 weeks of age. • All-in, all-out policy (no live birds moved on or off the premises since placement). 	<p>Negligible likelihood</p> <p>Low uncertainty</p>

Pathway	Comment	Assessment of likelihood of infection via this route following VRA
<p>Undisclosed infection in the UK:</p> <p>Indirect contact with an infected flock</p>	<ul style="list-style-type: none"> • Personnel & visitors - movements of area manager, staff, PVS, pest control contractor, electricians and drivers of the egg lorry and bedding deliveries. • No poultry held by staff members at home – company policy of no poultry/bird contact outside of work. • No history of recent overseas travel by staff. • Feed delivery – Feed delivered whenever needed straight in silos from delivery lorry via a hose. Potential spread between sheds via hose or driver. • Water – Mains water. • ABP collection – ABPs collected from outside the perimeter of IP. ABP lorries and drivers do not come onto the site. • Egg collection – Driver drives around perimeter of sheds to get to the egg store. Lorry wheels have C&D on entry/exit of IP. Driver wears own footwear and disposable protective clothing 	<p>Very low likelihood Medium uncertainty</p> <p>Very low likelihood Medium uncertainty</p> <p>Very low likelihood Medium uncertainty</p> <p>Negligible Low uncertainty</p> <p>Very low likelihood Medium uncertainty</p> <p>Very low likelihood Medium uncertainty</p>

Pathway	Comment	Assessment of likelihood of infection via this route following VRA
	<p>but wears own gloves.</p> <ul style="list-style-type: none"> • Bedding delivery – Bedding (bales of wood shavings wrapped in plastic) delivered periodically on pallets to outside of the sheds and then manually moved into shed lobbies by hand. Bales are not disinfected prior to getting into the houses. Last delivery 07/11/19. 	<p>Very low likelihood Medium uncertainty</p>
<p>Infection elsewhere in the world: Direct contact with an infected flock or wildfowl</p>	<ul style="list-style-type: none"> • No recent introduction onto the IP of live birds or hatching eggs/day old chicks. 	<p>Negligible likelihood Low uncertainty</p>

9. Assessment of the Likely Source

The most likely source of the outbreak is considered to be indirect contact with wild birds.

Whilst the overall assessment of biosecurity protocols for movements onto and off the IP did not identify a likely alternative source for introduction of virus onto the site, findings from the investigation suggest some potential deficiencies of biosecurity within the IP (e.g. egg trolley wheels not disinfected, mice seen in the poultry houses, leakage of the roof of House 2, the first affected poultry house, storage of wrapped bales of spare litter outside the houses, without disinfection before being transferred into houses) which may have led to introduction of virus from the environment into the poultry houses.

10. Spread Investigations – Potential and Probability of Spread

Spread windows as determined from tracing timelines:

1. Precautionary (OIE) 13/11/2019 to 18/11/2019
2. Likely 20/11/2019 to 30/11/2019
3. High risk 01/12/2019 to 07/12/2019

Veterinary risk assessment, including the consideration of biosecurity protocols, and additional tracings follow-up involving data gathering and data verification (record checks, telephone interviews, emails, written declarations), did not indicate any credible likely risk pathways for further spread of virus from the IP to other poultry premises.

A number of batches of hatching eggs originating from the IP in the precautionary lower risk spread tracing window had already been placed in setters in the associated hatchery, prior to detection of disease on the IP. Given observed high standards of biosecurity and traceability within the hatchery (including daily fumigation on the IP before consignment to the hatchery, daily fumigation in the egg store in the hatchery prior to setting, and inclusion of formalin fumigation within hatchers) veterinary risk assessments considered that the risk of spread of disease via hatching of these eggs was very low.

Three batches of day old chicks hatched from such eggs, along with others sourced from other breeding farms, were permitted to be licenced out of the hatchery to three rearing premises within the company structure (all movement were for all-in, whole single site repopulations). These were placed under restriction and subject to official APHA supervision for a period of 21 days following placement, with regular veterinary clinical inspections, checks of production records, and statistical sampling of all epidemiological groups on each premises (representative of all chicks on each site, not just chicks sourced from the IP derived eggs) towards the end of the monitoring period.

These three rearing sites have returned negative results and have had restrictions revoked.

A single batch of hatching eggs derived from the IP during the high risk spread tracing window had been delivered to the hatchery on 03/12/2019, but not yet placed in setters. These had been segregated from other eggs in the egg store on arrival at the hatchery and been subject to daily fumigation. These eggs were subsequently voluntarily surrendered for secure disposal under official APHA supervision. Following veterinary risk assessment, and supervised cleansing and disinfection of the hatchery after hatching of the last batch of chicks originating from eggs laid on the IP, restrictions on the hatchery were removed on 24/12/2019.

The last batch of second grade eggs originating from the IP were moved into separate storage outside the hatchery building itself on 22/11/19, and these were consigned for processing for human consumption on 26/11/19, which is before the estimated most likely date of introduction of virus onto the IP.

11. Surveillance in the LPAI Restricted Zone

Interrogation of APHA databases indicated the presence of two other premises (in addition to the IP itself) that were officially registered as keeping poultry species, within the 1km radius LPAI Restricted Zone around the IP. These holdings were subjected to veterinary clinical inspections, checks of available production and medicine records for any indications of flock level disease. Sampling on these premises was carried out 12/12/2019 with negative laboratory results (PCR testing was carried out on oro-pharyngeal and cloacal swabs, in addition to serology, and all undertaken at the sampling level of 60:60:60. As these were small flocks, this protocol resulted in all birds being sampled).

Foot patrols within the 1km LPAI Restricted Zone subsequently revealed a further five (previously unregistered) hobby flocks (the five containing a total of 27 chickens). These small flocks were also subject to immediate veterinary clinical inspections and checks of available production and medicine records.

In addition, owners on all the above premises were provided with advice and written guidance on (i) maintaining biosecurity and (ii) the statutory disease control requirements to be observed within the LPAI Restricted Zone, including (iii) the requirement to immediately report any suspicion of the presence of notifiable disease to APHA.

Epidemiological investigations did not reveal any potential contacts between these premises and the IP, other than geographical proximity.

Final veterinary clinical inspections and record checks (with sampling repeated where previously undertaken) were undertaken on these premises between 03/01/2020 – 07/01/2020 in order to support lifting of the LPAI Restricted Zone.

Following receipt of satisfactory reports of final veterinary clinical inspections (and negative laboratory results where applicable) and completion of all identified source and spread tracings investigations, the LPAI Restricted Zone was lifted at 17:00 on 08/01/2020.

12. Analysis of the Virus

The haemagglutinin gene of the virus from House 2, named A/chicken/England/032739/19 (H5N3), shares a common progenitor with the virus derived from the H5N1 LPAI chicken case in Scotland in January 2016, and hence the data supports indirect/direct introduction from wild birds, rather than undisclosed maintenance of the virus in poultry populations during the last three years.

Genetic data at full genome level also supports this interpretation, with all gene segments matching closely (nucleotide identities of full gene sequences in the range 98-99%) to those of contemporary viruses from Eurasian wild birds.

Molecular typing data confirmed that the viruses in houses 2 and 6 were identical.

13. INTERNATIONAL CONTEXT

According to ADNS (the EU's Animal Disease Notification System), there were five LPAI outbreaks in domestic poultry in Europe in 2019; three in Denmark (two were H5, one was H7), one in Italy (LPAI H7N3) and one outbreak (this outbreak) in the UK. There were two outbreaks in captive birds, and no reports in wild birds in Europe in 2019.

In Denmark, in February 2019, LPAI H5 was confirmed in a commercial poultry holding with approx. 7,000 organic laying hens. The second outbreak in Denmark was reported in March 2019 when LPAI H7 was identified in a holding that consisted of 3,300 mallards for restocking supplies of game. There had been no clinical signs of disease. The third outbreak in Denmark was reported in June 2019, and LPAI H5 was detected in a holding with 3,000 mallards for restocking supplies of game and hatching eggs, again with no clinical signs. In Italy, LPAI H7N3 was reported in broiler birds.

There were a further two outbreaks of LPAI reported in captive birds in France and Germany in 2019. In France, LPAI H5 was identified in a holding with 2,600 mallards in October. The birds showed no clinical signs. In April 2019, LPAI H5 was identified in Germany in a single non-commercial holding of 48 geese, 46 ducks and 25 chickens. There were relatively few highly pathogenic avian influenza (HPAI) virus outbreaks in Europe in winter 2018/19; when compared to H5N6 in the winter of 2017/18, and the exceptional H5N8 epizootic in 2016/17. In this winter season to date, HPAI H5 outbreaks have been reported in domestic poultry in Poland (December 2019), Slovakia and Hungary (January 2020), and in a wild bird in Poland (January 2020).

14. Public Health Impact

The advice from Public Health England (PHE) is that the risk to public health from the virus isolated is very low, and the Food Standards Agency has made clear that this avian influenza virus does not pose a food safety risk for UK consumers. Thoroughly cooked poultry and poultry products, including eggs, are safe to eat.

15. Remaining Uncertainty

There is no evidence to suggest that the IP (AIV 2019-01) 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.

The most likely hypothesis for the source of the LPAI virus and the route of introduction into the IP remains indirect contact with wild birds, and the uncertainty associated with this is considered to be low.

There is a continually present, albeit considered low, risk of further outbreaks of avian influenza (not limited to H5N3 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.

16. Concluding Remarks

The most likely source of infection is considered to be indirect contact with wild birds. Extensive epidemiological investigations did not give rise to any suspicion that disease was likely to have either originated from, or been spread onwards to, any further premises investigated in connection with the IP; either by known contact (source and spread tracings), or as a result of geographical proximity (i.e. their location within the 1km LPAI Restriction Zone).

Although the epidemiological investigation concluded that the most likely route of introduction of virus onto this IP was indirect contact with wild birds, an incursion such as this onto an individual premises remains a low likelihood event and is influenced by the effectiveness of the biosecurity measures that have been implemented on the particular site.

17. Acknowledgements

The views expressed in this report are those of the National Emergency Epidemiology Group (NEEG). However, we would like to express our thanks to the avian virology experts within APHA, members of the APHA National Wildlife Management Centre, the Cardiff APHA Specialist Service Centre Tracings Team and the many other APHA colleagues who have assisted with this investigation.

The NEEG is comprised of staff from APHA Service Delivery and Science Directorates.

National Emergency Epidemiology Group

14 January 2020

18. Appendices

Appendix 1: Tables summarising selected laboratory analyses

The intravenous pathogenicity index (IVPI): The OIE and UK National Reference Laboratory carried out an intravenous pathogenicity index test for the virus A/chicken/England/032739/19 (H5N3) and this was determined to have a value of zero – the lowest possible for the test.

Table 4: Bird level H5 PCR/shedding and serology results for all samples.

House	Date of sampling	Time of sample	PCR		Serology		
			No. tested	No. Pos	No. tested	No. Pos	No. Neg
2	06/12/19	TTE* ₁	8* ₂	4* ₂	n/a	n/a	n/a
2	07/12/19	Report case	20	1* ₃	19* ₄	17	2
5	09/12/19	Pre-cull	20* ₅	0	20	0	20
6	09/12/19	Pre-cull	20	2* ₅	20	0	20
1	10/12/19	Pre-cull	60	0	60	0	60
3	10/12/19	Pre-cull	60	0	60	0	60
4	10/12/19	Pre-cull	60	0	60	0	60

*₁ TTE samples = Testing for exclusion – 20 Oropharyngeal and 20 Cloacal swabs

*₂ PCR pools only

*₃ 1 positive to H5, 5 in total positive to Influenza A

*₄ 1 sample insufficient to test

*₅ 1 carcass also received – all tissue samples gave negative results

*₆ 2 positive to H5, 8 in total positive to Influenza A

Appendix 2: Estimated timeline and tracing windows

AIV 2019/01 Estimated timeline for source and spread of infection			
Source Tracing Window	Spread Tracing Window	Date	
Day 21		12/11/19	Start of precautionary source tracing window, as per OIE guidelines (-21d)
Day 20		13/11/19	Start of precautionary spread tracing window (source + 24h)
Day 19		14/11/19	
Day 18		15/11/19	
Day 17		16/11/19	
Day 16		17/11/19	
Day 15		18/11/19	
Day 14		19/11/19	Start of likely source tracing window (-14d)
Day 13	Day 1	20/11/19	Start of likely spread tracing window (source tracing window +24h)
Day 12	Day 2	21/11/19	
Day 11	Day 3	22/11/19	
Day 10	Day 4	23/11/19	
Day 9	Day 5	24/11/19	
Day 8	Day 6	25/11/19	
Day 7	Day 7	26/11/19	
Day 6	Day 8	27/11/19	
Day 5	Day 9	28/11/19	
Day 4	Day 10	29/11/19	
Day 3	Day 11	30/11/19	Start of high risk source tracing window (-3d) Most likely infection date for this outbreak
Day 2	Day 12	01/12/19	Start of high risk spread tracing window (source +24h)
Day 1	Day 13	02/12/19	
	Day 14	03/12/19	Onset of clinical signs (drop in egg production in House 2).
	Day 15	04/12/19	
	Day 16	05/12/19	
	Day 17	06/12/19	PVS Test to Exclude sampling
	Day 18	07/12/19	APHA investigation (DPR 2019/13) disease restrictions served and official sampling in House 2 (20:20:20 - 1/20 positive to H5 on PCR, 17/19 seropositive - 1 untestable - 5/20 in total positive on Influenza A PCR).
	Day 19	08/12/19	Egg production drop in Houses 5 and 6
	Day 20	09/12/19	Official sampling Houses 5 and 6 (20:20:20 - House 5 negative on PCR and serology, House 6 2/20 H5 PCR positive, 10/20 in total positive on Influenza A PCR, all seronegative).
	Day 21	10/12/19	LPAI confirmed (AIV 2019/01). Official sampling Houses 1, 3, 4 (60:60:60 - all negative on PCR and serology).
	Day 22	11/12/19	
	Day 23	12/12/19	
	Day 24	13/12/19	Culling commenced.
	Day 25	14/12/19	
	Day 26	15/12/19	

AIV 2019/01 Estimated timeline for source and spread of infection			
Source Tracing Window	Spread Tracing Window	Date	
	Day 27	16/12/19	Culling completed.
	Day 28	17/12/19	Preliminary C&D completed.
	Day 29	18/12/19	Preliminary C&D considered effective.
	Purple colour reflects source tracing window. Increased intensity of colour reflects increased likelihood of introduction on these dates.		
	Yellow colour reflects spread tracing window. Increased intensity of colour reflects increased likelihood 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 48-72 hours 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 04/12/2019 was the reported date of onset of first clinical signs on the IP (observed as a reduction in egg production in House 2), but following expert disease consultant advice and epidemiological analysis of (i) the laboratory results of all samples collected from the IP (including PVS samples taken on 06/12/2019), (ii) the clinical history and (iii) analysis of production records, it was agreed to set a precautionary date of onset of clinical signs as the 30/11/2019.

Appendix 3: Phylogenetic tree of the LPAI virus A/chicken/England/032739/19 (H5N3)



Appendix 4: Details of tracings assessments

The summary table below is based on data taken from APHA Cardiff Specialist Services Centre (SSC) Tracing Team records on 09/01/2020. This data describes the pathways and tracing subjects investigated by the outbreak tracing team; to identify premises from where the LPAI infection may have arrived onto 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.

Veterinary risk assessments were carried out to determine the level of risk associated to the different risk pathways either for source and/or spread. These were supported by tracing activities involving data gathering and data verification (record checks, telephone interviews, emails, written declarations).

The outcome of the VRAs indicated which locations to follow up for action: nine subjects were considered for investigation, of which only one required further tracing investigations with a visit by field staff. The estimated likelihood of exposure for these tracing investigations was nevertheless assessed as very low overall.

Table 5: Number of subjects investigated.

No.	Tracing subject	Tracing type – source and/or spread	Veterinary Risk Assessment outcome of likelihood as a risk pathway for source/spread of disease	Actions required	Final Outcome
1	Feed delivery	Both	Very Low with Medium uncertainty	No further action required	Tracing closed
2	Poultry training operative	Spread	Very Low with Medium uncertainty	No further action required	Tracing closed
3	Animal by-product collection	Both	Source – negligible with medium uncertainty Spread – very low with medium uncertainty	Precautionary veterinary inspection and records check at ABP disposal site.	Chicken carcasses all disposed of for maggot feeding, egg waste sent for rendering. Good

No.	Tracing subject	Tracing type – source and/or spread	Veterinary Risk Assessment outcome of likelihood as a risk pathway for source/spread of disease	Actions required	Final Outcome
					biosecurity on 4site and C&D of vehicle. No further action required – tracing closed
4	Private veterinary surgeon	Spread	Very Low with Medium uncertainty	No further action required	Tracing closed
5	Poultry company area manager	Both	Very Low with Medium uncertainty	No further action required	Tracing closed
6	Egg collection	Both	Very Low with Medium uncertainty	No further action required	Tracing closed
7	IP staff	Both	Very Low with Medium uncertainty	No further action required	Tracing closed
8	Electrician 1	Both	Very Low with Medium uncertainty	No further action required	Tracing closed
9	Electrician 2	Both	Very Low with Medium uncertainty	No further action required	Tracing closed

Appendix 5: Definitions of qualitative risk terms

Table 6: 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

Appendix 6: Definitions of uncertainty

Table 7: 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
<p>Low</p> <p>Further research is very unlikely to change our confidence in the assessed risk</p>	<ul style="list-style-type: none"> • 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
<p>Medium</p> <p>Further research is likely to have an important impact on our confidence in the risk estimate</p>	<ul style="list-style-type: none"> • 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
<p>High</p> <p>Further research is very likely to have an important impact on our confidence in the risk estimate</p>	<ul style="list-style-type: none"> • 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

Table 8: Matrix for the multiplication of two qualitative likelihoods (Gale et al., 2009)

Results of probability 2	Results of probability 1					
	Negligible	Very Low	Low	Medium	High	Very High
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Very Low	Negligible	Very Low	Very Low*	Very Low	Very Low	Very Low
Low	Negligible	Very Low*	Low	Low	Low	Low
Medium	Negligible	Very Low	Low	Medium	Medium	Medium
High	Negligible	Very Low	Low	Medium	High	High
Very High	Negligible	Very Low	Low	Medium	High	Very High

*If multiplying successive likelihoods together, particularly low likelihoods, a modified matrix may be used as given in Gale *et al.*, (2014) which allows for an improved estimation of risk accounting for basic mathematical principles; those likelihoods marked with an * are reduced to negligible.

Appendix 7: Summary of the Emergency Ornithology Field Assessment (EOFA) and a list of risk assessments and other measures carried out as part of investigations into potential source and further spread:

1. Summary of the Emergency Ornithology Field Assessment (EOFA) carried out by the APHA National Wildlife Management Centre.

Observations were made at dawn, dusk and during the day by two observers from vantage points to the N and SW of the IP, achieving a good description of birds moving around the site and in fields within 500m.

Few water-birds were found close to the IP, with the handful of significant waterbodies holding only small numbers of mainly resident species. As these were at the periphery of the assessment zone (AZ - radius of 10 km) it seems unlikely that they represent any significant source of infection.

Corvids (here specifically rooks, carrion crows and jackdaws), as well as gulls (here mainly black-headed and common gulls) were ubiquitous in fields across the AZ, exploiting the very many saturated / flooded areas as small mobile flocks.

Wild birds seen within 500m of the IP were unremarkable and entirely typical of the context (locale, landscape and season). A few small groups of birds, of mainly smaller species, were occasionally seen moving close to the IP, although none appeared to use the site specifically. A small flock of wood pigeon (170+) roosted, foraged and loafed in stubble fields, tree-lines and copses adjacent to the IP, but these did not appear to use the site specifically. Similarly, larger number of corvids could be seen in fields close to the IP, but other than a small number of overflights of the site, none of the activity was associated with the premises.

2. The list of veterinary risk assessments undertaken is as follows:

- 1) Veterinary Risk Assessment for the likelihood of transmission of LPAI H5N3 virus to and from Infected Premises AIV2019-01, via Animal By-Products collection associated fomites.
- 2) Veterinary Risk Assessment for likelihood of Source and Spread of LPAI H5N3 virus from AIV 2019-01) via Egg Collection Associated Fomites.
- 3) Veterinary Risk Assessment for the likelihood of transmission of LPAI H5N3 virus to and from Infected Premises AIV2019-01 via Visitors Associated Fomites.
- 4) Veterinary Risk Assessment for the likelihood of transmission of LPAI H5N3 virus to and from Infected Premises AIV2019-01 via IP Personnel Associated Fomites.
- 5) Assessment of risk level for Feed Deliveries tracings for AIV2019-01.
- 6) A Veterinary Risk Assessment Of The Likelihood Of The Spread Of LPAI H5 Virus Arising From The Movement On 12/12/2019 Of Day-Old Chicks From The Hatchery Associated With AIV 2019-01.

- 7) A Veterinary Risk Assessment Of The Likelihood Of The Spread Of LPAI H5N3 Virus Arising From The Movement On 19/12/2019 And 23/12/2019 Of Day-Old Chicks From The Hatchery Associated With AIV 2019-01.
- 8) A Veterinary Risk Assessment Of The Likelihood Of Spread Of LPAI H5N3 Virus Arising From The Movement Of IP1 Eggs/Chick Through The Hatchery Associated With AIV2019-01.