Animal &
Plant Health
Agency

Two low pathogenicity avian influenza outbreaks in Great Britain (H5N2 and H5N3)

United Kingdom

November 2020 to March 2021

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APHA is an Executive Agency of the Department for Environment, Food and Rural Affairs and also works on behalf of the Scottish Government, Welsh Government and Food Standards Agency to safeguard animal and plant health for the benefit of people, the environment and the economy.

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Executive summary

Two separate cases of low pathogenicity avian influenza (LPAI) cases were confirmed in Great Britain on 2 November 2020 and 27 March 2021 respectively, these were 2 completely unconnected, independently infected premises, one infected with H5N2 and one infected with H5N3 LPAI virus.

The H5N2 case was located in Kent close to the coast, in the south-east of England and the H5N3 case was located in Cheshire, approximately 380 km distant in the North West of England.

The infected premises in Kent was a small, family run, commercial business serving the hobby sector, it contained 554 birds of 9 different species. Various other livestock (goats, and alpacas) and pets (guinea pigs, rabbits, tortoises and ferrets) were present. The poultry were mainly of non-commercial native British breeds.

The infected premises in Cheshire was part of a large, integrated commercial poultry breeding company, with a medium-sized, pedigree, grandparent turkey breeding flock, supplying hatching eggs to an associated company owned hatchery.

Extensive epidemiological, tracings and genetic sequencing investigations revealed no evidence of spread to any other holdings containing susceptible species, nor to trading partners.

The two affected premises are considered to have become infected as a result of independent introductions, either direct or indirect, from wild birds.

The confirmation of a H5N2 low pathogenicity avian influenza virus in Kent on 2 November 2020 occurred after suspicion of virus circulation was aroused by a non-negative H5 serology result from a sample submitted as part of the UK's annual AI active surveillance programme. The HA from the resulting virus isolate had the highest identity with a mallard duck (*Anas platyrhynchos*) LP H5N6 virus in 2019.

A number of common risk factors for the introduction of infection to the Kent premises were revealed: The general lack of effective biosecurity, staff personal protective equipment discipline and proximity to water bodies.

The presence of H5N3 avian influenza virus in Cheshire was confirmed on 26 March 2021 as a result of an investigation that followed surveillance samples of turkeys taken under the 'Testing to Exclude' (TTE) scheme for avian influenza (part of the UK's early warning system)[1]. Following the initial TTE testing yielding positive influenza A virus results, the

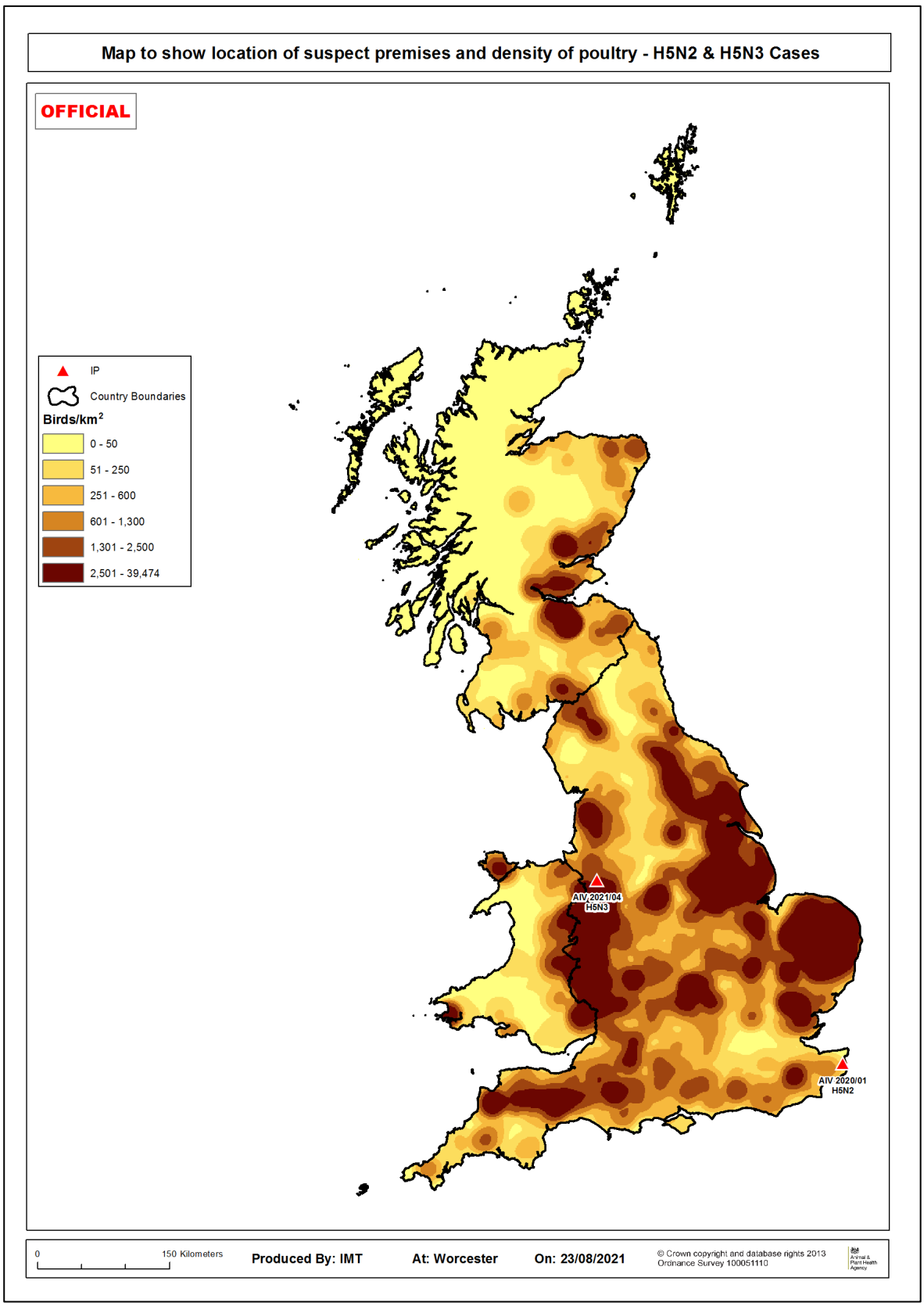
[1] Gibbens N, Brown IH, Irvine RM. Testing for exclusion of notifiable avian disease. *Veterinary Record* 2014; 174:534-535. doi: 10.1136/vr.g3412

case was immediately escalated by APHA to a statutory notifiable disease investigation. Analysis of official swab samples detected evidence of infection of H5N3. Low pathogenicity H5N3 was confirmed on 27 March 2021.

The biosecurity of the Cheshire premises was considered to be of a good standard, the introduction of infection was regarded as being most likely due to repeated flooding events occurring following heavy rainfall, causing pooling within internal corridors, and washing wild bird faecal matter into some of the houses.

Location of the LPAI H5N2 and H5N3 cases

Figure 1: location of the LPAI H5N2 & H5N3 cases



Analysis of the low pathogenicity H5N2 and H5N3 viruses

H5N2 virus – AIV 2020/01 Kent

Description of the virus H5N2

The detection of a low pathogenicity (LP) avian influenza virus (AIV) occurred after suspicion of virus circulation was aroused by a non-negative H5 serology result as part of the UK's annual AI active serological surveillance programme in poultry. From the official swab samples taken as a follow-up to the non-negative serology results, six tested positive for avian influenza A followed by subtyping PCRs for H5 and N2. Serological assessment yielded a pattern of reactivity across several avian species, suggesting exposure to virus and seroconversion in the absence of reported clinical disease.

No live virus isolate was recovered from swab material taken from birds on the infected premises. The HA could not be genetically typed further than the determination of a LP cleavage site sequence from official samples taken. However, environmental samples were taken from the site, as it was of research interest to gather material to assess potential viral shedding.

From this material, a faecal sample was positive for viral RNA and live virus was later recovered, from faecal material. The HA from the resulting isolate had the highest identity with a mallard duck (*Anas platyrhynchos*) isolate from an LP H5N6 virus in 2019.

Analysis of the virus H5N2

A single report case involving the detection of an H5N2 avian influenza A virus of low pathogenicity has been characterised. The report case involved investigation following serological positivity in surveillance samples taken under an active surveillance initiative for avian influenza in poultry. Non-negative results were obtained for H5 serology stimulating a disease investigation.

A total of 6 PCR positives were detected from swab samples submitted. From these positives, virus isolation was attempted but was unsuccessful. Further cull samples and repeat swab samples were negative by PCR. Serology on submitted samples yielded pattern of reactivity across species.

Amongst 124 samples, 93 were negative, and 31 samples were positive for serology across a range of titres against a standard H5N3 (A/teal/Eng/7394-2805/06) antigen. These results demonstrate exposure to virus and seroconversion in the absence of clinical disease.

The broad range of serological reactivity combined with the weakly positive PCR results suggested declining infection in a general picture of overall viral clearance. The range of species involved and biosecurity on the farm meant that environmental faecal samples were taken for molecular testing. From these samples a single faecal sample was positive by PCR. The sample was inoculated into embryonated fowl eggs for virus isolation. A viral isolate was recovered and named A/environmental faeces/England/AV1024/2020.

The recovered viral stock was processed for whole genome sequencing. The resulting sequence yielded data that demonstrated that the HA from A/faeces/England/AV1024/2020 had the highest identity with a mallard isolate from an H5N6 virus from Belgium in 2019 (A/Anas Platyrhynchos/Belgium/10811_6/2019 (H5N6)) whereby 98% sequence identity was observed.

Across other genes closest matches were from a variety of different avian influenza subtypes from geographically distinct locations and species, which typifies genetic profiles of such viruses with direct wild bird origin. The detection of a single isolate precludes meaningful assessment of transmission pathways.

Further, infection pressures and environmental contamination levels in such instances cannot be estimated, especially as these viruses circulate inapparently in wild bird populations causing sporadic detections in national surveillance programmes.

H5N3 virus – AIV 2021/04 Cheshire

Description of the virus H5N3

The haemagglutinin (HA) of this low pathogenicity avian influenza (LPAI) isolate had high genetic identity with an H5N3 LPAI virus from France in 2020 (A/mallard/France/20PO17917/2020). The remaining genes had sequence identities that closely match with isolates from different geographical regions and time frames reflective of genetic profiles of viruses contemporaneously circulating in wild birds.

Analysis of the virus H5N3

A single report case involving the detection of an H5N3 avian influenza A virus of low pathogenicity (LPAIV) has been characterised. The report case involved investigation of surveillance samples taken under the 'Testing to Exclude' (TTE) scheme for avian influenza, which is part of the UK early warning system for AI.

Following the initial TTE testing yielded positive AI virus results, the case was immediately escalated by APHA to a statutory notifiable disease investigation. Analysis of official swab samples detected evidence of infection of H5N3. Low pathogenicity H5N3 was confirmed on 27 March 2021.

Six epidemiological units were present across the infected premise. From sampling epidemiological units on the infected premise, within house 4, 2 cloacal swabs were positive and within house 3 a single oropharyngeal swab was positive for viral nucleic acid. One turkey was positive for viral RNA in both cloacal and oropharyngeal swabs.

Serological assessment demonstrated reactivity to H5N3 antigen in birds from across houses 3 and 4 with moderate serological responses. The PCR positive birds were serologically negative suggesting that they had recently been infected and would likely clear infection and seroconvert in time. A further bird was weakly seropositive against H5N3 antigen in house 1 although all birds in houses 1, 2 and 5 were negative for viral RNA.

This suggests that the infection may have spread to house 1 but had either been cleared without excretion and onward infection or had not yet managed to productively infect the birds tested. It is of importance to note that with the exception of a marginally reduced egg production, no clinical disease was seen in any turkeys at any stage during the investigation.

The detection of a single virus isolate precludes meaningful assessment of transmission pathways, whilst active infection was detected in the absence of overt clinical disease it had largely resolved at the time of report and epidemiological investigations failed to identify any onward transmission risk at the time or prior to report.

From a genetic perspective, few sequences are available for contemporary low pathogenicity avian influenza viruses, as they are rarely detected in poultry . However, the H5N3 detected in Cheshire shared common ancestors with other Eurasian poultry viruses including progenitor strains in contemporary wild bird viruses, consistent with a wild bird origin for this poultry incursion.

Public health impact

Food safety

The advice of the Food Standards Agency and Food Standards Scotland is that on the basis of the current scientific evidence, avian influenzas pose a very low food safety risk for UK consumers. Properly cooked poultry and poultry products, including eggs, remain safe to eat.

Likelihood of human infection

Public health officials undertook a risk assessment following confirmation of the H5N2 and H5N3 LPAI viruses and concluded that the risk to the general public was very low – given there have been no reported cases of human infection with H5N2 or H5N3 LPAI in the UK or Europe, and the low probability of exposure to infected birds.

Remaining uncertainty

There remains some uncertainty around the risk of disease incursion posed by wild birds, and when and where further cases or outbreaks may occur. There is continual evidence of ongoing threat from wild bird viruses since such LPAI strains are endemic in waterfowl populations in particular.

Therefore, we consider that there is an on-going low risk of another outbreak occurring in poultry on individual premises, but this likelihood will reduce during the warmer months when reservoir wild bird populations are much smaller and more dispersed. This risk is largely dependent on the level of biosecurity on the individual premises.

Concluding remarks

Extensive epidemiological investigations and routine disease surveillance activities did not detect the presence of infection in any further premises investigated in connection with the IPs, either by known contact (source and spread tracings), or as a result of proximity.

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

The World Organisation for Animal Health (OIE), Food and Agriculture Organization (FAO) International Reference Laboratory, UK National Reference Laboratory at APHA Weybridge has the necessary ongoing proven diagnostic capability for these strains of virus, whether low or high pathogenicity avian influenza, and continually monitors changes in the virus.

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’s Veterinary, Operations and Science Directorates

Low Pathogenicity Avian Influenza H5n2- AIV 2020/01 Kent

LPAI H5N2

Overview of the premises and the wider business

The infected premises (IP) was a small, family run, commercial business operated by two people. It comprised 15 acres and contained 554 birds of nine different species. Various other livestock (goats, and alpacas) and pets (guinea pigs, rabbits, tortoises and ferrets) were present. The poultry were mainly of non-commercial native British breeds.

Several activities took place:

1. Monthly purchases of 90 to 125 point-of-lay pullets that were then sold in small numbers to local keepers.
2. A retail pet supplies and feed business (including home deliveries), with a farm shop and storage facilities positioned amongst the poultry houses.
3. Rehoming of unwanted poultry and small pets.
4. A boarding service for poultry and small pets.
5. The door-step sale of eggs (but none sold during the preceding two months)
6. A horse livery yard.
7. A small number of sheep grazed on rented ground, separate from the main premises.

Description of the housing

The housing consisted of outdoor runs with huts. There were two main pens, one with a small pond, and another that housed mainly Indian Runner Ducks - although chickens had access to this area and were seen in the pen. Other small pens were scattered in various location across the premises. Chickens, geese, Guinea fowl, and some ducks, were free to roam over the whole site. Pigeons were free-flying. The canaries and finches were kept in an aviary.

Table 1: Species and number of each present

Species	Number of each present
Ducks	108
Geese	34
Chickens	319
Pigeons	69
Black swans	2
Rheas	2

Species	Number of each present
Guinea fowl	4
Canaries	14
Finches	2

Description of the surrounding area

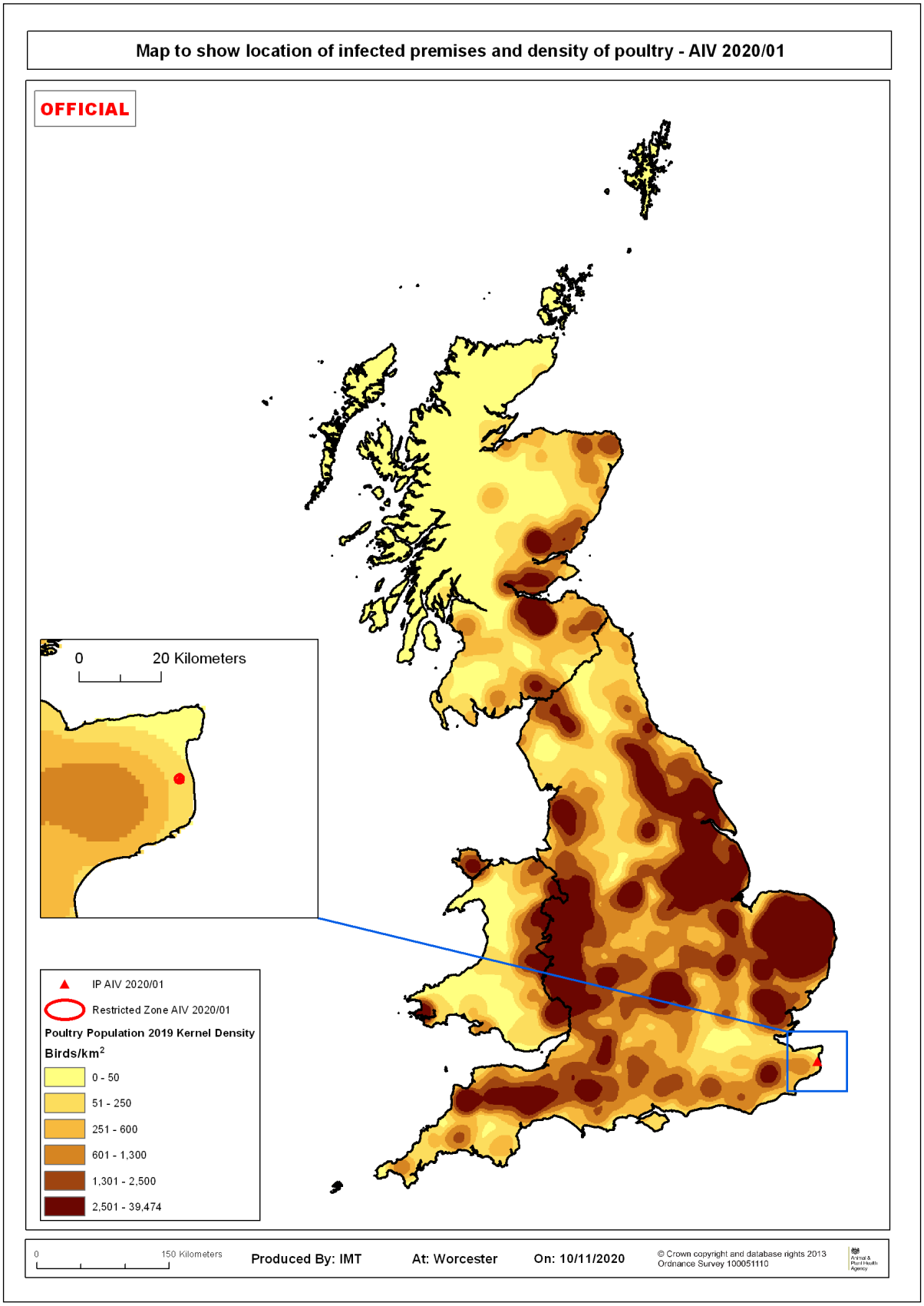
The premises was located within a low-to-medium poultry density area of Kent (see Figure 4). The only poultry flocks within one kilometre were ten small, hobby, poultry flocks.

Ornithological assessment

No expert ornithological assessment was carried out for this infected premises. However, it is relevant to note that the IP was located approximately 2 to 3 km from the East Kent coast and 1.5 km from an area of wetlands, these are part of the RSPB Worth Nature Reserve, where migrating birds are known to stop over.

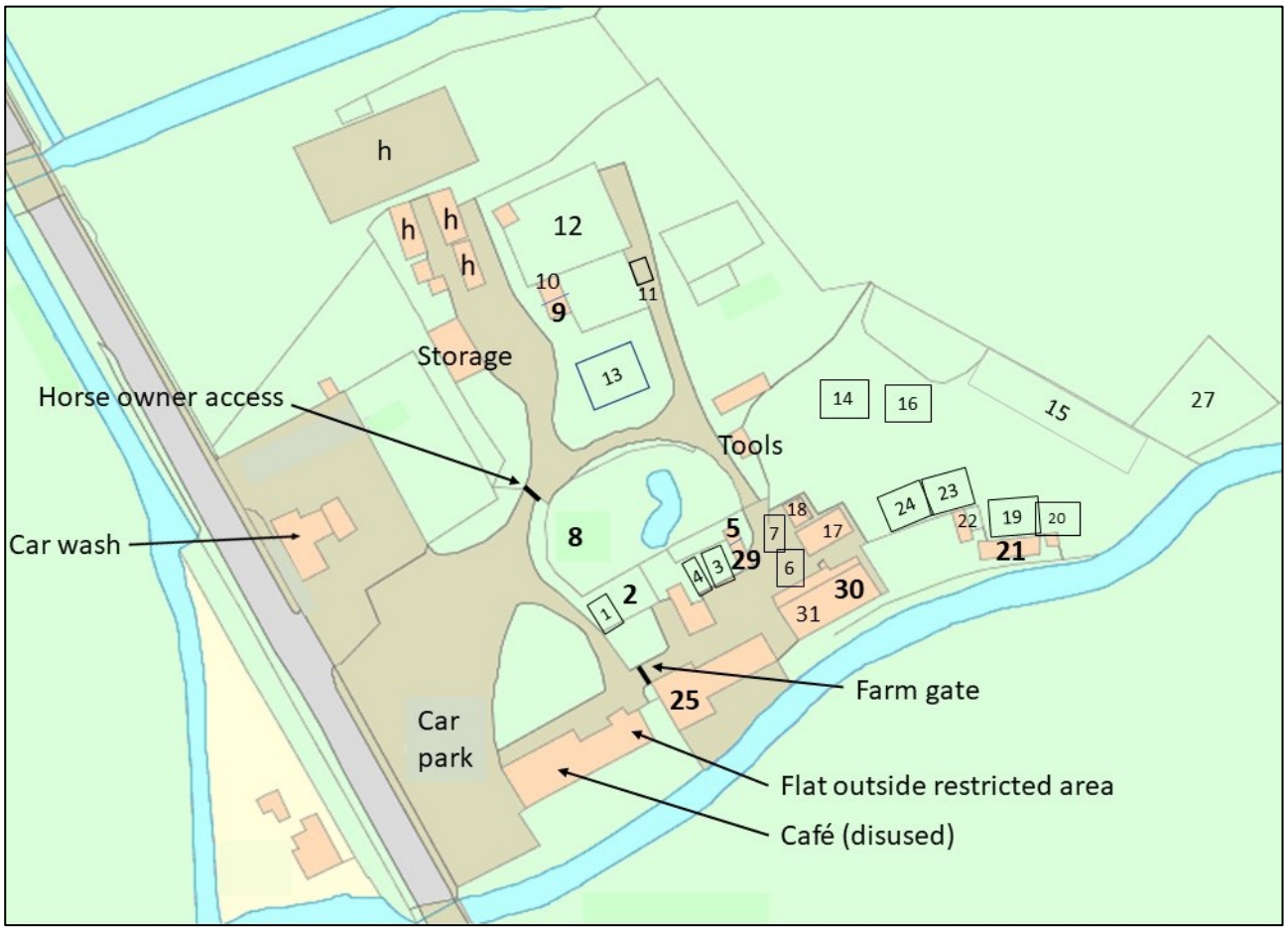
Location in Great Britain

Figure 2: Location of AIV 2020/01 in Great Britain



Plan of the infected premises

Figure 3: plan of the infected premises AIV 2020/01



Key for main features on the site (numbered in bold), other numbers are for smaller groups of birds.

#2 - 51 Indian Runner Ducks, 1 goose
#5 - 73 Mixed chickens and bantams
#8 - Large pen with pond. 10 geese, 53 ducks, 4 guinea fowl
#9 - 69 pigeons in loft
#21 - Aviary – 14 canaries, 2 Mexican House Finches
#25 - Domestic Dwelling House
#29 - Feed store
#30 - Pet and feed supply shop

Disease picture

A disease investigation was initiated following the detection of low positive antibody titres in three out of twenty duck serology samples, taken on 28 October 2020, as part of the routine annual UK avian influenza serological survey in poultry.

At the time of the APHA investigation visit on 30 October 2020, there was no clinical evidence of notifiable avian disease in any of the species on the farm. However, there were signs of ill-thrift in 4 seven week old chickens – 2 of the group had died within the previous week.

This was attributed to the effects of bullying from older chickens and a change of feed. This conclusion was supported by signs of recovery when feed was changed back to the original type, and these four sick birds later tested negative for AI.

Timeline

Tracings windows

Source tracings window:

Precautionary:	1 October 2020 to 7 October 2020
Likely:	8 October 2020 to 13 October 2020
High risk:	14 October 2020 to 21 October 2020

Spread tracings window:

Precautionary:	2 October 2020 to 8 October 2020
Likely:	9 October 2020 to 14 October 2020
High risk:	15 October 2020 to 30 October 2020

Most likely date of infection (Start of high-risk source tracing window)**:** 21 October 2020

Timeline

Table 2: timeline for AIV 2020/01

Source Tracing Window	Spread Tracing Window	Date	AIV 2020/01 ESTIMATED TIMELINE FOR SOURCE AND SPREAD OF INFECTION
Day 21		**01/10/20**	Start of precautionary source tracing window, as per OIE guidelines.
Day 20	Day 1	**02/10/20**	Start of precautionary spread tracing window (source + 24h).
Day 19	Day 2	03/10/20	
Day 18	Day 3	04/10/20	
Day 17	Day 4	05/10/20	
Day 16	Day 5	06/10/20	
Day 15	Day 6	07/10/20	
Day 14	Day 7	**08/10/20**	Start of likely source tracing window
Day 13	Day 8	**09/10/20**	Start of likely spread tracing window (source tracing window +24h).
Day 12	Day 9	10/10/20	
Day 11	Day 10	11/10/20	
Day 10	Day 11	12/10/20	
Day 9	Day 12	13/10/20	
Day 8	Day 13	**14/10/20**	Start of high risk source tracing window
Day 7	Day 14	**15/10/20**	Start of high risk spread tracing window (source +24h).
Day6	Day 15	16/10/20	
Day 5	Day 16	17/10/20	
Day 4	Day 17	18/10/20	
Day 3	Day 18	19/10/20	
Day 2	Day 19	20/10/20	
Day 1	Day 20	**21/10/20**	**Latest and most likely infection date for this outbreak based on current serology results available from AI survey bleed - single duck with antibody titre on threshold for significance and allowing for 7-14 days to seroconvert.**
	Day 21	22/10/20	
	Day 22	23/10/20	
	Day 23	24/10/20	
	Day 24	25/10/20	
	Day 25	26/10/20	
	Day 26	27/10/20	
	Day 27	**28/10/20**	AI survey bleed carried out non-negative serology in ducks
	Day 28	29/10/20	
	Day 29	**30/10/20**	Official APHA investigation **(DPR 2020/20)**. Restrictions served and sampling undertaken: 30 ducks & 30 chickens cloacal and oro-pharyngeal swabs
	Day 30	31/10/20	
	Day 31	01/11/20	Preliminary laboratory results detected Avian Influenza H5 viral genes by PCR testing in 4 out of 30 ducks and 2 out of 30 chickens. Decision to slaughter on suspicion pending further laboratory results (SOS AIV 2020 / 01).
	Day 32	**02/11/20**	Disease confirmed as **LPAI H5N2 (AIV 2020/01)**
	Day 33	03/11/20	Culling started.
	Day 34	04/11/20	
	Day 35	05/11/20	2 escaped guinea fowl culled
	Day 36	06/11/20	1 escaped chicken culled. Culling completed.
	Day 37	07/11/20	
	Day 38	08/11/20	
	Day 39	09/11/20	Preliminary C&D completed
	Day 40	**10/11/20**	Preliminary C&D considered effective
	Day 41	11/11/20	
	Day 42	12/11/20	
	Day 43	13/11/20	
	Day 44	14/11/20	
	Day 45	15/11/20	
	Day 46	16/11/20	
	Day 47	17/11/20	
	Day 48	18/11/20	
	Day 49	19/11/20	
	Day 50	20/11/20	
	Day 51	21/11/20	
	Day 52	22/11/20	
	Day 53	23/11/20	
	Day 54	24/11/20	
	Day 55	**25/11/20**	Preliminary C&D repeated following culling of an additional chicken and her brood that appeared on site
	Day 56	**26/11/20**	Preliminary C&D considered effective
	Purple colour reflects source tracing window. Increased intensity of colour reflects increased possiblity of introduction on these dates.		
	Yellow colour reflects spread tracing window. Increased intensity of colour reflects increased possiblity of spread from the on these dates.		

Assumptions

- Detection of viral nucleic acid in birds indicates that infection took place within the last 14 days, after this only antibody is present.
- Spread of infection within a flock is generally rapid once established, but can vary depending on virological, epidemiological and environmental factors.
- Assume earliest onset of detectable seroconversion is from 7-8 days post-infection.
- Incubation period is 2-14 days, up to 21 days from onset of earliest clinical signs for the purposes of the OIE Terrestrial Animal Health Code.
- Incubation period is generally considered most likely to be around 48-72 hours.

Investigations on the infected premises

Overview of biosecurity

There were no effective biosecurity measures practised on the holding. There was no regular disinfection of boots, or outer clothing, and no change of clothing between pens, or on entry to the domestic dwelling. Wild birds had access to the poultry, their food and water.

The walkway to the pens was hardcore, or damaged tarmac, on which there was pooling of rainwater and visible contamination with chicken and goose faeces. There was no documented rodent control on the site, although bait boxes were in use at various locations.

Chickens could access most parts of the premises and could even enter the on-site farm shop. Geese, Guinea fowl and pigeons were free flying, and reported to have been able to range beyond the boundaries of the infected premises. Carcases were disposed of by burning on-site in an unapproved, dustbin-type incinerator. Manure and litter were disposed of by burning on-site in a bonfire.

Overview of tracing activities

The following tracing events were investigated:

Source tracings

One high risk, live bird tracing and one very low risk, feed delivery were identified in the high-risk window. The high-risk bird premises was visited, placed under official restrictions, and sampled on 6 November 2020 with negative results.

Spread tracings

One high risk live bird tracing was visited, placed under official restrictions, and sampled on 5 November 2020 with negative results.

Further tracings were identified and assessed as being very low risk - four tracings associated with people, and seven feed-related tracings all within the high risk spread window, and one tracing for a bedding delivery within the likely spread window.

Source investigations

Hypothesis for the source

The most likely source for infection was considered to be direct or indirect contact with wild birds, with low uncertainty.

Assessment and evidence base for the likely source

The evidence for this was:

1. The lack of biosecurity measures allowing entry of virus from wild birds on vehicles, and people.

2. The attractiveness of the site to wild birds, because of the accessibility of feed, water and a pond, increasing the likelihood of close contact of potentially infected wild birds with poultry on the IP.

3. The proximity to the east Kent coast and the associated wetlands, where migrating birds are known to stop over.

4. The absence of any other viable risk pathways, as determined by epidemiological tracing activities.

5. The ranging of some poultry and birds outside the perimeter of the IP, where mixing with local waterfowl was likely.

Spread investigations

Assessment of the potential and likelihood of spread

Only one movement of live birds off the premises occurred within the spread tracing window, this was a group of three ducks moved off on 22 November 2020. These birds were inspected and sampled with negative results (tested using both PCR and serology).

The likelihood of spread from the IP by the movement of fomite was considered to be very low, because of the likely low environmental contamination.

A probable low viral output from the IP poultry was indicated by the apparent slow spread of infection within the premises, with low prevalence of infection in those birds sampled (PCR for H5 viral RNA - 6 positives out of 60 on 30 October 2020, and 2 out of 162 birds sampled on 3 and 4 November 2020), and low seroconversion (positive titres on 28 October 2020 in 3 out of 20 birds sampled, and on 4 November 2020 in 31 out of 105 birds sampled, with a wide range of titre levels).

Reported high rainfall and mild temperatures were likely to have diluted excreted virus, and increased the rate of viral decay. The results of environmental sampling for virus supported this assessment - viral RNA for H5 was detected in only one environmental faeces sample out of 15 samples collected across the premises.

Geese, Guinea fowl and pigeons were free flying, and reported to have been able to range beyond the infected premises. The pigeons were considered to have very low susceptibility, and were therefore considered very low risk, acting only as fomite spreaders of virus to other premises. The range of the geese and Guinea fowl is unknown, but it was considered likely that the geese mixing with waterfowl in the adjacent streams .

Ten premises with poultry were identified from either the national poultry register database and/or foot patrols within the one kilometre restricted zone. These were all small backyard keepers, with a range of 3 to 44 birds apiece.

One of these premises kept only pigeons. All were visited, and birds and records inspected. Sampling of 44 birds with negative results was undertaken on this premises because eggs were sold, qualifying it as a commercial operation. No indication of LPAI infection was found on any of the premises.

Remaining uncertainty

Movements of birds, livestock, people, vehicles, and other risk pathways were not recorded. on the small number of these were obtained from the owner’s memory, text messages and invoices. However, any risk was mitigated by a likely, very low risk of spread via fomites, due to the low level of virus excretion for this type of virus in these species.

There was also a heightened awareness of avian influenza in the area as a result of the national Avian Influenza Prevention Zone (AIPZ), and yet no subsequent H5N2 LPAI IPs were confirmed anywhere in Great Britain during the 2020 to 2021 season.

Low Pathogenicity Avian Influenza H5n3 Ip1 AIV 2021/04 (Cheshire)

LPAI H5N3

Overview of the premises and the wider business

The IP was part of a large, integrated commercial poultry breeding company, with a medium-sized turkey breeding flock, supplying hatching eggs to an associated company owned hatchery.

The site operated an all-in and all-out placement policy, the breeding birds had been placed at 22 weeks of age and these production groups normally remain on site until de-population at around 56 to 58 weeks of age.

Description of the housing

The birds were permanently housed within controlled environment poultry houses, and had no outdoor access.

Six houses were present (Houses 1 to 5 and the 'Stag House' – see site plan below in Figure 7). Houses 1to 4 were approximately 40 years old, with House 5 and the Stag House being approximately 15 years old.

However, all houses had been re-roofed approximately 12 months previously and the building fabric appeared to be in generally good condition, with no obvious breaches or defects in the structure. No obvious gross contamination of the roofs was observed, although a number of mature trees and conifers close to the houses could potentially act as roosting areas for wild birds.

All houses had solid sides, with the exception of House 5, which had mesh sides covered to approximately 25cm from the top edge. Ventilation was provided via weld mesh covered fans and vents.

The diameter of the mesh was sufficient to exclude entry by wild birds. The internal house walls were lined with solid plastic panels, for ease of cleaning between flocks, apart from House 5 where the walls were of unfinished breeze block construction.

Water was mains supplied and feed was provided via a sealed delivery system from feed silos located outside the houses.

Species and number of each present

Approximately 4,540 turkey breeders (stags and hens) had been placed between 25 November 2020 and 3 December 2020. The birds had been sourced from other local company-owned rearing sites.

Description of the surrounding area

The IP was located in a rural setting, immediately adjacent to a small village and within an area of medium poultry density. It was located very close to a busy main road with two lay-bys within approximately 100 m of the site perimeter.

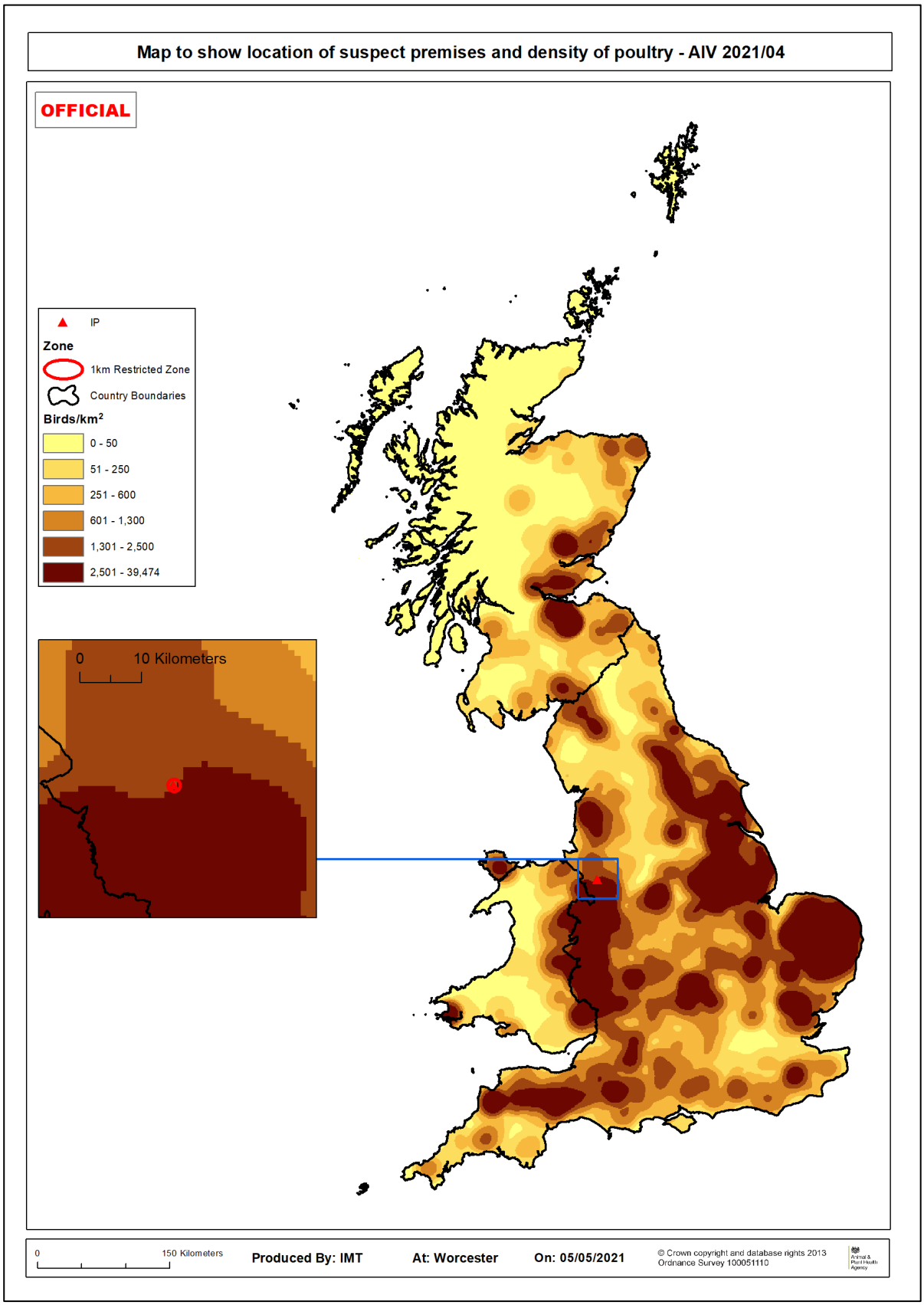
Apart from a nearby adjacent housing estate, the majority of surrounding land was fields used for rough grazing. There were no known immediately adjacent premises containing susceptible species, nor were there any large waterbodies nearby. Some ponds were present in nearby fields, but the extent of any use by wild bird species could not be determined.

Ornithological assessment

A specific ornithological assessment was not commissioned for this particular IP. However, during the epidemiological investigation pheasants, crows and pigeons were observed in the vicinity and it was reported that the site was regularly overflown by wild geese and that starlings were frequently observed.

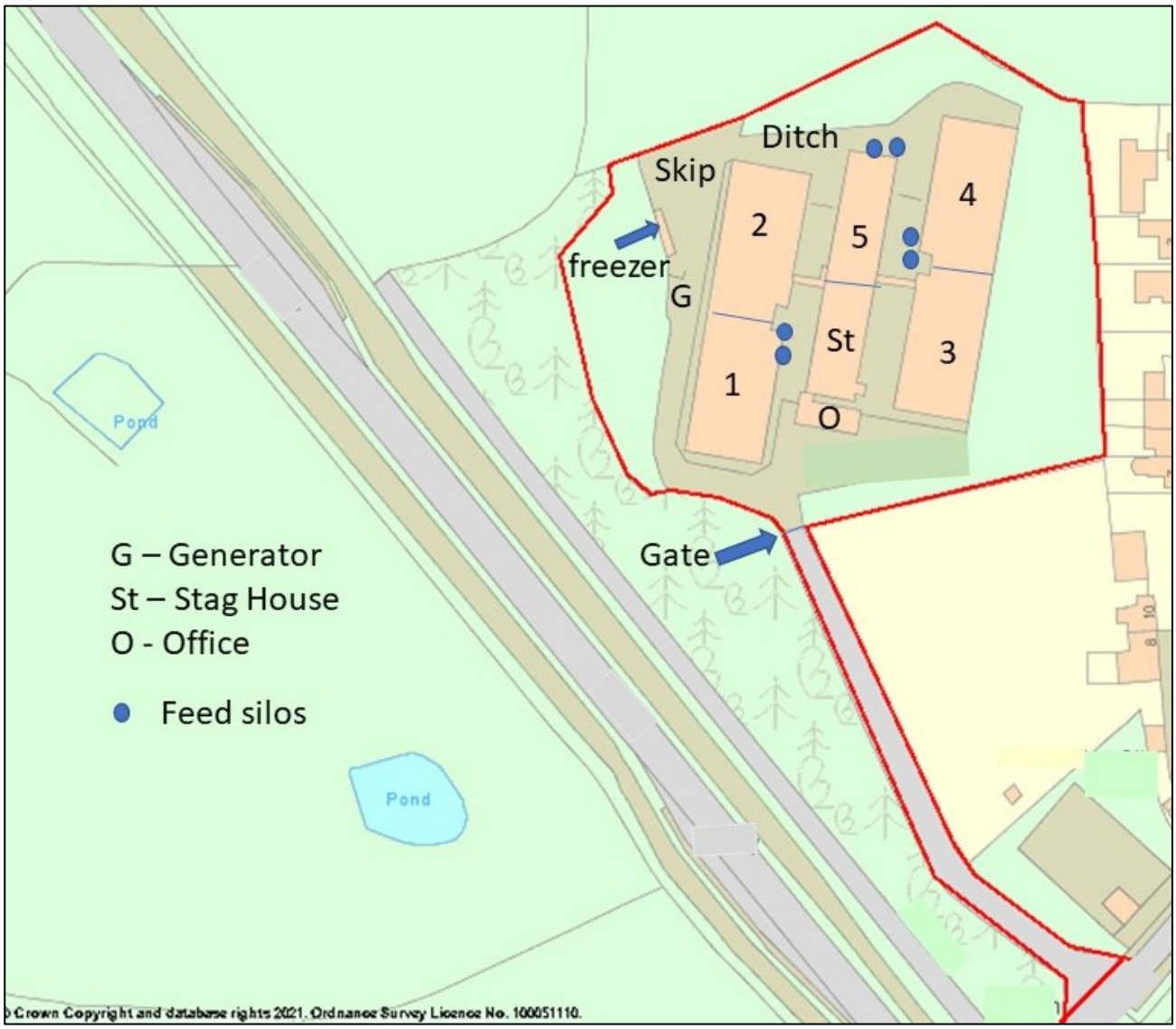
Location in Great Britain

Figure 4: location of AIV 2021/04



Plan of the infected premises

Figure 5: plan of AIV 2021/04



Timeline

Tracings windows

Source tracings window:

Precautionary:	17 February 2021 to 23 February 2021
Likely:	24 February 2021 to 6 March 2021
High risk:	7 March 2021 to 9 March 2021

Spread tracings window:

Precautionary:	18 February 2021 to 24 February 2021
Likely:	25 February 2021 to 7 March 2021
High risk:	8 March 2021 to 25 March 2021

Most likely date of infection (Start of high risk source tracing window): 7 March 2021.

The most likely date of infection was estimated on a precautionary basis following analysis of production records, alongside consideration of results of the serological testing undertaken, including the additional epidemiological samples taken following confirmation of disease.

Clinical picture

Clinical signs were first noted as a reduction in egg production observed from 12 March 2021 in House 3 of approximately 15% from the expected levels. This was initially attributed to 2 periods of power outage on 5 March 2021 and 8 March 2021 as a result of repairs required to a faulty generator.

All birds remained clinically healthy during this time, with no observed respiratory or behavioural signs, no changes in feed or water consumption, and no increase in mortality above expected levels since the flock was placed.

TTE samples (as part of UK early warning system) were submitted (as avian influenza was not suspected based on mild clinical presentation) from houses 3, 4 and the ‘Stag House’, and gave positive PCR results for Influenza A.

Restrictions were served on the premises and an official APHA veterinary investigation was initiated, with additional diagnostic sampling of these houses, being undertaken on 25 March 2021.

Following receipt of weakly positive PCR positive results for House 4, along with serologically positive results from all three houses sampled, the presence of avian influenza H5N3 was confirmed on 26 March 2021.

Following epidemiological assessment of the clinical picture and the initial laboratory results further epidemiological sampling was undertaken in Houses 1, 2 and 5 in order to investigate whether disease may have been present on the site for longer than the clinical picture and initial results indicated.

On 27 March 2021 cleavage site analysis of the H5N3 virus confirmed it to be of low pathogenicity.

Houses 2 and 5 returned seronegative results, with House 1 showing very low levels of seroconversion, with low antibody titres, in comparison to houses 3, 4 and the Stag House.

Consideration of all sample results received indicated that infection had most likely been first introduced into the Stag House (highest antibody titres) followed by houses 3 and 4 and finally house 1.

Following analysis of production records for all houses and consideration of the laboratory results, in particular the serological results, the most likely date of first introduction of infection onto the site was estimated, on a precautionary basis, as being 10 March 2021.

Timeline

Table 3: timeline for AIV 2021/03

Source Tracing Window	Spread Tracing Window	Date	
Day 21		**17/02/21**	Start of precautionary source tracing window, as per OIE guidelines (-21d).
Day 20		**18/02/21**	Start of precautionary spread tracing window (source + 24h).
Day 19		19/02/21	
Day 18		20/02/21	
Day 17		21/02/21	
Day 16		22/02/21	
Day 15		23/02/21	
Day 14		**24/02/21**	Start of likely source tracing window (-14d).
Day 13	Day 1	**25/02/21**	Start of likely spread tracing window (source tracing window +24h).
Day 12	Day 2	26/02/21	
Day 11	Day 3	27/02/21	
Day 10	Day 4	28/02/21	
Day 9	Day 5	01/03/21	
Day 8	Day 6	02/03/21	
Day 7	Day 7	03/03/21	
Day 6	Day 8	04/03/21	
Day 5	Day 9	05/03/21	Power outage for generator repairs.
Day 4	Day 10	06/03/21	
Day 3	Day 11	**07/03/21**	Start of high risk source tracing window (-3d). **Estimated most likely infection date for this outbreak.**
Day 2	Day 12	**08/03/21**	Start of high risk spread tracing window (source +24h). Power outage for generator repairs.
Day 1	Day 13	09/03/21	
	Day 14	**10/03/21**	**Precautionary onset of clinical signs following analysis of production records and consideration of serology results.**
	Day 15	11/03/21	
	Day 16	12/03/21	Onset of first clinical signs as initially reported - Slight egg drop reported in house 3.
	Day 17	13/03/21	
	Day 18	14/03/21	
	Day 19	15/03/21	
	Day 20	16/03/21	
	Day 21	17/03/21	
	Day 22	18/03/21	
	Day 23	19/03/21	
	Day 24	20/03/21	
	Day 25	21/03/21	
	Day 26	22/03/21	
	Day 27	23/03/21	APHA notified of non-negative serology results from private testing.
	Day 28	24/03/21	Testing To Exclude by PVS authorised - houses 3, 4 and Stag house sampled.
	Day 29	**25/03/21**	TTE results PCR positive for influenza A (Houses 3 and 4). APHA investigation and sampling (20:20:20) of houses 3, and Stag House (**DPR 2021/18**). Restrictions served.
	Day 30	**26/03/21**	Avian influenza H5N3 confirmed (**AIV 2021/04**). Stag House and House 3 PCR negative. House 4 two weak PCR positives. Seropositives in all 3 houses in majority of testable samples (Stag House 18/20, House 3 18/20, House 4 15/20).
	Day 31	27/03/21	Additional epi sampling (serology only, 20 samples per house) in Houses 1, 2 and 5. Houses 2 and 5 all seronegative, House 1 - 2/20 seropositive. Confirmed as **low pathogenicity.**
	Day 32	28/03/21	Culling commenced.
	Day 33	29/03/21	Culling completed.
	Day 34	30/03/21	Preliminary C&D completed 15:30.
	Day 35	31/03/21	Preliminary C&D considered effective 15:30.
	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		

Assumptions

- Detection of viral nucleic acid in birds indicates that infection took place within the last 14 days, after this only antibody is present.
- Spread of infection within a flock is generally rapid once established, but can vary depending on virological, epidemiological and environmental factors.
- Assume earliest onset of detectable seroconversion is from 7-8 days post-infection.
- Incubation period is 2-14 days, up to 21 days from onset of earliest clinical signs for the purposes of the OIE Terrestrial Animal Health Code.
- Incubation period is generally considered most likely to be around 48-72 hours.

Investigations on the infected premises

Overview of biosecurity

Initial appearances suggested that biosecurity on the site was of a reasonably high standard. The site was accessed via a long unmetalled driveway, leading to a gated main site entrance.

The outer perimeter of the site was enclosed with post and rail fencing, which was predominantly netted, or multi-stranded barbed wire, with an inner perimeter of chain-link fencing to a height of approximately 2 metres surrounding the poultry houses themselves.

Overall, the site appeared to be tidy and well-maintained with minimal presence of weeds or other vegetation seen. The immediate areas around the poultry houses were concreted, with some areas of interspersed gravel and hardcore leading to an onsite freezer, skip and the feed bins situated further towards the rear of the site. There appeared to very few features likely to attract wild birds to the immediate area near to the poultry houses.

Outside the entrance to the site itself was a small wooden hut containing the visitor record log and a knapsack sprayer for disinfection of vehicle wheels on entry to the site. All entrance gates to the areas surrounding the sheds were padlocked, with the exception of the entrance to the showers and offices.

All staff and visitors were required to shower-in to the main site containing the poultry houses and then put on site dedicated protective clothing.

The shower doors were externally locked using a keypad entry and there was good signage to deter unauthorised entry. There was CCTV at the front of the shower block, but at the time of the investigation it was out of order.

On entry to the poultry houses, there was a barrier to delineate where outside footwear should be discarded and replaced with dedicated internal footwear. Foot dips were present at each portal between sheds. All personnel had to enter the Stag House initially, and the sheds were then connected by internal corridors.

However, on further investigation a number of potential factors which could undermine the integrity of biosecurity were identified:

1. Whilst there were biosecurity protocols in place on this premises, they relied on visitors completing the visitor log unsupervised, and voluntarily adhering to the standard operating procedures in place. Gates at the beginning of the driveway from the public road were locked at night.
2. There was no drive-through wheel wash facility, and wheels would be likely to be heavily soiled from the dirt track approach to the site, thus rendering disinfection considerably less effective. Vehicles do not enter the primary biosecurity area. Both the egg collection vehicle and the feed truck are fitted with in-built wheel sprayers, which

are operated as they approach the farm and effectively spray the wheels all over with disinfectant.

3. It is known that the feed delivery driver wore his own overalls, and the egg collection driver used his own gloves.

4. Once through the shower block all staff and visitors would have access to all sheds at all times. The doors between sheds were often left open. For operational reasons the company operates the biosecurity of the site as a whole with all airspaces viewed as having the same status – given the nature of the operations on site and the layout of the farm it is considered be impractical to operate differently. The aim of the biosecurity on this site is to keep diseases out of the site as a whole.

5. When populated, the sheds themselves can only be accessed via the main entrances at the front of the stag house, via the shower rooms and office and communal kitchen area.

6. The shower doors were kept locked and under keypad entry only. However, the lack of a clear demarcation between clean and dirty areas could reduce efficacy of this biosecurity barrier and lead to compromise if items were passed between the areas prior to showering.

7. It was noted that although visitors shower in, the site staff were able to pass in and out of the houses in the same work clothes during the epidemiological investigation. This could have been as a result of relaxation in biosecurity protocols following confirmation of disease, but it could also be suggestive of a potentially more common practice leading to a potential route of entry for disease, for example, a fomite source if wild bird faeces contaminated footwear or clothing (staff are provided with colour coded boots for going outside and do not wear the boots they would wear within the housing, they also required to wear different footwear in the communal areas).

   Boots are changed within the initial access to the sheds, but contamination of communal areas could occur.

8. A period of five days freedom from any contact with poultry outside of the company (three days for contact with any other livestock species) was required for all visitors to the site. However, the company's own staff were permitted to move between different farms within 24 hours.

   The company policy is to visit one farm per day unless there is suspicion of disease when the interval between visits is extended to five days. There should therefore be no direct movement from one farm to another on the same day that involves accessing the internal bird spaces. The egg collection vehicle can visit more than one site per day,

but does not go anywhere other than the egg collection point, after which the egg store and external pad is cleaned and disinfected.

9. Trees surrounding the site could potentially attract birds such as starlings for roosting and there were tall conifers in close proximity to houses 3 and 4.

10. An area of water pooling was observed in front of house 3, with visible algal staining up to the doors, suggesting that this was not an isolated event, whilst there were rubber aprons on the doors, they were not flush against the ground in all places, and this could provide the potential for ingress of potentially contaminated external water into the poultry housing.

    It was confirmed that the rubber aprons on the outer doors are not the seal that prevents water ingress or fomite being blown under the doors. This is the function of a 45 cm high muck board behind the doors, which was close fitting to the ground with a rubber seal which seals the board to the ground. It was reported that no water ingress had occurred from this area.

11. Following periods of heavy rainfall, it was reported that an internal corridor floor drain floods and with the sloping concrete, this floodwater can flow into house 3 and soak the bedding near the door. This was reported to occur not infrequently (although no records were kept of such events) and whilst staff would apply approved disinfectant to any such areas and brush away floodwater this could represent another route for ingress of infection, as the source of the water is likely to be groundwater, which could have been contaminated by faecal material from wild birds.

    A drainage issue was reported as having developed in the weeks leading up to the outbreak. It was first seen during the extremely heavy rain and flooding seen in Cheshire in the late winter. During the cleansing and disinfection operation following the outbreak, a drainage problem was identified and corrected. The resulting floodwater did enter the bird pen, as it is surrounded by a solid concrete nest box plinth, but caused flooding in the pathways around it during intense rain.

12. On entry into the housing, dedicated footwear is used, but once inside the buildings the first access for entry and exit is into the Stag House. The corridor that floods is immediately after passage through the Stag House. In addition, whenever birds within the houses required to be culled (for example, for welfare reasons), their carcasses would be taken to the closest side access points. However, at the time of the investigation clear trails of dried blood were observed throughout the passageways.

    This could again have been a lapse following confirmation of disease, but if occurring more frequently could potentially contaminate footwear and lead to onward spread of disease between houses if any of the dead birds had been viraemic. It was reported

that dead birds are normally carried to two points on the farm that have facilities for boot changing in order that they may be taken to the dead bird storage unit.

Overview of tracing activities

A number of tracings were identified as detailed below:

1. Hatching eggs were collected at least three times a week and delivered to the company hatchery. This was identified as the highest risk spread tracing.

   The hatchery was 'designated' under relevant avian influenza legislation and as such subject to compliance with high standards of biosecurity and traceability. Following detection of disease on the IP the hatchery was placed under disease restrictions as a contact premises, and subjected to a thorough veterinary epidemiological investigation considering all aspects of its operation, including the standards of biosecurity and traceability practised. The company voluntarily decided to remove and destroy all hatching eggs present within the hatchery that had originated from the IP.

   Detailed veterinary risk assessment (VRA) was undertaken and concluded that given the observed high standards of hatchery operation and other biosecurity mitigations in place the likelihood of LPAI H5N3 spreading from the hatchery was very low (with medium uncertainty).

   Following satisfactory additional cleansing and disinfection at the hatchery following destruction of all eggs that had originated from the IP (under APHA supervision), and secure disposal of all associated hatchery waste, restrictions were lifted from the hatchery.

2. The vehicles and drivers involved in the egg collections were also identified. Their routes, any other potential bird contact and biosecurity protocols were all assessed, and this line of enquiry was deemed to be very low risk and no further action was taken.

3. The manager, six staff and five casual workers were traced. None had any other bird contacts, and all followed the company biosecurity protocols. On this basis, these tracings were closed.

4. The private company vet who visited to take samples did not undertake any other poultry visits for at least 48 hours after leaving the IP. This line of enquiry were closed.

5. There were no ABP tracings as all carcases and egg waste had been frozen and retained on the site.

6. An electrician had visited during the high risk source and spread window and a pest controller within the high risk spread only window. Both were contacted. Neither had entered the turkey houses, they followed biosecurity protocols and had no other same day bird contact. These lines of enquiry were closed.

7. There were three feed deliveries during the high risk spread tracing window. The driver and vehicle were identified. On each occasion the delivery was the last of the day and returned to the mill for a full cleaning and disinfection. The driver had no birds of his own. No further action taken.

8. There were no bedding deliveries during the high risk tracing window.

All tracing investigations in relation to this IP were satisfactorily completed and closed.

Source investigations

Hypothesis for the source

In the absence of any other plausible and likely high risk contacts, the most likely source of infection to this IP is considered to be indirect introduction from a wild bird source.

Assessment and evidence base for the likely source

Observation of flooding occurring within internal corridors following heavy rainfall, and also water pooling and algal staining around the doors to House 3 – floodwater is likely to be from an external groundwater source that could be potentially contaminated by a wild bird source.

Water could flow into House 3 and also contaminate internal corridors leading to subsequent fomite transfer to other areas (for example, the Stag House). There was wild bird activity observed in the surrounding area.

Direct introduction via domestic (or international) birds or products – negligible likelihood with low uncertainty: there were no recorded movements onto the premises of susceptible species or their products during the source window. No direct international links identified as a potential source for this IP.

Direct introduction from a wildlife source – negligible likelihood with low uncertainty: birds were permanently housed in well maintained, wildlife proof housing and no evidence of wild bird access to the interior of the houses nor evidence of vermin activity.

Indirect introduction via movements of personnel, vehicles or equipment (including egg collections) – tracings investigations and further assessments considered these to be of very low likelihood with low uncertainty.

Introduction via contaminated feed, bedding, or water – negligible likelihood with low uncertainty – no feed deliveries within the high risk source window, feed delivered via a sealed system and delivery driver has no direct contact with the birds, water is mains supplied and no deliveries of bedding onto the site during the high risk window.

Bales of bedding were plastic wrapped and stored on shrink wrapped pallets on which they were delivered. Although stored outside in the fenced areas between houses individual bales were disinfected before transfer inside the houses to replenish the bedding.

Any damaged bales which could potentially be contaminated were reported to be discarded and placed in the onsite skip, and evidence of this occurring was observed.

Whilst transfer of bedding bales into the houses could represent a potential additional route for introduction of infection this was considered less likely than other potential routes (and as above negligible for initial introduction onto the site itself).

Assessment of a short region of haemagglutinin (HA) gene sequence demonstrated that this virus clusters genetically with an H5N3 detected in the UK in 2019 (AV-19-033248) and a mallard in France from 2020 (A/mallard/France/20P017917/2020 (A/H5N3).

Intravenous Pathogenicity Index (IVPI) assessment of the UK virus in 2019 gave an IVPI of 0.0 (such as, no pathogenicity demonstrated). All these results together with mild clinical signs confirmed the virus to be LPAI virus.

Spread investigations

Assessment of potential and likelihood of spread

Overall, the highest potential risk of spread from the site was initially considered to be as a result of movement of hatching eggs to the company hatchery (and potential subsequent movement out of the hatchery of day old turkey poults and associated hatchery waste.

However, following detailed veterinary investigation of the hatchery and associated observed high levels of biosecurity, mitigations in place and traceability a detailed veterinary risk assessment concluded that the likelihood of onward transmission via this route was very low (with medium uncertainty).

Direct contact with other domestic susceptible species (or via products) – negligible likelihood with low uncertainty: no such movements occurred during the risk period.

Indirect contact via movements of personnel, vehicles or equipment (including egg collections) – tracings enquiries and further assessments considered these to be of very low likelihood with low uncertainty.

Onward transmission via wildlife was considered as very low likelihood with low uncertainty as the turkeys were permanently housed in well maintained buildings and the risk would be approaching negligible in comparison to the prevailing background level of infection in wild birds at the time.

Remaining uncertainty

The precise route of first introduction of LPAI H5N3 virus into the flock – whilst all the available evidence suggests that introduction of virus was most likely to have occurred via a source of contamination within the perimeter of the site (rather than being introduced from another external source) this cannot be conclusively proven.

Sources via ingress of contaminated water from the internal flooding drain or via water entering under incomplete door seals are considered most likely.

The results of serological sampling point to disease having been present in the Stag House for the longest period of time with subsequent rapid spread into Houses 3 and then 4. This may, however, be an anomaly resulting from the relative difference in sampling frames between the numbers of birds in the Stag House compared to Houses 3 and 4.

However, it is possible that increased handling of birds in the Stag House for breeding purposes could have led to more rapid spread of disease within this area compared to other areas, as well as the Stag House being a common route for entry and exit of the buildings and thus having the potential to be a route of introduction resulting from any other potential breaches in biosecurity due to other reasons.

However, results of serological sampling supported the precautionary most likely date of first clinical signs, and by extrapolation the most likely date of first infection, as estimated from analysis of production records.