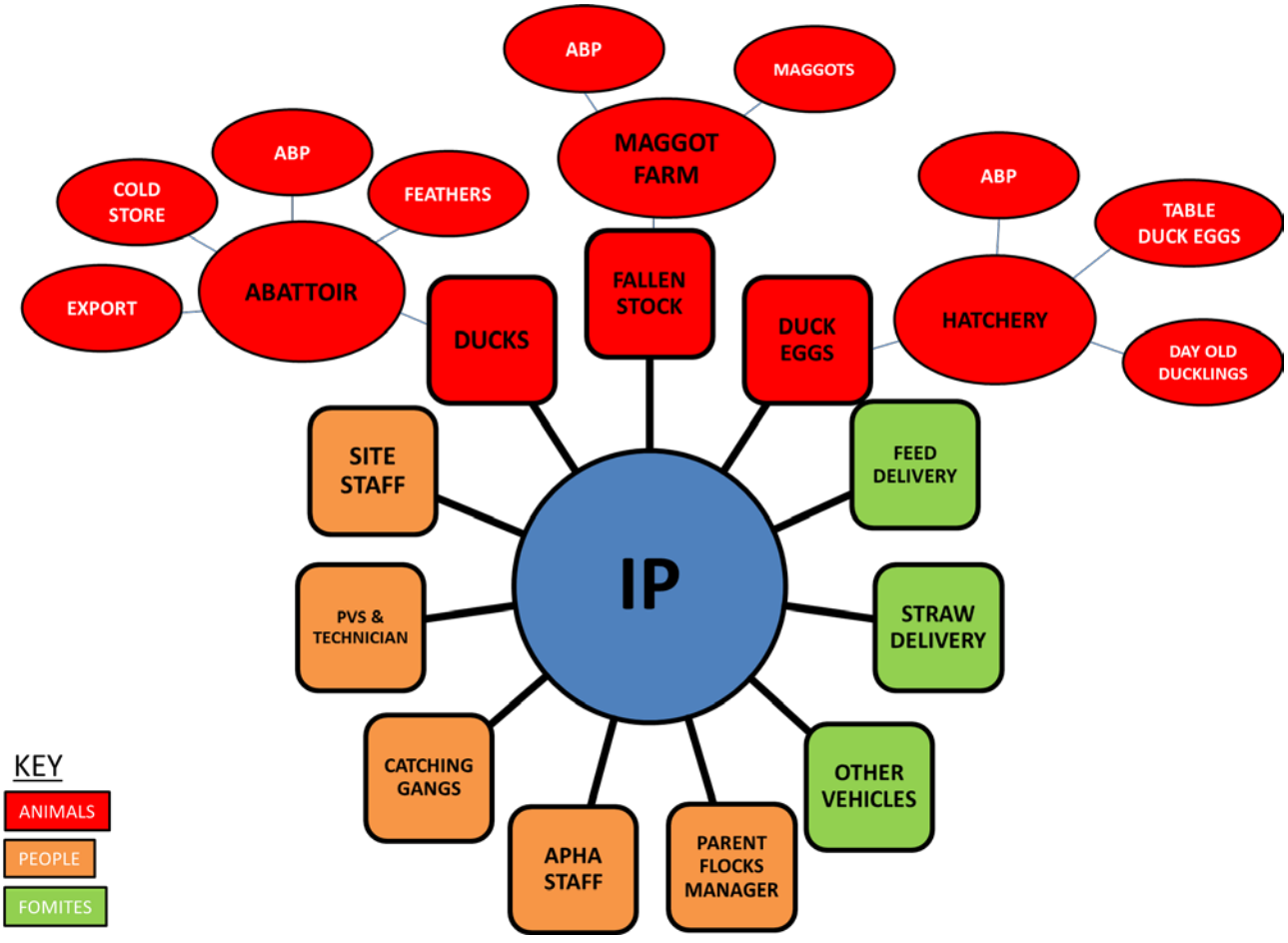
Animal & Plant Health Agency

EPIDEMIOLOGY REPORT

H5N8 Highly Pathogenic Avian Influenza outbreak (AIV2014/01) in breeding ducks

November 2014, England, UK



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January 2015

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1. Executive Summary

a. Highly Pathogenic Avian Influenza (HPAI) was confirmed on the 16th November 2014 on a duck breeding site in Yorkshire, which was designated as the Infected Premises (IP), reference number AIV2014/01. The IP was part of an integrated duck breeding and growing operation encompassing pedigree breeding through to commercial duck meat production, with 43 associated sites mainly situated in the Midlands or north of England. The company covers approximately half of annual UK duck production, and has a wide export market for its elite stock.

b. The virus that caused the outbreak is strain H5N8 HPAI, which is the same strain, with close sequence similarity, as that found in the outbreaks in the same time period in Germany in turkeys and ducks, in the Netherlands in chickens and ducks, and in Italy in turkeys. The same strain was recovered in a similar time frame from two (healthy) wild birds shot in Germany, from the faeces of wild birds in the Netherlands, a wild bird in the Russian Federation and has also been identified in apparently healthy wild birds, captive birds of prey fed on hunter killed wild birds and in an outbreak in a mixed backyard flock in the USA. The H5N8 HPAI virus has also been reported as the cause of many poultry outbreaks in East Asia in the past twelve months. The IP virus has been fully sequenced and the results suggest that it is still predominantly an avian-adapted virus, without any specific increased affinity for humans.

c. The most likely time that infection was estimated to have entered the infected flock is between 24th October and 6th November 2014. Initially there was uncertainty as to whether the other flock on the premises ('Site 1', which was routinely depopulated on 5th and 6th November 2014), was also infected. A significant drop in egg production had been noted in this flock before depopulation but this could have been due to routine management practices implemented to prepare the birds for slaughter. Further analysis of production and mortality data and initially negative PCR test results from eggs and feathers from this flock found that there was no conclusive evidence to suspect HPAI infection in these birds prior to depopulation and it was concluded there was a low probability that the flock was infected. However, early stage infection could not be ruled out, so the maximum precautionary tracing window up to the 8th October 2014 (21 days before the start of the egg drop observed on Site 1) was applied. Subsequently, further experimental analysis carried out on the feather samples yielded a positive result, implying that early stage HPAI infection was present in the flock before depopulation.

d. There is substantial uncertainty as to the source of infection for the IP, however all the available evidence suggests that indirect contact with infected wild birds (for example via their faeces) is the most likely source.

e. This assessment is based on: evidence that no poultry were brought onto the IP during the source window; that there is no evidence of infection in poultry in the local area; that there is no evidence of a direct industry-related connection to the cases in Germany, the Netherlands, Italy, USA or East Asia; the time of year of these outbreaks occurred with respect to wild bird migratory movements and the finding of this strain of virus in healthy wild birds in multiple countries are both supportive of a wild bird mediated introduction. Additionally the absence of other cases having been identified in the UK provides supporting evidence.

f. There are extensive personnel and other contacts within the company and the industry, and the ability of the virus to cause few clinical signs in ducks raised the

possibility that one or more associated duck premises were silently infected and were the source of infection. However extensive investigations have found no evidence of HPAI on these or any other holding in GB.

g. The IP itself has been assessed as being likely to be unattractive to wild birds and direct contact between wild birds and the farmed ducks themselves is thought to have been unlikely. However the moderate biosecurity on the IP, the known presence of this strain of virus in wild birds, and the location of the IP on the East Coast of England in the area where domestic poultry have been identified as being at risk of exposure to avian influenza infection in migratory wild birds, all support a hypothesis that the source was wild bird contamination of personnel, vehicles, or other equipment or consumables used on the farm.

h. Evidence from local surveillance completed in the protection zone and tracings of contacts with the IP indicates there has not been spread of infection from the IP into the local area or more widely. Premises at risk on which there are only species that may not show overt clinical signs (water fowl and some game birds) were subject to sampling with all results being negative.

i. In summary, following extensive investigations, no HPAI infection was found on any other UK premises and this outbreak was limited to a single IP. Preliminary cleansing and disinfection (C&D) of the IP was completed by 21st November 2014, and secondary C&D by 13th February 2015.

2. Introduction

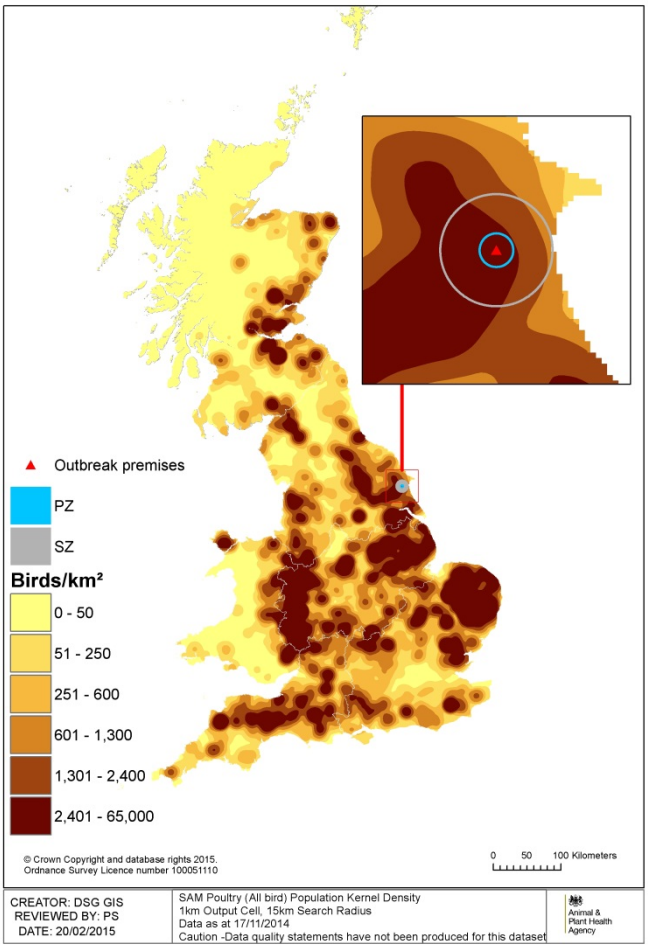
This report summarises all the epidemiological investigations carried out which seek to describe and explain the outbreak of H5N8 Highly Pathogenic Avian Influenza (HPAI) infection in breeding ducks on a premises in Yorkshire.

This report provides evidence to support the UK's claim to have controlled the outbreak and declare freedom from H5N8 HPAI to the EU and OIE; provides source material for the technical annex for UK co-financing claims to the EU; records logistics and technicalities of investigation and control to inform future resource planning, contingency plans and training requirements; and highlights gaps in our understanding of HPAI and so identifies areas for further research or other needs.

3. Description of the Infected Premises

The IP is situated in Yorkshire in the North East of England (see map below). It is a commercial indoor duck breeding site which routinely supplied hatching eggs to a company owned hatchery, primarily for commercial fattening on multiple sites in the UK, and occasionally for commercial vaccine production. Some of the eggs not suitable for hatching were supplied for human consumption via the UK market.

The farm held two groups of ducks in three sheds each. Sheds 1-3 (known as 'Site 1') contained 7499 ducks when first placed on the IP in June 2014. These ducks were depopulated on 5th and 6th November 2014 at the end of their second lay cycle. Sheds 4-6 (known as 'Site 2') contained 6779 ducks when first placed on the IP in December 2013. These were the birds in which HPAI was confirmed on 16th November 2014.



Map to show location of IP and density of poultry

The ducks were housed, with straw bedding and mains supply drinking water. Pre-mixed feed was delivered regularly into enclosed storage hoppers. Eggs were collected once daily by hand, washed in a solution of sodium hypochlorite, rinsed in a dip tank, held in a temperature controlled room and collected twice weekly by the hatchery. Waste duck carcases were collected and regularly taken to a maggot farm for disposal, with the last collection being on 13th November 2014. No waste straw, feed or manure had left the IP during the tracing periods.

Biosecurity on the site was moderate, with some separation between clean and dirty areas. However this was not complete and in addition the perimeter and one house were not fully secure to potential entry by wildlife. Further details of biosecurity procedures are provided in the detailed IP description at Appendix 1. Members of the Ornithological Expert Panel (OEP) undertook a visit around the IP and the protection and surveillance zones (PZ/SZ) on 18th November 2014 and reported an extremely low wild bird population being present at that time, and no evidence to suggest that more birds had used the site previously.

The area is poultry and pig dense with a large number of commercial premises as well as hobby farmers. Three backyard poultry flocks are present in the very near vicinity of the IP perimeter.

The IP is owned by an integrated duck breeding and growing operation encompassing elite pedigree breeding through to commercial duck meat production, from both company farms (16 sites) and contract producers (27 sites) (Appendix 5). The company is responsible for approximately half of annual UK duck production with production sites in central and northern England. Investigations have confirmed that the IP in Yorkshire was the only affected premises in UK, and in the company.

4. Timeline of key events

29/10/14	Drop in egg production in Sheds 1-3 (Site 1). This was an expected consequence of the management practices undertaken prior to the planned depopulation but experimental testing has subsequently revealed the presence of virus in waste feathers collected from within the Site 1 sheds, suggesting the presence of early HPAI infection in this flock at the time of depopulation.
05-06/11/14	Planned depopulation of Site 1 at end of lay cycle.
07-09/11/14	Keeper reports and examination of the egg production and mortality records show evidence of clinical disease developing over this period, starting with mild egg drop being first evident on the 8th November and progressing to severe egg drop and increasing mortality by the 10th November.
11/11/14	Private veterinary surgeon (PVS) visit to premises. Post mortem and clinical investigation - no suspicion of notifiable disease but clinical signs consistent with septicaemia and aspergillosis noted.
12/11/14	PVS test results indicate presence of bacterial infection. Soluble antibiotic started. Antibodies for Egg Drop Syndrome have also since been confirmed in these birds.
13/11/14	No further deterioration. Continued mortalities and reduced egg production.
14/11/14	PVS reported to APHA substantial increased mortality over 24 hours in Site 2 with no improvement in egg production. APHA applied restrictions verbally and a Report Case investigation was initiated. A Veterinary Officer visited the premises the same day and took initial samples for testing.
15/11/14	Initial serology negative for H5/H7 AI and ND; H5 PCR positive.
16/11/14	Sequencing results confirm high pathogenicity AI. N1 PCR negative. UK CVO confirmed presence of H5 HPAI. 3km and 10km protection zone (PZ) and surveillance zones (SZ) established.
17/11/2014	Repeat tests on the original samples gave positive serology results, based on a more sensitive test using H5N8 virus from the IP.
18/11/14	N-type of virus confirmed as N8; virus designated H5N8.
18-19/11/14	Depopulation of all birds in Site 2. Samples taken for epidemiological purposes at time of culling.
19-20/11/14	Preliminary cleansing and disinfection (C&D) undertaken and completed.
21/11/14	Preliminary C&D fully effective.

5. Investigations on the Infected Premises

HPAI was confirmed in the flock on Site 2. Laboratory results from the initial diagnostic sampling indicate that Sheds 4 and 5 were infected before Shed 6 and this is supported by the clinical evidence. Assessment of the serology and virus detection results from the diagnostic and subsequent epidemiological samples collected from the Site 2 flock indicate rapid spread through the flock with clearing of the virus as antibody developed. Expert opinion suggests the pattern seen was indicative of recent infection i.e. weeks rather than months (as shown in the timeline and tracing windows). The tropism of the virus and pathology findings support a non-severe infection in the majority of birds and expert opinion is that the presence of inter-current disease within the flock may have been responsible for the clinical presentation and mortalities. The consistent virus shedding via the cloaca shown in the test results supports effective faecal–oral transmission, particularly within this setting where the ducks were housed.

There was uncertainty around the infection status of the Site 1 flock (housed in sheds 1-3) which was depopulated on 5th – 6th November 2014. Therefore further investigation and analysis was carried out on the egg production data, egg hatchability data, flock mortality data and slaughterhouse inspection reports for the Site 1 flock, as

well as assessment of test results from eggs produced by this flock prior to depopulation, and waste feathers collected from within sheds 1-3 after depopulation.

The flock production data showed a significant drop in egg production starting on 29th October 2014. Field epidemiology investigations determined that routine management changes were implemented at the end of the laying cycle (restriction of feed and lighting), to prepare the birds for slaughter. Comparison with production data at the end of the 1st lay cycle for these birds and data from other similar flocks indicated that the reduction in egg production seen was consistent with that which could be expected following these routine management practices. Therefore the possibility that this reduced production was due to the presence of disease could not be differentiated from the expected reduction due to management practices.

Mortality data (both daily rates and cumulative mortality data) was within normal limits with no evidence suggesting clinical disease due to infection with HPAI virus. Ante and post mortem inspection data at slaughter revealed gross evidence of air saculitis, aspergillosis and septicaemia, similar to that observed in birds from Site 2, which could have masked any (more subtle) gross pathology suggestive of HPAI. Egg hatchability data did not provide any evidence that the birds on Site 1 could have been infected with HPAI for the lay date period approximately 8th - 18th October 2014, but the structure of this data made interpretation difficult. Further assessment was not possible due to the limited data availability.

The initial conclusions from this analysis were that, whilst subclinical infection with HPAI H5N8 in the Site 1 flock prior to depopulation could not be ruled out, the available evidence suggested that there was low probability that these birds were infected with HPAI. If infection was present, it was likely to be very early stage infection. However, it was still recommended to take a precautionary approach and maintain the extended source tracing window back to 8th October 2014 (21 days before the start of the egg drop observed in Site 1) for the following reasons:

- The value of production data as an indicator of HPAI disease in ducks is uncertain, given the low susceptibility of ducks to clinical disease due to HPAI in the absence of other concurrent disease;
- H5N8 infection in ducks is expected to cause a drop in egg production, but it is not known at which point, following onset of viraemia, that this would occur;
- Management practices and concurrent disease could have masked the onset of early clinical HPAI infection;
- The overall biosecurity on the premises was not sufficiently robust to mitigate the risk of infection being transmitted between the Site 1 flock and the Site 2 flock (the infected flock) prior to depopulation of the birds on Site 1;
- The initial feather testing yielded a negative result. However, in the absence of final confirmed negative egg testing and forward tracings results at the time of this initial analysis (November 2014), there was still sufficient uncertainty in the overall conclusions to justify the precautionary assumption of infection in these birds;
- There were some limitations in the data available for assessment which meant there was, at best, a low to medium level of uncertainty for the majority of the conclusions drawn.

PCR testing conducted on pooled waste feather samples collected from sheds housing the Site 1 flock post depopulation did not recover viral RNA. Although this test had not been validated and the sensitivity of this method was uncertain, expert advice was that feathers are a reliable sample for testing. However HPAI H5N8 infection in the birds on Site 1 could not be ruled out on these feather testing results alone.

Similarly, no viral RNA was detected in the limited number of eggs tested, implying that the Site 1 flock may not have been infected for the period from just prior to slaughter back to the earliest feasible lay date of 14th October (supported by hatchability data analysis). The hatching eggs tested from the site 2 flocks also failed to reveal the presence of virus, demonstrating that the value of a negative result in these circumstances was questionable and could not be taken as evidence of absence of infection. The effect of infection with H5N8 and related viruses upon the developing egg is unknown, although it is not unlikely that there would be rapid embryo death and increased numbers of infertile eggs. This could result in an unavoidable selection bias at the time of sampling due to routine removal of infertile/dead in shell eggs as part of normal hatchery management practices, reducing the number of eggs in the sampling pool that are more likely to have influenza present in the contents/embryos. Therefore, while a positive result would have been indicative of HPAI infection, the negative result was not considered to add substantially to the overall assessment of the infection status of the birds from Site 1.

Subsequently, further experimental analysis completed at the end of December 2014 on the feather samples collected from the Site 1 flock using an H5 PCR test specifically designed for the outbreak virus, yielded a positive result. Sequence analysis of this sample revealed it to be H5 HPAI. This experimental result suggests that the birds on Site 1 were infected with HPAI and supports the precautionary approach taken to maintain the extended tracings window based on the conclusions of the analysis of the production and other available data (which are unchanged by the feather result). The tracing windows were specifically designed to account for this possible eventuality, so are unaffected, and so there are no implications for the efficacy of the disease control operations or the final conclusions of the outbreak investigation.

6. Overview of tracing activities to investigate source and spread

Evidence based on the clinical picture, laboratory results and expert advice gave the following source and spread time windows:

- Most likely date of introduction of infection is between 24/10/14 – 6/11/14, with a maximum precautionary source period back to 8/10/14.
- Most likely potential for spread from the premises is between 25/10/14 - 14/11/14, maximum precautionary spread period is 9/10/14 - 21/11/14.

The tracing windows are based on the assumption that the infected premises was exposed to a single point source of infection; a most likely incubation period of 2-14 days and a maximum period of 21 days; with an additional precautionary period accounting for the possibility of undetected infection in Site 1 prior to depopulation (later supported by experimental analysis, see above). A diagrammatic representation of the timeline and likely time windows for source and spread is shown in Appendix 2.

The tracing work was extensive due to the presence of two flocks on site, with recent depopulation of one of these, numerous contacts by personnel (including poultry catching gangs which visited numerous other poultry premises) and the holding being part of an integrated company which conducts movement of flocks between sites, between 1st and 2nd lay cycles, and movement of eggs via a hatchery.

In total 271 tracing tasks were generated with 155 premises identified as potential contact premises via tracings. Investigations have been completed on all premises including clinical inspection, checks of production records and testing where indicated, with negative findings. This included investigation of the six premises most closely associated with the infected premises within the company structure. A diagram and map that show the extent of tracing activity, together with tables of tracing and stock numbers, are attached at Appendix 3.

In addition to tracings and visits to all premises in the PZ, awareness was raised in SZ premises and wider with a range of measures.

7. Source investigations

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

A summary of the sources of infection considered is shown in Table 1; definitions of qualitative risk terms and confidence levels are given in Appendix 4.

Table 1: Possible source of infection for the Infected Premises AIV 2014/01

Pathway	Comment	Assessment of likelihood of infection via this route
Direct introduction from wild birds	H5N8 HPAI has been detected in samples from one common teal (*Anas crecca*) and one mallard (*Anas playrhynchos)* in Germany and in two faecal samples from Eurasian wigeon (*Anas penelope*) in the Netherlands. The distribution, flight paths and normal movement periods of these and other waterfowl species in Northern Europe make this a plausible source of infection. One of the sheds on the IP had ventilation openings large enough for wild birds to pass through. Other sheds had mesh walls allowing very close (if not direct) contact between wild birds and poultry. However an expert ornithological assessment found that the IP site was not attractive to wild birds i.e. type of sheds, location and containment of feed etc. and there was an extremely low wild bird population around the IP.	Low likelihood but still possible. Moderate uncertainty

Pathway	Comment	Assessment of likelihood of infection via this route
Indirect introduction from wild birds	Whilst the ornithological assessment reported an extremely low wild waterfowl bird population in the vicinity of the IP, gulls were noted in adjacent fields and moorhens from a watercourse adjacent to the IP were seen wandering onto the site, with webbed feet marks being observed in front of the duck sheds. Equally, indirect introduction may have occurred via infected wild bird contamination from more distant areas. This remains a likely source, especially given the location of the IP on the east coast where there is a higher risk of wild bird exposure (see Appendix 6), the extensive number and type of movements of personnel and equipment both on to, and between sheds on, the IP and the incomplete biosecurity.	Most likely source although uncertainty remains about exact route of entry.
Undisclosed infection in the UK: Direct introduction by purchased birds	Investigations showed that no poultry or eggs were brought onto the premises during the source tracing period (08/10/14 – 6/11/14) or for a substantial time before.	Negligible
Undisclosed infection in the UK: Indirect contact with an infected flock	Investigations of poultry premises in the local area (PZ and SZ), and those with known links to the IP in the source tracing window have concluded with negative findings. This includes investigation and sampling where relevant at the company sites most closely connected with the IP. Reports of suspicion of notifiable avian disease on 25 other premises in GB during 2014, 16 of which were subsequent to this outbreak, were all negated.	Very low. Low uncertainty, however there were some premises which could not be sampled due to depopulation.
Introduction from contaminated product(s)	Drinking water supply was from the mains source and header tanks were covered . Feed was pre-mixed before being blown in to feed hoppers and distributed to birds. Straw was sourced from a site away from poultry – it had been stored in a stack that was not wild bird proof. However, the straw delivered to the IP had been taken from the middle of the stack and should not therefore have been subject to contamination by wild birds prior to being loaded onto the delivery vehicle The possibility of straw becoming contaminated (e.g. by wild bird faeces) during transport/unloading could not be entirely ruled out.	Negligible for drinking water or feed on IP. Risk of contamination of straw remains but assessed as low likelihood.

Pathway	Comment	Assessment of likelihood of infection via this route
Infected premises in DE or NL (or elsewhere in the world): Direct introduction by purchased birds	Birds on the IP were sourced from within the company's national production chain (i.e. not imported stock). Phylogenetic analysis suggests high degree of similarity (but not identical) between UK, DE and NL viruses. However the first confirmed cases in DE and the NL were in different poultry species and production systems (turkeys and chickens respectively) with no identified direct links.	Negligible as no birds had been introduced during the source tracing period. Low uncertainty - company structure and imports of poultry and eggs from Germany and NL to the UK show no route for contact with the IP
Infected premises in DE or NL (or elsewhere in the world): Indirect contact with an infected flock	No evidence of company links to the infected premises in DE or NL.	Unlikely source for the IP. Low uncertainty.

Further consideration of H5N8 HPAI in Europe and the role of wild birds

H5N8 HPAI was reported in four European Union Member States including the UK in November and December 2014. Germany reported three outbreaks in housed domestic poultry in two regions (North East [1, turkeys] and North West, [1, turkeys; 1 ducks]), two cases in healthy wild birds (teal, *Anas crecca* and Mallards, *Anas playrhynchos*) in the North East and East, and most recently 1 case in captive wild birds (white storks, *Ciconia ciconia*) at a zoo in North East Germany. The Netherlands reported 5 outbreaks in housed domestic poultry (4 in chickens, 1 in ducks; 2 outbreaks were epidemiologically related, the other three were separate incursions, according to the virus gene sequences) and two positive samples from wild bird faeces (Eurasian wigeon, *Anas penelope*). Italy reported a single outbreak in a commercial turkey farm in Veneto, a region with a large population of wild waterfowl.

During December 2014, the European Food Safety Authority convened a group of experts from affected member states (Netherlands, Germany and the UK) to examine the role of wild birds in greater detail and concluded that "while the entry of H5N8 HPAI into Europe may have been long distance transmission as a result of cross infection between different birds in north Eurasian breeding areas, the infection of housed poultry in Europe was more likely through indirect introduction via humans, vehicles, equipment, fomites, live animals and/or animal-derived products contaminated with virus from faeces of infected birds rather than through direct contact with infected wild birds" (EFSA, 2014 http://www.efsa.europa.eu/en/efsajournal/pub/3941.htm).

The spatial and temporal patterns of detection in poultry and wild birds, together with phylogenetic analyses indicating common ancestral viruses as recent as late summer 2014, support the likely introduction of H5N8 HPAI viruses into Europe via wild birds. The ecology and behaviour of migratory wild waterfowl that return to northern Europe from north-central Russia in late summer/autumn onwards provides a potential pathway. This hypothesis is further supported by phylogenetic analyses of viruses detected in both poultry and wild birds in eastern Asia. In addition detections in North

America of closely related H5 viruses support intercontinental dispersal via wild birds, particularly in the absence of epidemiological evidence identifying introduction pathways via the poultry sector or associated ancillary activities. Continued detections in Germany of H5N8 HPAI virus in wild waterfowl indicate ongoing presence of this virus in wild bird populations.

Assessment of likely source

The most probable source of the outbreak is indirect contact with wild birds, for example through faecal contamination of the environment which was then transferred into the duck sheds by means of contaminated fomites (e.g. personnel, equipment etc.). This assessment is based on the following key pieces of evidence:

- The IP itself has been assessed as unattractive to wild birds and direct contact is thought unlikely. However the moderate biosecurity on the IP (including observations of moorhens from an adjacent water-filled ditch entering the site), the known presence of this strain of virus in apparently healthy wild birds, and the location of the IP near the East Coast of England in the area where domestic poultry have been identified as being as at higher risk of exposure to avian influenza infection in migratory wild birds, all support a hypothesis that the source was wild bird contamination of personnel, vehicles, or other equipment or consumables used on the farm.
- There have been no other cases identified in domestic poultry in the UK despite raised awareness following confirmation of disease, active surveillance undertaken in the local area, on premises with known links to the IP and the ongoing passive surveillance programme with a legal requirement to report suspicion of avian influenza to APHA.
- There were no poultry or eggs brought onto the IP in the source window and there is also no evidence of contaminated product being brought on.
- There is no evidence of a direct connection to the cases in Germany (DE), the Netherlands (NL), Italy, USA or East Asia. The confirmed cases in DE and the NL in the same period were mostly in different poultry species and production systems, and investigations showed no links with any of them to the IP.

This conclusion that the incursion of AI into housed birds raises questions as to the role of housing as a protective measure to prevent such incursion. Our current state of knowledge and the range of possible explanations mean that we cannot be sure of the value of housing, nor can we rule out its possible protective effect. There are a number of possible explanations which have been postulated, which consider both the probability of exposure, and the subsequent potential for propagation and transmission. These include:

- Incursion of AI from wild birds is a rare event so determining any pattern is difficult;
- If the hypothesis that outdoor flocks have a higher probability of incursion via direct or indirect contact with wild birds than indoor flocks is true, the observed pattern of more incursions to indoor flocks may still be expected if many more indoor flocks are present across Europe (as is thought to be the case for some poultry types).

- There is much that we do not know about the interface between wild birds and poultry with regards to the epidemiology of avian influenza. Both the wild bird and poultry populations in Europe are highly heterogeneous with respect to demographics and other factors likely to influence avian influenza infection. This fact combined with variation in our ability to detect AI in different situations means finding "the signal through the noise" is a considerable challenge and the potential for undetected disease is not negligible;
- There may be differences in the amplification of virus within housed flocks versus outdoor flocks, affecting the potential for establishing and propagating infection;
- The ability of the virus to successfully adapt to a domestic poultry species may be enhanced by the constant availability of close susceptible contacts so the kinetics and dynamics of transmission in housed flocks may have a role, even for a highy pathogenic strain (i.e. HPAI).

8. Spread investigations

Potential routes of onward transmission both within and outside the company are shown in Table 2, together with comment on probability of transmission and action taken. In summary there was no evidence of any spread of infection from this IP.

Table 2: Possible spread of infection from the Infected Premises AIV 2014/01

Pathway	Comment	Assessment of likelihood of infection via this route
Direct contact of live birds on IP with infected domestic poultry on another holding	All bird movements off the IP were to slaughter, with the last movements off on 5th and 6th November 2014 (Site 1). There was potential for direct contact with free-range poultry (chickens and geese) adjacent to the IP, but all holdings with poultry in the PZ, including these, have been investigated with negative findings.	Negligible. Low uncertainty given the management of the holding.
Movements of poultry products off IP - eggs, meat, feathers	Ducks on Site 1 may have been infected shortly before depopulation (see above). All carcases from Site 1 were traced and destroyed. All the IP eggs were assessed as high risk for the potential onward transmission of disease, with possibility of fomite spread between eggs from the IP and those from other holdings in the same setters – all IP eggs, and all non-IP eggs that could potentially have been contaminated, were destroyed. All ducklings hatched from IP eggs laid during the risk period and that had moved out of the hatchery prior to confirmation of disease were traced and investigated with negative findings. Feathers were sent to a feather treatment plant; a risk assessment concluded that the risk of onward spread from feathers following treatment was negligible.	High likelihood that infected and/or contaminated eggs went to the hatchery. Medium likelihood that Site 1 carcases were infected.

Pathway	Comment	Assessment of likelihood of infection via this route
Movement of contaminated substrate off IP - manure, straw, carcasses	Waste duck carcases were moved from the IP to a maggot farm, and a Veterinary Risk Assessment was conducted on this move. Used straw/bedding had not been moved off the premises during the maximum spread tracing period. Some used bedding that had spilled out of the Site 1 sheds during depopulation was seen on the farm yard but this was addressed by APHA staff.	Negligible risk from carcases to maggot farm. Low risk from the used straw/bedding as remained on site and subject to C&D procedures following depopulation.
Indirect contact via personnel, equipment or vehicles	Documented contacts include o Catching team and drivers o Hatchery collection staff o Company area manager o IP staff and residents o Private Vet and technician o Feed delivery staff o Straw delivery dealer o Maggot farm collection driver o APHA staff Some traced premises had been depopulated prior to confirmation of disease on the IP. These premises were all investigated – some had subsequently re-populated with birds; some premises re-populated with ducks were sampled with negative results, others re-populated with galliform species had clinical inspections and checks of production records with no evidence of disease being found. Premises that had not re-populated had a veterinary risk assessment (VRA) of the standards of cleansing and disinfection undertaken in order to provide reassurance that virus, if present in the previous flocks, would have been destroyed and thus could not infect subsequent flocks. Results of these VRAs and signed owner declarations of C&D undertaken, gave reassurance that no virus persisted on these sites.	High likelihood of contact with infectious virus for first five of these. High uncertainty as to subsequent contacts with susceptible birds, however all have been traced and investigated with negative results.
Local spread into PZ and SZ	All PZ premises visited and investigated, and ducks, geese and game birds tested if not directly co-located and mixing with galliforms. No commercial waterfowl or captive game bird premises were found in the SZ. All PZ and SZ surveillance completed with negative results.	Medium risk with low uncertainty.
Indirect contact with wild birds	The ornithological field assessment noted the presence of gulls on fields adjacent to the property. Wild birds (which could act as bridge vectors) were scarce at the IP. Contact with contaminated litter is possible as some had spilt out of sheds into the farm yard. This was rectified as soon as restrictions were placed on the farm.	Low risk; moderate uncertainty.

9. Surveillance in the Protection and Surveillance Zones

A census to identify all premises containing poultry was undertaken in both the Protection and Surveillance Zones, in line with EU legislative requirements. There were 70 premises containing poultry identified within the 3-km radius of the outbreak (Protection Zone). The poultry on these premises together with their production and medicine records were inspected by APHA personnel (and tested where relevant) with negative findings. Guidance notes were also sent to these holdings to raise awareness and remind keepers of the restrictions that apply in this zone.

There were 138 premises with susceptible stock identified in total in the area between the 3km-10km radius of the outbreak (Surveillance Zone). Owners of premises were sent guidance notes to raise awareness and also remind keepers of the restrictions that apply in this zone. All commercial premises were also assessed for the presence of captive waterfowl or captive game birds (which may not express clinical disease) with instructions issued to visit and sample birds on such premises. This assessment found that no commercial captive waterfowl or game bird premises were present in the Surveillance Zone.

10. Analysis of the virus

The virus that caused the outbreak is an H5N8 HPAI strain, which is the same type as that found in the cases in Germany, The Netherlands and in Italy. It is also the same strain that was recovered from apparently healthy wild birds in Germany and the Netherlands and is the cause of ongoing epidemics in East Asia and North America. The virus isolated from the IP has been fully sequenced. Advanced phylogenetic analysis of the haemagglutinin gene of isolates from Germany, The Netherlands, UK and Italy suggests a high degree of similarly but there are some differences which makes direct contact between these cases unlikely. These viruses form a distinct cluster of European strains (not directly including those from Asia). Furthermore there is also high similarity to viruses detected in European wild birds since November 2014. Although these viruses are all closely related to those associated with outbreaks the precise origin pathways carry uncertainty; however the European viruses all appear to originate from a common ancestor dated to July 2014 onwards. These data taken together with the temporal and spatial patterns of detection in both wild birds and poultry support the independent presence of these viruses in wild birds which are therefore likely sources of initial introduction across the region.

Seven mutations have been identified compared to the WHO (CDC-USA) H5N1 reference strain. None of these mutations in isolation are considered to pose an increased zoonotic risk: on the contrary, there is a lack of a deletion in the NS1 at amino acid position 80-84 that is conserved among contemporary H5N1 viruses, possibly decreasing the zoonotic potential of the H5N8 virus.

Biological properties and pathogenicity for galliform poultry hosts were determined using the intravenous pathogenicity index test. A high pathogenicity virus was confirmed with all infected chickens dying within 48 hours (90% within 24 hours).

11. International context

Since January 2014, H5N8 HPAI has caused outbreaks in the Republic of Korea, Japan and China. Japan in particular has reported recent outbreaks and cases in wild birds, while in North East Russia a wild bird (Eurasian wigeon) tested positive in October 2014 (although only reported recently). The USA reported a case of H5N8 HPAI in two wild birds: a captive Gyrfalcon which had been fed on hunted wild waterfowl and an outbreak in backyard poultry in another region. Canada has reported 12 outbreaks of H5N2 HPAI which although considered initially unrelated, sequence analysis has shown the H5 gene to be the same as that from H5N8 HPAI viruses in the USA and Asia. It is understood that the virus responsible for the Canadian outbreaks is a re-assortment of the Asian H5N8 and an American N2 gene.

The increase in outbreaks in Asia associated with this new avian influenza virus is not unprecedented. What is somewhat unusual is that outbreaks have also occurred in two more regions (Europe and North America) within a short timeframe. Wild bird migration is likely to be implicated to a certain degree, but experts are still debating whether this is a long-range migration of infected birds, or infection occurring in staging areas along the migration routes (which may explain the similarity between the USA and European strains).

Once infection is present in wild birds within Europe, the risk of introduction into domestic poultry will depend on the prevalence and pattern of shedding in wild birds, and the level of biosecurity on the holdings and many other factors. Phylogenetic analyses suggest the viruses isolated from the European outbreaks are similar but not identical and therefore several introductions may have taken place, as opposed to direct spread between farms. This may indicate a certain level of infection in the wild bird population, which will be very difficult to assess by routine passive surveillance of wild birds found dead, as this virus has not always been associated with severe disease in wild waterfowl. In fact, infection has been detected in apparently healthy wild birds on multiple occasions in Europe, Asia and North America.

Long-range dissemination of infection can occur through movement of infected birds, products or contaminated equipment and there are many trade links between Asia and Europe. However, the majority are for export from Europe to Asia, which reduces the likelihood of this being the source of infection for all the affected premises in Europe. Nevertheless, there were questions about the movements of personnel from the UK IP to sister companies in Asia but this was ruled out as potential source of infection. Similarly, although there were transport links between UK, Germany and Netherlands none were identified as a significant risk pathway.

There was only one significant trade consignment from the IP to Europe – this was of the carcases from Site 1, from birds depopulated on the 5th November 2014. The Portuguese Competent Authorities were informed and the carcases were seized; the Authorities have confirmed they destroyed the carcases.

Other trade consignments were for hatching eggs from the Elite Hatchery and not directly connected to the IP. A detailed risk assessment concluded that there was a negligible probability that the elite side of the business was infected with HPAI. Receiving competent authorities were informed about the consignments for the purpose of transparency and good practice. No problems were reported on the hatchability of the eggs or of subsequent losses of birds.

12. Public health impact

Public Health England (PHE) determined the risk of H5N8 HPAI to the general public to be very low – given there have been no reported cases of human infection with H5N8 HPAI and the low probability of exposure to infected birds. PHE determined the risk to persons occupationally exposed to H5N8 HPAI to be slightly higher than the general public but still low (https://www.gov.uk/government/publications/avian-influenza-ah5n8-risk-assessment).
Both PHE and the Food Standards Agency (FSA) advised there was no risk to consumers from duck eggs or meat (http://www.food.gov.uk/news-updates/news/2014/13230/fsa-advice-about-avian-bird-flu).

13. Remaining uncertainty

There remains some uncertainty around the risk posed by wild birds and whether further cases or outbreaks may occur: there is evidence of virus still circulating in Europe and therefore we consider there is an increased risk of another outbreak (risk level is “low to medium” where “low” is an event that is rare but could occur and “medium” is an event which occurs regularly). The terminology for qualitative risk is subjective and difficult to quantify, but we consider that the risk has increased since November, when fewer outbreaks and fewer wild bird cases had been reported in Europe. While the bird migration to Europe from Russia/west-central Asia will have peaked in December, short distance movements around Europe may still occur, particularly if the weather turns colder in North Europe and birds may become highly aggregated due to freezing of surface water, increasing the potential for transmission of avian influenza viruses.

14. Concluding remarks

This was a complex epidemiological investigation due to the occurrence of disease on premises that were part of a large integrated company structure, including two hatcheries, a company owned slaughterhouse, rearing premises and a feather treatment plant. The extent of investigations needed was further increased by the depopulation of one of the flocks on site during the spread tracing window, resulting in tracing of numerous staff and vehicles associated with the catching gangs. The wide range of contacts and movements of ducks, eggs etc. necessitated a number of complex veterinary risk assessments as well as the high number of tracings reported. All potentially exposed premises were traced and investigated with negative findings.

Many premises traced as a result of the catching gangs in particular, had been depopulated prior to confirmation of disease on the IP and so could not be directly sampled to ascertain whether or not they may have been a source of disease for the infected premises. Investigation of all these premises was undertaken and where appropriate risk assessments and review of cleansing and disinfection protocols were carried out. No evidence of disease was found.

Surveillance for the presence of disease was further complicated by the potential for H5N8 HPAI to be clinically silent in otherwise healthy waterfowl (and some game bird)

species so there may not be significant production changes in the absence of intercurrent disease in species, resulting in the need for sampling and laboratory testing to confirm absence of infection.

Although our investigations suggest the most likely route of introduction for this infected premises was indirect contact with wild birds, an incursion such as this remains a low probability event. Extensive epidemiological investigations did not detect the presence of disease in any further premises investigated in connection with the IP either by known contact (source and spread tracings) or as a result of proximity (protection and surveillance zones). However, given the detection of H5N8 in wild bird species in northern Europe there remains an ongoing (considered low to medium) risk of further outbreaks occurring in the UK as a result of separate incursions of disease via wild bird sources.

National Emergency Epidemiology Group
January 2015

Acknowledgments
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 many colleagues who have assisted us.

15. Appendices

Appendix 1: Detailed description of IP

The IP is owned by an integrated duck breeding and growing operation situated in Yorkshire. It is a commercial indoor duck breeding site which routinely supplied hatching eggs primarily for commercial fattening to multiple sites and occasionally for commercial vaccine production. Six staff members worked on the site at the time of the outbreak.

There are a total of six wooden sheds for housing ducks with straw bedding and mains supply drinking water provided to the birds from covered header tanks located within each shed. Five of the sheds are naturally ventilated and have wire mesh at inlets that appears intact, protecting against wild birds. Shed 6 has controlled ventilation with inlets, through which wild bird access is potentially possible but considered unlikely and there have been no reports of wild bird sightings in the sheds. Shed 6 was also used as a straw and egg store and includes an egg cleaning area and office access. Pre-mixed feed was delivered weekly into enclosed storage hoppers.

Disinfectant dip was present at the entrance to the site office and at the entrance to each shed and door to bird area. Boot changes were apparently carried out between designated “dirty” (green boots) and “clean”/bird contact areas (white boots) of sheds. Outer clothing was not changed between sheds and gloves did not appear to be worn. The site perimeter was not fully secure against entry by wildlife with hedgerow for much of it and a water-filled ditch along the south perimeter, within 20 metres of the duck sheds. Moorhens were observed wandering onto the site from this ditch by APHA staff, and numerous webbed feet markings from these were noted in the area around the front of the sheds. Rodents are known to be a problem with mice seen during the APHA investigation on the IP. Additionally, during preliminary cleansing and disinfection following completion of culling and disposal operations, some rats were observed running from shed 3 to the ditch.

Members of the Ornithological Expert Panel (OEP) undertook a visit around the IP and PZ/SZ and reported an extremely low wild bird population at this time of year which has been validated with APHA visit findings. However, there is also a pond present in an adjacent field on which the site manager reported that mallard ducks breed during the springtime (although they were no longer reported to be present at the time of the investigation).

Sheds 1-3 (known as ‘Site 1’) contained 7499 ducks when first placed on the IP in June 2014. These ducks were depopulated on 05-06/11/14 at the end of their second lay cycle. Sheds 4-6 (known as ‘Site 2’) contained 6779 ducks when first placed on the IP in December 2013. Each shed had between 1500 and 2500 birds, divided between two or three pens with approximately 5375 birds in total at the time of cull.

Eggs were collected once daily by hand on plastic trays which were batched up and taken to the egg store and washing room at the end of Shed 6. Eggs were washed in a chlorine based wash, and placed on an adjacent table for drying and grading before being placed on trolleys of 3,200 eggs. These eggs were then placed in a temperature controlled room held at 15˚C.The eggs were collected twice weekly by the hatchery.

Waste duck carcases were collected and regularly taken to a maggot farm with the last collection on being on 13th November 2014. No waste straw, feed or manure had left the IP during the tracing windows.

The area is poultry and pig dense with a large number of commercial premises as well as hobby farmers. There are at least three backyard poultry flocks known to be in the very near vicinity of the IP perimeter.

Please see Appendix 5 for a diagram of the company structure.

Appendix 2: Estimated timeline and tracing windows

HPAI H5 AIV/2014/01
ESTIMATED TIMELINE FOR SOURCE AND SPREAD OF INFECTION

Source Tracing Window	Spread Tracing Window	Date	
		08/10/14	Earliest infection date for Site 1 based on clinical signs
		09/10/14	Earliest spread possible based on Site 1 infection
		10/10/14	
		11/10/14	
		12/10/14	
		13/10/14	
		14/10/14	
		15/10/14	
		16/10/14	
		17/10/14	start of precautionary Site 2 clinical signs incubation period (21 days)
		18/10/14	
		19/10/14	
		20/10/14	
		21/10/14	earliest infection date based on virology from Site 2 (10 days to get through house)
		22/10/14	
		23/10/14	
		24/10/14	start of typical inc period for clin signs Site 2; earliest infection date from Site 2 virology (7 days in-house spread)
		25/10/14	earliest spread given infection on 24th
		26/10/14	
		27/10/14	
		28/10/14	
		29/10/14	
		30/10/14	
		31/10/14	
		01/11/14	
		02/11/14	
		03/11/14	
		04/11/14	
		05/11/14	latest date of infection based on clinical signs incubation period (Site 1 starts depopulation)
		06/11/14	latest date of infection based on serology
		07/11/14	Most likely date of first clinical signs
		08/11/14	Clinical signs first clearly evident in production records
		09/11/14	
		10/11/14	
		11/11/14	PVS visit
		12/11/14	Antibiotics started
		13/11/14	
		14/11/14	Disease report - Restrictions served, diagnostic samples: virus present, seropositive only on senstive test
		15/11/14	
		16/11/14	
		17/11/14	
		18/11/14	Cull started, diagnostic samples taken, seropositive, only low virus
		19/11/14	
		20/11/14	Potential completion of C&D
		21/11/14	C&D 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 IP on these dates.		

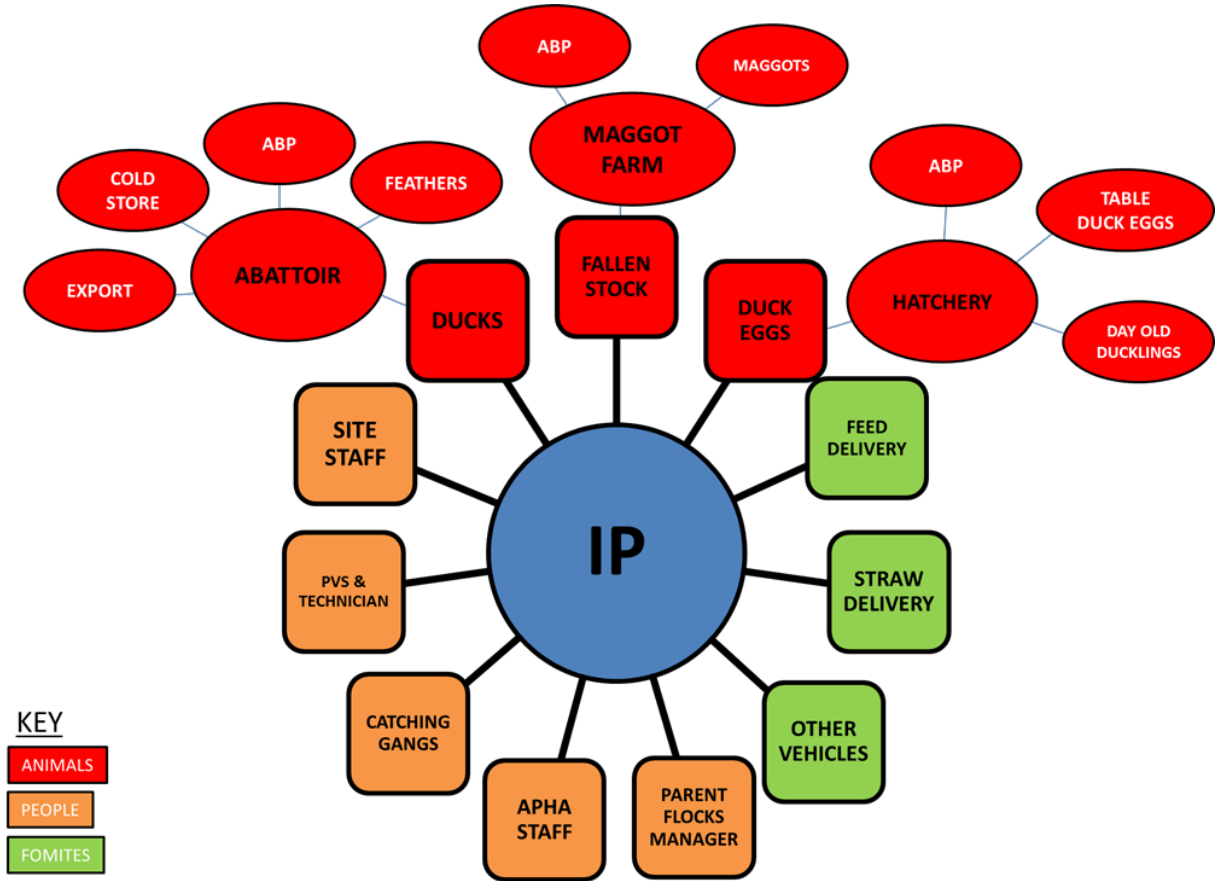
Assumptions

- point source infection, reasonable if water is mains, feed not mixed on farm
- presence of virus in birds indicates infection took place within the last 14 days, after this only antibody is present
- Spread of infection within a flock is rapid, estimate being used is 7-10 days (expert opinion) which is believed conservative
- Virology: if assume detect first or last bird infected, it got virus in previous 1-14 days, likely entered flock in the previous 7-10 day period
- Clincial signs: unlikely to be noticed until some number of birds affected, incubation period is 2-14 days, up to 21 days in statute

Appendix 3: Details of tracing and stock numbers in zones

The source and spread tracing windows covered 38 days from 8 October to 14 November 2014. Animals, animal products and people who had directly contacted the ducks on site were generally classified as high risk tracings. The tracing of two catching gangs and their associated people and equipment, who had very recently been involved in the depopulation of Site 1 of the IP, gave rise to a significant proportion of the total traced premises.

Figure to show range of tracing work carried out



Number of locations with tracings per region*

Region	Both	Source	Spread	Total traced locations of region
Midlands	23	20	25	68
North	38	28	16	82
South East	0	2	3	5
Total locations by trace type	**61**	**111**	**105**	**155**

*note that the total number of traced locations is lower than the sum of source tracing locations and spread tracings locations, because 59 locations had both a source and a spread tracing

Number of locations with spread tracings

Row Labels	Catching Gang	Area Manager	Eggs/Ducklings	ABP	Feed/Straw Delivery	IP Workers	Vets	Vehicle/People (Other)	Miscellaneous	
Midlands	25	5	7	2	6	0	0	12	6	
North	20	1	3	1	10	6	4	15	12	
South East	1	0	3	0	0	0	0	0	0	
Total locations per traced item	**46**	**6**	**13**	**3**	**16**	**6**	**4**	**27**	**18**	**139**

Number of locations with source tracings

Row Labels	Catching Gang	Area Manager	Eggs/Ducklings	ABP	Feed/Straw Delivery	IP Workers	Vets	Vehicle/People (Other)	Miscellaneous	Total
Midlands	22	5	2	1	4	0	5	11	6	56
North	24	1	2	1	7	6	15	16	11	83
South East	2							1		3
Total locations per traced item	**48**	**6**	**4**	**2**	**11**	**6**	**20**	**28**	**17**	**142**

Summary of stock and holdings in the zones

	Chicken	Turkey	Duck	Goose	Aviary Birds	Guinea Fowl	Partridges	Pheasant	Pigeon	Quail	Psittacines	Other Birds	Total Birds
Number of animals of that species in PZ	307529	17	72	50	83	4	0	0	426	15	1	10	308212
Number of holdings with that species in PZ	59	2	12	6	8	1	0	0	6	3	1	3	70
Number of animals of that species in SZ	758684	37576	138	26	58	0	80	530	163	25	1	113	797394
Number of holdings with that species in SZ	117	11	22	7	3	0	1	2	5	1	1	8	138

Notes: Premises and stock number calculated from an extract of CORE 2 taken on 26th January.
A duplication in the protection zone with 8 chickens and 3 ducks has been removed.
Total bird figure calculated by summing the total population for Aviary Birds, Cassowary, Chicken, Duck, Emu, Goose, Guinea Fowl, Kiwi, Ostrich, Partridges, Pheasant, Pigeon, Quail, Rhea, Turkey, Other Birds, Poultry, Wild Birds ,Birds of Prey and Psittacines.
The premises totals have been provided by the MIDAS team.
The protection zone figures have been removed from the figures given for the surveillance zone.

Appendix 4. Definitions for qualitative risk terms and level of confidence

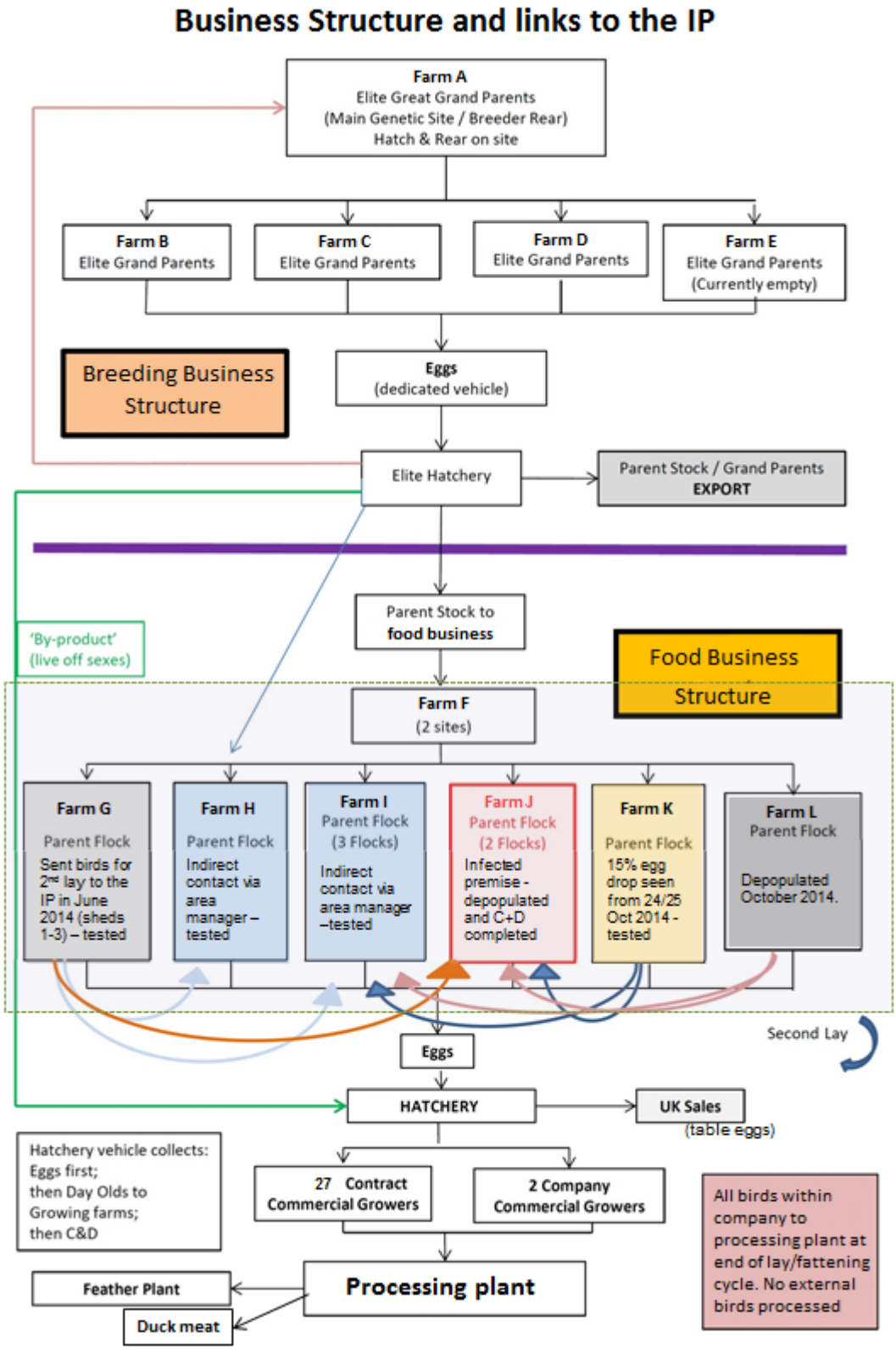
Table 1: Definitions for qualitative risk terms based on EFSA (2006) and OIE (2004)

Risk level	Definition
Negligible	Event is so rare, does not merit consideration
Very low	Event is very rare, but cannot be excluded
Low	Event is rare, but does occur
Medium	Event occurs regularly
High	Event occurs very often
Very high	Event occurs almost certainly

Table 2: Definitions for level of confidence in the risk estimate given the evidence used; based on definitions within (EFSA, 2006; ECDC, 2011, Spiegelhalter & Riesch, 2011)

Level of confidence	Definition
Unsatisfactory	Further research very likely to have impact on confidence of information and likely to change assessment
Satisfactory	Further research likely to have impact on confidence of information and may change assessment
Good	Further research unlikely to change confidence in the information

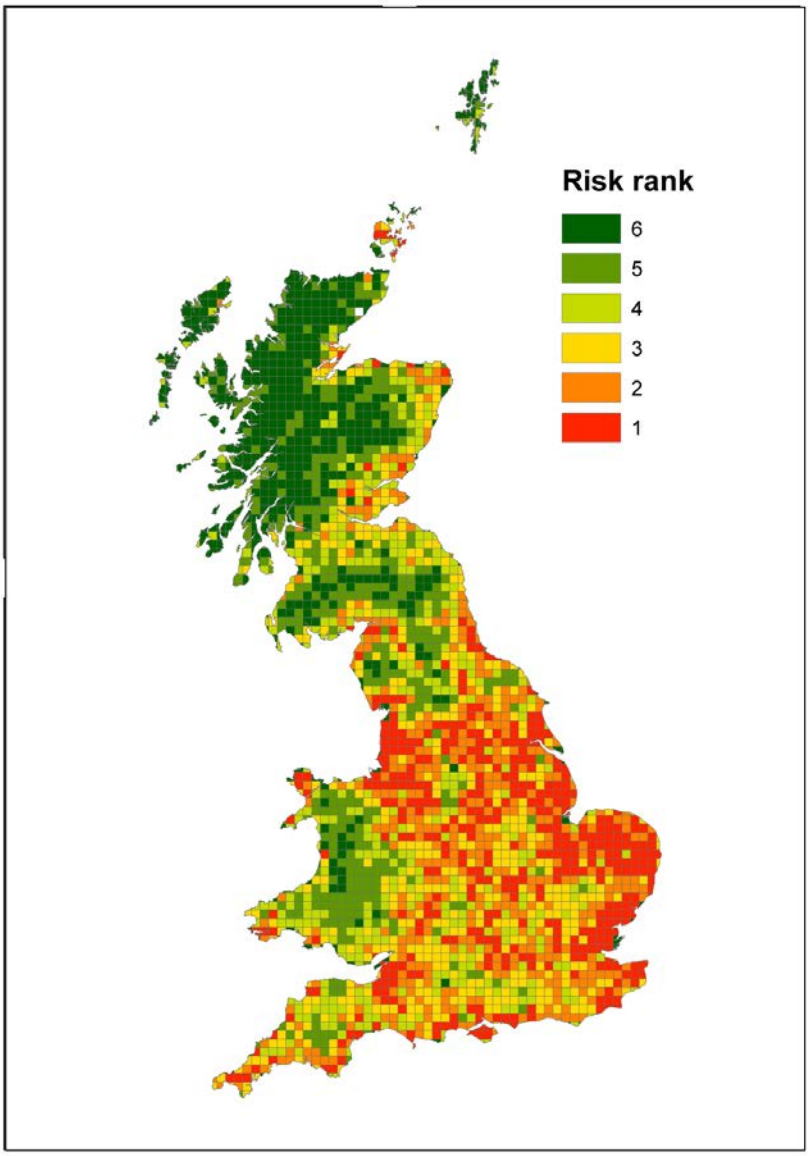
Appendix 5: Company structure



Appendix 6: Estimated incursion risk of H5N1 HPAI (wild birds to domestic poultry)

(NB unpublished, paper in preparation)

Using a semi-quantitative approach, the geographical areas where commercial poultry are at greatest risk of an incursion of H5N1 from wild birds was estimated for each 10 km square area within GB by overlaying the abundance of 24 migratory wild bird species that winter in GB and are deemed to have a high probability of exposure to H5N1 outside the European Union (EU) with the estimated density of commercial poultry. This map is similar to that published in 2007* but data on migratory birds and poultry distribution has been updated. Incursion risk is ranked 1 to 6 in order of high to low risk.



*****Snow LC, Newson SE, Musgrove AJ et al. Risk-based surveillance for H5N1 avian influenza virus in wild birds in Great Britain. Veterinary Record, 2007; 161 (23):775-81