Spatial and temporal analysis of the highly pathogenic avian influenza H5N1 outbreak in the Dombes Area, France, in 2006

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Abstract

In February 2006, the highly pathogenic H5N1 strain of the avian influenza virus was confirmed as being the cause of death for three Common Pochard Aythya ferina recovered at the same time from a pond in the Dombes, France. The subsequent epizootic lasted for 65 days in the Dombes region, with the last occurrence of the H5N1 strain of the virus confirmed on 18 April. Data recorded for dead wild birds collected on ponds and confirmed as being positive for the H5N1 strain of avian influenza were used to develop a pond-level analysis of the H5N1 outbreak. The outbreak was found to be limited mainly to Common Pochard and Mute Swan Cygnus olor, which were found dead at 20 of about 1,200 ponds in the Dombes. The outbreak was confined to an area of about 15 km in diameter, with two spatio-temporal clusters of ponds where birds had contracted the disease. The findings are used to assess the potential role played by wild waterfowl in avian influenza epidemiology and to suggest possible scenarios for the introduction and spread of the virus at a local scale based on analysis of its occurrence in the Dombes.

Key words: avian influenza, spatial analysis, temporal analysis, wild birds.
Since the first cases of Highly Pathogenic Avian Influenza (HPAI) H5N1 were reported in poultry in 1996, at which time efforts were made to eradicate the disease (Webster & Govorkova 2006), the H5N1 virus re-emerged in southeast Asia in 2003, resulting in sporadic human fatalities as well as mass mortality in the poultry industry (Peiris et al. 2004). This H5N1 strain is considered to be a good candidate for an influenza pandemic (Conly & Johnston 2004; Horimoto & Kawaoka 2001); attention therefore has focussed both on the mutation dynamics of this strain and on associated spatio-temporal patterns of HPAI outbreaks. The highly pathogenic H5N1 strain started to spread westwards in the summer of 2005, following an outbreak at Lake Qinghai, China, in April that year (Chen et al. 2005; Liu et al. 2005). The strain was found to be more lethal for wild birds than any previously recorded avian influenza virus (Chen et al. 2005; Liu et al. 2005). By the end of 2005, H5N1 outbreaks had occurred in both wild waterfowl and in domestic birds in Russia, Kazakhstan, Romania, Turkey and Ukraine, with Croatia and Mongolia additionally reporting cases for wild bird only (Webster & Govorkova 2006). Early in 2006, the HPAI H5N1 virus reached and spread throughout Western Europe (successively from Bulgaria, Italy, Germany, Austria, France and, almost simultaneously, Switzerland, United Kingdom and Bosnia) causing mortality most commonly in Mute Swan Cygnus olor. It has been suggested that the spread of the HPAI H5N1 virus could be due either to the movements of migratory birds or to the legal and illegal trade of birds and/or associated bird products (Kilpatrick et al. 2006; Gauthier-Clerc et al. 2007). Up to now, the spread of HPAI H5N1 virus within Europe remains poorly documented (Gaidet et al. 2007; Stakknecht & Brown 2007; Cromie et al. 2006), yet understanding how the virus propagates amongst wild birds is of paramount importance for controlling the disease in these populations.

On 13 February 2006 the HPAI H5N1 virus was first isolated in three Common Pochard Aythya ferina found dead on a pond in the Dombes, France (Hars et al. 2008). This was the first record of the disease in wild birds in France and the start date for this study. Ten days later, farm turkeys were found to be infected, but the subsequent epizootic which lasted for 65 days (i.e. until the H5N1 virus was last isolated from birds in the Dombes on 18 April 2006,) mainly concerned wild birds. During this period, the HPAI H5N1 virus was found in birds in a total of 42 swab pools (swab pools being swab samples grouped according to sample location) of which 39 were collected in the Dombes, two were recorded (on 27 February and 16 March) at Leman Lake, 100 km east of the Dombes, and one swan was found (on 28 February) on the Mediterranean coast, 300 km south of the Dombes (Hars et al. 2008; Le Gall-Recule et al. 2008). The 39 swab pools on the Dombes where birds tested positive for H5N1 consisted of 32 with Mute Swan cases, four with Common Pochard, one with a Grey Heron Ardea cinerea, one with a Buzzard Buteo buteo and one with a Greylag Goose Anser anser, giving a total of 67 dead wild birds of which 62 were collected on the ponds and five were collected either on roads or grazing areas.
next to the ponds. During the outbreak, a surveillance programme to monitor the spread of the disease was undertaken by staff of the National Game and Wildlife Service, who visited several ponds across the Dombes region every 1–5 days to allow early collection of bird carcasses. Between February and April 2006, The Dombes was the only region in France where the HPAI H5N1 virus was recorded in wild and domestic birds.

The aim of this paper was to perform a spatio-temporal analysis of incidence of HPAI in the Dombes, in order to provide some insight into the epizootology of the disease in this area. Ponds rather than individual birds therefore are treated as epidemiological units, and the analysis of the spread of HPAI H5N1 is at the pond scale.

Material and methods

Study area

The Dombes is located in the northern part of the Rhône Alpes region in France, ranging from 45°55’7”–46°12’20”N to 4°49’25”–5°14’59”E. It is a wetland mosaic, covering about 100,000 ha, and includes c. 1,200 ponds (Fig. 1). Pond size ranges from

Figure 1: The Dombes area (enlarged map in the circle at the left), France (map at the top right), at the county level with lines representing boundaries between counties. The region is a stopover and wintering site for many bird species along one of the two main bird migratory routes (arrows in bold fonts in the map of France) across France. It is a wetland mosaic of more than a thousand ponds (scattered spots in the enlarged map).
0.5–5.5 ha for half of them, while 10% of ponds exceed 22 ha. The mean distance between a pond and its nearest neighbouring pond is \( c. 370 \) m (s.d. = 358.53 m). The site is on one of the two main routes used by birds migrating across France (Fig. 1), and is also an important breeding and wintering site for many wild waterfowl species, especially Mallard *Anas platyrhynchos* and Common Pochard (Hars et al. 2008; Bernard & Lebreton 2007; Fouque et al. 2005). The site is a wetland of international importance under the criteria set down in the Convention on Wetlands of International Importance especially as Waterfowl Habitat (the “Ramsar Convention”) (Ramsar 2007), in that it receives > 20,000 waterfowl, including at least 1% of the biogeographic population for several Anatidae and Coot *Fulica atra* wintering in this area (Fouque et al. 2007). Mute Swan numbers have been growing exponentially in the Dombes (Benmergui et al. 2005), as in other parts of France and in Europe (Fouque et al. 2007). It is also close to the Bresse region, which has a large number of free-range poultry farms.

**Definition of case ponds and treatment of the data**

The way in which the swabs were collected does not allow a classical case study. When several dead birds were found on ponds or elsewhere, all were collected. Swab samples taken from the cloaca and trachea of these birds were then pooled according to birds’ recovery location. It was therefore impossible to distinguish, within a pool, which swab corresponded to which individual bird. Thus, we did not consider individuals as cases but investigated the ponds from which a pool of swabs was found to include positive cases. As some of the swabs were not collected on ponds, but for example on roads or in grazing areas, and as these particular swab pools mainly consisted of one swab of a non-Anatidae species, the data were not homogeneous across swab pools. For the HPAI outbreak analysed in this study, the data consisted of 32 (out of 39 for the whole of the Dombes) H5N1 laboratory-confirmed positive swab pools, corresponding to 62 birds found dead on 20 different ponds, collected by National Game and Wildlife officers between 13 February and 18 April 2006. Dead birds were recovered from some ponds on >1 day, which provided several swab samples for these sites. The two waterfowl species mainly affected by the outbreak were Mute Swan and Common Pochard. Cloacal and tracheal swab samples collected from dead birds were tested by the local authorised laboratory and confirmed as positive at the National Reference Laboratory of Afssa Ploufragran (Le Gall-Recule et al. 2008). These data were recorded for the duration of the outbreak (Fig. 2) according to the geographic coordinates of the pond where the samples were collected and entered into a Geographic Information System. Ponds where dead birds were confirmed as being positive for HPAI H5N1 at least once during the outbreak are defined as “case ponds” for this study.

**Spatial and temporal analyses**

The K-mean clustering routine in CrimeStat (Aldenderfer & Blashfield 1984; Fisher 1958) was used to identify clusters of case
**Figure 2:** Chronology of the 2006 H5N1 outbreak on ponds in the Dombes area. PL stands for the pond label with the first case pond labelled 1. Dark squares correspond to case ponds with dead Mute Swans and dashed ones indicate those with Common Pochard. Pond 20 was excluded from the analysis as it is isolated and distant from the clusters of case ponds (see Fig. 4).
ponds. A routine was developed and implemented in Matlab 7.0 to investigate space–time interactions and compute the relative risk of HPAI H5N1 occurring at case ponds, based on the Knox test (Knox 1964). This considered a case pond “i”, characterised by its space–time coordinates \((r_i, t_i)\), where \(r_i\) is the location and \(t_i\) the date of occurrence (i.e. the date of collection for dead birds confirmed positive for H5N1). For any pair “\((i,j)\)” of case ponds, characterised by the space distance, \(\Delta r_{ij} = |r_i - r_j|\), and time spacing, \(\Delta t_{ij} = |t_i - t_j|\), and given distance \(d\) and lag time \(t\), the pair of cases was said to be: 1) close both in space and time if \(\Delta r_{ij} < d\) and \(\Delta t_{ij} < t\), or 2) close in space and not close in time if \(\Delta r_{ij} < d\) and \(\Delta t_{ij} > t\), or 3) not close in space but close in time if \(\Delta r_{ij} > d\) and \(\Delta t_{ij} < t\), or 4) not close both in space and time when \(\Delta r_{ij} > d\) and \(\Delta t_{ij} > t\). This latter case was omitted as it was not relevant for cluster identification. For all pairs of case ponds considered, the relative risk (RRn) with respect to the above criteria “\(n\)” (i.e., \(n = 1, 2\) or \(3\)) was defined as the ratio of the observed number of pairs satisfying the criteria \(n\) to the expected number of pairs found by chance to be satisfying the same criteria (Tran et al. 2004). The expected number of pairs found by chance was obtained from observed data by distributing the dates of occurrence (locations) at random across the case locations (dates of occurrence) and averaging the calculated numbers over the number of trials. RRn > 1 indicates the relative risk (RR) of occurrence of a case pond \(t\) days and \(d\) kilometres away from the first case for \(n = 1\), \(d\) kilometres away from the first case for \(n = 2\), and \(t\) days after the first case for \(n = 3\). The three space-time interaction indices RR1, RR2 and RR3, corresponding respectively to criteria 1, 2 and 3, were computed for space distances \(d\) varying from 0.5–29 km (in 0.5 km steps) and lag times \(t\) varying from 1–66 days (in 2-day steps).

**Results**

**Description of the outbreak**

Not all of the 1,200 ponds were monitored throughout the outbreak, so the reported number of ponds with positive cases of HPAI H5N1 may have been higher than presented here. The results presented include an assumption that uncertainty regarding the number of positive ponds was of about 5%. Binomial tests therefore were used to provide a \(P\) value of \(\pm 5\%\) for each ratio, together with the corresponding 95% confidence intervals.

Dead birds were collected from ponds on 33 occasions on 24 days during the 65-day outbreak of the disease (Fig. 2). Of the 1,200 ponds in the study area, 20 (1.67%) were case ponds, with at least one bird tested positive for HPAI H5N1 during the outbreak (\(P = 0.05\), 95% C.I. = 1–2.6%). A rough estimation of the basic reproductive number \(R_0\) (the mean number of secondary case ponds generated from an index case) can be obtained in writing the epidemic size (total number of case ponds) \(N_s = 20\) as \(N_s = n_0 (1 + R_0 + R_0^2 + \ldots) = n_0/(1 - R_0)\), where \(n_0\) is the seed number of case ponds, \(n_0 R_0\) the number of new case ponds generated by \(n_0\) ponds, and so forth. Inverting this expression for \(R_0\), \(R_0 = 0.95\) for \(n_0 = 1\) and \(R_0 = 0.90\) for \(n_0 = 2\), with \(R_0\)
decreasing with $n_0$. The distribution of the number of times that dead birds were found to be infected on case ponds was once for 13 of the ponds, twice for four ponds (ponds 8, 9, 14, 15), three times for two ponds (ponds 2 and 10) and six times for one pond (pond 4). Apart from pond 5, all case ponds were >9 ha. An assessment of the first time that dead birds tested positive for H5N1 were found at each pond showed that pond-switching (i.e. the appearance or reappearance of the disease at a case pond) occurred on 25 occasions, with a mean time interval between the two successive events of 3.76 days (s.d. = 6.38 days) and mean distance of 8 km (s.d. = 4.42 km) corresponding roughly, for a two dimensional isotropic diffusion, to a diffusion constant of $\frac{(8*8)}{(4*3.76)} = 4.25 \text{ km}^2/\text{day}$.

### Space-time interactions

Calculated values of the relative risk of occurrence were $\leq 1$ both for RR1 (case occurrence close both in space and time) and for RR3 (case occurrence not close in space but close in time), but were $>1$ for two space-time windows for RR2 (i.e. where case occurrences are close in space but not close in time). RR2 increased and peaked at a spatial distance of 2–5 km between 25–45 days after the initial occurrence, and again at about 60 days, suggesting a spatial clustering of case ponds with occurrence of the disease occurring in two phases (Fig. 3).

### Cluster identification

Two non-overlapping clusters of case ponds A and B were identified about 5 km from

![Figure 3: Relative risk (RR) for pairs of cases close in space but not in time. The grey bar indicates RR values with highest RR in darker grey and lowest ones in white.](image-url)
each other (Fig. 4, left panel). Apart from
the first case pond (belonging to cluster A)
which concerned infected dead Common
Pochard, all infected dead birds in both
clusters A and B were Mute Swan. Fig. 4
(right panel) shows how the case ponds were
distributed over the clusters during the
course of the outbreak. It was observed that
11 ponds (labelled 1,4,5,6,8,9,10,13,15,17
and 18 in Fig. 2) belonged to cluster A;
H5N1 appeared here for the first time
between days 1–12, and again between days
24–32 of the outbreak. Five ponds (labelled
11, 12, 14, 16 and 19 on Fig. 2) belonged to
cluster B. These were infected between days
2–23, and again between days 25–64 of the
outbreak. One pond (pond 3; labelled C on
Fig. 4) was located on the western side of
cluster B. This pond is the furthest away
from the initial outbreak at pond 1. One
pond (pond 7; labelled D on Fig. 4) was
located on the western side of cluster A.

Most case ponds (60%) belonged to the
group centred < 5 km from the initial case

Figure 4: Left Panel: Cluster map of case ponds. Case ponds are shown in black and the two clusters A and B are delimited by ellipses around ponds. The distance between cluster centres is about 10 km. Pond C (the third one in the chronology of the outbreak course) belongs to cluster B. Pond 1 (the first case pond) and pond D (the seventh of the outbreak) are both included into cluster A.

Right Panel: Space – time and cluster – time distributions of cases. The vertical and bottom horizontal axes represent the spatial distance (km) and time interval (days) between the first case and the following ones, respectively. The upper horizontal axis corresponds to the number of case ponds ($n$) represented by a histogram. Circled letters stand for case ponds with the letter referring to the cluster they belong to.
pond (cluster A, if pond 7 labelled D in Fig. 4 is included in cluster A), while the remaining 40% of cases fell into cluster B (if pond 3 labelled C in Fig. 4 is included in cluster B), centred c. 12.5 km from the initial case pond (Fig. 4). The mean distance between case ponds within clusters was approximately 5 km (cluster A: mean = 6.18 km, s.d. ± 3.56 km; cluster B: mean = 5.38 km, s.d. ± 4.3 km).

Discussion

The outbreak of HPAI H5N1 in wild birds in the Dombes in 2006 followed a period of cold weather, during which wildfowl congregated in large numbers on large ponds. The outbreak was an epizooty of minor size, mainly involving wild birds. It was initially found in a few Common Pochard but had a greater effect on Mute Swans with more swans dying of the disease. The outbreak was fairly localised within the Dombes since it was confined to a circle of c. 15 km in diameter, with two clusters of case ponds c. 5km from each other.

Transmission of HPAI H5N1

At the time that the virus reached the Dombes area, waterfowl were highly concentrated on a small number of ponds, for two reasons. Firstly, in contrast to the summer, the occupation rate of ponds usually decreases down to 20% (i.e. during the winter season only 20% of the 1,200 ponds are occupied) as birds gather onto large unfrozen waterbodies (Bernard & Lebreton 2007). Secondly, the 2005/06 winter was particularly cold with a total of 97 days of sub-zero temperatures in the study area from 1 November 2005 to 30 April 2006. Freezing conditions result in an even higher concentration of birds on the remaining ice-free areas of water, and the areas of open water were at a minimum at the end of January 2006. Thus the weather not only led to a high concentration of birds but increased the likelihood of contact between them, which is favourable for the transmission of avian influenza viruses (Zhang et al. 2006; René & Bicout 2007).

Despite the conditions, the number of dead birds and case ponds recorded suggest that the 2006 episode was a minor outbreak, with a basic reproductive number R₀ lower than one. Assuming that contacts between birds were very likely, a R₀ < 1 would thus imply either that the virus was weakly contagious (i.e. the bird-to-bird viral transmission was feeble), that the infectiousness duration was quite short for birds which later recovered, and/or that the virus was so highly lethal that the mean time from contracting the virus to the death of the bird was very short.

To attempt an understanding of the low number of cases observed during the outbreak, we suggest that the heterogeneity in the response to H5N1 viral infection of different bird species could have played the key role. In this perspective, populations of birds in contact for H5N1 transmission during the outbreak would be considered as being composed of mixed bird species, which could be classified into three categories according to their response to H5N1 infection. The first category, “Victim Birds”, may be defined as being highly susceptible to viral infection with a short mean death time, and a high viral excretion level. Swans apparently behaved in this way...
in the present study. The second category, “Victim-Source Birds”, could be defined as being less susceptible than victim ones to viral infection, with low mortality rate. When infected, such birds would either be highly contagious with a high excretion level but of short duration, or poorly contagious with a low excretion level but of rather long duration. These two opposite situations are equivalent in terms of generating secondary cases. Common Pochard apparently behaved in this way in the present study. Thirdly, “Source-like Birds” would have a low susceptibility to viral infection with no associated mortality and very low morbidity. The infection for these birds would mainly be asymptomatic and their excretion level high enough to ensure infection of the other bird species. One of the key roles of these reservoir like birds would be to allow both persistence of viruses in the ecosystem and transmission of viruses to other bird species (Haydon et al. 2002). Published data suggest that the Mallard would be a likely candidate for this category (Munster et al. 2005; Keawcharoen et al. 2008). This proposed categorisation of birds may be seen as speculative, but may also help with developing hypotheses and shaping the framework for future epidemiological fieldwork and mathematical modelling studies on the role of bird heterogeneity in the propagation of avian influenza. Other authors have also suggested a similar approach (Hars et al. 2008).

Introduction and propagation of HPAI H5N1 in the Dombes

Earlier studies suggest that there were no HPAI H5N1 viruses circulating in waterfowl in the Dombes before the 2006 outbreak (Durand 2006). Several possible scenarios for the introduction and propagation of the virus can therefore be proposed. Scenario 1) is that multiple introductions occurred, with all infections originating elsewhere and infected cases being imported into the Dombes, with no transmission between birds within the Dombes during the outbreak. This scenario implies several migration waves or a wider migration wave of birds during the 64-day outbreak. According to ornithologists, such kinds of bird movements are unlikely and were not observed at all in the area during the episode. Scenario 2) is a single introduction of the H5N1 virus from elsewhere to a pond in cluster A, followed by a contagion of ponds in both clusters A and B. Scenario 3) is the simultaneous introduction of the H5N1 virus from elsewhere into both clusters A and B (given that the first case on 13 February in cluster A and the next on 18 February in cluster B are close in time), followed by a contagion process of nearby ponds in both clusters. Scenario 4) is a combination of early introductions of H5N1 viruses by reservoir-like species during the incoming migration in autumn 2005 and persistence of infectious viruses in the bird-environment ecosystem due to favourable weather conditions. The increase in bird concentrations during the cold period in January–February 2006 may have triggered the onset of the transmission-contagion process to victim and/or intermediate bird species such as Common Pochard and Mute Swans.

Whether the contagion process, for any of the three last scenarios, was intra-cluster,
inter-cluster or both simultaneously are all plausible and consistent with daily distances covered by birds (for instance ducks) to cover their resting and feeding requirements throughout the day (Tamisier 1978). In future assessment of the three scenarios, distances between ponds may be used as a proxy to assess the contact between infected units. The virus is known to survive in cold water (Stallknecht et al. 1990; Stallnecht et al. 1990), but propagation via cold water was not considered in the present study as it was not known exactly how ponds in the study area are inter-connected via canals or rivers. This would also be worth testing in a future analysis. Having some insight into the way in which the viruses were disseminated during the 2006 episode is of major importance for a better assessment of the risk of H5N1 spreading both spatially and temporally, not only in the study area but potentially for other areas. In the case of both intra- and inter-cluster contagion, the H5N1 infection spread with a diffusion constant of about 4.25 km²/day. Eventually, it was reported that two different H5N1 strains circulated in the Dombes during the outbreak (Le Gall-Recule et al. 2008). This finding may support the hypotheses of either two viral introductions with no mutations, or one single viral introduction with mutations. Further investigations on which strain infected which ponds would be very informative for describing the propagation of the H5N1 virus in the Dombes area in 2006.

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