Serological surveillance reveals patterns of exposure to H5 and H7 influenza A viruses in European poultry

Short running title: H5 and H7 influenza A in European poultry

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Ethics statement
The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. Ethical approval was not required, as this manuscript did not involve collection of original research data.
1. Summary

Influenza A viruses of H5 and H7 subtype in poultry can circulate subclinically, and subsequently mutate from low to high pathogenicity with potentially devastating economic and welfare consequences. European Union Member States undertake surveillance of commercial and backyard poultry for early detection and control of subclinical H5 and H7 influenza A infection. This surveillance has moved towards a risk-based sampling approach in recent years; however quantitative measures of relative risk associated with risk factors utilised in this approach are necessary for optimisation. This study describes serosurveillance for H5 and H7 influenza A in domestic and commercial poultry undertaken in the European Union from 2004 to 2010, where a random sampling and thus representative approach to serosurveillance was undertaken. Using these representative data, this study measured relative risk of seropositivity across poultry categories and spatially across the EU. Data were analysed using multivariable logistic regression. Domestic waterfowl, game birds, fattening turkeys, ratites, backyard poultry and the “other” poultry category holdings had relatively increased probability of H5 and/or H7 influenza A seropositivity, compared to laying-hen holdings. Amongst laying-hen holdings, free-range rearing was associated with increased probability of H7 seropositivity. Spatial analyses detected ‘hotspots’ for H5 influenza A seropositivity in western France and England, and H7 influenza A seropositivity in Italy and Belgium, which may be explained by the demographics and distribution of poultry categories. Findings suggest certain poultry category holdings are at increased risk of subclinical H5 and/or H7 influenza A circulation, and free-range rearing increases the likelihood of exposure to H7 influenza A. These findings may be used in further refining risk-based surveillance strategies, and prioritising management strategies in influenza A outbreaks.

2. Keywords

Avian, bird, influenza, poultry, risk, serosurveillance, virus
3. Introduction

Influenza A H5 and H7 subtypes infect birds and a range of mammals, including humans (Fouchier et al., 2003). In poultry, H5 and H7 influenza A viruses occur as either low pathogenicity or high pathogenicity strains. As clinical symptoms can vary with factors such as age, species, concurrent illness and environmental conditions, low pathogenicity infections may result in apparent disease, or remain undiagnosed as a mild illness or subclinical infection (particularly in waterfowl) (Alexander, 2000; Pillai et al., 2010). In contrast, high pathogenicity influenza A strains are typically clinically apparent in poultry, with mortality rates of 50 to 100%, although clinical signs may be less obvious in waterfowl (Alexander, 2007). Influenza A viruses, particularly low pathogenicity influenza A, can enter poultry flocks by direct or indirect contact with infected wild birds, or by secondary spread from other infected domestic poultry (Alexander, 2007). High pathogenicity influenza A strains occur as a result of mutation of low pathogenicity influenza A strains circulating in domestic poultry (Capua and Marangon, 2006). Outbreaks of H5 or H7 influenza A in poultry that are not detected and controlled in a timely manner can spread through poultry populations, ultimately resulting in severe economic losses for the affected poultry industry (Stegeman et al., 2004; Capua and Marangon, 2007). There can also be zoonotic implications to human exposure to some strains of influenza A in domestic poultry (Koopmans et al., 2004).

Whilst much has been published on outbreaks of influenza A involving clinical disease, less is understood about the epidemiology of subclinical infection. Wild waterfowl are a natural reservoir of influenza A viruses and hence have coevolved with these pathogens over millennia (Alexander, 2007). It is, therefore, unsurprising that subclinical H5 and H7 influenza A infection can occur in domestic waterfowl (ducks and geese) more readily than in gallinaceous poultry (chickens and turkeys) (Cooley et al., 1989; Perkins and Swayne, 2002). A study that analysed 2005–2007 EU influenza A surveillance data reported a higher relative risk of low pathogenicity influenza A seropositivity in ducks and geese, game birds, ratites and ‘others’ compared to chickens (Gonzales et al., 2010). Amongst chickens, laying-hens had increased risk of infection with high pathogenicity influenza A during an outbreak in the Netherlands, which was attributed to higher
contact rates between laying-hen farms (particularly by egg tray fomites) (Thomas et al., 2005). In New Zealand, backyard ducks have been demonstrated to be more likely to be seropositive for influenza A than backyard chickens (Zheng et al., 2010). Outdoor rearing of poultry, linked to many H5 and H7 influenza A outbreaks, is considered a risk factor for influenza A in areas inhabited by free-living birds (Koch and Elbers, 2006). Studies in Italy have found backyard flocks to be at high risk of influenza A infection and an important point of entry for influenza A into domestic poultry populations (Terregino et al., 2007; Cecchinato et al., 2010). However, a modelling study in the Netherlands suggested backyard flocks may be of little importance in outbreaks (Bavinck et al., 2009). This may indicate a geographical heterogeneity of risk, potentially associated with variation in poultry population demographics and management practices, or distribution and migratory pathways of wild birds (Olsen et al., 2006; Alexander, 2007). Holdings with low biosecurity practices (particularly use of surface water), multispecies production, and birds with longer life spans are also thought to be at increased risk of infection (European Commission, 2002, 2010).

European Union Member States undertake surveillance of commercial and backyard poultry for early detection and control of subclinical H5 and H7 influenza A infection (European Commission, 2007, 2010). This surveillance has moved towards a risk-based sampling approach in recent years; however quantitative measures of relative risk associated with risk factors utilised in this approach are necessary for optimisation. Additionally, such measures have utility for prioritisation of management actions in the case of outbreaks of influenza A in poultry. This study’s objectives were to: 1) describe the distribution of the European Union Influenza A serosurveillance in poultry, and of holdings found to be seropositive, from 2004 – 2010 (a period where representative sampling was undertaken in the surveillance program); 2) compare the odds of H5 and H7 influenza A seropositivity between holdings of different poultry categories; 3) compare the odds of H5 and H7 influenza A seropositivity between free-range and non-free-range holdings; and 4) test for the presence of significant spatial heterogeneity of seropositive holdings, and whether this could be explained by previously identified covariates.
4. Materials and Methods

4.1 Collation of influenza A serosurveillance data

Yearly cross-sectional data were collated from the EU influenza A surveillance program in poultry from the years 2004 to 2010. The 2004 to 2010 serosurveillance data were considered the most suitable for the purposes of this study as sampling strategies were designed to be representative of the poultry categories sampled during this time. From 2011 onwards, surveillance legislation strongly encouraged a risk-based sampling strategy (European Commission, 2010) and hence surveillance results are based less on representative sampling of the poultry populations since this time. The surveillance target population was EU commercial and backyard poultry. Twenty-five EU Member States contributed surveillance data over the entirety of the period considered, while two Member States contributed from 2007 onwards following joining the EU (Table 1). Eighteen poultry categories were sampled over that time: seven for the duration of the study period; an additional three broad categories sampled from 2004 to 2006 were subsequently divided into eight categories for sampling from 2007 to 2010 (Table 1).

EU legislation required poultry category sampling in each Member State, in each year, as follows:

i) the number of holdings to be sampled to ‘ensure the identification of at least one infected holding if the prevalence of infected holdings is at least 5%’, with 99% confidence in all turkey categories (2004 – 2010), all duck and geese categories (2005 – 2010), and quail (‘game birds’ category) (2006), or with 95% confidence in all other poultry categories (2004 – 2010), ducks and geese (2004) and quail (2004 – 2005, 2007 – 2010).

ii) the number of birds to be sampled per holding to ‘ensure 95% probability of identifying at least 1 seropositive bird if the number of seropositive birds is greater than or equal to 30%’ — 40 – 50 birds per holding from duck and geese holdings (2004 to 2010) and quail (2005 to 2010), or 5 – 10 birds per holding (or per shed on a multi-shed holding) from all other poultry categories (European Commission, 2002, 2005a, 2005b, 2007, 2010).
Sampling was required to be geographically stratified to be representative of the poultry population in the Member State. A representative sampling approach was required, employing random sampling of poultry category strata— however, the legislative guidelines did allow for some targeting of holdings that might be considered at higher risk of infection (European Commission, 2007, 2010). All serological detections were required to have been confirmed using haemagglutination inhibition testing as per the EU Diagnostic Manual. Tests were performed at the Member State’s National Reference Laboratory for Influenza A, with consistent methodology and using the designated antigens for haemagglutination inhibition testing supplied by the EU Reference Laboratory for Influenza A, as per EU legislative guidelines (European Commission, 2002, 2005a, 2005b, 2007, 2010). Antigens were selected to ensure broad reactivity with antibodies to the diversity of contemporary H5 and H7 Eurasian viruses. Serological detections were reported at holding level.

The EU Influenza A serosurveillance data were collated into serosurveillance ‘annual poultry category reports’, each summarising serological testing undertaken in a particular poultry category, EU Member State and year (e.g. breeder geese in Germany in 2008). The unit considered both descriptively and analytically was a ‘holding test’. A few Member States tested seronegative holdings more than once within a year, but this could not be specifically accounted for so all ‘holding tests’ were included in the analyses. Two binary outcomes were considered in the analyses: seropositive or seronegative for H5 influenza A, and seropositive or seronegative for H7 influenza A. Where holding test serology was reported as ‘inconclusive’, it was defaulted to seronegative unless confirmed by repeat serology, or virological investigation that could include PCR.

4.2 Comparison of the odds of H5 and H7 influenza A seropositivity between holdings of different poultry categories
Logistic regression was undertaken using the glmmTMB package (Brooks et al., 2017) in R v3.5.3 (R Core Team, 2019), to identify associations between either of the two outcomes (H5 or H7 influenza A serological status of a poultry holding test) and the exposure of interest (poultry category). Member State was included
as a random effect, and year as a fixed effect, to account for potential clustering of data. Laying-hens were chosen as the reference poultry category, due to both the relatively low proportion of seropositive holdings identified and the relatively consistent, proportionately substantial sampling strategies applied in this stratum across years and EU Member States. To improve poultry category data balance across years and EU member states, poultry categories that were subdivided for surveillance from 2007 to 2010 were collated to match groupings for 2004 – 2006 for the initial analyses (e.g. ‘domestic waterfowl’ included ducks and geese [2004 – 2006], and breeder ducks [2007 – 2010], breeder geese [2007 – 2010], fattening ducks [2007 – 2010] and fattening geese [2007 – 2010]). Modelling was then repeated considering the domestic waterfowl subcategories (data available 2007 – 2010), to enable assessment of whether certain subcategories of ‘domestic waterfowl’ particularly contributed to the relatively high odds of H5 and H7 influenza A seropositivity observed in this stratum. Testing for interaction was omitted from all models, due to the large number of strata constituting the exposure of interest.

4.3 Comparison of the odds of H5 and H7 influenza A seropositivity between free-range and non-free-range holdings
As no broiler holding tests were seropositive from H5 or H7 influenza A, only the laying-hen categories were used in analysis of free-range rearing as a risk factor for H5 or H7 influenza A seropositivity. Data on free-range rearing were only available for laying-hens in the years 2007 to 2010, so analyses comparing the odds of infection between free-range and non-free-range rearing were restricted to surveillance data from these years. Logistic regression was undertaken regarding the two outcomes (H5 or H7 serological status at a holding-test level), as per the poultry category analyses; the exception was that year was included as a random effect in the H5 model, as the model did not converge with year as a fixed effect.

4.4 Spatial heterogeneity of H5 and H7 seropositivity in the European Union
The software R v 3.3.2 (R Core Team, 2016) was used for the spatial analyses. To test for heterogeneity in the geographical distribution of the two outcomes, data from the most comprehensive years, covering the widest selection of Member States (2008 to 2010), were linked to the X and Y coordinates of the centroid location for each Member State NUTS (Nomenclature of Units for Territorial Statistics (Eurostat, 2015))
region from which results were present. In some instances, assigning a centroid location was problematic, either due to the use of historical NUTS regions or where centroids of coastal locations were not placed fully within a landmass, and so in these cases data were aggregated to a larger NUTS level or were moved within the nearest landmass. Two European non-Member States (Norway and Switzerland) also contributed data for the spatial analysis.

At each spatial location, the serosurveillance data were summarised as the number of holdings sampled, number of holdings H5 or H7 seropositive, and a binary outcome for whether the location had any H5 or H7 positive results. Spatial analyses were undertaken for all poultry categories combined, for gallinaceous poultry (chickens and turkeys) only, and for waterfowl (geese and ducks) only.

The first spatial analysis was completed to assess whether any spatial heterogeneity of H5 or H7 seropositivity was present that applied to the entire area, which may indicate the importance of local spread of infection amongst holdings. The spatial points of each regional centroid were examined for any significant spatial heterogeneity of results, by standardising residuals from a binomial model and estimating a variogram, using the add-in package geoR (Ribeiro Jr and Diggle, 2001). The variogram summarises the difference in results in a pair of observations as a function of their spatial separation and as such, it is a spatial analogue of the variance in more standard statistics (Clough et al., 2009). The results were plotted along with simulated spatially-uncorrelated Monte Carlo estimate envelopes, based on 99 permutations of the data values across the locations (Rowlingson and Diggle, 1993). If significant spatial heterogeneity was detected, covariates found to be associated with influenza A through the other sections of this study were added to the base variogram model to determine whether these negated the significant heterogeneity and thus may have explained any apparent spatial associations. For the purpose of this analysis, the effects of spatial closeness were deemed to be equal over land and sea, which allowed for all of the spatial locations to be used in the analysis, without limiting the analysis to a single landmass. Two sets of variograms were
selected for each analysis, one with the distance limits set at 5,000 km, and one set at 100 km. Variograms were also produced at other distances to further explore the results, where necessary.

The second spatial analysis was completed to detect individual geographical areas where significantly high risk of seropositivity was detected (hotspots). A map was produced using the sparr package (Davies et al., 2011) to present the log-relative risk of a region being labelled as H5 or H7 positive, and to highlight the ‘hotspots’ of exposure risk. To account for the lack of data at sea boundaries and the varying concentration of data points within areas, the analysis used an edge-corrected adaptive bandwidth to provide a focused estimation of risk in areas with a greater concentration of data points, whilst maintaining stable estimates in areas with few regions that submitted results. Each map presented the log-relative risk as 12 increasing ‘heat’ colours with two sets of contour lines representing the upper 5th and 25th percentile of risk.

5. Results

5.1 Description of holdings tested and patterns of seropositivity
A total of 1,314 serosurveillance ‘annual poultry category reports’ were submitted from 2004 to 2010, comprising 289,766 holding tests. There was considerable variation in poultry categories sampled across Member States and years (Supporting information Table 1). Where sampling was undertaken, the number of holdings sampled relative to the reported total number of holdings in that Member State was also variable (Supporting information Table 2). A marked peak in the overall number of holding tests undertaken occurred in 2007, attributable to exceptionally large sampling of backyard flocks in Romania (83,244 tests) and Bulgaria (8,667 tests) (Supporting information Figure 1). Consequently, backyard flocks were the most sampled poultry category (Figure 1), and the largest number of holding tests were undertaken in Romania (Supporting information Figure 2).

From 2004 to 2010, 570 H5 and/or H7 influenza A seropositive holdings were detected; 433 were H5 seropositive and 150 were H7 seropositive (13 holdings were seropositive for both subtypes concurrently).
The number of H5 and H7 seropositive holdings detected each year was variable across 2004 - 2010, with a peak in 2007 (Supporting information Figure 3).

H5 or H7 seropositive holdings were not detected in free-range broiler, non-free-range broiler or turkey breeder categories. Over 60% of holdings identified as H5 or H7 seropositive were of the five waterfowl poultry categories (78.5% of the identified H5 seropositive holdings, and 12.7% of the identified H7 seropositive holdings). The poultry categories with highest number of holdings identified as H7 seropositive were backyard flocks (27.3%) and fattening turkeys (22.0%) (Figures 2 and 3).

Thirteen EU Member States did not detect seropositive holdings for H5 or H7 influenza A during the study period, while four Member States detected H5 seropositive holdings but not H7 seropositive holdings. Sixty percent of the H5 seropositive holdings were detected in France and just under 70% of the H7 seropositive holdings were detected in Italy (Supporting information Figure 4).

In consideration of free-range and non-free-range rearing (2007 – 2010), neither H5 nor H7 influenza A seropositivity was identified in any broiler holdings. H5 influenza A antibodies were detected in three free-range (0.05%) and three non-free-range laying-hen holdings (0.01%), while antibodies to H7 influenza A were found in nine free-range laying-hen (0.14%) and 10 non-free-range laying-hen (0.04%) holdings (Figure 4).

### 5.2 Comparison of the odds of H5 and H7 influenza A seropositivity between holdings of different poultry categories

Associations were identified between both outcomes (H5 and H7 influenza A seropositivity) and poultry category (Tables 2 and 3). Holdings of the poultry categories domestic waterfowl (adjusted odds ratio (OR) = 58.0, 95% CI 30.7 – 109), gamebirds (adjusted OR = 10.2, 95% CI 5.15 – 20.3), “other” (adjusted OR = 6.26, 95% CI 2.82 – 13.9) and ratites (adjusted OR = 5.27, 95% CI 1.45 – 19.2) had relatively increased odds of H5 seropositivity. Meanwhile, holdings of domestic waterfowl (adjusted OR = 5.11, 95% CI 2.63 – 9.90), backyard flocks (adjusted OR = 6.07, 95% CI 3.41 – 10.8), “other” (adjusted OR = 3.34, 95% CI 1.77 – 6.44)
and fattening turkey (adjusted OR = 2.67, 95% CI 1.52 – 4.69) categories had relatively increased odds of H7 seropositivity (Table 2).

Amongst the domestic waterfowl subcategories, fattening geese, breeder duck and breeder geese holdings had increased odds of H5 seropositivity, compared to fattening duck holdings (Table 3).

5.3 Comparison of the odds of H5 and H7 influenza A seropositivity between free-range and non-free-range holdings
There was good evidence of an association between free-range rearing and H7 influenza A seropositivity in laying-hen holdings (adjusted OR = 30.1, 95% CI 2.71 – 334; p = 0.006). Meanwhile, there was insufficient evidence of an association between free-range rearing and H5 influenza A seropositivity (adjusted OR = 6.43, 95% CI 0.42 – 98.2; p = 0.18).

5.4 Spatial heterogeneity of H5 and H7 seropositivity in the European Union
The first set of spatial analyses utilised the data from all poultry categories from EU member states, and data from two European non-Member States. These non-Member States were Norway (222 holding tests from a range of poultry categories in 2009) and Switzerland (104 holding tests from 2009 and 64 holding tests from 2010, from free-range laying hen and backyard poultry categories). Figure 4 displays the centroid locations of the analysed population. The density of surveillance location centroids varies, from a sparse number of data point locations in Scandinavia, and island locations, to areas with many data points in Belgium, the Netherlands, Romania, Bulgaria and Hungary. The variogram analysis, using summarised results from 2008 to 2010, did not detect significant spatial heterogeneity of either H5 or H7 seropositivity results across the studied area, with all of the points of the estimated variogram within the simulation envelopes. The same pattern was found when the results were assessed from each of the three years individually. The variogram results for waterfowl and gallinaceous poultry for 2008 to 2010 also did not show any significant spatial heterogeneity. As no significant spatial heterogeneity was detected, the addition of covariates to the variogram models was not tested.
The risk plots for H5 seropositivity for the surveillance data collected from 2008 to 2010 showed a contour for the upper 5th percentile of risk around western France, England and a small localised cluster near the border of Norway and Sweden, with the 25th percentile covering France, Great Britain, northern Spain and Belgium, as well as a cluster around Norway and Sweden (Figure 5). The same pattern was shown in the individual year plots, although Norway was not included in the hotspot in 2010.

The H7 risk plot for 2008–2010 (Figure 6) showed the upper 5th percentile of risk covering a cluster around Italy and a cluster around Belgium, with the 25th percentile covering this and also parts of northern France, southern Netherlands and parts of Switzerland, Austria and Slovenia. The H7 plots for individual years did not show any hotspots in 2010, which may have been because fewer than 10 regions had seropositive results and this limited the power of the analysis to detect a hotspot. The 2009 plot displayed the Italy cluster, whereas the 2008 plot detected a cluster around northern France, Belgium and south-east England, with a small 25th percentile cluster in southern Italy.

The spatial relative risk plots for H5 and H7 for gallinaceous poultry from 2008 to 2010 may have been limited in ability to detect a hotspot by having fewer than 10 positive regions; however the H7 plot did show significant hotspots around Italy, Belgium and the Netherlands (Supporting information Figures 5 and 6). The duck and geese plots for the same time period showed a hotspot for H5 in western France and southern England (Supporting information Figure 7), whereas the H7 plot (Supporting information Figure 8) showed a hotspot around Belgium, northern France and south-east England. The H7 plot had fewer than 10 positive regions.

6. Discussion

6.1 Comparison of the odds of H5 and H7 influenza A seropositivity between holdings of different poultry categories, and between free-range and non-free-range holdings

The findings of this study suggest that domestic waterfowl, game birds, backyard flocks, fattening turkeys, ratites and the “other” poultry category holdings are at increased risk of subclinical exposure to H5 and/or
H7 influenza A, and provide corresponding measures of effect. These findings may be used to refine risk-based surveillance strategies, and for prioritising management actions in influenza A outbreaks.

The findings of this study are also useful for generation of hypotheses regarding causal relationships of H5 and or H7 influenza A exposure among different poultry categories in the European Union. Certain poultry categories may be more susceptible to H5 and/or H7 influenza A virus infection or at increased risk of exposure through specific transmission pathways, or a combination of both. Relatively higher efficiency of transmission of influenza A viruses of wild bird origin has been demonstrated in ducks compared to chickens (Pillai et al., 2010; Spackman et al., 2010), and a greater efficiency of transmission of influenza A strains of both wild bird origin and domestic poultry origin has been demonstrated in turkeys than chickens (Pillai et al., 2010). Similarly, a low pathogenicity influenza A strain of wild bird origin was demonstrated to replicate for a longer period in ducks than chickens, while low pathogenicity influenza A strains of domestic poultry origin replicated for longer periods in chickens than ducks (Mundt et al., 2009). These factors are influenced by the virus not needing to adapt to a different host species after transmission.

Another potential contributor to these apparent differences in the likelihood of H5 and H7 influenza A seropositivity between poultry categories may be a tendency for relatively mild H5 and H7 clinical signs amongst certain poultry categories, combined with management practices that may result in a lower likelihood of detection and elimination of infected flocks by passive surveillance. For example, considering the markedly increased odds of H5 seropositivity in domestic waterfowl, game birds and ratites: clinical symptoms of influenza A infection may be markedly less severe in these poultry categories than in gallinaceous poultry (Perkins and Swayne, 2002; Mutinelli et al., 2003; Perkins and Swayne, 2003; Kaleta and Honicke, 2004; Sturm-Ramirez et al., 2005). Further, some of these poultry categories may also be more likely to be managed in a way that reduces the sensitivity of detection of infection by passive surveillance, compared to laying-hens. In laying-hens, a detectable decline in egg production and feed intake associated
with influenza A infection may provide a relatively early prompt for disease investigation by passive surveillance (Henzler et al., 2003; de Wit et al., 2004).

Further, differences in production systems may also increase the likelihood of exposure to certain influenza A viruses in certain poultry category types. For example, while findings suggest that fattening turkey holdings had a relatively increased likelihood of exposure to H7 influenza A, this was not identified in turkey breeder holdings. This may be attributable to generally higher standards of biosecurity on turkey breeder holdings, given the higher value of the flocks. Amongst domestic waterfowl, the breeder category holdings appear to be at greatest risk of exposure to H5 and H7 influenza A viruses. This may be attributable to their relatively longer lifespan compared to those used for meat production, entailing higher probability of exposure to H5 or H7 influenza A. Consistent with this finding, H5 and H7 influenza A exposure was rarely detected in broiler holdings, which have the shortest production cycle of any poultry type by a substantial margin. A positive association between age and influenza A seropositivity has been previously identified in chickens (Woo and Park, 2008). Amongst the non-waterfowl poultry categories with relatively increased probability of H5 or H7 influenza A seropositivity, outdoor rearing is a common husbandry practice, which may increase the probability of exposure to influenza A by spillover of infection from wild birds. This corresponds with the increased probability of H7 seropositivity identified in free-range laying-hen holdings, compared to non-free-range.

The discordant H5 and H7 findings in certain poultry categories, and particularly the corroboration of relatively increased probability of H7 seropositivity in both free-range laying-hens and backyard flocks in the absence of increased probability of H5 seropositivity, raise questions about variation in virus epidemiology between the H5 and H7 subtypes. Certain poultry species may be more susceptible to infection with H7 than H5 subtypes, or vice versa. Alternatively, particularly in the case of free-range holdings, production of certain poultry categories may be concentrated in areas of the EU where a certain influenza A subtype dominates in the corresponding wild bird population. Findings of studies based in different areas of the USA similarly
support geographical heterogeneity in the risk of influenza A infection in domestic poultry (Donahue et al., 2011; Yendell et al., 2012; Madsen et al., 2013). However, these discordant findings could be attributable to a lack of statistical power in the study, given that the outcomes were relatively rare.

6.2 Spatial heterogeneity of H5 and H7 seropositivity in the European Union
Spatial analyses were used to explore the heterogeneity of spatial risk for influenza A and whether these results could be used to further inform risk-based surveillance strategies. The results of these analyses showed that ‘hotspots’ of risk were present for both H5 and H7 influenza A. The H5 results showed a relatively high level of risk centred on western France and England, with a secondary ‘hotspot’ of risk between Norway and Sweden, which was consistent over the three years examined. The similarity of the cluster around France and England for both the combined bird species plot and that related to ducks and geese, suggests that the results from ducks and geese are the main component of this risk cluster. This is consistent with the extensive outbreak of H5 high pathogenicity influenza A viruses in duck holdings in southwest France in 2015-2016 (Briand et al., 2017). The H7 risk ‘hotspots’ were located in Italy and Belgium. The Italian cluster may be related to the gallinaceous poultry surveillance results, particularly from 2009. The cluster around Belgium may be related to both bird species groupings, particularly the peak in results in 2008.

The presence of significant hotspots of H5 and H7 risk contrasted with the absence of any significant spatial heterogeneity assessed by the variogram analysis. The lack of significant association between the distance between data points and their results across the studied area suggests that any effect of local transmission or localised risk factors was not consistent across Europe. The results may highlight that the hotspots were present due to factors present in specific areas, such as high concentrations of flocks with certain risk factors for infection or in areas with a higher probability of contact with infected wild birds. The contrasting results from the two sets of analyses may also be related to the ability of the sparr analysis to account for data rich and data sparse areas within the analysis. The analyses also differed due to data aggregation, with the
variograms using a binomial model of the holding results at each regional location whereas the sparr plots used an outcome of whether a region had reported any seropositive results.

A scenario of spatial variation in the demographics of poultry populations, and possibly the wild bird reservoir, across Europe is consistent with the observed results and indicate that a spatially flexible perspective to influenza A is more appropriate than a ‘one size fits all’ approach. These results show a number of significant variations in influenza A exposure patterns in European poultry that can be used to enhance future surveillance and control plans.

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8. Conflict of Interest statement
The authors declare that there are none.

9. References


### 10. Tables

**Table 1**: EU Member States and poultry categories surveyed as part of the EU influenza A surveillance program 2004–2010

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</tr>
<tr>
<td>Poultry Category</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken breeders</td>
<td>Ducks &amp; Geese</td>
<td>Breeder ducks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey breeders</td>
<td></td>
<td>Breeder geese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fattening turkeys</td>
<td></td>
<td>Fattening ducks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Game birds$^1$</td>
<td></td>
<td>Fattening geese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratites$^2$</td>
<td>Broilers</td>
<td>Free-range broilers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backyard flocks</td>
<td></td>
<td>Non-free-range broilers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other$^3$</td>
<td>Laying-Hens</td>
<td>Free-range laying hens</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-free-range laying hens</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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$^1$ Game birds includes quail, pheasant, mallard duck, guinea fowl, partridge and meat pigeon holdings

$^2$ Ratites covers several orders of birds, including ostriches and emus

$^3$ The poultry category “others” includes zoo birds, show poultry, pullets, pigeon fanciers and breeders
Table 2: Associations between poultry category and H5 or H7 influenza A seropositivity in the EU, controlled for year and EU Member State

<table>
<thead>
<tr>
<th>Poultry category</th>
<th>H5 influenza A</th>
<th>H7 influenza A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Chicken Breeders</td>
<td>0.33</td>
<td>0.07 – 1.48</td>
</tr>
<tr>
<td>Broilers</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Laying-hens</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ratites</td>
<td>5.27</td>
<td>1.45 – 19.2</td>
</tr>
<tr>
<td>Fattening turkeys</td>
<td>1.16</td>
<td>0.43 – 3.18</td>
</tr>
<tr>
<td>Other</td>
<td>6.26</td>
<td>2.82 – 13.9</td>
</tr>
<tr>
<td>Backyard flocks</td>
<td>1.23</td>
<td>0.49 – 3.13</td>
</tr>
<tr>
<td>Game birds</td>
<td>10.2</td>
<td>5.15 – 20.3</td>
</tr>
<tr>
<td>Domestic waterfowl</td>
<td>58.0</td>
<td>30.7 – 109</td>
</tr>
</tbody>
</table>

1 Neither outcome was detected in turkey breeders
2 OR= odds ratio; CI= confidence interval
3 H5 influenza A was not detected in broilers

Table 3: Associations between category of ducks and geese and H5 or H7 influenza A seropositivity in the EU, controlled for year and EU Member State

<table>
<thead>
<tr>
<th>Domestic waterfowl subcategory</th>
<th>H5 influenza A</th>
<th>H7 influenza A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Fattening ducks</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Breeder ducks</td>
<td>5.41</td>
<td>3.36 – 8.70</td>
</tr>
<tr>
<td>Fattening geese</td>
<td>2.34</td>
<td>1.09 – 5.00</td>
</tr>
<tr>
<td>Breeder geese</td>
<td>11.7</td>
<td>6.93 – 19.7</td>
</tr>
</tbody>
</table>

1 OR = odds ratio; CI = confidence interval
11. Figure Legends

Figure 1: EU H5 and H7 influenza A serosurveillance 2004 – 2010: total number of holding tests performed, by poultry category. † denotes poultry categories sampled only 2004 – 2006; ‡ denotes poultry categories sampled only 2007 – 2010.
Figure 2: EU H5 and H7 influenza A serosurveillance 2004 – 2010: number of seropositive holdings detected, by poultry category. † denotes poultry categories sampled only 2004 – 2006; ‡ denotes poultry categories sampled only 2007–2010.
Figure 3: EU H5 and H7 influenza A serosurveillance 2007 – 2010: free-range and non-free-range broilers and laying hens.
Figure 4: Map of the location of NUTS regions used for spatial analysis, collected from all bird species from 2008 – 2010.
Figure 5: Plot of spatial relative risk of regional H5 influenza A serology results, created from binary regional results for all bird species results from 2008 – 2010. The thick contour line and white grid pattern represents the upper 5th percentile of risk; the dashed lines indicate the upper 25th percentile. This map is cropped to display significant findings in sufficient detail; please see Supporting Information for colour maps displaying findings across the entire sampling area.
Figure 6: Plot of spatial relative risk of regional H7 influenza A serology results, created from binary regional results for all bird species results from 2008 – 2010. The thick contour line and white grid pattern represents the upper 5th percentile of risk; the dashed lines indicate the upper 25th percentile. This map is cropped to display significant findings in sufficient detail; please see Supporting Information for colour maps displaying findings across the entire sampling area.