Low-pathogenic notifiable avian influenza serosurveillance and the risk of infection in poultry – a critical review of the European Union active surveillance programme (2005–2007)

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Background Since 2003, Member States (MS) of the European Union (EU) have implemented serosurveillance programmes for low pathogenic notifiable avian influenza (LPAI) in poultry. To date, there is the need to evaluate the surveillance activity in order to optimize the programme’s surveillance design.

Objectives To evaluate MS sampling operations [sample size and targeted poultry types (PTs)] and its relation with the probability of detection and to estimate the PTs relative risk (RR) of being infected.

Methods Reported data of the surveillance carried out from 2005 to 2007 were analyzed using: (i) descriptive indicators to characterize both MS sampling operations and its relation with the probability of detection and the LPAI epidemiological situation, and (ii) multivariable methods to estimate each PTs RR of being infected.

Results Member States sampling a higher sample size than that recommended by the EU had a significantly higher probability of detection. Poultry types with ducks & geese, game-birds, ratites and “others” had a significant higher RR of being seropositive than chicken categories. The seroprevalence in duck & geese and game-bird holdings appears to be higher than 5%, which is the EU-recommended design prevalence (DP), while in chicken and turkey categories the seroprevalence was considerably lower than 5% and with that there is the risk of missing LPAI seropositive holdings.

Conclusion It is recommended that the European Commission discusses with its MS whether the results of our evaluation calls for refinement of the surveillance characteristics such as sampling frequency, the between-holding DP and MS sampling operation strategies.

Keywords Avian influenza, European Union, LPAI, risk factors, surveillance.

Introduction Avian influenza (AI) is a contagious viral disease of various avian species. Avian influenza virus (Alv) strains of the subtypes H5 and H7 can be either of low or high pathogenicity. Highly pathogenic strains can cause high and progressive mortality in commercial poultry flocks and can lead to large outbreaks with severe economical consequences to poultry industries of affected countries. Highly pathogenic AI (HPAI) epidemics occurred either as a consequence of direct introduction of HPAI virus (UK 2007) or mutations from H5 or H7 Low Pathogenic AI (LPAI) viruses. The latter likely happened in the USA 1983, Mexico 1994, Italy 1999–2000, Chile 2002, The Netherlands 2003–2005 and UK 2008. Based on the risk of LPAI strains changing to HPAI, the World Organization for Animal Health (OIE) considers all Alv of the H5 or H7 subtypes a notifiable disease: Highly pathogenic notifiable avian influenza (HPNAI) and Low pathogenic notifiable avian influenza (LPAI).

In accordance with the OIE, early detection, notification, control and eradication of LPNAI has become compulsory in the European Union (EU). Early detection is mainly based on passive surveillance, which in general has proven...
Serological surveillance of poultry populations in MS were first carried out in 2003.\(^\text{17}\) The objective was to perform an initial screening to get a first idea of the extent of LPAI H5 and H7 introductions in different species of poultry as a precursor study for possible EU-wide surveillance programmes. Since then, the number of MS implementing surveillance and the number of samples tested have increased over the past years.\(^\text{18}\) However, despite the increase in participation of MS in the programme and the overall large amount of samples processed, there are still gaps in our knowledge of the epidemiological situation of LPNAI infections in the EU. For example, there is no quantitative estimation of the risk of each poultry type (PT) of being infected with LPNAI. This risk has been, so far, only qualitatively assessed.\(^\text{19}\) Additionally, there is the need to evaluate the current surveillance results in order to optimize the programme’s surveillance design.\(^\text{16,17}\) This paper analyses the EU surveillance activity carried out from 2005 to 2007, in particular addressing: (i) the MS adherence to the recommended sample size, (ii) the degree of concordance of the observed prevalence and the \textit{a priori} prevalence assumed in the surveillance design, and (iii) the risk of each PT of being infected with LPNAI in the programme.

### Methods

#### Sampling design

For serological surveillance, the EU sampling design targets the detection of at least one infected holding based on an \textit{a priori} prevalence [design prevalence (DP)] of AI infected holdings of 5% or more and a confidence level of 95% for each PT (layers, chicken breeders, broilers, etc.) except turkey, duck, geese and quail holdings. For the latter four PTs the sampling design is based on a confidence level of 99%. The number of birds to be sampled within a holding must ensure the detection of at least one positive bird assuming a DP of 30% and a confidence level of 95%. In simple numbers, at least 5 to 10 birds (except ducks, geese and quail) should be sampled per holding, and if there is more than one house within a holding it is recommended to sample at least five birds per house (this would increase the sample size accordingly to the number of houses in the holding). In case of ducks, geese and quails it is recommended to sample at least 40 to 50 birds per holding.\(^\text{15,16}\)

This higher number of birds sampled is due to the expected lower sensitivity of serological tests when testing these bird species.\(^\text{20}\)

#### Data analysis

The data used for the analyses are those submitted in 2005, 2006 and 2007 by MS to the European Commission and published in the official LPNAI surveillance reports.\(^\text{18,21,22}\)

To summarize and describe MS sampling operations, the ratio between the total number of holdings sampled by each MS for each PT and the minimum sample size required by the EU for each specific PT (ratio = holdings sampled/required sample size) was used as a descriptive indicator. A ratio equal to one indicates that the MS sampled the minimum required number of holdings, a ratio higher than 1 means that the MS sampled more than required and a ratio lower than 1 means that the MS sampled less holdings than required. The relation between this ratio and the probability of finding at least one positive holding was evaluated using a logistic regression model. The results of the model were interpreted as (adjusted by the covariates year and PT) summary odds ratios (sOR), of detecting at least one positive holding when the ratio >1 compared to a ratio ≤1 (Table 1). The goodness of fit of the model was assessed by residual analysis.

The seroprevalence of LPNAI in poultry holdings was modeled using a Poisson regression model where \(\ln(\text{number of holdings positive/holdings sampled})_j = b_0 + b_i \times \text{PT}_j + b_k \times \text{year of surveillance}_j\). Table 1 shows a description of the variables used in the model. In this model, \(\ln(\text{number of holdings positive})_j\) is the natural log of the number of PT\(_j\) positive holdings in MS\(_j\), \(\ln(\text{holdings sampled})_j\) is the natural log of the total number of PT\(_j\)
holdings sampled in MS_j. The estimated parameters were b_0 as the intercept, b_i as the regression coefficients (RC) of PT_i, b_k as the RC for Year_k. The parameters were exponentially expressed (e^{RC}) and the results interpreted as the prevalence ratio (PR) or relative risk (RR).\textsuperscript{23,24} The significance of the RCs was estimated by the Wald test. The goodness of fit of the model was assessed by residual analysis. Turkey breeders as well as all the data from France were removed from the final model, because in the residual analysis they were outliers.

To characterize the situation of poultry with respect to LPNAI in the EU, we calculated the observed seroprevalence in MS, but also the one tailed exact 95% Fisher’s upper confidence limits (UCL). The latter results were expressed as the maximum expected seroprevalence of LPNAI in each PT.

All the statistical analyses were performed using the software packages ‘R’\textsuperscript{25} for fitting the Poisson and logistic models and WinPepi\textsuperscript{26} for the UCL estimations.

Table 1. Description of the variables used in the multivariable (Logistic and Poisson) regression models

| Variable                  | Type               | Description                                                                 |
|---------------------------|-------------------|-----------------------------------------------------------------------------|
| Poultry type (PT)         | Categorical       | 1 = chicken breeders, 2 = commercial layers, 3 = broilers, 4 = turkey fatteners, 5 = turkey breeders, 6 = backyard, 7 = ducks & geese, 8 = game-birds, 9 = ratites, 10 = others\textsuperscript{1} |
| Year                      | Categorical       | Year of each survey: 2005, 2006 and 2007                                      |
| Samples                   | Numerical         | Total number of samples taken by each MS for each PT                         |
| Ratio sampled/required    | Categorical       | 1 = Ratio >1, 0 = Ratio \leq 1                                              |
| Member States\textsuperscript{3} | Categorical      | Every Member State (MS) of the EU                                             |
| Result                    | Numerical         | Number of positive holdings for each PT and MS each year of survey          |
| Detection                 | Categorical       | 1 = Detected, 0 = Not detected                                               |

\textsuperscript{1}Member States reported as others: pheasants, partridges, Zoo birds, quails, ostrich, Passeriformes, pigeons, ornamental birds and guinea fowl.

\textsuperscript{2}An initial evaluation showed no difference between Ratio = 1 and Ratio < 1, therefore these were joined as one factor for the final logistic model evaluating the relation between the amount of sampling and the probability of detection.

\textsuperscript{3}Member States were initially included as a categorical covariate in both, the logistic and the Poisson models. They were removed from the final models since no significant differences (between MS) were observed for this variable and the models fitted better when this variable was excluded.

Results

Member State’s surveillance sampling operation

The surveillance activities were carried out by each MS not in an homogenous way, main differences included the sample size and the selected PTs. Member States such as Denmark, Italy, Spain, Portugal, The Netherlands, Bulgaria and Romania sampled higher numbers of holdings (some MS like The Netherlands even sampled all the poultry holdings included in the surveillance) than the number requested by the EU guidelines (Table 2a,b).

The ratio holdings sampled/required sample size was used for describing MS sampling operations and their relation with the probability of detection. The results of the logistic model, showed a significant relation between the ratio >1 and the probability of detecting at least one positive holding. The estimated OR for the ratio >1 was 6.3 (95% CI: 3.2–16.3) compared to the ratio \leq 1, which means that the odds for detection of a positive holding were 6.3 times higher when the number of holdings sampled were higher than the EU required sampled size.

Table 2a,b summarize each country sampling operation for 2005–2007 surveillance. Member States were grouped in two groups: MS which detected at least one positive holding in at least 1-year surveillance (n = 14) (Table 2a) and those which did not detect any positive holding (n = 13) (Table 2b). Most MS with positive findings had a median overall ratio of holdings sampled/required sample size higher than 1 (approximately 50% of PTs targeted for sampling, were sampled in higher numbers than the required sample size), while most MS with only negative results had a median ratio equal or lower than 1 (Figure 1).
Table 2. Number of holdings and poultry types (PT) sampled during the surveillance performed from 2005 to 2007 by European Union Member States (MS) (a) at least one LPNAI positive holding was detected, (b) no LPNAI positive holding was detected

| Member State (MS) | 2005 | 2006 | 2007 |
|-------------------|------|------|------|
|                   | No of Holdings sampled | Median Ratio Holdings sampled/required | No of Holdings sampled | Median Ratio Holdings sampled/required | No of Holdings sampled | Median Ratio Holdings sampled/required |
| Belgium (BE)      | 6 (2)† | 355 | 0.8  | 8   | 7 (3) | 749 (894) | 0.9  | 4   |
| Czech Republic (CZ) | 3 (3) | 91 (220) | 10  | 5 (4) | 127 (240) | 1.0  | 10  |
| Denmark (DK)      | 5 (3) | 283 | 1.6  | 3   | 6 (4) | 611 (533) | 1.8  | 13  |
| Finland (Fi)      | 6 (4) | 238 (1891) | 1.0 | 6 (1) | 193 (1211) | 0.6  | 2   |
| France (FR)       | 5 (3) | 959 (7349) | 2.2  | 59  | 7 (5) | 1075 (9438) | 1.9  | 45  |
| Germany (DE)      | 4 (3) | 396 (104884) | 1.6  | 3   | 9 (4) | 1024 | 3.1  | 3   |
| Italy (IT)        | 7 (6) | 2373 (3195) | 4.0  | 7 (7) | 2335 (2818) | 4.0  | 2   | 10 (9) | 10239 (148436) | 11.6  | 27  |
| Netherlands (NL)  | 6 (6) | 5396 (2672) | 10.4 | 5 (5) | 6623 (2448) | 16.5 | 1   | 6 (5) | 2913 (2853) | 5.8   |
| Poland (PL)       | 5 (4) | 501 (816) | 1.6  | 2   | 6 (4) | 458 (725) | 1.2  | 1   |
| Portugal (PT)     | 6 (6) | 488 (2884) | 1.3  | 8 (7) | 3094 (239287) | 1.2 |
| Romania (RO)      | 2 (2) | 12 (12) | 1.0  | 2 (2) | 15 (15) | 1.0  | 1   |
| Spain (ES)        | 9 (8) | 2370 (13587) | 3.0  | 1   | 10 (9) | 7390 (32004) | 5.1  | 17  |
| Sweden (SE)       | 6 (3) | 169 (851) | 1.0  | 7 (6) | 181 (491) | 1.0  | 1   | 8 (4) | 203 (612) | 1.0   |
| United Kingdom (UK) | 7 (4) | 438 | 1.0  | 1   | 7 (4) | 452 (4055) | 1.0  | 3   | 7 (2) | 340 (4439) | 0.7   |
| Bulgaria (BG)     | 4 (3)† | 177 (1768) | 1.0  | 4 (3)† | 188 (1781) | 1.0  | 4 (4) | 177 (1749) | 1.1 |
| Cyprus (CY)       | 8 (2) | 71 | 0.4  | 8 (4) | 168 (8723) | 0.9  | 4 (4) | 177 (1749) | 1.1 |
| Estonia (EE)      | 1 (1) | 13 (13) | 1.0  | 2 (1) | 18 (13) | 1.0  | 4 (4) | 177 (1749) | 1.1 |
| Greece (EL)       | 6 (2) | 158 | 0.6  | 7 (3) | 360 | 1.4  | 9 (4) | 240 (149547) | 0.9 |
| Hungary (HU)      | 4 (3) | 527 (2785) | 1.6  | 5 (4) | 2679 (2075) | 1.4  | 9 (6) | 2676 | 1.0   |
| Ireland (IE)      | 5 (4) | 305 (398) | 1.3  | 5 (4) | 306 (397) | 1.2  | 7 (4) | 275 (394) | 1.0 |
| Latvia (LV)       | 5 (1) | 38 (43) | 0.7  | 6 (6) | 133 (432) | 1.0  | 6 (6) | 119 | 1.0   |
| Lithuania (LT)    | 8 (0) | 42 (174281) | 0.4  | 7 (1) | 175 | 0.5  | 6 (6) | 107 (33) | 2.7   |
| Luxembourg (LU)   | 2 (2) | 12 (12) | 1.0  | 2 (2) | 15 (15) | 1.0  | 4 (2) | 40 (771) | 0.9 |
| Malta (MT)        | 1 (1) | 63 (305) | 1.2  | 1 (1) | 71 (83) | 1.3  | 1 (0) | 30 (305) | 0.6 |
| Slovak Republic (SK) | 7 (1) | 134 (299) | 0.5  | 9 (4) | 430 (54827) | 0.9  | 10 (7) | 332 (332) | 1.3 |
| Slovenia (SI)     | 5 (4) | 187 (279) | 1.0  | 5 (3) | 150 (267) | 1.0  | 6 (4) | 149 (860) | 1.0 |

(a) At least one LPNAI positive holding was detected

(b) No LPNAI positive holding was detected

† Number of PT sampled to an equal or higher number of holdings than the required statistical sample size as stated in the EU surveillance guidelines.

The ratio of the number of holdings sampled/EU required sample size was estimated for each PT. The median of these ratios is reported per MS as a descriptive summary of the MS sampling operations.

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LPNAI in poultry and the PT risk of being infected

Since 2005, an increase in the number of holdings sampled by MS and an increase in the number of positive holdings were observed for most PTs (Table 3). A multivariable analysis which included each year of surveillance and the targeted PTs showed an increase in the overall PR of LPNAI in poultry, in 2006 and 2007 compared to 2005 (Table 4). When MS (including France) were also included as a variable in this analysis (Table 1), no significant differences in the PR between MS were observed. This variable was removed from the final model, in order to improve the fit of the model.

Positive holdings were detected in all PTs but turkey breeders (Table 3). Turkey breeders could not be included in the final Poisson model because the data of this PT did not fit the model. The results of the model, where “Chicken breeders” were used as reference, showed significantly higher RRs for waterfowl PTs, ratites and “others” while no significant differences in the RR was observed between chicken (breeders, layers, and broilers) and turkey PTs (Table 4).

Table 3. Number of Member States (MS) targeting each poultry type (PT) and number of holdings sampled during 2005–2007 surveys in the EU

| Poultry type (PT)       | 2005          | 2006          | 2007          |
|-------------------------|---------------|---------------|---------------|
|                         | MS sampling each PT | No. holdings sampled | No. positives (highest prevalence %)* | MS sampling each PT | No. holdings sampled | No. positives (highest prevalence %)* | MS sampling each PT | No. holdings sampled | No. positives (highest prevalence %)* |
| Chicken breeders        | 15 (12)†      | 2489          | 3 (4.2)       | 17 (11)       | 2130          | 1 (0.1)       | 22 (17)       | 2646          |
| Laying hens             | 25 (22)       | 5869          | 1 (1.5)       | 25 (22)       | 8537          | 5 (1.5)       | 27 (24)       | 9554          |
| Broilers                | 7 (3)         | 1967          |               | 11 (6)        | 2383          |               | 18 (11)       | 2875          |
| Turkey fatteners        | 20 (13)       | 2058          |               | 21 (13)       | 1981          | 1 (0.6)       | 22 (16)       | 3765          |
| Turkey breeders         | 10 (4)        | 251           |               | 10 (5)        | 150           |               | 15 (10)       | 409           |
| Backyard flocks         | 3 (1)         | 247           |               | 9 (8)         | 9051          | 2 (0.6)       | 15 (12)       | 99901         |
| Ducks & Geese           | 21 (14)       | 1795          | 68 (16.7)     | 22 (16)       | 2176          | 62 (33.3)     | 23 (16)       | 4096          |
| Game birds              | 13 (5)        | 756           |               | 18 (8)        | 1500          | 12 (2.3)      | 21 (14)       | 1927          |
| Ratites                 | 17 (5)        | 352           | 1 (11.1)      | 17 (8)        | 448           | 2 (2.2)       | 18 (7)        | 325           |
| Others†                 | 3 (2)         | 441*          | 1 (0.6)       | 1 (1)         | 649           | 6 (0.9)       | 9 (6)         | 1414          |
| **Total**               | 25            | 15784         |               | 25            | 29005         |               | 27            | 126912        |

MS, member states.

†Number of PT sampled to an equal or higher number of holdings than the required statistical sample size as stated in the EU surveillance guidelines.

§MS reported as others: pheasants, partridges, Zoo birds, quails, ostrich, Passeriformes, pigeons, ornamental birds and guinea fowl.

§France sampled 156 free-range chicken holdings which were reported as others.

*The highest apparent prevalence observed by PT in a specific MS.
LPNAI apparent prevalence in poultry compared to the programme’s design prevalence

The EU situation with respect to LPNAI in poultry was characterized taking the point estimates and the 95% UCL for the seroprevalence. In many cases the absence of positive detections was linked to a lower sample size, which led to high and uninformative UCL, therefore we only used the positive results for these characterization. Table 3 shows the highest estimated seroprevalence for each PT each year. For most PTs the estimated seroprevalence is lower than 5%. For the rest of PTs, UCLs were generally lower than 5%. For ducks & geese as well as game-birds had estimated prevalences higher than 5%. Most of the UCLs for ducks & geese as well as game-birds were higher than 5%. For the rest of PTs, UCLs were generally lower than 5% and in case of chicken layers the highest estimated UCL, within the 3 years surveillance, was 3.5%; which was observed in free range chickens in France 2005 (reported as “others”) (Figure 2).

Discussion

The overall Sensitivity (Se) of a surveillance system is a function of both the sampling scheme and the accuracy of the detection methods being employed. In the context of the EU LPNAI surveillance: sampling procedures (targeted PTs and risk regionalization), sample size, sampling frequency and the applied diagnostic assays will determine the Se of the system.

Member States had different sampling procedures, and were grouped in those with only negative findings and those which were able to detect LPNAI positive holdings. The latter group included MS with risk based surveillance programmes (Italy, Spain, Denmark, The Netherlands, etc.) which, in addition to sampling holdings in the whole country, carried out enhanced surveillance in identified high risk areas or PTs. For example, Denmark and The Netherlands sampled some specific PTs more than once a year (for instance free range layers and game-birds were sampled up to four times per year). Additionally, most MS with positive findings sampled more holdings than required by the EU (ratio holdings sampled/required sample size >1) (Figure 1) which was significantly associated with a higher probability of detection. On the contrary MS with only negative findings, sampled less or just the required number of holdings in each PTs. The latter may suggest that the sampling operations of these MS was not enough for detection.

Targeted surveillance to PTs with higher risk of being infected may improve the Se of the programme. In the EU targeted sampling has been based on qualitative evaluations which indicated waterfowl (ducks, geese, and game birds) as the poultry category with higher risk of being infected.
Our results not only confirm these qualitative observations but also provide quantitative estimations of the RR of waterfowl PTs of being infected with LPNAI compared to chicken and turkey poultry categories. The higher risk observed in waterfowl might be related to the species relatedness with migratory wild waterfowl, their long lifespan and the fact that these PTs are mainly kept outdoors which is associated with a higher risk of exposure to AIVs from the environment and wild fauna.\(^9,33,34\) Commercial layers were the most targeted PT. This PT was targeted assuming a higher risk due to their lifespan and their production characteristics (e.g. marketing of eggs), which sustained transmission during HPAI\(^35\) and LPNAI\(^36\) outbreaks. Our results do not show a significant higher risk for this PT compared to chicken breeders, which are expected to have lower risk due to the higher biosecurity measures implemented in this type of production. Outdoor or free range keeping of poultry is considered a major risk factor for introduction of AI into poultry.\(^19,33\) For this reason, MS targeted backyard flocks with increasing numbers every surveillance-year, and also targeted free range layer and broiler holdings in 2007.\(^18\) Due to the absence of sufficient data (differentiation of holdings as outdoor or indoor was only done in 2007) it was not possible to include an outdoor/indoor evaluation in the model. The low RR estimated for backyard and broilers, although they were not significant, might raise the question of the value of sampling these PTs. The short lifespan of broilers explains their low RR and it has been observed that virus introduction into backyard flocks occurred at a lower rate than into commercial holdings during the H7N7 HPNAI in 2003 in The Netherlands\(^37\) and apparently this PT may not play a risk in the transmission of AI to commercial farms in the EU.\(^19,37\) Therefore, it might be advisable to consider sampling these PTs and refine their sample size according to the risk they represent to each MS poultry industry.

It was observed that the expected prevalence in waterfowl PTs appears to be similar or higher than 5%, which is the EU programme’s between holdings DP. However, the expected prevalence in chicken and turkey PTs appears to be lower. The latter might explain the observed lower odds for detection when sampling the EU required holding sample size (ratio ≤ 1). If we take into account that some MS (n = 6) with only negative findings sampled a few or no duck & geese holdings,\(^18,21,22\) and the fact that no difference was observed in the PR between all MS when fitting the Poisson model, it can be expected that many of these negative MS might be detecting LPNAI introductions when using a lower than 5% DP.

Frequency of sampling is a key element of the overall surveillance Se. The current EU sampling frequency of once a year inherently will result in missed or in delayed detections, which might lead to the development of undesired mutations. The rapid mutation (2–3 weeks after introduction) observed in the last H7N7 HPNAI outbreak in the UK\(^8\) as well as evidence of multiple incursions (different times) of different LP or HPNAI, in Italy 2007\(^30\) and UK 2007,\(^11\) highlights the need for early detection. This can be achieved by a higher sampling frequency. Strategies such as that of Denmark and The Netherlands, where PTs considered of higher risk and/or located in higher risk areas are sampled in higher frequency than others, may contribute to improve the probability of early detection.

To conclude, the estimates of PT RRs for LPNAI reported in this study can be used for the risk based design of targeted surveillance,\(^38\) quantitative evaluations of the Se of surveillance programmes (e.g. scenario tree models\(^39\)) and risk assessments. It is recommended that the European Commission discusses with its MS whether the results of our evaluation calls for refinement of the surveillance characteristics such as sampling frequency, the between-holding DP (for chicken and turkey PTs), and MS sampling operation strategies.

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