Development of a seroprevalence map for avian influenza in broiler chickens from Comunidad Valenciana, Spain

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Summary

The aim of this study was to design and implement a seroprevalence map based on business intelligence for low pathogenicity notifiable avian influenza (LPNAI) in broiler chickens in Comunidad Valenciana (Spain). The software mapping tool developed for this study consisted of three main phases: data collection, data analysis and data representation.

To obtain the serological data, the authors analysed 8,520 serum samples from broiler farms over three years. The data were represented on a map of Comunidad Valenciana, including geographical information of flock locations to facilitate disease monitoring. No clinical signs of LPNAI were reported in the studied flocks. The data from this study showed no evidence of contact with LPNAI in broiler flocks and the novel software mapping tool proved a valuable method for easily monitoring the serological response to avian influenza, including geographical information.

Introduction

Avian influenza is a viral disease caused by type A *Orthomyxoviridae* (1). It is included in the list of notifiable diseases of the World Organisation for Animal Health (OIE), as well as in the list of notifiable diseases in the European Union. Influenza virus contains eight negative-sense RNA genome segments, with each one coding for one or more proteins (1).

The viral envelope is covered with glycoprotein projections of two types: haemagglutinin and neuraminidase (2). The haemagglutinin is the most important antigen of the virus and one of the determinants of infectious and pathogenic capacity; it facilitates the attachment and penetration of the virus into the host cell. The neuraminidase plays an essential role in the release of virus from infected cells and their diffusion through the respiratory system. It prevents the aggregation of viral particles and allows the virus to spread through respiratory secretions. The haemagglutinin represents 25% of the viral proteins, compared to 5% for the neuraminidase (3).

Avian influenza viruses are usually specific to their host but may be transmitted between individuals of closely related species and even human transmission has been reported (4). In domestic poultry, clinical signs reflect abnormalities in the respiratory, digestive, urinary and reproductive organs (5).

The active surveillance of this virus is based on sampling and serological testing of birds against the H5 and H7 strains. Vaccinating poultry against avian influenza is prohibited in Spain. However, in the event of serious risk, vaccination could be considered a useful tool to combat avian influenza, so there is a plan for emergency vaccination.

A useful, easy and inexpensive diagnostic tool to evaluate the contact of poultry flocks with low pathogenicity notifiable avian influenza (LPNAI) virus is serology. A seroprevalence map allows monitoring of the presence, distribution and evolution of animal diseases over time and space. Farm location is of interest because of the highly contagious potential of LPNAI viruses. Thus, developing such maps at specific intervals reveals the behaviour of these processes, which are linked to other risk factors, such as the movement of birds, the evolution of the weather and other ecological factors. A knowledge of these epidemiological parameters helps to control the disease and thus to minimise its occurrence.

The aim of this paper was to monitor and represent in a geographical form the serological response of broiler chickens from Comunidad Valenciana in Spain to LPNAI, using a specific commercially available enzyme-linked immunosorbent assay (ELISA) kit and a novel mapping tool. Geographical information on flock locations is useful when monitoring the serological response to LPNAI in large-scale studies. Farm location is also of interest because of the highly contagious potential of LPNAI.

Materials and methods

The study was conducted on broiler poultry farms in Comunidad Valenciana (Spain). The authors begin by describing the software tool, then define the associated serological tests performed. The software tool consisted of three main phases: data collection, data analysis and data representation.

During the first phase of data collection, the processes of Extract, Transform and Load (ETL) enabled the authors to obtain data from many different sources and to load it into a single database, to be analysed in another operating system. Thus, Oracle, Excel and xChek were integrated for this task.

For data analysis, the authors developed a computer application called On-line Analytical Processing (OLAP), allowing dynamic and geographic analysis through the use of multidimensional cubes

containing health information, and integrating the results for LPNAI on broiler farms in Comunidad Valenciana. A cube is a multidimensional database, in which the physical storage of data is performed in a multidimensional vector. We can consider OLAP cubes as an extension of the two dimensions of a spreadsheet into three or more dimensions, also called hypercubes. Finally, after obtaining the data using the ETL processes and analysing them using the OLAP tool, the next step was to represent them geographically. A geographical information system (GIS) was integrated with an open source server called GeoServer. This tool generates Spanish geographic information, such as communities, provinces, regions or towns. It associates data for each sample with the identification code of each geographic unit. This information is contained in the register of livestock holdings (*Registro General de Explotaciones Ganaderas*, or REGA) for each farm, thus establishing the relationship between GIS and OLAP. The data were categorised into different areas, identified by colour, and the values for each area could be obtained by clicking on it.

In the three-year period covered by this study, a total of 8,520 samples were analysed. Some 3,315 samples from 131 broiler farms were analysed in 2008; 2,925 samples were analysed from 173 farms in 2009, and 2,280 were taken from 145 broiler farms in 2010. These samples were collected from animals over 30 days old, with 15 sera being taken per flock. Venipuncture was performed with a needle or scalpel in the brachial vein and the blood was collected in a 5 ml glass tube (one tube per animal) to obtain approximately 3 ml of sample. The tubes were kept horizontally at room temperature until clot formation and then subsequently cooled until their arrival at the laboratory. Once in the laboratory, the samples were recorded using ORALIMS (Nobel Biocare AB, Gothenburg, Sweden), a program based on ORACLE. Each batch of 15 sera was assigned a registration number to maintain traceability throughout the analytic process and evaluation of the results. The samples were centrifuged at 2,500 revolutions per minute (rpm) for 5 min. Red blood cells were deposited in the bottom of the tube and the serum at the top. About

250 µl of each serum sample was collected in 96-well plates, which were identified with their corresponding registration number.

For the analysis, the authors used FlockChek (IDEXX Laboratories Inc., Westbrook, USA), an immunosorbent assay designed to detect influenza A virus nucleoprotein antibodies in sera. Titres were calculated as described by the manufacturer.

Results

Monitoring the health status of broiler farms is a tool for disease control used by veterinary technical services. In this study, a seroprevalence map for LPNAI in broilers of Comunidad Valenciana was designed and implemented to assess the disease situation in this territory. The authors used a computer application for dynamic and geographic OLAP analysis, with multidimensional cubes containing health information, and integrated the qualitative results from the LPNAI serology survey on broiler farms in Comunidad Valenciana. Using the software tool, the authors obtained seroprevalence maps of LPNAI on broiler farms in Comunidad Valenciana during 2008, 2009 and 2010 (Fig. 1). These maps graphically display the qualitative results (positive/negative) obtained by ELISA. The status of a sample is evaluated by the sample-to-positive ratio (S/P ratio):

$$S/P = \frac{\text{Sample mean absorbance} - \text{Negative control mean absorbance}}{\text{Positive control mean absorbance} - \text{Negative control mean absorbance}}$$

This tool could allow us to compare the humoral response to LPNAI in different geographical locations during this period, outlining the possible infection pressures.

The average age of animals sampled in this study was 42.34 days, with a standard deviation (SD) of 8.44, in 2008; 40.64 days with an SD of 8.11 in 2009; and 41.13 days with an SD of 8.95 in 2010. Table I shows the regions of Comunidad Valenciana with the number of flocks analysed, the number of sera tested, and the number of positive and negative results obtained. All results were negative.

Discussion

The consumption of poultry meat is expected to grow further in the coming years, with France, the United Kingdom, the Netherlands, Germany, Italy, Poland and Spain being the main producing countries within the (then) 27 Member States of the European Union (now 28) (6). Gonzáles *et al.* (7) recommended that the European Commission discuss the need to evaluate surveillance activities with its Member States to optimise the design of a surveillance programme for LPNAI in poultry. Spain conducts control measures against avian influenza, while taking into account the fact that legislation is frequently being updated, and that good coordination and communication between the animal health and public health authorities are crucial (8). Avian influenza outbreaks cause dramatic damage to the poultry industry, leading to bans on exportation and to intensive depopulation (9). The combination of on-farm analysis and preventive measures together with the development of seroprevalence maps are vital tools in the task of keeping poultry safe. Recent outbreaks of highly pathogenic notifiable avian influenza (HPNAI) have been reported on three poultry holdings in Italy: in Ostellato (in the province of Ferrara), Mordano (the province of Bologna), and Portomaggiore, close to Ostellato, in the region of Emilia-Romagna. These outbreaks occurred on 15, 21 and 23 August 2013, respectively (10). Although the precise source of infection has not been identified in most outbreaks of HPNAI, wild aquatic birds have been suggested as the most likely source of LPNAI viruses, which then convert to HPNAI viruses in many HPNAI outbreaks (excluding, for the moment, those caused by Asian-lineage H5N1 HPNAI viruses). However, the circulation of an LPNAI virus in industrialised poultry-rearing systems, although considered an important factor in the emergence of some HPNAI strains, is not an essential prerequisite for the genesis of an HPNAI virus (11).

The seroprevalence map developed in the present study can filter results by several metrics, such as time (year, quarter, month or day) or geography (province, region or town). If an outbreak of LPNAI occurred, it would be necessary to prevent the spread of infection by

carefully monitoring and restricting poultry movements and the movements of animal products that might be infected; strengthening biosecurity measures at all levels of poultry production through the cleaning and disinfection of infected premises; establishing protective and surveillance zones around the outbreak and, if necessary, by vaccination.

Thomas *et al.* (12) hypothesised that the introduction of HPAI into a flock, which occurred in the Netherlands in 2003, could have been caused by, in order of relative importance:

– poultry transports

– neighbourhood infection

– staff in the poultry industry who handle birds and enter poultry houses (e.g. farm helpers, information officials, egg transporters)

– infected wild birds

– the mechanical transfer of faeces of infected commercial poultry into new flocks by wild birds, vermin or pigs on mixed farms.

These authors suggested that a failure to control the disease was due to the fact that GIS data on farm locations were not available. The use of GIS is very important in determining risk factors. The literature reflects the use of GIS technology in the prevention of avian diseases in countries such as Italy and China. Ehlers *et al.* (13) applied this technology in the frame of a contingency plan implemented during the 1999–2001 avian influenza epidemic in Italy. Outbreaks in 1999–2000 in Lombardy and Veneto were mapped, using the interface between the infection incidence rate and the geographical information system called GRASS 5.0 (14). In China, Chang *et al.* (14) developed the IV Sequence Distribution Tool (IVDT), similar to GIS, which searches the coordinate data and draws the distribution map after the search. The basic local alignment search tool (BLAST) and other multiple sequence alignment tools run on clusters of supercomputers, and computational tasks are submitted by portable batch system (PBS). Furthermore, the system searches an influenza virus database (IVDB), using an established pipeline for biological databases which consists

of three hardware components: a World Wide Web server, a database server, and a server for sequence analysis. The system is based on a MySQL relational database, and the front end consists of a set of JSP scripts running on a TomCat Web server. The Q-Filter and search engine were developed using Java.

At the global level, Boulos and Burden (15) applied a Web-based Really Simple Syndication (RSS) feed of avian influenza news based on a complete web page (this was essentially pre-AJAX – the Asynchronous JavaScript and XML), with the Google Maps code and data embedded within it. The precursor of this system was designed by the Food and Agriculture Organization of the United Nations (FAO), which, in 1994, developed a Web-based global animal health information system called EMPRES-i, which was applied to HPAI, among other diseases (16). This system was developed using various information sources, such as the official websites of the OIE, World Health Organization, European Commission, governments and Ministries of Agriculture. In addition to these official sources, data are drawn from mailing lists such as ProMed, GPHIN, or AI-watch and news items on avian influenza are traced through Internet research.

This system has been linked to a global information system application to provide a visual representation of disease distribution patterns and to explore the relationship between location, environment and disease. Furthermore, exploratory spatial data analysis (ESDA) is used to seek patterns indicating clusters of cases or areas with elevated incidence, or to highlight associations between the magnitude of disease and other risk factors. The GIS supports ESDA through the calculation of spatial explorative measures, including the creation of smoothed surfaces derived from interpolation techniques applied to punctual variables.

Vietnam, in collaboration with the French Agricultural Research Centre for Development (CIRAD), developed the GIS and Agent-based Modelling Architecture (GAMA) simulation platform (17), which aims to provide field experts, modellers and computer scientists with a complete modelling and simulation development environment

for building spatially explicit multi-agent simulations. This platform has two main applications. One involves modelling the spread of avian influenza in a province of North Vietnam to simulate the poultry value chain of a whole province using geolocalised data, and to use this information to optimise a monitoring network. A second application, conducted with the Institute for Marine Geology and Geophysics (VAST, Hanoi), involves an interactive simulation used to support decision-making during urban post-disaster situations. This application relies on geolocalised data as well, and requires tools that enable interaction between the user and the simulation.

Conclusions

The analysis of broiler results using this system demonstrates the presence and real-time evolution of antibodies against the virus, taking into account spatial distribution and temporal evolution.

This seroprevalence map, applied to broiler chickens in Comunidad Valenciana and based on business intelligence (the techniques and tools for transforming raw data into meaningful and useful information for business analysis purposes), could be an effective tool for Veterinary Services to control and prevent LPNAI.

The use of seroprevalence maps during 2008, 2009 and 2010 showed, in real time, that there was no contact with LPNAI viruses on broiler farms in Comunidad Valenciana and demonstrated the usefulness of this approach for monitoring the serological response, including geographical information.

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Table I

Regions of Comunidad Valenciana: number of flocks, number of samples and qualitative results obtained

Region	No. of flocks			No. of samples			No. of negative results			No. of positive results		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Alcalatén	9	3	3	135	45	45	135	45	45	0	0	0
Alto Maestrazgo	12	7	9	180	105	135	180	105	135	0	0	0
Alto Palancia	0	1	0	0	15	0	0	15	0	0	0	0
Bajo Maestrazgo	50	44	35	750	660	525	750	660	525	0	0	0
Bajo Segura	0	1	1	0	15	15	0	15	15	0	0	0
Campo de Turia	0	1	0	0	15	0	0	15	0	0	0	0
Canal de Navarrés	0	0	2	0	0	30	0	0	30	0	0	0
Condado de Cocentaina	5	14	8	75	210	120	75	210	120	0	0	0
Costera	1	1	0	15	15	0	15	15	0	0	0	0
Hoya de Alcoy	3	0	0	45	0	0	45	0	0	0	0	0
Hoya de Buñol	4	1	1	60	15	15	60	15	15	0	0	0
Huerta Sur	2	1	1	30	15	15	30	15	15	0	0	0
Los Serranos	5	6	0	75	90	0	75	90	0	0	0	0
Marina Alta	4	1	3	60	15	45	60	15	45	0	0	0
Plana Alta	52	47	40	780	705	600	780	705	600	0	0	0
Plana Baja	1	2	0	15	30	0	15	30	0	0	0	0
Puertos de Morella	1	1	0	15	15	0	15	15	0	0	0	0
Ribera Alta	6	5	2	90	75	30	90	75	30	0	0	0
Safor	27	28	21	405	420	315	405	420	315	0	0	0
Utiel – Requena	4	3	3	60	45	45	60	45	45	0	0	0
Valle de Albaida	35	28	23	525	420	345	525	420	345	0	0	0
Total	221	195	152	3,315	2,925	2,280	3,315	2,925	2,280	0	0	0

2008

2009

2010

Fig. 1

Seroprevalence maps of low pathogenicity notifiable avian influenza in broiler chickens in the regions of Comunidad Valenciana, Spain, 2008–2010

Regions in green are those in which results were negative (a sample-to-positive [S/P] ratio less than or equal to 0.5). There were no positive results (an S/P ratio higher than 0.5) during this period. Regions in white are those in which no sampling took place