https://doi.org/10.17221/145/2020-VETMED

Dynamic paramyxovirus type 1 seroprevalence maps in broilers in the Valencian Community (eastern Spain) during a five-year period (2008–2012)

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Citation: Garcia Bover C, Soriano del Castillo JM, Cortes Moniz V, Sevilla-Navarro S, Marin Orenga C, Catala-Gregori P (2022): Dynamic paramyxovirus type 1 seroprevalence maps in broilers in the Valencian Community (eastern Spain) during a five-year period (2008–2012). Vet Med-Czech 67, 471–478.

Abstract: Newcastle disease is a devasting disease in poultry production worldwide, thus it is important to implement control measures to avoid entrance of the disease and its spread in the field. In this context, the aim of this study was to design and implement a seroprevalence map based on business intelligence for avian paramyxovirus type 1 (APMV-1) in broilers in the Valencia Community (eastern Spain). This tool consists in software mapping based on data collection, data analysis and data representation. In order to obtain the serological data, 12 495 sera from 131 broiler farms over 5 years were analysed (2008–2012). The data were represented on a map of the Valencian Community including geographical information of flock locations to facilitate disease monitoring. No clinical signs of APMV-1 were reported in the studied flocks. The data from this study showed no evidence contact with APMV-1 in broiler flocks and the novel software mapping tool as a valuable method for easily monitoring the serological response to avian paramyxovirus type 1 (APMV-1) including geographical information.

Keywords: geographical information system (GIS); mapping; haemagglutination inhibition; Newcastle disease (ND); poultry

Newcastle disease (ND) is one of the most important diseases isolated from herbivorous waterfowl and livestock. It is an especially devastating disease in poultry production worldwide listed as a mandatory reporting disease to the World Health Organisation for Animals (OIE). ND is caused by an avian paramyxovirus serotype-1 (APMV-1) that could cause up to 100% morbidity and mor-

https://doi.org/10.17221/145/2020-VETMED

tality (Triosanti et al. 2018). The virus strains could be classified in three different pathotypes from less to more virulent as lentogenic, mesogenic, or velogenic, according to the mean death time in chicken embryos. The introduction of the Newcastle disease virus (NDV) into domestic poultry production could have severe economic consequences as a result of the decreasing production, the cost of implementing control measures and the trade restrictions established due to an outbreak (Brown and Bevins 2017). NDV is a virus primarily transmitted via inhalation or ingestion of the virus shed in faeces and respiratory secretions, and also transmitted through the egg to the hatching chick (Roy and Venugopalan 2005; Brown and Bevins 2017). Moreover, the virus is highly stable on multiple types of materials and temperatures (Olesiuk 1951), such as fomites or poultry carcasses, skin or bones (Brown and Bevins 2017); for this reason, control of ND is one of the most important targets worldwide (Miller et al. 2015; Elmberg et al. 2017; Schirrmacher 2017).

The recent increase in the number of ND epidemics reported in Spain and in other European Union (EU) member countries along with the failure to identify the source of the Spanish epidemics caused concern over the vulnerability of Spain to this disease (Sanchez-Vizcaino et al. 2010). One of the useful, easy-to-use and inexpensive tools to evaluate the contact with the virus is the serological technique. For the analysis, the technique of haemagglutination inhibition (HI) was conducted as described in the Manual of the OIE about terrestrial animals (OIE 2019).

Several avian orthomyxo- and paramyxoviruses, such as avian influenza virus, Newcastle disease virus, adenovirus, egg drop syndrome ’76 virus and infectious bronchitis virus, cause the haemagglutination of the red blood cells in birds. This haemagglutination usually occurs as a result of the presence of natural receptors (haemagglutinin) on the virus surface. Also, the seroprevalence map allows the monitoring of the presence, distribution and evolution of animal diseases over time and space. In addition, the broiler farm location is interesting point to be known due to the highly contagious potential of APMV-1. Therefore, the development of these maps with a certain frequency will reveal the behaviour of these processes that are probably linked to other risk factors, such as the origin and movement of birds, the evolution of the weather or biosecurity measures. The knowledge of these epidemiological parameters by veterinarians helps to control the disease and, thus, minimise its occurrence.

In Europe, the OIE provides “Disease distribution maps” (https://web.oie.int//downld/PROC2020/A_ANIMAL_HEALTH.pdf), including ND, by countries and by a six-month period or year. This monitoring tool could be improved by adding selectable factors as real-time information, poultry production type, and focusing up to smaller geographical unities (communities, provinces or regions). Currently, there is no such tool available to veterinarians in Spain.

The aim of this paper was to monitor and geographically represent the serological response of broilers from the Valencian Community (Spain) to APMV-1 with the objective to be a useful tool to control this disease by veterinary technical services. It is possible to know if there has been contact with the virus through the serology, to represent that seroprevalence in a dynamic map and, thus, improve the control of the disease.

MATERIAL AND METHODS

A total of 131 non-vaccinated broiler farms from the Valencian Community (Spain) were included in this study. The software tool is described and then the associated serological tests performed are defined. The software tool consisted of three main phases: data collection, data analysis and data representation.

The data were obtained from many different sources during the first phase of the data collection, using the processes of extract, transform and load (ETL). Then, the data were loaded into a single database, to be analysed in another operating system. Thus, Oracle (v9.2; Oracle Corporation, USA), Excel (v2010; Microsoft, USA) and xChek (V.1; IDEXX Co. Ltd., USA) were integrated for this task.

A computer application called on-line analytical processing (OLAP) was developed for the data analysis which allows a dynamic and geographic analysis through the use of multidimensional cubes containing health information, integrating the results for ND titres on broiler farms in the Valencian Community.

A total of 12 495 samples were obtained and analysed over five-year period (2008–2012). In 2008,

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3 345 samples were analysed from 131 broiler farms; in 2009, 2 925 samples were analysed from 173 broiler farms; in 2010, 2 280 samples were analysed from 145 broiler farms; in 2011, 2 430 samples were analysed from 147 broiler farms; and in 2012, 1 515 were taken from 127 broiler farms.

All the individuals sampled ranged from 35 to 42 days old. From each flock, 15 sera samples were collected through the punction of the brachial vein. Around 5 ml of blood was collected from each individual. Then, the tube was kept horizontally at room temperature until clot formation and cooled until its 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 (15 sera) was registered to maintain the traceability throughout the analytic process and evaluation of the results. All the samples were centrifuged at 870 *g* for 5 min and 250 µl of each serum sample was collected from the top and introduced into 96 "V" bottom-shaped wells.

The haemagglutination inhibition (HI) technique was performed with each sera obtained. First, the plates with bottom in "V" were codified and 25 µl of phosphate buffered saline (PBS) were dispensed into the plate wells, then 25 µl of test sera were added in the first well of each row, 25 µl of the controls (positive and negative) were added to the V-bottom plate and after, 1 : 2 serial dilutions were made from the first well. Twenty-five microlitres (25 µl) of the four unities of the haemagglutination activity (4UHA) antigen were dispensed, then the plates were stirred for 30 s on a horizontal shaker plate and incubated for 30 min at room temperature. Afterwards, 25 µl of the red blood cell suspension (diluted to 1%) were dispensed in all the plates, the plates were stirred for about 30 s on the horizontal shaker plate and incubated for 40 min at room temperature. The highest dilution of the serum causing complete inhibition of the antigen 4UHA is called the HI titre. The plates were tilted to obtain the agglutination. Inhibition occurred when the red blood cell stream was observed to be at the same rate as the control wells (positive serum, virus/antigen and PBS controls). The validity of the results should be assessed against a negative control serum, which should not give a titre > 1/4 (> 2^2 when expressed as the reciprocal), and a positive control serum for which the titre should be within one dilution of the known titre. The HI titre was the highest dilution of the serum causing the complete inhibition of antigen 4UHA. HI titres can be considered positive if there is inhibition at a serum dilution of 1/16 (2^4) or more, against the antigen 4UHA.

RESULTS

To prevent the entrance of any infectious diseases in a poultry farm, it is necessary to establish proper tools to assess the health status of the animals. In this study, a seroprevalence map for APMV-1 in broilers from the Valencian Community was designed and implemented to assess the disease situation in this territory. With this aim, a computer application for the dynamic and geographic OLAP analysis, with multidimensional cubes containing health information, which integrated the quantitative results (ND titres) from the APMV-1 serology survey on the broiler farms in the Valencian Community was applied.

Using the software tool, a seroprevalence maps of APMV-1 on the broiler farms in the Valencian

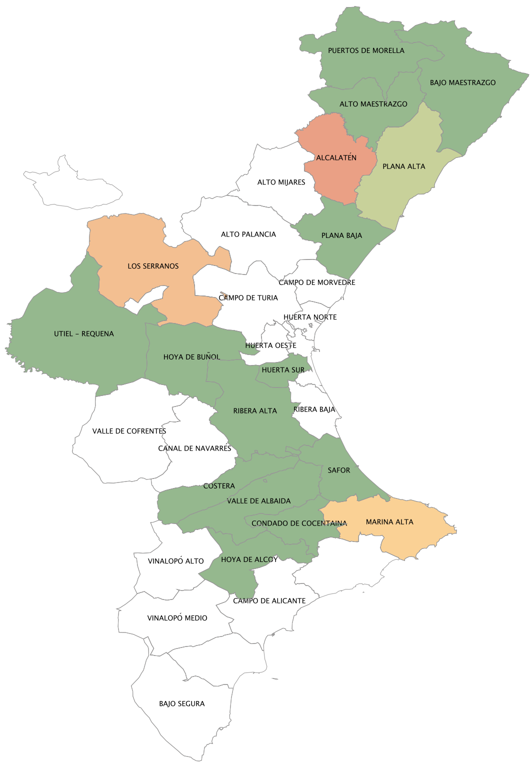

Figure 1. Newcastle disease map of the regions of the Valencian Community (Comunidad Valenciana) for the broilers in 2008

The HI titre was the highest dilution where the highest, intermediate and lower seroprevalence levels are represented in red, yellow and green colour, respectively

https://doi.org/10.17221/145/2020-VETMED

Figure 2. Newcastle disease map of the regions of the Valencian Community (Comunidad Valenciana) for the broilers in 2009

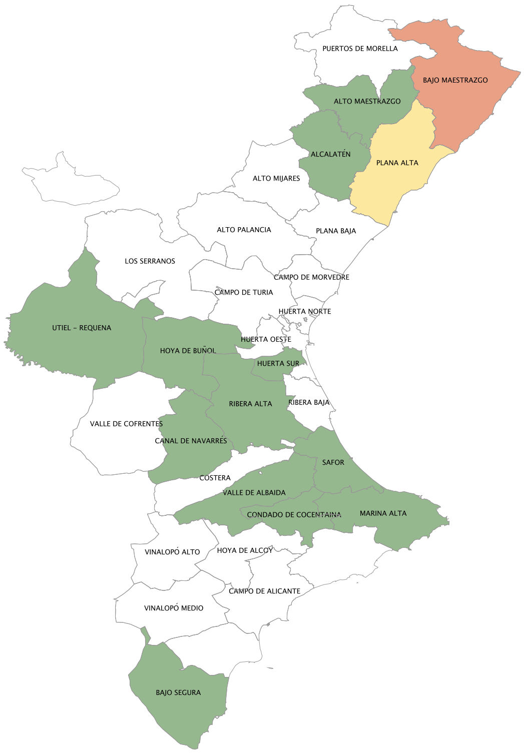

Figure 3. Newcastle disease map of the regions of the Valencian Community (Comunidad Valenciana) for the broilers in 2010

Figure 4. Newcastle disease map of the regions of the Valencian Community (Comunidad Valenciana) for the broilers in 2011

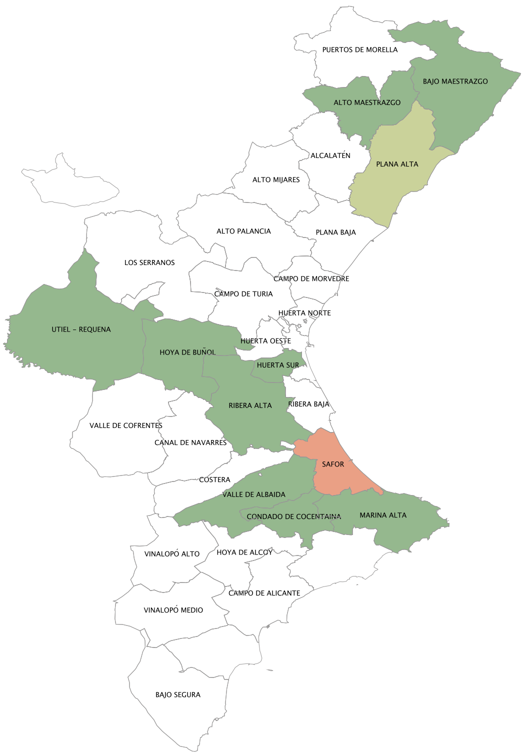

Figure 5. Newcastle disease map of the regions of the Valencian Community (Comunidad Valenciana) for the broilers in 2012

https://doi.org/10.17221/145/2020-VETMED

Community was obtained in 2008, 2009, 2010, 2011 and 2012 (Figures 1, 2, 3, 4 and 5, respectively), where the highest, intermediate and lower seroprevalence levels are represented in red, yellow and green colour, respectively.

Moreover, the results have been further grouped by regions, as a geographical unit. This colour coding is dependent on the values obtained in the analysed period. So, the responses against APMV-1 for this period in the different geographical regions can be compared, outlining any possible infection pressures. The absolute value of the humoral response must be considered for the correct interpretation. This tool could allow veterinary technical services to compare the humoral response to APMV-1 in different geographical locations during this period, outlining the possible infection pressures.

The average age of the animals sampled in this study was: 42.34 days with a standard deviation (SD) of 8.44 days in 2008; 40.64 days with an SD of 8.11 days in 2009; 41.13 days with an SD of 8.95 days in 2010; 41.74 days with an SD of 13.27 days in 2011; and 40.49 days with an SD of 10.81 days in 2012.

Tables 1, 2, 3, 4 and 5 show the three regions of the Valencian Community with the number of flocks analysed, the number of sera tested and the average HI titres in 2008, 2009, 2010, 2011 and 2012, respectively.

Therefore, the only positive result during the study period was in Bajo Segura in 2009. It was determined as a false positive by molecular means [polymerase chain reaction (PCR)]. Probably, a cross-reaction with another paramyxovirus, like PMV-3 or PMV-7, was the cause of this HI result. These results indicate that, for the analysed period, there was no contact with the virus in the broiler farms in the Valencian Community.

Table 1. Regions of the Valencian Community (Comunidad Valenciana): Registration number, number of samples and qualitative results obtained in 2008

Region	2008		
	number of batches	number of samples	average HI titre
Alcalatén	10	150	0.37
Alto Maestrazgo	13	195	0.00
Bajo Maestrazgo	50	750	0.00
Condado de Cocentaina	5	75	0.00
Costera	1	15	0.00
Hoya de Alcoy	3	45	0.00
Hoya de Buñol	4	60	0.00
Huerta Sur	2	30	0.00
Los Serranos	5	75	0.30
Marina Alta	4	60	0.25
Plana Alta	52	780	0.09
Plana Baja	1	15	0.00
Puertos de Morella	1	15	0.00
Ribera Alta	6	90	0.00
Safor	27	405	0.00
Utiel – Requena	4	60	0.00
Valle de Albaida	36	540	0.00
Total	224	3 360	–

HI = haemagglutination inhibition

Table 2. Regions of the Valencian Community (Comunidad Valenciana): Registration number, number of samples and qualitative results obtained in 2009

Region	2009		
	number of batches	number of samples	average HI titre
Alcalatén	3	45	0.00
Alto Maestrazgo	7	105	0.00
Alto Palancia	1	15	0.00
Bajo Maestrazgo	44	660	0.00
Bajo Segura	1	15	5.27
Campo de Turia	1	15	0.00
Condado de Cocentaina	14	210	0.00
Costera	1	15	0.00
Hoya de Buñol	1	15	0.00
Huerta Sur	1	15	0.00
Los Serranos	6	90	0.00
Marina Alta	1	15	0.00
Plana Alta	47	705	0.00
Plana Baja	2	30	0.00
Puertos de Morella	1	15	0.00
Ribera Alta	5	75	0.00
Safor	28	420	0.00
Utiel – Requena	3	45	0.00
Valle de Albaida	29	435	0.00
Total	196	2 940	–

HI = haemagglutination inhibition

https://doi.org/10.17221/145/2020-VETMED

Table 3. Regions of the Valencian Community (Comunidad Valenciana): Registration number, number of samples and qualitative results obtained in 2010

Region	2010		
	number of batches	number of samples	average HI titre
Alcalatén	3	45	0.00
Alto Maestrazgo	9	135	0.00
Bajo Maestrazgo	35	525	0.04
Bajo Segura	1	15	0.00
Canal de Navarrés	2	30	0.00
Condado de Cocentaina	8	120	0.00
Hoya de Buñol	1	15	0.00
Huerta Sur	1	15	0.00
Marina Alta	3	45	0.00
Plana Alta	40	600	0.02
Ribera Alta	2	30	0.00
Safor	21	315	0.00
Utiel – Requena	3	45	0.00
Valle de Albaida	23	345	0.00
Total	152	2 280	–

HI = haemagglutination inhibition

Table 4. Regions of the Valencian Community (Comunidad Valenciana): Registration number, number of samples and qualitative results obtained in 2011

Region	2011		
	number of batches	number of samples	average HI titre
Alto Maestrazgo	8	120	0.00
Bajo Maestrazgo	36	540	0.08
Bajo Segura	1	15	3.07
Canal de Navarrés	1	15	0.00
Condado de Cocentaina	12	180	0.00
Costera	1	15	0.00
Hoya de Alcoy	1	15	0.00
Hoya de Buñol	1	15	0.00
Huerta Sur	1	15	0.00
Los Serranos	1	15	0.00
Marina Alta	3	45	0.00
Plana Alta	40	600	0.01
Ribera Alta	4	60	0.00
Safor	23	345	0.00
Utiel – Requena	4	60	0.00
Valle de Albaida	25	375	0.00
Total	162	2 430	–

HI = haemagglutination inhibition

Table 5. Regions of the Valencian Community (Comunidad Valenciana): Registration number, number of samples and qualitative results obtained in 2012

Region	2012		
	number of batches	number of samples	average HI titre
Alto Maestrazgo	7	105	0.00
Bajo Maestrazgo	24	360	0.00
Condado de Cocentaina	3	45	0.00
Hoya de Buñol	1	15	0.00
Huerta Sur	1	15	0.00
Marina Alta	3	45	0.00
Plana Alta	30	450	0.05
Ribera Alta	1	15	0.00
Safor	15	225	0.22
Utiel – Requena	1	15	0.00
Valle de Albaida	15	225	0.00
Total	101	1 515	–

HI = haemagglutination inhibition

DISCUSSION

Within the 27 member states of the European Union (EU), Spain was the fourth producer in 2020 and 2019, and the third from 2015 to 2018 behind Poland, Germany and France (AVEC 2021). From 2000 to 2009, in the European Union member states, ND viruses virulent for chickens have been detected in wild birds, domesticated pigeons and poultry (Alexander 2011). The law applicable in controlling ND in the European Union is Directive 92/66/CEE (CEC 1992). Because this disease is highly transmissible, the EU policy aims to achieve early detection and prevent the spread or ensure its rapid eradication by euthanising the animals. Traceability and surveillance are necessary to carry out this premise to determine the source of the infection and the routes of the disease spread.

The risk of introducing disease pathogens into a country and the spread of the agent within a country depends on a number of factors including import

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controls, movement of animals and animal products and the biosecurity applied by livestock producers. In a previous study performed in southern Spain, the highest incidence of APMV-1 to APMV-3 in wild birds was recorded in the summer, whereas the prevalence reached its maximum in the domestic species in the winter (Maldonado et al. 1994). An adequate contingency plan is an important instrument in the preparation for and the handling of an epidemic. The EU legislation requires that all Member States draw up a contingency plan which specifies the national measures required to maintain a high level of awareness and preparedness and is to be implemented in the event of a disease outbreak (Westergaard 2008).

The most effective approaches to control the spread of APMV-1 include strict biosecurity measures, continuous surveillance and the eradication of infected flocks. The rapid expansion of the poultry industry worldwide in restricted geographical areas and the severe economic losses due to APMV-1 outbreaks has led the Spanish government to implement new monitoring tools (Ministerio de Agricultura, Alimentación y Medio Ambiente 2014). Monitoring the disease status of broiler farms is a tool needed for disease control by veterinary technical services. This dynamic seroprevalence map for AMPV-1 applied in broilers of the Valencian Community could be an effective tool to assist with the ND disease control, which may help veterinarians and public officials to be better prepared to combat this disease. Maps of the seroprevalence for avian influenza and *Mycoplasma gallisepticum* have been published using the described monitoring tool in this paper (Garcia et al. 2014; Garcia et al. 2016).

Conflict of interest

The authors declare no conflict of interest.

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Received: July 10, 2020
Accepted: May 13, 2022
Published online: July 22, 2022