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Development of a seroprevalence map for *Mycoplasma gallisepticum* in broilers and its application to broilers from Comunidad Valenciana (Spain) over the course of two years (2009–2010)

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ABSTRACT: The aim of this study was to design and implement a Seroprevalence Map based on Business Intelligence for *Mycoplasma gallisepticum* (*M. gallisepticum*) in broilers in Comunidad Valenciana (Spain). To obtain the serological data we analysed 7363 samples from broiler farms over 30 days of age over the course of two years (3813 and 3550 samples in 2009 and 2010, respectively, from 189 and 193 broiler farms in 2009 and 2010, respectively). Data were represented on a map of Comunidad Valenciana to include geographical information of flock location and to facilitate the monitoring. Only one region presented with average ELISA titre values of over 500 in the 2009 period, indicating previous contact with *M. gallisepticum* in broiler flocks. None of the other regions showed any pressure of infection, indicating a low seroprevalence for *M. gallisepticum*. In addition, data from this study represent a novel tool for easy monitoring of the serological response that incorporates geographical information.

Keywords: *Mycoplasma gallisepticum*; broiler; seroprevalence map; ELISA, Comunidad Valenciana

Mycoplasma gallisepticum (*M. gallisepticum*) is a bacterium causing poultry disease that is listed by the World Organisation for Animal Health (OIE) and is a communicable disease in Spain (Orden ARM/831/2009). It belongs to the genus *Mycoplasma* from the class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae (Ley 2008). *M. gallisepticum* is particularly important in chickens and turkeys as a cause of respiratory disease and can elicit a decline in the production of meat and eggs (Bradbury et al. 2001; Pillai et al. 2003). In poultry, infection is transmitted vertically through infected eggs and horizontally through contact between birds, by aspiration of contaminated dust, drinking water, contaminated utensils and through intermediaries such as humans (Marois et al. 2002). The incubation period in experimental conditions can vary from six to 21 days, but it is apparently quite difficult to determine the precise time of infection in natural conditions as infected birds may be asymptomatic for several days or months before experiencing stress (Dingfelder et al. 1991). The clinical signs of *M. gallisepticum* in poultry vary from subclinical infection to obvious respiratory symptoms such as sneezing, conjunctivitis, and cough (Pillai et al. 2003). Unilateral or bilateral sinusitis can also be a feature, particularly in turkeys and game birds, and the infraorbital sinuses may become so swollen that the eyelids become closed. Conjunctivitis with eye discharge foam is also a common feature in turkeys and game birds, and sometimes chickens. Affected turkeys often exhibited spotted feathers on the wings as a result of attempts to remove exudate from the eyes. The severity of the disease is strongly influenced by the degree of secondary infection with viruses such as Newcastle disease and infectious bronchitis, and bacteria such as *Escherichia coli* (Gross 1990; Fischer et al. 1997).

Serology represents a useful, easy and inexpensive diagnostic tool to evaluate contact with the

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bacteria. Serologically derived information linked to time and space can be integrated in order to create a seroprevalence map, allowing monitoring of the presence, distribution and evolution of animal infections over time and space. Farm location is of interest because of the contagious potential of *M. gallisepticum*. Therefore, the periodical development of these maps will reveal the behaviour of these processes and their link to other risk factors such as origin and movement of birds, evolution of the weather or biosecurity measures. Information regarding these epidemiological parameters helps veterinarians to control the disease and minimise its occurrence.

In Europe, the OIE provides "Disease distribution maps", including for *M. gallisepticum*, by country and by six-month period or month of the year. This tool could be improved by adding selected factors such as real-time information, poultry production type, and by narrowing the focus to lower geographical units (communities, provinces or regions). Currently, there are no similar tools which include these features available for veterinarians to control *M. gallisepticum* in Spain.

The aim of this work was to develop and obtain a seroprevalence map for *M. gallisepticum* in broilers from Comunidad Valenciana (Spain) with the objective of establishing a useful tool that can be used by veterinary technical services to control disease.

MATERIAL AND METHODS

The study was conducted in *M. gallisepticum* non-vaccinated broiler poultry farms in Comunidad Valenciana (Spain). We will begin by describing the software tool, and will 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 us to obtain data from many different sources and to load it into a single database, to be analysed in another operating system. Thus, Oracle and × Chek were integrated for this task.

For data analysis, we developed a computer application called on-line analytical processing (OLAP), allowing dynamic and geographical analysis through the use of multidimensional cubes containing health information, with the integration of results for *M. gallisepticum* titres 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. 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 geographical information, such as communities, provinces, regions or towns. It associates data for each sample with the identification code of each geographical 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 two-year period covered by this study, a total of 7363 samples were analysed. Some 3813 samples from 189 broiler farms were analysed in 2009 and 3550 were taken from 193 broiler farms in 2010. These samples were collected from animals over 30 days old, with 15 samples being taken per flock. Venipuncture was performed with a needle or scalpel in the brachial vein and the blood was collected into 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 samples was assigned a registration number to maintain traceability throughout the analytic process and facilitate evaluation of the results. The samples were centrifuged at 748 g for 5 min. Red blood cells were deposited in the bottom of the tube and the blood serum at the top. About 250 µl of each sample were collected in 96-well plates, each identified with a corresponding registration number.

For the analysis, we used FlockChek (IDEXX Laboratories Inc., Westbrook, USA), a serological test designed to detect *M. gallisepticum* antibodies. Titres were calculated as described by the manu-

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Table 1. Reference titres from IDEXX for *M. gallisepticum*

Type of bird	Titre	Interpretation
Broilers > 30 days	< 500	unvaccinated and uninfected
	≥ 500	vaccinated, seroconversion or field challenge

facturer. The interpretation for broilers using this kit is described in Table 1.

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 *M. gallisepticum* in non-vaccinated broilers of Comunidad Valenciana was designed and implemented to assess the disease situation in this territory. We used a computer application for dynamic and geographical OLAP analysis, with multidimensional cubes containing health information, and integrated the quantitative results (*M. gallisepticum* titers) from the *M. gallisepticum* serology survey on broiler farms in Comunidad Valenciana.

Using the software tool, we obtained seroprevalence maps of *M. gallisepticum* on broiler farms in Comunidad Valenciana over the course of 2009 and 2010 (Figures 1 and 2), with the lowest prevalences represented in green, the highest in red and the intermediate values in yellow, grouped by regions, as a geographical unit. The status of a sample was evaluated by calculating the sample-to-positive ratio (S/P ratio):

$$S/P = (\text{sample mean absorbance} - \text{negative control mean absorbance})/(\text{positive control mean absorbance} - \text{negative control mean absorbance})$$

This colour coding is dependent on the values obtained in the period analysed. Therefore, responses against *M. gallisepticum* for this period in different geographical regions can be compared, and possible infection pressure can be identified. The absolute value of the humoural response must be taken into account for correct interpretation.

Titres over 500 are considered to be indicative of contact with *M. gallisepticum*. As no broiler vac-

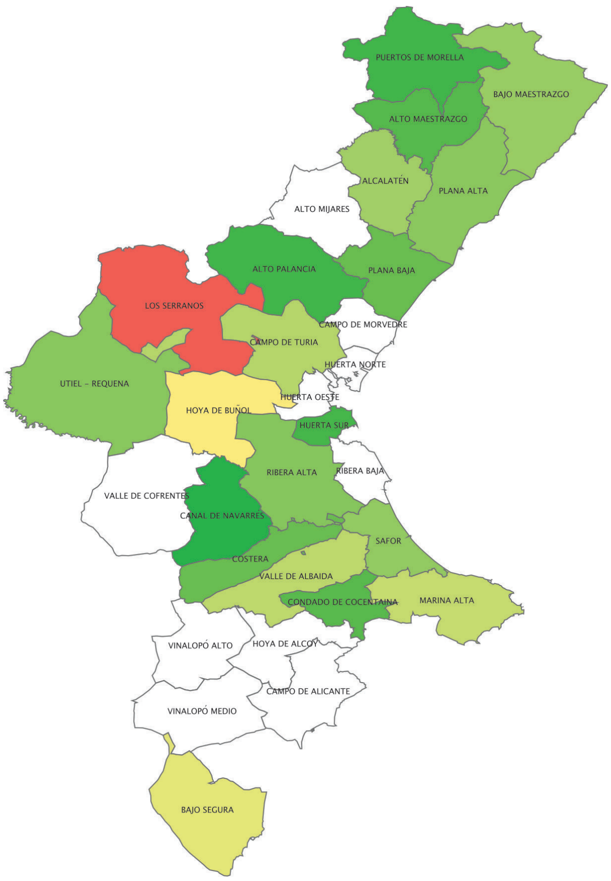

Figure 1. *M. gallisepticum* disease map of regions of Comunidad Valenciana for broilers in 2009

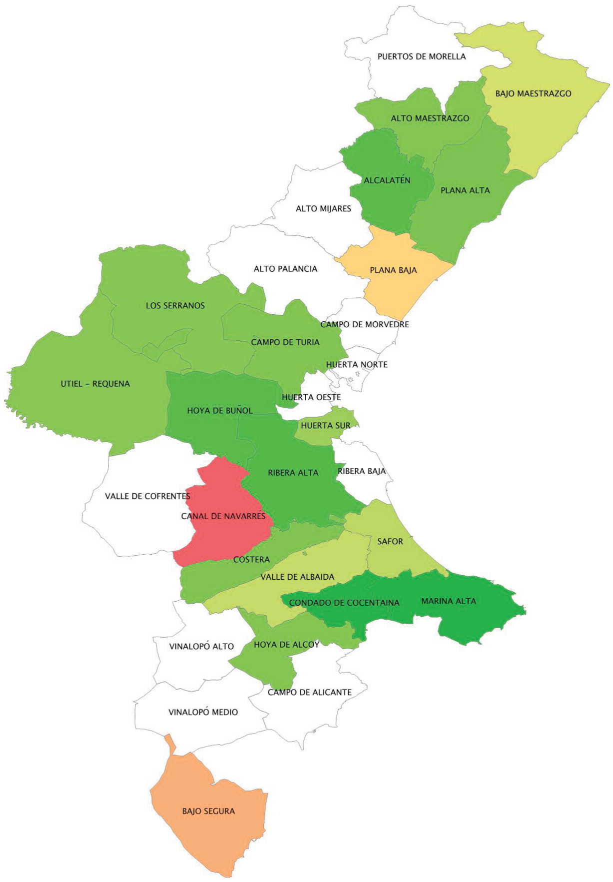

Figure 2. *M. gallisepticum* disease map of regions of Comunidad Valenciana for broilers in 2010

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cination against *M. gallisepticum* was performed in Comunidad Valenciana, 2009 results indicated some pressure of infection in farms from the "Los Serranos" region. Farms from this region were probably previously infected with the agent, as the average value was 605. In 2010, none of the regions studied presented average values indicative of infection. In general, a low level of *M. gallisepticum* infection pressure was detected in broiler farms in the present study.

The average age of animal sampled in this study was 41.73 days, with an SD of 7.29, in 2009 and 42 days with an SD of 7.79, in 2010.

Table 2 shows the three regions of Comunidad Valenciana with the number of samples, average titre and number of farms.

DISCUSSION

The use of GIS is important for the determination of risk factors. In the literature, examples of the use of GIS technology in the prevention of avian diseases include Avian Influenza in several countries such as Italy and China (Ehlers et al. 2003; Mannelli et al. 2006; Chang et al. 2007). However, until now, there has been no real-time informatics tool implemented to improve disease surveillance for *M. gallisepticum* in broilers in Comunidad Valenciana. A map of the seroprevalence for avian influenza has been published using the monitoring tool described in this paper (Garcia et al. 2015).

The most effective approaches to control the spread of *M. gallisepticum* include strict biosecurity measures, continuous surveillance and eradication of infected flocks. The rapid expansion of the poultry industry worldwide in restricted geographical areas and the severe economic losses due to *M. gallisepticum* outbreaks (Gharaibeh and Hailat 2011), have necessitated the implementation of new monitoring tools.

The *M. gallisepticum* infection pressure in broiler flocks was low, which is probably due to good biosecurity practices, including vaccination of broiler breeders. In contrast, previous studies in backyard

Table 2. Regions of Comunidad Valenciana, number of samples, average titre, maximum titre and number of farms studied

Region	Number of samples		Average titre		Number of farms	
	2009	2010	2009	2010	2009	2010
Alcalaten	75	72	205	79	4	4
Alto Maestrazgo	196	166	111	114	11	11
Alto Palancia	15		86		1	
Bajo Maestrazgo	582	588	195	193	34	34
Bajo Segura	15	15	282	328	1	1
Campo de Turia	44	31	226	112	2	1
Canal de Navarres	16	46	43	450	1	2
Condado de Cocentaina	200	126	116	40	11	9
Costera	30	15	136	110	1	1
Hoya de Alcoy		15		113		1
Hoya de Bunol	35	42	313	77	2	3
Huerta Sur	16	16	90	139	1	1
Los Serranos	167	105	605	117	8	6
Marina Alta	11	40	246	40	1	3
Plana Alta	669	684	179	105	41	43
Plana Baja	60	75	135	275	3	4
Puertos de Morella	75		87		3	
Ribera Alta	153	119	172	72	7	6
Safor	409	294	187	168	22	20
Utiel-Requena	576	665	176	118	9	15
Valle de Albaida	469	436	237	178	26	28

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chickens from a region of Argentina (Xavier et al. 2011), and in broilers from Egypt (Osman et al. 2009), showed high *M. gallisepticum* prevalence values.

In the present study, sampled animals were over 30 days of age in order to avoid maternal antibodies in the ELISA test, although maternal antibody titres against *M. gallisepticum* were depleted by 10 days of age in a recent study (Gharaibeh and Mahmoud 2013), with a half-life of 4.9 days and a coefficient of variation (CV; the ratio of the standard deviation to the mean) of 13.4%. This BI-based seroprevalence Map for *M. gallisepticum* applied in broilers of Comunidad Valenciana could be an effective tool in the control this disease, and may be useful for veterinarians and public officials.

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