

The use of dynamic prevalence maps to assess the prevalence of *Salmonella* in broiler flocks in the Valencian Community, Spain

This paper (No. 16102018-00130-EN) has been peer-reviewed, accepted, edited, and corrected by authors. It has not yet been formatted for printing. It will be published in December 2018 in issue 37 (3) of the *Scientific and Technical Review*

C. García ^{(1)*}, J.M. Soriano ⁽²⁾, V. Cortés ⁽¹⁾, S. Sevilla-Navarro ⁽¹⁾, C. Marín ⁽³⁾ & P. Catalá-Gregori ⁽¹⁾

(1) Centre of Poultry Quality and Animal Feed of the Valencian Community (CECAV), C/ Nules nº 16. 12539, Alquerías del Niño Perdido, Castellón, Spain

(2) Food & Health Laboratory, Institute of Materials Science, University of Valencia, C/ Catedrático José Beltrán, 2 46980 Paterna, Spain

(3) Biomedical Sciences Institute, Cardinal Herrera University (CEU), CEU Universities, Valencia, Spain

*Corresponding author: c.garcia@cecav.es

Summary

The aim of this study was to design and implement a dynamic prevalence map for managing the data collected within the framework of the *Salmonella* spp. national control plan in broiler flocks, using a dataset from the Valencian Community, Spain. Such a map would be a useful tool for veterinarians seeking to define control options.

The development of the mapping tool for this study involved three main phases: data collection, data analysis and data representation. The data used were obtained from the results of the National Control Programme (based on European Commission [EC] Regulation No. 2160/2003).

The *Salmonella* prevalence data were represented on a map of the Valencian Community, which included geographical information on flock locations to facilitate the interpretation of the results from monitoring.

The average prevalence of *Salmonella* spp. was 2.74% from 2009 to 2013. Moreover, the proportion of broiler flocks that remained positive for the target serovars according to EC Regulation No. 2160/2003 (*S. Enteritidis* and/or *S. Typhimurium* [including monophasic *S. Typhimurium*]) was less than 1%. Over the five-year period of the study (2009–2013), the area of Bajo Maestrazgo had the highest level of *Salmonella* spp. prevalence every year, thereby validating the usefulness of this mapping tool. The aim of the study is to detect geographical ‘high pressure’ *Salmonella* zones over time and make suggestions on where efforts should be focused in this region to reduce future *Salmonella* spp. prevalence.

Local Veterinary Services could, due to the map, identify the infection pressure of *Salmonella* in the geographical areas where farms are located. Proximity to higher prevalences may imply an increased risk of flock contamination. Although annual data are represented, data can be presented in real time with regular frequency (daily, weekly, monthly or quarterly) or even over periods of several years. Therefore, the dynamic prevalence map provides extremely useful epidemiological information to veterinarians on the prevention of *Salmonella*.

Keywords

Broilers – Dynamic prevalence map – EC Regulation No. 2160/2003 – ISO 6579 – *Salmonella* spp. – Valencian Community.

Introduction

Salmonella is recognised as an important food-borne zoonotic pathogen of economic significance in both animals and humans (1). In 2016, a total of 94,530 confirmed salmonellosis cases were reported by the 28 European Union (EU) Member States, resulting in an EU notification

rate of 20.4 cases per 100,000 of the population (2). The monitoring and control of food-borne diseases as well as food-hygiene requirements and food-safety criteria are regulated by EU legislation. Since 2009, EU Member States have implemented national control programmes for *Salmonella* spp. in broiler flocks, in accordance with European Commission (EC) Regulation No. 2160/2003 (3). The EU definitive target for broiler flocks is defined in EC Regulation No. 200/2012 (4) as the maximum annual percentage of broiler flocks remaining positive for the target serovars (*S. Enteritidis* and/or *S. Typhimurium* [including monophasic *S. Typhimurium*]) of 1% or less (2). The minimum detection requirements in broiler flocks include the sampling of flocks within three weeks of the birds being moved to the slaughterhouse, taking at least two faeces samples from two pairs of boot/sock swabs per flock (4).

Information on *Salmonella* prevalence linked to time and space could be integrated to create a dynamic prevalence map (DPM), allowing for the presence, distribution and evolution of positive flocks to be monitored over time. Developing these DPMs with a certain frequency will reveal the epidemiology of *Salmonella* prevalence. Moreover, this tool could be improved by adding information on risk factors, such as the origin and movement of birds, the evolution of the weather and/or biosecurity measures. Knowledge of these epidemiological parameters by veterinarians could help to control the disease and minimise its occurrence.

The World Organisation for Animal Health (OIE) provides world disease distribution maps for several diseases (5), including salmonellosis (*S. Abortusovis*). These maps are produced using data provided by countries and can be created for a six-month period or for a specified month or year period. This tool could be improved by adding selected factors, such as real-time information or poultry production type, and by narrowing the focus of the geographical units examined to, for example, communities, provinces or regions. While maps of seroprevalence for avian influenza and *Mycoplasma gallisepticum* have been published using the monitoring tool described in this paper (6, 7), to the authors' best knowledge, there are no available mapping tools for

veterinarians to control one of the most important food-borne pathogens, *Salmonella* spp., in Spain.

In this context, the aim of this study was to apply and assess the information contained in a prevalence map for *Salmonella* spp. in broilers in the Valencian Community, Spain, with the objective of establishing a useful tool for veterinary technical services in their attempts to control the bacteria.

Materials and methods

The study was conducted in broiler poultry farms located in the Valencian Community. Firstly, the software application developed for this study will be described, and secondly, the associated microbiological analysis to be performed will be defined.

Development and application of an appropriate software tool

Three main phases were chronologically undertaken for this software tool: data collection, data analysis and data representation. During the first phase (data collection), the process of extract, transform, load (ETL) enabled the authors to obtain data from many different sources and to input it into a single database, to be analysed in another operating system. Online analytical processing (OLAP) was used for the analysis of this data using Lite Explorer (Litebi, Valencia, Spain). This software provided a dynamic and geographical analysis of the data with multidimensional cubes containing prevalence information, and integrates *Salmonella* spp. results from broiler farms in the Valencian Community. In the cube or multidimensional database, the data is stored in a multidimensional vector. An OLAP cube (also called a hypercube) should be considered as an extension of the two dimensions of a spreadsheet, and can be more than three dimensions. After obtaining the data using the ETL processes and analysis using the OLAP tool, the next step was to represent them geographically. The Geographic Information System (GIS) was integrated with an open source server for sharing geospatial data called GeoServer (www.geoserver.org). This tool generated Spanish geographical

information, such as communities, provinces, regions or towns. Data for each sample was associated with the identification codes of each geographical unit. This information is documented in the Spanish General Registry of Farms (REGA) for each farm, thus establishing a relationship between GIS and OLAP (8). The data was categorised by area, colour-coded and showed the number of positives in each region by clicking on it (analysed samples). A total of 15,168 flocks were sampled over five years (2009–2013). The average sampling age with its standard deviation (SD), the number of flocks and the number of farms are indicated in Table I. A sample of two pairs of socks for each flock of each farm was taken. Data from 100% of the farms in the Valencian Community were provided. Therefore, if the sample was positive, the flock was also considered to be positive (EC Regulation No. 2160/2003). Of all flocks sampled, 399 were positive for *Salmonella*.

Microbiological sampling and culturing protocols

To assess the *Salmonella* status of the flocks, two samples of faeces were collected from boot swabs within three weeks of depopulation of the poultry building. The sampling strategy was in accordance with EC Regulation No. 2160/2003. *Salmonella* spp. was detected by identifying the presence or absence of this microorganism in a particular sample (two pairs of socks). Prevalence was calculated as a percentage of the number of positive flocks among the total flocks tested. For *Salmonella* isolation, boot swabs samples were placed directly into 500 ml sterile sample jars, and analysed according to ISO 6579:2002 Annex D (9, 10). To know the degree of compliance with the legislation regarding target serotypes, the *Salmonella* strains isolated were serotyped in accordance with the Kauffman–White–Le–Minor scheme.

Results

The software tool created DPMs for *Salmonella* spp. in broiler farms in the Valencian Community during 2009, 2010, 2011, 2012 and 2013 (Figs 1, 2, 3, 4 and 5, respectively).

These maps graphically display prevalence data in colour-coded form. The green regions in the maps indicate flocks which tested negative for *Salmonella* spp. Those flocks which tested positive for *Salmonella* spp. range in colour from yellow to orange (yellow indicating lower values and orange higher values). This colour coding depended on the values obtained in the period analysed. The results for *Salmonella* spp. were compared in different geographical regions for the same period.

The region that had the highest prevalence of *Salmonella* spp. in 2009, 2010, 2011, 2012 and 2013 was Bajo Maestrazo. DPM results showed that to decrease the pressure exerted by *Salmonella* spp., veterinary technical services should be focused on the Bajo Maestrazo region. Less than 1% of flocks were contaminated with the serovars *S. Enteritidis* and *S. Typhimurium* (including monophasic *S. Typhimurium*) during the whole period of the study, being 0.19%, 0.04%, 0.39%, 0.23% and 0.23% in 2009, 2010, 2011, 2012 and 2013, respectively.

Discussion

The consumption of poultry meat is expected to show further growth in the coming years. The main poultry-meat-producing countries within the EU are France, the United Kingdom, the Netherlands, Germany, Italy, Poland and Spain (11). In the period from 2008 to 2015, there has been a statistically significant decreasing trend in salmonellosis cases in the EU and the European Economic Area (EEA), however, salmonellosis remains the second most common zoonosis in humans in the EU with 94,530 confirmed cases and 1,067 food-borne outbreaks reported in 2016 (2).

The prevention of *Salmonella* spp. contamination of poultry products requires detailed knowledge of the main sources associated with its presence in the production system (12). The most important risk factors for *Salmonella* spp. contamination of the flocks at the end of the rearing period were: the *Salmonella* spp. status of: the poultry building after cleaning and disinfection, day-old chick flocks and feed from feeders. Hence, the whole production chain needs to be controlled to eradicate the bacteria from primary production (12).

An outbreak of *S. Typhimurium* took place in south-west England during April and May 2003. As a result, a matched case-control study was implemented, together with an environmental investigation and a GIS analysis (13). The author of the study suggested that alternative methods should be considered in support of traditional epidemiological investigation. Several authors implemented DPM in their epidemiology studies as an alternative method to improve *Salmonella* prevalence investigations, as was presented by Vico and Mainar-Jaime (14). Nevertheless, this new tool is less useful if data are not actualised in real time. The use of GIS is important to determine risk factors (15, 16). The literature reflects the use of GIS technology in the prevention of avian diseases, such as avian influenza in several countries, including the People's Republic of China and Italy (17, 18, 19). However, as yet, no real-time computer-based mapping tool has been used to improve the disease surveillance of *Salmonella* spp. in broilers flocks from the Valencian Community.

Since 2009, when the national control programmes began being implemented in Europe, the prevalence of *Salmonella* has been reduced to levels below the community target (1%), due to great efforts made by poultry farmers and public administrations. However, a ceiling of control has now been reached that cannot be improved upon. Thus, positive cases are still being declared and more sophisticated tools are needed to improve the control measures for the epidemiological situation in order to prevent and detect outbreaks.

Conclusions

The analysis of broiler results using this system suggests that *Salmonella* presence can be detected and its real-time evolution tracked, taking into account spatial distribution and temporal evolution. This prevalence map, applied to broiler chickens in the Valencian Community and based on business intelligence tools/techniques (the techniques and tools for transforming raw data into meaningful and useful information for business analysis purposes), could possibly be an effective tool for Veterinary Services in reducing *Salmonella* spp. prevalence.

The use of prevalence maps during 2009, 2010, 2011, 2012 and 2013 showed, in real time, that the region with the highest prevalence of *Salmonella* spp. was Bajo Maestrazo and its control for the bacteria in this region is necessary to reduce *Salmonella* prevalence in the Valencian Community. The map proves the usefulness of this approach to monitoring *Salmonella* spp. prevalence and of the inclusion of geographical information.

Acknowledgements

This work was partially supported by the Centre of Poultry Quality and Animal Feed of the Valencian Community (CECAV) and the Valencian Poultry Association (ASAV).

References

1. Hugas M. & Beloeil P.A. (2014). – Controlling *Salmonella* along the food chain in the European Union: progress over the last ten years. *Eurosurveillance*, **19** (19), pii=20804. doi:10.2807/1560-7917.ES2014.19.19.20804.
2. European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2017). – The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA J.*, **15** (12), e05077. doi:10.2903/j.efsa.2017.5077.
3. European Commission (EC) (2003). – EC Regulation No. 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents. *Off. J. Eur. Union*, **L 325**, 1–15. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R2160&from=EN> (accessed on 28 May 2018).
4. European Commission (EC) (2012). – Commission Regulation (EU) No. 200/2012 of 8 March 2012 concerning a Union target for the reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium in flocks of broilers, as provided for in EC Regulation

No. 2160/2003 of the European Parliament and of the Council. *Off. J. Eur. Union*, **L 71**, 31–36. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0200&from=EN> (accessed on 28 May 2018).

5. World Organisation for Animal Health (OIE) (2012). – Disease outbreak maps. World Animal Health Information Database (WAHIS Interface), Version 1. Available at: www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home (accessed on 13 July 2018).

6. García C., Soriano J.M. & Catalá-Gregori P. (2014). – Development of a seroprevalence map for avian influenza in broiler chickens from Comunidad Valenciana, Spain. *Rev. Sci. Tech. Off. Int. Epiz.*, **33** (3), 947–955. doi:10.20506/rst.33.3.2332.

7. García C., Soriano J.M. & Catalá-Gregori P. (2016). – 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). *Vet. Med.*, **61** (3), 136–140. doi:10.17221/8764-VETMED.

8. Spanish Ministry of Agriculture Fisheries and Food (MAPAMA) (2018). – Spanish General Registry of Farms (REGA). Registration: Comprehensive Animal Traceability System (SITRAN). Available at: www.mapa.gob.es/es/ganaderia/temas/trazabilidad-animal/registro/default.aspx (accessed on 11 October 2018).

9. International Organization for Standardization (ISO) (2002). – Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. ISO, Geneva, Switzerland.

10. International Organization for Standardization (ISO) (2007). – ISO 6579:2002/Amd 1: 2007. Annex D: detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. ISO, Geneva, Switzerland.

11. European Commission (EC) (2016). – COM(2016) 182 final. Report from the Commission to the European Parliament and the

Council on the impact of genetic selection on the welfare of chickens kept for meat production. EC, Brussels, Belgium, 14 pp. Available at: <https://ec.europa.eu/transparency/regdoc/rep/1/2016/EN/1-2016-182-EN-F1-1.PDF> (accessed on 11 October 2018).

12. Marin C., Balasch S., Vega S. & Lainez M. (2011). – Sources of *Salmonella* contamination during broiler production in Eastern Spain. *Prev. Vet. Med.*, **98** (1), 39–45. doi:10.1016/j.prevetmed.2010.09.006.

13. Boccia D., Oliver C.I., Charlett A., Bennett S., Orr H., Sarangi J. & Stuart J. (2004). – Outbreak of a new *Salmonella* phage type in South West England: alternative epidemiological investigations are needed. *Communic. Dis. Public Hlth*, **7** (4), 339–343. Available at: <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.566.4308&rep=rep1&type=pdf> (accessed on 28 May 2018).

14. Vico J.P. & Mainar-Jaime R.C. (2012). – Serological survey of *Salmonella* spp. infection in finishing pigs from northeastern Spain and associated risk factors. *Span. J. Agric. Res.*, **10** (2), 372–382. doi:10.5424/sjar/2012102-446-11.

15. Aung M.M. & Chang Y.S. (2014). – Traceability in a food supply chain: safety and quality perspectives. *Food Control*, **39**, 172–184. doi:10.1016/j.foodcont.2013.11.007.

16. Bosona T. & Gebresenbet G. (2013). – Food traceability as an integral part of logistics management in food and agricultural supply chain. *Food Control*, **33** (1), 32–48. doi:10.1016/j.foodcont.2013.02.004.

17. Chang S., Zhang J. [...] & Wang J. (2007). – Influenza Virus Database (IVDB): an integrated information resource and analysis platform for influenza virus research. *Nucleic Acids Res.*, **35** (Suppl. 1), SD376–SD380. doi:10.1093/nar/gkl779.

18. Ehlers M., Möller M., Marangon S. & Ferre N. (2003). – The use of Geographic Information System (GIS) in the frame of the contingency plan implemented during the 1999–2001 avian influenza

(AI) epidemic in Italy. *Avian Dis.*, **47** (S3), 1010–1014.
doi:10.1637/0005-2086-47.s3.1010.

19. Mannelli A., Ferrè N. & Marangon S. (2006). – Analysis of the 1999–2000 highly pathogenic avian influenza (H7N1) epidemic in the main poultry-production area in northern Italy. *Prev. Vet. Med.*, **73** (4), 273–285. doi:10.1016/j.prevetmed.2005.09.005.

Table I**Details of the sampling age and number of flocks and farms included in this study**

Period	Average age	SD age	Number of flocks	Number of farms
2009	36.45	9.66	2,125	420
2010	38.82	3.96	2,493	416
2011	35.24	9.55	2,812	421
2012	29.25	8.23	3,378	437
2013	29.30	7.05	4,360	423

SD: standard deviation

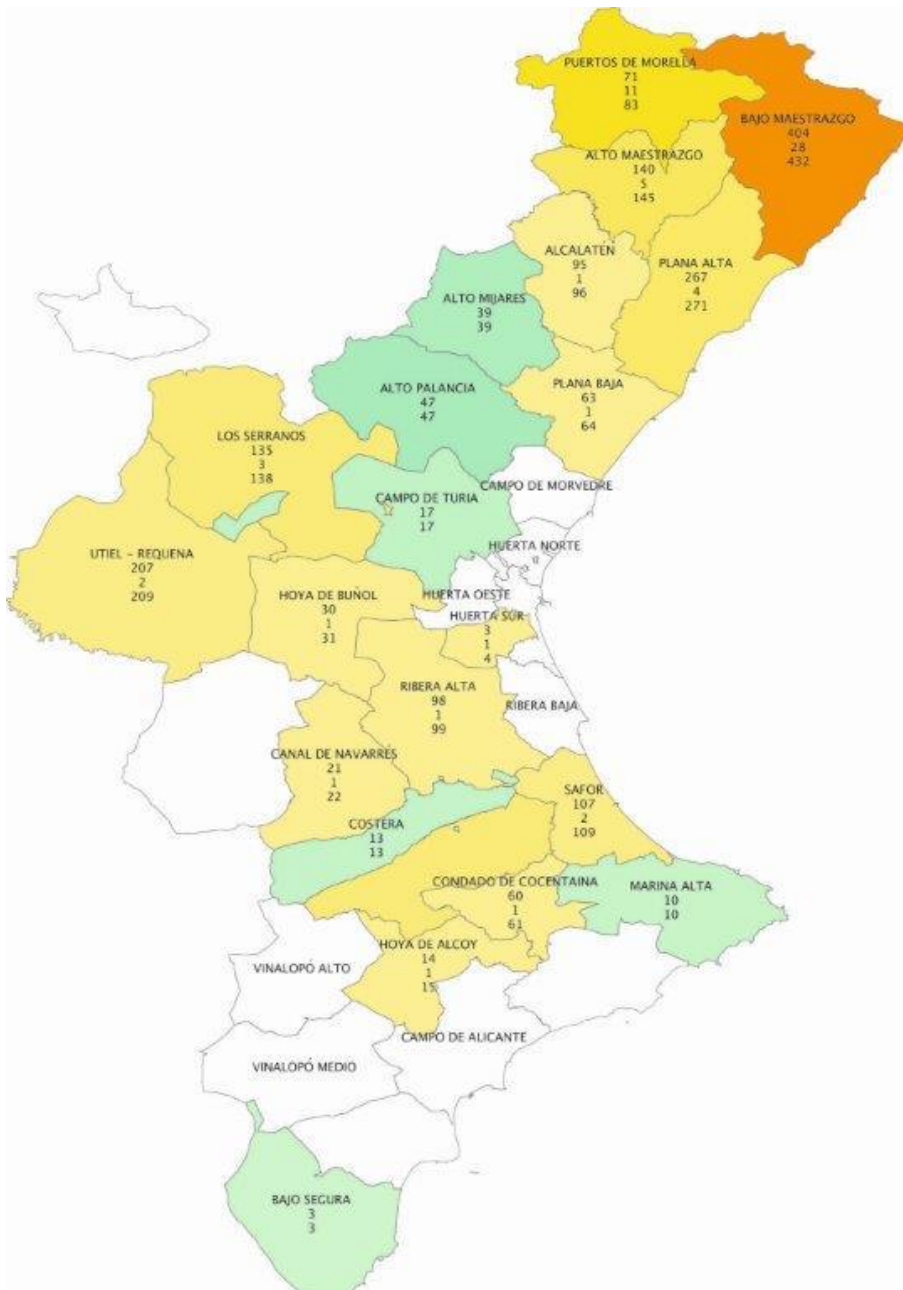


Fig. 1

Dynamic prevalence map of *Salmonella* spp. in the regions of the Valencian Community, Spain, for broiler flocks in 2009

Green: flocks which tested negative for *Salmonella* spp.
 Yellow to orange: flocks which tested positive for *Salmonella* spp., yellow indicating lower values and orange higher values
 This colour coding depends on the values obtained in the period analysed

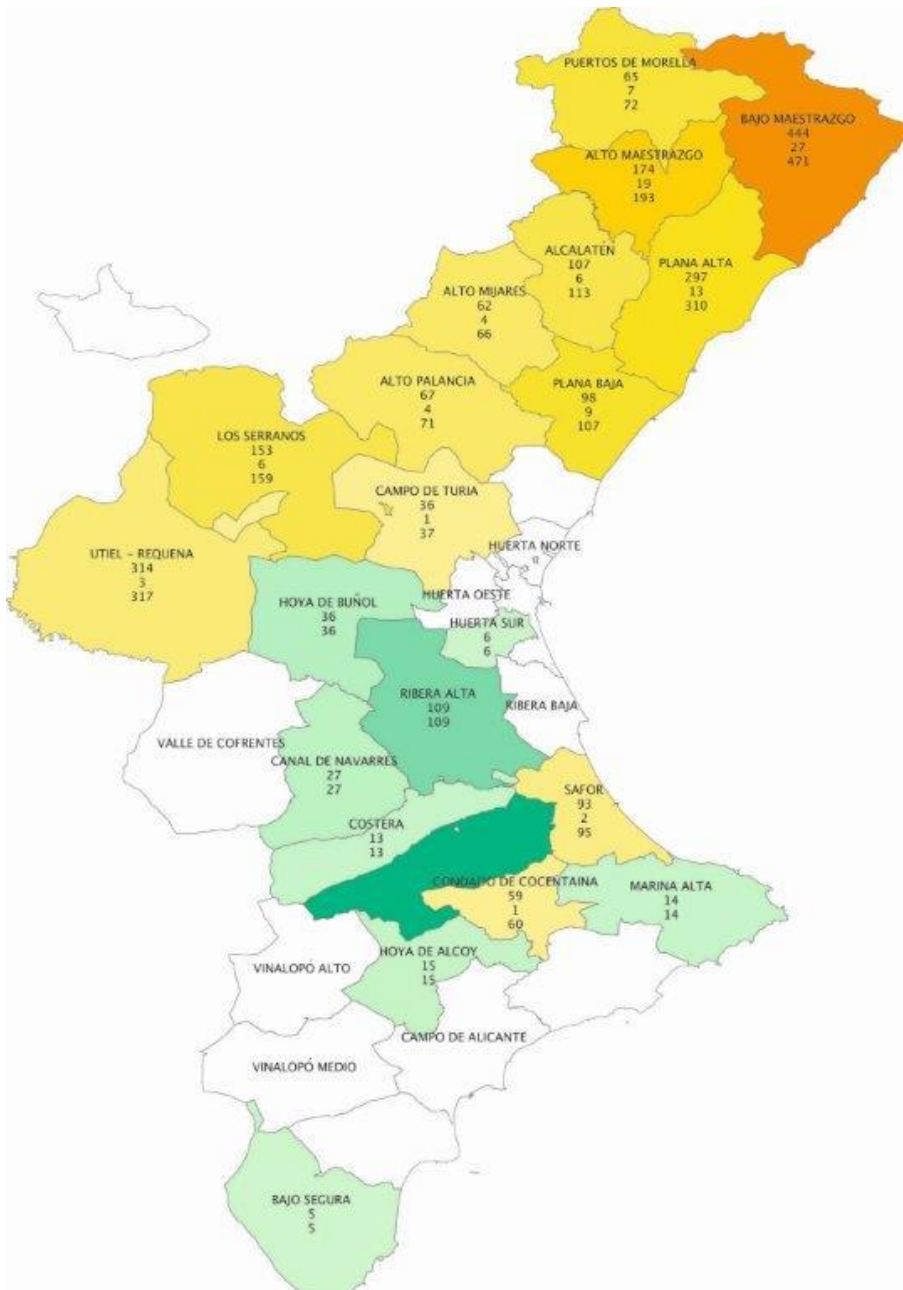


Fig. 2

Dynamic prevalence map of *Salmonella* spp. in the regions of the Valencian Community, Spain, for broiler flocks in 2010

Green: flocks which tested negative for *Salmonella* spp.

Yellow to orange: flocks which tested positive for *Salmonella* spp., yellow indicating lower values and orange higher values

This colour coding depends on the values obtained in the period analysed

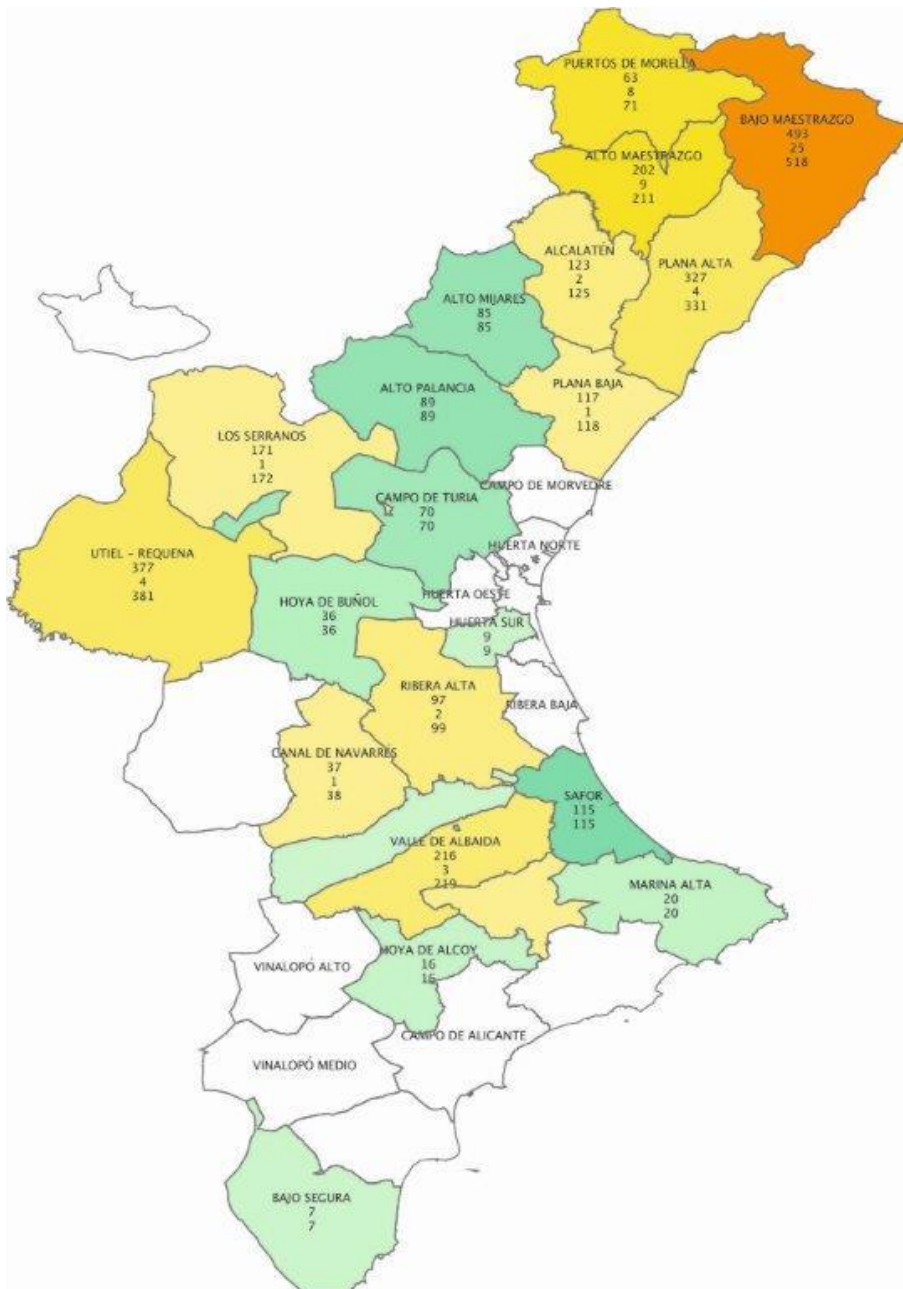


Fig. 3

Dynamic prevalence map of *Salmonella* spp. in the regions of the Valencian Community, Spain, for broiler flocks in 2011

Green: flocks which tested negative for *Salmonella* spp.

Yellow to orange: flocks which tested positive for *Salmonella* spp., yellow indicating lower values and orange higher values

This colour coding depends on the values obtained in the period analysed

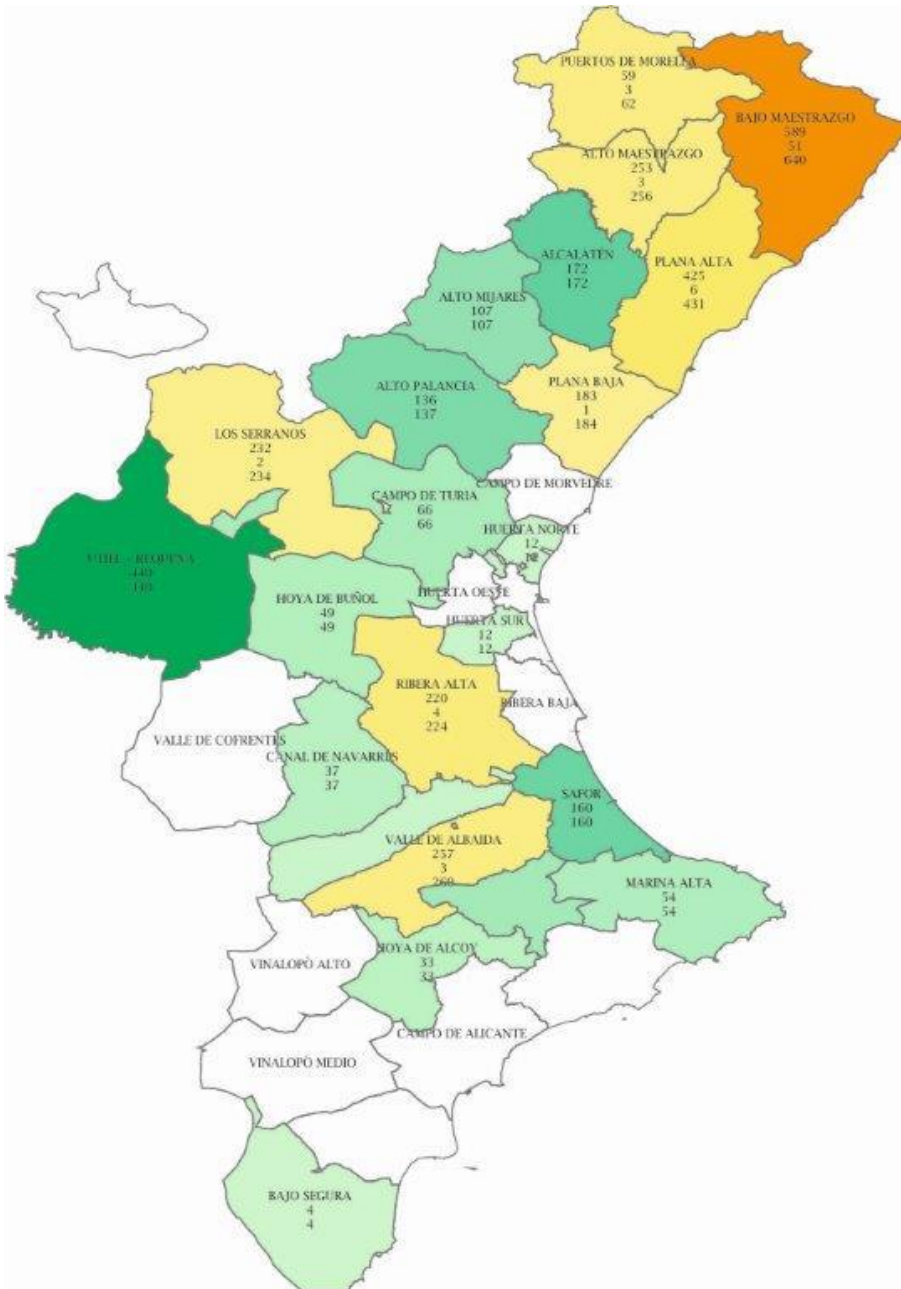


Fig. 4

Dynamic prevalence map of *Salmonella* spp. in the regions of the Valencian Community, Spain, for broiler flocks in 2012

Green: flocks which tested positive for *Salmonella* spp.
 Yellow to orange: flocks which tested positive for *Salmonella* spp., yellow indicating lower values and orange higher values
 This colour coding depends on the values obtained in the period analysed

