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Evolution of *Salmonella* spp. isolated compared to those of *Campylobacter* spp. in faecal samples for 12 years

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ABSTRACT

Introduction. The Autonomous Community of Galicia has adopted DECREE 216/2011 on health standards for poultry production, in addition to the Spanish national programs. However, no program has yet been implemented to eradicate campylobacteriosis, which shares the same reservoir. The aim of this study was to compare the evolution of *Salmonella* spp. isolates with respect to those of *Campylobacter* spp. in faecal samples received by the Microbiology Department.

Material and methods. A retrospective descriptive comparative study was conducted through the Laboratory Information System (SIL) of *Salmonella* spp. isolated against *Campylobacter* spp. in faeces between 2011 and 2022 at the Lucus Augusti University Hospital (HULA), Lugo, Spain.

Results. A total of 35,704 stool samples were analysed, of which 3,045 were positive. 751 Salmonella spp. were isolated. Statistical differences were observed in the annual distribution ($p<0.01$), with a clear turning point in 2018. Five hundred and five patients required hospital care, especially in 2014 with 72 patients (69%). On the other hand, 1,587 *Campylobacter* spp. were isolated. Required hospital care 1,002 patients during the study, with a peak in 2019 with 111 cases (62%)

Conclusion. The reduction of salmonellosis cases and the maintenance of campylobacteriosis cases are directly related to the implementation of DECREE 216/2011. This, in turn, has reduced the pressure on hospitals in the HULA health area. Therefore, we believe that the ONE Health concept is being strengthened in the area studied.

Keywords: campylobacteriosis; salmonellosis; one health,

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Evolución de *Salmonella* spp. aislados en comparación con los de *Campylobacter* spp. en muestras de heces durante 12 años

RESUMEN

Introducción. La Comunidad Autónoma de Galicia ha adoptado el DECRETO 216/2011 sobre normas sanitarias para la producción avícola, además de los programas nacionales españoles. Sin embargo, todavía no se ha implementado ningún programa para erradicar la campilobacteriosis, que comparte el mismo reservorio. El objetivo de este estudio fue comparar la evolución de *Salmonella* spp. con respecto a *Campylobacter* spp. aislados en las muestras fecales recibidas por el Departamento de Microbiología.

Material y métodos. Se realizó un estudio comparativo descriptivo retrospectivo a través del Sistema de Información de Laboratorio (SIL) de *Salmonella* spp. contra *Campylobacter* spp. aislados en heces entre 2011 y 2022 en el Hospital Universitario Lucus Augusti (HULA), Lugo, España.

Resultados. Se analizaron un total de 35.704 muestras de heces, de las cuales 3.045 resultaron positivas, donde resultaron aislados 751 *Salmonella* spp. se observaron diferencias estadísticas en la distribución anual ($p<0,01$), con un claro punto de inflexión en 2018. Requirieron atención hospitalaria 505 pacientes, especialmente en 2014 con 72 pacientes (69%). Por otra parte, fueron aislados 1.587 *Campylobacter* spp. Requirieron atención hospitalaria 1.002 pacientes durante el estudio, con picos en 2019 con 111 casos (62%)

Conclusión. La reducción de los casos de salmonelosis y el mantenimiento de los casos de campilobacteriosis están directamente relacionados con la implementación del DECRETO 216/2011. Esto, a su vez, ha reducido la presión hospitalaria en el área de salud del HULA. Por lo tanto, creemos que el concepto ONE Health se está fortaleciendo en el área estudiada.

Palabras clave: campilobacteriosis, salmonellosis, concepto "one health"

INTRODUCTION

Salmonellosis, an enteric disease caused by the *Salmonella* bacteria, contaminates food and typically results in self-limiting colitis; however, it can be complicating and fatal in up to 16% of patients [1]. Globally, there are an estimated 535,000 cases/year of invasive disease, with the highest incidence occurring in sub-Saharan Africa. It is more common in children under 5 years of age and adults over 70 years of age [2]. The challenge of controlling this disease, characterized by stomach involvement, has persisted through the ages for public health managers. Knowledge of the relationship between this bacterium and animal flesh, such as poultry, pork, and milk [3], has led to efforts focused on handling pre-cooked foods containing these components. This policy has successfully reduced outbreaks of salmonellosis associated with catering [4]. However, the prevalence of this disease remains a public health problem, as it is one of the most frequently isolated microorganisms in the feces of patients with gastrointestinal disorders [5,6]. For these reasons, new control programs aim to attack the food chain at its source, including milk producers and chicken farms.

In Spain, in addition to national programs for the control of *Salmonella* in poultry farms [6], actions are taken at the level of autonomous communities. In the autonomous community of Galicia, particularly in the area of Lugo, where both industrial and artisanal poultry farms are prevalent, programs such as DECREE 216/2011 of November 10 have been implemented. This decree establishes zootechnical and health standards for artisanal poultry production and the Galician Register of Artisanal Poultry Production, focusing on animal welfare and the control of specimens carrying *Salmonella* spp. in order to reduce its transmission to the food chain [7]. Action that was later raised to the national level with DECREE 637/2021.

On the other hand, campylobacteriosis is a zoonosis caused by the same reservoirs as salmonellosis poultry, farm animals, and contaminated animal products. Its most common clinical manifestation is gastroenteritis [8]. However, complications such as Guillain-Barré syndrome and reactive arthritis may occur in immunocompromised, pregnant, or extremely elderly patients, leading to serious long-term consequences [9]. Over the last decade, it has become the most common gastrointestinal pathogen, higher than other recognized pathogens such as *Shigella* spp., *Salmonella* spp. with *C. jejuni* and *C. coli* species being the most common [10], responsible for approximately 2.5 million cases of gastroenteritis per year in the US alone and 16 million cases worldwide. The dramatic increase in North America, Europe and Australia is alarming, and data from regions of Africa, Asia and the Middle East indicate that campylobacteriosis is endemic in these areas, especially in children [9]. Yet, public health authorities have not implemented a program to eradicate this pathogen at the source of contamination, similar to *Salmonella* spp.

The aim of this study was to compare the evolution of *Salmonella* spp. isolates with respect to those of *Campylobacter* spp. in the stool samples received by the Microbiology Service of the Lucus Augusti University Hospital in Lugo after the implementation of local *Salmonella* spp. control programs. This allowed us to infer their effectiveness in terms of public health.

MATERIAL AND METHODS

This study was a retrospective descriptive comparative analysis conducted through the Laboratory Information System (SIL), focusing on *Salmonella* spp. isolates versus *Campylobacter* spp. in faeces. Only the initial isolate from each patient was considered, covering the period from 2011 to 2022 at the Microbiology Service of Lucus Augusti University Hospital (HULA), Lugo, which serves approximately 250m000 registered inhabitants.

The isolation protocol involved culturing on XLD agar BD Difco® (Becton Dickinson France S.A., Le Pont de Claix, Francia), incubated at 37ºC for 24 hours, and selective selenite broth BD Difco® (Becton Dickinson France S.A., Le Pont de Claix, Francia). Subsequently, subculturing was performed on selective Salmonella and Shigella agar BD Difco® (Becton Dickinson France S.A., Le Pont de Claix, Francia) at 37ºC for 24 hours, along with the use of selective agar plates for Campylobacter BD Campylobacter Agar (Becton Dickinson France S.A., Le Pont de Claix, Francia) under microaerophilic conditions at 42°C for 48 hours.

Identification of isolates was accomplished using the MALDI-TOF Biotyper® system (Bruker Daltonics, Bremen, Germany) or panels for MicroScan microbiology systems (Beckman Coulter, Brea, CA, USA). The serotyping of *Salmonella* spp. was conducted using antisera, including Salmonella polyvalent H:g,m, H:i, and antiserum Salmonella monovalent O:4,5 O: 6,7,8 and O:9 (BIO-RAD).

Statistical analyses were performed using the $\chi 2$ and ANOVA tests with the SPSS program. The study of age distribution involved categorizing individuals into six groups: Group 1 (0 to 5 years), Group 2 (6 to 15 years), Group 3 (16 to 30 years), Group 4 (31 to 65 years), Group 5 (66 to 80 years old), and Group 6 (over 80 years old).

RESULTS

A total of 35,704 stool samples were analysed in the study, the distribution by years from 2011 to 2022 was 2,213, 2,544, 2,741, 2,970, 2,969, 2,918, 2,947, 3,226, 3,541, 2,874, 3,136 and 3,625 samples, of which 3,045 were positive, ranging from 287 to 229 positives per year. 751 *Salmonella* spp. were isolated with an incidence per 100,000 inhabitants year (2011 to 2022) of 25, 30, 31, 43, 33, 34, 33, 18, 22, 14, 16 and 14 episodes and an average monthly frequency of 5 episodes. There was a higher frequency of isolation in the summer months (July, August, and September) ($p<0.01$), with a total of 92, 92, and 107 accumulated strains per month, respectively (Figure 1). Statistical differences were also observed in the annual distribution ($p<0.01$), with a clear turning point from the year 2018 in the global isolation of *Salmonella* spp. and the year 2013 a signif-

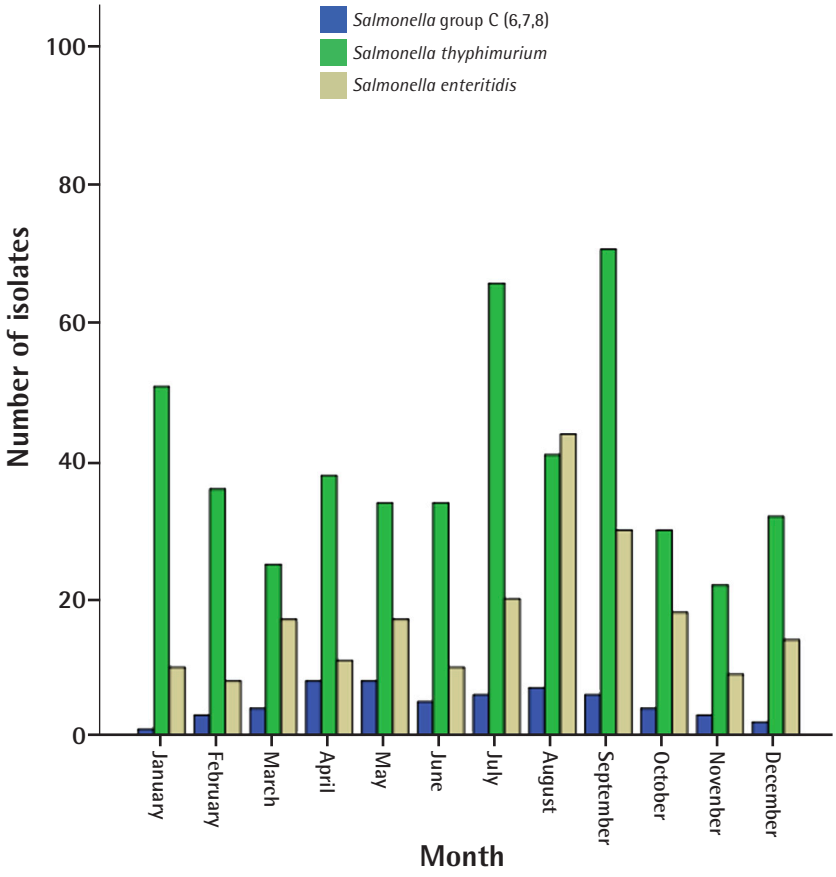

Figure 1 | **Cumulative distribution of *Salmonella* spp. by month**

icant decrease in the isolation of *Salmonella enteritidis* was observed. The mean age was 30 years, ranging from 0 to 102 years (Table 1). Regarding the distribution by sex, no significant differences were observed (p=0.62), although it was more frequent in males (413, 55%), consistent with other published series [11].

The cumulative distribution by age group was significantly more frequent (p=0.02) in group 1 with a total of 268 cases, in a range of 43-2. This decrease was statistically significant (p=0.002). Group 2 was next, with 241 cases, in a range of 18-3, although this decrease was not statistically significant (p=0.1) (Table 2). During the study, a total of 505 patients required hospital care, with the year with the highest number of visits being 2014 with a total of 72 patients (69%) and the year with the lowest number of visits being 2020 with 18 patients (54%). Group 5 and group 6 had the highest number of cases that required hospital care with 85 and 49 cases respectively (83%), followed by group 4 with 108 cases (81%).

On the other hand, a total of 1587 *Campylobacter* spp. were isolated, with an incidence per 100,000 inhabitants year (2011 to 2022) of 45, 60, 62, 63, 56, 49, 49, 69, 79, 63, 54 and 51 episodes and an average frequency of 11.25 episodes per month. No statistically significant differences were observed either in the distribution by year or by accumulated months (p=0.48) (Figure 2). The mean age was 24 years, ranging from 0 to 94 years, and no statistically significant differences were observed in the distribution by sex (p=0.33). However, similar to *Salmonella* spp., they were more frequent in males (936, 59%). A more detailed distribution is shown in Table 1.

Regarding the accumulated age range, group 1 was significantly more frequent (p< 0.01), with a total of 887 cases within a range of 75-28 cases per year, although this decrease was not statistically significant (p=0.10). Group 2 had the next highest frequency with 275 cumulative episodes within a range of 15-30 cases per year. A total of 1,002 patients required hospital care during the study, with peaks in 2019 with 111 cases (62%) and in 2017 with 64 cases treated (57%). By age group, group 1 was the most common with 390 cases (55%), followed by group 4 with 176 treated patients (75%). Although a significant difference (p < 0.01) was observed in the need for hospital care of patients over 80 years of age,

Table 1 **Distribution of *Salmonella* spp. and *Campylobacter* spp. per year**

	Salmonella spp.					*Campylobacter* spp.		
Year	Group C (6,7,8)	*S. paratyphi*	*S. typhimurium*	*S. enteritidis*	Total	*C. jejuni*	*C. coli*	Total
2011	0	0	46	19	65	102	0	102
2012	0	0	55	18	73	135	0	135
2013	5	0	61	11	77	140	0	140
2014	7	0	83	14	104	142	0	142
2015	12	0	38	29	79	126	0	126
2016	5	0	48	28	81	115	0	115
2017	4	0	37	37	78	112	0	112
2018	3	0	29	10	42	144	12	156
2019	10	0	35	7	52	166	13	179
2020	6	0	17	10	33	135	8	143
2021	4	0	19	13	36	114	8	122
2022	4	3	14	12	31	108	7	115
Total	60	3	480	208	751	1,539	48	1,587

with 78 patients treated (86%) compared to 12 of non-hospital origin. Refer to Table 2 for more details.

Regarding the *C. coli* isolates, a total of 48 patients were detected, being significantly more frequent in group 1 with 11 cases (p=0.02) in total, in the distribution by sex, men were more frequent (27/21), and 31 patients (64%) needed hospital care.

DISCUSSION

This study reflects the evaluation of *Salmonella* spp. isolates in comparison to *Campylobacter* spp. in the health area attached to HULA, after implementing the control plan DECREE in the health area of the province of Lugo. In our study, the sex distribution of both *Salmonella* spp. and *Campylobacter* spp. was very similar to that found in other series [10-12].

The age group corresponding to 0-5 years turned out to be the group in which both *Salmonella* spp. and *Campylobacter* spp. were most frequently isolated, as reported in other studies [11-13]. The age group with the highest number of complications and hospitalizations for *Salmonella* spp. was those over 30 years of age, although this data does not agree with other series in our country where the age group with the highest number of complications is from 65 years of age [11,12].

On the other hand, in the case of campylobacteriosis, the group of children aged 0-5 years were the most hospitalized in absolute terms, and in terms of frequency within the age group, the over-80s were the most affected, which is in line with the published literature [10].

The months with the highest incidence in the case of *Salmonella* spp. were the summer months, especially September. In our study, *Salmonella typhimurium* was the most common species, contrary to other Spanish studies [12], since it is usually a more common species in the USA, Mexico, or Taiwan [15-17], followed by *Salmonella enteritidis*, but in the years prior to the study, such as in 2005 or 2006, *S. enteritidis* was the predominant species in our environment. This change is possibly due to the fact that *S. enteritis*, unlike *S. typhimurium*, has the capacity for transovarian transmission and by eliminating laying hens carrying *Salmonella* spp., the number isolated from humans was also reduced. For campylobacteriosis, no seasonality could be demonstrated, as in the work of Ruiz de Alegría-Puig et al [13], although a decrease in incidence was observed in the month of March. The most common species was *Campylobacter jejuni*, as in other European series [13, 14], although in our case we must take into account that as of 2017 mass spectrometry was introduced for the identification of *Campylobacter* spp., therefore, prior to these years we may have an underdiagnosis of *Campylobacter coli*.

Similar programs have been launched in different European regions, such as Denmark or Sweden, where the control of laying hens carrying *Salmonella* spp. has produced significant reductions in the incidence of human foodborne salmonellosis, a decrease that can also be see in our studio [18,19].

CONCLUSION

The results of this study seem to indicate how action at source in the food industry, with the detection and eradication of chickens carrying *Salmonella* spp., is reflected in the reduc-

Table 2 Distribution by year and group age range and isolated microorganism

Year	*Salmonella* spp. Group age range (years)						*Campylobacter* spp. Group age range (years)					
	0-5	6-15	16-30	31-65	65-80	>80	0-5	6-15	16-30	31-65	65-80	>80
2011	25	15	6	10	6	3	55	13	4	15	8	7
2012	35	12	4	10	12	0	79	23	7	18	5	3
2013	36	16	5	10	8	2	67	28	10	16	9	10
2014	43	18	6	9	11	17	73	30	7	15	9	8
2015	30	10	7	19	11	2	62	22	7	14	9	12
2016	27	14	5	15	9	11	66	15	8	10	11	5
2017	21	21	5	17	7	7	53	19	5	18	11	6
2018	10	11	3	10	6	2	69	19	16	23	24	5
2019	20	8	1	9	11	3	64	28	17	38	20	12
2020	11	3	4	5	6	4	58	26	11	23	18	7
2021	2	8	3	12	5	6	33	25	22	20	14	8
2022	8	5	0	7	9	2	28	27	20	23	10	7

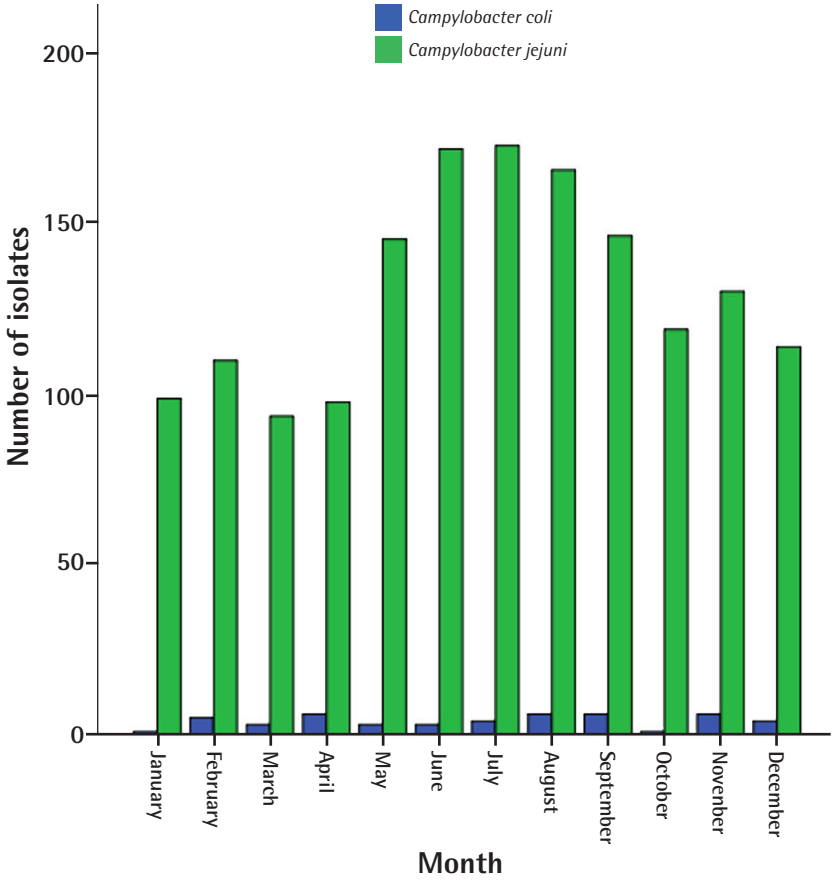

Figure 2 Cumulative distribution of *Campylobacter* spp. by month

tion of cases of human salmonellosis, especially *S. enteritidis* and especially in the vulnerable population. This also reduces the pressure on hospitals, so we believe that in this case the ONE Heldh concept is reinforced in the area studied. Although this ecological niche was occupied by *S. typhimurium*, which is not associated with transovarial transmission. Therefore, the overall decrease in cases must take into account other environmental and hygienic factors of the population. Unlike what happened with *Campylobacter* spp. which does not have transnovarian transmission, but due to contamination of faecal origin, a stagnation of isolates was observed, with a discrete and non-significant decrease. Possibly due to the change in population hygiene habits and the lack of institutional response. Please note that data from 2020 and later may be influenced by changes in habits due to the COVID-19 pandemic.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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