



## Demographic characterization and spatial cluster analysis of human *Salmonella* 1,4,[5],12:i:- infections in Portugal: A 10 year study



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### ABSTRACT

*Salmonella* 1,4,[5],12:i:- is presently considered one of the major serovars responsible for human salmonellosis worldwide. Due to its recent emergence, studies assessing the demographic characterization and spatial epidemiology of salmonellosis 1,4,[5],12:i:- at local- or country-level are lacking. In this study, a analysis was conducted over a 10 year period, from 2000 to the first quarter of 2011 at the Portuguese National Laboratory in Portugal mainland, with a total of 215 *Salmonella* 1,4,[5],12:i:- serotyped isolates obtained from human infections by a passive surveillance system. Data regarding source, year and month of sampling, gender, age, district and municipality of the patients were registered. Descriptive statistical analysis and a spatial scan statistic combined with a geographic information system were employed to characterize the epidemiology and identify spatial clusters. Results showed that most districts have reports of *Salmonella* 1,4,[5],12:i:-, with a higher number of cases at the Portuguese coastland, including districts like Porto (n = 60, 27.9%), Lisboa (n = 29, 13.5%) and Aveiro (n = 28, 13.0%). An increased incidence was observed in the period from 2004 to 2011 and most infections occurred during May and October. Spatial analysis revealed 4 clusters of higher than expected infection rates. Three were located in the north of Portugal, including two at the coastland (Cluster 1 [RR = 3.58,  $p \leq 0.001$ ] and 4 [RR = 10.42  $p \leq 0.230$ ]), and one at the countryside (Cluster 3 [RR = 17.76,  $p \leq 0.001$ ]). A larger cluster was detected involving the center and south of Portugal (Cluster 2 [RR = 4.85,  $p \leq 0.001$ ]). The present study was elaborated with data provided by a passive surveillance system, which may originate an underestimation of disease burden. However, this is the first report describing the incidence and the distribution of areas with higher risk of infection in Portugal, revealing that *Salmonella* 1,4,[5],12:i:- displayed a significant geographic clustering and these areas should be further evaluated to identify risk factors in order to establish prevention programs.

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### Introduction

The prevalence of *Salmonella* serovars are constantly changing in many European countries [1] and, in 2010, the European Food Safety Authority (EFSA) Panel on Biological Hazards published a scientific opinion alerting for the increasing number of outbreaks in the European Union member states promoted by “*Salmonella* Typhimurium-like” strains [2].

*Salmonella* 1,4,[5],12:i:- is considered a monophasic variant of serovar Typhimurium (1,4,[5],12:i:1.2) due to genotypic similarities between the two serovars [3,4], being characterised by a lack of the *fljB* gene expression, which encodes the second phase flagellar antigen [3]. Serovar Typhimurium is the second most common serovar associated with human cases of *Salmonella* infection in the European Union (EU), exceeded only by serovar Enteritidis [1]. On the other hand, serovar 1,4,[5],12:i:- was seldom isolated before the mid-1990s but is now among the top 3 most prevalent serovars isolated from humans in EU [4].

Public health methods applied to the surveillance of *Salmonella*, and in particular of *Salmonella* 1,4,[5],12:i:- strains, may help to monitor disease development, reduce morbidity and mortality and improve health, avoiding unnecessary regulatory measures [5]. In the medical field, the application of geographic information

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systems (GIS) has become extremely useful in understanding the bigger picture of disease's dissemination. These methods associated with others techniques, including spatial statistics, are important tools for public health maintenance, as they allow to identify risk areas requiring fast intervention, promoting the rationalization of prevention's procedures and also the reduction of costs [6]. Therefore, this study aimed to perform the demographic characterization of salmonellosis 1,4,[5],12:i:- cases and to use spatial analysis combined geoprocessing techniques in order to identify spatial clusters for *Salmonella* 1,4,[5],12:i:- infection, evaluating high-risk areas and providing useful information to understand the spread and epidemiology of this serovar in Portugal.

## Methods

### Data source: study population and area

In Portugal, salmonellosis is a notifiable disease and is defined as the isolation of *Salmonella* spp. (excluding *Salmonella* Typhi or Paratyphi) from an appropriate clinical sample, namely stool, urine and blood, collected from patients with or without clinically-compatible signs and symptoms. Medical doctors are obliged to report by post any confirmed or suspected case of salmonellosis, including nontyphoidal salmonellosis, to the local health authority of the municipality where the case lives [7].

In this study, 215 *Salmonella* 1,4,[5],12:i:- isolates were obtained from cases reported from 2001 to the first quarter of 2011 in mainland Portugal were included. All *Salmonella* were previously isolated at the National Health Institute Doutor Ricardo Jorge (INSA), serotyped using the slide agglutination method according to the Kauffmann–White scheme, and included different sources, namely feces, blood, peritoneal fluid and urine [8]. Additionally, information including, source, year and month of sample, gender, age, district and municipality of the patients were registered.

Isolates were obtained from patients at Portugal mainland, representing an area of 89.015 km<sup>2</sup>, corresponding 96.6% of the Portuguese national territory, with 10.047.083 inhabitants [9]. For administrative purpose, this territory is divided into 18 Districts, as follows: Aveiro, Beja, Braga, Bragança, Castelo Branco, Coimbra, Évora, Faro, Guarda, Leiria, Lisboa, Portalegre, Porto, Santarém, Setúbal, Viana do Castelo, Vila Real and Viseu. The Eurostat-based Nomenclature of Territorial Units for Statistics (NUTS) system subdivides Portugal mainland in 308 municipalities [9].

### Statistical analysis

#### Descriptive statistics

Descriptive statistical analyses were performed using SPSS 21.0 software (IBM Corporation, New York, USA). For statistical purposes, age was grouped in three different classes according to National Statistics Institute [9], namely: young (less than 15 years), adult (16–64 years) and elderly (higher than 65). Age mean, median, mode, range and standard deviation were also determined.

#### Spatial analysis

*Salmonella* 1,4,[5],12:i:- reports were geocoded at the municipality level. The centroids of each municipality were determined using the open-source Quantum Geographic Information System (QGIS) software. Spatial clustering of *Salmonella* 1,4,[5],12:i:- cases was analyzed using spatial scan statistics [10]. Statistical procedures were carried out in SaTScan software using a purely spatial Poisson model. The following data were considered for analysis: the number of positive cases in each municipality, the resident population within each municipality according to the 2011 Portuguese census, and the Cartesian coordinates of the centroids of each municipality included in the survey. The model was first run

**Table 1**

Distribution of *Salmonella* 1,4,[5],12:i:- clinical cases per district and gender in Portugal from 2000 and to the first quarter of 2011.

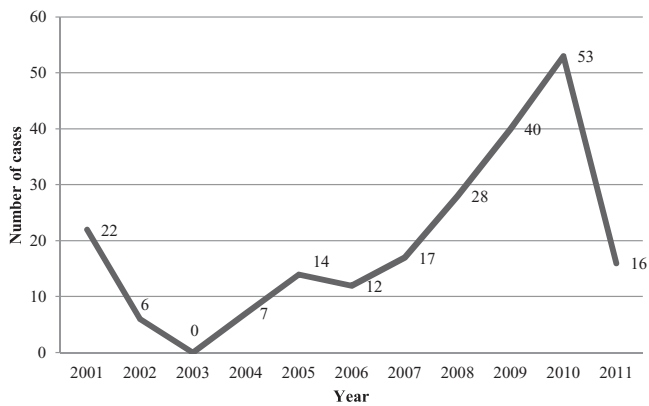
District	Gender	Not registered				Total
		Count	F	M	% of total	
Aveiro	Count	0	21	7	28	
	% of total	0,0%	9,8%	3,3%	13,0%	
Beja	Count	0	3	4	7	
	% of total	0,0%	1,4%	1,9%	3,3%	
Braga	Count	0	15	12	27	
	% of total	0,0%	7,0%	5,6%	12,6%	
Bragança	Count	0	2	0	2	
	% of total	0,0%	0,9%	0,0%	0,9%	
Castelo Branco	Count	0	2	6	8	
	% of total	0,0%	0,9%	2,8%	3,7%	
Évora	Count	0	2	12	14	
	% of total	0,0%	0,9%	5,6%	6,5%	
Faro	Count	0	0	1	1	
	% of total	0,0%	0,0%	0,5%	0,5%	
Leiria	Count	1	1	0	2	
	% of total	0,5%	0,5%	0,0%	0,9%	
Lisboa	Count	0	16	13	29	
	% of total	0,0%	7,4%	6,0%	13,5%	
Porto	Count	2	24	34	60	
	% of total	0,9%	11,2%	15,8%	27,9%	
Santarém	Count	0	2	3	5	
	% of total	0,0%	0,9%	1,4%	2,3%	
Setúbal	Count	0	8	16	24	
	% of total	0,0%	3,7%	7,4%	11,2%	
Vila Real	Count	0	3	4	7	
	% of total	0,0%	1,4%	1,9%	3,3%	
Viseu	Count	0	0	1	1	
	% of total	0,0%	0,0%	0,5%	0,5%	
Total	Count	3	99	113	215	
	% of total	1,4%	46,0%	52,6%	100,0%	

using the default maximum spatial cluster size of 50% of the total study population to ensure statistical power. The maximum-size parameter was then set at 10% to check for the presence of extreme small risk areas, possibly masked by the 50% scanning window. The number of Monte Carlo replications to estimate the statistical significance of the most likely cluster was set at 9999 iterations. A *p* value <0.05 was considered statistically significant.

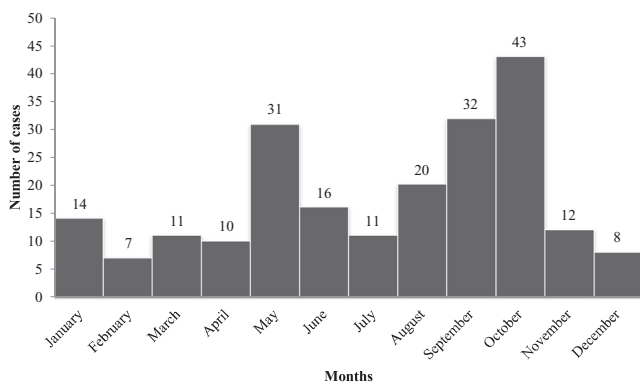
## Results

The majority of *Salmonella* 1,4,[5],12:i:- isolates were obtained from feces (*n* = 185, 86%), followed by unknown sources (*n* = 16, 7.6%), blood (*n* = 8, 3.7%), blood and feces (*n* = 3, 1.4%), peritoneal fluid (*n* = 1, 0.5%), blood and urine (*n* = 1, 0.5%) and urine (*n* = 1, 0.5%). Regarding the cases' location, the majority was reported in Porto (*n* = 60, 27.9%), followed by Lisboa (*n* = 29, 13.5%), Aveiro (*n* = 28, 13.0%), Braga (*n* = 27, 12.6%) and Setúbal (*n* = 24, 11.2%). On the other hand, single cases were reported in Faro at 2010 and Viseu at 2007, as well as, two-single cases in Bragança at 2008 and 2009 and in Leiria at 2002 and 2009 (Table 1).

The distribution through years of *Salmonella* 1,4,[5],12:i:- infections in Portugal from 2001 to the first quarter of 2011 reveal that 2010 was the year with the higher number of reports (*n* = 53, 24.6%), with an increasing trend in the number of cases from 2004 to 2010 (Fig. 1). The seasonal variation was also evaluated during the period of study and is shown in Fig. 2. Most of the infections occurred between May and October, with the highest and the lowest



**Fig. 1.** Trend in Portugal of the number of cases per year of *Salmonella* 1,4,[5],12:i:- from 2001 to the first quarter of 2011.



**Fig. 2.** Number of clinical cases per month of *Salmonella* 1,4,[5],12:i:- from 2001 to the first quarter of 2011.

Legend: Months were pooled into seasons, which were defined as Spring (March–May), Summer (June–August), Autumn (September–November) and Winter (December–February).

number of cases being diagnosed in October (43 infections) and February (seven infections), respectively.

Information regarding the patient's gender by district is revealed in Table 1. However, in three patients, one from Leiria and two from Porto, gender was not possible to register.

Patient's age was registered from 199 individuals and revealed that young individuals were the most affected with 69.3% ( $n = 149$ ) of *Salmonella* 1,4,[5],12:i:- cases, followed by adults with 14.0% ( $n = 30$ ) and elderly with 9.3% ( $n = 20$ ). Descriptive statistical analyses showed that mean age was 17 years with a median and mode of 3 and 1 year, respectively. Additionally, standard deviation was 25.9 years with a minimum age of 0 years, a newborn, and maximum age of 90 years.

Spatial analysis revealed 4 clusters of higher than expected infection rates, including two located in the north coastland area involving the districts of Porto and Aveiro (Cluster 1 and 4, respectively), one at the countryside involving the districts Bragança Viana Real, Viseu and Guarda (Cluster 3), and a larger cluster located in the center and south of Portugal which included the districts Castelo Branco, Leiria, Santarém, Lisboa, Portalegre, Évora, Setúbal and Beja (Cluster 2) (Fig. 3). Only clusters one, two and three were statistically significant, attributing a risk of infection of 4, 5 and 18 times higher, respectively, for a individual located in the delimited perimeter compared to other individual outside these areas. The number of cases, observed/expected ratio, relative risk and  $p$  value for each cluster can be found in Table 2.

## Discussion

The first worldwide report on the isolation of *Salmonella* 1,4,[5],12:i:- was from Portugal in 1986 in poultry [11]. Since this first report, a rapid increase in *Salmonella* 1,4,[5],12:i:- isolation was observed on an international scale, becoming the third most common serovar related with humans infections in 2015 in the EU [4].

Data presented in this study represents a passive monitoring based on clinical samples submitted to the national reference laboratory, which often underestimates the number of cases, since not every individual with salmonellosis goes to a physician due to its self-limiting course [12,13]. In addition, young individuals usually present more severe symptoms of infection and because of that, are more likely to be tested than adults [12,13]. Thus, a limitation to this approach is the probable selection bias [13].

In the present study, as many other reports [14,15], *Salmonella* was commonly isolated from stool samples. Others sources are scarcely documented and to our knowledge, this report include the first isolation of *Salmonella* 1,4,[5],12:i:- from peritoneal fluid, highlighting an unusual source of isolation, particularly important in clinical settings [16].

It is important to refer that positive stool samples for enteric pathogens can be found in asymptomatic patients, many weeks after the acute infection episode [17]. Some patients with nontyphoidal *Salmonella* infections may develop a chronic carrier state, characterized as a positive stool or urine culture for *Salmonella* at 12 months following the diarrhoeal illness. Although chronic carriage of nontyphoidal *Salmonella* occurs infrequently, approximately 0.15% in healthy adults and 3.9% in children, it represents an important mechanism of person-to-person transmission. Additionally, antimicrobial administration has not been proven to enhance the clearance of infection and may actually increase the duration of asymptomatic shedding [18].

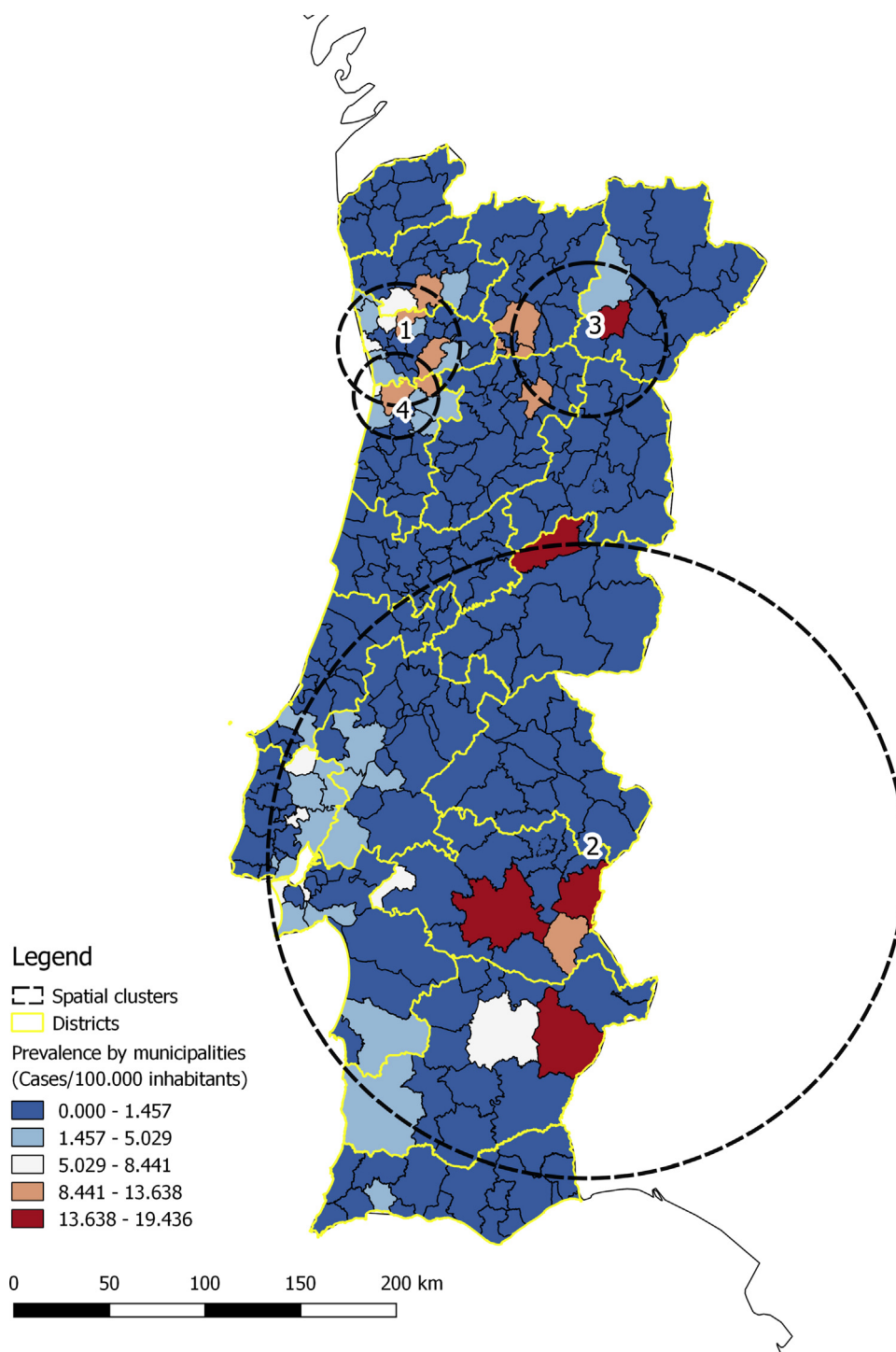
The age distribution of individuals with *Salmonella* 1,4,[5],12:i:- infections in the present study is in agreement with others reports [13,14,19], where most infections caused by this serovar are observed in young individuals. While this study detected *Salmonella* 1,4,[5],12:i:- infections more frequently in men, others reports also identified both men or women frequently affected [14,19].

Although in the period of this study an increasing annual incidence was observed, the number of reported isolates is low, which may be attributed to a decreasing in the amount of salmonellosis cases reported in the EU during this period, including Portugal [2].

In this country, most districts have reports of *Salmonella* 1,4,[5],12:i:-, which suggests a wide distribution from the north to south of the country. A higher prevalence in the Portuguese coastland was observed, which the higher human population density in these areas may explain and because patients are more likely to go to a doctor if gastrointestinal symptoms are presented. It is worth noting that some districts are classified without reports, which do not necessarily represent the absence of the disease, especially when there is evidence of underreporting due to its self-limiting course.

Rates of human *Salmonella* 1,4,[5],12:i:- infection appear to be higher in summer and early autumn, with most cases occurring between May and October, being this last month the one with the higher number of cases. In several studies, *Salmonella* infections among humans generally peak in summer months [20,21]. The reasons of these seasonal differences are not entirely known and could be related to combination of factors, including seasonal human behaviors [20], the parallel *Salmonella* shedding trends by animal reservoirs [22] and environmental variations influencing the pathogen virulence or persistence [20].

Cluster analysis of *Salmonella* 1,4,[5],12:i:- infections showed the existence of areas with a high number of cases, especially in



**Fig. 3.** Prevalence and spatial clusters of *Salmonella* 1,4,[5],12:i:- cases in Portugal, 2000 to the first quarter of 2011.

Legend: Prevalence of *Salmonella* 1,4,[5],12:i:- per 100,000 inhabitants, by municipalities according to the Eurostat-based Nomenclature of Territorial Units for Statistics system. Spatial clusters results based on a purely spatial Poisson model using the SatScan™ software.

**Table 2**

Spatial cluster of high *Salmonella* 1,4,[5],12:i:- infection rates in mainland Portugal from 2000 to the first quarter of 2011.

Cluster	Number of cases	Number of municipalities involved	Annual cases per 100,000 habitants	Expected cases	Overlap	Observed/expected	Relative risk	Log likelihood ratio	p Value
1	109	22	4.8	48.02	4	2.27	3.58	41.188036	≤0.001
2	84	109	4.9	46.70	No	1.80	4.85	27.495199	≤0.001
3	10	18	49.8	0.99	No	10.14	17.76	16.440983	≤0.001
4	4	5	37.5	0.55	1	7.28	10.42	5.071314	0.230

districts located at the northwest, northeast, center and southeast of Portugal. This observation suggests an occurrence of non-random cases, confirmed by the representation of three high rate clusters, which may reveal an increased exposure to human *Salmonella* 1,4,[5],12:i:- infection in these areas.

Interestingly, cluster 1 located at northwest of Portugal presents the highest number of human *Salmonella* 1,4,[5],12:i:- infections. This cluster contains two districts, Porto and Aveiro, with only 22 municipalities involved. Individuals in these areas present a risk about 4 times higher to acquire this infection than inhabitants of other municipalities. Although it may exist several reasons to explain this spatial cluster, one possible explanation is because pig farms are prevalent in these locations, especially in Aveiro [23]. Since pigs can be reservoirs for this serovar, this may justify the spatial cluster and the high frequency of cases [24,25]. This reason may also be valid for cluster 2, as some of the districts included like Beja, Leiria and Santarém, are also the important locations for pig production in Portugal [23].

In the public health perspective, spatial clusters analyzes may reveal the space dimensions of the diseases processes. Identification of *Salmonella* 1,4,[5],12:i:- cases that are concentrated in a specific location in Portugal may be essential for the efficient distribution of resources for prevention and treatment of this notifiable disease.

## Conclusions

As far as we know, this is the first report describing the incidence and the presence of areas with a higher risk for human *Salmonella* 1,4,[5],12:i:- infections in Portugal. Although passive surveillance may represent an underestimation of disease burden, they provide valuable information on incidence and trends that could aid public health authorities in developing prevention and control programs. There is a need to better understand the demographic, geographic, and seasonal factors associated with the increase of *Salmonella* 1,4,[5],12:i:- infections and to provide evidence-based information for policy makers to prioritize future efforts in addressing the increasing number of infections.

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## Competing interests

None declared.

## Ethics approval

Not required.

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