Universidade de Lisboa

Faculdade de Medicina Veterinária

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Acknowledgments

I wish to start this work by giving a word of appreciation and friendship to those who helped me throughout this journey in Copenhagen.

In Portugal I met Professor Doctor Sara Pires, on a Conference about "*Salmonella* in Pigs" at the Veterinary Medicine Faculty where I listened to her speaking with such determination and knowledge that I became inspired to pursue my research in Epidemiology and Food Safety.
The warm welcome and the trust that Professor Sara gave to my master project, my topic and my dream, made this experience complete. I want to thank Professor Sara, for accepting to be my supervisor in Copenhagen, for all her lessons, shared experiences and guidance, always allying scientific knowledge with wisdom.

The same gratitude words I also extend to Tine Hald, our group leader, to all researchers at the Epidemiology and Risk Modeling and Zoonosis Centre, for their constant support and encouragement.

To my co-supervisor, Professor Doctor Virgílio Almeida, I wish to express my gratitude for his enthusiasm about my work and learning, his guidance, representing a crucial link between Universities.

To Dr. Jorge Machado, from the National Reference Laboratory Doutor Ricardo Jorge, for the data shared and availability.
To Dr. Graça Ribeiro from the Microbiology Department of the University of Coimbra Hospital and all health professionals for their advice and information provided for this thesis.
To all my dearest colleagues from Veterinary Medicine Faculty of the University of Lisbon, especially those from 2007-2012 class, for all the moments shared, with experiences and friendships that will be forever cherished…you all know who you are!

Last but not least, to my family for their incredible support and love. You are all always in my heart.
To all of you that made reality the words of Pythagoras "between friends everything is at everybody's disposal" I will always keep you in my heart.

Abstract

A modelling approach to estimate the incidence of salmonellosis in humans in Portugal

Estimates of foodborne illness are relevant for setting food safety priorities and making public health policies. *Salmonella spp*. is one of the most important pathogens causing foodborne disease. In 2010, despite its progressive decrease in the European Union (EU), the incidence of salmonellosis was still 21.5 cases per 100,000 inhabitants, with a total of 99,020 confirmed cases of human salmonellosis reported by the 31 countries of the EU Member States (MSs).

The objectives of this study are to estimate the burden of illness of salmonellosis in Portugal, to account for the domestically acquired, foodborne illness, while identifying data gaps and areas for further research. Estimates of illness due to *Salmonella* were based on data from 2000-2010 of the Portuguese reported laboratory-based surveillance system, relevant international literature and the Portuguese census population for 2010.
The model approach - Burden of Illness (BoI) - defined as the impact of a health problem in an area or population measured by the incidence or number of cases, required accounting for underreporting and underdiagnosis, and estimating the proportion of illness domestically acquired and through foodborne transmission. The uncertainty was accounted with Monte Carlo simulations with 100,000 iterations, generating a mean estimate and 90% credible interval, using the program @Risk 6 (Palisades Corporation). The estimated number of salmonellosis cases is approximately 93 (52,04 –111,23) times higher than the reported from surveillance (239 cases of salmonellosis in 2010). It was estimated that there were 22,201 (11,476 – 35,956) episodes of domestically acquired foodborne Salmonellosis in Portugal for 2010. The multipliers for underreporting and underdiagnosis in Portugal were of 1 and 111.23, respectively.
This was the first study to estimate foodborne salmonellosis cases in Portugal.

Key-words: Burden of Illness, BoI, modelling, human salmonellosis, Portugal.

Resumo

A análise de dados referentes à incidência de doenças de origem alimentar é crucial para a melhoria da segurança alimentar e para a revisão das políticas de Saúde Pública num país. A *Salmonella spp.* é um dos agentes etiológicos de zoonoses transmitidas por alimentos de maior relevância em todo o mundo. Em 2010, apesar da redução progressiva da sua incidência na União Europeia (EU), ainda se atingem valores de 21,5 casos de salmonelose por 100.000 habitantes nos 31 Estados-Membro (EM) da UE.

Este estudo teve por objectivos: calcular a "Carga de Doença" causada por *Salmonella spp.* em Portugal; estimar os casos de salmonelose domesticamente adquiridos e de origem alimentar; e identificar lacunas no sistema de vigilância existentes, propondo linhas de investigação futuras. Os dados obtidos para calcular a incidência de salmonelose em Portugal, num período de tempo entre 2000 e 2010, foram recolhidos no sistema de vigilância de índole laboratorial; em artigos científicos e no recenseamento da população Portuguesa em 2010. O modelo criado, descrito como o que estima a "Carga da Doença", caracteriza o impacto de um problema de saúde numa área ou população, através da incidência ou do número de casos observados. Faz ajustamentos para possíveis casos que não são reportados pelo sistema de vigilância e para casos que, apesar de existirem, não foram diagnosticados. Termina por calcular a proporção de casos de salmonelose contraídos por via alimentar e adquiridos domesticamente, incorporando a incerteza que os resultados possam acarretar. A incerteza foi calculada pelo método Monte Carlo, no programa @Risk 6 (Palisade Corporation), com simulações de 100.000 iterações, criando uma média estimativa e intervalos de credibilidade de 90%.

O modelo de "Carga de Doença" gerou ocorrências de casos de salmonelose, 93 (52,04 – 111,23) vezes superiores aos reportados pelo sistema de vigilância vigente (239 casos de salmonelose em 2010). Em 2010, foram estimados 22,201 (11,476 – 35,956) episódios de salmonelose transmitida por via alimentar e adquirida domesticamente. Os "multiplicadores" obtidos para a "subnotificação" e o "subdiagnóstico" foram, respectivamente, de 1 e 111,23.

Este trabalho, pioneiro no cálculo da incidência de casos de salmonelose em Portugal por exposição alimentar, pretende ser um contributo válido e rigoroso para o estudo científico de uma doença, de grande incidência e da maior importância, em saúde pública.

Palavras-chave: Burden of Illness, BoI, modelo, salmonelose humana, Portugal.

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List of Abbreviations

BoI – Burden of Illness

C DC – Centers for Disease Control and Prevention (Atlanta).

CrI – Credible Interval

DGAV – Direcção Geral de Alimentação e Veterinária (National Authority for Animal Health)

DGS – Direcção Geral de Saúde – Portugal (National Authority for Human Health)

DTU –Technical University of Denmark (National Food Institute)

ECDC – European Centre for Disease Prevention and Control (Stockholm).

EEA – European Economic Area

EFSA – European Food Safety Authority.

ELSTAT – Hellenic Statistical Authority

EU – European Union

FDA – Food and Drug Administration

FoodNet – Foodborne Disease Active Surveillance

GP – General Practitioner

GP - sentinel – General Practitioner sentinel Web (Rede de Médicos Sentinela – Portugal)

HCIDC –Hellenic Centre for Infectious Diseases Control

INSA – National Laboratory Doutor Ricardo Jorge

LNIV – Laboratório Nacional de Investigação Veterinária – Portugal (National Laboratory for Veterinary Research)

MSs – Member States

NRL – National Reference Laboratory for *Salmonella*

PC – Personal Communication

SSI – Statens Serum Institute – Denmark

STEC – Shiga toxin-producing Escherichia coli

UC – University of Copenhagen (Faculty of Life Sciences)

UCSP – Healthcare Personalized Units – Portugal

UK – United Kingdom

US – United States

USDA – FSIS – US Department of Agriculture's Food Safety and Inspection Service

USF – Health Centres

UTL – Technical University of Lisbon.

WHO – World Health Organization

1. Introduction

1.1. Foreword

Every research starts with a work plan, to structure ideas and to get to the aim of our question. Mine started by coming to Denmark's Technical University, Department of Food Safety (DTU Food), in the National Food Institute for a period of time of four months, from March until June of 2013, to develop my final year thesis as a Veterinary Medicine master student, with an ERASMUS SMP scholarship.

When this project started we wanted to assess the impact of the increase in consumption of imported food products on the occurrence of human salmonellosis. For that we defined a time frame of ten years, from 2000 to 2010, for which it was necessary to know the incidence of salmonellosis in each year and relate it to the change in consumption patterns due to the increase of imports/exports and migration/travel-related cases.

However, due to the lack of Portuguese data, the previous plan was revised and altered to focus on determining the incidence of human salmonellosis in the Portuguese population.

This thesis is divided in six parts. The first part describes *Salmonella spp*. as an important foodborne pathogen, explaining different sources and ways of transmission. Also, it summarizes the pathogen's reported incidence in the European Union, considering in particular differences between Member States surveillance systems, focusing on Denmark and Portugal. The second part refers to the objectives of the thesis, and the third part the methods utilized to determine the incidence of salmonellosis in Portugal. The fourth and fifth parts encompass the results and their discussion. The conclusions are in the sixth and final part of this work.

This project allowed me to reinforce my knowledge in Food Safety/Epidemiology. Also, during my internship at DTU Food, I had the opportunity to present my project to a diverse audience of professionals at the Epidemiology and Risk Modeling and Zoonosis Centre groups.

I also enrolled in a three-week course on Quantitative Microbiological Risk Assessment at DTU, and attended to the Med Vet Net Conference 2013 that took place in Copenhagen, in

24 and 25th of June, with the main theme "One Health, one medicine: sharing challenges for combating zoonosis".

The second part of my internship also took place in Copenhagen, between October and December 2013, at the National Food Institute, with an extended ERASMUS scholarship.

I've continued developing my thesis about salmonellosis in the Portuguese population, a burden of Illness study, in order to improve the statistical approach, with a deeper understanding of the results.

During this second part of my internship I also assisted to some of the final meetings of the seven-year project of the Foodborne Disease Burden Epidemiology Reference Group (FERG) to estimate the global Disability Adjusted Life Years (DALYs) for several pathogens causing diarrhea. DALYs is a common metric used to express the human disease burden and enables comparisons and accounts for morbidity and mortality (WHO, 2006).

I am very grateful for these opportunities to work in Denmark, at the National Food Institute, where I had the privilege to work and learn with renowned experts of Food Safety and Zoonosis fields.

On the 15th of December 2014, as a master student, in my University, the University of Lisbon – Veterinary Medicine Faculty, I was invited by Professor Doctor Virgílio Almeida for a session integrated on 5th year's Subject of Risk Analysis, where I presented my research on "A modelling approach to estimate the incidence in humans in Portugal". This was a most enriching experience with great feedback.

With this thesis I wanted to study the incidence in the human Portuguese population. However, the estimated 10 million Portuguese inhabitants has a significant specificity: 2 million are immigrants – a diaspora that is reminiscent of the epic age of the discoveries, especially since 1498, year of Vasco da Gama's arrival to India, that signals the beginning of the Modern Age.

From all over the world, Europe, Africa, America, Asia and Oceania, there are Portuguese and they return to their country for variable periods of time, per year. Those who return

from other European countries stay normally in Portugal in August and bring back friends, neighbors, and work colleagues that give a multicultural air to all Portugal, from North to South, from Littoral to villages and mountainous areas of the Portuguese interior regions.

Moreover, it is estimated that 22 million tourists visit Portugal per year, for an average of 15 to 20 days.

These people that stay in Portugal temporarily, with different social and food consumption patterns, drink and eat and as the autochthone people are in danger of getting infected by *Salmonella spp.*

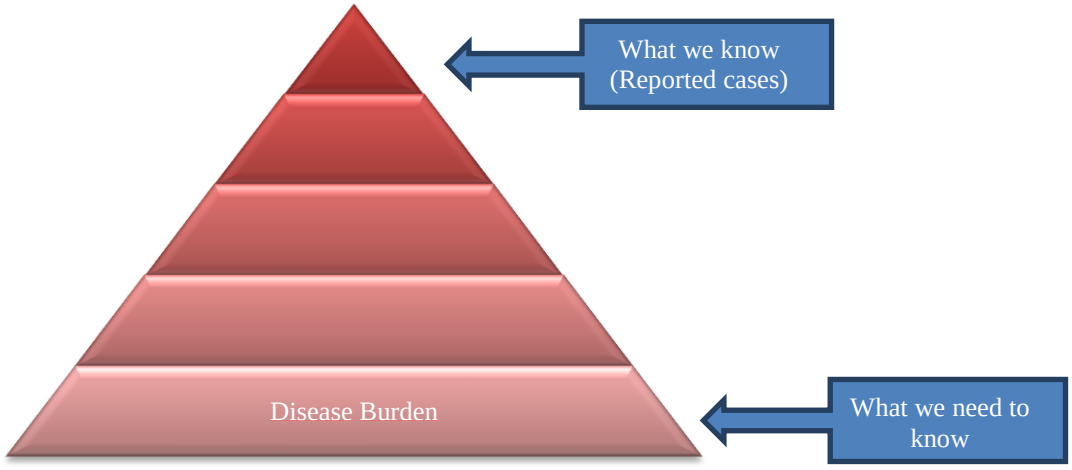
Therefore, for these reasons we couldn't name this study "A modelling approach to estimate the incidence of Salmonellosis in the human Portuguese population", and with accuracy it was entitled as "A modelling approach to estimate the incidence of Salmonellosis in humans in Portugal".

1.2. Foodborne Illnesses

Foodborne illnesses have long been considered an international public health challenge (World Health Organization [WHO], 2012). They are recognized worldwide for their easy spread and transmission through a wide variety of sources, as well as for their implications in human health. In recent years, international travel, migration, and growing industrialization and trade of foods have contributed to increase the frequency of foodborne diseases. It is estimated that food and waterborne diarrheal diseases are responsible for 2.2 million deaths per year worldwide, 1.9 million of which are children (WHO, 2012).

Despite increased awareness and political attention, the true burden of foodborne diseases is to a wide extent still unknown (WHO, 2012). This scarceness of knowledge is mainly due to the lack of identification and reporting of foodborne disease cases that ends on an important difference between the surveillance evidence and the true occurrence of a disease in a population (Figure 1).

Figure 1. ”The Unknown Burden” surveillance pyramid (adapted from WHO, 2012), representing the underreporting of foodborne diseases. The top of the pyramid represents the cases that are captured by public health surveillance, whereas the bottom represents the *true* incidence of the disease in a population.



Several studies have made efforts to estimate the total burden of foodborne diseases in various countries (Scallan et al., 2010; Thomas et al., 2013; Kubota et al., 2011; Hall et al., 2005; Vaillant et al., 2005; Adak, Meakins, Yip, Lopman, & O'Brien, 2005). In all studies, *Salmonella spp*. was identified to be one of the pathogens with highest incidences in the population. In the United States (US), it was estimated that *Salmonella spp*. caused 1.0 million foodborne illnesses, 11% of the total 3.6 million foodborne illnesses caused by bacteria (Scallan et al., 2011b). With a population of 299 million habitants *circa* 2006 and an estimated annual number of *Salmonella spp*. cases of 1,027,561, the incidence of salmonellosis in the US population was 3436.7 cases per 100,000 inhabitants (Scallan et al., 2011b).

In Canada, Thomas et al. estimated for 2006 a total number of 87,510 foodborne cases of salmonellosis, corresponding approximately to 16.2% of the total 541,495 foodborne illnesses caused by the 18 bacterial pathogens studied. Salmonella was the third most frequent pathogen in Canada (Thomas et al, 2013). With a population of 325 million people *circa* 2006 and the estimated annual number of *Salmonella spp*. cases of 87,510, the incidence of salmonellosis in the Canadian population was 269.3 cases per 100,000 inhabitants (Thomas et al., 2013).

In Japan, the burden of acute gastroenteritis and foodborne illnesses caused by several pathogens including *Salmonella* and the estimated numbers of episodes of acute

gastroenteritis were 209 per 100,000 habitants per year at the Miyagi Prefecture region (Kubota et al., 2011). With a population of 236 million inhabitants and the estimated annual number of *Salmonella spp*. cases of 254,000, the incidence of salmonellosis in the Japanese population was 1076.3 cases per 100,000 inhabitants (Kubota et al., 2011).

In Australia, a total of 81,000 cases of foodborne salmonellosis was estimated to occur *circa* 2000, approximately 8.5% of the 950,000 million foodborne illnesses caused by the eleven studied pathogens. *Salmonella* was the third most important bacterial pathogen causing illness in the population (Hall et al., 2005).

In Europe, studies carried out on France, Greece and Denmark revealed also the importance of *Salmonella spp*. and its transmission through food (Vaillant et al., 2005; Gkogka, Reij, Havelaar, Zwietering & Gorris, 2011; Haagsma et al., 2012). In France, a study of 13 foodborne bacterial pathogens, revealed *Salmonella spp*. as the most important pathogen causing foodborne illness, ranging between 30,598 and 41,139 cases from a total 51,269 and 81,927 cases (Vaillant et al., 2005). The French population was 60,185,831 in 1999. The incidence of salmonellosis in the French population was considered to be between 50.8 cases per 100,000 and 68.4 cases per 100,000 inhabitants. In Greece, Gkogka et al. (2011) estimated for 1996 – 2006 a total of 369,305 (95% credible interval [CrI] 68,283 – 910,608) illnesses per million inhabitants per year, attributable to contaminated food. Regarding reported/estimated cases, salmonellosis was responsible for the majority of the cases after ill-defined intestinal infections, with a total of 3,793 (95% CrI 750 – 8,350) illnesses. The incidence of foodborne salmonellosis in Greece was estimated to be 74 cases per 100,000 habitants (95% CrI 22 – 128) (Gkogka et al., 2011). In Denmark, Haagsma et al. (2012) estimated an incidence of foodborne salmonellosis of 165 cases per 100,000 inhabitants.

These studies show that *Salmonella spp*. is a very important food safety problem worldwide. By comparing incidence estimates we observe differences between countries that can be explained by differences on the surveillance scheme of *Salmonella spp.*, on different population sizes and on the methods used to correct underreporting. Therefore, it is important to estimate the burden of salmonellosis in Portugal to improve its surveillance and to provide decision makers with supplementary data that will help them to make better decisions to mitigate the transmission of foodborne pathogens to the Portuguese consumers.

1.2.1. *Salmonella spp*.

Salmonella spp. is a well-recognized zoonotic pathogen of economic significance in animals and humans. In humans it causes a disease called salmonellosis (European Food Safety Authority [EFSA], 2005). The genus Salmonella is divided into two species: *S. enterica* and *S. bongori*. *S. enterica* is further split into six subspecies, and most *Salmonella* belong to the subspecies *S. enterica* subsp. *enterica* (EFSA, 2013).
There are more than 2,600 serovars of zoonotic *Salmonella* and the prevalence of each serovar may change over time (EFSA, 2013). In this thesis, only *Salmonella enterica* will be considered. Non-typhoid Salmonella will be referred as *Salmonella spp.*

1.2.1.1. *Salmonella spp*. in humans

Humans are infected mainly via fecal-oral route (also animals), and it results in different symptoms and disease syndromes (enterocolitis and bacteremia/septicemia).

The main clinical manifestation of salmonellosis is acute gastrointestinal illness. On severe cases septicemia may occur (Pires, 2009). Symptoms are usually mild and characterized by onset fever, abdominal pain, sometimes nausea. The average incubation period is 12-36 hours. Most infections are self-limiting with a short duration (EFSA, 2013). Severe cases are associated with persistent diarrhea that may last more than seven days, dehydration, and septicemia and require treatment with antimicrobials.

Unfortunately, salmonellosis has also been related to long-term and sometimes chronic sequelae (e.g. reactive arthritis). The mortality rate is less than 1% of the total reported cases (EFSA, 2013).
Despite all serovars being genetically related, their pathogenicity and virulence differs.

The severity of *Salmonella spp*. infection is dependent upon several variables, such as (1) the virulence of the *Salmonella* serovar and its survival on the environment; (2) their capability of causing infection (pathogenicity); (3) the response human immune system and (4) antimicrobial resistances.

Many studies characterize *Salmonella spp*. in humans by establishing mild and severe cases. A mild case is defined as an individual with acute diarrheal illnesses (AGI) that experienced three or more loose stools in 24 hours or any vomiting in the past 28 days, excluding patients with chronic conditions (e.g. Crohn's disease or irritable bowel

syndrome), absence of bloody diarrhea and the duration of illness was less or equal to 7 days (Thomas et al., 2006). A severe case is defined as an individual with acute bloody diarrhea or diarrhea that lasted longer than 7 days (Thomas et al., 2006).

1.2.1.2. Sources of salmonellosis

The reservoir of Salmonella is the intestinal tract of a wide range of domestic and wild animals, which may contaminate a variety of foodstuffs of animal and plant origin (EFSA, 2013).

The main sources and routes of transmission can be separated in four categories (Pires, 2009): (1) foodborne transmission (the most frequent); (2) contact with live animals (direct or indirect (fomites); (3) environmental transmission (mainly through contaminated water); (4) person-to-person transmission (especially in the elderly groups in the population, immunocompromised people and children in day care).

Human salmonellosis is mainly due to foodborne transmission (Pires, 2009), and it is often related with inadequate cooking of contaminated products, cross-contamination between food items or inadequate storage temperatures. Factors related to food consumption also influence the frequency of human infection and those are: (1) the amount of food consumed; (2) cross-protection of *Salmonella spp*. from low water activity and high fat contents to gastric acid-stress (Aviles, Klotz, Smith, Williams & Ponder, 2013); (3) buffering capacity of the food at the time of the meal; and (4) nature of contamination (Pires, 2009).

To better understand the most important sources of human salmonellosis in a country, methods to attribute cases to food-animal sources, thus quantifying the contribution of different sources to the burden of human illness of foodborne pathogens, should be applied. This process is defined as "source attribution". It produces information that is crucial to identify and prioritize effective food safety interventions (Pires et al., 2009).

1.3. Incidence of human salmonellosis in the European Union

The overall incidence of human salmonellosis at the EU has decreased from 31.1 in 2007 to 21.5 cases per 100,000 inhabitants in 2010 (EFSA, 2012). In 2010, a total of 99,020 confirmed cases of human salmonellosis were reported by 27 MSs. The incidence of salmonellosis in the different countries varied considerably in this period, and these

differences may reflect a true variability on the incidence disease, differences on the efficiency of the national reporting systems or a combination of both scenarios. In 2010, the country-incidence within the EU ranged from zero in Liechtenstein to 78.1 cases per 100,000 inhabitants in Czech Republic (EFSA, 2012) (Table 1).

Table 1. Reported cases of human salmonellosis from 2006 until 2010 and notification rate for confirmed cases in the EU (EFSA, 2012).*

Country	2010				2009	2008	2007	2006
	Report Type[1]	Cases	Confirmed cases	Confirmed cases / 100,000	Confirmed cases			
Austria	C	2179	2179	26.0	2775	2312	3386	4787
Belgium	C	3169	3169	29.2	3113	3831	3915	3630
Bulgaria	A	1217	1153	15.2	1247	1516	1136	1056
Cyprus	C	137	136	16.9	134	169	158	99
Czech Republic	C	8456	8209	78.1	10480	10707	17655	24186
Denmark	C	1608	1608	29.1	2130	3669	1648	1662
Estonia	C	414	381	28.4	261	647	428	453
Finland	C	2422	2422	45.3	2329	3126	2738	2576
France	C	7184	7184	11.1	7153	7186	5313	6008
Germany	C	25306	24833	30.4	31395	42885	55399	52575
Greece	C	300	299	2.6	403	792	706	890
Hungary	C	6246	5953	59.4	5873	6637	6578	9389
Ireland	C	356	349	7.8	335	447	440	420
Italy	C	2730	2730	4.5	4156	6662	6731	6272
Latcia	C	951	881	39.2	798	1229	619	781
Lithuania	C	1962	1962	58.9	2063	3308	2270	3479
Luxembourg	C	211	211	42.0	162	153	163	308
Malta	C	160	160	38.7	125	161	85	63
Netherlands[2]	C	1447	1447	13.6	1205	1627	1224	1644
Poland	A	9732	9257	24.3	8521	9148	11155	12502
Portugal	C	207	205	1.9	220	332	438	387
Romania	C	1291	1285	6.0	1105	624	620	645
Slovakia	C	5171	4942	91.1	4182	6849	8367	8191
Slovenia	C	363	363	17.7	616	1033	1336	1519
Spain[3]	C	4420	4420	38.4	4304	3833	3842	5117
Sweden	C	3612	3612	38.7	3054	4185	3930	4056
United Kingdom	C	9670	9670	15.6	10479	11511	13557	14124
EU Total		**100921**	**99020**	**21.5**	**108618**	**134579**	**153837**	**166819**
Iceland	C	34	34	11.0	35	134	93	114
Liechtenstein	C	-	-	-	-	-	1	14
Norway	C	1370	1370	25.7	1235	1941	1649	1813
Switzerland[4]	C	1179	1179	15.1	1298	2031	1778	1768

1. **A:** aggregated data report; **C**: case-based report.
2. Sentinel system; notification rates calculated with an estimated population coverage of 64%.
3. Notification rates calculated with estimated population coverage of 25%.
4. Switzerland provided data directly to EFSA

* Data from 2011 are available at link: www.efsa.europe.eu/en/search/doc/2597.pdf

The age distribution of *Salmonella* cases in 2010 is closely similar as the ones seen in previous years, with the highest notification rates (i.e. the number of incident cases in 2010 divided by the population at risk, according to each age groups, in each country) from the age groups of 0-4 years (112.7 / 100.000) and 5-14 years (35.1 /100.000), followed by 15-24 years and 25-44 year olds.

As in previous years, in 2010 the two most common *Salmonella* serovars were *S.* Enteritidis and *S.* Typhimurium, with 45.0% and 22.4% of all known serovars reported in human cases, respectively.

In 2010, approximately 63% of the cases of human salmonellosis in the EU were reported as domestically acquired, being at the same level as for 2009, with 62.4% of cases. Also, at a similar level, the cases acquired abroad were 10.9% in 2010 and 10.5% in 2009 (EFSA, 2012). For 26.0% of cases there was no information on whether the cases were acquired domestically or abroad (Table 2).

Table 2. Distribution of confirmed salmonellosis cases in humans by reporting countries and origin of infection (domestic / imported) in 2010 (adapted from the annual report of zoonosis and foodborne pathogens from EFSA, 2012). *

Country	Domestic (%)	Imported (%)	Unknown (%)	Total (n)
Austria	97.2	2.8	0	2179
Belgium	-	-	100	3169
Bulgaria	-	-	100	922
Cyprus	-	-	100	136
Czech Republic	98.1	1.9	0	8209
Denmark	42.9	35.5	21.6	1608
Estonia	93.7	6.3	0	381
Finland	13.1	83.8	3.1	2422
France	-	-	100	7184
Germany	88.3	7.3	4.4	24833
Greece	90.6	2.0	7.4	299
Hungary	99.8	0.2	0	5953
Ireland	41.3	36.1	22.6	349
Italy	-	-	100	2730
Latvia	100	0	0	881
Lithuania	-	-	100	1962
Luxembourg	77.7	3.8	18.5	211
Malta	100	0	0	160
Netherlands	88.7	11.3	0	1447
Poland	99.9	0.1	0	9732
Portugal	0.5	0	99.5	205
Romania	16.5	0	83.5	1285
Slovakia	99.1	0.9	0	4942
Slovenia	-	-	100	363
Spain	100	0	0	4420
Sweden	22.5	73.8	3.7	3.612
United Kingdom	24.9	32.2	42.9	9670
EU Total	**63.1**	**10.9**	**26.0**	**99264**
Iceland	23.5	50	26.5	34
Liectenstein	-	-	-	-
Norway	15.1	65.5	19.4	1370

1. Aggregated data for Bulgaria and Poland include all reported cases.

* Data from 2011 are available at link: www.efsa.europe.eu/en/search/doc/2597.pdf

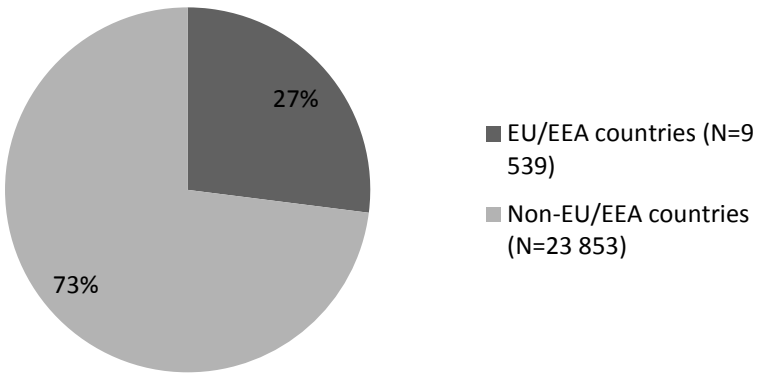
These data may not reflect a true picture of the proportion of cases associated to international travel. As in previous years, three Nordic countries (Finland, Sweden and Norway) reported the highest proportions of imported (travel-associated) cases of salmonellosis, respectively, 83,8%, 73,8% and 65,5%, in 2010 (EFSA, 2012). Except for these three Nordic countries, *Salmonella spp.* infections seem to have been domestically acquired in the majority of the other countries. However, we can always debate the fact

that data on domestic/imported reported cases are often scarce or non-existent, which may deviate the proportion of the distribution between imported and domestic cases.

In Sweden, detailed studies on the epidemiology of travel-associated non-typhoidal salmonellosis have been performed (Ekdahl et al., 2005; de Jong & Ekdahl, 2006; Havelaar et al., 2012). Ekdahl (2005) and De Jong and Ekdahl (2006) published estimates on the underreporting of salmonellosis and campylobacteriosis, based on the risk of illness of returning Swedish travelers for the period 1997-2003. Havelaar & Ivarsson estimated the risk of illness of returning Swedish travelers for the period 2005-2009, and provided new incidence estimates for all EU 27 MSs, by anchoring to a Dutch population-based study. According to these studies, relative risks for travelers were highest when travelling to Southern Europe and to the Eastern Mediterranean.

However, recent published data by the European Centre for Disease Prevention and Control (ECDC), the EU agency with a mandate to operate surveillance networks and to identify, assess, and communicate current and emerging threats to human health from communicable diseases, shows that from 2007 until 2009, 38,510 cases were travel-related salmonellosis from other countries. This represents 14% of cases with known history of travelling (N=257 825, pooled data). Travel-related salmonellosis cases was pointed out for 33,392 reported cases, of which 73% (23 853 cases) were acquired in non-EU countries and 27% (9 539 cases) originated from another EU/ European Economic Area (EEA) country (Figure 2).

Figure 2. Origin of travel-related non-typhoidal salmonellosis cases as reported by EU/EEA countries, from 2007 until 2009, from a total of 33 392 Salmonella travel-related reported cases (adapted from European Centre for Disease Prevention and Control [ECDC], 2012).



Source: Austria, Cyprus, Czech Republic, Denmark, Estonia, Finland, Germany, Greece, Hungary, Ireland, Latvia, Luxembourg, Malta, the Netherlands, Portugal, Slovakia, Spain, Sweden, United Kingdom; non-EU countries: Iceland and Norway.

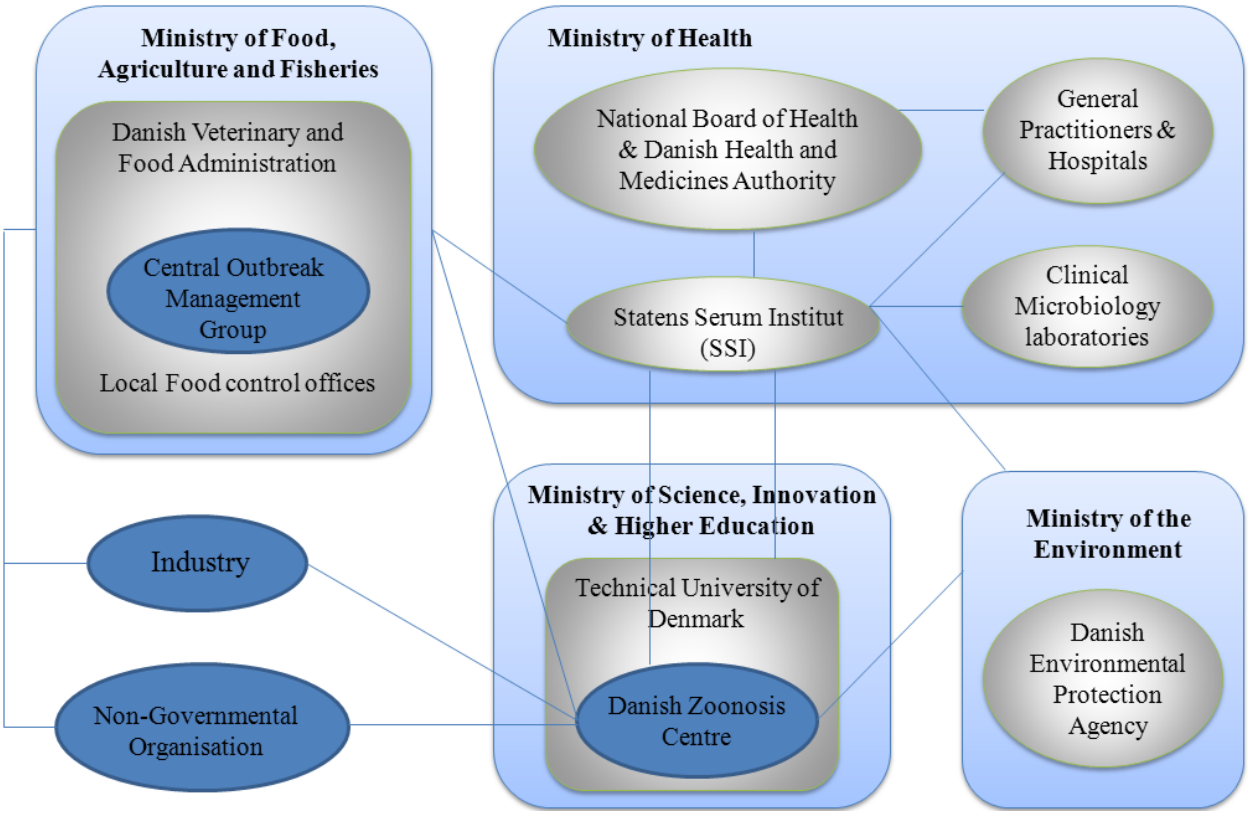
The most commonly reported non-EU countries associated with *Salmonella* infection were Thailand, Turkey and Egypt, with 6 380, 3 692 and 2 701 cases, respectively, corresponding to a total of 38% of all imported cases with known destination, from 2007 until 2009 (ECDC, 2012).

1.3.1. *Salmonella* surveillance in Denmark

1.3.1.1. Surveillance of *Salmonella* in humans

In Denmark, the surveillance system is integrated, e.g., there is collaboration between national and regional authorities, the industry and non-governmental organizations (Figure 3).

Figure 3. Overview of the Danish surveillance system, with the monitoring and investigation for reporting infectious pathogens in humans (Anonymous, 2013).



The diagnosis and reporting of foodborne disease episodes involves laboratory analysis of stool samples, identification of the pathogen (e.g. *Salmonella*), subtyping of the isolate (e.g. with serotyping), notification of cases and storage of information at a national data base, managed by the National Public Health Institute, Statens Serum Institute (SSI).

Physicians report individually notifiable zoonotic diseases to the Danish Health and Medicines Authority and the Department of Epidemiology at SSI. The diagnosis is carried out by SSI, the Reference Laboratory for enteric pathogens and responsible for the laboratory based surveillance conducted in Denmark, and by 14 clinical microbiology laboratories. SSI serotypes all human *Salmonella* isolates, forwarding all S. Enteritidis and S. Typhimurium to the National Food Institute at the Technical University of Denmark for phage typing. It is one of the three – serotyping, phage typing and antimicrobial resistance susceptibility test – types of phenotypic method, and is an important tool for the classification of *Salmonella*, reflecting differences between organisms with the same serotype, but different susceptibilities to infection. Testing for antimicrobials susceptibility is performed at SSI using the tablet diffusion method (Neo-Sensitabs, A/S Rosco, Roskilde, Denmark) (DANMAP, 2007) (Pires, 2009).

Information regarding travelling abroad before the onset of symptoms is available for a proportion of the reported cases. General practitioners ask their patients if they travelled abroad in the seven-day period before disease onset. Until 2007, data on international travelling were very often incomplete. Since then SSI has been interviewing retrospectively a significant fraction of the patients without travel information reported by their general practitioners. The information is then stored, processed and analyzed at the Danish Zoonosis Centre, a network involving three institutions, the National Food Institute, the Danish Veterinary and Food Administration and the Statens Serum Institut. As part of this overall strategy to gather more information, Müller, Korsgaard and Ethelberg (2012) performed a population-based telephone survey in Denmark to determine the incidence of AGI (Müller, Korsgaard & Ethelberg, 2012).

1.3.1.2 Incidence of human salmonellosis in Denmark

The incidence of human salmonellosis in Denmark increased in the mid 80's. The following years until late 90's, three distinct waves of salmonellosis frequency were observed. Then, since 1997, there has been a steadily decreasing trend (Pires, 2009). The number of reported cases of salmonellosis in the country decreased from 3,669 in 2008 to 1,207 in 2012 corresponding to an incidence of 21.6 cases per 100,000 inhabitants in 2012 (EFSA, 2014).

From 2007 until 2010 two waves of salmonellosis were recorded. The number of reported cases of salmonellosis in the country increased from 1,648 in 2007 to 3,669 in 2008, corresponding to an incidence of around 70 cases per 100,000 inhabitants in 2008, almost two times higher than in 2007. This scenario reflects the occurrence of foodborne outbreaks in 2008, not a trend. In 2009 and 2010, however, the number of notified cases of salmonellosis started decreasing from 2,130 to 1,608 cases (EFSA, 2012). S. Enteritidis has been the most frequently isolated serovar in humans, followed by *S.* Typhimurium. In 2010, 35.5% of the salmonellosis cases were due to international travelling, in opposition to 45% in 2007.

1.3.2. *Salmonella* surveillance in Portugal

1.3.2.1. Surveillance of *Salmonella* in humans

The diagnosis of human salmonellosis in Portugal is done by the Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA, the National Reference Laboratory of the Ministry of Health) and by an unknown number of public and private microbiology laboratories. Human cases of salmonellosis are reported to INSA, the reference laboratory for enteric pathogens infections, and responsible for the laboratory based surveillance. Serotyping of all human *Salmonella* isolates is performed at the National Reference Laboratory (Laboratório Nacional de Investigação Veterinária, LNIV) and at the INSA, Department of Infectious Diseases (EFSA 2010 report on trends and sources of zoonoses for Portugal, 2010). Phage typing of *S.* Enteritidis and *S.* Typhimurium began in 1999, but it isn't routinely performed (EFSA, 2010). Antimicrobial susceptibility testing of *Salmonella* is performed at the National Reference Laboratory for *Salmonella* (NRL for *Salmonella*), using disk diffusion method in Mueller Hinton plates (EFSA, 2010). Furthermore, INSA reports to the Portuguese National Authority for Animal Health (DGAV – Direcção-Geral de Alimentação e Veterinária), responsible for notifying Salmonellosis cases to EFSA.

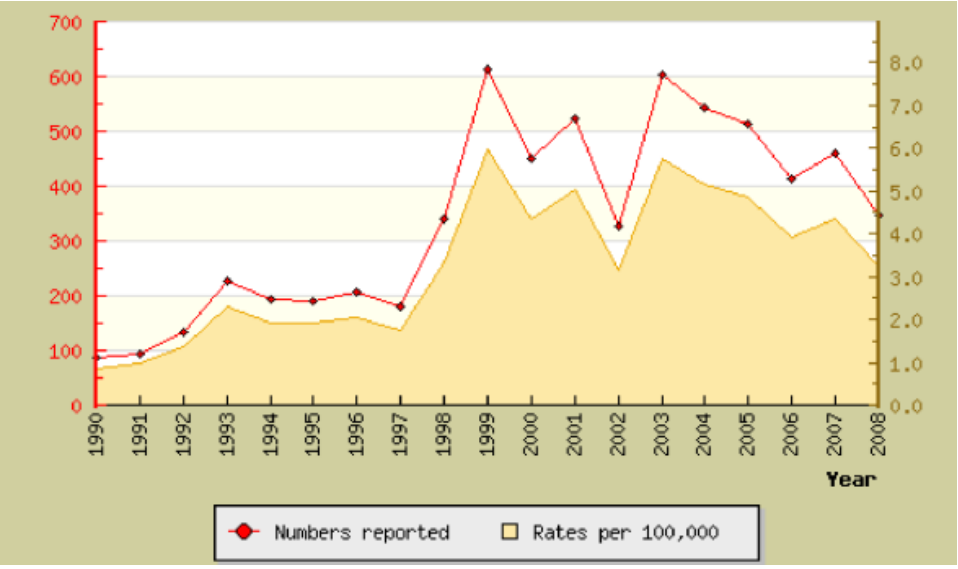
Information on international travelling data before the onset of illness is not collected by routine by general practitioners. Hence, the relative proportion of reported cases with unknown travel-history is 99.5% (Table 2) (EFSA, 2012). However, there has been an effort focused on travelers visiting tropical countries, especially on children ranging from 2 months-old until 16 years old of age. Silva, Figueiredo and Varandas (2009) estimated that 21.8% (from a total of 174 diagnosed children) of Portuguese children became ill while travelling abroad, mainly to African countries such as Angola (47.1%), Guinea-Bissau (33.3%) and India (31.6%).

1.3.2.2. Incidence of human salmonellosis in Portugal

Since 1951, that Salmonella spp. is notifiable in Portugal. Afterwards, with the implementation of the European programs for *Salmonella spp*. control, it was observed that at the beginning of the 80's the incidence of salmonellosis increased. In Portugal, as in many other countries, *S*. Enteritidis was the most common serotype, with a proportion of persons affected 8 / 100,000 population in 1986 (Bernardo, 1991).

Portuguese data are mostly for foodborne outbreaks and prevalence surveys. They revealed that from 1984 to 1989 occurred 14.8% of gastroenteritis cases. From 1990 to 1991, 34% of all gastroenteric diseases were food-related outbreaks (Berger, 2014) (Figure 4).

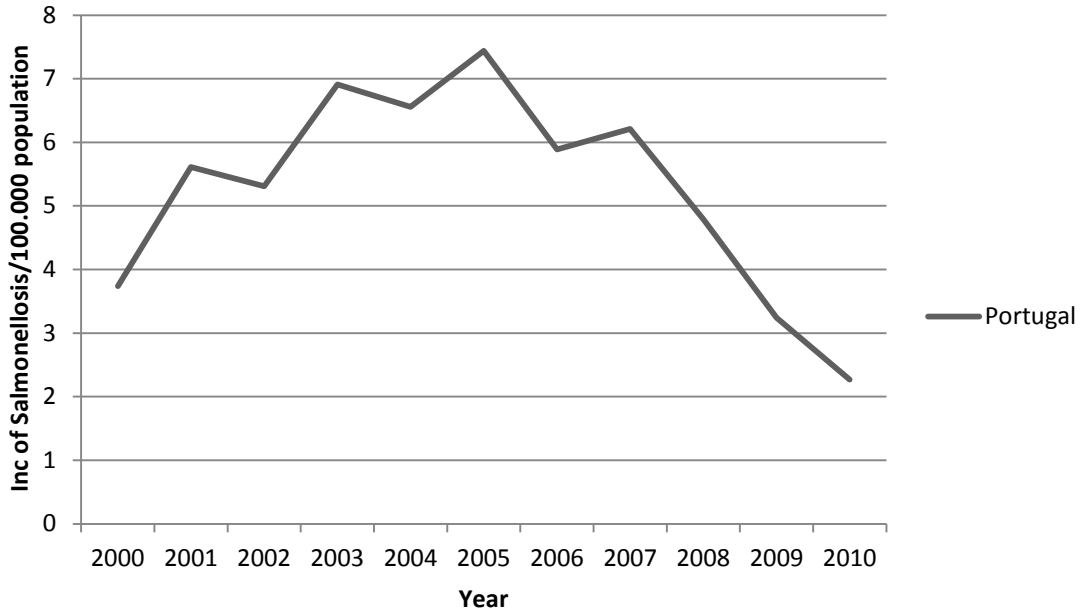
Figure 4. Portuguese salmonellosis cases from 1990 until 2008 (Berger, 2014)



In the 90's the incidence of salmonellosis increased, which was a reflection of the EU *Salmonella spp*. control programs in poultry and broiler production and eggs that reinforced the mechanisms for disease diagnosis and notification.

In the following decade, public health surveillance did not identify any trend since it was very random. If any, it shows less and less capture of cases, and these were mainly outbreaks (Figure 5).

Figure 5. Incidence of human salmonellosis per 100,000 inhabitants from 2000 to 2010 (Instituto Nacional de Saúde Dr. Ricardo Jorge [INSA], 2013).



Despite the surveillance system weaknesses, since 2007 there has been a steadily decreasing trend. The number of reported cases of salmonellosis in Portugal decreased from 659 in 2007 to 239 in 2010, corresponding to an incidence of around 2 cases per 100,000 inhabitants in 2010. *S.* Enteritidis has been the most isolated serovar in humans, followed by *S.* Typhimurium, except for 2010, when *S.* Typhimurium was the most frequent serovar in humans. In 2010, 32.6% of the reported cases were caused by *S.* Enteritidis and around 35% by *S.* Typhimurium (Instituto Nacional de Saúde Dr. Ricardo Jorge [INSA], 2013). It was estimated for 2010, zero cases of international travel.

1.3.3. Underreporting

Estimating the frequency of salmonellosis cases in a population is a nuclear task in evaluating the burden of illness of any pathogen. In most industrialized countries, Salmonella spp. and other foodborne pathogens are subject to surveillance. However, data from passive surveillance programs are frequently underreported.

Also, most ongoing surveillance systems for foodborne disease depend upon symptomatic patients consulting a primary care general practitioner. Without this step, illness is unlikely to be recorded in any official statistics. The loss of data at various points along the surveillance chain from patient, through laboratory tests, to official statistics is generally described as a pyramid (Figure 1) (Wheeler et al., 1999). Disease in the community forms the base of the pyramid, while the cases that reach official statistics form the apex.

In 2010, Portugal reported 239 laboratory-based salmonellosis cases, a number that is considered to be extremely low, especially when comparing to countries that have less than half of the Portuguese population, such as Denmark, for example.

To understand the reporting process in Portugal, it was attempted to compare the reported confirmed cases to EFSA, from 2000 to 2010, with the reported *Salmonella* infection laboratory-confirmed cases from INSA and the National Authority for Human Health (DGS – Direcção Geral de Saúde), which revealed major discrepancies between reports. This way, data are considered inconsistent and there is a problem on the reporting process to EFSA.

Furthermore, in Portugal, data related to patients visiting their general practitioner, and practitioners requesting stool samples from a patient with symptoms of foodborne disease were inexistent. This does not imply that patients do not seek medical care when they are sick foodborne related infections or that general practitioners do not regularly request stool sample analysis for foodborne pathogens. It only means that is lacking coordination and defined protocols designed to increase the odds of *Salmonella* laboratory isolation and identification. Therefore, it can be assumed that the number of salmonellosis cases is higher than those reported to surveillance.

1.3.3.1. Population surveys

Information on patient-behavior, specifically on the proportion of patients that seek medical care, can be obtained through population surveys (Van Cauteren, De Valk, Vaux, LeStrat & Vaillant, 2012; Kubota et al., 2011; Müller et al., 2012). Population surveys are used by researchers for diverse purposes. They can have different designs, such as retrospective, cross-sectional or prospective. People involved in this type of survey are randomly selected to participate: either households or individuals within households are randomly contacted and selected. These surveys can be performed by: telephone; in presence; mail or email; depending on (1) where to conduct the survey; (2) the sample size; (3) time-frame; (4) season (some pathogens are most frequent in certain periods of the year); (5) ethics.

Population surveys are a cost-effective way to improve knowledge about population characteristics and patterns, and can target specific periods of time, age ranges and genders, as well as gathering more detailed information about a specific pathogen.

In Denmark, to estimate the burden of acute gastrointestinal illness, Müller et al. (2012) performed a cross-sectional population-based telephone survey in 2009, by evidence from

National Health registries or by literature review. In the telephone survey 1,853 people were interviewed, with 206 people fitting the case definition (diarrhea) and providing information for analysis. Of these, 198 reported non-bloody diarrhea and 5 bloody-diarrhea in the 28 days before the interview. Symptomatic participants were also asked about duration of disease, care-seeking behavior, stool sample collection and absence of work (Müller et al., 2012). The individuals were randomly selected and provided information of gender, age, symptoms, seasonality, etc. Women and children between 0-9 years-old were most affected by acute gastrointestinal illness.

This study adds to the picture of foodborne and gastrointestinal illness in Denmark. The data regarding care-seeking and the proportion of patients with a stool sample submitted for analysis will help to fill a gap in the efforts to estimate the size of each layer in the surveillance pyramid for gastrointestinal infections in Denmark. In this case the data obtained were not pathogen specific, but the data onset, when seen in the context of national surveillance data, register studies, model studies and even serological studies can assist in the efforts to calculate cost and disease burden of gastrointestinal illness in Denmark (Müller et al., 2012). Policy making and food safety preventive measures can target specific groups, since the high incidence of acute gastrointestinal illness in children suggests that hygiene should be improved particularly in day-care and similar institutions for children.

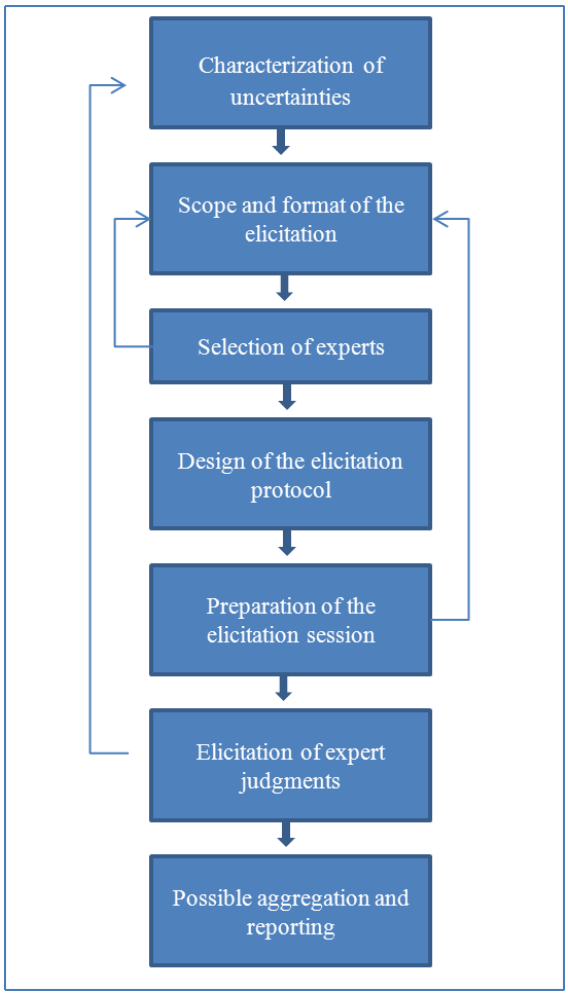
1.3.3.2. Expert elicitations

Expert elicitation can be a good alternative, when Burden of Illness studies haven't been assessed. Expert elicitations are described as the synthesis of opinions conducted by a panel of different specialists (e.g. epidemiologists, microbiologists, general practitioners, and veterinarians), where there is uncertainty due to insufficient data or when such data is unattainable because of lack of resources. Expert elicitation generally quantifies uncertainty.

Theoretically, the organization of a formal expert elicitation is described as a seven-step procedure, since the characterization of the uncertainties, that can scope and format the elicitation, and choose a panel of experts accordingly to the subject in hand, followed by the design of the elicitation protocol, the brainstorming of the experts judgments in a pre-

determined session, from which possible aggregation and reporting are expected (Figure 6).

Figure 6. A theoretical approach of a seven-step schematic procedure to organize a formal expert elicitation.



Since 1989, a group of general practitioners, exclusively voluntary, working in Health Centers (Unidades de Saúde Familiar – USF) or in Healthcare Personalized Units (Unidades de Cuidados de Saúde Personalizados – UCSP) of the Portuguese National Health System, created a General Practitioners-Sentinel Web. Its main objectives are: (1) to estimate incidence rates for different illnesses with public health impact, improving the surveillance system; (2) identify and investigate potential outbreaks in the community; (3) create and manage a database helping to improve epidemiological research and policy making.

New cases of disease are notified weekly and added to the practitioner's list of patients. Then those reports are reviewed and shared nationally and internationally. However, this GP-sentinel web, only reports systematically cases of influenza, hypertension, Diabetes

Mellitus and acute myocardial infarct. This type of Web can be considered expert elicitation, but was not useful for our study because it did not produce data regarding Salmonellosis on the Portuguese population (INSA, 2014).

1.4. Active surveillance system – US surveillance system

The notification of human salmonellosis confirmed cases depends, among other things, upon an on-going surveillance system in the country of origin. Both Denmark and Portugal have a so-called "passive" surveillance system, which rely mostly upon reports of foodborne diseases from clinical laboratories to regional/local or national health departments, and then to EFSA or ECDC.

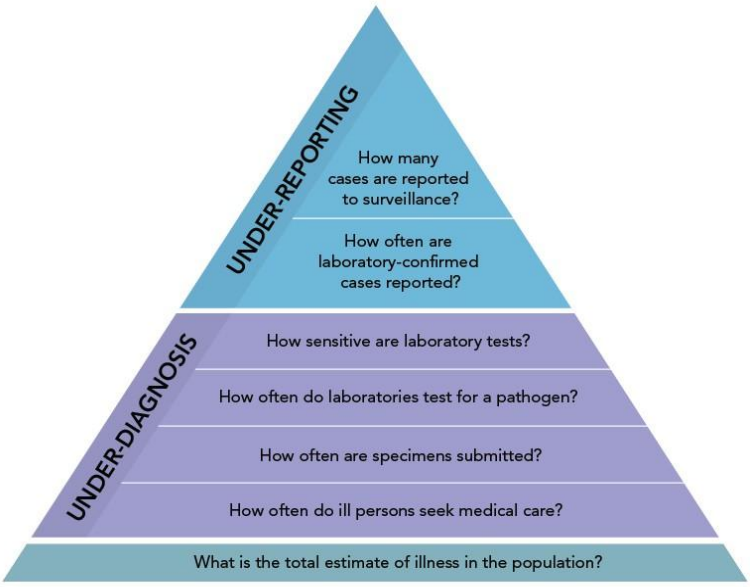
On the other hand, as an example, in the United States (US) the surveillance system is "active", based on a program, since 1996, and called Foodborne Disease Active Surveillance Network (FoodNet). FoodNet is a network that includes collaborative efforts from Centre of Disease Control (CDC), the US Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS), and the Food and Drug Administration (FDA).

FoodNet conducts surveillance at 10 US health departments for foodborne pathogens such as *Campylobacter spp*., *Cryptosporidium spp*., *Cyclospora cayetanensis*, Shiga toxin-producing *Escherichia coli* (STEC) O157, STEC non-O157, *Listeria monocytogenes*, *Shigella spp*., *Yersinia enterocolitica*, *Salmonella enterica* serotype *Typhi* and nontyphoidal *Salmonella spp*. (Scallan, 2007).

The US clinical laboratories are regularly contacted from FoodNet personnel (either weekly or monthly, depending on the size of the laboratory) to ascertain laboratory-confirmed cases occurring within their surveillance area. Those clinical laboratories are identified through state licensing lists and physician surveys, and are audited at least twice per year to ensure that all cases of disease under surveillance are ascertained and that changes in incidence are not a result of surveillance artifacts.

Without an active surveillance program it is difficult to have precise estimates of the human health burden of a foodborne illness in a specific country. With limited resources, assessing the burden of foodborne illness is difficult for regulatory agencies and policy-makers. This aspect has been revolutionized by adopting a paradigm known as the "Burden of Illness pyramid" (Figure 7).

Figure 7. The underreporting and underdiagnosis in a laboratory-based surveillance system capturing foodborne illnesses, represented in a "Burden of Illness pyramid" adapted from Thomas et al. (Thomas et al., 2013).



The several missing surveillance steps, necessary for a case to be ascertained by laboratory-based surveillance (seeking medical care, stool sample submission, and laboratory testing) until the reporting to a public health agency, that go undetected, can be extrapolated from lab-confirmed cases, from the top of the Burden of Illness (BoI) pyramid to estimate the overall burden of disease in the community, from the bottom of the BoI pyramid (Figure 4). Subsequently, the impact of a health problem in an area or population can be observed.

2. Objectives

The global aim of this study was to estimate the incidence of salmonellosis in the human Portuguese population.

The specific objectives were:

(1) To estimate the burden of illness of human salmonellosis in Portugal;

(2) To investigate the underreporting and underdiagnosis in the health system;

(3) To identify potential ways to improve the public health and health care system.

3. Materials and methods

3.1. Burden of Illness methodology

To estimate the incidence or number of human cases in a population, while accounting for the surveillance system gaps, e.g. the underreporting and under-ascertainment, we have applied a Burden of Illness model (BoI model). *Salmonella spp.* was selected due to its public health significance, better data availability in Portugal than for other pathogens and also because cases of disease are estimated to be largely foodborne.

3.1.1. Data requirements

To estimate the incidence of foodborne salmonellosis in the Portuguese population, required data included:

(1) The reported cases to existent surveillance (regional and provincial);

(2) Travel-related data;

(3) Proportion foodborne transmission.

3.1.2. Modelling approach

The approach used to estimate the annual number of salmonellosis cases in the Portuguese population, consisted on scaling up the laboratory-confirmed salmonellosis cases, while adjusting for the undercounts (surveillance system "gaps") represented by underreporting and underdiagnosis (Figure 8).

Figure 8. Modelling approach to estimate the total incidence of salmonellosis (adapted from Thomas et al., 2013). *

* Available at http://www.phac-aspc.gc.ca/efwd-emoha/efbi-emoa-eng.php#php

In order to estimate the total incidence of disease by Salmonella spp. we have computed multiplication factors that corrected the reported number of cases for underreporting and underdiagnosis by reconstructing the "Burden of Illness pyramid".

Portuguese data available were mostly from 2000-2009 and all estimates were based on the 2010 Portuguese population (10.5 millions).

3.1.3. Multiplication factors or multipliers

A multiplication factor or multiplier is the inverse of a proportion, calculated to account for the underreporting or underdiagnosis between subsequent surveillance steps in a BoI pyramid. These multipliers were represented in the model as the inverse of the proportion of care seeking, stool submissions, laboratory testing and tests sensitivity. Then they were applied to the annual number of reported *Salmonella* cases. The domestically acquired foodborne *Salmonella* cases in the Portuguese population were the annual number of reported *Salmonella* cases minus the travel-related cases, multiplied by the proportion of foodborne cases.

3.1.3.1. Underreporting multiplier

All laboratories are required to report notifiable disease to DGS (National Authority for Human Health). Since the number of reports from INSA and DGS are very similar, we assume in the model that Portugal reports all the notifiable salmonellosis cases and we set 1 for the underreporting multiplier. However, it is unknown if all cases tested and diagnosed are reported by the laboratories.

3.1.3.2. Underdiagnosis multiplier

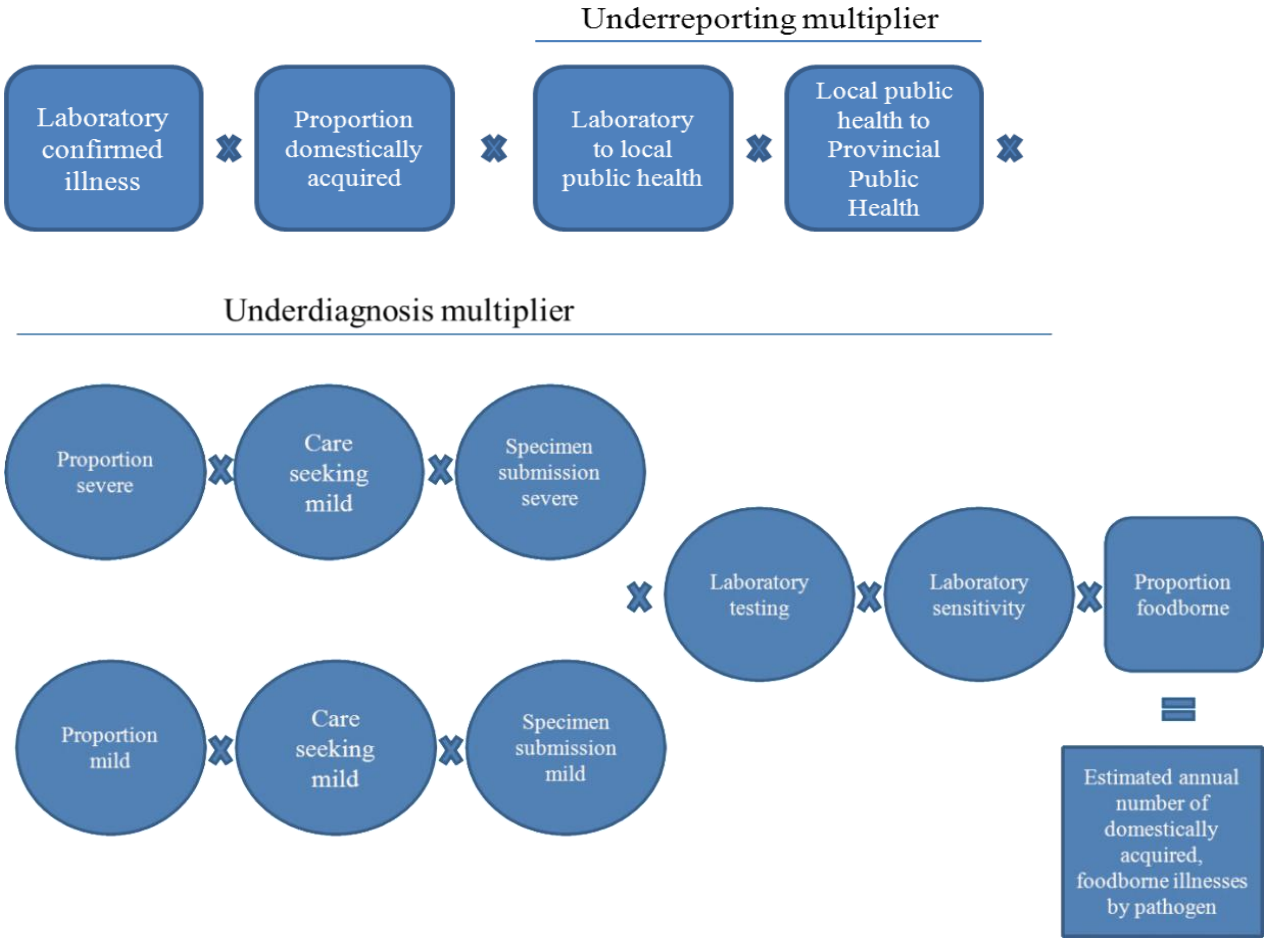
To adjust for underdiagnosis, taking into account the variations of all the surveillance steps in medical care seeking, specimen submission, laboratory testing and test sensitivity, we developed a multiplier for *Salmonella spp.*

To adjust for medical care seeking and specimen submission, we pooled data from our sources using expert elicitations and literature data from the US and French population

surveys (Scallan et al., 2011; Van Cauteren et al., 2012). These proportions were separated from laboratory confirmed infections of persons who had severe illness (e.g. bloody diarrhea) and mild illness (e.g. non-bloody diarrhea) and seek medical care and stool sample submission rates for bloody and non-bloody diarrhea, for severe and mild cases of salmonellosis. The percentage of laboratories that routinely tested for specific pathogens and test sensitivity were also corrected on the underdiagnosis multiplier. This way and through subsequent multiplications of all these ratios, the underdiagnosis multiplier for Portugal was 111.2.

Further multipliers were applied to scale the total community cases to the domestically acquired cases and foodborne cases (Figure 9).

Figure 9. Schematic of the general model used to estimate the number of cases of pathogens for which laboratory confirmed illnesses were scaled up (adapted from Thomas et al., 2013).



All multipliers were represented by probability distributions to account for the degree of uncertainty in them (Table 3).

Table 3. Distributions used for each model inputs and summary statistics

Model Inputs	**Minimum (min.); Most Likely (m.l.); Maximum (max.)**
Proportion of travel-related cases	Pert (0.07; 0.11; 0.15)
Proportion of severe cases	Pert (0.023; 0.17; 0.318)
Care seeking for severe cases (bloody diarrhea)	Pert (0.19; 0.35; 0.51)
Care seeking for mild cases (non-bloody diarrhea)	Pert (0.05; 0.10; 0.25)
Specimen submission for severe cases	Pert (0.077; 0.36; 0.62)
Specimen submission for mild cases	Pert (0.05; 0.10; 0.25)
Laboratory testing	Pert (0.94; 0.97; 1)
Laboratory test sensitivity	Pert (0.60; 0.70; 0.90)
Proportion foodborne	Pert (0.91; 0.94; 0.96)

3.1.4. Uncertainty analysis

To account for the uncertainty of the inputs and to compute the range of the final estimates, we run a Monte Carlo simulation with 100,000 iterations on the software @Risk 6 (Palisade Corporation).

Portuguese data were always used when available to select distributions. When data were scarce we used surrogate data from other countries and expert elicitations. All model inputs were defined as *Pert* distributions. Pert distributions are a type of beta distribution that allows specifying a minimum, a maximum and a most likely value.

In the model, minimum, maximum and most likely values were used and assumed to be within the 95% CI of (i) 7% - 15% for the proportion of travel-related cases, (ii) 2.3% - 31.8% for the proportion of *Salmonella* infections that caused bloody diarrhea, (iii) 19% - 51% for the proportion of infected persons who sought medical care for bloody diarrhea and 5% - 25% for non-bloody diarrhea, (iv) 7.7% - 62% for the proportion of general practitioners who asked for bacteriological exams on cases of bloody diarrhea and 5% - 25% for non-bloody diarrhea, (v) 60% - 90% for the sensitivity of the stool culture methods and 94% - 100% for laboratory testing, and (vi) 91% - 96% for the proportion of *Salmonella* foodborne infections (Table 4).

Table 4. Uncertainty analysis for "The Portuguese Model".

MODEL INPUTS	UNCERTAINTY	DATA SOURCES
Proportion of travel-related cases	7% - 15%	Surrogate data (Scallan et al., 2011)
Proportion of severe cases	2.3% - 31.8%	Portuguese data
Care seeking for severe cases (bloody diarrhea)	19% - 51%	Surrogate data (Scallan et al., 2011b)
Care seeking for mild cases (non-bloody)	5% - 25%	Expert elicitations
Specimen submission for severe cases	7.7% - 62%	Surrogate data (Scallan et al., 2011b; Van Cauteren et al., 2012)
Specimen submission for mild cases	5% - 25%	Expert elicitations
Laboratory sensitivity	60% - 90%	Surrogate data (Scallan et al., 2011b)
Laboratory testing	94% - 100%	Surrogate data (Scallan et al., 2011b)
Proportion foodborne	91% - 96%	Surrogate data (Scallan et al., 2011b)

In the final output, described in the model as "the total domestic foodborne salmonellosis cases", uncertainty is estimated by using the function "RiskPoint" of @Risk, and bounded by a 90% credible interval (CrI), with upper and lower limits that account for variability and uncertainty of the data. This means that 90% of the time the true value of the estimate falls within the upper and lower values.

The uncertainty in the model is expressed by the cumulative effect of the uncertainty that each model inputs encompasses.

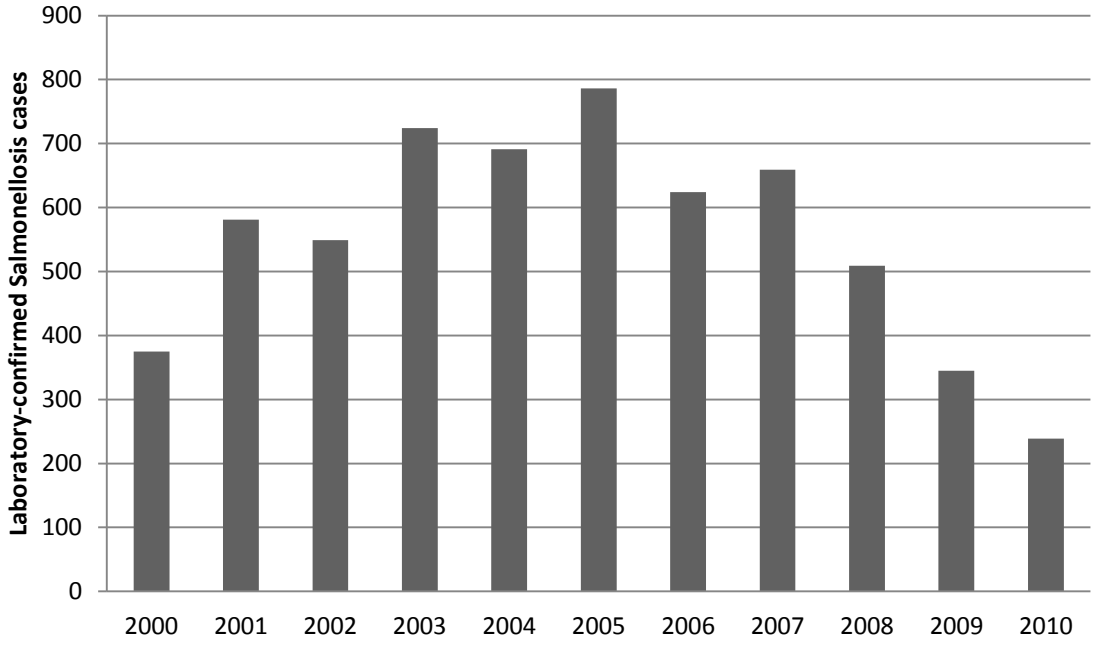
The following sections describe the approach used to estimate salmonellosis cases in Portugal.

3.1.5. Portuguese data sources

3.1.5.1. Portuguese Laboratory confirmed cases

Data for laboratory confirmed cases were obtained from the Portuguese laboratory-based surveillance from 2000 to 2010. Data were provided by INSA (INSA, 2013. http://www.insa.pt/sites/INSA/English/Pages/NationalHealthInstituteDoutorRicardoJorge.aspx assessed on May 2013). Between 2000 and 2010, INSA received 6082 stool samples from different laboratories of several regions of Portugal for serovar studies. To estimate the true incidence of foodborne and domestically acquired salmonellosis per year, it was required to know the total number of new cases of salmonellosis reported in Portugal. This was 239 cases in 2010 (Figure 10).

Figure 10. Total number of laboratory confirmed cases per year reported from 2000 to 2010 by the National Health Institute Doutor Ricardo Jorge (INSA, 2013).



3.1.5.2. Proportion of mild and severe cases

To estimate the proportion of patients with severe illness, data were collected from three scientific articles based on retrospective studies carried out at North Region (i.e. Oliveira de Azemeis, with patients of age groups ≤12 years old; Penafiel, with patients of age group ≤15 years old, but the majority of cases were on 1-3 years old) and Centre Region (i.e. Coimbra, with age groups ≤13 years old). These indicators were all generated for pediatric populations from 0 to 12 years old. Patients attended the Emergency Units of three

different hospitals: S. Miguel Hospital (Oliveira de Azeméis); Tâmega e Sousa Hospital (Penafiel); and Pediatric Hospital of Coimbra (Coimbra) (Figure 11).

Figure 11. Map of Portugal highlighting regions – North and Centre of Portugal – where data concerning the severity of cases of foodborne illness were collected.

The northern region of Portugal accounted for approximately 50% of the severe cases of salmonellosis during the period of 2000 until 2009 (Table 5). In Oliveira de Azeméis, from a total of 58 patients with Salmonellosis, only 17% show severe salmonellosis symptoms (Costa et al., 2004). In Penafiel, out of a total of 173 patients with symptoms characterized according to the level of dejections (diarrhea), 55 patients scored a "high" level of dejections per day, accounting for a proportion of 31.8% of salmonellosis severe cases (Almeida et al., 2012). In Coimbra, only 2.3% cases were of bloody diarrhea, from a total 30 salmonellosis cases (Rodrigues, Alves, Alves & Lemos, 2007).

These proportions of severe cases were used as data inputs in the model, accounting for uncertainty by defining a *Pert* distribution with a minimum of 2.3%, a most likely value of 17% and a maximum of 31.8%. Hence, it was estimated that in Portugal, the proportion (e.g., most likely value) of patients with severe cases of salmonellosis was 22%.

Table 5. Proportion of severe salmonellosis cases, according to different characterizations of severe symptoms, in pediatric population, from three different hospitals.

Portuguese Region	Symptoms	No of cases (%)	Total cases per Region (%)	References
North (Oliveira de Azeméis)	Mucous and/or bloody diarrhea	17	48.8	Costa et al., 2004
North (Penafiel)	High level of dejections (diarrhea) per day	31.8		Almeida et al., 2012
Centre (Coimbra)	Bloody diarrhea	2.3	2.3	Rodrigues et al., 2007

Assuming that most of the patients with mild symptoms do not seek a practitioner, the proportion of patients with mild cases was obtained, as on a simplified probabilistic method, by subtracting the proportion of severe salmonellosis cases (22%) to an assumed total of 100 cases. Therefore, the proportion of mild salmonellosis cases was 78% for the Portuguese population.

3.1.5.3. Medical care seeking (mild and severe)

Although, it was impossible to gather a panel of specialists and to implement formal expert elicitation methods, general practitioners from different health centers were asked (personal interview) for their personal opinion on care seeking behavior.

On the basis of their opinion, we estimated the pathogen-specific proportions of patients with laboratory-confirmed salmonellosis who had severe illness (e.g., bloody diarrhea) and then we used it as surrogates for severe and mild cases of salmonellosis.

3.1.5.4. Sample submission

Since Portuguese data were unavailable, data related to stool sample submission for severe cases were based on surrogate data from a French population survey, conducted by telephone between May 2009 and April 2010 (Van Cauteren et al., 2012) and on the US FoodNet survey, performed from 2000 to 2007 by the active surveillance system (Scallan et al., 2011b). The French study included 10,080 persons, from which 559 individuals reported 596 episodes of illness with gastrointestinal symptoms (diarrhea or vomiting), plus their description of healthcare-seeking behavior for acute gastroenteritis (AG). This data allows for a more accurate interpretation of the information derived from existing

provider-based AG surveillance systems (Van Cauteren et al., 2012). 1 out of 3 cases consulted a practitioner for their illness. They also concluded that prolonged cases and cases affecting young children were more likely to consult a practitioner. Van Cauteren et al. (2012) found that only 7.7% of the patients were requested for a stool sample. This proportion shows the magnitude of underestimation of the true burden of AG via provider-based and laboratory-based surveillance systems. A high underreporting level was detected for cases aged between 30-64 years in provider-based surveillance systems, since this age group seeks less medical care than children and the elderly (Van Cauteren et al., 2012).

US estimates were based on the proportion of survey respondents who submitted a stool specimen among persons with bloody diarrhea who sought medical care. This value was 36% (stool sample submission rates for bloody diarrhea) according to Scallan et al. (2011). The US estimates were higher than the French. These differences may be due to different surveillance systems for foodborne pathogens, for example the US has an active surveillance system covering approximately 15% of the US population, or due to different methods used to correct for underdiagnosis, or both the mentioned situations

Specimen submissions for mild cases are expected to be lower than for the severe ones. These estimates were based on expert elicitations by the head of the Department of Clinical Analysis of the Hospitals of the University of Coimbra (HUC) and also from the interviewed general practitioners. The cases of mild illness with submissions of stool samples were about 10%, although opinions varied between 5% and 25%.

3.1.5.5. Proportion of Laboratory samples tested

Surrogate data for the proportion of collected samples that were tested for *Salmonella spp*. were retrieved from Scallan et al. (2011), whom found that 93% of the samples were tested for *Salmonella spp*.

3.1.5.6. Laboratory test sensitivity

Laboratory methods to isolate and to identify *Salmonella* isolates are standardized, and sensitivity and specificity are likely to be similar across countries, namely on reference laboratories.

We used Scallan et al. (2011) data as surrogate for Portugal. The authors identified variations in specimen collection, specimen transport procedures and considered potential laboratory errors. According to their studies, the stool sample culture method was

estimated to be 70% sensitive for detecting *Salmonella* from patients with bloody and non-bloody diarrhea, severe and mild cases of illness.

3.1.5.7. Proportion of travel-related cases

Data on patients that got sick while travelling abroad were incomplete for the Portuguese population. A study published in 2009 on the Journal of Travel Medicine (Silva et al., 2009) estimated the incidence and the risk factors for traveler's diarrhea in Portuguese-born children between August 2002 and May 2007. "Diarrhea" was defined as a change on the usual stool consistency with one or more unformed bowel movements (Silva et al., 2009). Most of non-European travels were to Portuguese-speaking countries, such as Angola, Mozambique, Cabo Verde, São Tomé, Guinea-Bissau and Brazil. Out of 174 travelers, 38 had diarrhea (21.8%) (Table 6).

Table 6. Travel-related diarrhea cases collected at D. Estefânia Hospital from August 2002 to May 2007 (Silva et al., 2009).

		No of travel-related cases according to years of age and country of destiny				
Country	**No of travelers**	0-2y (N=75)	3-6y (N=50)	7-14y (N=47)	15-20y (N=8)	**Total**
Angola	34	10	5		1	16
Guinea-Bissau	9	1	1	1		3
India	19	5		1		6
S. Tomé	21	1		2		3
Mozambique	22		2			2
Cabo-Verde	12	1				1
Brazil	12	1				1
Other countries	57	5	1			6
Total	174	24	9	4	1	38

Comparing the Portuguese estimate for traveler's diarrhea with other international published studies that report proportions of travel-related cases for salmonellosis between 11% and 26% (Table 7), the value 21.8% was considered higher than a *real* value for traveler's diarrhea caused only by *Salmonella spp*. Silva et al. (2009) measured all types of traveler's diarrhea without distinguishing *Salmonella* cases.

Table 7. Proportion of travel-related cases from abroad studies (adapted from the New Zealand study of Cressey et al., 2011).

	Proportion of travel-related cases (%)				
Country	New Zealand[1]	US[2]	Canada[3]	Netherlands[4]	England and Wales[5]
Salmonella spp.	18	11	26	14	12

[1](Lake et al., 2010); [2](Scallan et al., 2011); [3](Thomas et al., 2013); [4](Havelaar et al., 2008); [5](Hall et al., 2005); [6](Adak et al., 2002)

This way, and assuming that the Portuguese proportion of travel related salmonellosis may be similar to Netherlands (14%) and/or to England and Wales (12%), and considering that in 2010 the tendency to get salmonellosis while travelling abroad is to diminish and that the US estimates are the lowest from the literature review, data from US surveillance system was used as surrogate for Portugal. The proportion of travel-related cases of *Salmonella* infection, described as cases reporting travel outside the US within seven days of illness onset, was 11%.

3.1.5.8. Proportion of foodborne cases

Information on the proportion of cases transmitted through food was also not available from Portuguese data.

Foodborne transmission can be accessed through source attribution studies and it has been estimated for few countries worldwide (Pires, 2013). Several studies from England and Wales (Adak et al., 2002), Australia (Hall et al., 2005), the Netherlands (Havelaar, Galindo, Kurowicka & Cooke, 2008), France (Vaillant et al., 2005), the US (Scallan et al., 2011b), Canada (Thomas et al., 2013) and New Zealand (Cressey & Lake, 2011) estimated the proportion of foodborne salmonellosis. These estimates varied between 60% and 94% (Table 8).

Table 8. Mean proportion of foodborne salmonellosis cases obtained from other studies estimates (adapted for Salmonella spp. from New Zealand study by Cressey et al., 2011).

	Mean proportion foodborne salmonellosis cases (%)					
Country	New Zealand[1]	US[2]	Canada[3]	Netherlands[4]	Australia[5]	England and Wales[6]
Salmonella spp.	60	94	80	64	87	92

[1](Cressey et al., 2011); [2](Scallan et al., 2011); [3](Thomas et al., 2013); [4](Havelaar et al., 2008); [4](Adak et al., 2002)

We decided to use surrogate data from Scallan et al. (2011) to represent the proportion of Salmonella infections that were caused by food in Portugal. Since this is a recently reviewed publication we chose US's value, and also because Scallan's estimates were

obtained from surveillance data (Scallan et al., 2011), contrary to some studies that based their estimates on expert elicitations and literature comparisons.

3.1.5.9. Model parameters

The model consists of sets of country-specific and pathogen-specific parameters. Table 9 provides an overview of these parameters.

Table 9. Parameters used in the "pyramid" reconstruction model (adapted from Haagsma et al., 2012).

Symbol	Description	Source/Distribution
Country-specific parameters		
N	Total population per year	Data
n_R	Number of reported cases per year	Data
t	Probability of being infected with *Salmonella spp.* while travelling abroad	Pert (t_1;t_2;t_3)
n_D*	Number of cases that got infected within the country	$n_R - t$
a	Probability of a patient to have a: • Severe case	Pert (a_1; a_2; a_3)
	• Mild case	$1 - a$
b	Probability of seeking medical care with a: • Severe case (bloody diarrhea)	Pert (b_1; b_2; b_3)
c	• Mild case (non-bloody diarrhea)	Pert (c_1; c_2; c_3)
d	Probability of submitting a stool sample of a patient with: • Severe case	Pert (d_1; d_2; d_3)
e	• Mild case	Pert (e_1; e_2; e_3)
f	Probability of being infected with *Salmonella spp*. through food consumption	Pert (f_1; f_2; f_3)
Pathogen-specific parameters		
g	Probability of testing for *Salmonella spp.* in stool samples for patients visiting a GP	Pert (g_1; g_2; g_3)
h	Sensitivity of laboratory analysis for *Salmonella spp*.	Pert (h_1; h_2; h_3)
M	Multiplier	$\frac{1}{(a * (1-a) * b * c * d * e * f * g * h)}$

GP, General Practitioners;
* Domestic salmonellosis cases

All parameters followed *pert* distributions, and all were based on different data sources as described above. The combination of country-specific and pathogen-specific parameters results on an overall multiplier (*M*).

4. Results

Estimates were developed from stochastic models, using probability distributions to describe a range of plausible values for all model inputs, thus reflecting its uncertainty.

The final output, excluding the number of *Salmonella* cases acquired during international travel and the proportion transmitted by food, was the number of *Salmonella* cases that were domestically acquired and foodborne. This output was estimated in the model as a point estimate with 90% CrI and integrated the overall multiplier used to adjust for underdiagnosis and underreporting in Portugal.

4.1. Total *Salmonella spp.* human cases in Portugal

We estimated that 22,207 cases (90% CrI: 11,476 – 35,956) were domestically acquired and were foodborne salmonellosis cases occurred in Portugal in year 2010. These estimates are shown on Table 10.

Table10. Model final estimates for 2010.

Pathogen	Total cases	Total domestic cases	Total domestic foodborne
Salmonella spp.	23,676 (90% CrI: 9,972 – 42,327)	1,459	22,207 (90% CrI: 11,476 – 35,956)

4.1.1. Total domestically acquired and foodborne salmonellosis cases

Of the total number of cases of illness due to a particular microbial organism, a percentage of the cases may be acquired in another country (travel-related). There is also potential for infection to be acquired from a variety of sources other than food (e.g. water, animal contact or infected people). In order to determine the proportion of the total illness that is domestically acquired and due to food, it is necessary to have an estimate of the percentage of cases that are travel related and the percentage of cases that were acquired from food, rather than another source.

From the average Portuguese population of 10,5 million (10,511,523 million), the domestically acquired and foodborne salmonellosis cases were estimated to be within the 90% CrI of 11,476 – 35,956, with an average value (using the function "RiskMean") of 22,207 cases per year.

4.1.2. Incidence of *Salmonellosis* in Portugal

The incidence of domestic acquired salmonellosis cases in Portugal was estimated to be 184.91 cases per 100,000 habitants in 2010.

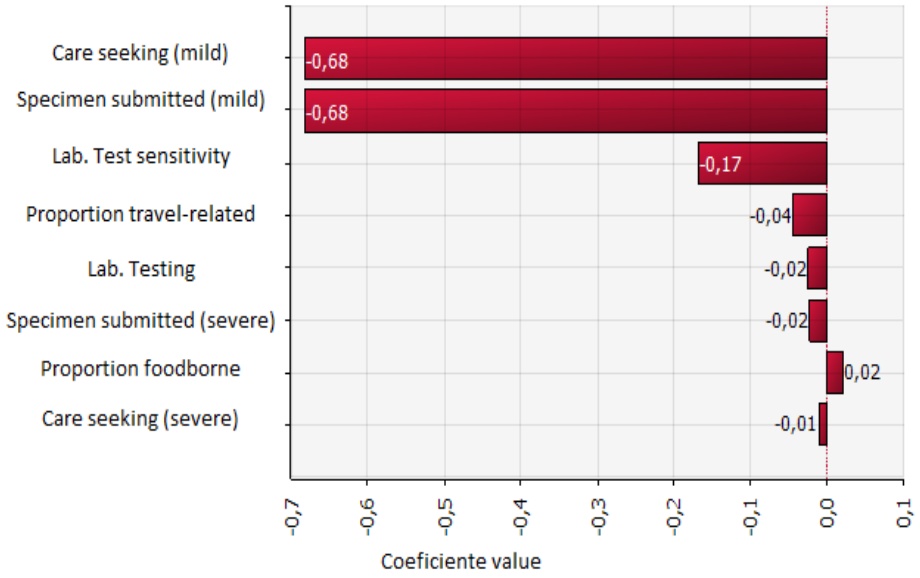
4.2. Sensitivity analysis

A sensitivity analysis was conducted to examine the impact of changes in the model inputs.

The variables investigated were (1) care seeking on severe and mild cases, (2) specimen submission on severe and mild cases, (3) proportion of travel-related cases, (4) proportion of foodborne salmonellosis, (5) laboratory testing, and (6) the sensitivity of the laboratory test.

We have used a "tornado" analysis (@Risk, Palisade corporation), where the effect of the variables on the output is represented by the coefficient value (Figure 12).

Figure 12. Sensitivity analysis illustrated in a "tornado" type of graph showing the main sources of uncertainty (@Risk 6, Palisade Corporation).



A negative coefficient represents that the bigger the variable the less the multiplier, because the multiplier is estimated as the reverse of all multiplied variables. From the sensitivity analysis, it was observed that the input values used for "care seeking of mild cases" and "specimen submission of mild cases" influenced mostly the multiplier, increasing its value and therefore increasing the incidence of Salmonellosis cases.

The range of estimates in the sensitivity analysis is represented on Table 9. It is based on a Pert distribution in which the output changed for: (1) the proportion of travel related cases with 21,483 - 22,962 domestic and foodborne cases; (2) the proportion of infected patients who sought medical care for severe cases with 21,945 - 22,408 domestic and foodborne cases; and (3) mild cases with 12,964 - 36,475 domestic and foodborne cases; (4) the proportion of practitioners who demanded cultures on severe cases with 21,825 - 22,681 domestic and foodborne cases; (5) mild cases with 13,008 - 36,428 domestic and foodborne cases; (6) the sensitivity of stool culture methods with 19,478 - 25,091 domestic and foodborne cases; (7) the frequency of laboratory testing for *Salmonella* with 21,668 - 22,541 domestic and foodborne cases; (8) the proportion of salmonellosis acquired through food with 21,752 - 22,552 domestic and foodborne salmonellosis cases (Table 11).

Table 11. Output lower and upper limits for each input distributions (Pert distributions), using @Risk software (Palisade Corporation).

		Change in output statistics for total domestic foodborne cases (point estimates for 90% CrI)	
Model variables	**Pert distribution (max; most likely; min values)**	**Lower limit**	**Upper limit**
Proportion of travel-related cases	7; 11; 15 %	21,483	22,962
Care seeking (severe cases)	19; 35; 51 %	21,945	22,408
Specimen submission (severe cases)	7.7; 36; 62 %	21,825	22,681
Care seeking (mild cases)	5; 10; 25 %	12,964	36,475
Specimen submission (mild cases)	5; 10; 25 %	13,008	36,428
Laboratory test sensitivity	60; 70; 90 %	19,478	25,091
Laboratory testing	94; 97; 100 %	21,668	22,541
Proportion of foodborne salmonellosis cases	91; 94; 96 %	21,752	22,552

4.2.1 Scenario analysis

The following alternative scenarios were carried out based on the sensitivity analysis. Three input distributions had the most relevant impact on the final output: (1) the proportion of travel-related salmonellosis cases; (2) the care seeking and (3) specimen submission for the mild salmonellosis cases (Table 12).

Table 12. Alternative scenarios for the model input distributions and their impact on the output.

Model variables	Domestic and foodborne cases of Salmonellosis per year for alternative scenarios (95% CrI)	Coefficient value for each alternative scenario
Baseline	22,207	0
Proportion travel-related cases (max=0.218)	21,930	-0,0055
Care seeking for mild cases (max =0.30)	21,306	-0,0180
Specimen submitted for mild cases (max=0.30)	21,292	-0,0183

On the first scenario, it was assumed that the maximum value for the input distribution of the proportion of travel-related cases were those published for the travelers of Portuguese pediatric population, with a proportion of 21.8% of travel-related diarrhea. The estimated number of domestic and foodborne salmonellosis per year decreased from 22,207 to 21,930 (95% CrI).

On the second scenario, it was assumed that a maximum of 30% of patients with mild illness sought medical care, in contrast with the 25% used on the model. The estimated number of domestic and foodborne salmonellosis cases was reduced from 22,207 to 21,930 (95% CrI).

On the third scenario, it was assumed a maximum of 30% of patients with mild salmonellosis that submitted a stool sample for bacteriological diagnosis, instead of the 25% entered into the model. The estimated number of domestic and foodborne salmonellosis cases was 21,292 in comparison with 22,207 (95% CrI).

5. Discussion

This *Burden of Illness* (*BoI*) study is the first attempt to quantify the foodborne *Salmonella spp.* burden in Portugal.

BoI studies are considered of great importance, particularly when public health surveillance information is scarce or incomplete. *BoI* studies take a simple approach to estimate the overall impact of an illness in a population and have been used to improve the understanding of the clinical and economic impact of a disease. Additionally, these studies may be complemented with cost-benefit studies, to define where resources should be allocated within the food chain, to reduce the burden of illness in the population in the most efficient way.

In this thesis, we applied a BoI model to assess the public health impact of salmonellosis in the Portuguese population.

Firstly, by exploring the health impact of salmonellosis, including assessment of the country incidence and the proportion of severe cases of the illness, BoI analysis allow for the estimation of disease incidence even if data are lacking. Secondly, BoI analysis afford an additional insight into the frequency and consequences of salmonellosis by assessing the use of health-care resources and detailing the components involved. This information is policy-relevant in the sense that it increases awareness and may provide a better understanding of the specific contribution of salmonellosis to the overall health-care system, of the impact of each surveillance step to the health-care system and the particular role of every intervenient (general practitioners, patients, laboratories), crucial to the management and the control of the zoonosis.

Moreover, epidemiological results derived from BoI studies are very useful to predict future trends when, for example, they are combined with demographic projections. For example, ageing populations or high level of pediatric populations may be responsible for a significant increase on salmonellosis incidence, since they are usually the major immunocompromised groups and thus more affected by pathogens.

It should be taken into account that the number of people infected varies, in each country or region, among other reasons, according to cultural consumption patterns, age distribution patterns, season and the quality of the national health system of the country. For instance, peaks on the number of reported *Salmonella* cases normally occur in August-September with a rapid decline in winter months (EFSA, 2012). This trend is common for

all age groups supporting the influence of temperature on the multiplication of bacteria in foods and the environment (EFSA, 2012).

In Portugal, the number of domestically acquired foodborne cases of salmonellosis in 2010, after applying the underdiagnosis and underreporting multipliers, was estimated as 22,207 (90% CrI: 11,476-35,956) which corresponds to an incidence of 189.91 cases per 100,000 habitants.

This incidence estimate is approximately 100 (95% CrI: 52.04-111.3) times higher than the reported from the laboratory-based surveillance.

The major constraint for this study was data availability, above all concerning the number of reported salmonellosis cases and the proportion of patients that seek medical care. Therefore, it was necessary to make several assumptions: (i) most of the patients that sought medical care were assumed to suffer from a severe episode of salmonellosis; (ii) although data for the frequency of severe cases of salmonellosis was collected in the North and Centre regions it was assumed to be representative of country regions; (iii) we assumed that the standards of health and surveillance systems of the US and France, from which we used surrogate data, were similar; (iv) expert elicitations were used to cope with data gaps.

Mild manifestations of salmonellosis were less detected and notified in the model final outcome because we assumed that most of the patients who seek medical care at the hospital or at a medical center have severe episodes of salmonellosis.

To overcome the barrier of lack of data for mild cases, expert elicitations were applied as reliable solution. According to the expert opinion of practitioners of the North and Centre regions of Portugal, mild cases account for 10% of the total cases of salmonellosis. This elicitation, when compared with other studies estimates, was considered to be valid for Portugal. Yet, there are factors that condition the elicitation design and execution that may influence considerably the results, such as: (i) the types of uncertainties of the inputs; (ii) the use of elicited information and (iii) the resources available (time, money). In general, the major limitations of expert elicitations can be described as follows: (1) overconfidence; experts might be overconfident in their ability to provide an answer when faced with known questions; (2) data availability: the occurrence of rare events may be overestimated, whereas the occurrence of common events may be underestimated; (3) anchoring and adjustment: experts when asked a series of questions, might give an adapted answer when

regarding the question answered previously, which might not occur if questions are asked independently, and (4) representativeness bias, which applies when a series of events, either of random or non-random basis, are evaluated with subjective judgments of whether this sequence appears to be random or not.

Despite the verified lack of data for all regions in Portugal and the fact that most population is situated in Littoral zones (e.g. Lisbon), data for the frequency of severe salmonellosis cases was obtained mainly from the North and Centre regions of the country and the model uses these data as representative of the national population. However, other studies, e.g. the US, revealed that surveillance was performed for only 10 sites of the country and, despite this, also considered it to be a good representation of the population.

Also, most data for *Salmonellosis* cases obtained were from children and youngsters, ranging especially from 0 to 12 years. These represented all population. However, most Salmonellosis cases are more frequent on children, youngsters and elderly or immunocompromised people.

Assuming other country's data as surrogate for Portugal, in this study US and France, needs to be viewed with caution, because surrogate's data includes variables that are country-specific and its direct application to the Portuguese population may lead to distorted conclusions. Nevertheless, the US model has been reviewed several times since their first estimates and we considered it to be a reliable source of inputs to our BoI. French data was used mostly due to the diversity of consumptions patterns of the French consumers that were assumed to be similar of the Portuguese consumers.

Having in mind all these assumptions, the development of a sensitivity analysis was necessary to comprehend how they would interfere with the output and which variables had the greatest impact on the final estimates. This analysis showed that the most important sources of uncertainty were the proportion of patients that sought medical care and that were asked to submit a stool sample, and the proportion of foodborne salmonellosis cases.

Furthermore, data from laboratory surveillance, published data and expert elicitations were used to construct sequential multipliers, which *per se* constitute another source of uncertainty.

The underreporting multiplier was set to be of 1, similarly to the US multiplier for bacterial pathogens, assuming that all laboratory surveillance reports to DGS (National Authority for Human Health) represent all the notifiable salmonellosis cases in the country.

Portugal has a laboratory-based surveillance system where much information is lost throughout the surveillance pyramid. As a consequence, not all salmonellosis cases are detected. Although it is unlikely that public health agents and policy makers can keep track of every single case of salmonellosis, it is also unknown whether a patient with acute gastrointestinal disease in Portugal is more or less likely to present to the medical system and potentially become a case notification.

As for the underdiagnosis multiplier of 111.2, it is important to acknowledge that it is unknown whether factors embodied in the underdiagnosis multipliers (the proportion of severe cases, the probability of severe and mild cases presenting to the medical system, sensitivity and specificity of the laboratory testing system) are applicable to Portugal.

Also, to estimate the underdiagnosis multiplier a number of *Pert* distributions were sequentially applied, with wide credible intervals of 95%, which implies a high level of uncertainty. The fewer data values we have, the broader a distribution becomes and more uncertainty follows. *Pert* distributions were chosen because they are considered the most suitable to express expert elicitations (Vose, 2008), because they are less strict than triangle distributions and especially to avoid the over consideration of the values of the distribution tails.

Despite all, the underdiagnosis multiplier is a representation of the laboratory-based surveillance system and most of all the lack of data concerning mild cases of salmonellosis in the Portuguese population, which is also observed in other countries, from US (Scallan, Griffin, Angulo, Tauxe & Hoekstra, 2011a), Canada (Thomas et al., 2013) to European countries such as France (Van Cauteren et al., 2012), and therefore accepted.

Previous studies conducted in different countries estimated multipliers to correct for underreporting and underdiagnosis of *Salmonella spp*. These estimates vary substantially (for example from 5 in the UK to 51 in Greece) as vary the methods used to compute them (Table 13).

When we compare our estimates with results from studies conducted in countries considered similar to Portugal (e.g. Mediterranean countries), our estimates are higher than the Greek for *Salmonella spp*. These differences may be explained by variations in the

surveillance system, for example in Greece the reported cases of illnesses that were mainly foodborne were for the larger part collected from the Hellenic Statistical Authority (ELSTAT) and the Hellenic Centre for Infectious Diseases Control (HCIDC). HCIDC is representative of hospitalizations or visits to practitioners and are a combination of laboratory-confirmed and symptom-based cases as it collects information on: (1) notified cases from hospital microbiologic laboratories and district health authorities; (2) performs active surveillance on gastroenteritis through physicians' reports (Gkogka et al., 2011).

Also for Denmark, when comparing estimates for countries considered similar (e.g. northern European), Danish estimates are slightly higher than the UK for *Salmonella* spp., and generally similar to the Dutch estimates. These disparities may be explained by differences in the surveillance programs (for example, surveillance in the Netherlands does not cover the entire population) and on the methods used to correct for underreporting which varied substantially (Table 13).

Table 13. Overview of multiplication factors estimated to correct for underreporting of *Salmonella spp* (Pires, 2014).

Country	**Study**	**Multiplier (Multiplication factor)**
		***Sallmonella spp*.**
Denmark	Havelaar et al., 2012	4.4
Greece	Gkogka et al., 2011	51.45 (3.2 – 99.7)
UK	IID2, 2012	4.7 (1.2 – 18.2)
Denmark	Haagsma et al., 2012	24.7 (5.2 – 64.7)
Japan	Kubota et al., 2011	74 (35.8 – 140.7)
Australia	Hall et al., 2008	13.06 (6.37 – 67.83)
Canada	Thomas et al., 2013	12.7
US	Scallan et al., 2011	29.3
Netherlands	Bouwknegt et al., PC*	7.2
New Zealand	Cressey et al., 2011	29.3
Denmark	Pires, 2014	7.2
Portugal	(our study)	**111.2**

*PC: Personnal Communication; non-published.

Havelaar et al. estimated the true Swedish incidence of salmonellosis in 2009 (i.e. the number of new cases in a specified country) by combining several data sources, with a set of simplifying assumptions. Since Sweden is acknowledge to be a "free-*Salmonella*" country, the main objective was to estimate the incidence of *Salmonellosis* cases for the Swedish population obtained while travelling abroad. Data were extracted from the Swedish infections disease surveillance system and relied on laboratories and practitioners reporting diagnosed cases to the Swedish Institute for Communicable Disease Control. The

relative risks to Swedish travelers are predictive of risks for the local population. The risks in Swedish travelers were estimated by combining two databases, both containing data at country level: the reported cases of Salmonellosis in travelers returning from destination countries and the number of journeys undertaken to these countries, respectively (Havelaar et al., 2012). The true disease incidence rate (i.e. the number of incident cases in 2009 divided by the population at risk in each country) was then estimated by expressing all risks to Swedish travelers concerning the risk of travelling to The Netherlands, and multiplying this relative risk with the incidence rate from a population-based study from The Netherlands. The disease incidence was estimated by multiplying the incidence rate with the population in each country. The underreporting factor per country was calculated as the ratio between the estimated true incidence and the reported incidence.

Havelaar et al. (2012) estimated that the true incidence rate was 4,310 cases per 100,000 inhabitants. The underreporting factor or multiplier was estimated at 57.5 (95% CrI 9.0 – 172), but ranged from 0.4 for Finland to 2,080 for Portugal (Havelaar et al., 2012).

This way, when comparing the BoI Portuguese incidence of 189.91 cases per 100,000 inhabitants with the incidence estimated by Havelaar et al. (2012) of 4,310 cases per 100,000 inhabitants, we found a major model gap, mainly due to the fact that Havelaar et al. did not use data from the Portuguese population and estimated those cases by extrapolating the Swedish cases, and therefore there is an overestimation of number of *Salmonellosis* cases in Portugal. This way, we consider that our improvements on data sources and the adjustments we made on the multipliers led to less uncertainty in our model. Therefore, the *BoI* Portuguese model seems to be a more realistic representation of the country's surveillance system for human salmonellosis.

Furthermore, when comparing the Portuguese incidence of 184.91 *Salmonellosis* cases per 100,000 inhabitants with the Danish incidence of 165 *Salmonellosis* cases per 100,000 inhabitants, we acknowledge that our model is valid and not very far from the reality. Despite the differences between the two countries in terms of food consumption patterns (Mediterranean habits vs Nordics habits); surveillance systems: Denmark's surveillance system for foodborne pathogens integrates human health with animal health and information is easily collected and organized, whereas the Portuguese one is mainly a laboratory type of surveillance system, e.g. diseases are diagnosed and reported but there is a deficient correlation between Animal and Human Health Authorities.

Other designs of Burden of Illness studies, such population surveys, would be a possible alternative to overcome data limitations and improve surveillance system, since they may provide a history of the population's behavior towards an illness. They are capable of monitoring the incidence of foodborne diseases of local and national public health importance, either performed by age and gender groups and/or by mild/severe manifestation of the illness, being a reliable source to compare data available for salmonellosis and to state a correlation between laboratory-confirmed cases and the surveillance's notification system.

It is a fact that data are scarce and often very difficult to locate, thus economic and political issues arise to this matter, and so if a country such as Portugal that has the double of tourists than its actual population and considering the impact of foodborne outbreaks, should a country communicate its real number of salmonellosis cases?

6. Conclusion

Since this was the first BoI study for Portugal, the current model outputs relied upon important assumptions. While its methodology is sound and appropriate, data to inform some of our model parameters were sparse. This led to a certain amount of uncertainty on our estimates, which may be reflected or not within our credible intervals. An update of this study with Portuguese data would be very useful.

Despite all, the final results of this study reflect the Portuguese surveillance system for human salmonellosis. Therefore they may help DGAV to reformulate Public Health policies and investments. Moreover they may be used to anchor other epidemiological analysis (e.g. evaluation of the economic impact of foodborne diseases in the Portuguese population) and this BoI study is an excellent example of the potential behind the cooperation between public health, veterinary and food safety experts to improve control and surveillance-based strategies for food borne diseases.

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