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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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2018

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Acknowledgements

I would like to thank, firstly, to Professor Telmo for introducing me to Epidemiology, risk analysis, R and, finally, to Sara. For making it possible for me to undertake an interns
To Sara for being the best supervisor one could wish for. For receiving me in Copenhagen with friendship, making it feel like home. And for being such an inspiration as a researcher. Thank you for all the knowledge and all the time you spent making me a more enlightened person.
To all the Risk Benefit Group at DTU for a warm welcome and for the sharing of knowledge. A special thank you to Maarten for accepting me in his risk assessment course and for being such a great teacher.
To Steen Ethelberg at SSI for helping me gather sound information to develop this study.
To Letícia for all the good times spent throughout Copenhagen. For sharing our Portuguese/Brazilian office. I, Sara and Letícia made Portuguese the most spoken language at DTU! Essentially, for making Copenhagen such a warm place with her Brazilian ways. Thank you also to Michael, Jan, Miguel and João for the fun times.
I will always be thankful for all the friendships college brought me. To Fábio for being there in the best and worst of times. For all the time spent roaming through college and through Lisbon, the best adventures ever. To Ingrid for teaching me the word “proactivity” and for being such a great example of it. To Lara, Chambel, Adriana, Bia, Neves and Patrícia for making college worthwhile.
To my Podin friend, Joana, for being the amazing person she is. For the shared love of Ireland, Star Wars, travelling, “things that are so bad it kind of make them good” and our annual little visits to each other. Thank you for all the fun, mostly fun, we had in the last… Let’s say couple of years so we do not sound old.
To Rosário for being my friend since middle school, for the shared love of books, all the great times and also for always making me see reason.
To my childhood friends: Marina, Ana, Salomé, Joana, Inês, Daniela e Patrícia. If you are still here, I must have been doing something right!
Now, for the most important people in my life, my family, I am afraid I am not a good enough writer to give thanks, but I will try.
To my amazing parents for being the most incredible human beings, for supporting me, always, and for giving me everything I needed (even if I thought I did not). The biggest thank you the world has ever seen.
To my sister, Teresa, for being like a second mom, I know I am a high maintenance baby! To my brother, Afonso, for teaching me the meaning of patience (he will love this one) and being a more or less bearable big brother. To Filipa, the new addition to the family, my sister in law. Thank you for all the support and for being a true sister (also, for putting up with my brother!).
To my grandmother, Maria, for being the best example of a woman I could possibly have had. To all my other grandparents, I had an amazing childhood and I miss you all. To my aunt for always being there.
And finally, to my four-legged family: Pastor, Larbos and Fusca. Love is not even enough to describe it.

Abstract

Burden of disease of four foodborne pathogens: a harmonized approach in Denmark

Consumption of contaminated food products and exposure to a variety of health hazards can lead to a wide spectrum of foodborne diseases (FBD). The true impact of these diseases is still unknown worldwide. Burden of illness (BoI) and burden of disease (BoD) studies can be developed in order to help decision makers implement intervention and control measures to improve food safety systems.

This thesis describes an integrated model to estimate the public health impact of four zoonotic foodborne pathogens in Denmark in 2016 – *Campylobacter* spp., *Salmonella* spp.*, Yersinia enterocolitica* and verocytotoxin-producing *Escherichia coli (VTEC).*

The applied model consisted of two general components: a BoI study, which was applied to estimate total incidence of these pathogens in the Danish population, accounting for underdiagnosis and under-reporting; and a BoD study, which built on the first and estimated disease burden in terms of the disability adjusted life year metric (DALYs). It also describes the model developed to estimate the BoD of yersiniosis – the first developed in Denmark and in Europe.

Total incidence estimates point to 66,202 cases of illness, with *Campylobacter* contributing the most (51,225 cases), and *Yersinia* the least (1,860 cases). The total BoD is 2,290 DALYs. Ranking in first place with the highest burden is campylobacteriosis, followed by salmonellosis, yersiniosis and VTEC infections, with 30, 8, 1 and 0.8 DALYs per 100,000 inhabitants, respectively. Gastroenteritis was the sequela which born the highest burden, when compared to long-term sequelea.

Total incidence estimates for all four pathogens show that children under five years old have the highest incidence when compared to other age groups, while BoD estimates regarding *Campylobacter* and *Salmonella* show the highest burden on elderly people, which can be explained by the high number of fatal cases estimated for that age group. Still, those two diseases have a considerable high burden on young children, as does yersiniosis and VTEC infections.

Differences in methodological approach used to estimate total incidence and the BoD makes comparison among countries difficult. The burden of these preventable diseases is still considerable, even in developed countries like Denmark. Understanding the contribution of each cause to the burden of FBD and incorporating estimates into policy development worldwide will enable efficient and effective interventions and improvements throughout all the food chain.

Keywords: Burden of Illness, Burden of Disease, DALYs, *Y. enterocolitica, Campylobacter, Salmonella*, VTEC.

Resumo

Impacto na saúde pública de quatro agentes patogénicos de origem alimentar: uma abordagem harmonizada na Dinamarca

O consumo de alimentos contaminados e a exposição a fatores de risco pode causar um alargado espectro de doenças de origem alimentar. O verdadeiro impacto destas doenças é ainda desconhecido. Estudos de *burden of illness* (BoI) e *burden of disease* (BoD) podem ser desenvolvidos, facilitando a implementação de medidas de intervenção e controlo por parte das autoridades, com o intuito de melhorar os sistemas de segurança dos alimentos.

Esta tese descreve um modelo integrado para estimar o impacto na saúde pública de quatro agentes zoonóticos transmitidos por alimentos – *Campylobacter* spp., *Salmonella* spp.*, Yersinia enterocolitica* e *Escherichia coli* verocitotoxinogénica (VTEC)*.*

O modelo aplicado é composto por duas componentes: um estudo de BoI para estimar a incidência total destes agentes patogénicos na população dinamarquesa, tendo em conta o grau de sub-diagnóstico e sub-notificação; e um estudo de BoD que contabiliza o impacto destas doenças utilizando uma medida universal designada: disability adjusted life year (DALYs). Também está descrito o modelo concebido para estimar o impacto da yersiniose – o primeiro a ser desenvolvido quer na Dinamarca, quer na Europa.

A incidência total estimada aponta para 66,202 casos de doença; com Campylobacter dando um maior contributo (51,225 casos) e Yersinia o menor (1,860 casos). A totalidade do impacto destas quatro doenças foi de 2,290 DALYs. O maior impacto na saúde pública é causado por campilobacteriose, seguida de salmonelose, yersiniose e infeções por VTEC, com 30, 8, 1 e 0.8 DALYs por 100,000 habitantes, respetivamente. A gastroenterite foi a sequela com maior impacto, quando comparada com sequelas de longa duração.

As estimativas de incidência total para os quatro agentes patogénicos mostram que crianças com menos de cinco anos têm uma maior incidência, comparando com outras faixas etárias, enquanto as estimativas de *BoD* para Campylobacter e Salmonella mostram um maior impacto em idosos. Ainda assim, essas duas doenças têm um considerável impacto em crianças, como o têm a yersiniose e as infeções VTEC.

As diferenças na abordagem metodológica utilizada para estimar a incidência total e o BoD dificultam a comparação entre países. O impacto dessas doenças evitáveis é ainda considerável, mesmo em países desenvolvidos como a Dinamarca. Compreender a contribuição de cada causa para o peso das doenças transmitidas por alimentos e incorporar estimativas no desenvolvimento de políticas em todo o mundo permitirá intervenções e melhorias eficientes e efetivas em toda a cadeia alimentar.

Palavras-chave: Peso de Doença, Peso Global de Doença, DALYs, *Y. enterocolitica, Campylobacter, Salmonella*, VTEC

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List of abbreviations

BoI – Burden of illness

DALY – Disability adjusted life year

DTU – Denmark Technical University

DW – Disability weight

EEA – European Economic Area

EFSA – European Food Safety Authority

EU – European Union

FBD – Foodborne diseases

GBD – Global Burden of Disease

GBS – Guillain-Barré Syndrome

GE – Gastroenteritis

GP – General practitioner

IBD – Inflammatory bowel disease

IBS – Irritable bowel syndrome

MiBa – Danish Microbiological Database

Pbd – Proportion of bloody diarrhea cases

PCSb – Probability of seeking medical care for bloody diarrhea patients

PCSnb – Probability of seeking medical care for non-bloody diarrhea patients

Ph – Proportion of hospitalized cases

PRR –Probability of reporting a positive laboratory result

PSSb – Probability of submitting a stool sample for analysis for bloody diarrhea cases

PSSh – Probability of submitting a stool sample for analysis for hospitalized patients

PSSnb – Probability of submitting a stool sample for analysis for non-bloody diarrhea cases

PTP – Probality of testing for pathogen in the sample

ReA – Reactive arthritis

Sen – Sensitivity of laboratory analysis

SSI – Statens Serum Institute

US – United States of America

VTEC – verocytotoxin-producing E. coli

WHO – World Health Organization

YLD – Years lived with disability

YLL – Years of life lost

Internship Report

As part of the Integrated Master's Degree in Veterinary Medicine from the Faculty of Veterinary Medicine (FMV), University of Lisbon, I completed two internships with a total duration of nine months.

The first internship took place at the FMV, from mid-September to January. I was supervised by Professor Telmo Nunes and acquired skills that were essential to prepare for my second internship in Denmark. I learned how to perform statistical data analysis in R and did literature research on Burden of Illness and Burden of Disease studies, as well as on Yersinia. Still in Lisbon, I started adapting a BoI model to R, which enabled me to extend my initial designated tasks for my second internship.

In Denmark I spent five months at the National Food Institute, Technical University of Denmark (DTU-Food), where I developed a model to estimate the burden of disease of yersiniosis and adapted previously developed models concerning other three diseases (which was only possible due to the work I developed under the supervision of Professor Telmo Nunes).

Under the supervision of Dr. Sara Monteiro Pires, I learned how stochastic disease models are built to estimate the true incidence of foodborne diseases and learned how to apply the Disability Adjusted Life Years metric to estimate the Burden of foodborne pathogens. I worked with R and adapted previously built models in @Risk to C language. I also had the opportunity of contributing to the Annual Report on Zoonoses in Denmark, writing an article on the Disease Burden of Yersiniosis in the country.

I was able to interact with a wide range of researchers from different backgrounds, learn about their projects and be inspired by different ways of accessing problems.

I enrolled in a three week PhD course, taught by Dr. Maarten Nauta and Dr. Sofia Duarte, on Quantitative Microbiological Risk Assessment, where I learned about food pathways and how to apply deterministic and stochastic models regarding growth, inactivation and cross contamination of foodborne pathogens, using Excel and @Risk.

While in Denmark, I also had the opportunity to interact with an expert on Epidemiology from Statens Serum Institut, Dr. Steen Ethelberg, who helped me gather information to inform my yersiniosis' model and enlightened me on how the surveillance of human zoonotic diseases was performed in Denmark.

Another enriching experience was doing a presentation to another expert, this time on Yersinia, from the Danish Agriculture and Food Council. I met with Dr. Marianne Sandberg, first to present my ideas regarding yersiniosis' model and second to try and gather data to develop a source attribution model for this disease. Although there was no data available, I left this meeting with new knowledge and ideas.

Lastly, I had the opportunity to attend to several meetings of the Toxoplasma Group, which gathered experts from all Nordic countries, in order to understand how to tackle this parasitic disease, where I witnessed a true One Health approach, meeting researchers from all backgrounds.

At the end of my internship I presented my work on Yersinia to everyone at the Risk Benefit Group, which enabled me to practice my oral presentation skills, as well as to think critically in order to answer all the questions related to the study developed.

I. Literature review

1. Introduction

In the United Nations Agenda for Sustainable Development, the second goal to achieve by the year 2030 is to “End hunger, achieve food security and improved nutrition and promote sustainable agriculture”. Ensuring that all people have access to safe, nutritious and sufficient food is the first step to accomplish that goal. This dissertation will focus on foodborne diseases, which is a main obstacle in the road towards achieving food safety.

Foodborne diseases (FBD) have long posed a threat to public health. In this changing World, the types, severity and impact of these illnesses have been in constant mutation, varying between individuals, communities, countries and regions (World Health Organization [WHO], 2015).

Through the consumption of contaminated foods, people are exposed to a variety of foodborne health hazards and can acquire a wide spectrum of illnesses, caused by a range of agents of bacterial, viral, parasitic, prionic or chemical nature (WHO, 2015; Tauxe, Doyle, Kuchenmüller, Schlundt, & Stein, 2010; Tauxe, 2002). These illnesses are often acute and self-limiting (diarrhea and vomiting), but can also be severe and chronic, such as kidney and liver failure, neurological disorders, and non-communicable diseases, like cancer, reproductive and immunological problems (WHO, 2014) .

Many foodborne pathogens have animals as main reservoirs, thus enabling transmission to humans through various exposure routes, including environmental from primary production sources, and foodborne transmission due to contamination at different points in the food chain (Pires, 2014).

To join the multitude of current foodborne agents, new and re-emerging hazards, with different sources of contamination, have brought new challenges to food safety in the last decade (WHO, 2014).

With the globalization of food trade, people expect a wider variety of foods, which led to a modification in the food production, distribution and consumption. With this, contaminated food products can reach people living in different countries throughout the world, spreading more easily and reaching global consequences (WHO, 2014). Another reason for this accelerated spread is the growing easiness for travelling long distances (Tauxe, 2010).

As expected, FBD not only influence people’s health, but have also a negative impact on economy. The burden on the health-care systems, trade and tourism, food and agricultural sectors, brings significantly high damages to economic productivity and threatens the livelihood of people (Food and Agriculture Organization [FAO], World Organization for Animal Health [OIE], & WHO, 2010).

Veterinary doctors play a key part on the implementation of preventive and control measures, either concerning animal diseases, zoonoses or food hygiene and safety.

Therefore, on September 29, 2004 the Wildlife Conservation Society gathered a group of health experts from around the world for a symposium on the current and potential movements of diseases among human, domestic animal, and wildlife populations. With this event, the “One World, One Health” initiative was born, proposing 12 recommendations, The Manhattan Principles, for the application of a more holistic approach for the prevention of epidemic/epizootic disease and the maintenance of ecosystems integrity (FAO, OIE, WHO, United Nations System Influenza Coordination, United Nations Children’s Fund [Unicef], The World Bank, 2008).

They concluded that, to overcome all the hardships caused by zoonoses and animal diseases, while trying to ensure the biological integrity of the Earth for future generations, it would require interdisciplinary and cross-sectoral approaches to disease prevention, surveillance, monitoring, control and mitigation, as well as to environmental conservation (FAO, OIE, WHO, United Nations System Influenza Coordination, Unicef, The World Bank, 2008).

With this vision, cooperation and strong partnership is expected between human and veterinary medicine, among other scientific-health and environmentally related disciplines.

2. Estimating total incidence of foodborne pathogens

To help decision makers implement prevention, intervention and control measures in order to improve food safety systems, ranking FBD’ impact is essential (WHO, 2015).

Although the importance of FBD is recognized globally, accurate data on its epidemiology, causative agents and its relative impact on public health is lacking (WHO, 2015). In order to give good guidance for the development and implementation of food safety policies, to achieve effective and efficient food safety systems and thereby protect consumers and improve public health, the first step is to estimate the total disease burden and etiology of these illnesses (WHO, 2015).

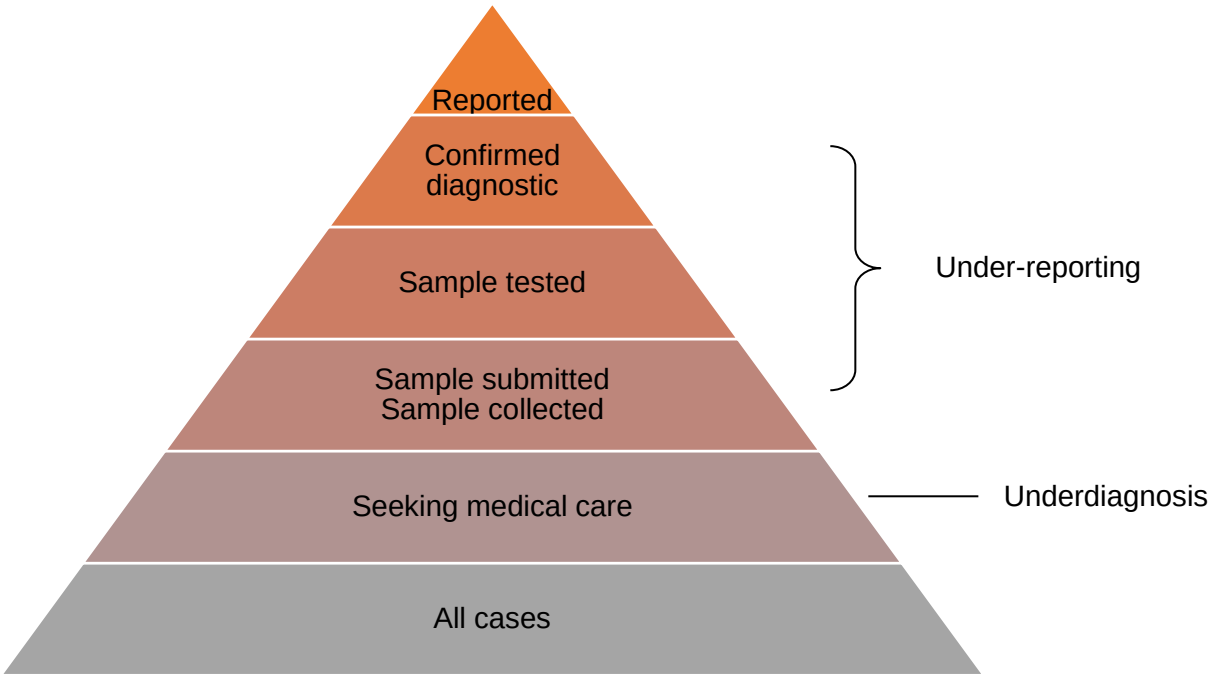
The burden of foodborne diseases reflects the number of cases occurring on the population. For many pathogens, such data can be retrieved from national public health surveillance systems, which can be both active and passive, as well as from outbreak surveillance. But, even with a surveillance system in place, the reported laboratory-confirmed cases are known to be largely underestimated (Scallan *et al*., 2011).

Data collected by surveillance systems represent only the “tip of the iceberg” and leads to a wrong picture on how these diseases impact public health (Pires, 2014). That happens because some ill persons do not seek medical care; some physicians do not request and submit a stool sample to a clinical laboratory to be tested; the clinical laboratory might not be able to isolate and identify the causative pathogen; and failures to report positive cases to

the public health surveillance system might occur (WHO, 2015), leading to under-reporting and/or underdiagnosis (Figure 1).

Underdiagnosis is a consequence of the health care system's failure to capture cases in the community that do not seek medical care, while under-reporting, the consequence of not achieving diagnosis, classification or notification of cases that have sought care (Haagsma, Polinder, Stein, & Havelaar, 2013b).

Figure 1 - The foodborne diseases' surveillance pyramid. The tip of the pyramid represents pathogen-specific cases reported to public health surveillance, whereas the base represents all cases caused by that pathogen occurring in the country in a given year. (Adapted from Pires, 2014).



2.1. Surveillance of human disease in Denmark

Public health surveillance systems are used to collect data in order to inform the authorities about epidemic and other health problems in a community. The data collected can be used to identify the magnitude and distribution of health events, to detect and monitor changes in infectious agents, and to evaluate control measures (Centers for Disease Control and Prevention [CDC], 2012).

In Denmark, human cases due to foodborne zoonotic pathogens are reported to Statens Serum Institut (SSI) through different channels depending on the disease (Anonymous, 2017):

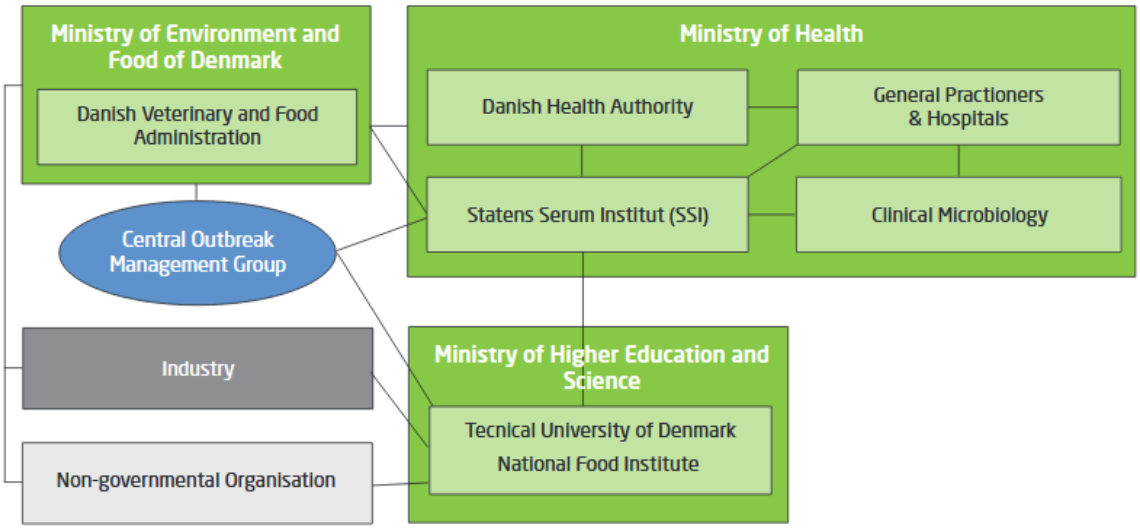
- Notifiable through the laboratory surveillance system: *Salmonella, Campylobacter, Yersinia*, verocytotoxin-producing *E. coli* (VTEC) and *Listeria.*

- Individually notifiable zoonotic pathogens: *Chlamydia psittacci* (ornithosis), *Leptospira* (Weils disease), *Mycobacterium*, Bovine Spongiform Encephalopathy (BSE) prions (var. Creutzfeldt-Jakob Disease), VTEC and Lyssavirus (rabies).

- Non-notifiable zoonotic pathogens: *Brucella*.

The general practitioners (GP) report individually notifiable zoonotic diseases to the Danish Health Authority and the Department of Infectious Disease Epidemiology at SSI. They also send samples from suspected cases to one of the official clinical microbiology laboratories. There are twelve clinical microbiological departments covering the whole of Denmark. Most are located at a hospital and receive samples from the surrounding GPs (Espenhain, 2013). Positive cases diagnosed are reported through the laboratory surveillance system to the Unit of Gastrointestinal Infections at SSI. The laboratories must report positive results within one week. In addition to this, all *Salmonella* and VTEC isolates are sent to the reference laboratory, also at SSI, for further sero and genotyping. The results are recorded in the Register of Enteric Pathogens (MiBa) and cases are reported as episodes, i.e. each patient-infectious agent combination is only recorded once in a six-month period (Anonymous, 2017). The diagram of the collaboration between the authorities, the industry and non-governmental organizations is described by Figure 2. This inter-relationship is a successful example of a One Health approach, including a multidisciplinary team of scientists, which works independently, either from the State (policy makers and authorities) or other stakeholders, ensuring transparency. Also, stakeholders, such as industry, and the primary production sector (represented by the Danish Veterinary and Food Administration) are parts of this approach. This is an example of a holistic approach to food safety, quite unique in the world.

Figure 2 - Overview of the monitoring and outbreak investigation network for reporting infectious pathogens in humans, animals, foodstuffs and feed-stuffs in Denmark, 2016 (Anonymous, 2017).



2.2. Population, Physician and Laboratory surveys

Surveys are a valuable tool to understand the behavior of the different groups that influence under-reporting and underdiagnosis (Flint *et al*., 2005).

As mentioned before, underdiagnosis contributes to the underestimation of cases of FBD. People with gastrointestinal illness often do not seek medical care and therefore are not diagnosed. Those cases never reach the surveillance system and are not accounted for in the health reports.

Population surveys can be used to understand patient-behavior and assess the proportion of people that do not seek medical care, which will then be used to correct the reported number of cases, getting closer to the true incidence of foodborne pathogens (Müller, Korsgaard, & Ethelberg, 2012; Scallan *et al*., 2006).

General practitioners' knowledge, submission and interpretation of clinical specimen test results have a marked influence on public health surveillance, outbreak detection and patient management (CDC, 2012). Undetected cases of foodborne illness and misinterpretation of laboratory test results can delay treatment, reporting and outbreak detection (Clogher *et al*., 2012; Schmutz *et al*., 2017; Van Cauteren *et al*., 2015).

Even though many foodborne infections are self-limiting and do not require treatment, to successfully manage patient disease, the physician should properly diagnose it. Also, the use of antimicrobial drugs in mild or moderate cases of foodborne illness is relatively common, despite guide lines recommending their use only for severe cases (Rosner, Werber, Höhle, & Stark, 2013). Knowledge of which diagnostic tests to order and their right

interpretation, as well as correct treatment approaches, is needed to achieve better patient outcomes. However, laboratory testing methods are constantly changing, therefore both open communication between physicians and microbiologists and educational interventions are a main step to achieve best practices (Clogher *et al.*, 2012).

Physician surveys can be useful to understand their perception and practices (Wong *et al.*, 2004) and will help to reconstruct the surveillance pyramid, assessing the proportion of physicians that do not request and submit a stool sample (Schmutz *et al.*, 2017; Van Cauteren *et al.*, 2015).

At last, in a laboratory-based surveillance, clinical microbiology laboratories are the key. It is through the identification and notification to the public health authorities of culture-confirmed infections that recognition of both foodborne outbreaks and sporadic cases, and epidemiological understanding overtime is achieved. Thereby, to interpret laboratory-based surveillance data, laboratory testing and reporting procedures must be considered (Voetsch *et al*., 2004).

Differences between laboratories' routine testing practices and their reporting systems might contribute to variations in the incidence rate of reported cases of foodborne illnesses, adding to under-reporting.

Laboratory surveys might therefore help to characterize current practices and monitor changes in methodologies overtime (Voetsch *et al.*, 2004).

2.3. Burden of foodborne illness studies

Both in Europe and outside, several countries have conducted burden of illness (BoI) studies in an attempt to estimate the true incidence of foodborne illnesses in their population (Cressey & Lake, 2011; Haagsma *et al*., 2013a; Hall *et al*., 2005; Scallan *et al*., 2011; Thomas *et al*., 2013; Vaillant *et al*., 2005).

Table 1 shows an overview of the countries performing these studies, the number of pathogens considered and the estimation of incidence per 100,000 population.

Even though all studies follow similar methodologies, there are differences in details of the surveillance pyramid reconstruction approach and on the number of pathogens included, highlighted by Table 1, therefore the results of these studies cannot directly be compared (Haagsma *et al.*, 2013a).

The above being true, I would like to emphasize that all these studies have considered *Campylobacter* spp*.,* non-typhoidal *Salmonella* spp., verocytotoxin-producing *Escherichia coli* (VTEC) (although many grouped it with other *E. coli* pathotypes) and *Yersinia enterocolitica*, considering them relevant causes of FBD.

Table 1 - Burden of illness studies by country, number of studied pathogens and estimated incidence per 100,000 population.

Country	Number of pathogens	Estimated Incidence/100,000 population	Reference
Australia	16	8,056	Hall *et al.*, 2005
Canada	30	4,923	Thomas *et al.*, 2013
France	23	450	Vailant *et al.*, 2005
Germany	7	1,522	Haagsma *et al*., 2013a
Italy	2	276	Haagsma *et al.*, 2013a
Netherlands	6	6,649	Haagsma *et al*., 2013a
Poland	4	2,671	Haagsma *et al*., 2013a
Sweden	5	2,014	Haagsma *et al*., 2013a
United Kingdom	6	7,201	Haagsma *et al.*, 2013a
United States of America	31	3,100	Scallan *et al*., 2011
New Zealand	24	12,900	Cressey & Lake, 2011

In eight out of eleven countries, *Campylobacter* spp. is the pathogen with the highest incidence, when compared to *Salmonella* spp. VTEC and *Y. enterocolitica* (Table 2), ranking from first to third, when compared with all foodborne hazards included in its respective study. (Hall *et al*., 2005; Cressey & Lake, 2011; Haagsma *et al*, 2013a; Thomas *et al*., 2013).

In the United States (US) and in France, *Salmonella* spp., compared to the other three pathogens, is the highest incident pathogen, occupying the second and fourth position, respectivily, according to Scallan and colleagues (2011) and Vailant and colleagues (2005).

Regarding *Yersinia enterocolitica,* New Zealand estimated more cases of this pathogen than *Salmonella* spp. (Cressey & Lake, 2011)*,* which is not seen in the other studies, where *Yersinia* occupies lower positions in the ranking than both *Campylobacter* and *Salmonella* (Hall *et al*., 2005; Vailant *et al.*, 2005; Scallan *et al*., 2011; Haagsma *et al.*, 2013a; Thomas *et al*., 2013). Although in Australia *Yersinia* is one of the pathogens with lower incidence (Hall *et al.*, 2005), in countries like Canada, France and the US (that considered a larger number of pathogens), *Yersinia* is the 7th and 8th most incident pathogen (Thomas *et al*., 2013; Vailant *et al.*, 2005; Scallan *et al.,* 2011), showing that it is not a negligible cause of FBD.

VTEC was only considered separatly in Canada and France. Nonetheless, verocytotoxin-producing *E. coli* is an important cause of FBD and should not be discarded.

Table 2 - Estimated incidence of *Campylobacter* spp.*, Salmonella* spp., VTEC and *Y. enterocolitica* and their ordinal position among other foodborne pathogens by country.

Country	Campylobacter	Salmonella	VTEC	Yersinia
Australia	208,000 3rd	81,000 4th	-	1.620 14th
Canada	145,350 3rd	87,510 4th	33,350 6th	25,915 7th
France	12,796 – 17,322 5th	30,598 – 41,139 4th	373 – 747 10th	655 – 1,909 8th
Germany	515,000 1st	430,000 2nd	-	86,000 5th
Italy	156,000 1st	34,000 2nd	-	-
Netherlands	322,000 2nd	55,000 3rd	-	20,000 4th
Poland	765,000 1st	242,000 2nd	-	9,600 3rd
Sweden	122,000 1st	40,000 2nd	-	9,500 3rd
United Kingdom	2,500,000 1st	563,000 2nd	-	55,000 5th
United States of America	845,024 4th	1,027,561 2nd	-	97,656 8th
New Zealand	190,092 2nd	22,570 5th	-	29,715 4th

3. Burden of Disease studies

The impact of a disease can be measured by its incidence and/or the number of deaths (mortality) caused by it in a population (Devleesschauwer *et al.*, 2015), therefore burden of foodborne illness studies could be used to rank diseases. However, using only these two population health measures, the impact of FBD on human health is not depicted accurately (Devleesschauwer *et al.*, 2015). In fact, a disease might have a very high incidence but cause very low burden due to its mild symptoms, or because it is mostly asymptomatic. On the other hand, a disease can cause few cases per year, but have high mortality rate. How to

compare, for example, an incidence of 300,000 cases, low severity and 0% mortality with a disease causing three cases with high severity and 33% mortality?

Also, ignoring the age at which people die due to a specific disease, can be problematic. What if two diseases have the same incidence and the same mortality, but the deaths in one occur in younger people? Likewise, if those population health measures are the same, but one disease only causes acute symptoms (i.e. have short duration) and the other chronic life-long sequelae?

To overcome these limitations, the Disability Adjusted Life Year (DALY) metric was developed (Murray & Lopez, 1996) and is currently the most used metric to estimate the burden of disease (WHO, 2015), a concept developed in the 1990s. By incorporating morbidity, mortality and disability in one metric, the DALY enables the comparison and ranking between diseases for all regions of the world (Murray & Lopez, 1996).

The concept of the DALY metric is simple: it represents the years lived with disability (YLD), i.e. years lived with decreased quality of life, and the years of life lost (YLL) due to premature death as a consequence of a given disease or condition, at the individual or population level (Anonymous, 2017).

3.1. Foodborne disease model

Risk factors increase the probability of having a disease by facilitating the exposure to biological, chemical or physical hazards. The course of disease is then characterized by distinct health states, with possibly different severity levels (Devleesschauwer *et al*., 2014b). Therefore, a disease model, also called health-outcome tree, provides a qualitative representation of all potential disease progression pathways throughout time (Mangen *et al*., 2013).

Depending on the perspective of the study, there are three disease model approaches relevant to burden of disease studies regarding foodborne diseases (Devleesschauwer *et al*., 2014b; Haagsma *et al*., 2013b; Mangen *et al*., 2013):

I. Outcome-based disease models

This approach assigns different health states of disease, independent of the etiology (Figure 3). For example, a disease model for the burden of diarrhea might describe different severity levels, as well as different health-outcomes occurring sequentially in time (e.g. reactive arthritis), which contribute to YLDs, and diarrhea related death, contributing to YLLs (Devleesschauwer *et al*., 2014b; Haagsma *et al*., 2013b; Mangen *et al*., 2013).

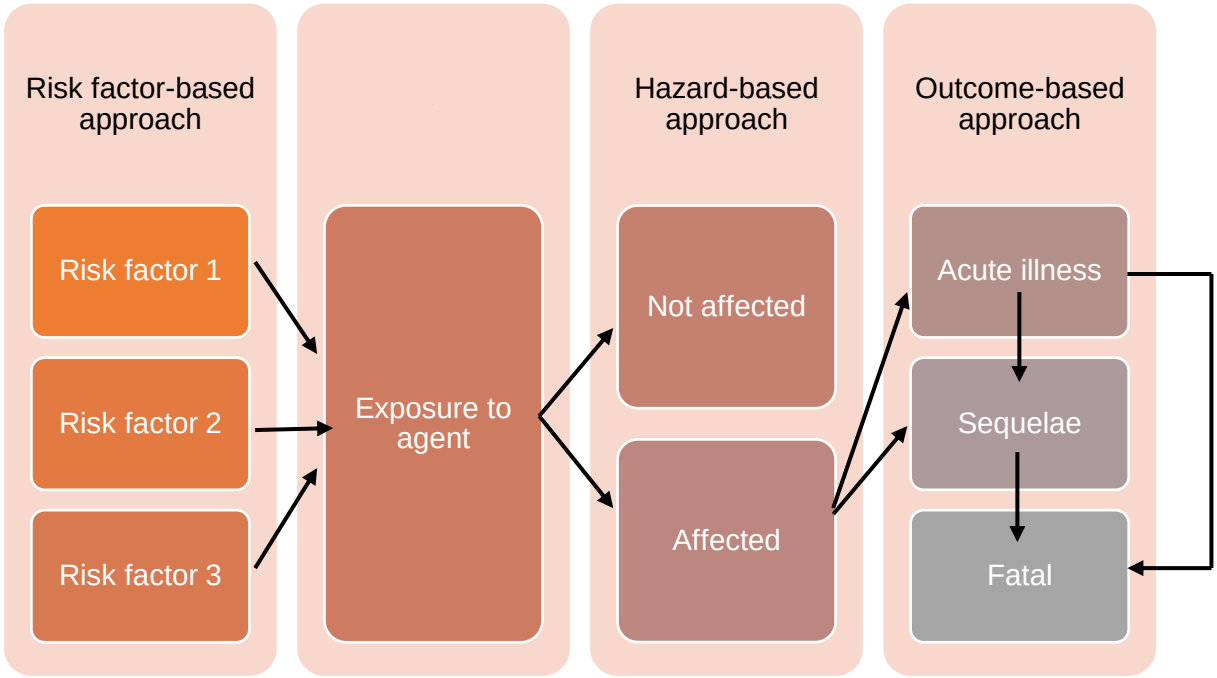
II. Hazard-based disease models

This approach represents all relevant health-outcomes, acute and/or chronic, that can be attributed to a single agent (Figure 3). Being so, the starting point using a hazard-based disease model is illness caused by the exposure to biological, chemical or physical hazards (Devleesschauwer *et al*., 2014b; Haagsma *et al*., 2013; Kretzschmar *et al.*, 2012).

III. Risk factor-based disease models

The third possible disease model used to estimate the burden of foodborne diseases associates different health states with risk factors that can influence exposure to causative agents of foodborne illness (Figure 3) (Devleesschauwer *et al*., 2014b; Haagsma *et al*., 2013). As an example, a disease model for unsafe water would include the health-outcomes associated with feco-oral agents (Clasen *et al*., 2014; Prüss-Ustün *et al.*, 2014).

Figure 3 - Foodborne disease model depicting the three possible approaches: Risk factor-based, Hazard-based and Outcome-based (Adapted from Haagsma, 2013b).



3.2 Disability weights

The disability weight (DW) concept was developed in order to reflect the impact of health states causing different disabilities and to compare morbidity and mortality (Murray & Lopez, 1996). Disability is, in this context, defined as any short or long-term loss of health (Salomon *et al*., 2015).

A DW for a health state is scaled from zero, indicating full health, to one, which implies that the health state is equivalent to death (worst possible health state) (Salomon *et al*., 2015).

In the DALY calculation, the DWs are a crucial component. By multiplying the number of people affected by a health-outcome with the time of its duration and the correspondent DW, the number of years lived with disability can be calculated (Murray & Lopez, 1996).

This value is based on judgements of possibly three sets of respondent groups. Namely, health-care professionals, individuals who experience a specific health state and the general public (Salomon *et al*., 2012).

The Global burden of disease study of 1996 (GBD) used a panel of health-care professionals on the basis that they would have knowledge of a large set of health states and would be able to perform comparative unbiased judgements. However, most of the studies using DW use the responses of the general public, under the argument that the views of the general public are relevant in comparative assessments that inform public policy (Salomon *et al.*, 2012).

3.3 Social weighting

In the course of developing the DALY metric, the first GBD study applied two social value choices: time discounting and age weighting (Murray & Lopez, 1996). These two factors were applied in attempt to address two questions:

'Are lost years of healthy life valued more at some ages than others?'

Age weighting reflects that individuals have different roles and changing levels of dependency and productivity with age (Murray & Lopez, 1996). Its application means that, depending of an individual's age, time will be valued differently, specifically, youngest and oldest ages are given less weight (Pires, 2014).

'Is a year of healthy life gained now worth more to society than a year of healthy life gained sometime in the future?'

Time discounting is based on a standard practice in economic analysis, which entails that benefits now are preferable to a benefit gained later in time (Murray & Lopez, 1996). By applying this social weighting function, we consider that future life years are less valuable than those lived today (Devleesschauwer *et al*., 2014a; M. Pires, 2014).

These two social weighting functions have not been universally accepted and, in recent years, have not been applied by burden of disease studies (Institute for Health Metrics and Evaluation [IHME], 2016; WHO, 2015).

4. Yersiniosis

4.1. *Yersinia* spp.

The genus *Yersinia,* of the family *Enterobacteriaceae,* comprises three major human pathogens and several non-pathogenic species. The pathogenic species are *Yersinia pestis,* the causative agent of the plague, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis,* which are both enteropathogens, causing yersiniosis (European Food Safety Authority (EFSA), 2007).

Of these three species, *Y. pestis* is neither foodborne nor found in Europe. Regarding the two enteropathogenic species, *Y. enterocolitica* is the most frequent cause of human disease in Europe, since *Y. pseudotuberculosis* cases are rare and are mostly found in North-Eastern Europe, mainly, Finland and Russia (EFSA, 2007; Jalava *et al*., 2004). In 2015, 99.5% of the reported cases of yersiniosis in the European Union/European Economic Area (EU/EEA) were caused by *Y. enterocolitica* (EFSA, 2016).

4.2. *Yersinia enterocolitica*

Y. enterocolitica is a Gram-negative, facultative anaerobic rod (occasionally coccoid). It does not form a capsule or spores and it is nonmotile at 35–37 °C, but motile at 22–25 °C (Bottone *et al.*, 2005). In addition, it has the capacity of growing at refrigeration temperatures (Van Damme, De Zutter, Jacxsens, & Nauta, 2017). Within this species there is enough biochemical heterogeneity to establish six biotypes and different O-antigen specificity for several (more than 48) serotypes (Nesbakken, 2015).

Bio and serotyping of *Y. enterocolitica* are crucial, as its pathogenic potential varies greatly between bio/serotype combinations (Table 3) (Nesbakken, 2015). Harboring a virulence plasmid and having the High Pathogenicity Island, enables the differentiation of three groups of *Y. enterocolitica* pathogenicity: highly pathogenic, pathogenic and non-pathogenic (EFSA, 2007).

Although biotype 1A is regarded as non-pathogenic, recent studies have provided conflicting reports on the subject (EFSA, 2016). In a literature review, Bottone (2015) concluded that 1A strains may cause symptomatic infections in patients with underlying disorders (immunosuppression). In a study that compared *Y. enterocolitica* 1A isolated from patients and asymptomatic carriers, it was concluded that more research is needed to prove that this strain is a primary cause for human yersiniosis and not only a secondary finding (Stephan *et al*., 2013).

Table 3 - Pathogenic potential of *Y. enterocolitica* associated with bio and serotype (Adapted from EFSA, 2007).

Biotype	Serotypes	Virulence for humans	Frequency in Europe	Pathogenicity Determinants
1A	Numerous (O:8; O:5; O:7; O:13;…)	NP	++++[n]	None
1B	O:8; O:21; O:13; O:7;…	HP	≈0	Both
2	O:9; O:5,27	P	++ to +++	pYV
3	O:3; O:5,27		+	pYV
4	O:3	P	++++	pYV
5	O:3; O:2,3; O:1,2,3	P	≈0	pYV

[n]: From 0 to ++++ indicates the frequency of each subgroup. NP: non-pathogenic; HP: highly pathogenic; P: pathogenic; pYV: virulence plasmid.

4.2.1. Epidemiology of *Yersinia enterocolitica*

Numerous animals, animal-derived food products, vegetables and water sources have been identified as sources of *Y. enterocolitica*, however the understanding of its epidemiology is still incomplete (Nesbakken, 2015; Rosner, Stark, & Werber, 2010).

There is often a strong correlation between the bio/serotype of strains isolated from humans and the ones isolated from pigs in the same geographical area (EFSA, 2007; Fredriksson-Ahomaa, Stolle, Siitonen, & Korkeala, 2006; Kapperud, 1991; Rosner, Stark, Höhle, & Werber, 2012; Tauxe, 2002), therefore pork is considered the main source of pathogenic *Y. enterocolitica* (Van Damme *et al*., 2017).

The most frequently reported bio/serotype combination since the beginning of EU's yersiniosis surveillance has been 4/O:3 (EFSA, 2016). The main reservoir of this strain is the pig, which can asymptomatically carry the pathogen in the intestinal tract, lymph nodes and tonsils, which is the major source of contamination (Fredriksson-Ahomaa, Björkroth, Hielm, & Korkeala, 2000; Laukkanen-Ninios, Fredriksson-Ahomaa, & Korkeala, 2014).

Case control studies of sporadic cases of yersionisis conducted in Norway (Ostroff *et al*., 1994) and in Germany (Rosner *et al*., 2012) have identified consumption of pork as an important risk factor for *Y. enterocolitica* infections.

This pathogen has also been isolated from tonsils and fecal samples of wild boars (Fredriksson-Ahomaa, Wacheck, Koenig, Stolle, & Stephan, 2009; Nesbakken, 2015). They are also a significant concern regarding yersiniosis, because of poor slaughter hygiene (EFSA, 2013).

Cattle can be asymptomatic carriers of serotype O:9 (EFSA, 2016), although case-control studies have not identified beef as a source of yersiniosis (Nesbakken, 2015). The link

between cattle and humans has been milk and dairy products, which have been connected with outbreaks of yersiniosis (EFSA, 2007; Nesbakken, 2015).

Water and vegetables have also been associated with sporadic cases and outbreaks of *Y. enterocolitica* infections (Macdonald *et al*., 2011; MacDonald *et al.*, 2016; Ostroff *et al.*, 1994).

Other source of yersiniosis are rodents, reservoirs of biotype 1B (serotypes O:8 and O:21) in Japan, and potentially in North America (EFSA, 2007). Dogs and cats might also be vehicles of this pathogen (Stamm, Hailer, Depner, Kopp & Rau, 2013).

4.2.2. Control of *Y. enterocolitica* in the food chain

Y. enterocolitica can survive and grow at temperatures as low as -5 ºC *(*Laukkanen-Ninios *et al*., 2014). Therefore this bacterium has the ability to survive and propagate at refrigeration temperatures, which implies that it has to be controlled in the food chain (Nesbakken, 2015; Van Damme *et al*., 2017).

I. Pig meat chain

Because pigs are the major known source of *Y. enteroclitica* infection worldwide, the control of this foodborne pathogens throughout all the meat chain is essential to ensure the protection of consumers (Nesbakken, 2015).

Lowering the prevalence of *Y. enterocolitica* in farms would also lower the contamination at slaughterhouses, helping to reduce yersiniosis' cases caused by contaminated pork (Laukkanen-Ninios *et al.*, 2014). Therefore, identifying control measures at farm level is the first step to reduce yersiniosis' burden.

At farm level, some risk factors have been identified as contributors for seropositive herds, namely:

1. Buying animals from herds with an unknown carrier state for human pathogenic *Y. enterocolitica* (Skjerve, Lium, Nielsen, & Nesbakken, 1998; Virtanen, Salonen, Laukkanen-Ninios, Fredriksson-Ahomaa, & Korkeala, 2012);
2. Buying piglets from more than one farm (Virtanen *et al.,* 2012; Vilar, Virtanen, Heinonen & Korkeala, 2013; Virtanen, Nikunen & Korkeala, 2014);
3. Use of non-municipal water sources and having a continuous production (instead of applying an all-in/all-out strategy) (Vilar *et al*., 2013).

According to a Norwegian study, it is possible to establish clusters of pig herds free of pathogenic *Y. enterocolitica*, and keep them free from this foodborne pathogen for many

years (Nesbakken, Iversen, & Lium, 2007), however it may not be an economically feasible practice (Laukkanen-Ninios *et al*., 2014).

Laukkanen-Ninios and collaborators (2014) have also concluded that there is insufficient data on how to reduce the prevalence of this pathogen at farm level and that more studies regarding infection's dynamic on farms are required.

During slaughter and dressing procedures, human pathogenic *Y. enterocolitica* from the oral cavity and/or intestinal content of pigs, may contaminate both the carcasses and the environment in the slaughterhouses (Nesbakken, 2015; Van Damme *et al*., 2015), therefore control measures are needed to prevent it (Laukkanen-Ninios *et al*., 2014).

One way to reduce contamination at environmental level would be to slaughter pigs at an older age (135 days or older), when the secretion of the pathogen in feces decreases, however, at this age, pigs still carry the pathogen in high concentrations in the tonsils (Nesbakken, Iversen, Eckner & Lium, 2006), so the major source of contamination remains (Laukkanen-Ninios *et al*., 2014).

During the slaughter process, scalding, singeing, bagging of the rectum after bunging, and removal of the head with tonsils and tongue intact are the phases that can reduce carcass contamination with *Y. enterocoli*tica (Nesbakken, Nerbrink, Røtterud & Borch, 1994; Laukkanen *et al*., 2008; Laukkanen-Ninios *et al*., 2014; Van Damme *et al.*, 2015, 2017).

Although various control measures during slaughter and dressing procedures have been identified, the best way to reduce contamination at the slaughterhouse due to *Y. enterocolitica* is to improve hygiene practices and use the bagging of the rectum (Laukkanen-Ninios *et al*., 2014; Nesbakken, 2015). The separation of the head with tonsils and tongue intact, even though consisting in an efficient control measure, would require changes in many slaughter lines (Laukkanen-Ninios *et al*., 2014).

Regarding meat inspection, compulsory procedures that involve incisions in the submaxillary lymph nodes in order to detect tuberculosis, represent a cross-contamination risk (Nesbakken, Eckner, Høidal & Røtterud, 2003). EFSA also recommends a visual *post mortem* inspection to avoid this risk factor on its 'Scientific opinion on the public health hazards to be covered by inspection meat (swine)' (EFSA, 2011).

After slaughter, control measures seem ineffective, since *Y. enterocolitica* can survive and grow during cold storage and under modified atmospheres (Laukkanen-Ninios *et al*., 2014; Nesbakken, 2015).

II. Consumer phase

The correct handling of pork, hygienic practices during food preparation and adequate cooking of meat in both domestic and restaurant kitchens are of vital importance to reduce the number of cases of yersiniosis (Nesbakken, 2015).

Many people are not aware of basic food safety practices, as well as the risks associated with the consumption of animal derived food products (Laukkanen-Ninios *et al.*, 2014). Therefore, consumers need to be informed and educated regarding food safety procedures of food handling, storage and preparation of food, which can be achieved by sharing information in a more appealing and disseminated way (Langiano *et al*., 2012; Losasso *et al.*, 2012; Nesbitt *et al*., 2014).

4.3. Clinical manifestations of yersiniosis

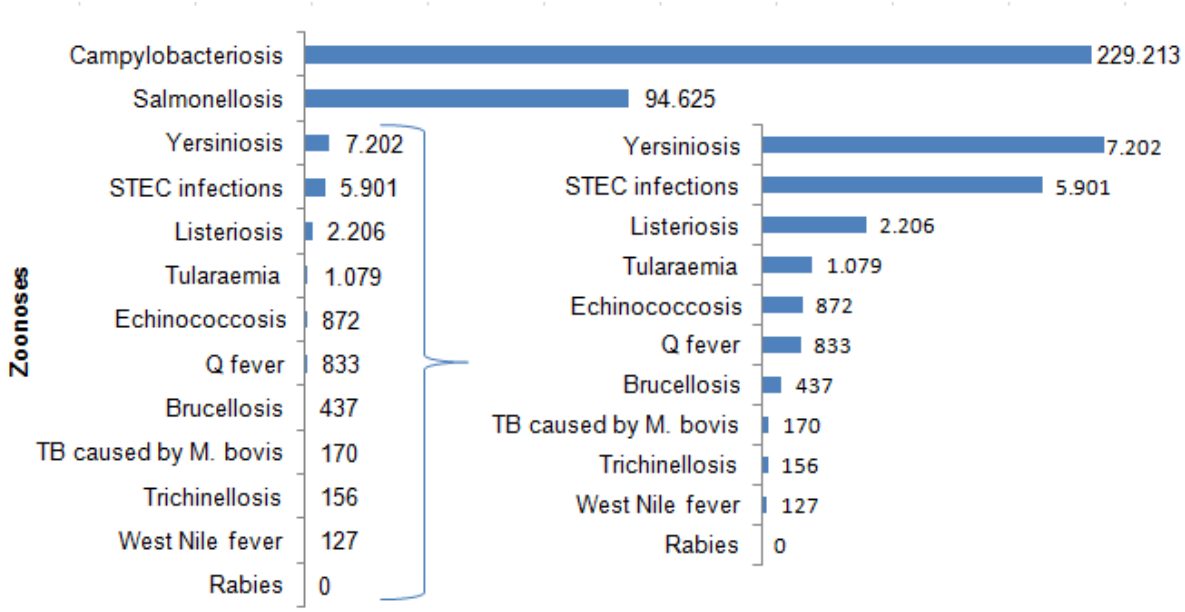
Yersiniosis' most common presentation is gastroenteritis with self-limiting diarrhea associated with mild fever and abdominal pain; however, moderate and bloody diarrhea can also occur (Bottone, 1997; Helms, Simonsen, & Molbak, 2006; Huovinen *et al.*, 2010; Nesbakken, 2015; Ostroff *et al*., 1992; Rosner *et al*., 2013; Schiellerup, Krogfelt, & Locht, 2008; Stolk-Engelaar & Hoogkamp-Korstanje, 1996; Zheng, Sun, Lin, Mao, & Jiang, 2008).
In addition, in older children and young adults, symptoms may resemble those of appendicitis (pseudoappendicitis), due to an infection limited to the right fossa iliaca, causing terminal ileitis or mesenteric lymphadenitis (Azghari *et al*., 2016; Bottone, 1997; Nesbakken, 2015; Rosner *et al*., 2013; Shorter, Thompson, Mooney, & Modlin, 1998; Stolk-Engelaar & Hoogkamp-Korstanje, 1996; Van Noyen, Selderslaghs, Bekaert, Wauters, & Vandepitte, 1991; Zheng *et al*., 2008) .In a cohort study, yersiniosis patients were more than 70 times more likely to report an appendectomy than the reference group (RR 73.5 [95% CI: 9.9 – 544.0]; $p<0.001$) (Rosner *et al*., 2013).
Yersiniosis can also cause long-term extra-intestinal complications, such as reactive arthritis (Bottone, 1997; Hannu, Inman, Granfors, & Leirisalo-Repo, 2006; Helms *et al*., 2006; Huovinen *et al*., 2010; Nesbakken, 2015; Ostroff *et al.*, 1992; Rosner *et al*., 2013; Schiellerup *et al.*, 2008; Stolk-Engelaar & Hoogkamp-Korstanje, 1996; Townes *et al.*, 2008; Zheng *et al.*, 2008) and erythema nodosum (Bottone, 1997, 2015; Helms *et al.*, 2006; Nesbakken, 2015; Rosner *et al.*, 2013; Stolk-Engelaar & Hoogkamp-Korstanje, 1996; Zheng *et al.*, 2008). In addition, Irritable bowel syndrome, a chronic gastrointestinal disorder, is also a possible sequela of this disease (Helms *et al*., 2006; Ostroff *et al*., 1992; Porter *et al*., 2013; Rosner *et al*., 2013, Schwille-Kiuntke, Frick, Zanger, & Enck, 2011).
Y. enterocolitica can also cause septicemia (Azghari *et al*. 2016, Bottone, 1997, 2015; Helms *et al*., 2006; Stolk-Engelaar & Hoogkamp-Korstanje, 1996; Zheng *et al*., 2008), especially in immunosuppressed persons and those in iron overload or being treated with desferrioxamine (Bottone, 1997).

4.4. Yersiniosis in the European Union and Denmark

Yersiniosis was the third most reported zoonosis in the European Union for the year 2015 (Figure 4), and has been so since 2005 (EFSA, 2016; EFSA, 2009).

In the EU/EEA, the notification rate of yersiniosis for the year 2015 was 2.2 cases per 100,000 population, which was 6.8% higher than the year before. Although there was an increase in 2015, for the last eight years there has been a statistically significant decrease in the reported cases of this disease (EFSA, 2016).

Figure 4 - Reported numbers of confirmed human zoonoses cases in the EU, 2015. (Adapted from EFSA, 2016)



Twenty six member states reported this disease in 2015 (Table 4). Portugal reported it for the first time. Finland, Denmark and Check Republic had the highest country-specific notification rates in 2015. Lithuania has been decreasing the number of reported cases since 2011, when it was the country with the highest notification rate; the opposite has happened with Denmark, with a steady increase since 2011, having the second highest country-specific notification rate in 2015 and 2014 (9.54 and 7.71 cases per 100,000 population, respectively); while Finland has showed a fairly constant prevalence of yersiniosis, having the highest notification rate in 2012-2015.

Only 0.5% of the reported cases were caused by *Y. pseudotuberculosis* in 2015 (EFSA 2016).

Table 4 - Reported cases of yersiniosis and notification rates in the EU/EEA, by country and year, 2011–2015 (Adapted from EFSA, 2016).

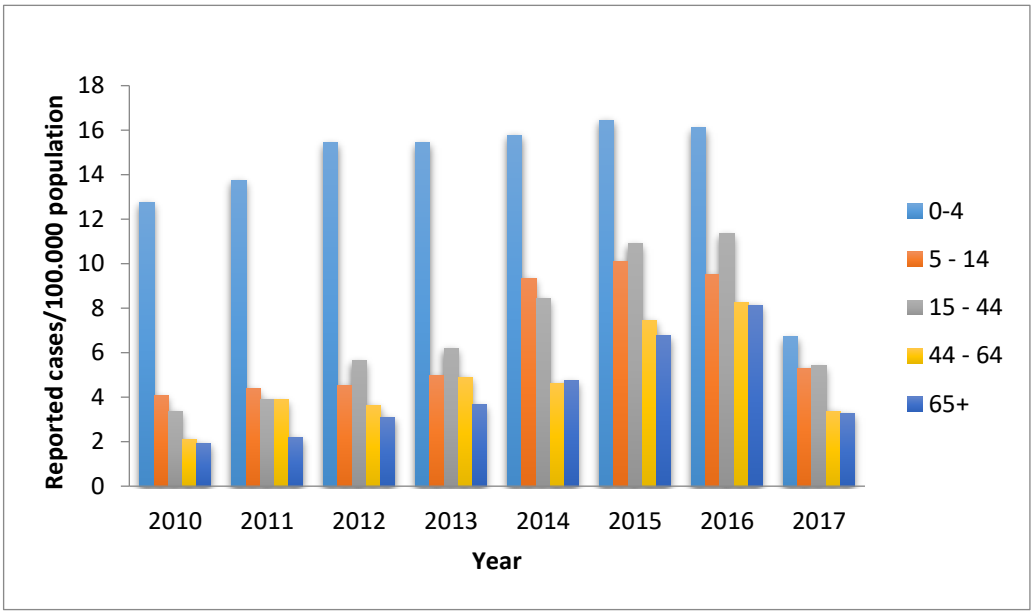
Country/Year	2015		2014		2013		2012		2011	
	Confirmed cases & rates		Confirmed cases & rates		Confirmed cases & rates		Confirmed cases & rates		Confirmed cases & rates	
	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Austria	118	1,38	107	1,26	158	1,87	130	1,55	119	1,42
Belgium[a]	350	-	309	-	350	-	256	-	214	-
Bulgaria	12	0,17	20	0,28	22	0,30	11	0,15	4	0,05
Croatia	16	0,38	20	0,47	0	0,00	0	0,00	-	-
Cyprus	0	0,00	0	0,00	1	0,12	0	0,00	0	0,00
Czech Republic	678	6,39	557	5,30	526	5,00	611	5,82	460	4,39
Denmark	540	9,54	434	7,71	345	6,16	291	5,22	225	4,05
Estonia	53	4,04	62	4,71	72	5,45	47	3,55	69	5,19
Finland	582	10,64	579	10,62	549	10,12	565	10,46	554	10,31
France [a]	624	-	574	-	430	-	314	-	294	-
Germany	2739	3,37	2470	3,06	2 579	3,15	2 690	3,29	3 381	4,21
Greece [b]	-	-	-	-	-	-	-	-	-	-
Hungary	41	0,42	43	0,44	62	0,63	53	0,54	93	0,93
Ireland	13	0,28	5	0,11	4	0,09	2	0,04	6	0,13
Italy [a]	16	-	18	-	25	-	14	-	15	-
Latvia	64	3,22	28	1,40	25	1,24	28	1,37	28	1,35
Lithuania	165	5,65	197	6,69	262	8,82	276	9,19	370	12,12
Luxembourg	15	2,66	19	3,46	15	2,79	28	5,33	14	2,74
Malta	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00
Netherlands[b]	-	-	-	-	-	-	-	-	-	-
Poland	172	0,45	212	0,56	199	0,52	201	0,52	235	0,62
Portugal	24	0,23	-	-	-	-	-	-	-	-
Romania	25	0,13	32	0,16	43	0,22	26	0,13	47	0,23
Slovakia	224	4,13	172	3,18	164	3,03	181	3,35	166	3,08
Slovenia	10	0,48	19	0,92	26	1,26	22	1,07	16	0,78
Spain [d]	432	2,07	436	2,08	243	1,73	221	1,89	264	2,26
Sweden	245	2,51	248	2,57	313	3,28	303	3,20	350	3,72
United Kingdom	44	0,07	58	0,09	59	0,09	54	0,09	59	0,09
EU Total	**7 202**	**2,20**	**6 619**	**2,06**	**6 472**	**2,05**	**6 324**	**2,05**	**6 983**	**2,33**
Iceland	1	0,03	3	0,92	0	0,00	-	-	-	-
Norway	76	1,47	211	4,13	55	1,09	43	0,86	60	1,22

In Denmark, yersiniosis is notifiable through the laboratory surveillance system and, even though biotype 1A is still considered non-pathogenic, all *Y. enterocolitica* biotypes causing human infection are reported. In 2016, with 572 reported cases of yersiniosis, Denmark reached the highest notification rate for the past seven years, reporting 10 cases per 100,000 population (Anonymous, 2017).

Danish children under five years old are the most affected by this disease (Figure 5), although cases in other age groups also occur. Since 2010, there has been a consistent increase in the number of reported cases. However, in 2014 and 2015 more than half (61%)

were of biotype 1A (SSI, 2016). Also, in 2016, 70% of the reported cases were of the non-pathogenic strain (Anonymous, 2017).

Figure 5 - Age-specific cases of yersiniosis per 100,000 population in Denmark, 2010 - 2017. Cases from 2017 are from January to August. (SSI, 2017).



Improvements of the surveillance system (automatic data capture) are believed to contribute to the observed increase from 2014 to 2015, which can also be related to the evolution of laboratory methods, as an increasing number of patients is diagnosed with biotype 1A, leading to a mismatch between the number of registered cases and the real number of disease cases (SSI, 2016).

Most EU countries do not report biotype 1A, Denmark being responsible for 98.8% of the reports in 2015. EFSA concludes that the 6.8% increase of yersiniosis cases was due to improvements in surveillance systems in Denmark and Spain and because Portugal and Croatia started to report (EFSA, 2016).

II. Objectives

The main purpose of this study was to estimate the disease burden of yersiniosis, campylobacteriosis, salmonelosis and VTEC infections in the Danish population for the year 2016, using a harmonized approach.
To achieve that goal, specific objectives were delineated:

1. Adapt a previously developed model in @Risk 6.0 (palisade Corporation, 2014) to R 3.3.2 (R Core Team, 2016), in order to estimate the true incidence of yersiniosis, campylobacteriosis, salmonellosis and VTEC infections, by accounting for underdiagnosis and under-reporting in the Danish population;

2. Gather data to quantify the under-reporting and underdiagnosis of yersiniosis in the Danish population;

3. Identify all potential health-outcomes associated with yersiniosis and estimate the probability of their occurrence given infection;

4. Develop a health-outcome tree for yersiniosis, using the information gathered in objective three;

5. Update the data and adapt the calculations necessary to estimate the probability of each health-outcome of campylobacteriosis, salmonellosis and VTEC infections to R, using previously developed health-outcome trees;

6. Use the DALY calculator (Devleesschauwer *et al*., 2016), a graphical interface for probabilistic DALY calculation developed in R, to calculate DALYs, YLDs and YLLs for each health-outcome of each of the four studied diseases, using the data and estimates collected in objectives one to five;

7. Compare and rank these diseases regarding their public health impact in Denmark.

III. Material and methods

Four pathogens were selected for this study. *Yersinia enterocolitica, Campylobacter* spp.*, Salmonella* spp.*,* and VTEC. They were selected due to data availability and due to their public health significance (assessed in terms of incidence in the population).

1. Estimating the incidence of four foodborne pathogens

1.1 Public health surveillance data

All the pathogens selected for this study are of mandatory notification through the Danish laboratory surveillance system. Results are recorded in the Register of Enteric Pathogens (MiBa) maintained by SSI. Everyone can then consult all data regarding every notifiable human disease in Denmark through SSI website (www.ssi.dk). This data give information regarding the year (since 2001) and month in which the case occurred, gender and age group of the individuals affected, the geographic region and if it was a travel-related infection. For *Salmonella* spp. and VTEC there is also information regarding the agent's serotype.
The website was accessed (on February 2017) to retrieve information on the number of cases of human campylobacteriosis, sallmonelosis, VTEC infections and yersiniosis reported through the Danish surveillance system in the year 2016.
All the surveillance data were aggregated in six age categories (0 to 4 years old, 5 to 14 years old, 15 to 44 years old, 45 to 64 years old and 65 years old or more) and by gender (male and female) to account for differences in different age and sex groups (Annex I).

1.2. Modelling approach

To estimate the total incidence of the four selected pathogens in Denmark, the surveillance pyramid was re-constructed as described by Haagsma and colleagues (2013) and by Pires (2014), using a model that scales up the reported number of cases, caused by each pathogen, correcting it for underdiagnosis and under-reporting.
The model described here is multiplicative, meaning that successive sets of non-pathogen specific and pathogen-specific parameters are applied by multiplication to obtain proportional increases in the number of reported cases (Figure 6). With this approach, a multiplication factor (the inverse of a proportion) was estimated for each pathogen, which was then applied to the number of reported cases.

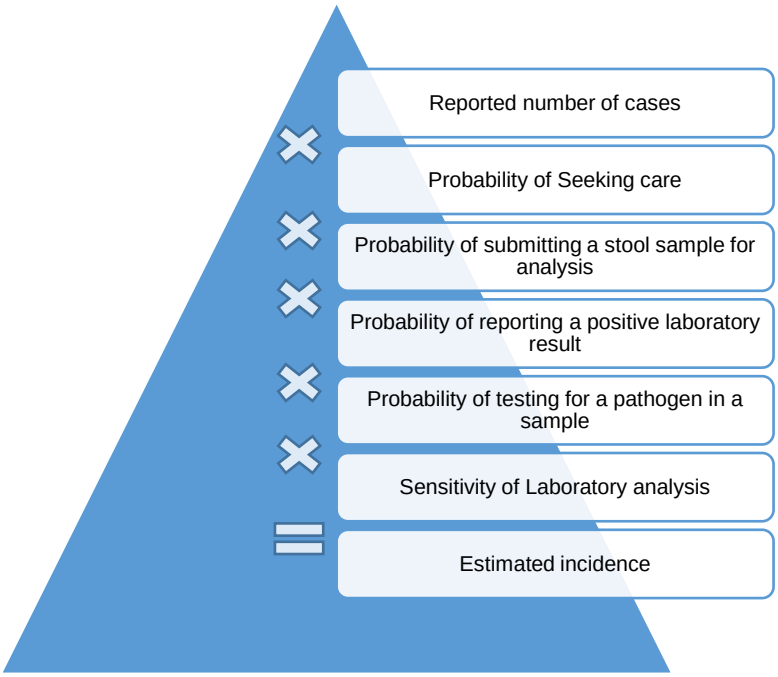
The non-pathogen specific and pathogen specific parameters, the inputs in the model, were informed by a population-based telephone survey applied to the Danish population in 2009

(Müller *et al.*, 2012), by evidence from National Health Registries, by literature review or expert elicitation.

In the telephone survey 1,853 people were interviewed and 206 met the case definition (diarrhea or vomit, with no underlying disorders). Of these, 155 reported having non-bloody diarrhea and five having bloody-diarrhea in the 28 days prior the interview. Participants that met the case definition were also asked about duration of disease, care seeking behavior, stool sample collection and absence from work or normal activities (Müller *et al.*, 2012).

To account for uncertainty in the model, probability distributions describing a range of plausible values for each parameter were used.

Figure 6 - Schematic of the modelling approach to estimate the total incidence of foodborne pathogens in Denmark.



1.1.1. Non-pathogen specific parameters

There are three non-pathogen specific parameters and they are the same for *Campylobacter* spp., *Salmonella* spp., VTEC and *Yersinia enterocolitica* (Table 5).

I. Probability of seeking medical care

This parameter is divided in two: the probability of seeking medical care when having non-bloody diarrhea (a mild case of gastroenteritis) and the probability of seeking medical care when having bloody diarrhea (severe case). It is so, due to the fact that it is more likely that people will consult a GP when severely ill.

Pires (2014) assumes that non-bloody diarrhea cases with short duration (i.e. one to two days) are likely to be viral infections and less likely to seek medical care, and that cases with longer duration (three or more days) would most likely correspond to bacterial infections and a higher probability of seeking care. Therefore, the data from the telephone survey was stratified and analyzed in order to calculate two different underdiagnosis multipliers: one for viral foodborne infections and one for bacterial ones. Consequently, the data available to estimate underdiagnosis of bacterial diseases was reduced, as only 40 participants reported having diarrhea for 3 or more days. This assumption was not made for bloody-diarrhea cases.

II. Probability of submitting a stool sample for analysis

This parameter is divided in three: probability of submitting a stool sample for analysis for non-bloody diarrhea cases, for bloody diarrhea cases and for hospitalized patients.
The two first sub-parameters were estimated with the information from the telephone survey on the proportion of interviewed participants having a stool sample taken and submitted. For the hospitalized cases it was assumed that the proportion would be higher (Pires, 2014).

III. Probability of reporting a positive laboratory result

This parameter was defined on the basis of the proportion of cases that have been reported in MiBa in the years of 2009 to 2013 and on the National Registry for Foodborne Pathogens (Steen Ethelberg, Personal Communication). This proportion applies to all cases, regardless of its severity.

The parameters informed by data from the telephone survey were defined as Beta distributions. This probability distribution was chosen because it can describe the uncertainty or random variation of a probability, fraction or prevalence (Vose, 2008). In this case, it describes the uncertainty about various binomial probabilities, in which given a number, s, of positive responses for one variable and given the total number, n, of interviewed persons within that category, we have α set to the value (s + x) and β set to (n – s + y), where Beta(x,y) is the prior.
The probability of submitting a stool sample for analysis for hospitalized cases was informed by expert elicitation and therefore we used a PERT (or BetaPERT) distribution. This probability distribution is a version of the Beta distribution and requires three parameters, namely a minimum, a most likely and a maximum value. It is used for modelling expert

estimates, where one is given the expert's minimum, most likely and maximum guesses (Vose, 2008).

Table 5 - Non-pathogen specific parameters used to estimate the true incidence of Campylobacteriosis, Salmonelosis, VTEC infections and yersiniosis in Denmark.

Notation	Description	Distribution	Reference
	Probability of seeking medical care		
PCS_{nb}	Non-bloody diarrhea	~ Beta(14;26)*	Müller *et al*., (2012)
PCS_{b}	Bloody diarrhea	~ Beta(4;3)**	Müller *et al*., (2012)
	Probability of submitting a stool sample for analysis		
PSS_{nb}	Non-bloody diarrhea	~ Beta(7;16)	Müller *et al*., (2012)
PSS_{b}	Bloody diarrhea	~ Beta(2;3)	Müller *et al*., (2012)
PSS_{h}	Hospitalized patients	~ Pert(0.3;0.7;0.9)	Pires (2014)
	Probability of reporting a positive laboratory result		
PRR	All cases	~ Beta(9;1)	MiBa

*Data from cases that reported having diarrhea for three or more days.
** Data from patients that reported having bloody diarrhea, regardless of duration.

1.1.2. Pathogen-specific parameters

There are four pathogen-specific parameters (Table 6), which combined with the probability of submitting a stool sample for analysis and the probability of reporting a positive laboratory result will account for the under-reporting associated to campylobacteriosis, salmonellosis, VTEC infections and yersiniosis.

I. Probability of testing for a pathogen in a stool sample

Campylobacter spp.*, Salmonella* spp. and *Yersinia enterocolitica* are the three most common foodborne pathogens in Denmark and are included in the standard testing protocol of gastroenteritis patients (Steen Ethelberg, Personal communication).
Therefore, the probability that a laboratory will test for one of these three pathogens is the same, as well as higher when compared to VTEC.
This probability is lower for VTEC because historically there is less awareness regarding this pathogen, the laboratory testing methods have changed overtime (Clogher *et al.*, 2012) and because of it being included later, and at different times, in laboratories' routine procedures throughout the country (Pires, 2014).

II. Sensitivity of laboratory analysis

The sensitivity of a laboratory test measures the proportion of positive results that are correctly identified as such, which translates as the ability of the test to identify correctly affected individuals. This parameter varies between laboratory methods and thus, also varies between pathogens.

In the same way as all the other parameters, this one was defined as a probability distribution. For *Campylobacter* spp. and *Salmonella* spp. a triangular distribution was used. This distribution constructs a triangular shape from the three input parameters (a minimum, a most likely and a maximum). It is commonly used due to the intuitive nature of its defining parameters and speed of use, offering considerable flexibility due to its shape (Vose, 2008). The information to define this parameter for *Y. enterocolitica* was obtained by expert elicitation, therefore we used a PERT distribution, using the expert minimum, most likely and maximum estimates as distribution's parameters.

III. Proportion of bloody diarrhea in cases and proportion of hospitalized cases

The probabilities of a patient having bloody diarrhea and of being hospitalized are related to the severity of disease and vary between pathogens.

In a literature review by Pires (2014) the proportion of cases with bloody diarrhea is substantially higher for VTEC, followed by *Salmonella* spp. and *Campylobacter* spp., while the proportion of hospitalized cases, although following the same ranking, presents similar values between the three pathogens.

Both these parameters for yersiniosis were also informed by a literature review.

For the proportion of bloody diarrhea, data from a prospective case-case comparison study performed in Denmark (Schiellerup *et al.*, 2008), which concluded that reactive joint pain after gastro-intestinal (GI) infection is positively correlated to severity of GI symptoms, were used. From the 2,105 surveyed patients, 91 had yersiniosis, 17 of which presented severe gastro-enteritis (GE), defined by bloody diarrhea and/or hospitalization.

For the proportion of hospitalized cases data were used from a case-control study also performed in Denmark (Helms *et al.*, 2006), which determined short and long-term odd ratios of hospitalization due to GE. Among 52,121 patients (compared with a reference group of 587,720 persons from the general population) 3,922 had yersinniosis, of which 368 were hospitalized.

Table 6 - Pathogen-specific parameters used to estimate the true incidence of *Campylobacteriosis, Salmonelosis,* VTEC infections *and yersiniosis* in Denmark.

Notation	Description	Distribution	Reference
	Yersinia-specific		
P_{TP}	Probability of testing for *Yersinia* in sample	~ Beta(9.9;0.1)	S. Ethelberg, PCº
Sen	Sensitivity of laboratory analysis	~ Pert(0.7;0.76;0.88)	E. Moller Nielsen PCº
P_{bd}	Proportion of bloody diarrhea in cases	~ Beta(18;75)	Schiellerup *et al.* (2008)
P_{h}	Proportion of hospitalized cases	~ Beta(369;3,555)	Helms *et al*. (2006)
P_{A1}	Proportion of biotype 1A	70%	S. Ethelberg PCº
	Campylobacter-specific		
P_{TP}	Probability of testing for *Campylobacter* in sample	~ Beta(9.9;0.1)	S. Ethelberg, PCº
Sen	Sensitivity of laboratory analysis	~ Triang(0.7;0.76;0.82)	Haagsma *et al.*, (2013a)
P_{bd}	Proportion of bloody diarrhea in cases	~ Beta(4.74;21.3)	Haagsma *et al*., (2013a)
P_{h}	Proportion of hospitalized cases	~ Beta(2,221;15,771)	Espenhaim, (2013)
	Salmonella-specific		
P_{TP}	Probability of testing for *Salmonella* in sample	~ Beta(9.9;0.1)	S. Ethelberg, PCº
Sen	Sensitivity of laboratory analysis	~ Triang(0.85;0.88;0.91)	Haagsma *et al.*, (2013a)
P_{bd}	Proportion of bloody diarrhea in cases	~ Beta(2.34;3.81)	Haagsma *et al.*, (2013a)
P_{h}	Proportion of hospitalized cases	~ Beta(5,811;22,085)	Espenhaim, (2013)
	VTEC-specific		
P_{TP}	Probability of testing for VTEC in sample	~ Beta(4;6)	S. Ethelberg, PCº
Sen	Sensitivity of laboratory analysis	~ Beta(7;3)	Haagsma *et al.*, (2013a)
P_{bd}	Proportion of bloody diarrhea in cases	~ Beta(2.79;0.73)	Haagsma *et al.*, (2013a)
P_{h}	Proportion of hospitalized cases	~ Beta(165;424)	Espenhaim, (2013)

ºPC: Personal communication

IV. Proportion of biotype 1A on the yersiniosis' reported cases

As mentioned before, Denmark registers and reports all positive stool samples of *Y. enterocolitica.* However, biotype 1A is considered non-pathogenic for humans. Of the 572 cases that presented a positive stool sample for *Y. enterocolitica* in 2016, 70% belonged to that biotype. Therefore, they were excluded to better depict the incidence of this disease.

Although all data were stratified by gender and age groups, access to that information regarding the cases of biotype 1A was not possible. Therefore, it was assumed that these cases would be equally distributed among all gender and age group categories. As such, only 30% of the reported cases for each category were considered, accounting for a total of 172 cases of yersiniosis.

1.1.3. Model for re-constructing the surveillance pyramid

After gathering all public health surveillance data regarding campylobacteriosis, salmonellosis, VTEC infections and yersiniosis and defining all parameters needed as input for the model, an overall multiplying factor was estimated to correct the reported cases to the true incidence of each selected pathogen in Denmark in the year 2016.

The defined parameters were combined in different steps (Table 7), which were all modelled using R 3.3.2 (R Core Team, 2016), using Monte Carlo simulation. The model was run for 20,000 iterations.

Monte Carlo simulation is a computer simulation technique that randomly selects a single value from each of the probability distributions that define the parameters of our model. Since the parameters are combined using various calculation steps, each single value will then be used to calculate a mathematical solution (the multiplying factor, the output). Subsequently, each result is stored, and the sequence can be repeated several number of times (iterations). Values with higher probabilities are more likely to occur and will be stored more frequently. At the end of the simulation run, the values for the multiplying factor can be analyzed in various ways: using graphs like a histogram or a cumulative distribution graph that depict the shape and range of the uncertainty in the model output or analyzed statistically.

Table 7 - Variables and calculations to re-construct the foodborne pathogens' surveillance pyramid.

Notation	Description	Calculation
N	Number of reported cases	Data (reported cases)
N_a*	Number of cases excluding biotype 1A	$N * (1 - P_{A1})$
N_h	Number of hospitalized cases	$N_a * P_h$
NCS	Number of cases that seek care	$N_a – N_h$
PCS	Probability of seeking medical care	$(1 – P_{bd}) * PCS_{nb} + P_{bd} * PCS_b$
PSS	Probability of submitting a stool sample for analysis	$(1 – P_{bd}) * PSS_{nb} + P_{bd} * PSS_b$
T_{nh}	Total number of non-hospitalized cases	NCS * 1/(PCS * PSS * PRR * PTP * Sen)
T_h	Total number of hospitalized cases	$N_h * 1/(PSS_h * PRR * PTP * Sen)$
T	Total number of cases	$T_{nh} + T_h$
M	Multiplier	T/Na

* This step is only taken into account when applying the model for yersiniosis data. Therefore, when considering the other three selected pathogens, whenever it reads “Na” it should be considered N, as in the reported cases for each disease.

1.3. Sensitivity analysis

The aim of a sensitivity analysis was to investigate the inter-relationships between inputs, the non-pathogen specific and pathogen-specific parameters, and the output, the multiplying factor.

A rank order correlation was used on the data that had been generated from input distributions and data calculated for the selected output, assuming that all the input parameters have either a purely positive or negative statistical correlation with the output. A rank order correlation is a statistical way of measuring an ordinal association, i.e. the relationship between rankings of different variables or different rankings of the same variable, to different observations of a particular variable (in this case the output). This analysis

replaces each collected value by its rank among other values generated for that input or output, and then calculates the Spearman's rank order correlation coefficient, ρ, between each input and the output (Vose, 2008).

The Spearman's rank order correlation coefficient, ρ, is a non-parametric statistic (meaning that the statistic correlation is not affected by the type of mathematical relationship between the variables) for quantifying the correlation relationship between two variables (Vose, 2008).

The sensitivity analysis was performed using @Risk 7.5 (Palisade Corporation, 2017).

1.4. Scenario Analysis

Four different alternative scenarios for each disease model were carried on, based on the sensitivity analysis. The increase on the different probabilities was assumed to be one which could be achieved if real measures were taken to decrease underdiagnosis and under-reporting.

For the *Campylobacter* spp. and *Y. enterocolitica*'s disease models the alternative scenarios were done with the following changes:

1) For the probability of submitting a stool sample for non-bloody diarrhea cases the scenario consists of increasing this probability to 40% (on the population survey 21 respondents went to the GP and had non-bloody diarrhea, but only six samples were submitted: 29%).
   Therefore,
   $$PSSnb \sim Beta(9.4; 13.6)$$
2) For the probability of seeking care for non-bloody diarrhea cases the scenario also consists on increasing this probability to 40% (on the population survey 38 respondents had non-bloody diarrhea, but only 13 went to a GP: 34%).
   Therefore,
   $$PCSnb \sim Beta(16.2; 23.8)$$
3) For the probability of submitting a stool sample for bloody diarrhea cases there was also an increase to 40% (three respondents went to the GP and had bloody diarrhea and only one sample was taken: 33%).
   Therefore,
   $$PSSb \sim Beta (2.2; 2.8)$$
4) On the fourth scenario all the previous changes were combined.

For the *Salmonella* spp.'s disease model the alternative scenarios were done with the following changes:

1) For the probability of submitting a stool sample for bloody diarrhea cases there was also an increase to 40% (three respondents went to the GP with bloody diarrhea and only one sample was taken: 33%).
   Therefore,
   $$PSSb \sim Beta(2.2; 2.8)$$
2) For the probability of submitting a stool sample for non-bloody diarrhea cases the scenario consists of increasing this probability to 40% (on the population survey 21 respondents went to the GP and had non-bloody diarrhea, but only six samples were submitted: 29%).
   Therefore,
   $$PSSnb \sim Beta(9.4; 13.6)$$
3) For the probability of seeking care for bloody diarrhea cases the scenario consists on increasing this probability to 50% (on the population survey seven respondents had bloody diarrhea, but only three went to a GP: 43%).
   Therefore,
   $$PCSb \sim Beta(4.5; 2.5)$$
4) On the fourth scenario all the previous changes were combined.

Finally, for the VTEC's disease model the alternative scenarios were done with the following changes:

1) For the probability of submitting a stool sample for bloody diarrhea cases there was also an increase to 40% (three respondents went to the GP with bloody diarrhea and only one sample was taken: 33%).
   Therefore,
   $$PSSb \sim Beta(2.2; 2.8)$$
2) For the probability of testing for VTEC in the sample the alternative scenario increased this probability to 45% (through an expert's personal communication only 3 out of 10 stool samples would be tested for VTEC: 37.5%)
   Therefore,
   $$PTP \sim Beta(4.6; 5.4)$$

3) For the probability of seeking care for bloody diarrhea cases the scenario consists on increasing this probability to 50% (on the population survey seven respondents had bloody diarrhea, but only 3 went to a GP: 43%)

Therefore we have,

PCSb ~ Beta(4.5; 2.5)

4) On the fourth scenario all the previous changes were combined.

2. Estimating the burden of disease of four foodborne pathogens

The disability-adjusted life year (DALY) metric was used to estimate the burden of disease of *Campylobacter* spp.*, Salmonella* spp.*, Yersinia enterocolitica* and VTEC.

The DALY is a summary measure of population health, commonly used in disease burden assessment studies (Murray & Lopez, 1996; GBD's Disease and Injury Incidence and Prevalence collaborators, 2016). DALYs are conceptually simple, representing the years of life lost due to decreased quality of life and/or premature death as a consequence of a disease or condition, measured at individual or population level.

DALYs aggregate morbidity and disability, expressed as years lived with disability (YLD), and mortality, expressed as years of life lost (YLL), into a single figure, calculated as:

$$DALY = YLD + YLL$$

YLD represents the healthy time lost while living with a disease or condition and is calculated as,

$$YLD = \sum (ni \times ti \times dwi)$$

Where, n is the number of incident cases of health-outcome i, t is the average duration of i until remission or death and dw is the disability weight assigned to i.

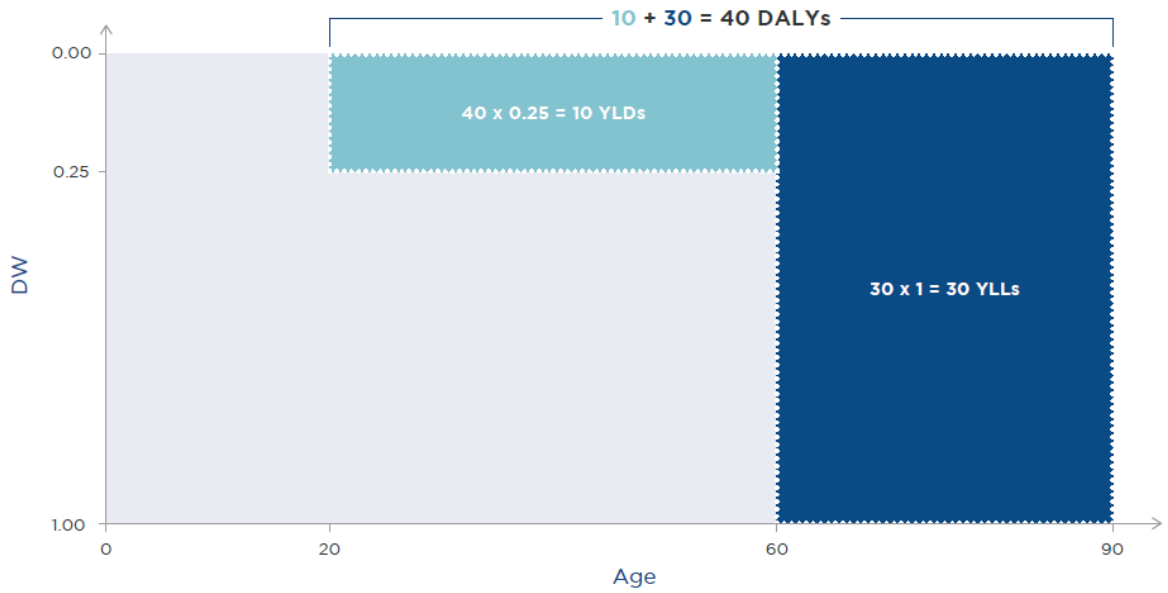
YLL represents the time lost due to premature death and is calculated with the following formula,

$$YLL = \sum (di \times e)$$

Where, d is the number of deaths due to health-outcome i in a certain period of time and e is the residual life expectancy at the age of death.

Figure 7 represents a theoretical example of the calculation of DALYs. It portrays the life of an individual born with perfect health and, at age 20, a given event (e.g. foodborne disease) decreases his or her life by 25% (disability weight of 0.25). Thereafter, the person lives in this new health state for another 40 years, dying prematurely at age 60. The burden associated with the disease for this individual (total DALYs) is calculated by summing the YLD and YLL due to premature death (his/her dying age compared with the life expectancy of the population).

Figure 7 - Theoretical example of disability adjusted life years (DALYs).



In this study, DALYs were calculated by a hazard-based and incidence-based approach. Therefore, this calculation required the identification of all health-outcomes associated with campylobacteriosis, salmonellosis, yersiniosis and VTEC infections and the estimation of their probability of occurrence.

2.1. Health-outcomes of four foodborne pathogens

The disease outcomes of foodborne infections and the probabilities of developing these outcomes after infection can be described in an outcome tree. Figures 8 to 11 represent the outcome trees for *Campylobacter* spp., *Salmonella* spp., *Y. enterocolitica* and VTEC infections.

The outcome trees currently used for *Campylobacter* spp., *Salmonella* spp. and VTEC infections were developed on the basis of a literature review of other burden of disease studies and of studies associating specific outcomes with foodborne infections, previously described by Pires (2014). Because the association between some of the health-outcomes considered then and its association with foodborne infections has been disputed (Jess *et al*, 2011), this study excluded one of the health-outcomes previously considered, namely Inflammatory bowel disease (IBD).

The health-outcome tree for yersiniosis was developed on the basis of a literature review of studies associating specific health-outcomes with *Y. enterocolitica* infections.

A thorough literature search was made using search engines such as: google and google scholar, science direct and the DTU online library, using key-words such as: *Yersinia, Y. enterocolica*, clinical manifestations and sequelae. A preliminary selection was made by selecting studies developed between 1990 and 2017 and by interest on the title. From this

research a total of 66 articles were selected. From those, 17 had relevant information regarding yersiniosis' health-outcomes.
These 17 studies depicted different types of methodologies:

- Six literature reviews (Azghari *et al.*, 2016; Bottone, 2015; Bottone, 1997; Hannu, *et al*., 2006; Schwille-Kiuntke, *et al.*, 2011)
- Five case-control studies (Helms *et al.*, 2006; Huovinen *et al.*, 2010; Ostroff *et al*., 1992; Rosner *et al.*, 2013; Van Noyen, *et al*., 1991)
- Three case-case comparison studies (Schiellerup *et al*., 2008; Stolk-Engelaar & Hoogkamp-Korstanje, 1996; Zheng *et al*., 2008)
- One retrospective cohort study (Porter *et al*., 2013)
- One case report (Shorter *et al*., 1998)
- One population-based telephone survey (Townes *et al.*, 2008)

On chapter 4.3 (Clinical manifestations of yersiniosis) all health-outcomes of yersiniosis were identified and refer all these studies.
To our knowledge, a health-outcome tree for yersiniosis had thus far not been developed, and only one study estimated the burden of this disease (Lake, Cressey, Campbell, & Oakley, 2010), although only considering two possible health-outcomes (reactive arthritis and gastroenteritis).

Figure 8 - Outcome tree for *Campylobacter* spp. Outcomes with dashed white lines are currently not considered in the model.

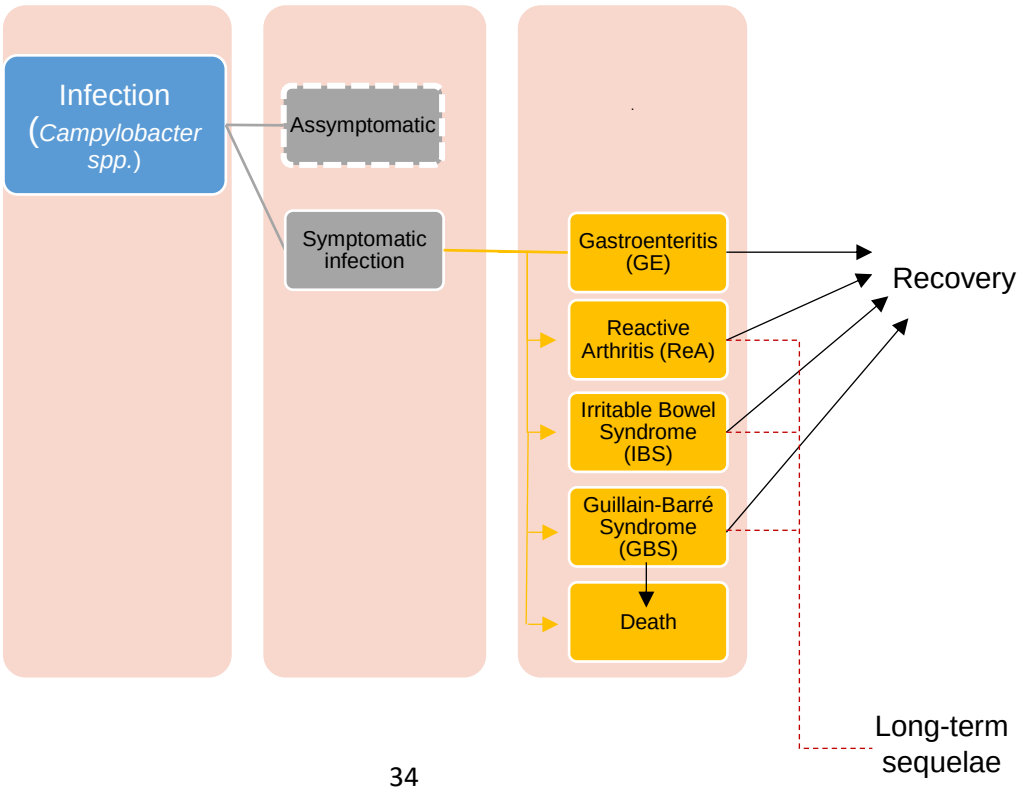


Figure 9 - Outcome tree for non-typhoidal *Salmonella* spp. Outcomes with dashed white and black lines are currently not considered in the model.

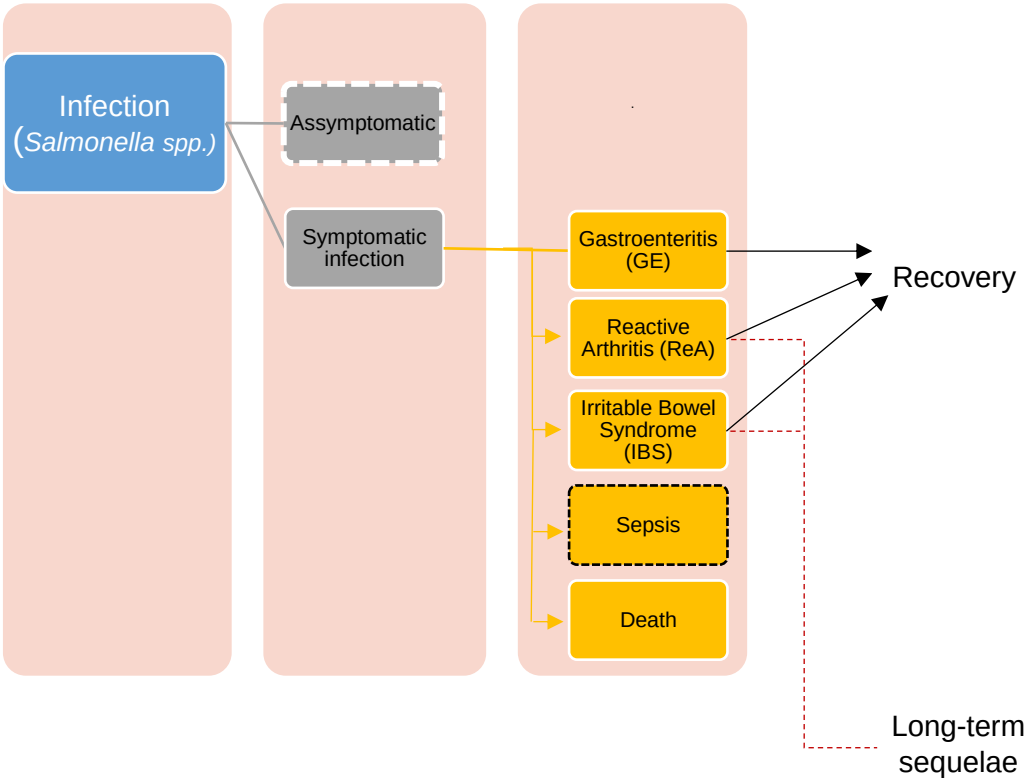


Figure 10 - Outcome tree for *Y. enterocolitica.* Outcomes with dashed white and black lines are currently not considered in the model.

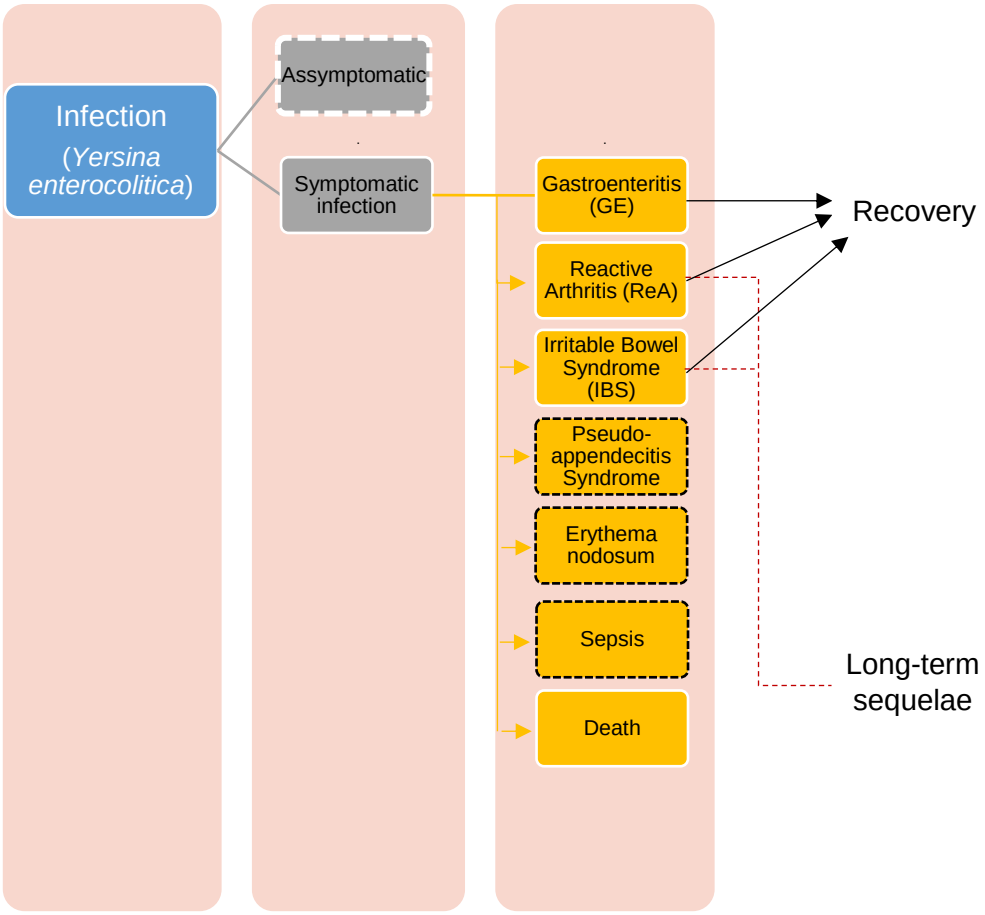
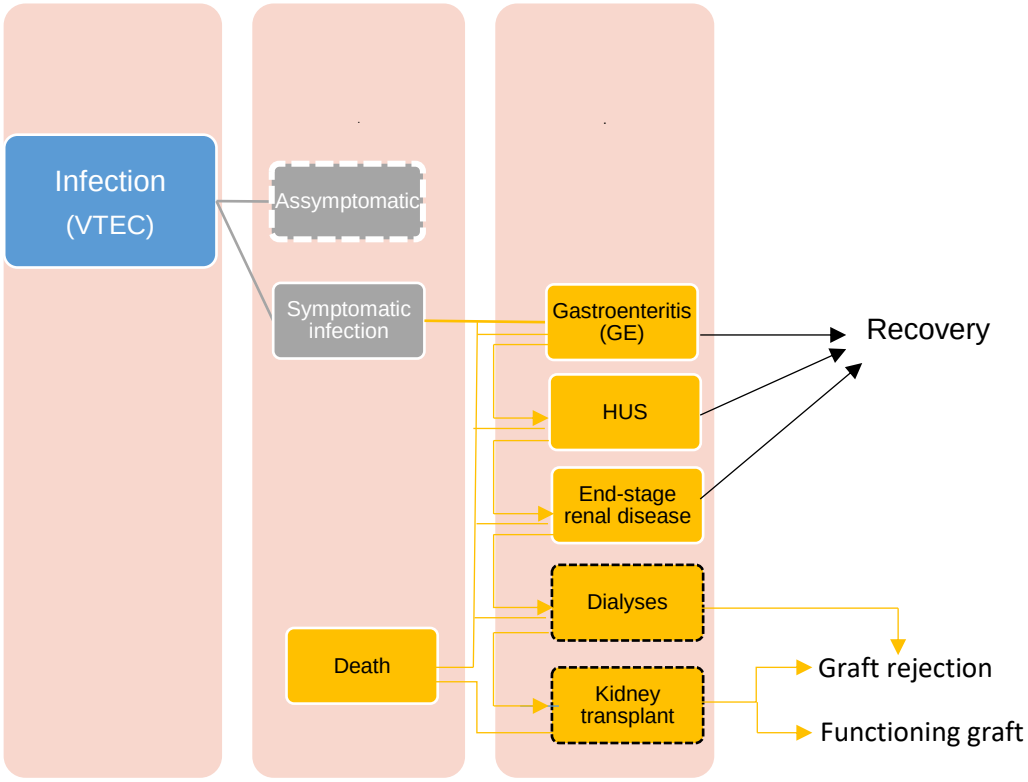


Figure 11 - Outcome tree for VTEC. Outcomes with dashed white and black lines are currently not considered in the model.



Due to lack of consistent data, pseudo-appendicitis syndrome and erythema nodosum were not considered in yersiniosis' model (Anonymous, 2017). Also, in consistency with the model previously developed by Pires (2014) for *Salmonella* spp., sepsis was not considered for *Y. enterocolitica*'s disease model. Dialyses and kidney transplant (VTEC's health-outcomes) were also not accounted for due to lack of sound data (Pires, 2014).

After all health-outcomes were identified, the probabilities of their occurrence given infection were collected through a literature review. The uncertainty associated with the input data was considered by including them in the model as probability distributions, using the Pert distribution.

I. Gastroenteritis (GE)

The input used to estimate the burden of GE for each foodborne pathogen was the total incidence calculated as mentioned through chapter one of the Material and methods section of this thesis (see Tables 15 to 18). The input data were aggregated by age and sex (applying the same age groups used to calculate total number of cases) and incidence by 1,000 population.

II. Reactive arthritis (ReA)

For *Campylobacter* spp.*, Salmonella* spp. and *Y. enterocolitica* the incidence of ReA after infection was calculated based on the probability of developing ReA for patients with GE visiting a general practitioner, the probability of seeking care for a patient with ReA and the probability of hospitalization for ReA patients who visit a GP (Table 9).

Under the assumption that a case with ReA that does not visit a GP has mild symptoms of ReA; that a case that visits a GP due to his/her symptoms of ReA has a moderate form of ReA; and that patients who are hospitalized have a severe form of ReA, the incidence of mild, moderate and severe ReA was estimated as follows.

Initially, a multiplying factor only accounting for under-reporting (Table 8) was applied to the reported cases in order to estimate the number of cases that visited a GP but were not reported to the Danish surveillance system.

The probability of developing ReA for patients with GE visiting a GP was then multiplied by the previous estimation (cases seeking medical care not captured by the surveillance system) and by the probability of seeking care for patients with ReA, thus estimating the number of cases with GE that visited a GP and developed ReA. In the subsequent step, the number of cases developing ReA but not visiting a GP was calculated by subtracting the modes of the number of cases visiting a GP and developing ReA to the number of cases developing ReA (either visiting a GP or not). Finally, the number of hospitalized patients with ReA who visited a GP was calculated by the multiplication of the number of cases with ReA visiting a GP by the probability of hospitalization for ReA patients who visited a GP.

Table 9 presents the collected input for this health-outcome and data sources.

Table 8 - Variables and calculations to re-construct the foodborne pathogens' surveillance pyramid, accounting only for under-reporting.

Notation	Description	Calculation
N	Number of reported cases	Data (reported cases)
PSS	Probability of submitting a stool sample for analysis	$(1 - P_{bd}) * PSS_{nb} + P_{bd} * PSS_{b}$
T	Total number of cases	N * 1 / (PSS * PRR * PTP * Sen)
M	Multiplier	T/N

Table 9 - Description of Reactive arthritis' input and data sources.

Pathogen	Input			Reference
	PRGE	PRGP	PRH	
Campylobacter spp.	~ Beta(46;565)	~ Beta(10;37)	~ Beta(2;45)	(Havelaar *et al*., 2012; Pires, 2014)
Salmonella spp.	~Pert(0.023;0.08;0.15)			(Havelaar *et al*., 2012; Pires, 2014)
Y. enterocolitica	~ Beta(22;71)			(Havelaar *et al*., 2012; Pires, 2014; Schiellerup *et al*., 2008)

PRGE: probability of developing ReA for patients with GE visiting a general practitioner.
PRGP: probability of seeking care for a patient with ReA.
PRH: probability of hospitalization for ReA patients.

III. Irritable bowel syndrome (IBS)

IBS is considered a long-term sequela of *Campylobacter* spp.*, Salmonella* spp. *and Y. enterocolitica* infections.

The probability of developing post-infectious IBS (Haagsma, Siersema, De Wit, & Havelaar, 2010), defined by ~ Pert (7.2; 8.8; 10.4), was applied to the total incidence of each pathogen in order to estimate the incidence of IBS, which was calculated per 1,000 population and stratified by gender and age group.

IV. Guillain-Barré syndrome (GBS)

The Guillain-Barré syndrome is a long-term and possibly fatal sequela associated with *Campylobacter* spp. infections.

To estimate its incidence, the number of reported cases of campylobacteriosis was combined with the probability of developing GBS given infection, defined as ~ Beta (60; 29,942) (Pires, 2014). After, the point estimate for the probability of developing GBS for each age group

(Annex II) was multiplied, resulting in the estimation of GBS' incidence calculated per 1,000 population and stratified by gender and age group.

V. Hemolytic uremic symdrome (HUS) and End-stage renal disease (ESRD)

The burden of disease of HUS was estimated based on the reported incidence of HUS for the year 2016 (Annex II), under the assumption that all cases of VTEC associated HUS were diagnosed and reported.

End-stage renal disease is a consequence of HUS, therefore ESRD's incidence was calculated by applying the probability of its development given HUS, defined by ~ Beta (24; 712), to the incidence of HUS (Pires, 2014).

VI. Mortality

Disease by all four foodborne pathogens can lead to mortality.

The excess mortality risk associated with the infections caused by three of these four pathogens was calculated using the mean value for the relative mortality associated with: *Campylobacter* spp., 1.86, *Salmonella* spp., 2.85, and *Y. enterocolitica*,, 2.10, (Helms, Vastrup, Gerner-smidt, & Mølbak, 2003), using the following formula:

$$\text{Excess mortality risk} = e^{(\log OR - 1)}$$

Where, OR is the relative mortality.

In the absence of Danish data, these multipliers were applied to Dutch data for the age-specific mortality risk by all causes (Annex II), stratified by gender and age groups, and finally applied only to the laboratory-confirmed cases, assuming that they reflect the most severe cases of disease (Pires, 2014).

Regarding the mortality associated with VTEC infections, the input data used was derived from surveillance data: three deaths for the last 15 years (Flemming Scheutz, Personal Communication) and the total number of reported cases in that time frame, 2,180 cases. With these data, a probability distribution was defined, ~ Beta (4; 2,178), and applied to the number of reported cases of VTEC for the year 2016.

Mortality was calculated per 1,000 population and the uncertainty with these input data was not considered in the model.

There is also another health-outcome which can be fatal, the Guillain-Barré syndrome, which is associated with *Campylobacter* spp. infections. To estimate the number of deaths caused by this syndrome, the probability of dying from the GBS given infection, ~ Pert (0.01; 0.02; 0.05) (Pires, 2014), was applied to the number of GBS cases estimated. It was calculated per 1,000 population and stratified by gender and age group. The uncertainty associated with

these input data was considered by including them in the model using a Pert distribution. Table 10 summarizes all health-outcomes' input and data sources (except ReA, showed in Table 9).

Table 10 - Description of health-outcomes' input and data sources.

Health-outcome	Input				Reference
	Campylobacter	Salmonella	Yersinia	VTEC	
IBS	~ Pert (7.2; 8.8; 10.4)			NA	*(*Haagsma *et al*., 2010)
GBS	~ Beta (60; 29,942)		NA		Pires (2014)
HUS	NA			Incidence	Surveillance
ESRD	NA			~Beta (24; 710)	Pires (2014)
Mortality	1.86 GBS: ~Pert(0.01; 0.02; 0.05)	2.85	2.10	~ Beta (4; 2178)	(Helms *et al*., 2003; Pires, 2014; Statistics Netherlands' data used as surrogate)

IBS: Irritable bowel syndrome; GBS: Guillain-Barre syndrome; HUS: Hemolytic uremic syndrome; ESRD: End-stage renal disease

2.2. Disability weights and duration of health-outcomes

The disability weight (DW) reflects the impact of a health condition in terms of health-related quality of life, and has a value ranging from 0, indicating full health, through 1, indicating worst imaginable health state.

DWs were retrieved from the Global burden of disease for the year 2013 study *(Salomon et al*., 2015). When the DW for a specific health-outcome was not available, a proxy DW was used from an outcome which has similar health effects. When DWs for specific health-outcomes differentiated between multiple degrees of severity, the overall DW on the basis of the proportion of cases that presented these degrees in Denmark (estimated based on BoI estimates) were calculated. For example, three severity levels of gastroenteritis were considered: mild, moderate and severe, each with a correspondent DW and a proportion of the totality of cases.

Data on the duration of each health-outcome were collected through a literature review.

Tables 11 to 14 summarize all the DW and durations of the different health-outcomes caused by each of the four foodborne pathogens.

Table 11 - Duration and disability weights for *Campylobacter* spp. health-outcomes.

Health-outcome	Duration (years)	Disability weights				Reference
GE	0.008 [0.003; 0.019]	Mild: 0.074 95% CI: [0.049; 0.104]	Moderate: 0.188 95% CI: [0.125; 0.264]	Severe: 0.247 95% CI: [0.164; 0.348]	*Overall: ~ Pert(0.0817; 0.05; 0.123)	(Salomon *et al*., 2015)
ReA	0.608219178	Not visiting a GP: 0.023	Visiting a GP: 0.115	Hospitalized: 0.186		(Haagsma, Havelaar, Janssen, & Bonsel, 2008)
IBS	5	0.042				(Havelaar *et al.*, 2012)
GBS	Life-long	Mild: 0.090	Severe: 0.280	Residual symptoms: 0.160		(Havelaar, de Wit, van Koningsveld, & van Kempen, 2000)

GE: Gastroenteritis; ReA: Reactive arthritis; IBS: Irritable bowel syndrome; GBS: Guillain-Barre syndrome.

Table 12 - Duration and disability weights for *Salmonella* spp. health-outcomes.

Health-outcome	Duration (years)	Disability weights				Reference
GE	0.008 [0.003; 0.019]	Mild: 0.074 95% CI: [0.049; 0.104]	Moderate: 0.188 95% CI: [0.125; 0.264]	Severe: 0.247 95% CI: [0.164; 0.348]	*Overall: ~ Pert(0.0817; 0.05; 0.123)	(Salomon *et al*., 2015)
ReA	0.608219178	Not visiting a GP: 0.023	Visiting a GP: 0.115	Hospitalized: 0.186		(Haagsma *et al*.,2008)
IBS	5	0.042				(Havelaar *et al*., 2012)

GE: Gastroenteritis; ReA: Reactive arthritis; IBS: Irritable bowel syndrome

Table 13 - Duration and disability weights for *Y. enterocolitica's* health-outcomes.

Health-outcome	Duration (years)	Disability weights				Reference
GE	0.04 [0.003; 0.08]	Mild: 0.074 95% CI: [0.049; 0.104]	Moderate: 0.188 95% CI: [0.125; 0.264]	Severe: 0.247 95% CI: [0.164; 0.348]	*Overall: ~ Pert(0.09; 0.06; 0.1)	(Rosner *et al*., 2013; Salomon *et al*., 2015)
ReA	0.608219178	Not visiting a GP: 0.023	Visiting a GP: 0.115	Hospitalized: 0.186		(Haagsma *et al*., 2008; Schiellerup *et al.*, 2008)
IBS	5	0.042				(Havelaar *et al*., 2012)

GE: Gastroenteritis; ReA: Reactive arthritis; IBS: Irritable bowel syndrome

Table 14 - Duration and disability weights for VTEC's health-outcomes.

Health-outcome	Duration (years)	Disability weights				Reference
GE	0.019 [0.014; 0.027]	Mild: 0.074 95% CI: [0.049; 0.104]	Moderate: 0.188 95% CI: [0.125; 0.264]	Severe: 0.247 95% CI: [0.164; 0.348]	*Overall: ~ Pert(0.0817; 0.05; 0.123)	(Majowicz *et al*., 2014; Salomon *et al*., 2015)
HUS	0.077 [0.038; 0.115]	0.21				(Kirk *et al*., 2015)
ESRD	Life-long	0.573 [0.397; 0.749]				(Havelaar *et al*., 2012; Pires, 2014)

GE: Gastroenteritis; HUS: Hemolytic uremic syndrome; ESRD: End-stage renal disease

2.3. Life expectancy

Life expectancy at a specific age can be derived from country-specific life tables (if available), or standard life tables with fixed life-expectancy (Pires, 2014).

To calculate YLL the WHO "Standard Life Table for Years of Life Lost" was chosen (WHO, 2017), which was developed based on the projected frontier period life expectancy and life table for the year 2050 (Annex II).

To calculate YLD the Danish life expectancy estimates for 2015 – 2016 were chosen (Statistics Denmark, accessed March 6th, 2017) (Annex II).
The different choices of life expectancy tables are based on two principles. First, to calculate YLL it is important to account for an individual highest possible longevity, so the burden of life lost can be measured at its maximum. And second, when calculating YLD (some health-outcomes can be life-long) the interest is on accounting for the actual years individuals in a certain population live with a specific disease or condition.

2.4. The Disability Adjusted Life Year model

To calculate the total DALYs associated with the four selected foodborne pathogens in Denmark for the year 2016, the incidence of all considered health-outcomes was estimated and combined with all variables described above.
Total years lived with disability (YLD), years of life lost (YLL) and overall DALYs for each sequela of each disease were calculated by applying a stochastic model using a Graphical User Interface for calculating DALYs and performing uncertainty and sensitivity analyses, the DALY calculator, accessible in the R statistical programming environment (Devleesschauwer *et al*., 2016).

IV. Results

1. Burden of disease of four foodborne pathogens

1.1. Total incidence of disease by four foodborne pathogens

The total estimated multiplier to correct reported cases to the real number of cases occurring in the Danish population in 2016 varied between pathogens (Tables 15 to 18). This multiplier was lower for *Salmonella* spp. (7.7), and highest for VTEC (19.7), while for *Campylobacter and Y. enterocolitica* the estimations were similar (11.0 and 10.9, respectively). These mean that for each case captured by the Danish public health surveillance system, around 8, 11 and 20 people fell ill due to *Salmonella* spp.*, Campylobacter* spp. *and Y. enterocolitica,* and VTEC infections, respectively.

In 2016, 4,674 cases of campylobacteriosis, 1,068 cases of salmonellosis, 172 cases of yersiniosis caused by pathogenic biotypes of *Y. enterocolitica* and 250 cases of VTEC infections were reported to the Danish public health surveillance system. When accounting for under-reporting and underdiagnosis, estimates suggest that a total of 51,225 cases of campylobacteriosis, 8,197 cases of salmonellosis, 1,860 cases of yersiniosis and 4,920 cases of VTEC occurred in Denmark in 2016.

Table 15 - Estimated incidence due to *Campylobacter* spp. infections in Denmark, 2016 (cases per 100,000 population).

	Reported/ 100,000		**Multiplier**	**Estimated total incidence / 100,000 (Median [95% CI])**	
	Male	**Female**		**Male**	**Female**
Total Population	75.8	86.7	11.0 [5.97; 23.8]	832.8 [453.0; 1804.9]	952.4 [518.1; 2064.0]
Age group					
0 – 4	78.3	102.8		860.3 [468.0; 1864.6]	1129.4 [614.4; 2447.7]
5 – 14	28.5	51.3		313.2 [170.4; 678.8]	563.8 [306.7; 1221.8]
15 – 44	92.0	99.3		1010.4 [549.6; 2189.7]	1090.8 [593.4; 2364.1]
45 – 64	79.9	100.0		877.5 [477.3; 1901.7]	1098.6 [597.6; 2381.0]
65+	63.4	62.3		717.9 [390.5; 1555.9]	684.7 [372.5; 1484.0]

Table 16 - Estimated incidence due to *Salmonella* spp. infections in Denmark, 2016 (cases per 100,000 population).

	Reported/ 100,000		Multiplier	Estimated total incidence / 100,000 (Median [95% CI])	
	Male	**Female**		**Male**	**Female**
Total Population	19.2	17.9	7.7 [3.7; 18.1]	147.6 [71.5; 348.7]	137.6 [66.7; 325.2]
Age group					
0 – 4	38.5	43.5		295.6 [143.3; 698.5]	333.8 [161.8; 788.5]
5 – 14	13.5	20.4		103.8 [50.3; 245.2]	156.7 [75.9; 370.1]
15 – 44	15.3	14.6		117.3 [56.9; 277.2]	111.8 [54.2; 264.1]
45 – 64	20.8	16.5		159.6 [77.4; 377.1]	127.0 [61.5; 300.0]
65+	23.5	18.2		180.5 [87.5; 426.5]	139.7 [67.7; 330.0]

Table 17 - Estimated incidence due to *Yersinia enterocolitica* infections in Denmark, 2016 (cases per 100,000 population).

	Reported/ 100,000		Multiplier	Estimated total incidence / 100,000 (Median [95% CI])	
	Male	**Female**		**Male**	**Female**
Total Population	3.6	2.4	10.9 [6.0; 23.3]	38.9 [21.5; 83.2]	26.1 [14.4; 55.8]
Age group					
0 – 4	4.1	5.6		44.7 [24.7;95.7]	60.8 [33.6; 130.1]
5 – 14	2.5	3.2		26.8 [14.8; 57.5]	35.3 [19.5; 75.5]
15 – 44	4.2	2.5		46.0 [25.4; 98.5]	27.7 [15.3; 59.3]
45 – 64	3.2	1.7		35.2 [19.4; 75.3]	18.6 [10.2; 39.7]
65+	3.2	1.8		35.1 [19.4; 75.1]	19.2 [10.6; 41.2]

Table 18 - Estimated incidence due to VTEC infections in Denmark, 2016 (cases per 100,000 population).

	Reported/ 100,000		Multiplier	Estimated total incidence / 100,000 (Median [95% CI])	
	Male	**Female**		**Male**	**Female**
Total Population	5.0	3.7	19.7 [6.0; 105.2]	97.7 [29.7; 522.5]	73.6 [22.4; 393.5]
Age group					
0 – 4	22.2	25.5		436.8 [132.9; 2335.9]	502.5 [153.0; 2687.5]
5 – 14	4.4	4.3		86.8 [26.4; 464.1]	86.8 [26.4; 464.1]
15 – 44	3.1	3.0		60.5 [18.4; 323.7]	58.8 [17.9; 314.4]
45 – 64	4.1	1.9		80.3 [24.4; 429.4]	36.5 [11.1; 195.0]
65+	5.6	1.9		109.8 [33.4; 587.3]	36.2 [11.2; 198.0]

Figures 12 to 15 depict the estimated total incidence per 100,000 population segregated by gender and age group.

Figure 12 - Estimated incidence of campylobacteriosis per 100,000 population segregated by gender and age group, Denmark, 2016 (error bars refer to the 95% confidence interval).

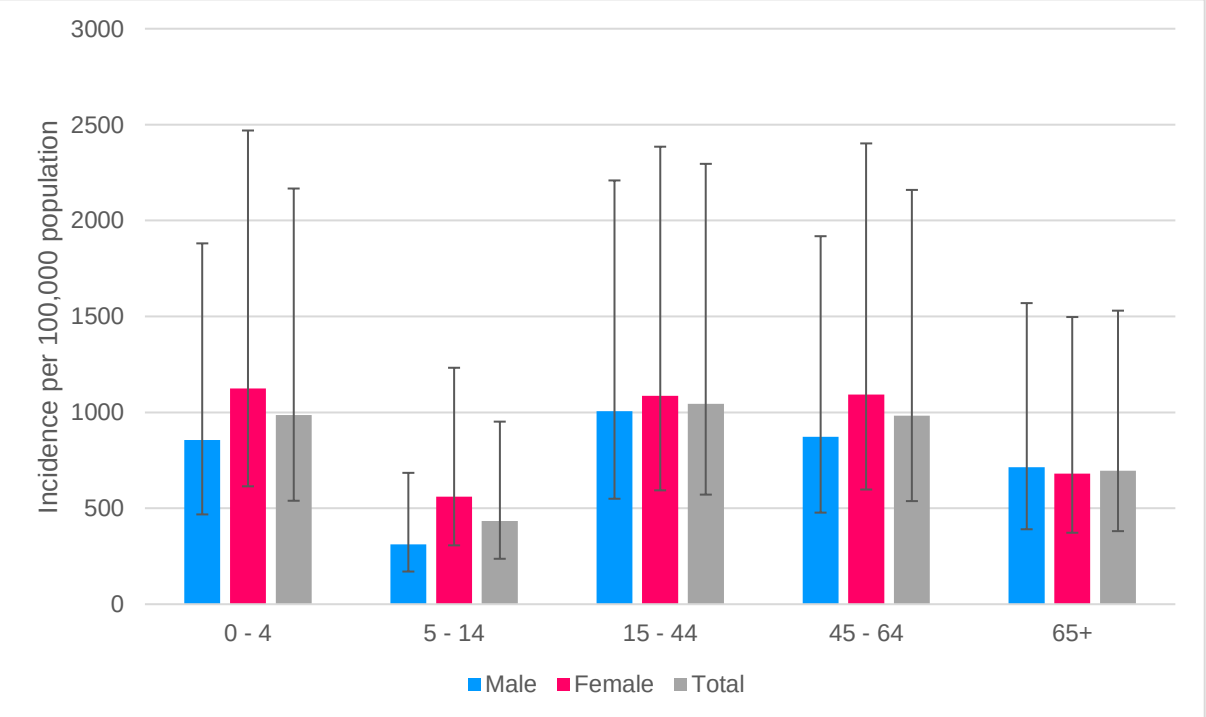


Figure 13 - Estimated incidence of salmonellosis per 100,000 population segregated by gender and age group, Denmark, 2016 (error bars refer to the 95% confidence interval).

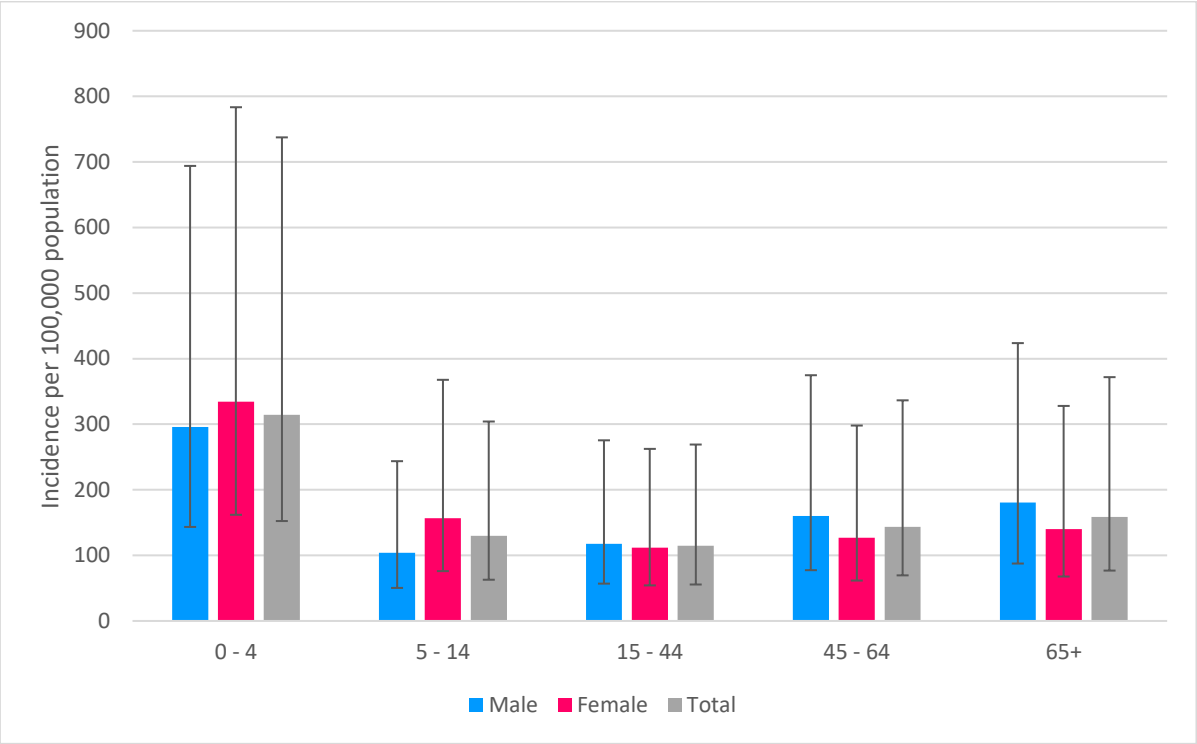


Figure 14 - Estimated incidence of yersiniosis per 100,000 population, segregated by gender and age group, Denmark, 2016 (error bars refer to the 95% confidence interval).

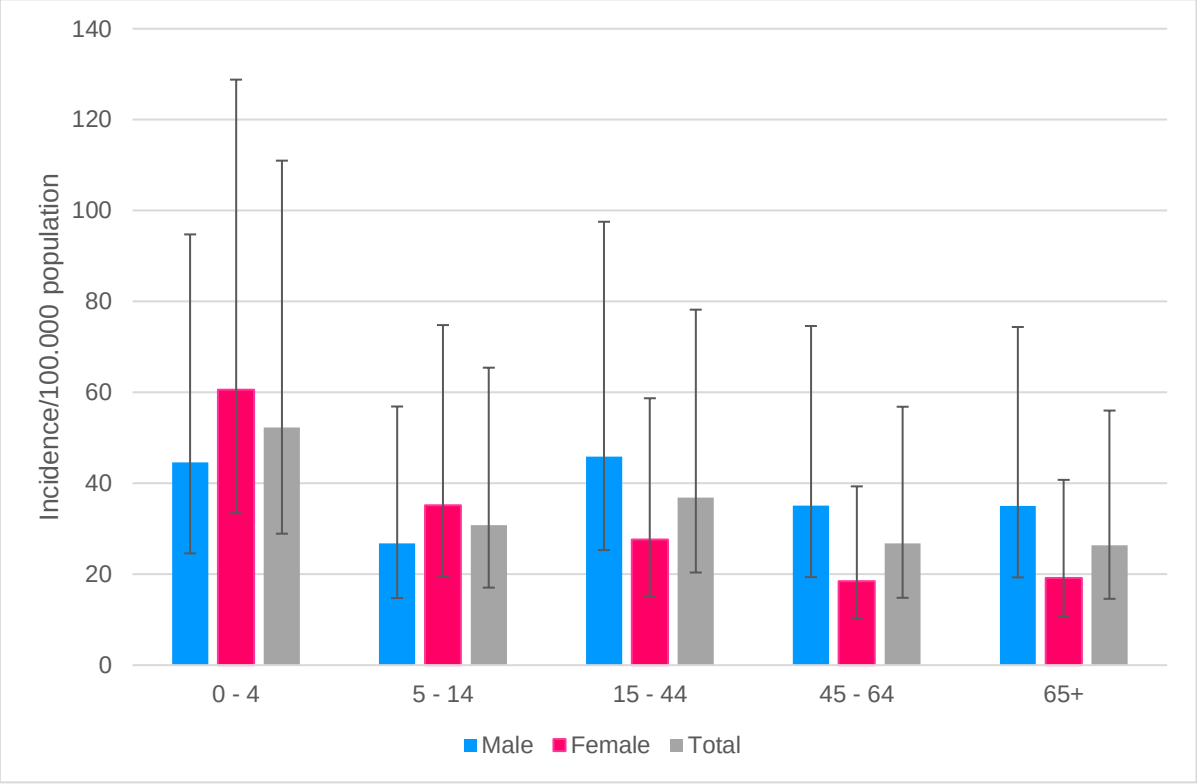
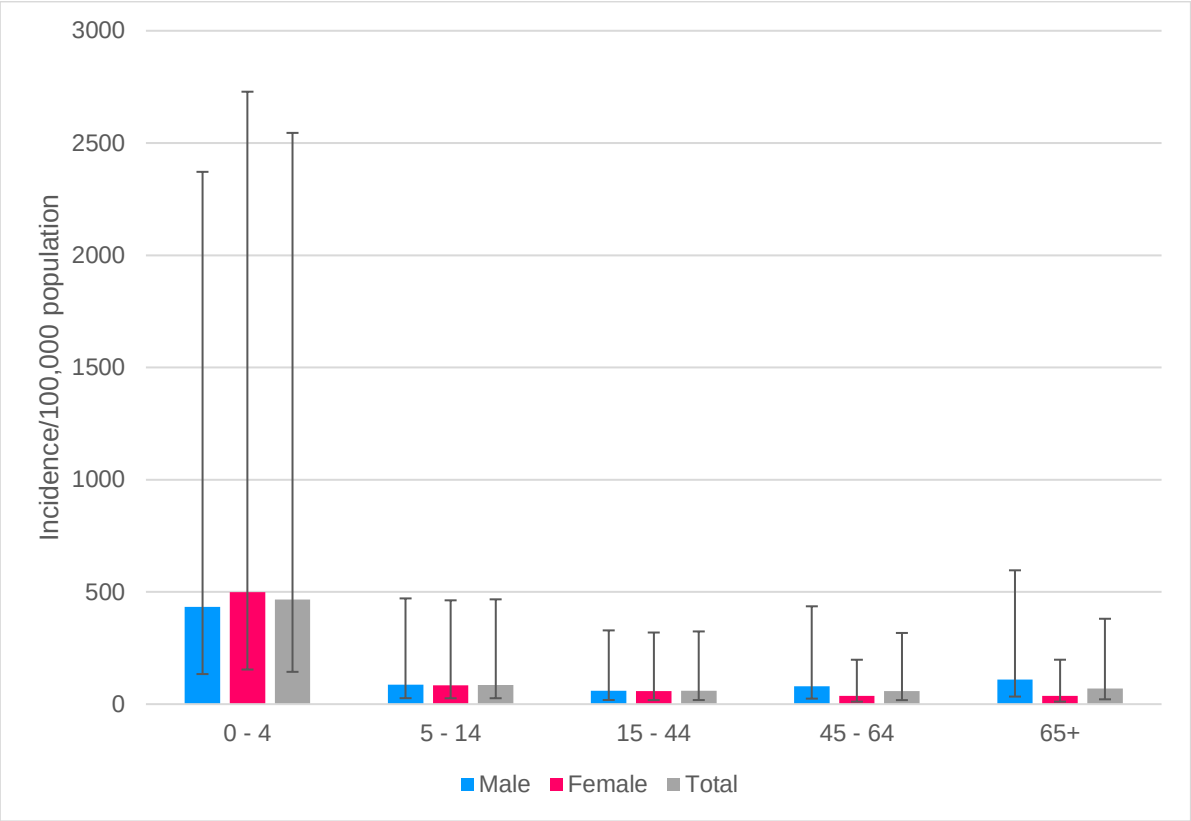


Figure 15 - Estimated incidence of VTEC per 100,000 population, segregated by gender and age group, Denmark, 2016 (error bars refer to the 95% confidence interval).



The incidence of all pathogens was highest in children under five years of age, except for yersiniosis, where the highest incidence is in male individuals with ages between 15 to 44 years old.

For *Campylobacter* spp. the difference between age groups is less marked, as all age groups have similarly high incidences (except for the 5 to 14 and 65 years old or more age groups). Campylobacteriosis has a higher incidence in females than in males, regardless of age group, except in the oldest one.

When discarding children under five years old, where incidence is markedly higher for both genders, salmonellosis' incidence increases with age group in the male population and stays constant in the female population.

Regarding yersiniosis, while in the female population the incidence was substantially higher in children under 5 and decreased with age, in males the variation of cases in the population was less marked.

VTEC has a fairly low incidence in all age groups except in children under five.

The uncertainty of all estimates was large, especially for VTEC.

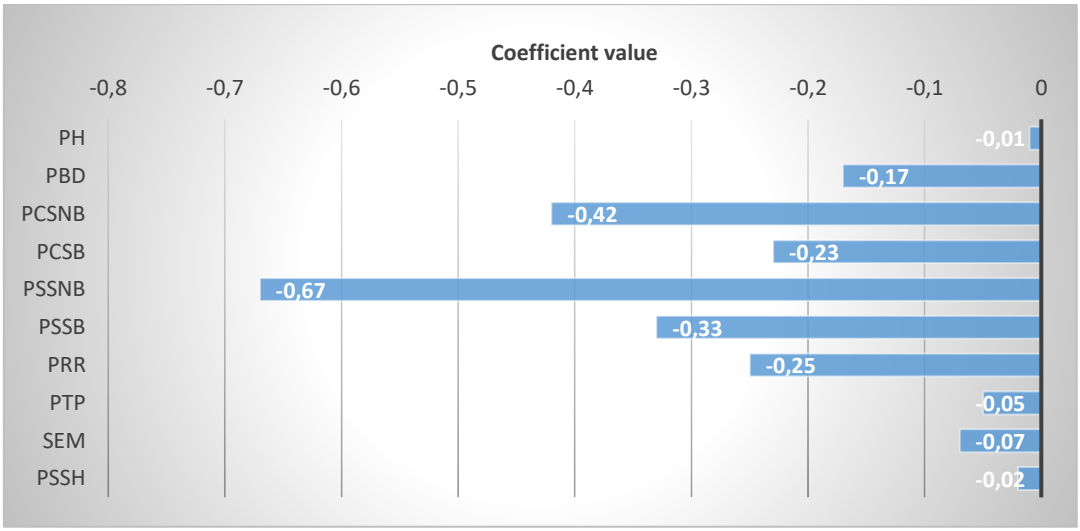
1.1.2. Sensitivity analysis

The sensitivity analyses were done separately for all four disease models.

All the variables and its contributions to the four disease models were evaluated: 1) proportion of hospitalized cases (Ph), 2) Proportion of bloody diarrhea in cases (Pbd), 3) Probability of seeking care for non-bloody diarrhea cases (PCSnb), 4) Probability of seeking care for bloody diarrhea cases (PCSb), 5) Probability of submitting a stool sample for analysis for non-bloody diarrhea cases (PSSnb), 6) Probability of submitting a stool sample for analysis for bloody diarrhea cases (PSSb), 7) Probability of reporting a positive laboratory result (PRR), 8) Probability of testing for the pathogen in sample (PTP), 9) Sensitivity of laboratory analysis (SEN), and 10) Probability of submitting a stool sample for analysis for hospitalized patients (PSSh).

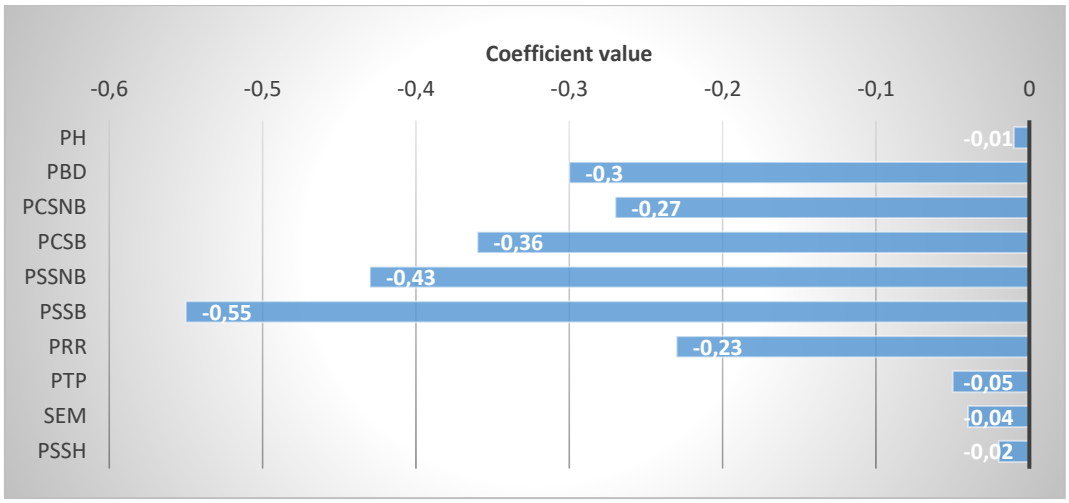
Figures 16 to 19 show the Spearman's rank order correlation coefficients of each variable, demonstrating their effect on the output (multiplying factor).

Figure 16 – Sensitivity analyses of *Campylobacter* spp.'s disease model illustrated in a "tornado" type of graph, showing the main sources of the model's uncertainty. The abbreviations of the input variables are on the vertical axis.



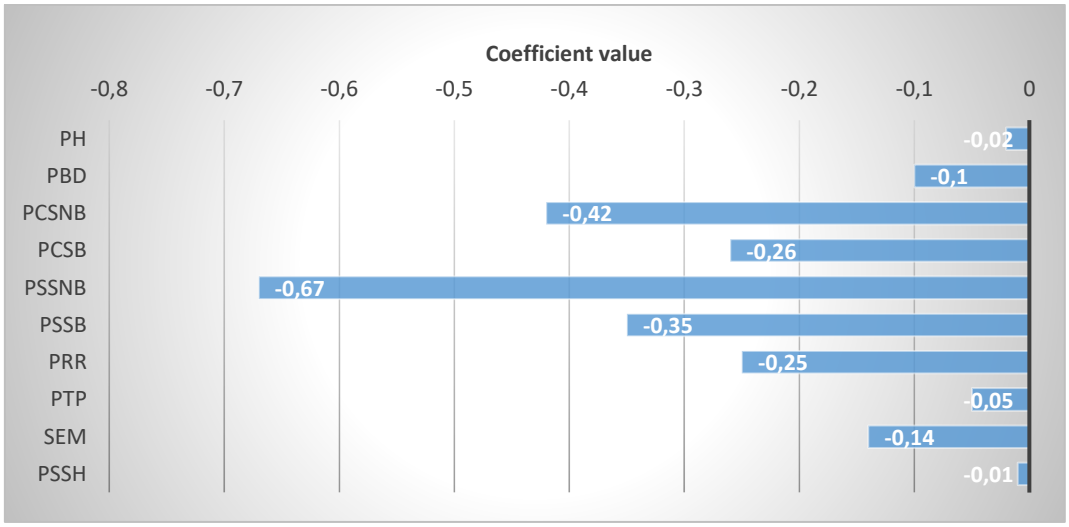
PH: proportion of hospitalized cases; PBD: Proportion of bloody diarrhea in cases; PCSNB: Probability of seeking care for non-bloody diarrhea cases; PCSB: Probability of seeking care for bloody diarrhea cases; PSSNB: Probability of submitting a stool sample for analysis for non-bloody diarrhea cases: PSSB: Probability of submitting a stool sample for analysis for bloody diarrhea cases: PRR: Probability of reporting a positive laboratory result; PTP: Probability of testing for the pathogen in sample; SEM: Sensitivity of laboratory analysis and PSSH: Probability of submitting a stool sample for analysis for hospitalized patients.

Figure 17 – Sensitivity analyses of Salmonella spp.'s disease model illustrated in a "tornado" type of graph, showing the main sources of the model's uncertainty. The abbreviations of the input variables are on the vertical axis.



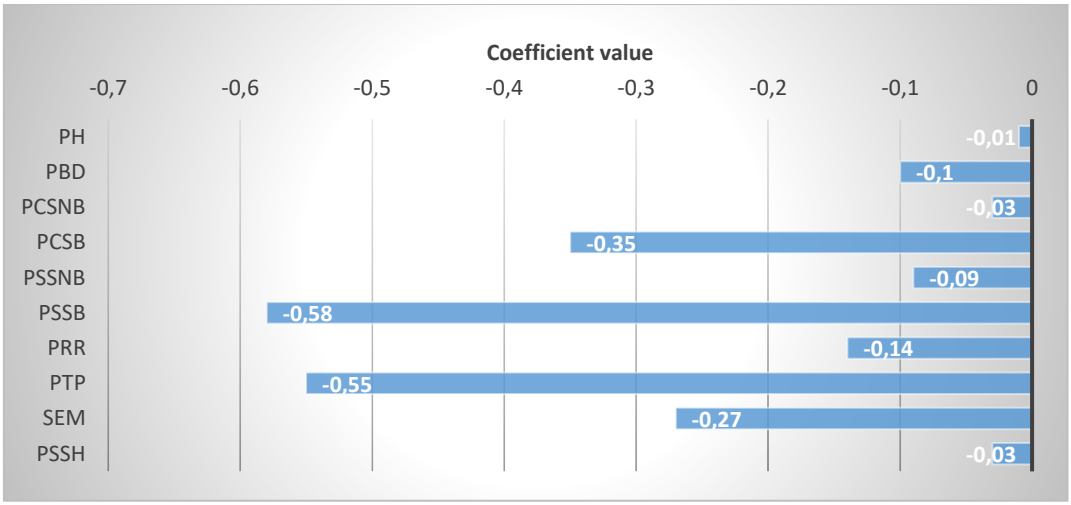
PH: proportion of hospitalized cases; PBD: Proportion of bloody diarrhea in cases; PCSNB: Probability of seeking care for non-bloody diarrhea cases; PCSB: Probability of seeking care for bloody diarrhea cases; PSSNB: Probability of submitting a stool sample for analysis for non-bloody diarrhea cases: PSSB: Probability of submitting a stool sample for analysis for bloody diarrhea cases: PRR: Probability of reporting a positive laboratory result; PTP: Probability of testing for the pathogen in sample; SEM: Sensitivity of laboratory analysis and PSSH: Probability of submitting a stool sample for analysis for hospitalized patients.

Figure 18 – Sensitivity analyses of *Y. enterocolitica*'s disease model illustrated in a "tornado" type of graph, showing the main sources of the model's uncertainty. The abbreviations of the input variables are on the vertical axis.



PH: proportion of hospitalized cases; PBD: Proportion of bloody diarrhea in cases; PCSNB: Probability of seeking care for non-bloody diarrhea cases; PCSB: Probability of seeking care for bloody diarrhea cases; PSSNB: Probability of submitting a stool sample for analysis for non-bloody diarrhea cases: PSSB: Probability of submitting a stool sample for analysis for bloody diarrhea cases: PRR: Probability of reporting a positive laboratory result; PTP: Probability of testing for the pathogen in sample; SEM: Sensitivity of laboratory analysis and PSSH: Probability of submitting a stool sample for analysis for hospitalized patients.

Figure 19 – Sensitivity analyses of VTEC's disease model illustrated in a "tornado" type of graph, showing the main sources of the model's uncertainty. The abbreviations of the input variables are on the vertical axis.



PH: proportion of hospitalized cases; PBD: Proportion of bloody diarrhea in cases; PCSNB: Probability of seeking care for non-bloody diarrhea cases; PCSB: Probability of seeking care for bloody diarrhea cases; PSSNB: Probability of submitting a stool sample for analysis for non-bloody diarrhea cases: PSSB: Probability of submitting a stool sample for analysis for bloody diarrhea cases: PRR: Probability of reporting a positive laboratory result; PTP: Probability of testing for the pathogen in sample; SEM: Sensitivity of laboratory analysis and PSSH: Probability of submitting a stool sample for analysis for hospitalized patients.

A negative correlation coefficient indicates that for an increasing value of an input variable, the multiplier will get lower values. Therefore, if, for example, the probability of submitting a stool sample increases, the multiplier will decrease, decreasing the uncertainty in the model.

From the sensitivity analysis on *Campylobacter* spp. and *Y. enterocolitica*'s disease models it was observed that the input values for the probability of submitting a stool sample for non-bloody diarrhea cases, the probability of seeking care for non-bloody diarrhea cases and the probability of submitting a stool sample for bloody diarrhea cases influenced mostly the multiplier, increasing its value and the uncertainty in the model.

In *Salmonella* spp.'s case the input variables that influenced the multiplying factor the most were the probability of submitting a stool sample for bloody diarrhea and non-bloody diarrhea cases and the probability of seeking care for bloody diarrhea cases.

Finally, on VTEC's disease model the input variables influencing the multiplier the most were the probability of submitting a stool sample for bloody diarrhea cases, the probability of testing for the pathogen in the sample and the probability of submitting a stool sample for bloody diarrhea cases.

1.1.3. Scenario Analysis

An overview of the results for the scenario analysis is shown on Table 19. As expected, there is a decrease on the multiplying factor in all scenarios, with a substantial one on scenario four, which encompasses all the changes in all the other three scenarios.
For campylobacteriosis and yersiniosis, scenarios two and three (that increased the PCSnb and PSSb, respectively) have similar decreases, whereas for salmonellosis it is the first, which also changed the PSSb, and second (PSSnb) scenarios.
Regarding VTEC's multiplying factor, all scenarios show different decreases, with the third scenario (PCSb) having the least impact, which also occurs with scenario three (PCSb) for *Salmonella* spp. multiplier.

Table 19 – Median and 95% confidence intervals (95% CI) for the multiplier and each of the four scenarios of each foodborne pathogen's disease model.

	Multiplier	**Scenario 1**	**Scenario 2**	**Scenario 3**	**Scenario 4**
	Median [95% CI]	Median [95% CI]	Median [95% CI]	Median [95% CI]	Median [95% CI]
Campylobacter spp.	11 [5.9; 24.4]	8.4 [5; 17]	9.9 [5.5; 20.2]	9.9 [5.5; 20.5]	6.8 [4.1; 12.9]
Salmonella spp.	7.7 [3.8; 18]	6.4 [3.1; 14.1]	6.4 [3.4; 13.3]	7.3 [3.5; 16.7]	5.1 [2.8; 10.3]
Y. enterocolitica	10.9 [6; 23]	8.5 [4.9; 16.5]	9.4 [5.4; 19.7]	9.6 [5.5; 19.4]	6.7 [4.1; 12.3]
VTEC	19.6 [5.9; 102.7]	13.8 [4.8; 61]	15.4 [5; 71.8]	17.8 [5.5; 90.8]	9.6 [3.7; 35.3]

Figures 20 to 23 show the empirical cumulative distribution function of the multiplier and each scenario considered, for each of the four foodborne pathogens. A shift to the left means a decrease in the multiplying factor according to each scenario.

Figure 20 – Empirical cumulative distribution function of Campylobacteriosis' multiplier (blue) and the first (pink), second (green), third (red) and fourth (orange) scenarios.

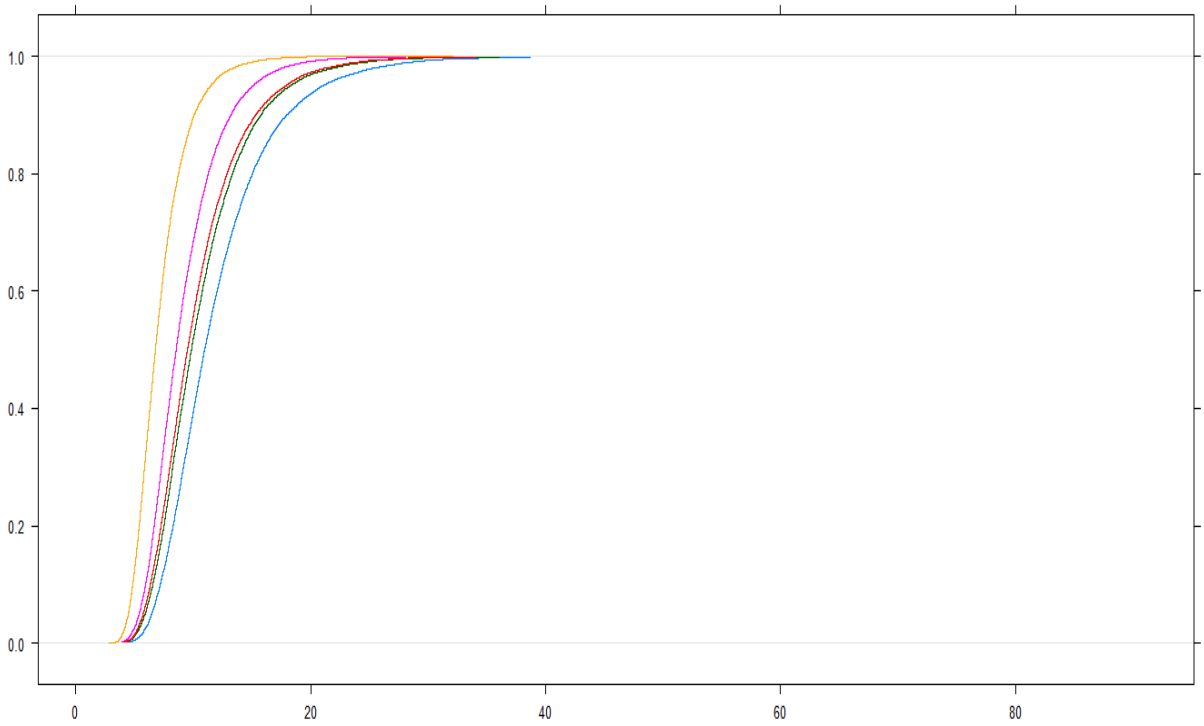


Figure 21 – Empirical cumulative distribution function of salmonellosis' multiplier (blue) and the first (pink), second (green), third (red) and fourth (orange) scenarios.

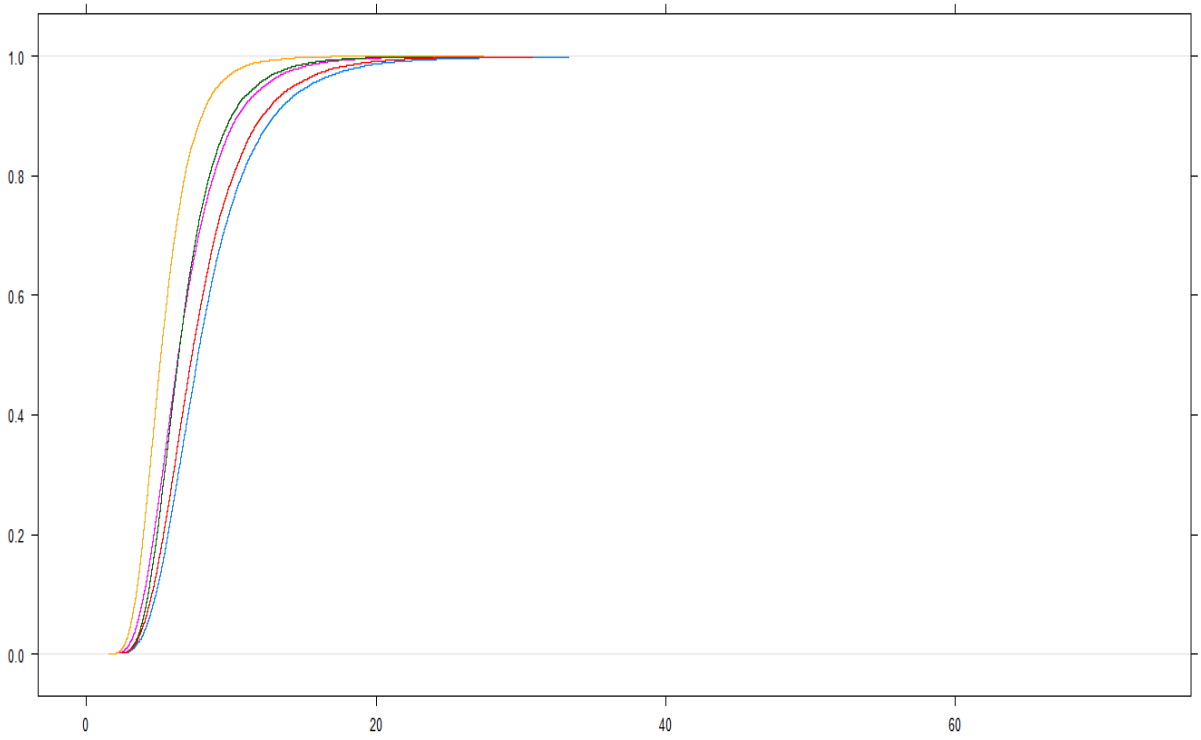


Figure 22 – Empirical cumulative distribution function of yersiniosis' multiplier (blue) and the first (pink), second (green), third (red) and fourth (orange) scenarios.

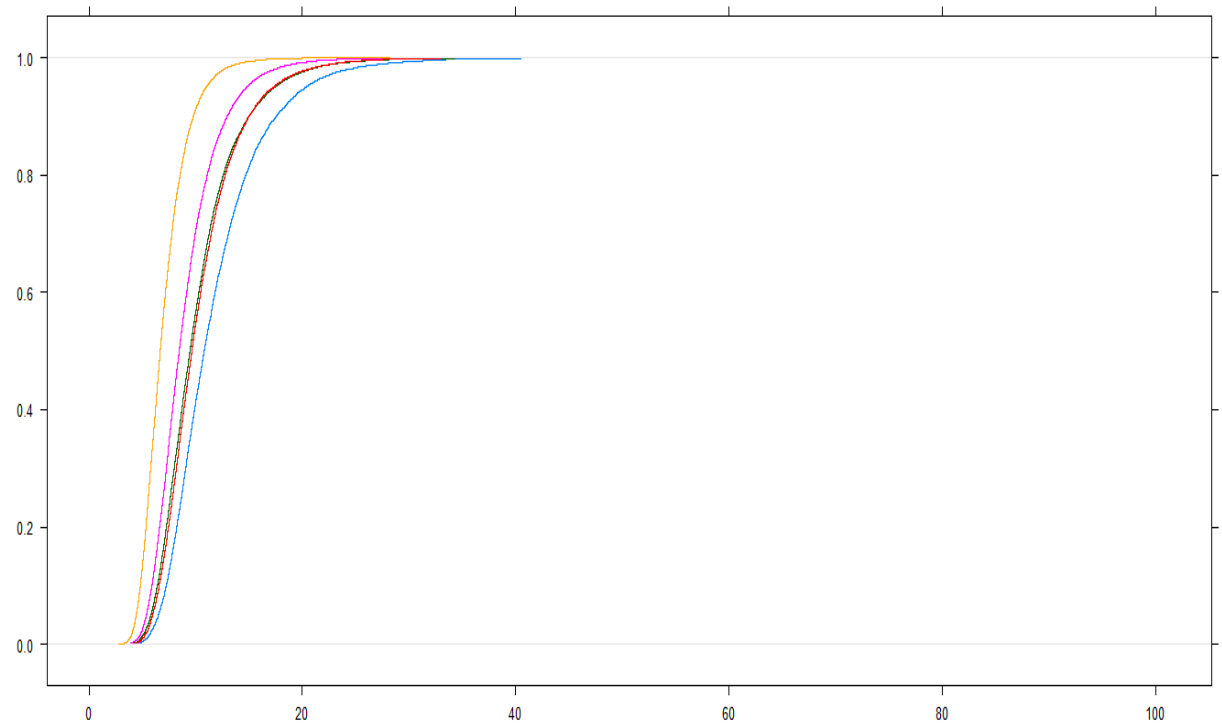
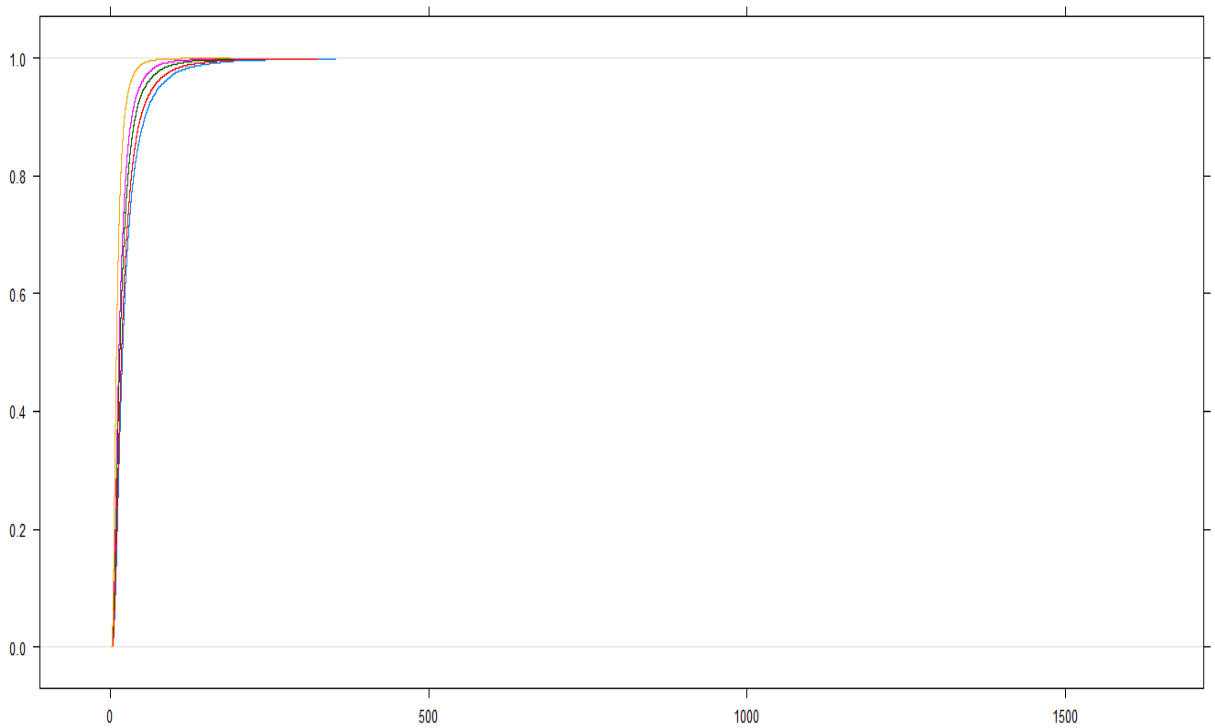


Figure 23 – Empirical cumulative distribution function of VTEC's multiplier (blue) and the first (pink), second (green), third (red) and fourth (orange) scenarios.



1.2. Disability Adjusted Life Years for four foodborne pathogens

The overall estimated burden of disease was higher for *Campylobacter* spp. with 1,751 DALYs. For *Salmonella* spp. a total of 432 DALYs were estimated, followed by an estimation of 63 and 44 DALYs for *Y. enterocolitica* and VTEC, respectively (Table 20).

Figure 24 shows the separate contribution of YLLs and YLDs for each pathogen's total burden. Both *Campylobacter* spp. *and Y. enterocolitica* have a relative contribution of YLDs of around 60%. For *Salmonella* spp. and VTEC the estimations point for a higher burden related to YLLs (around 60% of the total DALY estimation).

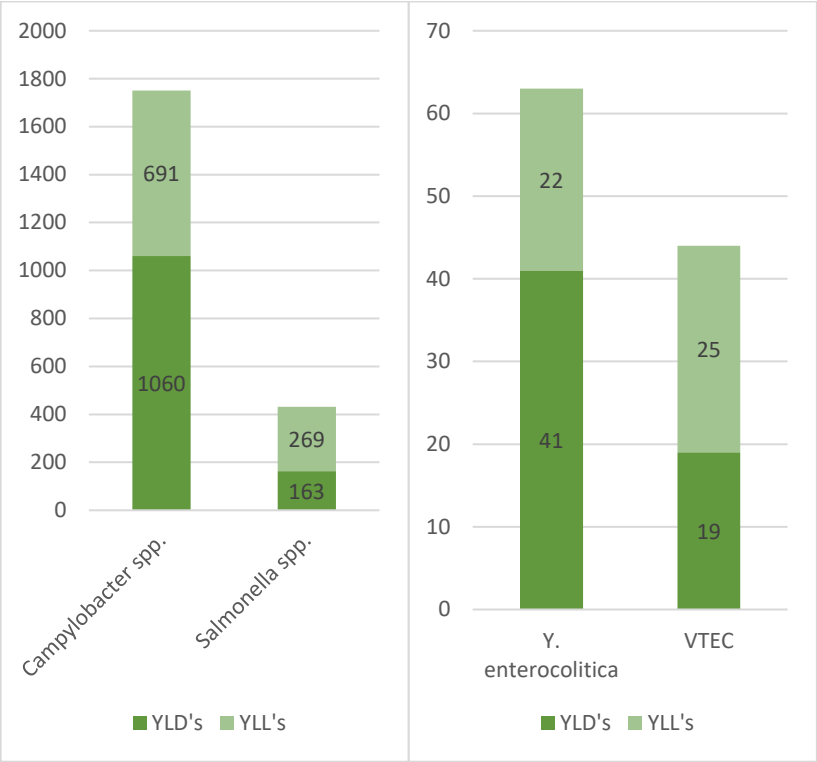
Tables 21 to 24 show the results of total DALYs, YLD and YLL caused by each different health-outcome associated with the different foodborne pathogens, while Figure 25 depicts their relative contribution to the total burden of disease. Gastroenteritis is the major contributor for the disease burden for all pathogens except *Campylobacter* spp.*,* for which irritable bowel syndrome occupies that place. Regarding reactive arthritis, its contribution to the burden of *Salmonella* spp. is lower than that of *Campylobacter* spp. and *Y. enterocolitica.*

Table 20 - Estimated total DALYs, YLL and YLD for *Campylobacter* spp.*, Salmonella* spp.*, Y. enterocolitica* and VTEC in Denmark, 2016.

	Campylobacter spp.		***Salmonella*** spp.		***Y. enterocolitica***		**VTEC**	
Reported cases	4674		1068		172		250	
	Median	**95% CI**	**Median**	**95% CI**	**Median**	**95% CI**	**Median**	**95% CI**
Total cases	59,677	[49,617–71,781]	9,711	[8,119-11,524]	2,130	[1,813-2,523]	6,952	[4,445-10,107]
Deaths	59	-	26	-	2	-	0	[0 - 1]
DALY Total	1,751	[1,697-2,255]	432	[425-440]	63	[59 - 69]	44	[36 - 53]
DALY/100,000	30		7.5		1.1		0.8	
YLD	1,060	[1,010–1,112]	163	[156-171]	41	[37 - 47]	19	[13 - 27]
YLL	691	[687-696]	269	-	22	-	25	[19 - 32]

Note: the DALY calculator uses the estimated total incidence as input for the model. The input is defined as a PERT distribution with most likely value the median and minimum and maximum value the 95% percentiles. Even though the estimates were based on the BoI estimates described on chapter 2, the use of a probability distribution in another stochastic model leads to different results on the estimated total cases for each pathogen.

Figure 24 - Disease burden of four foodborne pathogens in Denmark for the year 2016. YLL and YLD components are shown separately.



Guillain-Barré syndrome has a small contribution to *Campylobacter* spp. burden, even though it has the potential of causing severe and fatal illness. In the estimations for 2016, GBS caused no deaths and all its burden amounts to YLD.

Hemolytic uremic syndrome also has a low contribution to VTEC burden, which is approximately 0% for the 6 reported cases for the year 2016. Although estimations point for only 1 case of end-stage renal disease, its contribution is of 16%, causing a burden of seven DALYs.

Table 21 - Estimated total DALYs, YLL and YLD associated with different health-outcomes of *Campylobacter* spp. infection in Denmark, 2016.

	Gastroenteritis		**Reactive Arthritis**		**Irritable bowel syndrome**		**Guillain- Barré Syndrome**	
	Median	**95% CI**	**Median**	**95% CI**	**Median**	**95% CI**	**Median**	**95% CI**
DALY	711	[695 – 734]	175	[126 – 234]	845	[817 – 872]	16	[15 - 17]
YLD	41	[25 – 64]	175	[126 – 234]	845	[817 – 872]	16	[15 - 17]
YLL	670	-	0	-	0	-	0	-
Deaths	59	-	0	-	0	-	0	-
Cases	53,467	[43,508 – 65,578]	1,036	[747 – 1,393]	4,026	[3,891 – 4,153]	1,141	[1,098 1,183]

Table 22 - Estimated total DALYs, YLL and YLD associated with different health-outcomes of *Salmonella* spp. infection in Denmark, 2016.

	Gastroenteritis		Reactive Arthritis		Irritable bowel syndrome	
	Median	**95% CI**	**Median**	**95% CI**	**Median**	**95% CI**
DALY	276	[273 – 279]	23	[18 – 29]	133	[129 – 137]
YLD	7	[4 – 10]	23	[18 – 29]	133	[129 – 137]
YLL	269	-	0	-	0	-
Deaths	26	-	0	-	0	-
Cases	8,639	[7,066 - 10,452]	439	[381 – 505]	633	[615 – 652]

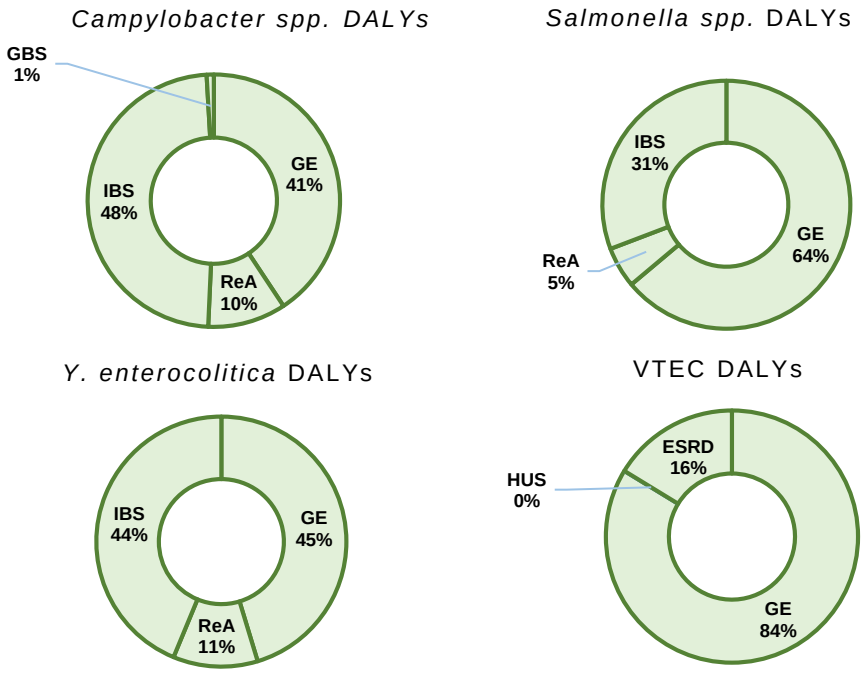
Table 23 - Estimated total DALYs, YLL and YLD associated with different health-outcomes of *Y. enterocolitica* infection in Denmark, 2016.

	Gastroenteritis		Reactive Arthritis		Irritable bowel syndrome	
	Median	**95% CI**	**Median**	**95% CI**	**Median**	**95% CI**
DALY	29	[24 – 34]	7	[6 – 9]	28	[27 – 29]
YLD	7	[3 – 13]	7	[6 – 9]	28	[27 – 29]
YLL	22	-	0	-	0	-
Deaths	2	-	0	-	0	-
Cases	1,833	[1,508 - 2,212]	177	[158 – 197]	131	[127 – 136]

Table 24 - Estimated total DALYs, YLL and YLD associated with different health-outcomes of VTEC infection in Denmark, 2016.

	Gastroenteritis		Hemolitic uremic syndrome		End-stage renal disease	
	Median	**95% CI**	**Median**	**95% CI**	**Median**	**95% CI**
DALY	36	[24 – 51]	0	-	7	[6 – 10]
YLD	11	[6 – 19]	0	-	7	[6 – 10]
YLL	25	[18 - 32]	0	-	0	-
Deaths	0	[0 - 1]	0	-	0	-
Cases	6,929	[4,484 - 10,093]	6	-	1	[0 – 1]

Figure 25 – Relative contribution of DALYs caused by different health-outcomes to the total burden of campylobacteriosis, salmonelosis, yersiniosis and VTEC infections in Denmark, 2016.



GBS: Guillain-Barré Syndrome; GE: Gastroenteritis; IBS: Irritable bowel syndrome; ReA: Reactive arthritis; HUS: Hemolytic uremic syndrome; ESRD: End-stage renal disease.

The total DALY estimates by age group and gender per 100,000 population (Figures 26 to 29) show a higher burden of disease for older people (65 years old or more) for campylobacteriosis and salmonellosis, whether for yersiniosis and VTEC infections, children under five years old have the highest burden, with the last disease causing a substantially higher burden in that population.

The burden of disease for campylobacteriosis and for salmonellosis is higher in the female population, except for people aged between 15 and 44 years old with *Salmonella* spp. infections. For both yersiniosis and VTEC infections there is no defined trend regarding sex.

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Figure 26 - Distribution of total burden of campylobacteriosis in age groups and sex in Denmark, 2016 (total DALYs per 100,000 population).

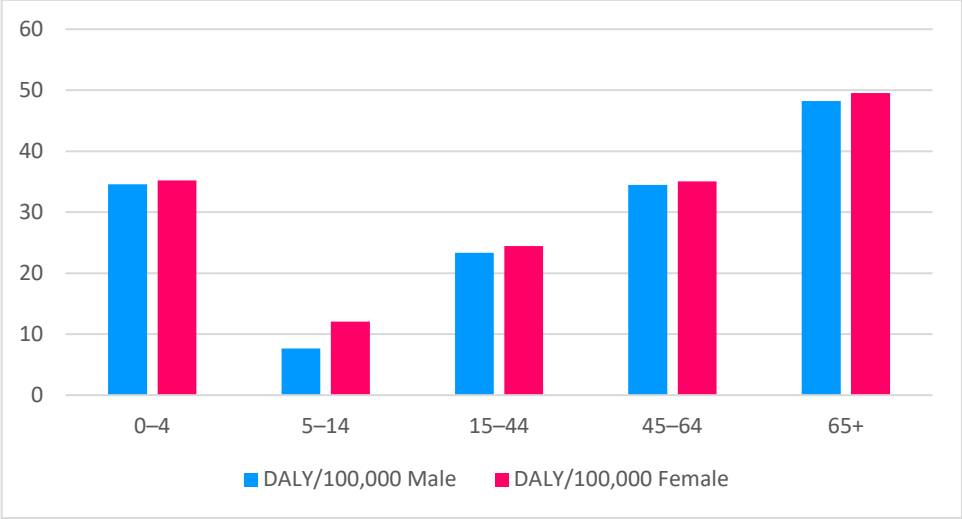


Figure 27 - Distribution of total burden of salmonellosis in age groups and sex in Denmark, 2016 (total DALYs per 100,000 population).

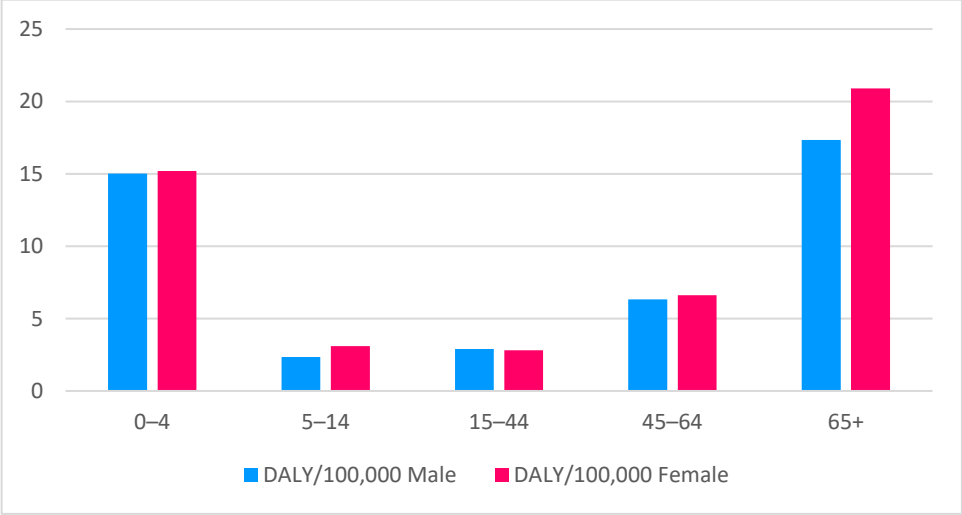


Figure 28 - Distribution of total burden of yersiniosis in age groups and sex in Denmark, 2016 (total DALYs per 100,000 population).

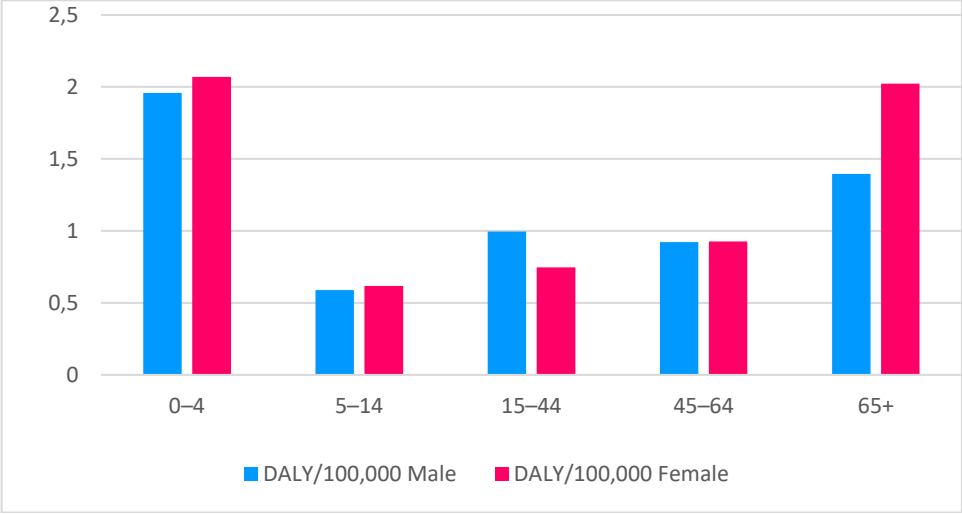
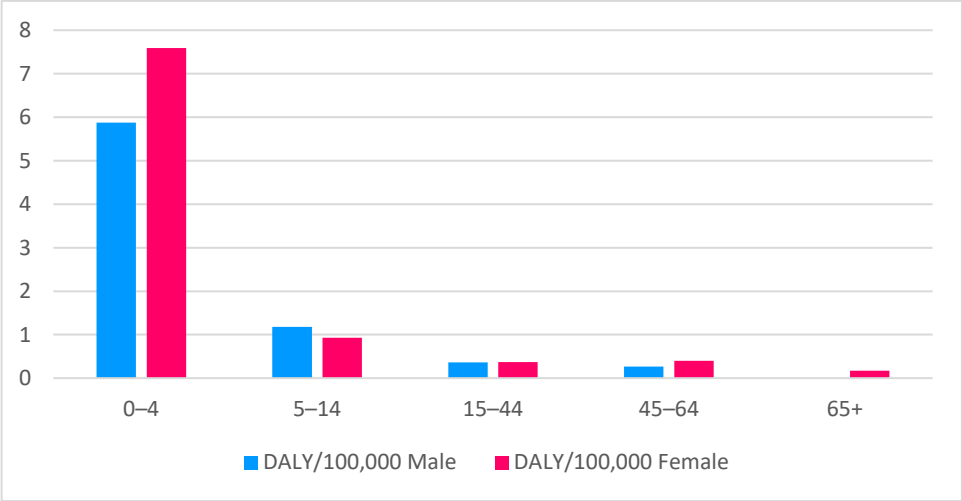


Figure 29 - Distribution of total burden of VTEC infections in age groups and sex in Denmark, 2016 (total DALYs per 100,000 population).



Tables 25 to 28 show the detailed burden, cases and deaths estimates stratified by age group and sex. All the foodborne pathogens caused a higher number of deaths in the oldest age group (people older than 64 years old), except VTEC which did not lead to fatal cases in the year 2016.

Table 25 – Estimated total DALYs, YLLs, YLDs, total cases and deaths associated with *Campylobacter* spp. infections in Denmark, 2016 by age group and sex.

	DALY		**YLD**		**YLL**		**Cases**		**Deaths**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
0–4	53	51	29	34	24	17	1,953	1,501	0	0
5–14	26	39	24	38	2	1	2,155	1,292	0	0
15–44	258	262	228	235	30	27	14,003	12,794	1	0
45–64	262	265	148	175	114	90	9,554	7,940	3	2
65+	242	294	70	80	172	214	3,969	4,886	23	29

Table 26 – Estimated total DALYs, YLLs, YLDs, total cases and deaths associated with *Salmonella* spp. infections in Denmark, 2016 by age group and sex.

	DALY		**YLD**		**YLL**		**Cases**		**Deaths**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
0–4	23	22	9	10	14	12	592	512	0	0
5–14	8	10	7	10	1	0	616	425	0	0
15–44	32	30	26	24	6	6	1,472	1,483	0	0
45–64	48	50	24	19	24	31	1,172	1,398	1	1
65+	87	124	18	17	69	107	843	1,226	9	14

Table 27 – Estimated total DALYs, YLLs, YLDs, total cases and deaths associated with *Y. enterocolitica* infections in Denmark, 2016 by age group and sex.

	DALY		YLD		YLL		Cases		Deaths	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
0–4	3	3	2	2	1	1	108	83	0	0
5–14	2	2	2	2	0	0	101	76	0	0
15–44	11	8	10	7	1	1	390	545	0	0
45–64	7	7	5	4	2	3	186	305	0	0
65+	7	12	4	3	3	9	125	225	1	2

Table 28 – Estimated total DALYs, YLLs, YLDs, total cases and deaths associated with VTEC infections in Denmark, 2016 by age group and sex.

	DALY		YLD		YLL		Cases		Deaths	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
0–4	9	11	3	6	6	5	1,093	901	0	0
5–14	4	3	2	1	2	2	407	395	0	0
15–44	4	4	1	1	3	3	914	909	0	0
45–64	2	3	1	1	1	2	394	853	0	0
65+	0	1	0	1	0	0	255	922	0	0

V. Discussion

This dissertation describes an integrated model to estimate the public health impact of four foodborne pathogens in Denmark for the year 2016. It also describes the model developed to estimate the burden of disease of yersiniosis, which is the first developed in the country and in Europe. These burden of disease estimates are used to rank foodborne pathogens at the national level and are used to provide evidence to the National Food and Veterinary Administration under a project running at the Denmark Technical University (DTU) Food.
The applied model consisted of two general components: a burden of illness study, which was applied to estimate the total incidence of these pathogens in the Danish population for that time frame; and a burden of disease study, which built on the first component and estimated disease burden in terms of the disability adjusted life year metric. These two components are discussed separately, and a general discussion follows.

1. Burden of Illness of four foodborne pathogens

Results show that campylobacteriosis was the most frequent infection by foodborne pathogens in Denmark in 2016, with an estimated incidence of 889 cases per 100,000 inhabitants, followed by salmonellosis, VTEC infections and yersiniosis with 143, 85 and 32 cases per 100,000 population, respectively.
Although this ranking follows the ranking of reported incidence, estimates show a large and variable degree of under-reporting for each pathogen. The pathogen with the largest degree of under-reporting and underdiagnosis (which translates as the biggest difference between reported and estimated total cases) was VTEC, with a multiplying factor of approximately 20. *Campylobacter* spp. and *Y. enterocolitica* had similar underreporting factors of around 11, which leaves *Salmonella* spp. with the lowest multiplier, of around eight.

1.1. Limitations of the modelling approach

1.1.1. Surveillance system

The basic input data of the BoI model were the number of cases reported to the Danish surveillance system, for each pathogen.
All these data were aggregated by age group and sex, but for yersiniosis the biotypes causing disease needed to be taken into account because one biotype is considered non-pathogenic. As a consequence, the proportion of cases caused by pathogenic biotypes of *Y. enterocolitica* needed to be considered in the model, but this could not be categorized by age or sex (i.e. only the percentage of the total cases was known). Therefore, a reduction to 30% of the total reported cases which was caused by the pathogenic biotypes was applied equally

to all age and sex groups. As a consequence, sex and/or age groups with relatively more or less notified cases caused by pathogenic biotypes of *Y. enterocolitica* might have been under-represented or over-represented, respectively. Such bias may have influenced the age and sex-distribution of the burden of yersiniosis estimates.

Regarding VTEC-associated hemolytic uremic syndrome cases, it was assumed that all cases were captured by the Danish surveillance system. However, it is likely that some HUS diagnosed cases were not linked to VTEC infections, which means that the incidence currently used may be underestimated (Pires, 2014). More data is needed to improve the disease model in order to capture the total incidence of VTEC infections.

1.1.2. Non-pathogen and pathogen specific parameters

Several of the model's variables were informed by a population-based telephone survey conducted in 2009 that attempted to determine the incidence of acute gastrointestinal illness in Denmark (Müller *et al*., 2012).

Although the methods were sound, the study collected data from a relatively low number of gastroenteritis patients, therefore data to inform some of the model's parameters were sparse.

Through the sensitivity analysis performed, almost all of the variables contributing the most to the model's uncertainty were those informed by this study (Figures 17 to 20). An update of this study would be useful in order to improve future estimates.

Also, all parameters used to correct the number of reported cases to total incidence, by accounting for under-reporting and underdiagnosis, were defined without any specific age and sex-distribution, due to insufficient data to inform these specifications. This led to an age and sex-independent multiplying factor for each of the four foodborne pathogens.

Being possible that people seeking medical care can have different behaviors according to age groups and sex, as well as that different approaches from general practitioners regarding different individuals exist (younger children, for example), it could be interesting to gather data and develop an age and sex-specific multiplying factor.

Regarding pathogen-specific parameters, even though the symptoms of mild uncomplicated disease by all four pathogens are similar, general practitioners and laboratories are more likely to test for *Salmonella* spp. in a stool sample, due to a positive public health impact caused by the implementation of various EU-level and Danish prevention and control measures, which brought awareness to this pathogen (ECDC, 2015). Whether VTEC's differences in the diagnostic methodology and principles for testing (Espenhain, 2013), which translated in a lower probability of testing for VTEC in a stool sample, affect its multiplier in a negative way, as shown by the sensitivity analysis (Figure 20).

1.2. Scenario analysis

The scenario analysis showed that the improvements chosen for the parameters that influenced negatively the multiplying factor the most would decrease the median value of the model's output, as well as its 95% confidence intervals.

The decrease of the multiplier would translate an improvement of the reporting and diagnosis capacities, which means that the Danish surveillance system would capture more cases of disease in the population. This scenario analysis could therefore be used to encourage Danish food authorities and policy makers to bring awareness to the general population to seek medical care when having a foodborne illness and to doctors and clinical microbiology laboratories to increase their stool sample requests and specific-pathogen testing.

On the other hand, the decrease of the 95% confidence intervals would mean that the uncertainty around the estimates was lower, and therefore food authorities and policy makers would have more certain results to develop new public health measures if needed.

1.3. Comparability with other studies

Several countries have previously conducted studies to estimate multipliers to correct for under-reporting and underdiagnosis. Estimates varied extensively, from seven in Denmark (Pires, 2014) to 111 in Portugal for *Salmonella* spp. *(*Morgado*,* 2015*),* as did the methods applied to inform the different models used in each study.

Even though both previous Danish estimates used the same disease model and the same population-based telephone survey done by Muller and colleagues (2012), they have reached different multipliers (Table 29). That can be explained by the assumption to consider gastroenteritis cases with duration shorter than three days as viral infections, which was a central assumption of this study when informing the different model's parameters.

When comparing Pires (2014) and this study, all multipliers show similar results for *Campylobacter* spp. and *Salmonella* spp*.,* which was expected since most disease model parameters were defined in the same way. The incongruence between the two VTEC's multipliers can be explained by an oversight on the previous Danish estimates, only found after publishing (Pires, Personal communication). An important addition of this study was the development of the model for yersiniosis, which has not been done before in an exclusively national study.

Regarding the American, Canadian, New Zealander and Portuguese studies, all used the same disease model for the different pathogens assessed. The model here described had differences compared to the one used in these studies, namely considering the probability of submitting a stool sample for analysis for hospitalized patients and the proportion of hospitalized patients. They also accounted for the proportion of travel-related and of

foodborne cases. The study from New Zealand used the American multipliers as surrogate, while the others informed their model's parameters based on country-specific data.

In this study's modelling approach, it was considered that hospitalized patients would have an increased probability of a sample being taken when compared with patients with non-bloody diarrhea and bloody diarrhea, under the assumption that hospitalized patients would have more severe symptoms than the other cases.

This model did not account for the proportions of travel-related and foodborne cases. In the Danish approach, after estimating total incidence and DALYs for each pathogen, Pires (2014) combines the DALYs estimates with source attribution estimates from pathogen-specific studies. A more complete overview of concepts and methods regarding source attribution, can be found at Pires *et al*., (2009).

Table 29 – Overview of multiplying factors estimated to correct for under-reporting and underdiagnosis of *Campylobacter* spp*., Salmonella* spp*., Y. enterocolitica* and VTEC infections in different countries worldwide.

Country	**Reference**	**Multiplying factor**			
		Campylobacter spp.	***Salmonella*** spp.	***Y. enterocolitica***	**VTEC**
AUS	(Kirk *et al*. 2014)	10.5	7.4	7.4	NA
CAN	(Thomas *et al*., 2013)	27.2	26.1	39.3	20.1*
DK	(Haagsma *et al*., 2013)	29.0	17.0	20.0	NA
DK	(Pires, 2014)	12.0	7.2	NA	31.2
GRE	(Vaillant *et al*., 2005)	274.8	51.45	NA	NA
NZ	(Lake *et al*., 2010)	30.3	29.3	122.8	NA
PT	(Morgado, 2015)	NA	111.2	NA	NA
UK	(Tam *et al*., 2012)	9.3	4.7	NA	7.4
USA	(Scallan *et al*., 2011)	30.3	29.3	122.8	26.1*
DK	**This study**	**11.0**	**7.7**	**10.9**	**19.7**

AUS: Australia; CAN: Canada; DK: Denmark; GRE: Greece; NZ: New Zealand; PT: Portugal; UK: United Kingdom; USA: United States of America. *Estimates only including VTEC O157. NA: not available.

In this dissertation, the source attribution step was not included due to lack of data for *Y. enterocolotica* and VTEC. Even though attempts to collect data were done, there are no food estimates for *Y. enterocolitica* and VTEC (in Denmark or elsewhere).

Differences in underreporting factors between countries are expected to occur due to variations among population behavior, health care systems, laboratories methodologies and surveillance systems. In addition, the differences between modelling approaches and on parameters' definitions are also important variables that contribute to these differences and make it difficult to compare results from different countries, or even between different studies. To overcome this problem, a harmonized and well-defined approach would need to be developed.

2. Burden of disease of four foodborne pathogens

The ranking of the DALY estimates of the four foodborne diseases considered, followed the same order as the estimates of total incidence, except for yersiniosis and VTEC infections. Therefore, Campylobacteriosis caused the higher burden, 1,751 DALYs, followed by salmonellosis with 432, yersiniosis with 63 and VTEC infections with 44 DALYs.

This ranking was to be expected since all diseases have similar mild symptoms and *Campylobacter* spp.*, Salmonella* spp. and *Y. enterocolitica* have similar sequelae.

Although campylobacteriosis may cause Guillain-Barré syndrome, which is a long-term and potentially severe sequela, only 16 DALYs, born by 1,141 cases, were attributed to this disease. This might be explained by a substantially higher number of mild cases of this disease. Also, the burden of campylobacteriosis was 4 times bigger than the burden of salmonellosis, even though its total incidence was 6 times higher, which can be explained by the large number of mild cases of gastroenteritis which cause a very low burden.

Regarding yersiniosis and VTEC infections' ranking positions, although the last has more severe sequelae than yersiniosis, those health-outcomes are not frequent, accounting for a lower number of DALYs.

Although having a relatively high disability weight (0.21), the hemolytic uremic syndrome had a very low burden (approximately zero). This can be explained by the low number of reported cases developing this sequela. When applying the formula to calculate YLD with the most likely duration for this health-outcome (0.077 years) the result obtained is 0.9 DALYs. Since a PERT distribution was applied to the duration of HUS symptoms, this number can be lower, and therefore appear to be an absolute 0 (the DALY calculator only shows absolute values).

Still, the total VTEC burden may be an underestimate because under-reporting was not considered for VTEC associated HUS cases.

2.1. Health-outcome trees

To develop the first health-outcome tree for yersiniosis a literature review was carried out, in which several health-outcomes were identified in addition to the ones included in the disease model to estimate the burden of yersiniosis.

Although there was evidence that all identified sequelae could be caused by *Y. enterocolitica*, lack of significant and sound data to inform the disease model made it impossible to include these in the burden of disease estimates. Therefore, the burden of yersiniosis might have been underestimated.

Also, for *Campylobacter* spp. and *Salmonella* spp. a change in the health-outcome trees was made (when compared to Pires (2014)), excluding IBD. In a nationwide Danish study from SSI, conclusions indicate that there is no relation between these two pathogens and IBD, but that such infections are more often detected in patients suffering from IBD, because they have many samples taken (Jess *et al*., 2011). Recent studies have also excluded this sequela from their health-outcome trees (de Noordhout *et al*., 2017; Van Lier *et al*., 2016).

In order to improve and increase available evidence on the link between foodborne infections and sequelae, studies such as cohort studies can be developed. These studies could help identify all potential health-outcomes associated with different foodborne infections, estimate probabilities of occurrence and revise currently used health-outcome trees

2.2. Disability weights

To inform the parameter defining the different sequelae, estimates from the Global Burden of Disease of 2013 were used (Salomon *et al*., 2015). However, DWs for many health-outcomes were lacking, and therefore data from different studies was used (Haagsma *et al*., 2008; Havelaar *et al*., 2000; Havelaar *et al*., 2012; Kirk *et al*., 2015). Different studies had assumed different disability weights for the same sequelae, which brought awareness to the validity and comparability of this model parameter, when comparing different burden of disease studies.

To improve this disparity and to develop disability weights for a larger range of sequelae, new studies could be carried out.

2.3. Comparability with other studies

Several burden of disease studies have been developed by countries and by different organizations (Table 30) in order to estimate the burden of foodborne diseases and to rank them and give information on how public health interventions could be prioritized.

There is only one study estimating the burden of yersiniosis (Lake *et al*., 2010). Their approach only includes gastroenteritis and reactive arthritis as health-outcomes of this

disease. Also, this study did not develop a health-outcome tree for this disease and assumed that aside from GE only ReA could contribute to the burden of yersiniosis. It is not clear if a literature review was carried out or if the authors' decision was based on expert opinions.

Even though not considering IBS as a sequela of yersiniosis, the estimates point to a burden twice as high when compared to this study estimates. In another study developed in New Zealand, the estimations of total incidence of yersiniosis were of 718 cases per 100,000 population (Cressey & Lake, 2011). The estimates for Denmark in the year 2016 were of 9 cases per 100,000 inhabitants. This can explain why the burden of yersiniosis is so high in New Zealand.

Lake and colleagues (2010) included GE, ReA, IBD and GBS (only for campylobacteriosis), when considering the burden of *Salmonella* spp. and *Campylobacter* spp.. They do not account for the burden of IBS. Their estimates point to a higher burden of campylobacteriosis when compared to all the other studies, and one of the lowest for salmonellosis. The first estimate can be explained by the adding of an extra sequela of campylobacteriosis (IBD) when compared to this study. Regarding salmonellosis, total incidence estimated might be behind this low burden.

Both studies from the Netherlands used the same health-outcomes as this study for each pathogen, but also included IBD as a sequela of campylobacteriosis and salmonellosis. The estimations are very similar when comparing the two Dutch studies, which can be explained by the application of exactly the same disease model, informed by the same parameter's data.

When comparing the burden of salmonellosis to the Danish burden, estimates have come to similar results. The same does not happen when comparing campylobacteriosis and VTEC's burden. It is important to note that Dutch estimates for VTEC only include VTEC O157, and therefore differences were to be expected.

Belgium has the lowest burden estimates for *Salmonella* spp., which can be explained by differences in the health-outcome trees used when compared to other studies. Both IBS and IBD were not included in the model developed. Furthermore, Noordhout and colleagues (2017) aknowledge that when accounting for under-reporting in their model, the mulplying factor used was particularly low for Belgium (3.5), when compared with the one used in the previous Danish estimates (7.2) (Pires, 2014). And therefore, when compared with the multiplier estimated in this study (7.7).

The burden of campylobacteriosis estimated in this study, when compared to Belgium and WHO estimates for Europe is substantially high. The explanation can lie behind the differences in health-outcome trees. Both WHO and the Belgium study do not consider IBS as a sequela of campylobacteriosis. Although, when subtracting the DALYs caused by IBS, campylobacteroisis will still cause 15.8 DALYs per 100,000 population, which is still

considerably higher than the other estimates. Potential differences in the incidence of *Campylobacter* spp. might be behind this higher burden.

Table 30 – Comparison of burden of disease estimates for Denmark 2016 and other countries and Europe. Total DALYs per 100,000 population.

Country/ Region	**Reference**	**DALYs per 100,000 population**			
		Campylobacter spp.	***Salmonella*** spp.	***Y. enterocolitica***	**VTEC**
BEL	(Noordhout *et al*., 2017)	9	0.9	NA	NA
DK	(Pires, 2014)	28.4	6.9	NA	2.02
JPN	(Kumagai *et al*., 2015)	4.8	2.5	NA	NA
NED	(Havelaar *et al*., 2012)	19.8	7.7	NA	0.7*
NED	(Van Lier *et al*., 2016)	19.9	8.2	NA	NA
NZ	(Lake *et al*., 2010)	37.9	4.5	2.2	NA
EUR	(WHO, 2015)	9	8	NA	NA
DK	**This study**	**30**	**7.5**	**1.1**	**0.8**

*Estimates only include VTEC O157. NA: not available.

Lastly, comparing both Danish estimates, similar results have been achieved regarding campylobacteriosis and salmonellosis, which means the model applied is consistent and sound.

The differences between VTEC's burden estimates are related to the multiplying factor considered, which was only corrected after publishing.

Overall, it is clear that differences in the methodological approach used to estimate the burden of disease of foodborne pathogens, makes comparison among countries difficult. Developing both more precise under-reporting and underdiagnosis multiplying factors and health-outcome trees could help improving the comparability of studies among countries.

3. Age and gender distribution of the burden of foodborne illness and disease

Total incidence estimates for all four pathogens show that children under five years old have the highest incidence when compared to other age groups. When comparing gender

differences, females with campylobacteriosis seem to be more affected. For yersiniosis, a decrease in the total incidence of cases with age is noticeable when looking at the female population. Still regarding burden of illness results, campylobacteriosis show a high incidence from ages 15 to 64, while VTEC have low incidences in all age groups beside children under five years of age.

When looking at the burden of disease estimates, both *Y. enterocolica* and VTEC have the highest number of DALYs in the youngest age group. On the other hand, for *Campylobacter* spp. and *Salmonella* spp. the highest burden falls in the oldest age group. That can be explained by the high number of fatal cases estimated in elderly people, when compared to the others. Still, children under five have a considerable high burden caused by these two foodborne pathogens.

In other studies, the public health impact of diarrheal diseases, including those caused by pathogens commonly transmitted through foods, has been found to be higher for young children (Havelaar *et al*., 2012; GBD's Diarrhoeal Diseases Collaborators, 2017; WHO, 2015). Diarrheal diseases have also been associated with increased mortality, especially in the oldest age group, which includes people older than 65 years old (GBD's Diarrhoeal Diseases Collaborators, 2017).

Because disease may be more severe in these age groups due to higher vulnerability or lower immune status, this evidence enhances the importance of control and prevention measures to reduce the burden of foodborne diseases in the population.

VI. Conclusion

This study presents the first Danish burden of disease of yersiniosis, the second worldwide, and the second estimates for campylobacteriosis, salmonellosis and verocytotoxin-producing *E. coli* infections in Denmark.

Campylobacteriosis was the disease with the highest burden, followed by salmonellosis, yersiniosis and VTEC infections.

The uncertainty around presented estimates shows that improved data availability and a global harmonized approach would increase the certainty of these results, as well as its comparability among countries.

Still, they show that the burden of these preventable diseases is still considerable, even in high-income countries like Denmark. Understanding the contribution of each cause to the burden of foodborne diseases and incorporating these estimates into policy development worldwide will enable efficient and effective interventions and improvements throughout all the food chain and therefore improving public health.

In addition, there are few burden of disease country-specific studies for foodborne diseases, most in high-income countries. These countries already have very well-developed surveillance systems and approaches to increase food safety, but are trying to better them. Other countries with less organized surveillance systems and higher or unknown incidence of foodborne diseases, would profit with the development of burden of illness and burden of disease studies. Ranking diseases on their public health burden, identifying specific food/animal sources using source attribution studies and performing risk assessment throughout the food chain, would facilitate food authorities' decision process.

Why not Portugal?

VII. References

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VIII. Annexes

Annex I

Table 31 – Reported cases of campylobacteriosis in Denamrk, 2016, aggregated by sex and age group.

Age group	**Reported cases**		
	Female	Male	Total
0 – 4	120	149	269
5 – 14	97	166	263
15 – 44	1017	1064	2081
45 - 64	607	756	1363
65 +	328	370	698
Total	2169	2505	4674

Table 32 – Reported cases of salmonellosis in Denmark, 2016, aggregated by sex and age group.

Age group	**Reported cases**		
	Female	**Male**	**Total**
0 – 4	59	63	122
5 – 14	46	66	112
15 – 44	169	156	325
45 - 64	158	125	283
65 +	118	108	226
Total	550	518	1068

Table 33 – Reported cases of VTEC in Denmark, 2016, aggregated by sex and age group.

Age group	**Reported cases**		
	Female	**Male**	**Total**
0 – 4	34	37	71
5 – 14	15	14	29
15 – 44	34	32	66
45 - 64	31	14	45
65 +	28	11	39
Total	142	108	250

Table 34 – Reported cases of *Yersinia enterocolitica* in Denmark, 2016 aggregated by sex and age group.

Age group	Reported cases		
	Female	**Male**	**Total**
0 – 4	21	27	48
5 – 14	28	35	63
15 – 44	156	91	247
45 - 64	82	43	125
65 +	54	35	89
Total	341	231	572

Table 35 – Danish Population in 2016, aggregated by sex and age group.

Age group	Danish population		
	Female	**Male**	**Total**
0 – 4	144,883	153,180	298,063
5 – 14	323,368	340,137	663,505
15 – 44	107,1204	110,5425	2,176,629
45 - 64	755,699	759,701	1,515,400
65 +	593,437	501,735	1,095,172
Total	2,888,591	2,860,178	5,748,769

Annex II

Table 36 - Probability of developing Guillain-Barré syndrome for each age group.

Age group	Probability of developing GBS
0 - 4	0,11
5 - 14	0,08
15 - 24	0,10
25 - 64	0,57
65 +	0,14

Table 37 – Reported cases of hemolytic uremic syndrome in Denmark, 2016, aggregated by sex and age group.

Age group	Reported cases	
	Female	Male
0 – 4	3	1
5 – 14	0	1
15 – 44	0	0
45 - 64	0	1
65 +	0	0

Table 38 – Age-specific mortality risk by all causes in the Netherlands, 2012.

Age group	Mortality risk
0	0,00325
1-4	0,00025
5-9	0,0001
10-14	0,00011
15-19	0,0002
20-24	0,00028
25-29	0,00032
30-34	0,00047
35-39	0,0006
40-44	0,001
45-49	0,00171
50-54	0,00292
55-59	0,00486
60-64	0,00753
65-69	0,01196
70-74	0,01924
75-79	0,03391
80-84	0,06292
85-89	0,11268
90+	0,2419

Table 39 – Danish average life expectancy table for the years 2015-2016 (Adapted from Danish statistics).

Age	Men	Women
0	78.61	82.53
1	77.97	81.84
5	74	77.88
10	69.03	72.9
15	64.06	67.93
20	59.11	62.97
25	54.23	58.02
30	49.36	53.07
35	44.49	48.16
40	39.68	43.26
45	34.93	38.43
50	30.33	33.69
55	25.92	29.11
60	21.73	24.7
65	17.88	20.53
70	14.23	16.53
75	10.91	12.8
80	7.91	9.49
85	5.52	6.76
90	3.75	4.51
95	2.59	3.04

Table 40 – World Health Organization' life table for calculating years of life lost (YLL) (Adapted from WHO, 2017).

Age	SEYLL*
0	91.94
1	91.00
5	87.02
10	82.03
15	77.04
20	72.06
25	67.08
30	62.11
35	57.15
40	52.20
45	47.2
50	42.3
55	37.4
60	32.6
65	27.8
70	23.15
75	18.62
80	14.41
85	10.70
90	7.60
95	5.13

* Standard expected years of life lost