UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

BURDEN OF DISEASE ESTIMATION USING DOSE-RESPONSE MODELS BASED ON *E.COLI* QUANTIFICATION IN READY-TO-EAT MEALS OF INSTITUTIONAL CANTEENS

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ORIENTADORA:
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Acknowledgments/Agradecimentos

À minha orientadora, Professora Ana Rita Henriques, por ter acreditado neste trabalho desde o início, pela confiança, muitas vezes mais do que eu própria, que seria capaz de o desenvolver. Por todo o apoio, boa disposição, disponibilidade, empenho, e pela amizade que se criou com a qual espero sempre poder contar. Uma inspiração para mim enquanto pessoa e por acreditar que sou capaz de ir mais além.

Ao Professor Telmo Nunes, pela ajuda indispensável com análises necessárias para este trabalho e pelas conversas proveitosas sobre o delineamento do mesmo.

A todos aqueles que direta ou indiretamente contribuíram para a realização deste trabalho prestando todos os esclarecimentos necessários.

À Professora Isabel Fonseca, por toda a amizade e conselhos amigos ao longo do meu percurso académico, por me ter incentivado sempre a querer saber mais e por me ter incutido o espírito e rigor científico desde as aulas de Parasitologia I.

Ao Professor David Ramilo, por me ter recebido no laboratório de Parasitologia durante o curso, por me ter iniciado na área da investigação, e por ser uma inspiração para mim enquanto cientista e professor.

À Lídia Gomes, pela paciência, conselhos amigos e por todos os momentos de boa disposição.

À Professora Esmeralda Delgado por toda a confiança, incentivo, apoio e palavras amigas ao longo do curso.

À Professora Graça Pires pela constante simpatia e palavras amigas que muito prezo.

À Ana, Joana, Mafalda e Maura por todos os momentos bem passados, companheirismo, apoio, risos altos nos corredores e por tornarem cada dia mais leve com a boa disposição constante. Foi espetacular ter feito este curso ao vosso lado.

Ao grupo da ‘salinha’ C3.37 por todos os momentos bem passados e interajuda.

Aos meus pais e avós por todo o apoio incondicional e por estarem sempre presentes.

A todos acima, o meu sincero obrigada, porque mais do que um curso, são também as pessoas que conhecemos ao longo do mesmo que fazem o curso e o percurso valer a pena.

Resumo

No ano de 2015, a Organização Mundial de Saúde coordenou uma iniciativa internacional para estimar a carga global de doença de origem alimentar, através da publicação *Estimates of the Global Burden of Foodborne Diseases*. De acordo com estimativas recentes, em 2010, *E.coli* produtora de toxina Shiga (STEC) foi responsável por 12 953 anos de vida ajustados por incapacidade (DALYs), enquanto *E.coli* enterotoxigénica (ETEC) foi responsável por 2 084 229 DALYs e *E.coli* enteropatogénica (EPEC) por 2 938 407 DALYs. Estes grupos de *E.coli* estão associados a gastroenterite humana com severidade variável, no entanto, STEC pode ainda desencadear síndrome hemolítico urémico e doença renal de estadio terminal. Este trabalho teve como objetivo estimar a carga de doença de origem alimentar anual associada a infeção por STEC, por ETEC e por EPEC a partir dos resultados de quantificação microbiológica efetuada a refeições prontas a consumir servidas em cantinas institucionais no período de 2018 a 2019. Para isso, os resultados das contagens de *E.coli* foram compilados e organizados numa base de dados. O número estimado de casos de doença resultou da aplicação de modelos de dose-resposta para a estimativa do risco, através da metodologia de avaliação quantitativa de risco microbiológico, tendo também permitido o cálculo dos DALYs. Construíram-se dois cenários diferentes com base na quantidade de refeição consumida e também considerando a ocorrência de *E.coli* STEC, ETEC e EPEC. Uma análise de sensibilidade foi efetuada para cada um dos modelos utilizando o método de Sobol.

Tendo em conta o cenário de consumo duma refeição diária com 450 gramas, a estimativa da carga de doença associada ao consumo das referidas refeições foi de $4{,}99 \times 10^{-3}$ DALYs/pessoa/ano para infeção por STEC, $2{,}82 \times 10^{-4}$ DALYs/pessoa/ano para infeção por ETEC e $7{,}91 \times 10^{-6}$ DALYs/pessoa/ano para infeção por EPEC. De acordo com a análise de sensibilidade, o fator que mais contribuiu para a variabilidade nos modelos STEC foi a ocorrência do grupo de *E.coli,* no modelo ETEC foi o número de pessoas expostas ao perigo e no modelo EPEC foram as concentrações de *E.coli*.

Palavras-chave: Carga de doença, *Escherichia coli,* anos de vida ajustados por incapacidade, alimentos prontos a consumir, avaliação quantitativa de risco microbiológico.

Abstract

In 2015 the World Health Organization launched an initiative to estimate the global burden of foodborne disease, through the release of the *Estimates of the Global Burden of Foodborne Diseases*.

According to the most recent estimate, in 2010, Shiga toxin-producing *E.coli* (STEC) was responsible for 12,953 disability adjusted life years (DALYs), enterotoxigenic *E.coli* (ETEC) for 2,084,229 DALYs and enteropathogenic *E.coli* (EPEC) for 2,938,407 DALYs. These *E.coli* groups are associated with the onset of gastroenteritis with different severities, however, STEC can be associated with other sequelae such as hemolytic uremic syndrome and end stage renal disease. This work aimed to estimate the annual foodborne burden of disease associated with STEC, ETEC and EPEC infection based on results of *E.coli* quantification in ready-to-eat meals of institutional canteens from 2018 to 2019. Results were compiled and organized in a database, and the number of expected cases of disease resulted from the application of dose-response models for risk assessment using quantitative microbial risk assessment methodology which also allowed DALYs calculation. Two different scenarios were built based on the ingested meal portion and also based on the occurrence of *E.coli* STEC, ETEC and EPEC. A sensitivity analysis was performed for each model using Sobol method.

Assuming a scenario in which a whole meal portion (450 grams) was consumed daily, the calculated burden was 4.99×10^{-3} DALYs/person/year for STEC infection, 2.82×10^{-4} DALYs/person/year for ETEC infection and 7.91×10^{-6} DALYs/person/year for EPEC infection. Regarding the sensitivity analysis, the factors that most contributed to the overall output variability were the occurrence of the *E.coli* group for the STEC model, the number of people exposed to the hazard for the ETEC model and the *E.coli* concentrations for the EPEC model.

Keywords: Burden of disease, *Escherichia coli,* Disability Adjusted Life Years, ready-to-eat foods, Quantitative Microbial Risk Assessment.

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List of Abbreviations

AIEC. Adherent-invasive *E.coli*

APEC. Avian pathogenic *E.coli*

CFR. Case Fatality Ratio

cfu. colony forming unit

CI. Credibility Interval

DAEC. Diffusely adherent *E.coli*

DALY. Disability Adjusted Life Years

DW. Disability Weight

EAEC. Enteroaggregative *E.coli*

EHEC. Enterohemorrhagic *E.coli*

EIEC. Enteroinvasive *E.coli*

EPEC. Enteropathogenic *E.coli*

ESRD. End Stage Renal Disease

ETEC. Enterotoxigenic *E.coli*

ExPEC. Extraintestinal pathogenic *E.coli*

FAO. Food and Agricultural Organization of the United Nations

HUS. Hemolytic Uremic Syndrome

InPEC. Intestinal pathogenic *E.coli*

NMEC. Neonatal meningitis-associated *E.coli*

OIE. World Organisation for Animal Health

PCR. Polymerase Chain Reaction

QMRA. Quantitative Microbial Risk Assessment

SEPEC. Sepsis-causing *E.coli*

STEC. Shiga toxin-producing *E.coli*

Stx. Shiga toxin

UPEC. Uropathogenic *E.coli*

WHO. World Health Organization

YLD. Years Lived with Disability

YLL. Years of Life Lost

Internship Report

From August to November 2021 an internship under the supervision of Professor Ana Rita Henriques took place at the Faculty of Veterinary Medicine of the University of Lisbon. Some of this time was spent on the institution referred in this dissertation gathering and organizing the data necessary for the development of the project.

Several courses were taken on the R program to learn how to code in R programming language. During the rest of the internship, all the coding necessary for the development of the present work was developed and the analysis performed were co-supervised by Professor Telmo Nunes. Intensive literature review was performed scoping for articles and information that allowed the building of the input data necessary for the development of the final output.

1. Introduction

The World Health Organization (WHO) works closely with the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (OIE) and other international organizations to ensure food safety along the entire food chain from production to consumption (WHO 2020a).

Foodborne diseases are an important cause of mortality and morbidity worldwide, but the full extent and cause of unsafe food is unknown (Aidara-Kane et al. 2016). To fill this gap, in 2015, the WHO together with other partnered institutions launched an initiative to estimate the global burden of foodborne disease, by supporting countries in the assessment of their own burden of foodborne disease, increasing the commitment to implement food safety standards, and ultimately raising awareness for the magnitude and dimensions of this global problem (WHO 2015; Aidara-Kane et al. 2016). Foodborne illnesses are infectious or toxic in nature and can be caused by bacteria, viruses, parasites or chemical substances such as naturally occurring toxins, persistent organic pollutants and heavy metals that enter the body through contaminated food or water (WHO 2020a). Additionally, physical hazards such as foreign objects are also important but are not subject to international food safety standards (Aidara-Kane et al. 2016). These hazards can be an inherent constituent of the food or result from unintentional addition during food production, processing or preparation (Hoffmann and Scallan 2017). The most common pathogens responsible for foodborne illness in the United States, according to the CDC (2020), are *Norovirus, Salmonella, Clostridium perfringens*, *Campylobacter* and *Staphylococcus aureus* and the ones most likely to cause hospitalization are *Clostridium botulinum*, *Listeria monocytogenes*, *Escherichia coli* and *Vibrio*. Although many of these agents cause diarrhea and vomiting, a universal and consensual way to fully characterize the clinical outcomes of all foodborne diseases is still to be proposed as there is no single clinical syndrome for all of them (Hoffmann and Scallan 2017).

Risk assessment is a well-defined scientific process that comprises several steps to characterize the potential hazards and the associated risks to life and health condition that results from the exposure through food over a specific period of time (Aidara-Kane et al. 2016). This type of approach constitutes the basis for an informed policy development, allows for the prioritization and implementation of risk management measures for hazard control and fundamental reduction and prevention of foodborne diseases (Aidara-Kane et al. 2016).

Regarding the burden of disease, Disability Adjusted Life Years (DALY) is recognized as the ultimate measure for quantifying the population health impact of foodborne diseases and while estimating DALYs is an inspirational goal, any step towards is valuable (Pires et al. 2021).

This dissertation's main aim is to perform a burden of disease estimation in institutional canteens food consumers, using *Escherichia coli (E.coli)* quantification results from routine food analysis by classical microbiological methods, discriminating all possible clinical outcomes associated with *E.coli* infection.

2. Literature Review

2.1. Overview of foodborne disease

Foodborne disease is one of the main causes of global mortality, which accounts for about 600 million illnesses and 42.000 deaths annually (Havelaar et al. 2015). Foodborne diseases can result from the presence in food of pathogens, such as virus, fungi, bacteria, prions, parasites, but also chemicals and foreign objects that can be transmitted to humans (Scallan et al. 2011; Pouliot and Wang 2018).

In the WHO European Region, it is estimated that 23 million people get sick from eating contaminated food every year, resulting in 4654 deaths and more than 400.000 Disability Adjusted Life Years (DALYs) (WHO 2017). The most frequent cause of foodborne illness are diarrheal agents, among which the most common are *Norovirus* and *Campylobacter* spp. Non-typhoidal *Salmonella* spp. is responsible for the majority of deaths (WHO 2017). Some of these agents can be zoonotic, being transmitted from non-human animals to humans (WHO 2020b). In the European Union, campylobacteriosis, salmonellosis, Shiga toxin-producing *E.coli* (STEC) infections and yersiniosis were the most frequently reported zoonosis in 2019 (EFSA 2021). The consumption of animal products has increased due to the increase in human population, *per capita* income, consumer demands, such as higher protein content in the diet, and is expected to continue rising (Dhama et al. 2013). The growing tendency on the demand of animal products will therefore increase intensive animal production, processing of products and global food trading (Heredia and García 2018). Regarding animal products, meat and poultry products are responsible for the majority of foodborne illnesses (Heredia and García 2018; Nganje et al. 2021).

According to the European Union One Health Zoonoses Report, in 2019, infection from Shiga toxin-producing *E.coli*, also known as STEC, was the third most reported zoonosis in the European Union (EFSA 2021). A total of 7775 infections were reported, of which, only one case was notified by Portugal. Two hundred and twenty-three cases were assigned to foodborne outbreaks, with fifty resulting in hospitalization and one death. A study of STEC outbreaks with foodborne origin in European member states between 2010-2018, concluded that beef and products thereof, water, vegetables, juices, and other products thereof, as well as milk and cheese, were the main vehicles for these infections (EFSA 2021). It is estimated that, annually, STEC is responsible for 2.801.000 infections worldwide,

3890 hemolytic uremic syndrome cases, 270 end stage renal disease cases and 230 deaths (Majowicz et al. 2014). However, there are more *E. coli* pathotypes that contribute to the burden of foodborne diarrheal disease. With regard to the WHO Estimates of The Global Burden of Foodborne Diseases, the most recent to date, it is estimated that, globally, STEC is responsible for 12.953 foodborne DALYs, enterotoxigenic *E.coli* (ETEC) for 2.084.229 and enteropathogenic *E.coli* (EPEC) for 2.938.407, in the year of 2010 (WHO 2015).

2.2. *Escherichia coli*

The genus *Escherichia* and *E.coli* species have been recognized for over a century (Donnenberg 2013). *E. coli* is a gram negative, facultative anaerobe, non-sporulating rod that belongs to the *Enterobacteriaceae* family; it is able to ferment sugars through lactose fermentation with the production of acid and gas (Feng 2013).

This organism mainly inhabits the lower intestinal tract of warm-blooded animals and is also present in the environment as a result of wastewater effluent and fecal contamination (Jang et al. 2017).

It is mainly present in the colon and cecum of vertebrates, residing in the mucus layer and shed into the intestinal lumen and excreted with the feces (Poulsen et al. 1994; Nawrocki et al. 2020).

The host-*E.coli* relationship begins at birth with the colonization of newborns with maternal *E.coli* present in fecal matter and as a result of subsequent handling (Blount 2015). In fact, *E.coli* becomes more abundant in the mother's microbiome during pregnancy in order to increase the chances for newborns contamination (Koren et al. 2012). However, with the increasing rates of cesareans and increased hospital hygiene practices, colonization by *E.coli* has decreased and this leads to broader microbiome changes, such as an increased colonization by *Staphylococcus aureus* (Blount 2015). This association since early human development benefits the host with the production of menaquinone (vitamin K) and riboflavin (vitamin B_2) (Blount 2015; Nawrocki et al. 2020). Moreover, the host-*E.coli* association has numerous other benefits beyond vitamin production, such as intestinal environment modulation, blocking other pathogens from colonizing the gut and also playing a role on the structure and function of epithelial cells, which is crucial for a healthy microbiome development (Blount 2015; Tomas et al. 2015).

The concentration of *E.coli* in human feces can range from 10^7-10^9 cfu per gram and 10^4-10^6 in domestic animals (Smith 1978; Penders et al. 2006; Tenaillon et al. 2010).

The classification of *E. coli* species is based on its antigenic composition based on the Kauffman classification, in which somatic (O) and flagellar (H) antigens are determined to insert the species in a serogroup and serotype, respectively (Whitfield and Roberts 1999; Fratamico et al. 2016). So far, 186 O and 53 H antigens have been recognized (Fratamico et al. 2016). Moreover, *E. coli* can be classified in intestinal pathogenic *E.coli* (InPEC) and

extraintestinal pathogenic *E.coli* (ExPEC) (van der Hooft et al. 2019). *E.coli* strains isolated from infections outside the intestinal tract can be classified in uropathogenic *E.coli* (UPEC), neonatal meningitis-associated *E.coli* (NMEC), sepsis-causing *E.coli* (SEPEC) and avian pathogenic *E.coli* (APEC) (Köhler and Dobrindt 2011; Sarowska et al. 2019; Kathayat et al. 2021). Detection of specific virulence-associated genes or combinations thereof allows the distinction between pathogenic, non-pathogenic and ExPEC *E.coli*. (Köhler and Dobrindt 2011; van der Hooft et al. 2019). ExPEC, in general, can be present in the intestinal microbiota of healthy population and once gaining access to locations outside of the gut, it is able to colonize the niche and cause disease. (Köhler and Dobrindt 2011; Sarowska et al. 2019).

Although most of *E.coli* strains are commensal organisms in the intestine, some *E. coli* pathotypes harbor virulence factors making them pathogenic, diarrheagenic or enterovirulent (Heredia and García 2018). This particular group of pathogenic *E.coli* is classically sub-divided into categories (Table 1), being responsible for gastrointestinal infections: enteropathogenic *E.coli* (EPEC), Shiga toxin-producing *E.coli* (STEC), enterotoxigenic *E.coli* (ETEC), enteroaggregative *E.coli* (EAEC), enteroinvasive *E.coli* (EIEC), diffusely adherent *E.coli* (DAEC) and adherent-invasive *E.coli* (AIEC) (Croxen et al. 2013; Heredia and García 2018). Enterohemorrhagic *E.coli* (EHEC) is considered a subset of pathogenic STEC strains (Feng 2013).

The common clinical outcome for all groups of intestinal pathogenic *E.coli* is gastroenteritis and can differ in the characteristics of diarrhea. Moreover, many risk factors can influence the development of bacterial gastroenteritis, such as age (lack of immunity to certain pathogens in young children, and physical and biological alterations in the elderly), gastric acidity, intestinal dysmotility, immunosuppression, genetic predisposition, use of antibiotics, overcrowded living conditions and poor sanitation (Ranasinghe and Fhogartaigh 2021).

Table 1. Characteristics and virulence genes of diarrheagenic *E.coli*. Adapted from Cabrera-Sosa and Ochoa (2020). In bold are the main virulence genes used for the diagnosis of each pathotype; CF, colonization factors.

Intestinal Pathogenic *E.coli* (InPEC)		
Pathotype	**Diarrhea's Characteristics**	**Virulence genes**
EPEC	Watery; acute or persistent	***eae, bfp***
ETEC	Watery; acute	**LT, ST**, and CF genes
STEC	Bloody; acute	***stx1, stx2, eae***, *ehx, fliC*
EAEC	Watery; acute or persistent	***aggR, astA, aatA***, *aaiC, aap, set1A*
EIEC	Bloody; acute	***ipaH, ial1***
DAEC	Watery; acute or persistent	Afa/Dr adhesin genes: ***daaC, daaD***, *daaE*, ***afaB***, *afaC*
AIEC	Watery; acute or persistent	No specific gene reported

2.2.1. Enteropathogenic *E. coli*

EPEC was the first group identified as diarrheagenic *E. coli* and is of more importance in children, especially those under two years old in developing countries (Cabrera-Sosa and Ochoa 2020). Transmission occurs via fecal-oral route through contaminated food, water or fomites (Donnenberg 2013). To date, humans and domestic animals are considered the main strain hosts (Denamur et al. 2021). Clinical signs often include watery diarrhea that sometimes can be bloody, with little or no fever lasting for a few days, although in severe cases it can last up to 14 days. Infection and exposure to EPEC seems to build immunity, which may explain the lower incidences in other ages (Feng 2013). Infection of adults is associated with the ingestion of large inoculum (Landraud and Brisse 2010). Despite the lower incidence, meta-analysis studies suggest that in adults EPEC incidence in developed nations is severely underreported, or not even reported, when compared with other pathogens (Carlino et al. 2020). This type of *E.coli* colonizes the small intestine mucosa and induces an attaching and effacing lesion in the enterocytes, although the way that it causes diarrhea is not well understood (Cabrera-Sosa and Ochoa 2020). Additionally, it has been reported that this type of *E.coli* can exhibit resistance to a large range of antibiotics (Rodrigues et al. 2019; Eltai et al. 2020).

2.2.2. Enterotoxigenic *E.coli*

ETEC is an important cause of diarrhea in all ages and a common cause of traveler's diarrhea; Clinical signs include watery diarrhea, without blood or mucus, that can be accompanied by fever, abdominal cramps, vomiting and is usually self-limiting, mild and brief, but severe forms can also occur. Infections generally result from consumption of contaminated food or water (Feng 2013). Similarly to EPEC, ETEC colonizes the small intestine mucosa by attachment to the intestinal epithelium, followed by the production of enterotoxins, that can be heat-labile (LT) or heat-stable (ST); ETEC strains may produce only one or both types of the toxin and several other pathogenic factors, such as colonization factors (Nazarian et al. 2012; Alerasol et al. 2014; Mirhoseini et al. 2018). Humans, pigs and cattle are the known hosts of the strain (Denamur et al. 2021)

2.2.3. Shiga toxin-producing *E.coli*

In 1898 Kiyoshi Shiga discovered the bacteria responsible for bacillary dysentery and named it *Bacillus dysenterie*. In the years that followed, several researchers isolated more strains, creating the *Shigella* genus that included four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. However, in 1944, strains of *E.coli* capable of invading the colon mucosa in a way that was similar to *Shigella* were identified and named enteroinvasive *E. coli* (EIEC) (Kupfer 2018).

Shigella is known for causing dysentery which consists of frequent, painful passage of stools that consist of mucus, blood, inflammatory cells and fecal matter with humans being the only known reservoir, and infection occurs by fecal-oral or human-to-human transmission (Bennish and Ahmed 2020). Children with ages 1-4 are the most affected by enteric shigellosis (Kotloff et al. 2013). However, more deaths occur in adults and older children because the immunity acquired is serotype specific, which makes adults and children susceptible to disease caused by other serotypes with which they have not previously contacted with (Naghavi et al. 2017; Bennish and Ahmed 2020). Of all *Shigella* species*, S. somnei* is the main cause of Shigellosis in wealthy and industrialized countries. However, the most problematic and pathogenic is *S. dysenteriae* type 1, causing the most severe disease*,* as it is capable of producing the Shiga toxin as well (Bennish and Ahmed 2020).

Shigella differs from *E.coli* in biochemical properties, as they are non-motile, lysine decarboxylase negative, do not form gas from carbohydrates fermentation, do not ferment lactose or show late lactose fermentation, and are unable to produce hydrogen sulfide (Bliven and Lampel 2017). With the development of molecular technologies and genome sequencing, it became clear that *Shigella* and EIEC were very similar and should be classified in one genus. Nowadays, *Shigella* and *E.coli* are considered to be unique genomospecies, and are so related that should be part of the same genus *Escherichia* (Beld and Reubsaet 2012; Devanga Ragupathi et al. 2018).

2.2.3.1. Shiga toxin

Shiga toxin (Stx) is one of the most potent bacterial toxins known. It can be found typically in *S. dysenteriae* 1 and in some *E.coli* serogroups (Stx1 or Stx2). Variations of the toxin can be found and isolated and therefore can be subdivided into groups that share the same antigen properties. Stx2 has the same mode of action as Stx/Stx1, however *E.coli* strains that encode Stx2 are more prone to cause a more severe disease than those carrying Stx1 (Melton-Celsa 2014). Stx1 is virtually identical to Stx with only one amino acid residue that differs, whereas Stx2 is only 60% similar to Stx and is immunologically distinct. However, all Stx isoforms share the same general structure and mechanism of action (Engedal et al. 2011). The presence of these genes that encode the toxin are acquired through a lambdoid bacteriophage and classify the *E.coli* strain as Shiga toxin-producing *E.coli* (STEC) (Croxen et al. 2013).

The toxin structure is constituted by an A subunit, responsible for the enzymatic action of the toxin, formed by two subunits, A_1 and A_2, connected by a disulfide bridge, and by B subunit, responsible for the binding to Gb3 receptors in host cells (Melton-Celsa 2014).

The B subunit is responsible for the selective binding to the Gb3 receptor located in the plasma membrane of target cells and allows for the cellular uptake and intracellular transport of the toxin. The Gb3 receptor is expressed mostly in kidney epithelium and endothelium, microvascular endothelial cells of the intestinal *lamina propria*, platelets, subsets of germinal centre B lymphocytes and, in less extent, monocytes and derived cells, neurons, and endothelial cells of the central nervous system (Engedal et al. 2011).

Once in the cytoplasm, the A subunit inhibits protein synthesis and acts in the cell, specifically the ribosomes, where it induces a response called “ribotoxic stress response” which is pro-inflammatory, pro-apoptotic and leads to cell death (Gallegos et al. 2012; Melton-Celsa 2014). Besides binding to the receptor, the B subunit presents other biological activities such as cytoskeletal remodeling, retrograde trafficking of the toxin, stimulation of von Willebrand factor secretion, activation of apoptotic cascades and possibly the signaling of Toll-like receptor-4 (Jandhyala et al. 2012).

2.2.3.2. Hemolytic Uremic Syndrome

One of the possible outcomes of the biological activity of Shiga toxin is the development of a medical condition known as Hemolytic Uremic Syndrome (HUS). After destruction of the colonic mucosa and disruption of the intestinal barrier, the toxin enters the systemic circulation, reaching target organs and binding to Gb3 receptors on microvascular endothelial cells which are mainly present in microvascular glomeruli (Joseph et al. 2020). Thrombotic microangiopathy, hemolytic anemia, thrombocytopenia and acute renal damage are the clinical signs and conditions that characterize the syndrome (Canpolat 2015).

HUS caused by infection of Shiga toxin-producing *E.coli* and *Shigella dysenteriae* is responsible for 90% of childhood HUS and mainly affects children younger than 5 years of age, but adults can also develop the disease, despite the much lower incidence (Ohanian et al. 2011 May; Manrique-Caballero et al. 2020; Travert et al. 2021). In adults it is mainly associated with epidemics and outbreaks. One of the most important complications of HUS development is the possible progression to end-stage renal failure and long-term renal impairment (Mayer et al. 2012). End-stage renal disease and permanent neurologic damage can happen in patients that survive the acute phase of HUS (Rahal et al. 2012). Most deaths associated with HUS occur in older people, particularly those with age above 60 (Travert et al. 2021). The most common serotype in HUS is *E. coli* O157:H7 although other HUS-causing serogroups have been reported, as O26, O45, O103, O111, O113, O121 and O145 (Noris and Remuzzi 2005; Tenaillon et al. 2010; Ohanian et al. 2011 May; Salvadori 2013; Castro et al. 2019; Manrique-Caballero et al. 2020; Alconcher et al. 2021).

In 2011, an unusual high number of adult HUS cases arose after a foodborne outbreak in Germany, and the agent responsible was *E. coli* O104:H4, classified as enteroaggregative hemorrhagic *E. coli*. Of 3816 affected people, 845 developed HUS, and

54 died (Frank et al. 2011). Most of the patients involved in this outbreak were adults with a median age of 42 years (Beutin and Martin 2012; Canpolat 2015). The presence of the Stx2 variant of the toxin has been more commonly associated with the development of HUS following infection and severe disease, and can be related to the variant's greater ability to enter the bloodstream, as it is more potent than Stx1 (Fuller et al. 2011).

The causative agent of the outbreak, *E. coli* O104:H4, corresponded to a hybrid of STEC/EAEC. Over the years, several hybrid strains have been reported, such as STEC/ETEC strains, that carried *stx*$_2$ and *sta* genes, belonging to livestock but also to human clinical isolates (Johura et al. 2017; Bai et al. 2019). STEC/ExPEC O80:H2 hybrid has been reported by Mariani-Kurkdjian et al. (2014) and EPEC/EAEC and ETEC/EPEC have also been reported (Liebchen et al. 2011; Hazen et al. 2017). Moreover, hybrids of ExPEC/InPEC have also been described (Lindstedt et al. 2018). The insurgence of these hybrids can be explained by the presence of mobile genetic elements, such as phages or plasmids, that carry virulence markers for a specific pathotype and allow for horizontal gene transfer, leading to the emergence of new hybrid pathotypes (Bai et al. 2019).

2.2.3.3. EHEC O157:H7

Escherichia coli O157:H7 and other STEC serotypes can be detected in a variety of animal species, with cattle being the main reservoir of strains that are highly pathogenic to humans (Chekabab et al. 2013). Consumption of contaminated food remains the main cause of infection, although contact with manure, animals and infected people are also responsible for the appearance of cases but at a much lower frequency (Ferens and Hovde 2011; Vidovic and Korber 2016). Exposure to undercooked meat, inadequately pasteurized dairy products or direct contact with animals or contaminated fomites are the main causes for zoonotic transmission (Erickson and Doyle 2007) . EHEC can cause disease in newborn calves and colonizes the gut of adult bovines that can be asymptomatic carriers of the pathogen, acting as important sources of contamination for food and the environment (Chase-Topping et al. 2008). This asymptomatic carriage can be explained by the lack of Gb3 receptors in vascular cells, and therefore the toxin is unable to bind to the gastrointestinal blood vessels (Pruimboom-Brees et al. 2000). Besides cattle, the strain O157:H7 has also been detected in wild birds, pigeons, chickens, horses, and rabbits, although it is not yet clear if these are actual hosts or merely infected animals because of contact with the agent (Money et al. 2010). Some of them are capable of shedding *E. coli* in levels higher than 10^4 cfu/g and are known as "super-shedders" (Munns et al. 2015). In a study conducted by Ballem et al. (2020) a STEC prevalence of 27% was found in dairy cattle in Portugal; *stx*1 genes were found in 18% of the isolates, *stx*2 in 51.9% and both *stx*1 and *stx*2 in 30.1%. From the 72 serotypes detected, 31 have been associated with human infection and 13 have been associated with hemolytic uremic syndrome (Ballem et al. 2020).

According to Erickson and Doyle (2007) shedding can be responsible for contamination of other carcasses with STEC during slaughter and produce can be contaminated with the agent after application of contaminated manure in the fields, contaminated irrigation or processing water, poor workers hygiene and poor equipment sanitation. Cross-contamination can also occur, as O157:H7 can survive for long periods of time in stainless steel and plastic and these surfaces can act as sources of contamination during food processing (Erickson and Doyle 2007).

E.coli O157:H7 remains the most common serotype to cause HUS throughout the world and is the serotype of which most data has been generated (Davis et al. 2014; Alconcher et al. 2021). STEC strains, which includes O157:H7, remain an important cause of morbidity and mortality with associated loss of life years and diminished health-related quality of life (Rivas et al. 2014). Clinical manifestations can range from asymptomatic, non-bloody diarrhea, hemorrhagic colitis and HUS (Gyles 2007). The development of HUS may result in death or end-stage renal disease (ESRD). Patients with ESRD are initially treated with peritoneal dialysis or hemodialysis, and, in last resource, may need kidney transplantation (Palermo et al. 2009).

According to Rivas et al. (2014) some of the risk factors for STEC human infection are dietary behaviors regarding beef consumption, but also other products, such as fresh produce or sprouts, as well as cattle management practices at the abattoir, cross-contamination in lairage areas, and the existence of “super-shedders” in a herd.

2.4. Laboratorial detection of *E.coli*

According to the Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea (2017), clinical presentations suggestive of infectious diarrhea caused by STEC include visible blood in stool, abdominal pain, severe abdominal pain, and often grossly bloody stools (occasionally non bloody, and minimal or no fever). If a STEC infection is suspected, laboratory diagnosis should include a stool sample for O157:H7 culture and Shiga toxin immunoassay or Nucleic Acid Amplification Test (NAAT) for Shiga toxin genes.

As described by Mueller and Tainter (2021), all pathotypes show bacterial growth on MacConkey agar and present indole production. STEC can also ferment sorbitol and for identification purposes bacteria can be grown on a sorbitol containing media. However, non-O157:H7 EHEC strains that do not ferment sorbitol have been identified by Polymerase chain reaction (PCR). Specific pathogens can be identified with the use of PCR-based assays. Molecular diagnosis relies in the detection of pEAF plasmid or BFP factor in EPEC, in the detection of the heat stable (*estA*) and heat labile (*eltB*) toxin genes of ETEC, and in the identification of *stx1* and *stx2* NAAT for EHEC/STEC (Sjöling et al. 2006; Anderson and Tarr 2018; Mueller and Tainter 2021).

Current standard methods for *E.coli* O157:H7 diagnosis include a culture-based most probable number (MPN) and genotyping, *i.e*, pulse-field gel electrophoresis (PFGE), but these methods are considered time-consuming, labour intensive and have a low sensitivity (Rani et al. 2021). Other methods, such as PCR and its variants: real-time PCR (qPCR), multiplex PCR and nested PCR are considered to have a higher sensitivity, but also present disadvantages, such as low specificity, inability to distinguish between viable and culturable and viable but non-culturable together, and high cost (Rani et al. 2021).

In food, bacterial culture-based methods are used as the gold standard for *E.coli* O157:H7 which present relatively low sensitivity when compared to other methods (Rani et al. 2021). PCR provides a more rapid and sensitive detection of bacteria than the standard plate counting method, which requires days for accurate detection (Zhang et al. 2021). Enzyme-linked immunosorbent assay (ELISA) can also be used to detect viable pathogens, despite cross-reactivity and poor specificity (Sunwoo et al. 2006; Rani et al. 2021). According to the International Organization for Standardization (ISO), ISO 16649:2018 describes the horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* in food and animal feeding stuffs. *E.coli* O157 will not be detected by these ISO methods, as it is considered β-glucuronidase-negative, although there have been reports on the emergence and presence of new O157 phenotypes that are β-glucuronidase-positive (Hayes et al. 1995; Nagano et al. 2004; Ogura et al. 2018). Therefore, European Commission Regulation (EU) 2073/2005 of 15 November 2005 and following amendments on microbiological criteria of foodstuffs recommends using ISO/TS 13136:2012 for the detection of Shiga toxin-producing *E.coli* (STEC) and determination of O157, O111, O26, O103, O145 serogroups by real-time PCR (European Commission 2020) .

Recently, in the past decade, methods like isothermal amplification, biosensor, Raman spectrophotometry, paper-based analytical devices and smartphone based digital methods have been developed for *E.coli* O157:H7 detection in food and water matrixes, as a way to overcome conventional methods (Kumar et al. 2019; Reali et al. 2019; Rani et al. 2021).

Regarding the environmental presence of *E.coli*, populations have been found in sand, soil, and sediments, as well as in association with macrophytic algae and periphyton, and potential pathogenic *E.coli* can survive and grow in natural environments (Ishii and Sadowsky 2008; Sadowsky and Whitman 2011; Jang et al. 2017). The methods used for environmental *E.coli* enumeration are culture-based methods, as the membrane filtration technique with selective growth media, defined-substrate technology and the most probable number (MPN) technique, have been used to enumerate *E.coli* from recreational waters and other water bodies (Jang et al. 2017).

2.5. Quantitative Microbial Risk Assessment

The risk analysis framework can be divided into three main parts: risk assessment, risk management and risk communication. The risk assessment consists of four basic steps: i) hazard identification, ii) exposure assessment, iii) hazard characterization and iv) risk characterization (Figure 1). Risk management includes consideration of several factors beyond the risk itself, such as social and economic factors as well as solutions to the inherent problem. Risk communication is the process of opinion change between individuals, groups and institutions (Gerba 2015). Risk assessment can be used in veterinary sciences in the scope of food safety and international trading frameworks (Stärk and Salman 2001).

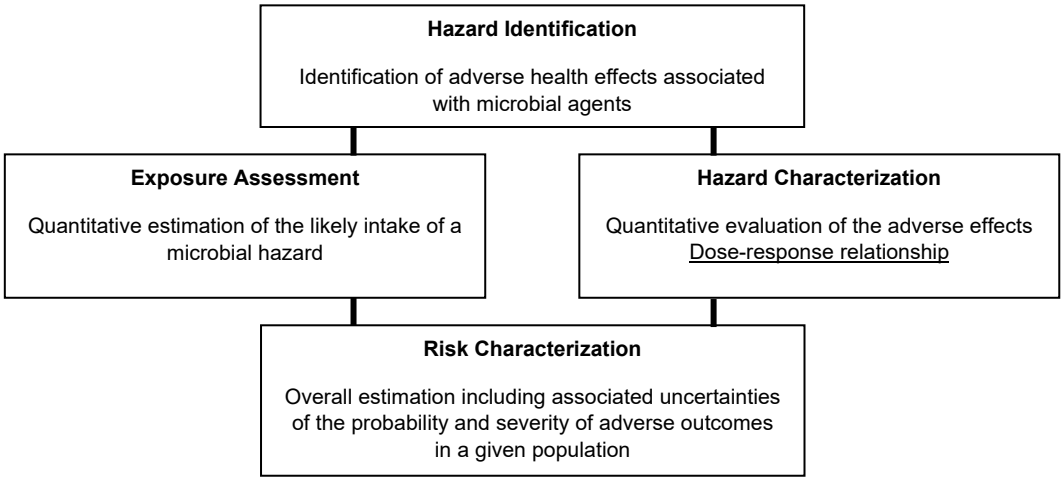

Figure 1. Microbial risk assessment components according to the Codex Alimentarius Commission. Adapted from Boone et al. (2010).

Quantitative Microbial Risk Assessment (QMRA) is a scientifically based process, branch of the risk analysis, that estimates the adverse health effects from exposure to microorganisms (Boone et al. 2010). Human dose-response models and predictive microbiology are two main components for the application of QMRA, as it aims to predict the consequences from exposure to infectious agents (Havelaar et al. 2008; Haas et al. 2014). The combination of this risk analysis method with burden of disease methodologies, introduced by WHO for the Global Burden of Disease project, can become extremely powerful and provide several social and economic advantages (Havelaar et al. 2008). Models developed for the purpose of QMRA consist of mathematical or schematic representation of a food safety problem and often require information from literature, but also need data from surveillance programs, laboratories, disease outbreak investigations, food consumption surveys and other relevant factors (Boone et al. 2010). Alongside the scientific

basis, value judgements and assumptions are often necessary and unavoidable (Boone et al. 2010).

QMRA can use deterministic or stochastic approaches, where the later uses probability distributions to describe variables. The stochastic approach is considered to be the most representative of the real world, despite being the most difficult to generate (FAO and WHO 2006). Probabilistic risk assessments can be determined using Monte Carlo analysis. A single 'point-estimate' value from each of the probability distributions assigned for each input parameter is randomly selected, and each random single selected value is used to calculate a mathematical solution defined by the risk assessment model and the result is stored. This sequence is repeated several times (iterations) with a different set of values for the inputs selected at each iteration. The more likely to occur values according to the defined probability distribution are selected more frequently. The result is a frequency distribution for the output of interest that represents the combined ranges and frequencies of the input parameters (Lammerding and Fazil 2000).

To perform the hazard characterization dose-response models are commonly used. Data used to build dose-response models is usually from human volunteer feeding studies, as they often provide the most direct measure of human response. However, ethical problems arise with the use of this method for pathogens that can cause life threatening diseases, and overall, the population used in these studies is generally healthy adults, so higher risk populations are usually not accounted (Buchanan et al. 2000; Strachan et al. 2005). Animal models can be used for extrapolation of results, but several factors influence the outcome, such as the difference in immune and physiological responses, and the quantitative relationship between infectivity, morbidity, and mortality for each species (Buchanan et al. 2000). Nowadays, epidemiological investigations constitute one of the sources for building these models, like the model built for *E.coli* O157:H7 by Strachan et al. (2005) that was used in this study.

Mathematical models have been used to describe the dose-response relationship. Two of the most used models are the exponential (1), and the approximated Beta-Poisson (2) (Buchanan et al. 2000).

$$P_i(d) = 1 - e^{-rd} \quad (1)$$

$$P_i(d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha} \quad (2)$$

Initially introduced by Haas (1983), the exponential model assumes that the probability of a pathogenic agent to cause infection is independent of the dose, while the Beta-Poisson assumes that infectivity is dose dependent. With the use of dose-response models, a relationship between the level of microbial exposure and the occurrence of an adverse effect can be established (Strachan et al. 2005). Dose-illness models belong to the

family of hit-theory models (FAO 2003). Single-hit models consider that when a host ingests one cell of a pathogenic microorganism, the probability that the pathogen will survive all barriers and colonize the host has a non-zero value of p_m. The Beta-Poisson model is based on further assumption on the distribution of the pathogens in the inoculums, and on the probability value; when the probability of starting an infection differs for any organism in any host, and is assumed to follow a beta-distribution, then the Beta-Poisson model can be applied (FAO 2003).

Uncertainty and variability are inherent to the biological process and also QMRA. Uncertainty arises from the lack of knowledge and may be related to the model used to characterize the risk and the models used to provide values (Membré 2016). Variability relates to the existing differences between individuals within a population, but also to microbial strains, and batches of products (Membré 2016). While variability does not disappear with more data collection, uncertainty can be reduced by obtaining more information, although that's not always possible (Membré 2016).

Creating different and alternative scenarios regarding QMRA has the purpose of exploring different mitigation strategies, but also allows to explore the extremes of input variables along with their associated uncertainties (Boone et al. 2010).

2.6. Burden of Disease

The population health metric disability-adjusted life years (DALY) concept was developed as a health indicator for the first Global Burden of Disease study under a joint exercise by WHO and the World Bank, in 1990. The concept of DALY implies that every person is born with a certain number of life years potentially lived in optimal health (Devleesschauwer et al. 2014a). These healthy years can be reduced through living with illness or dying before the reference life expectancy, therefore DALY is used as a representation measuring the losses of healthy life (Devleesschauwer et al. 2014a). Years of Life Lost (YLL) expresses the years lost due to a specific cause of death and Years Lost due to Disability (YLD) represents the occurrence of health conditions in a population that weighted for the severity of each health condition (Hilderink et al. 2020). Each of the previous factors can be calculated with the use of the following equations (3;4;5):

$$YLD = Number\ of\ cases\ x\ Duration\ till\ remission\ or\ death\ x\ Disability\ weight \quad (3)$$

$$YLL = Number\ of\ deaths\ x\ Life\ expectancy\ at\ the\ age\ of\ death \quad (4)$$

$$DALY = YLD + YLL \quad (5)$$

According to Devleesschauwer et al. (2014b) several steps are required to guide a burden of disease investigation. Defining the population and period of time, which can be a specific year or a range of years, is necessary so that the average burden of that time period can be calculated. A disease model should be built, also known as outcome tree,

schematically representing different health states associated with the cause of the disease burden. Regarding these models, three different approaches can be used such as outcome-based disease models, hazard-based disease models and risk factor-based disease models. Data collection requires, in general, demographical, epidemiological and disease severity data. The duration of disease can be obtained from hospital registers or literature review and the disability weights (DW) from global burden of disease studies. Data adjustment and DALY calculations are the final steps. DALY can therefore be obtained by summing the YLLs and YLDs for each health state in the disease model (Figure 2).

Figure 2. Graphical representation of the parameters used for DALY calculation. Adapted from Thomsen (2018).

Nowadays, the DALY metric has innumerous applications and has been used to estimate the burden of diseases such as cardiovascular, oncological, infectious diseases, and many others (Henriques et al. 2017; Coates et al. 2020; Mubarik et al. 2021)

Disability weights (DW) are a measure of the severity of consequences of a particular health condition for the physical, psychological and social functioning of patients on a scale from 0 to 1, ranging from “no effects” to “adverse serious effects”, including death (Hilderink et al. 2020). These values are based on how the majority of people perceive living with a specific disease or condition and reflect the severity of diseases and disease stages (Thomsen 2018). Nowadays, DW values are easily accessible for different health conditions and have been obtained from diverse national burden of disease studies (WHO 2015).

Life expectancy for the calculation of YLL is usually obtained from life expectancy tables for the population that is being studied (WHO 2015).

3. Materials and Methods

3.1. Data Collection

E. coli counts of ready-to-eat meals (n=473) served in Portuguese institutional canteens (N=30) from February 2018 to December 2019 were gathered in a database. The canteens were dispersed through national territory and the regular consumers were considered to be healthy adults. Food samples were analyzed using ISO 16649-2:2001 method for *E. coli* quantification in colony-forming units per gram (cfu/g) of food sample. Counts under 10 cfu/g were considered satisfactory, so all values of 10 and >10 cfu/g were an input to the model.

3.2. Study Design

The main goal of this study was to estimate the risk inherent to the consumption of ready-to-eat meals from the assessed canteens regarding *E.coli* infection. Together with the risk, a projection of number of illness cases and number of cases per health outcome was aimed. After determining the possible clinical outcomes for each type of infection, burden of disease (DALYs) was calculated. Three groups of *E.coli* were selected: Shiga toxin-producing *E.coli* (STEC), enterotoxigenic *E.coli* (ETEC) and enteropathogenic *E.coli* (EPEC) (WHO 2015). Two scenarios were built based on hypotheses on consumption values, one scenario considered a consumption of 450 grams of food and was named “450g” and a second scenario considered a consumption of 225 grams of food and was named “225g”. Other two scenarios, “Worst Case” and “Adjusted”, were considered within each consumption scenario. Scenario “Worst Case” considered that all cfu belonged to one *E.coli* group, while scenario “Adjusted” added the prevalence of each group within total *E.coli* counts. The prevalence of each *E.coli* group within the total counts was obtained through literature review, since PCR analysis was not performed. For risk and number of cases estimation, dose-response models were obtained from previous articles and the parameters that allowed for the use of the approximated Beta-Poisson equation were chosen. The proportion and probability of developing each health outcome was based on indicators from metanalyses conducted by WHO Burden Studies (WHO 2015). All QMRA and Disability Adjusted Life Models were performed using R software version 1.4.1103 (R Core Team 2020, Vienna, Austria) and all distributions and credible intervals with 95% credibility were obtained using 100.000 iterations.

The beta distributions built and used throughout this study were performed according to Vose (2008) where the probability of success (p) can be determined by combining the observed number of trials (n) and the number of success trials (s). The distributions were modulated by altering the respective variables of the following equation (6).

$$p = Beta\ (s + 1, n - s + 1)\ (6)$$

3.3. Exposure Assessment

Several assumptions were made due to the lack of real data. Assumptions about the number of people, age groups and ingested grams of food were unavoidable. The number of ingested cfu was also dependent of laboratory detection and methods used to perform the analysis. In an attempt to better characterize the risk, the burden, and to insert some variability in the consumption pattern, two doses were considered resulting from different size portions ingested per meal. Scenario “450g” represents the consumption of the whole meal, *i.e.*, 450 grams, while scenario “225g” represents the consumption of 225 grams (half a meal). The population considered to be exposed to the hazard included 150 people, per day, with the 25-29 year-old group being the most frequent age range within the assessed population.

Data from the considered two-year period was gathered and fitted into a distribution as a form of retrospective study of the concentrations of *E.coli* in ready-to-eat meals. The fitting of the data was made using “fitdistrplus” package (R core team 2020). A lognormal distribution was chosen as data’s best fit ~lognormal (0.74, 0.46). A final distribution representing the *E.coli* concentrations of the assessed time frame of two years was obtained (Figure 3).

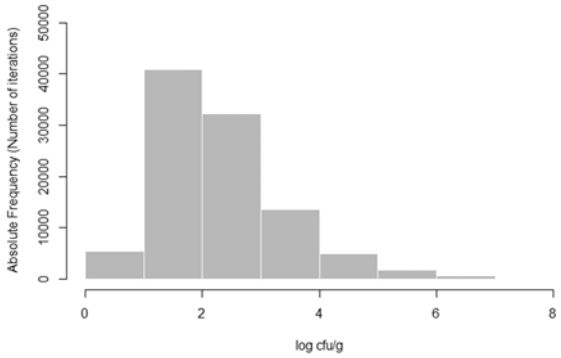

Figure 3. Histogram of *E.coli* concentrations in log cfu/g in meals obtained during the assessed two-year period.

For dose-response application, the total number of ingested cfu is necessary and constitutes the input for the dose-response model. Therefore, since the results of laboratory analysis were in cfu per gram of food sample, a multiplication of these results by the total of grams ingested by the people exposed gives the number of total cfu ingested. The first dose is equivalent to the whole meal, 450g, consumption of while the second dose results from the consumption of half a meal, 225g.

The median dose (95% CI) obtained representative of the dose of ingested *E. coli* cfu was 4.75 (3.50; 7.81) of *E. coli* log cfu for the first scenario (Figure 4), while for the second scenario, a median dose (95%CI) of 4.44 (3.20; 7.51) log cfu of *E. coli* was estimated (Figure 5).

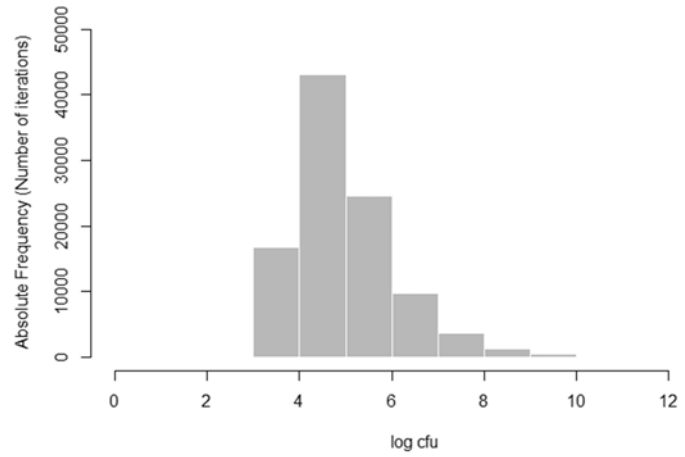

Figure 4. Histogram of ingested dose of *E.coli* (in log cfu) considering a 450g meal portion.

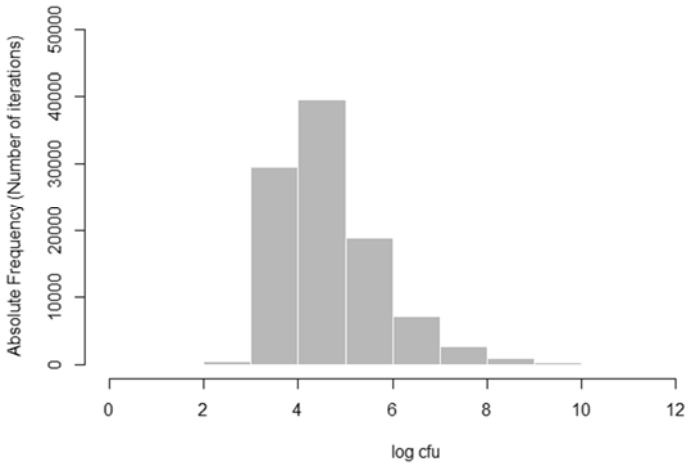

Figure 5. Histogram of ingested dose of *E.coli* (in log cfu) considering a 225g meal portion.

3.3.1. Prevalence of contaminated portions

Prevalence of *E.coli* in ready-to-eat meals was estimated from the number of positive samples that were found in routine microbiological monitoring. A total of 30 in 473 samples tested above 10 cfu/g. Therefore, a prevalence of 6.34% unsatisfactory meals was obtained. The prevalence of contaminated portions can be described by a beta distribution ~ Beta (31, 444).

3.3.2. Prevalence of STEC, ETEC and EPEC

To assess the prevalence of each pathotype group within general *E.coli* counts, a literature review was made for articles that executed molecular diagnosis for the specific groups from samples, mainly foodstuff, that had positive bacterial growth in *E.coli* culture. By doing this, an estimation of the prevalence of each group can be made. A summary of the chosen articles, description of number of samples, prevalence of each group and all used data can be consulted in table 2. For ETEC and EPEC, to improve the estimative, the data used as an input to the model resulted from the combination of data from two different articles as a way to increase the number of samples and have a more significant result. For STEC only one article was used due to the lack of additional data.

Table 2. Summary of the prevalence of each *E.coli* group found through literature review. Three groups were chosen, STEC, ETEC and EPEC. The line distribution used represents the parameter input to the model.

Pathotype	**STEC**	**ETEC**		**EPEC**	
Number of samples	5162	5162	559	5162	459
Number of positive samples for *E.coli*	409	409	219	409	144
Number of positive samples for the pathotype	5	1	36	44	39
Reference	(Canizalez-Roman et al. 2013)	(Canizalez-Roman et al. 2013)	(Zhang, Wu, Zhang, Lai, et al. 2016)	(Canizalez-Roman et al. 2013)	(Zhang, Wu, Zhang, and Zhu 2016)
Total number of samples	5162	5721		5621	
Total number of positive samples for *E.coli*	409	628		553	
Total number of positive samples for the pathotype	5	37		83	
Distribution used	~ Beta (6; 405)	~ Beta (38; 592)		~ Beta (84; 471)	

3.4. Hazard Characterization: Dose-Response Models

A total of four dose-response models were chosen to represent the three *E.coli* groups selected with two dose-response models used to represent STEC. All models used to calculate the risk were Beta-Poisson, with equation (7) used for STEC and EPEC and equation (8) used for ETEC.

$$P_i(d) = 1 - (1 + \frac{d}{\beta})^{-\alpha} \quad (7)$$

$$P_i(d) = 1 - \left[1 + \frac{d}{N_{50}}\, x\, \left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \quad (8)$$

Both of the above equations are Beta-Poisson equations, where α, β and N_{50} are specific parameters of the Beta-Poisson models with N_{50} being the dose at which 50% of the population is expected to be affected.

Parameters for dose-response models were obtained through literature review and the selected parameters can be found in table 3.

Table 3. Parameters chosen for dose-response model application.

Parameter		STEC	ETEC	EPEC
α	Model "O157"	0.0571	7.54 x 10^{-2}	0.221
	Model "Shigella"	0.162		
β	Model "O157"	2.2183	-	3.11 x 10^{6}
	Model "Shigella"	15.86		
N_{50}		-	1.7 x 10^{6}	-
Reference		(Strachan et al. 2005)	(Enger 2015)	(Strachan et al. 2005)

The models described were applied to the ingested doses and the output can be defined as the risk or the probability of illness.

For the STEC scenario, similarly to Strachan et al. (2005), three dose-response models were applied for the purpose of evidencing the available dose-response models that include a different host species, a different pathogen and another that was built using data from several outbreaks that occurred and that were mainly foodborne. According to Strachan et al. (2005) the *Shigella* model was the most similar dose-response model for the data and model obtained from the O157 data from outbreaks, mainly foodborne. From these three models, only two were used in the following steps of the study. The first was built with data from the specific strain O157:H7, and the second used a surrogate pathogen using *Shigella* spp. data, while the third used data from a different host species, a rabbit. For further

reference, the first will be referred as model “O157” and the second, model “Shigella”. The *Shigella* model is the result of administering different doses of two species, *S. dysenteriae* and *S.flexnerii*, to human subjects. The *E.coli* O157 rabbit model used data pooled from a study of infecting white rabbits with a bacterial suspension through an oral catheter. According to Strachan et al. 2005, the *E.coli* O157 rabbit model underestimates the risk of illness for doses $<10^5$. The data from figures 4 and 5 show that the median for the ingested dose distribution is ≈ 4.7 log and ≈ 4.4 log, considering both doses, making the rabbit model not suitable to be used for further concept application. Therefore, the rabbit model is not used in any point beyond the determination of the risk.

Representative histograms of the different dose-response models, considering a portion size of 450g are presented in figure 6.

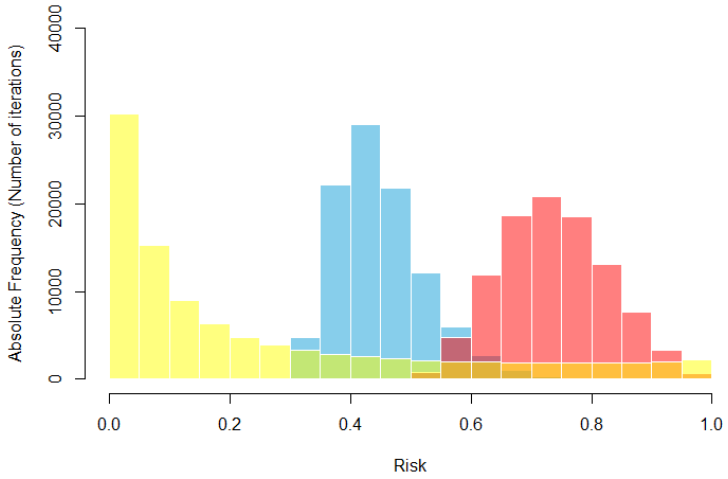

Figure 6. Histograms of the three dose-response models applied for risk estimation for STEC scenario “450g”. Results generated using the O157 model are presented in blue, data from the Shigella model is presented in red and data from the rabbit model is presented in yellow.

Table 4. Median values of the estimated risk for the STEC, ETEC and EPEC scenarios for all the models used with a credibility interval of 95% for scenario “450g”.

	Model	Median (95% CI) of Risk
STEC	Model “O157”	0.44 (0.34; 0.63)
	Model “Shigella”	0.73 (0.58; 0.92)
	Rabbit Model	0.12 (0.008; 0.94)

Table 4 (continued). Median values of the estimated risk for the STEC, ETEC and EPEC scenarios for all the models used with a credibility interval of 95% for scenario "450g".

ETEC	0.35 (0.20; 0.62)
EPEC	3.91×10^{-3} (2.26×10^{-4}; 0.49)

The values presented in table 4 represent the probability of illness development considering the ingested doses represented in figure 4. Considering the obtained results, the "Shigella" model presents a higher value of risk, followed by the "O157" model and the rabbit model. As explained above, the rabbit model will not be considered for the calculation of number of cases as it underestimates the risk of illness for concentration below 5 log and for describing the response in a different species.

For the ETEC scenario, only one dose-response model was applied (Figure 7). The same for the EPEC scenario (Figure 8).

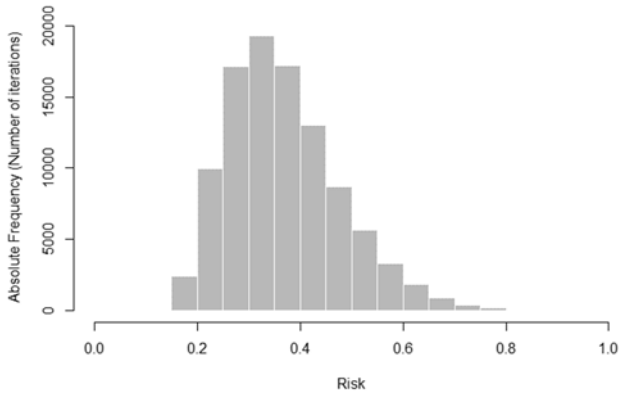

Figure 7. Histogram of the dose-response model applied for the ETEC scenario "450g".

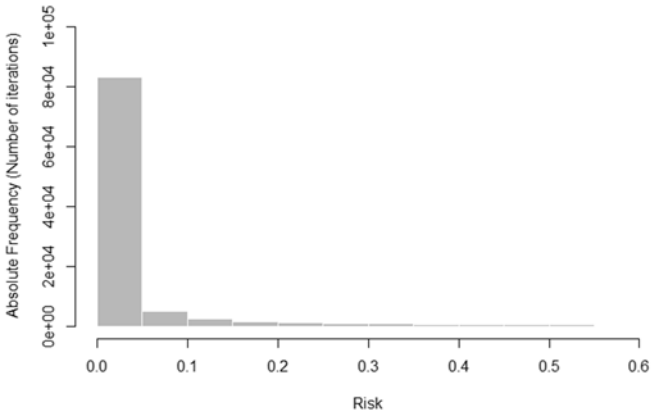

Figure 8. Histogram of the dose-response model applied for the EPEC scenario "450g".

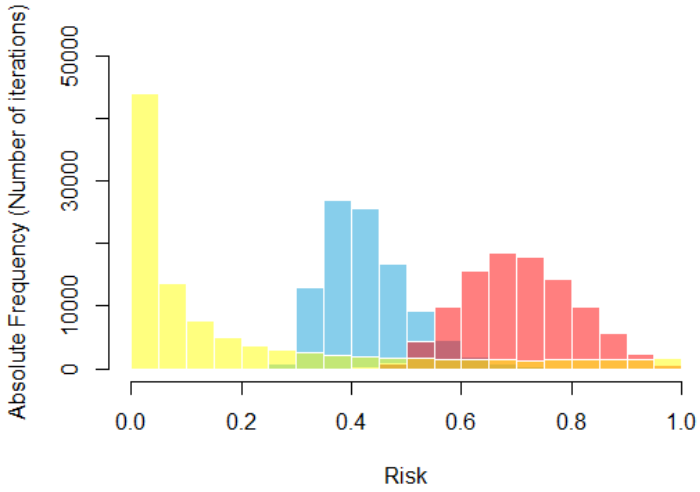

Figure 9. Histograms of the three dose-response models applied for STEC scenario "225g". The "O157" model is represented in blue, "Shigella" model is represented in red and the "rabbit model" is represented in yellow.

Table 5. Median values of the risk for the STEC, ETEC and EPEC scenarios for all the models used with a credibility interval of 95% for scenario "225g".

	Model	Median (95% CI) of Risk
STEC	Model "O157"	0.42 (0.31; 0.61)
	Model "Shigella"	0.70 (0.53; 0.91)
	Rabbit Model	0.07 (4.25×10^{-3}; 0.92)
	ETEC	0.32 (0.16; 0.60)
	EPEC	1.97×10^{-3} (1.13×10^{-4}; 0.42)

Representative histograms of the different dose-response models, considering a portion size of 225g are presented in figure 9. The values presented in table 5 represent the probability of illness development considering the ingested doses represented in figure 5. For the ETEC scenario, only one dose-response model was applied (Figure 10) and the same for the EPEC scenario (Figure 11).

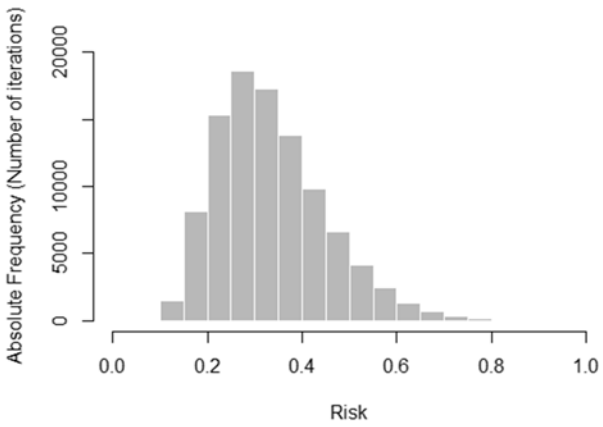

Figure 10. Histogram of the dose-response model applied for the ETEC scenario "225g".

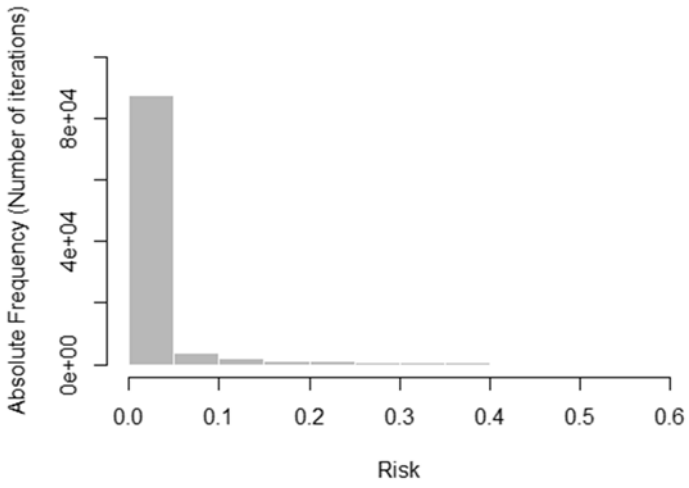

Figure 11. Histogram of the dose-response model applied for the EPEC scenario "225g".

3.5. Risk Characterization

Risk can be defined as the probability of developing an adverse outcome due to the ingestion of a certain dose and constitutes the output of the dose-response model. Determination of expected number of illness cases per year can be obtained through multiplication of the median risk of illness by the total eating occasions for that specific

population. Based on the model developed for *Listeria monocytogenes* by (Pérez-Rodríguez et al. 2017), the following equation (9) was developed for determining the expected number of cases of *E.coli* infections where $\bar{R}$ is the marginal risk, P_{cp} the prevalence of contaminated portions, P_g the prevalence of each group of *E.coli*, N the number of eating occasions and PE the number of people exposed:

$$Number\ of\ cases = \bar{R}\ x\ P_{cp}\ x\ P_g\ x\ N\ x\ PE \quad (9)$$

Table 6. Factor description and input parameters.

Factor Description	Input
$\bar{R}$ **(Marginal Risk)**	Median risk value resulting from the dose-response model application
P_{cp} **(Contaminated Portions)**	~ Beta (31;444)
P_g **(Prevalence of *E. coli* pathotype)**	~ Beta (6;405); ~ Beta (38; 592); ~ Beta (84; 471)
N **(Number of eating occasions)**	365
PE **(People Exposed)**	150

3.6. Burden of Disease Estimation

Burden of disease estimation included calculation of expected number of cases, YLDs, YLLs and DALYs for each health outcome, as well as total DALYs and DALYs/person/year.

3.6.1. Health Outcomes for *E.coli* infection

An outcome tree describes the probability of developing a specific disease outcome after infection. It is a schematic representation of all possible outcomes following infection. Outcome trees were designed for each pathotype of *E.coli* following literature review and disease models described by WHO (2015). However, some of the possible outcomes were not considered due to the lack of data or lack of adjustment to the concept. Asymptomatic infections are not considered, since the used dose-response models have disease as the output, so each dose is related to the onset of a clinical sign, such as diarrhea. The outcome tree for STEC was adapted from the outcome tree for STEC developed by Monteiro Pires et al. (2020). The health outcome schemes used for the STEC infection and for the ETEC/EPEC infection are illustrated in figures 12 and 13, respectively. The outcomes accounted for the model are delineated with a solid line and the not accounted are delineated with a dashed line.

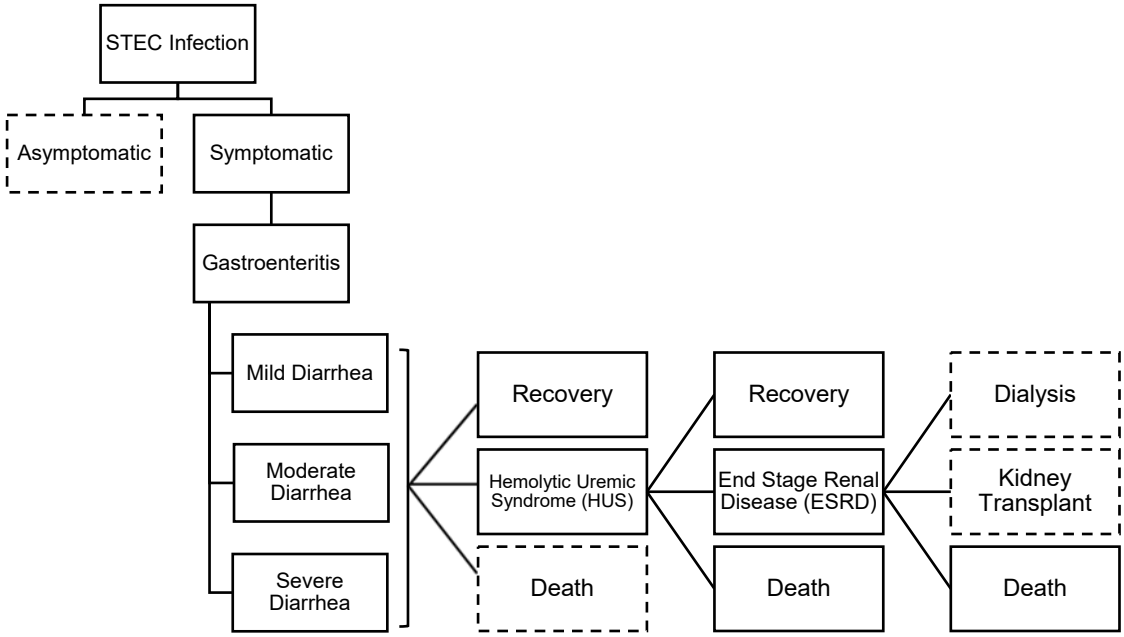

Figure 12. Outcome tree for STEC infection. Adapted from WHO (2015); Monteiro Pires et al. (2020).

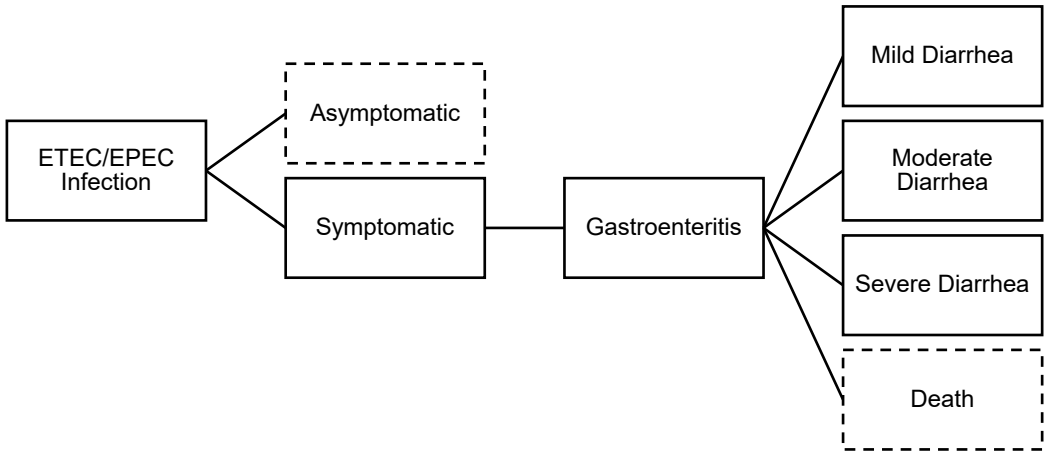

Figure 13. Outcome tree for ETEC/EPEC infection. Adapted from WHO (2015).

The probabilities of developing each and specific health outcomes (Table 7) were retrieved from Global Burden of disease studies by WHO (2015). Each probability was applied to each health outcome, and knowing the exposed population, it was possible to estimate the number of people expected to develop each type of condition. Two probabilities for HUS development were presented in the WHO Estimates of the Global Burden of Foodborne Disease (WHO 2015), *i.e.,* 0.8% for serogroup O157 and 0.03% for non-O157.

The first probability was chosen considering that the dose-response model used for STEC was built with data from O157 outbreaks.

Table 7. Probabilities (%) of developing a specific health outcome considered in this study. Adapted from WHO (2015).

Health Outcome (%)	*E.coli* Infection		
	STEC	**ETEC**	**EPEC**
Mild Diarrhea	80	91	91
Moderate Diarrhea	18	8.5	8.5
Severe Diarrhea	2	0.5	0.5
Hemolytic Uremic Syndrome (HUS)	0.8	-	-
End Stage Renal Disease (ESRD)	0.024	-	-

3.6.2. Disability weights (DW), duration of health outcomes, case fatality ratios (CFR) and life expectancy

The disability weights considered and the duration of the health outcomes that were retrieved from WHO Estimates of the Global Burden of Foodborne Diseases (WHO 2015) are summarized in table 8. Case Fatality Ratio (CFR) is the proportion of persons with a particular condition who die from that condition (CDC 2012). CFR was only considered in the Hemolytic Uremic Syndrome (HUS) and End Stage Renal Disease (ESRD) outcomes of the STEC infection, and no deaths were considered for ETEC and EPEC infections, since Portugal belongs to WHO subgroup EUR A for which WHO Estimates of the Global Burden of Foodborne Diseases estimated no deaths (WHO 2015).

Table 8. DWs, duration and CFRs for each possible health outcome regarding all three *E.coli* pathotypes considered in this study.

Health Outcome	DW	Duration (days)	CFR (%)
STEC induced mild diarrhea	0.061	7	-
STEC induced moderate diarrhea	0.202	7	-
STEC induced severe diarrhea	0.281	7	-
ETEC/EPEC induced mild diarrhea	0.061	2.8	-

Table 8 (continued). DWs, duration and CFRs for each possible health outcome regarding all three *E.coli* pathotypes considered in this study.

ETEC/EPEC induced moderate diarrhea	0.202	2.8	-
ETEC/EPEC induced severe diarrhea	0.281	2.8	-
Hemolytic Uremic Syndrome (HUS)	0.210	28	3.7
End Stage Renal Disease (ESRD)	0.573	Lifelong disability	20

The life expectancy table from WHO (2019) used for the calculation of YLL can be consulted in the Annex 1. The population exposed to the risk belonged primarily to the 25-29 years age group so the corresponding value of expectation of life at age 25-29 from the life expectancy table was used (Annex 1).

3.6.3. The Disability Adjusted Life Model

For model application, the incidence (number of cases) considered for YLD calculation was the projected number of cases that resulted from the multiplication of the risk obtained from the application of the dose-response model by the number of people exposed. Number of deaths resulted from the application of CFR to the expected number of cases. The burden of all health outcomes was estimated by combining all variables described above. Final DALYs were obtained through the summatory of all health outcomes DALYs that resulted from the summatory of YLDs and YLLs for all health outcomes, according to the following formula:

$$DALYs = \sum YLD\ (number\ of\ cases\ x\ duration\ x\ disability\ weight) + \sum YLL\ (number\ of\ deaths\ x\ life\ expectancy) \quad (10)$$

3.6.4. Sensitivity Analysis

In this work, the Sobol method was followed to perform the sensitivity analysis to determine the factors that most contributed to the final DALYs.

Inputs were selected according to “WHO Estimates of the Global Burden of Foodborne Disease” (WHO 2015). Uniform distributions were applied to each factor for which variability could be considered. Additional variability was expressed using uniform distributions in the number of people exposed to the hazard and the portion size of each meal. In this analysis, each parameter is considered to range over some finite interval

between (0,1) after rescaling (Zhang et al. 2015). All the information and respective input distributions are described in detail and summarized in annex 2.

Sensitivity analysis was performed using “sensitivity” package (Saltelli 2002) in R software (R core team 2020).

4. Results

4.1. Expected number of cases per health outcome

4.1.1. Scenario “450g”

Table 9. Expected annual number of cases for STEC infection scenario “450g”.

Number of cases per health outcome (Median [95%CI])			
Scenario “Worst Case”			
Health Outcome		**Model “O157”**	**Model “Shigella”**
Gastroenteritis	Mild Diarrhea	1244.17 [863.23; 1716.49]	2077.81 [1441.63; 2866.60]
	Moderate Diarrhea	279.94 [194.23; 386.21]	467.51 [324.37; 644.98]
	Severe Diarrhea	31.10 [21.58; 42.91]	51.95 [36.04; 71.66]
Hemolytic Uremic Syndrome (HUS)	Recovery	11.98 [8.31; 16.53]	20.01 [13.88; 27.61]
	Death	0.46 [0.32; 0.64]	0.77 [0.53; 1.06]
	Total	12.44 [8.63; 17.16]	20.78 [14.42; 28.67]
End Stage Renal Disease (ESRD)	Death	0.07 [0.05; 0.10]	0.12 [0.09; 0.17]
	Total	0.37 [0.26; 0.51]	0.62 [0.43; 0.86]
Total number of illness cases		1555.21 [1079.04; 2145.61]	2597.26 [1802.04; 3583.25]
Scenario “Adjusted”			
Health Outcome		**Model “O157”**	**Model “Shigella”**
Gastroenteritis	Mild Diarrhea	16.99 [6.33; 37.90]	28.38 [10.57; 63.30]
	Moderate Diarrhea	3.82 [1.42; 8.53]	6.39 [2.38; 14.24]
	Severe Diarrhea	0.42 [0.16; 0.95]	0.71 [0.26; 1.58]
Hemolytic Uremic Syndrome (HUS)	Recovery	0.16 [0.06; 0.37]	0.27 [0.10; 0.61]
	Death	6.29×10^{-3} [2.34×10^{-3}; 0.01]	0.01 [3.91×10^{-3}; 0.02]
	Total	0.17 [0.06; 0.38]	0.28[0.11; 0.63]
End Stage Renal Disease (ESRD)	Death	1.02×10^{-3} [3.80×10^{-4}; 2.27×10^{-3}]	1.70×10^{-3} [6.34×10^{-4}; 3.80×10^{-3}]
	Total	5.10×10^{-3} [1.90×10^{-2}; 1.14×10^{-2}]	8.51×10^{-3} [3.17×10^{-3}; 1.90×10^{-2}]
Total number of illness cases		21.24 [7.91; 47.38]	35.48 [13.22; 79.13]

Table 10. Expected annual number of cases for ETEC infection scenario "450g".

Number of cases per health outcome (Median [95%])		
Scenario "Worst Case"		
Health Outcome		**ETEC model**
Gastroenteritis	Mild Diarrhea	1137.75 [789.40; 1569.67]
	Moderate Diarrhea	106.27 [73.74; 146.62]
	Severe Diarrhea	6.25 [4.34; 8.62]
Total number of illness cases		1250.28 [867.47; 1724.92]
Scenario "Adjusted"		
Health Outcome		**ETEC model**
Gastroenteritis	Mild Diarrhea	67.72 [41.91; 105.77]
	Moderate Diarrhea	6.33 [3.91; 9.88]
	Severe Diarrhea	0.37 [0.23; 0.58]
Total number of illness cases		74.42 [46.06; 116.23]

Table 11. Expected annual number of cases for EPEC infection scenario "450g".

Number of cases per health outcome (Median [95%])		
Scenario "Worst Case"		
Health Outcome		**EPEC model**
Gastroenteritis	Mild Diarrhea	12.61 [8.75; 17.39]
	Moderate Diarrhea	1.18 [0.82; 1.62]
	Severe Diarrhea	0.07 [0.05; 0.10]
Total number of illness cases		13.86 [9.61; 19.11]
Scenario "Adjusted"		
Health Outcome		**EPEC model**
Gastroenteritis	Mild Diarrhea	1.90 [1.25; 2.77]
	Moderate Diarrhea	0.18 [0.12; 0.26]
	Severe Diarrhea	0.01 [6.89 x 10^{-3}; 0.02]
Total number of illness cases		2.09 [1.38; 3.04]

4.1.2. Scenario "225g"

Table 12. Expected annual number of cases for STEC infection scenario "225g".

Number of cases per health outcome (Median [95%CI])			
Scenario "Worst Case"			
Health Outcome		**Model "O157"**	**Model "Shigella"**
Gastroenteritis	Mild Diarrhea	1180.04 (818.74; 1628.02)	1988.15 (1379.42; 2742.90)
	Moderate Diarrhea	265.51 (184.22; 366.30)	447.33 (310.37; 617.15)
	Severe Diarrhea	29.50 (20.47; 40.70)	49.70 (34.49; 68.57)
Hemolytic Uremic Syndrome (HUS)	Recovery	11.36 (7.88; 15.68)	19.15 (13.28; 26.41)
	Death	0.44 (0.30; 0.60)	0.74 (0.51; 1.01)
	Total	11.80 (8.19; 16.28)	19.88 (13.79; 27.43)
End Stage Renal Disease (ESRD)	Death	0.07 (0.05; 0.10)	0.12 (0.08; 0.16)

Table 12 (continued). Expected annual number of cases for STEC infection scenario "225g".

	Total	0.35 (0.25; 0.49)	0.60 (0.41; 0.82)
Total number of illness cases		1475.05 (1023.43; 2035.02)	2485.19 (1724.28; 3428.63)
Scenario "Adjusted"			
Health Outcome		**Model "O157"**	**Model "Shigella"**
Gastroenteritis	Mild Diarrhea	16.12 (6.00; 35.95)	27.16 (10.12; 60.57)
	Moderate Diarrhea	3.63 (1.35; 8.09)	6.11 (2.28; 13.63)
	Severe Diarrhea	0.40 (0.15; 0.90)	0.68 (0.25; 1.51)
Hemolytic Uremic Syndrome (HUS)	Recovery	0.16 (0.06; 0.35)	0.26 (0.10; 0.58)
	Death	5.96×10^{-3} (2.22×10^{-3}; 1.33×10^{-2})	0.01 (3.74×10^{-3}; 2.24×10^{-2})
	Total	0.16 (0.06; 0.36)	0.27 (0.10; 0.61)
End Stage Renal Disease (ESRD)	Death	9.67×10^{-4} (3.60×10^{-4}; 2.16×10^{-3})	1.63×10^{-3} (6.07×10^{-4}; 3.63×10^{-3})
	Total	4.84×10^{-3} (1.80×10^{-3}; 1.08×10^{-2})	8.15×10^{-3} (3.03×10^{-3}; 0.02)
Total number of illness cases		20.15 (7.51; 44.94)	33.94 (12.64; 75.71)

Table 13. Expected annual number of cases for ETEC infection scenario "225g".

Number of cases per health outcome (Median [95%])		
Scenario "Worst Case"		
Health Outcome		**ETEC model**
Gastroenteritis	Mild Diarrhea	1026.43 (712.16; 1416.10)
	Moderate Diarrhea	95.88 (66.52; 132.27)
	Severe Diarrhea	5.64 (3.91; 7.78)
Total number of illness cases		1127.95 (782.60; 1556.15)
Scenario "Adjusted"		
Health Outcome		**ETEC model**
Gastroenteritis	Mild Diarrhea	61.10 (37.81; 95.42)
	Moderate Diarrhea	5.71 (3.53; 8.91)
	Severe Diarrhea	0.34 (0.21; 0.52)
Total number of illness cases		67.14 (41.55; 104.85)

Table 14. Expected annual number of cases for EPEC infection scenario "225g".

Number of cases per health outcome (Median [95%])		
Scenario "Worst Case"		
Health Outcome		**EPEC model**
Gastroenteritis	Mild Diarrhea	6.34 (4.40; 8.74)
	Moderate Diarrhea	0.59 (0.41; 0.82)
	Severe Diarrhea	0.03 (0.02; 0.05)
Total number of illness cases		6.97 (4.83; 9.61)
Scenario "Adjusted"		
Health Outcome		**EPEC model**

Table 14 (continued). Expected annual number of cases for EPEC infection scenario "225g".

Gastroenteritis	Mild Diarrhea	0.95 (0.63; 1.39)
	Moderate Diarrhea	0.09 (0.06; 0.13)
	Severe Diarrhea	0.01 (3.46×10^{-3}; 7.65×10^{-3})
Total number of illness cases		1.05 (0.69; 1.53)

4.2. Burden of disease of *E.coli* infections

4.2.1. Scenario "450g"

Burden of disease results can be presented as total DALYs, representing the total burden of disease in a given population, but also by dividing the burden of disease per person, and since this is an annual risk estimation, the final output can be presented as DALYs/person/year. Each DALY equals to 1 year of healthy life year lost, therefore the presentation in days is also possible.

Table 15. YLDs, YLLs and DALYs of STEC infection scenario "450g".

Scenario "Worst Case"				
Health Outcome			**Model "O157"**	**Model "Shigella"**
Gastroenteritis	Mild Diarrhea	YLD	1.46 [1.01; 2.01]	2.43 [1.69; 3.35]
		YLL	0	0
		DALY	1.46 [1.01; 2.01]	2.43 [1.69; 3.35]
	Moderate Diarrhea	YLD	1.08 [0.75; 1.50]	1.81 [1.26; 2.50]
		YLL	0	0
		DALY	1.08 [0.75; 1.50]	1.81 [1.26; 2.50]
	Severe Diarrhea	YLD	0.17 [0.12; 0.23]	0.28 [0.19; 0.39]
		YLL	0	0
		DALY	0.17 [0.12; 0.23]	0.28 [0.19; 0.39]
Hemolytic Uremic Syndrome (HUS)	YLD		0.20 [0.14; 0.28]	0.33 [0.23; 0.46]
	YLL		26.28 [18.23; 36.25]	43.89 [30.45; 60.55]
	DALY		26.48 [18.37; 36.53]	44.22 [30.68; 61.01]
End Stage Renal Disease (ESRD)	YLD		21.31 [14.78; 29.40]	35.58 [24.69; 49.09]
	YLL		4.26 [2.96; 5.88]	7.12 [4.94; 9.82]
	DALY		25.57 [17.74; 35.28]	42.70 [29.63; 58.91]
Total DALYs			54.76 [37.99; 75.54]	91.44 [63.45; 126.16]
Scenario "Adjusted"				
Health Outcome			**Model "O157"**	**Model "Shigella"**
	Mild Diarrhea	YLD	0.02 [7.41×10^{-3}; 0.04]	0.03 [0.01; 0.07]
		YLL	0	0
		DALY	0.02 [7.41×10^{-3}; 0.04]	0.03 [0.01; 0.07]
	Moderate Diarrhea	YLD	0.01 [0.01; 0.03]	0.02 [0,01; 0.06]
		YLL	0	0
		DALY	0.01 [0.01; 0.03]	0.02 [0,01; 0.06]

Table 15 (continued). YLDs, YLLs and DALYs of STEC infection scenario "450g".

	Severe Diarrhea	YLD	2.29×10^{-3} [8.53×10^{-4}; 5.11×10^{-3}]	3.82×10^{-3} [1.42×10^{-3}; 8.53×10^{-3}]
		YLL	0	0
		DALY	2.29×10^{-3} [8.53×10^{-4}; 5.11×10^{-3}]	3.82×10^{-3} [1.42×10^{-3}; 8.53×10^{-3}]
Hemolytic Uremic Syndrome (HUS)	YLD		2.74×10^{-3} [1.02×10^{-3}; 6.11×10^{-3}]	4.57×10^{-3} [1.70×10^{-3}; 1.02×10^{-2}]
	YLL		0.36 [0.13; 0.80]	0.60 [0.22; 1.34]
	DALY		0.36 [0.13; 0.81]	0.60 [0.23; 1.35]
End Stage Renal Disease (ESRD)	YLD		0.29 [0.11; 0.65]	0.49 [0.18; 1.08]
	YLL		0.06 [0.02; 0.13]	0.10 [0.04; 0.22]
	DALY		0.35 [0.13; 0.78]	0.58 [0.22; 1.30]
Total DALYs			0.75 [0.28; 1.67]	1.25 [0.47; 2.79]

For scenario "450g" the estimated total number of cases for the STEC infection is higher when using model "Shigella" and in the scenario "Worst Case" (Table 9). For STEC disease (Table 15) model "O157" projects a total of 54.76 (37.99; 75.54) DALYs and 0.37 (0.25; 0.50) DALYs/person/year (Table 21), corresponding to ≈133 days of healthy life lost per person. Model "Shigella" projects a total 91.44 (63.45; 126.16) DALYs and 0.61 (0.25; 0.50) DALYs/person/year, which corresponds to ≈223 days of healthy life lost per person for scenario "Worst Case" (Table 15). For scenario "Adjusted", the model "O157" projects a total of 0.75 (0.28; 1.67) DALYs and 4.99×10^{-3} (1.86×10^{-3}; 1.11×10^{-2}) DALYs/person/year (Table 21), corresponding to ≈ 2 days of healthy life lost per person per year. The model "Shigella" projects a total of 1.25 (0.47; 2.79) DALYs and 8.32×10^{-3} (3.10×10^{-3}; 1.86×10^{-2}) DALYs/person/year (Table 21) for model "Shigella", corresponding to ≈ 3 days per person per year.

Table 16. YLDs, YLLs and DALYs of ETEC infection scenario "450g".

		Scenario "Worst Case"	
Health Outcome			**ETEC model**
Gastroenteritis	Mild Diarrhea	YLD	0.53 [0.37; 0.73]
		YLL	0
		DALY	0.53 [0.37; 0.73]
	Moderate Diarrhea	YLD	0.16 [0.11; 0.23]
		YLL	0
		DALY	0.16 [0.11; 0.23]
	Severe Diarrhea	YLD	0.01 [0.01; 0.02]
		YLL	0
		DALY	0.01 [0.01; 0.02]
Total DALYs			0.71 [0.49; 0.98]
		Scenario "Adjusted"	

Table 16 (continued). YLDs, YLLs and DALYs of ETEC infection scenario "450g".

Health Outcome			ETEC model
Gastroenteritis	Mild Diarrhea	YLD	0.03 [0.02; 0.05]
		YLL	0
		DALY	0.03 [0.02; 0.05]
	Moderate Diarrhea	YLD	0.01 [0.01; 0.02]
		YLL	0
		DALY	0.01 [0.01; 0.02]
	Severe Diarrhea	YLD	8.02×10^{-4} [4.96×10^{-4}; 1.25×10^{-3}]
		YLL	0
		DALY	8.02×10^{-4} [4.96×10^{-4}; 1.25×10^{-3}]
Total DALYs			0.04 [0.03; 0.07]

For ETEC disease (Table 16) scenario "Worst Case" projects a total of 0.71 (0.49; 0.98) DALYs and 4.74×10^{-3} (3.29×10^{-3}; 6.54×10^{-3}) DALYs/person/year (Table 21) which corresponds to approximately 259 days of healthy life are lost per person per year. Scenario "Adjusted" obtained the result of 0.04 (0.03; 0.07) total DALYs and 2.82×10^{-4} (1.74×10^{-4}; 4.40×10^{-4}) DALYs/person/year (Table 21) which equals ≈ 15 days of healthy life lost per person per year.

Table 17. YLDs, YLLs and DALYs of EPEC infection scenario "450g".

Scenario "Worst Case"			
Health Outcome			**EPEC model**
Gastroenteritis	Mild Diarrhea	YLD	0.01 [4.09×10^{-3}; 0.01]
		YLL	0
		DALY	0.01 [4.09×10^{-3}; 0.01]
	Moderate Diarrhea	YLD	1.82×10^{-3} [1.27×10^{-3}; 2.25×10^{-3}]
		YLL	0
		DALY	1.82×10^{-3} [1.27×10^{-3}; 2.25×10^{-3}]
	Severe Diarrhea	YLD	1.49×10^{-4} [1.04×10^{-4}; 2.06×10^{-4}]
		YLL	0
		DALY	1.49×10^{-4} [1.04×10^{-4}; 2.06×10^{-4}]
Total DALYs			7.87×10^{-3} [5.46×10^{-3}; 0.01]
Scenario "Adjusted"			
Health Outcome			**EPEC model**
Gastroenteritis	Mild Diarrhea	YLD	8.89×10^{-4} [5.86×10^{-4}; 1.30×10^{-3}]
		YLL	0
		DALY	8.89×10^{-4} [5.86×10^{-4}; 1.30×10^{-3}]
	Moderate Diarrhea	YLD	2.75×10^{-4} [1.81 x 10-4; 4.01×10^{-4}]
		YLL	0
		DALY	2.75×10^{-4} [1.81 x 10-4; 4.01×10^{-4}]
	Severe Diarrhea	YLD	2.25×10^{-5} [1.48×10^{-5}; 3.28×10^{-5}]
		YLL	0
		DALY	2.25×10^{-5} [1.48×10^{-5}; 3.28×10^{-5}]
Total DALYs			1.19×10^{-3} [7.83×10^{-4}; 1.73×10^{-3}]

EPEC disease (Table 17) presented the lowest estimated burden with 7.87 x 10^{-3} (5.46 x 10^{-3}; 0.01) total DALYs and 5.25 x 10^{-5} (3.64 x 10^{-5}; 7.24 x 10^{-5}) DALYs/person/year (Table 21) for scenario "Worst Case" which corresponds to approximately 3 days of healthy life year lost per person per year. For the "Adjusted" scenario a total of 1.19 x 10^{-3} (7.83 x 10^{-4}; 1.73 x 10^{-3}) DALYs, 7.91 x 10^{-6} (5.22 x 10^{-6}; 1.15 x 10^{-5}) DALYs/person/year (Table 21) are projected which translates into approximately 0.4 days of healthy life lost per person per year.

4.2.2. Scenario "225g"

Table 18. YLDs, YLLs and DALYs of STEC infection scenario "225g".

Scenario "Worst Case"				
Health Outcome			**Model "O157"**	**Model "Shigella"**
Gastroenteritis	Mild Diarrhea	YLD	1.38 (0.96; 1.90)	2.33 (1.61; 3.21)
		YLL	0	0
		DALY	1.38 (0.96; 1.90)	2.33 (1.61; 3.21)
	Moderate Diarrhea	YLD	1.03 (0.71; 1.42)	1.73 (1.20; 2.39)
		YLL	0	0
		DALY	1.03 (0.71; 1.42)	1.73 (1.20; 2.39)
	Severe Diarrhea	YLD	0.16 (0.11; 0.22)	0.27 (0.19; 0.37)
		YLL	0	0
		DALY	0.16 (0.11; 0.22)	0.27 (0.19; 0.37)
Hemolytic Uremic Syndrome (HUS)	YLD		0.19 (0.13; 0.26)	0.32 (0.22; 0.44)
	YLL		24.92 (17.29; 34.39)	41.99 (29.14; 57.93)
	DALY		25.11 (17.42; 34.65)	42.31 (29.36; 58.38)
End Stage Renal Disease (ESRD)	YLD		20.21 (14.02; 27.88)	34.05 (23.62; 46.97)
	YLL		4.04 (2.80; 5.58)	6.81 (4.72; 9.39)
	DALY		24.25 (16.83; 33.46)	40.86 (28.35; 56.37)
Total DALYs			51.93 (36.03; 71.65)	87.50 (60.71; 120.71)
Scenario "Adjusted"				
Health Outcome			**Model "O157"**	**Model "Shigella"**
Gastroenteritis	Mild Diarrhea	YLD	0.02 (0.01; 0.04)	0.03 (0.01; 0.07)
		YLL	0	0
		DALY	0.02 (0.01; 0.04)	0.03 (0.01; 0.07)
	Moderate Diarrhea	YLD	0.01 (0.01; 0.03)	0.02 (0.01; 0.05)
		YLL	0	0
		DALY	0.01 (0.01; 0.03)	0.02 (0.01; 0.05)
	Severe Diarrhea	YLD	2.17 x 10^{-3} (8.09 x 10^{-4}; 4.84 x 10^{-3})	3.66 x 10^{-3} (1.36 x 10^{-3}; 8.16 x 10^{-3})
		YLL	0	0
		DALY	2.17 x 10^{-3} (8.09 x 10^{-4}; 4.84 x 10^{-3})	3.66 x 10^{-3} (1.36 x 10^{-3}; 8.16 x 10^{-3})

Table 18 (continued). YLDs, YLLs and DALYs of STEC infection scenario "225g".

Hemolytic Uremic Syndrome (HUS)	YLD	2.60 x 10^{-3} (9.67 x 10^{-4}; 5.79 x 10^{-3})	4.37 x 10^{-3} (1.63 x 10^{-3}; 9.76 x 10^{-3})
	YLL	0.34 (0.13; 0.76)	0.57 (0.21; 1.28)
	DALY	0.34 (0.13; 0.77)	0.58 (0.22; 1.29)
End Stage Renal Disease (ESRD)	YLD	0.28 (0.10; 0.62)	0.47 (0.17; 1.04)
	YLL	0.06 (0.02; 0.12)	0.09 (0.03; 0.21)
	DALY	0.33 (0.12; 0.74)	0.56 (0.21; 1.24)
Total DALYs		0.71 (0.26; 1.58)	1.20 (0.45; 2.67)

For scenario "225g", the estimated total number of STEC infection cases (Table 12) follows the pattern presented in scenario "450g" with a higher number of estimated cases using model "Shigella". Model "O157" for scenario "Worst Case" (Table 18) projects a total of 51.93 (36.03; 71.65) DALYs and 0.35 (0.24; 0.48) DALYs/person/year (Table 21) which corresponds to ≈ 126 days of healthy life lost per person per year, and model "Shigella" projects a total of 87.50 (60.71; 120.71) DALYs and 0.58 (0.40; 0.80) DALYs/person/year (Table 21), corresponding to ≈ 213 days of healthy life lost per person per year. Regarding scenario "Adjusted" (Table 18), model "O157" projects a total of 0.71 (0.26; 1.58) DALYs and 4.73 x 10^{-3} (1.76 x 10^{-3}; 1.05 x 10^{-2}) DALYs/person/year (Table 21), i.e., approximately 1.73 days, while model "Shigella" projects a total of 1.20 (0.45; 2.67) DALYs and 7.97 x 10^{-3} (2.97 x 10^{-3}; 1.78 x 10^{-2}) DALYs/person/year (Table 21), i.e, approximately 2.9 days of healthy life lost per person per year.

Table 19. YLDs, YLLs and DALYs of ETEC infection scenario "225g".

		Scenario "Worst Case"	
Health Outcome			**ETEC model**
Gastroenteritis	Mild Diarrhea	YLD	0.48 (0.33; 0.66)
		YLL	0
		DALY	0.48 (0.33; 0.66)
	Moderate Diarrhea	YLD	0.15 (0.10; 0.20)
		YLL	0
		DALY	0.15 (0.10; 0.20)
	Severe Diarrhea	YLD	0.01 (0.01; 0.02)
		YLL	0
		DALY	0.01 (0.01; 0.02)
Total DALYs			0.64 (0.44; 0.88)
		Scenario "Adjusted"	
Health Outcome			**ETEC model**
Gastroenteritis	Mild Diarrhea	YLD	0.03 (0.02; 0.04)
		YLL	0
		DALY	0.03 (0.02; 0.04)
	Moderate Diarrhea	YLD	0.01 (0.01; 0.01)
		YLL	0

Table 19 (continued). YLDs, YLLs and DALYs of ETEC infection scenario "225g".

		DALY	0.01 (0.01; 0.01)
	Severe Diarrhea	YLD	7.24×10^{-4} (4.48×10^{-4}; 1.13×10^{-3})
		YLL	0
		DALY	7.24×10^{-4} (4.48×10^{-4}; 1.13×10^{-3})
Total DALYs			0.04 (0.02; 0.06)

For the ETEC infection (Table 19), the model projects a total of 0.64 (0.44; 0.88) DALYs and 4.27×10^{-3} (2.97×10^{-3}; 0.01) DALYs/person/year (Table 21), corresponding to approximately 1.56 days of healthy life lost per person per year for scenario "Worst Case", while for scenario "Adjusted" a total of 0.04 (0.02; 0.06) DALYs is projected and 2.54×10^{-4} (1.57×10^{-4}; 3.97×10^{-4}) DALYs/person/year was estimated (Table 21), which corresponds to ≈ 0.09 days of healthy life lost.

Table 20. YLDs, YLLs and DALYs of EPEC infection scenario "225g".

Scenario "Worst Case"			
Health Outcome			**EPEC model**
Gastroenteritis	Mild Diarrhea	YLD	2.97×10^{-3} (2.06×10^{-3}; 4.09×10^{-3})
		YLL	0
		DALY	2.97×10^{-3} (2.06×10^{-3}; 4.09×10^{-3})
	Moderate Diarrhea	YLD	9.17×10^{-4} (6.37×10^{-4}; 1.27×10^{-3})
		YLL	0
		DALY	9.17×10^{-4} (6.37×10^{-4}; 1.27×10^{-3})
	Severe Diarrhea	YLD	7.51×10^{-5} (5.21×10^{-5}; 1.04×10^{-4})
		YLL	0
		DALY	7.51×10^{-5} (5.21×10^{-5}; 1.04×10^{-4})
Total DALYs			3.96×10^{-3} (2.75×10^{-3}; 0.01)
Scenario "Adjusted"			
Health Outcome			**EPEC model**
Gastroenteritis	Mild Diarrhea	YLD	4.47×10^{-4} (2.95×10^{-4}; 6.51×10^{-4})
		YLL	0
		DALY	4.47×10^{-4} (2.95×10^{-4}; 6.51×10^{-4})
	Moderate Diarrhea	YLD	1.38×10^{-4} (9.12×10^{-5}; 2.01×10^{-4})
		YLL	0
		DALY	1.38×10^{-4} (9.12×10^{-5}; 2.01×10^{-4})
	Severe Diarrhea	YLD	1.13×10^{-5} (7.46×10^{-6}; 1.65×10^{-5})
		YLL	0
		DALY	1.13×10^{-5} (7.46×10^{-6}; 1.65×10^{-5})
Total DALYs			5.96×10^{-4} (3.93×10^{-4}; 8.69×10^{-4})

Considering the EPEC infection (Table 20), the model projects a total of 3.96×10^{-3} (2.75×10^{-3}; 0.01) DALYS and 2.64×10^{-5} (1.83×10^{-5}; 3.64×10^{-5}) DALYs/person/year, equivalent to ≈ 0.009 days of healthy life lost per person per year in scenario "Worst Case", and for scenario "Adjusted" a total of 5.96×10^{-4} (3.93×10^{-4}; 8.69×10^{-4}) DALYs and 3.98 x

10^{-6} (2.62 x 10^{-6}; 5.79 x 10^{-6}) DALYs/person/year, which corresponds to 0.0015 days of healthy life lost per person per year.

4.2.3. Summary of Results

A summary of total DALYs that resulted from DALYs/person/year can be consulted in table 21.

Table 21. Summary of final output DALYs/person/year.

			DALYs/person/year (Median 95% CI)
Scenario "450g"	**Scenario "Worst Case"**	**STEC (model "O157")**	0.37 (0.25; 0.50)
		STEC (model "Shigella")	0.61 (0.25; 0.50)
		ETEC	4.74 x 10^{-3} (3.29 x 10^{-3}; 6.54 x 10^{-3})
		EPEC	5.25 x 10^{-5} (3.64 x 10^{-5}; 7.24 x 10^{-5})
	Scenario "Adjusted"	**STEC (model "O157")**	4.99 x 10^{-3} (1.86 x 10^{-3}; 0.01)
		STEC (model "Shigella")	8.33 x 10^{-3} (3.10 x 10^{-3}; 1.86 x 10^{-2})
		ETEC	2.82 x 10^{-4} (1.74 x 10^{-4}; 4.40 x 10^{-4})
		EPEC	7.91 x 10^{-6} (5.22 x 10^{-6}; 1.15 x 10^{-5})
Scenario "225g"	**Scenario "Worst Case"**	**STEC (model "O157")**	0.35 (0.24; 0.48)
		STEC (model "Shigella")	0.58 (0.40; 0.80)
		ETEC	4.27 x 10^{-3} (2.97 x 10^{-3}; 0.01)
		EPEC	2.64 x 10^{-5} (1.83 x 10^{-5}; 3.64 x 10^{-5})
	Scenario "Adjusted"	**STEC (model "O157")**	4.73 x 10^{-3} (1.76 x 10^{-3}; 0.01)
		STEC (model "Shigella")	7.97 x 10^{-3} (2.97 x 10^{-3}; 1.78 x 10^{-2})
		ETEC	2.54 x 10^{-4} (1.57 x 10^{-4}; 3.97 x 10^{-4})
		EPEC	3.98 x 10^{-6} (2.62 x 10^{-6}; 5.79 x 10^{-6})

The overall burden of *E.coli* infections is the highest if the pathogen is a Shiga toxin producer, even when the proportion is adjusted, which is expected given its virulence and pathogenic effects.

Different health outcomes have different contributions to the total burden of disease.

The major contributions to DALYs of STEC disease were HUS and ESRD (Figure 14). Although these two clinical outcomes present the lowest number of cases, the severity

and consequences associated with its occurrence are much higher, with also higher values of DW and case-fatality ratios.

For the ETEC/EPEC infection, mild diarrhea is the major cause of the associated burden (Figure 15).

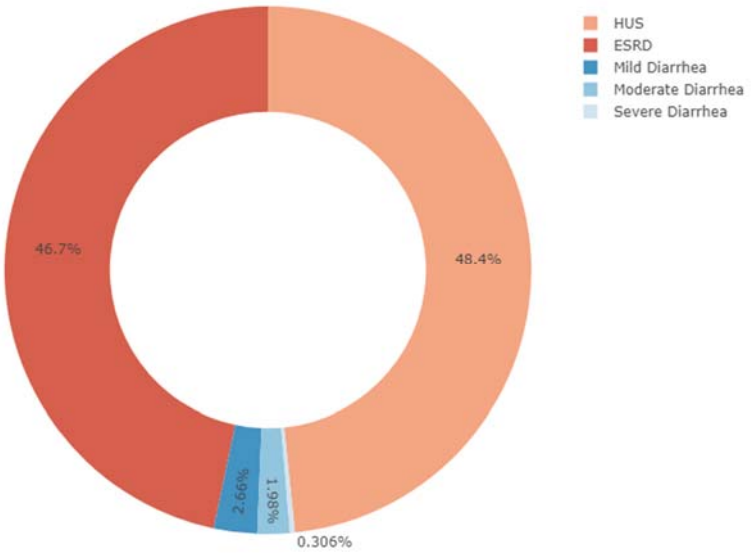

Figure 14. Contribution of clinical outcomes for DALYs in STEC infection.

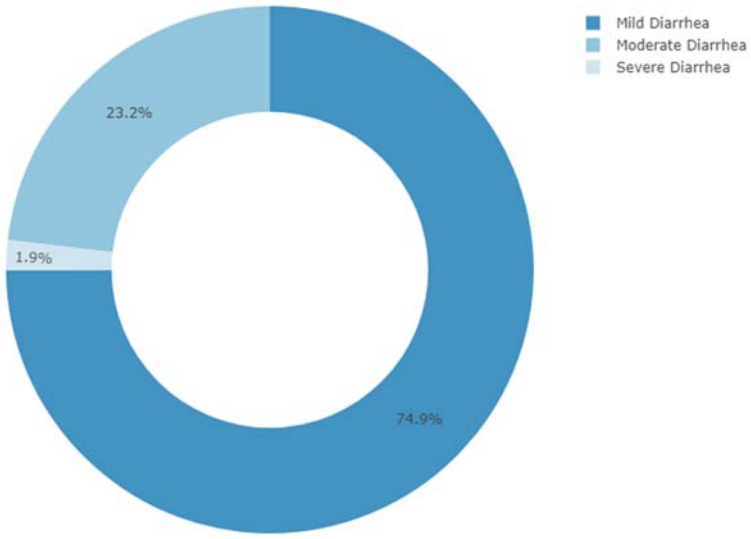

Figure 15. Contribution of clinical outcomes for DALYs in ETEC/EPEC infection.

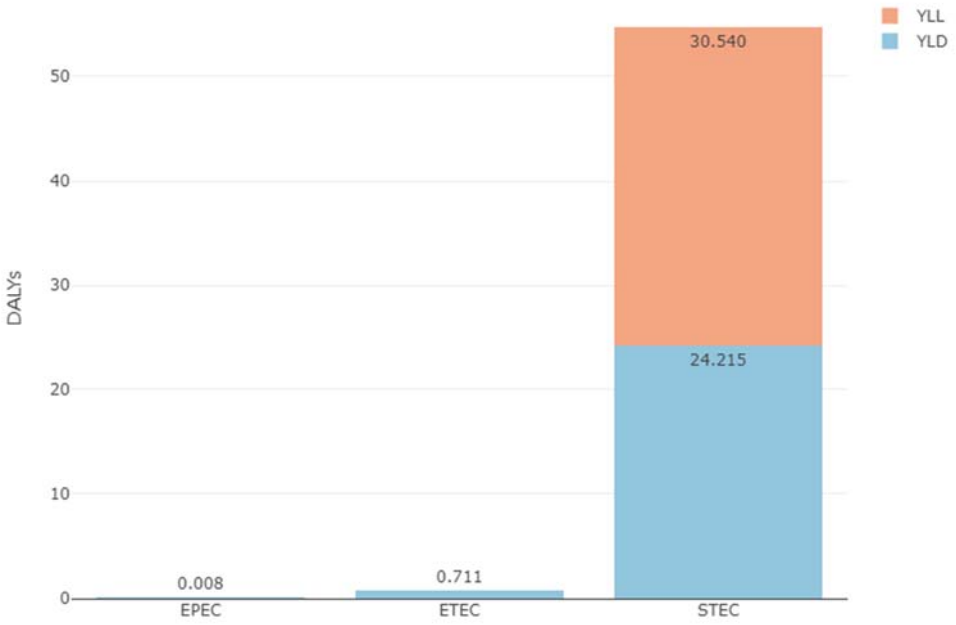

Figure 16. YLD and YLL contribution to DALYs in scenario “450g”: scenario “Worst Case”.

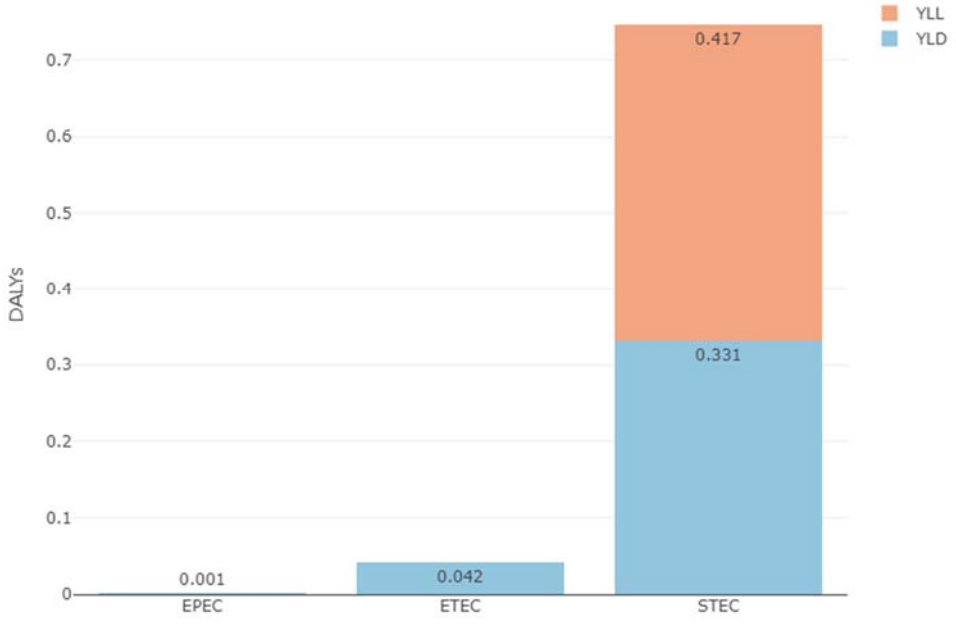

Figure 17. YLD and YLL contribution to DALYs in scenario "450g": scenario "Adjusted"

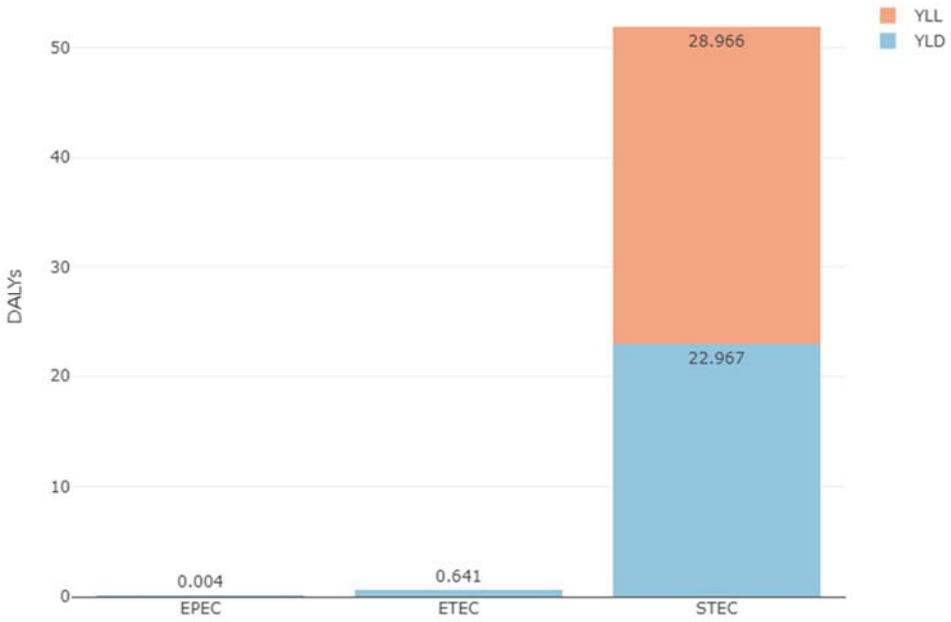

Figure 18. YLD and YLL contribution to DALYs in scenario "225g": scenario "Worst Case".

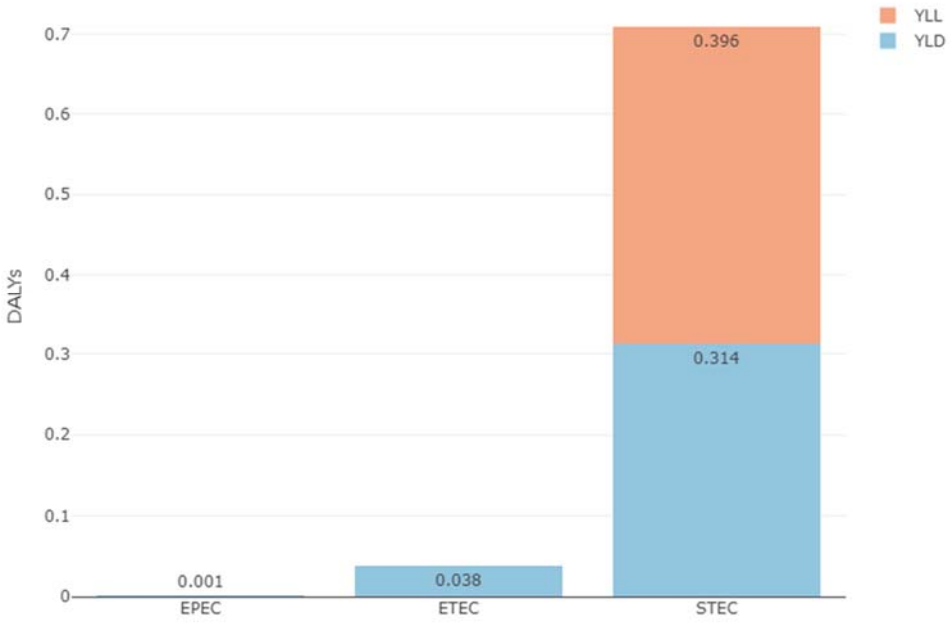

Figure 19. YLD and YLL contribution to DALYs in scenario "225g": scenario "Adjusted".

The contribution of YLD and YLL for the total DALYs considering scenario "450g" is presented in figures 16 and 17, while in figures 18 and 19 the contribution of YLD and YLL for the total DALYs considering scenario "225g". For both scenarios of STEC infection, YLL has the highest contribution to DALYs, despite the lower values in number of cases, because in all scenarios and outcomes the number of deaths < 1; however, in scenario "450g": "Worst Case": "Shigella", a death by HUS is projected, as well as one case of ESRD.

4.3. Sensitivity Analysis

Sensitivity analysis results for the STEC “O157” model (Figure 20) and STEC “Shigella” (Figure 21) reveal that for DALYs calculation the two models had very similar sensitivity results, with the factors contributing the most to the overall model variability being prevalence of *E.coli* group, followed by the number of people exposed to the hazard. In the “Shigella” model (Figure 21), the prevalence of contaminated portions had a slightly higher contribution than the *E.coli* concentrations when compared to the “O157” model.

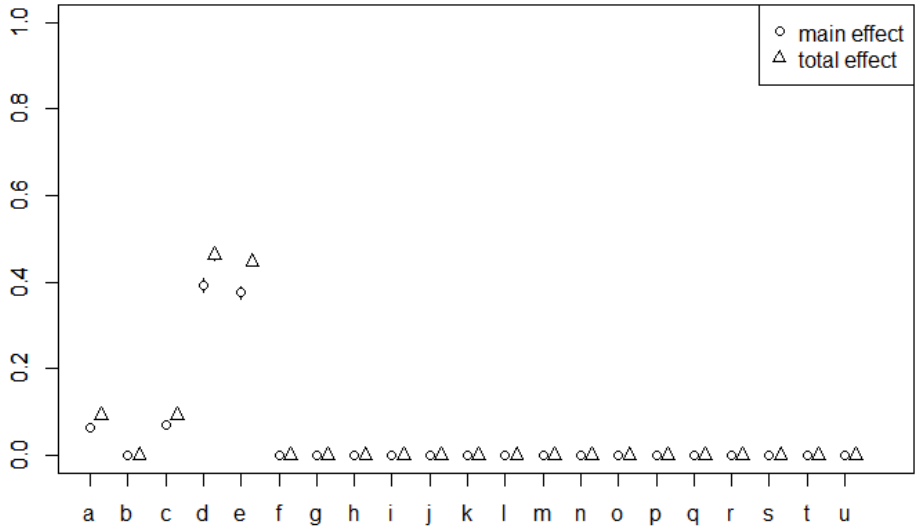

Figure 20. Sensitivity analysis for the STEC model “O157”. Input parameters as follows:
a- *E.coli* concentrations; b- meal portion; c- prevalence of contaminated portions; d- prevalence of *E.coli* group; e- number of people exposed to the hazard; f- duration of STEC induced diarrhea; g- probability of developing STEC induced mild diarrhea; h- DW for mild diarrhea; i- probability of developing STEC induced moderate diarrhea; j- DW for moderate diarrhea; k- probability of developing STEC induced severe diarrhea; l- DW for severe diarrhea; m- probability of developing HUS; n- DW for HUS; o- duration of HUS; p- probability of developing ESRD; q- DW for ESRD; r- duration of ESRD; s- probability of death by HUS; t- probability of death by ESRD; u- expectation of life at age group 25-29.

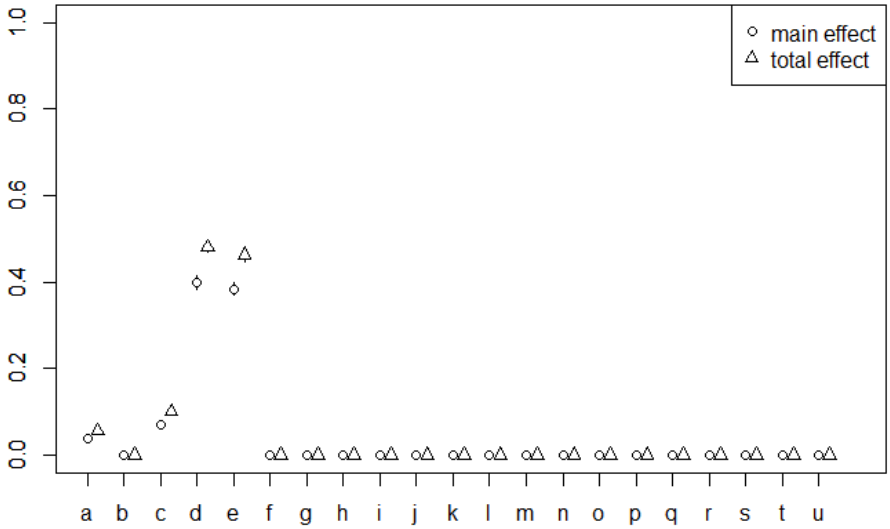

Figure 21. Sensitivity analysis result for the STEC model "Shigella". Input parameters as follows:
a- *E.coli* concentrations; b- meal portion; c- prevalence of contaminated portions; d- prevalence of *E.coli* group; e- number of people exposed to the hazard; f- duration of STEC induced diarrhea: g- probability of developing STEC induced mild diarrhea; h- DW for mild diarrhea; i- probability of developing STEC induced moderate diarrhea; j- DW for moderate diarrhea; k- probability of developing STEC induced severe diarrhea; l- DW for severe diarrhea; m- probability of developing HUS; n- DW for HUS; o- duration of HUS; p- probability of developing ESRD; q- DW for ESRD; r- duration of ESRD; s- probability of death by HUS; t- probability of death by ESRD; u- expectation of life at age group 25-29.

In the DALY calculation using ETEC model (Figure 22), the factors that influenced the most the output were the number of people exposed to the hazard, and the *E.coli* concentrations. Prevalence of contaminated portions and prevalence of *E.coli* group had lower contributions to the output, contrarily to the results obtained in the STEC models described above, in which the prevalence of *E.coli* group was the most influential in variability. In the ETEC model, variability in DW for mild diarrhea also had a contribution to the overall variability of the model, in contrast with the STEC models, for which none of the parameters from the YLD equation had a significant impact.

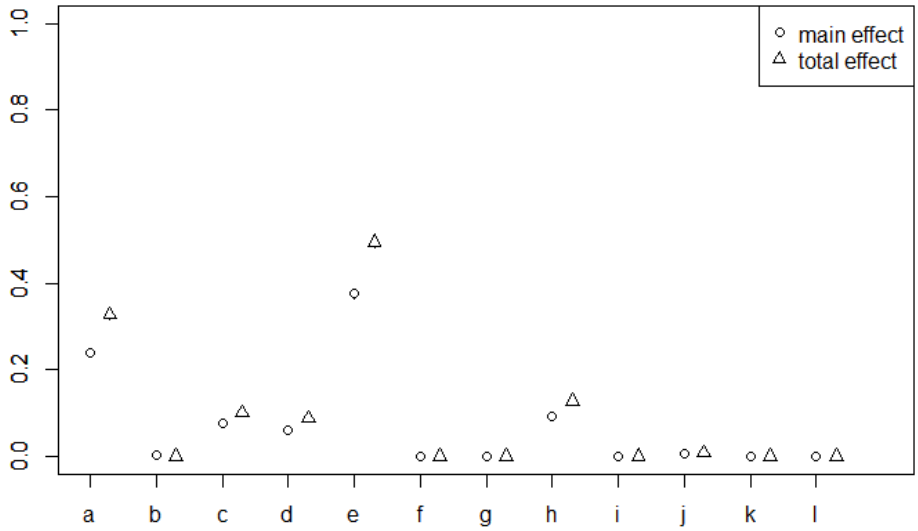

Figure 22. Sensitivity analysis for the ETEC model. Input parameters as follows:
a- *E.coli* concentrations; b- meal portion; c- prevalence of contaminated portions; d- prevalence of *E.coli* group; e- number of people exposed to the hazard; f- duration of ETEC induced diarrhea; g- probability of developing ETEC induced mild diarrhea; h- DW for mild diarrhea; i- probability of developing ETEC induced moderate diarrhea; j- DW for moderate diarrhea; k- probability of developing ETEC induced severe diarrhea; l- DW for severe diarrhea

Considering the EPEC model for DALYs calculation (Figure 23), *E.coli* concentrations was the factor that most contributed to DALYs final output variability, followed by the number of people exposed to the hazard and DW for mild diarrhea had a very low contribution.

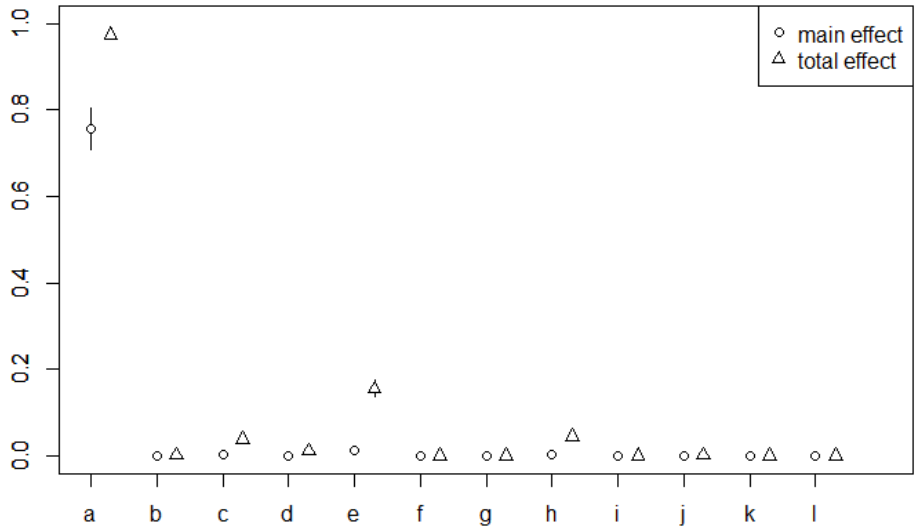

Figure 23. Sensitivity analysis result for the EPEC model. Input parameters as follows:
a- *E.coli* concentrations; b- meal portion; c- prevalence of contaminated portions; d- prevalence of *E.coli* group; e- number of people exposed to the hazard; f- duration of EPEC induced diarrhea; g- probability of developing EPEC induced mild diarrhea; h- DW for mild diarrhea; i- probability of developing EPEC induced moderate diarrhea; j- DW for moderate diarrhea; k- probability of developing EPEC induced severe diarrhea; l- DW for severe diarrhea.

5. Discussion

In this dissertation, a scenario-based approach was used to estimate the effects and burden of disease of foodborne *E.coli,* based on routine microbiological analyses results of ready-to-eat meals served in institutional canteens. Due to its genetic and phenotypic diversity, *E. coli* presents different clinical outcomes in the human host; to better represent this reality, in this work several scenarios were considered. Also, the inexistence of a single *E.coli* dose-response model, describing possible host-pathogen interactions, made the use of several dose-response models necessary, since PCR of food sample isolates was not routinely performed by the food laboratory responsible for processing the ready-to-eat meals assessed in this study.

These type of projections can have an important impact in companies, institutions and health services, since it allows to foretell the consequences of abnormal pathogen counts. These consequences include the number of cases, decreased quality of live, life-threatning conditions, and also economical consequences, as the economical impact can be predicted from the expected number of cases, as well as from DALYs. In this approach, some limitations need to be considered, such as the use and selection of dose-response models. Several models are available for a specific type of *E.coli*. Most of these models were obtained from human feeding exposure to the pathogen (Strachan et al. 2005). Ethical constraints arise from this type of approach, which is no longer used (Strachan et al. 2005). Currently, most dose-response models use epidemiological data from outbreaks, which requires the collaborative work of companies, laboratories, technicians and health services. In these types of dose-response models, different age-groups responses can be included, although when age-groups are not coincident with the ones observed in the exposed population, limitations occur, as children and adult response may differ and the clinical outcome may be dependent on the demographic of the affected population (Sperandio and Hovde 2015). However, not all existing *E.coli* models are built based on human response, and models using a surrogate animal model can also be considered in a general manner in dose-response models (Buchanan et al. 2000). The use of animal models must be carefully considered, as their response to the pathogen might differ from the human one, causing an under or overestimate of the risk, as represented and projected in the first example describing Shiga toxin-producing *E.coli* infection. In this work, because no data from outbreaks that might have occurred in the considered institutional canteens was available, and due to the fact that no further typification of *E.coli* isolates was performed whenever countings were above 10 cfu/g, the “Adjusted” scenario was chosen as the model that best represented reallity in this particular setting. Epidemiological investigation associated with laboratory work can be of extreme importance to assess clinical and food isolates, disclosing

the cause of the outbreak. Health outcome trees can be complex, and some outcomes cannot be projected because of the lack of investigation data. In this work, deeper approaches in burden of disease could be done if a better characterization of the population was possible, such as the detailed ages and age groups, as well as outcomes.

QMRA does not allow for a precise estimate of cases, due to the uncertainty that exists along the food chain and in the process of modelation (Havelaar et al. 2008). According to Nauta et al. (2001) these estimates are higher than expected considering the epidemiological estimates from population-based cohort studies. One of the possible causes of this overestimation is the lack of consideration of the acquired immunity to certain pathogens (Nauta et al. 2007), and also the fact that the majority of dose-response models available describe high levels of infection, resulting from high doses, while low doses remain to be investigated, so the uncertainty associated with the results can increase (Enger 2015). In this work, data collected from a food laboratory database was used, in which ready-to-eat meals were analyzed in order to quantify *E.coli* colonies using a culture method according to an ISO standard. The available data did not allow to discriminate countings inferior to 10 cfu, some of the possible solutions were to replace the non-detects with zero, log-linear extrapolation or substituting the non-detections with the limit of detection, but there is still a lack of agreement on how results below the limit of detection should be treated (Owens et al. 2020). Also, the used models, namely Beta-poisson, are single-hit models, so doses of one single cell could have the potential to cause illness, but this element could not be applied, specially to STEC O157:H7, as the infectious dose is considered to be less than 100 organisms (Smith et al. 2014).

These type of uncertainties enhance the need for interaction between epidemiology and QMRA. The epidemiological approach should be country specific to increase the chances of better adjustement of projected values to reality. As described in WHO Estimates of the Global Burden of Foodborne Diseases (2015), values of mortality differ between countries and group of countries, and so does the ages and population dimensions. However, country's characteristics and generalization allows them to be placed in groups with similar attributes, such as the groups defined by WHO (WHO 2015). This could enable the use of analogous data between countries, when groups of data are missing or do not exist. The application of this concept could be of great use in burden of disease projections, similarly to the one developed in this work.

The knowledge on the genetic diversity present in the genus *Escherichia* and *Escherichia coli* species is progressively increasing and previously thought to be different genus bacteria, *i.e Shigella,* is now being questioned. The adding and discovery of new *E.coli* intestinal pathogenic phenotypic groups in recent years urges the need for a better and more clear classification of this species (Yu et al. 2021). Together with the emergence of new

hybrids, horizontal gene transfer, difficulties arise with the use of the classical classification (Yu et al. 2021). If analyses beyond the classic culture techniques are not performed, *E.coli* that are thought to be harmless and belonging to a non pathogenic group can carry virulence genes that are typically not associated with a specific phenotype. According to the Annual report of the Scientific Network on Microbiological Risk Assessment 2020 (EFSA 2020) the importance of STEC serogroup typing is decreasing, while detection of virulence gene patterns is becoming increasingly relevant. Another example is the existence of typical and atypical EPEC, being the later thought to be closer to STEC in genetic characteristics, serotypes, toxins production, reservoirs and other epidemiological aspects; EPEC are present in a variety of places such as food, animal species, and environment, but humans are considered its main reservoir (Trabulsi et al. 2002; Rios et al. 2019). The presence of genes by itself does not translate into *in vivo* pathogenicity, since there are a variety of environmental factors that influence gene expression. Considering ETEC, factors such as bile, pH, bicarbonate, osmolarity, glucose and intestinal oxygen availability modelate gene regulation (Crofts et al. 2018). Regarding STEC, lactic acid, butyric acid, formic acid, probiotic bacteria, colicins, microcins and vitamin B_{12} have been proposed as factors that regulate Stx expression (Nawrocki et al. 2020). The diversity of these factors within the hosts, the overall health condition and the uncertainty associated with QMRA methods contribute to possible variations of the calculated risk, number of cases and respective clinical outcome.

Additionally, sensitivity analysis allows to identify the parameter or set of parameters that influenced the most the DALYs output, providing an insight into which specific input or set of inputs contributed the most to the variability of the model (Saltelli et al. 2000). The application of this analysis is necessary to understand the input-output relationship; determining to which extent uncertainty in model parameters contributes to the overall variability in the model output; identifying the important and influential parameters that drive model outputs and magnitudes; and also to guide future experimental designs (Saltelli et al. 2000; Mokhtari and Frey 2005; Saltelli 2008; Kiparissides et al. 2009). There are several commonly used global sensitivity analysis methods such as: Weighted average of local sensitivity analysis (WALS); Partial rank correlation coefficient (PRCC); Multi-parametric sensitivity analysis (MPSA); Fourier amplitude sensitivity analysis (FAST) and Sobol (Zhang et al. 2015). In this work, the Sobol method was followed to perform the sensitivity analysis, since according to Zhang et al. (2015) it allows for: discrete inputs; model independence; non-linear input-output relationship; non-monotonic input-output relationship; robustness; reproducibility; ability to apportion the output variance; higher order interaction of parameters and quantitative measure of ranking. Regarding sensitivity analysis results, the factors which variability contributed the most to the variability of the final burden in the STEC models was

prevalence of each *E.coli* group, and this can be associated with the infectious dose for STEC which is considered to be less than 100 cfu for O157:H7 (Smith et al. 2014). Therefore, *E.coli* concentrations do not cause a considerable variability because infection or illness is estimated to occur at a relatively low number of ingested microorganisms. The result for the STEC models differs from the results obtained for the EPEC model in which *E.coli* concentrations were the factor with the highest impact and it could also be related to the number of organisms that are necessary to initiate an infection or illness. For EPEC induced infection or illness it is postulated that a large inoculum, approximately 10^8-10^{10} bacteria are necessary to cause infection in adults (Mellies et al. 2007; Landraud and Brisse 2010), consequently, the variability in *E.coli* concentrations has the highest influence in the number of cases and DALYs. The same can be applied to the ETEC model in which concentrations above 10^8 cfu are required to cause ETEC induced infection (Daniels 2006), validating *E.coli* concentrations as the second most important factor after the number of people exposed to the hazard.

To date, no studies were found trying to estimate the risk of *E.coli* illness and disease burden based on dose-response models in ready-to-eat meals at the point of service.

Other studies attempted to estimate the probability of illness with the use of *E.coli* dose-response models. A study by O'Flaherty et al. (2019) estimated the probability of illness from antibiotic resistant *E.coli* associated with the consumption of lettuce irrigated with surface water. Since no dose-response models for antibiotic resistant *E.coli* were available, an EPEC model was used to calculate the mean probability of illness from exposure to antibiotic sensitive *E.coli,* and the range of the obtained mean probability value was 1.46×10^{-9} – 1.88×10^{-2} per 100g of lettuce. The probability of illness by *E.coli* O157:H7 associated with the consumption of raw fresh produce in India was also estimated by Kundu et al. (2018) with values ranging between 18-59%. A systematic review by Owens et al. (2020) of QMRA in public drinking water using the same risk estimation approach refers that, from all the possible pathogens and available data, *E.coli* was the most commonly used and analysed bacterial pathogen, with the most common used pathotypes being O157 and ETEC. Regarding these type of studies, almost half included the calculation of population disease burden, while others remained solely on probabilities of infection. Those that calculated the burden using 10^{-6} DALY/person/year as the reference level of risk, obtained values that ranged between 10^{-8} and 10^{-1} for the burden of *E.coli* infections. Following the same line of studies, similar applications have been made to estimate the risk of illness from beef products, and enterohemorrhagic *E.coli* is one of the most searched and analysed hazards (Tesson et al. 2020). Risk characterization models can give results in the form of incidence, mortality, illness risk, outbreak risk, severity of outcomes or DALY. In this work, DALYs were the model's final output and its utility could go beyond determining the outcome severity and

health weight in the population. Financial impact could also be determined by knowing the mean cost of hospitalization for an individual, as well as to determine the global cost of a contamination during a specific step in the food chain, similarly to what is proposed by Tesson et al. (2020) for the meat chain.

The presence of abnormal generic *E.coli* counts in ready-to-eat meals can be an indicator of poor hygiene and sanitation and may indicate the risk of contamination with serotype O157, as described in the Microbiological quality guideline for ready-to-eat foods (2009). Although *E.coli* can also be found in the environment, it is postulated that it has primarly intestinal origin, and therefore it is still advocated as an indicator of faecal contamination and poor hygiene (Metz et al. 2020). These ready-to-eat meals were miscellaneous, including salads but also composed meals with meat or fish. There is no mandatory European regulation or national law to comply with regarding microbiological criteria in composed ready-to-eat meals. The Commission Regulation (CE) No. 2073/2005 of 15 November 2005, and following amendments, on microbiological criteria for foodstuffs establishes limit values for *E.coli* quantification in certain types of food products such as meat, meat products and fish, but no values are established for ready-to-eat meals (European Commission 2020). According to this regulation, regarding *E.coli* in meat products, the sanitary status of products can be defined by determination and quantification of m and M parameters that represent the threshold value for the number of bacteria and the maximum value for the number of bacteria, respectively (European Commission 2020). As such, three categories are described, being: satisfactory if the logarithmic mean is under the m parameter, acceptable if it is between m and M, and non satisfactory if it is higher than M. As an example, for minced meat the limit values are 50 cfu/g and 500 cfu/g for m and M and for cheese that is made from milk that has undergone thermal treatment the limit values are 100 cfu/g and 1000cfu/g and the reference method is ISO 16649-1 or 2 (European Commission 2020).

At the national level, microbiological guidelines have been proposed by the National Health Institute - INSA (INSA 2019). In these guidelines, *E.coli* counts < 10 cfu/g are considered satisfactory in ready-to-eat meals, (INSA 2019).

The origin of high generic *E. coli* counts in food samples could be multiple, *i.e.*, cross-contamination from raw materials, from the food-producing environment, including staff, or from inadequate thermal treatment (INSA 2019). Contaminated vegetables, meat and other foodstuffs could be possible vehicles, as well as contamination from a human source, since *E.coli* is one of the most abundant bacteria in the gut of humans and animals. Regarding STEC, contamination of ready-to-eat foods via cross-contamination from raw or undercooked meat products is an important cause of foodborne infections (Public Health England 2018). Therefore, good manufacturing and hygiene practices, such as preventing

contamination of animal carcasses during slaughter, correct cooking of meat products, pasteurisation of milk and dairy products, and good personal hygiene, including appropriate handwashing, are key measures to prevent and control *E. coli* along the food chain (Hawker et al. 2012; Public Health England 2018).

Without further investigation of *E.coli* occurrence and typification, assumptions about the most probable pathotype cannot be made with confidence, as in a scenario approach.

The “Adjusted” scenario was built as an attempt to adapt the prevalence of each *E.coli* group considered in this study. Therefore, the final output values of DALYs for the “Adjusted” scenario can be considered as the ones closer to reality, representing the expected burden for the three considered groups of *E.coli.* Additionally, according to the sensitivity analysis, the size of the meal portion did not contribute significantly to the final output in any of the used models, which can explain the small difference in the results of scenario “450g” and “225g”.

Investigation procedures beyond generic classification of *E. coli* can impact the overall risk management and health outcomes, because whenever a STEC infection is confirmed, healthcare treatment should be specific and appropriate, as this is crucial for the success of most of the newly developed therapeutics (Mühlen and Dersch 2020). According to a meta-analysis on the use of antibiotics, a deeper identification of the *E.coli* group is relevant, especially in the case of STEC infection, as a significant association of the use of antibiotics and the risk of developing HUS was found (Freedman et al. 2016; Public Health England 2018). The recommended therapy today is mainly supportive, though in recent years novel therapy approaches - as monoclonal antibodies, antisera directed against Shiga toxin, toxin receptor analogs, and a possible vaccination strategy - is being evaluated *in vitro* and in animal models (Mühlen and Dersch 2020).

Nowadays, new laboratory methods are arising for determining the possible origin of *E.coli* strains found in food, such as Single Nucleotide Polymorphism (SNP)-based genotyping and Whole Genome Sequencing (WGS). SNP can correlate the geographical and genetic relationship of *E.coli,* allowing to identify the potential source, as well as to provide a theoretical basis for monitoring and control of this important foodborne pathogen (Liu et al. 2020). WGS can determine the whole genome by sequencing all DNA of one organism, enabling a greater precision in the surveillance of foodborne pathogens for a quicker and efficient response to foodborne outbreaks (CDC 2016; Therrien et al. 2021). WGS also allows to disclose an entire spectrum of pathogen information, such as toxin variant, serotype, sequence type and virulence factors (EFSA 2020).

The growing evidence of new hybrids, possible clinical outcomes following *E.coli* infection, and difficulties associated with its detection, reinforce the need for adequate implementation of food safety management systems, as well as regular verification

procedures, such as audits and inspections, since generic *E.coli* counts remain a fine indicator of good hygiene practices (Ekici and Dümen 2019). The occurrence of this potential foodborne pathogen can have a great impact on consumer's health, ranging from mild clinical conditions, such as self-limiting diarrhea, to long-term sequelae, life-long disabilities, and ultimately, death.

Knowledge on the burden of *E.coli* infections keeps evolving and recent evidence suggests that the AIEC pathogenic group is involved in the pathogenesis of inflammatory bowel disease, particularly Crohn's disease (Palmela et al. 2018), so the true long-term burden may have more than the previously predicted consequences, or may never be truly known.

6. Conclusion

In this work, a burden of disease estimation was performed based on generic and routine *E.coli* counts obtained from ready-to-eat foods collected in institutional canteens during a 2-year period.

The estimated burden of disease varied according to the type of scenario, and a lower ammount of ingested food was associated with lower risk and burden of disease. Considering the ingestion of the whole meal, STEC infection is expected to have a burden of 4.99×10^{-3} DALYs/person/year, ETEC infection of 2.82×10^{-4} DALYs/person/year, and EPEC infection of 7.91×10^{-6} DALYs/person/year, equivalent to approximately 2 days of healthy life lost, 0.1 days of healthy life lost and 0.003 days of healthy life lost per person per year, respectively. Mild diarrhea was the most common expected clinical outcome of infection with the considered *E.coli* groups, and no cases of hemolytic uremic syndrome or end stage renal disease are expected to arise from the consumption of this study ready-to-eat meals. Although these health metrics estimates seem low and present mild severity, especially when compared to other foodborne pathogens, they should not be disregarded in any circumstance, particularly in an institutional environment as was the case in this study. Additionally, considering the results obtained from sensitivity analysis, occurrence of the STEC group within total *E.coli* counts was the factor that contributed the most to the output variability in both STEC models outputs, while in the ETEC model the number of people exposed to the hazard explained most variability, and in the EPEC model *E.coli* concentrations contributed the most to the output variability.

Although a long way has yet to be wandered, this study draws attention to an innovative approach that contributes to a better understanding of *E.coli* in ready-to-eat foods, and its potential consequences and impact in consumers health, by combining quantitative microbial risk assessment and health metrics estimates.

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8. Annexes

8.1. Annex 1

Table 22. Life expectancy table for the year 2019 for Portugal. Adapted from WHO (2020).

Indicator	Age group	Both Sexes
Expectation of life at age x	< 1 years	81.57420886
	1-4 years	80.82533621
	5-9 years	76.87564665
	10-14 years	71.90253472
	15-19 years	66.9317005
	20-24 years	61.99687119
	25-29 years	57.08524021
	30-34 years	52.19320158
	35-39 years	47.30965483
	40-44 years	42.46168708
	45-49 years	37.70902792
	50-54 years	33.08839878
	55-59 years	28.63464499
	60-64 years	24.34322125
	65-69 years	20.21990929
	70-74 years	16.21677938
	75-79 years	12.4345432
	80-84 years	9.034845008
	85+ years	6.114170105

8.2. Annex 2

Table 23. Description of the input parameters used for sensitivity analysis for STEC "O157" and "Shigella" models.

"*E.coli* Concentrations"	10 ^ (~ Lognormal (0.74; 0.46))
"Portion size"	~ Uniform (225; 450)
"Prevalence of contaminated portions"	~ Beta (31;444)
"Prevalence of *E.coli* STEC"	~ Beta (6;405)
"Number of people exposed"	~ Uniform (150; 800)
"Probability of STEC induced mild diarrhea"	0.8
"DW for mild diarrhea"	~ Uniform (0.04; 0.09)
"Duration of STEC induced diarrhea (in years)"	~ Uniform (0.01; 0.03)
"Probability of STEC induced moderate diarrhea"	0.18
"DW for moderate diarrhea"	~ Uniform (0.13; 0.30)
"Probability of STEC induced severe diarrhea"	0.02
"DW for severe diarrhea"	~ Uniform (0.18; 0.40)
"Probability of STEC O157 induced HUS cases"	0.008
"DW for HUS"	~ Uniform (0.14; 0.30)
"Duration of HUS (in years)"	~ Uniform (0.04; 0.12)
"Probability of death due to HUS"	0.037
"Probability of ESRD after HUS"	0.00024
"DW for ESRD"	~Uniform (0.40; 0.75)
"Duration of ESRD (in years)"	57
"Probability of death due to ESRD"	0.2
"Expectancy of life at age 25-29"	57

Table 24. Description of the input parameters used for sensitivity analysis for the ETEC model.

"*E.coli* Concentrations"	10 ^ (lognormal (0.74; 0.46))
"Portion size"	~ Uniform (225; 450)
"Prevalence of contaminated portions"	~ Beta (31;444)
"Prevalence of *E.coli* ETEC"	~ Beta (6;405)
"Number of people exposed"	~ Uniform (150; 800)
"Probability of ETEC induced mild diarrhea"	0.91
"DW for mild diarrhea"	~ Uniform (0.04; 0.09)
"Duration of ETEC induced diarrhea"	0.01
"Probability of ETEC induced moderate diarrhea"	0.085
"DW for moderate diarrhea"	~ Uniform (0.13; 0.30)
"Probability of ETEC induced severe diarrhea"	0.005
"DW for severe diarrhea"	~ Uniform (0.18; 0.40)

Table 25. Description of the input parameters used for sensitivity analysis for the EPEC model.

"*E.coli* Concentrations"	10 ^ (lognormal (0.74; 0.46))
"Portion size"	~ Uniform (225; 450)
"Prevalence of contaminated portions"	~ Beta (31;444)
"Prevalence of *E.coli* EPEC"	~ Beta (84; 471)
"Number of people exposed"	~ Uniform (150; 800)
"Probability of EPEC induced mild diarrhea"	0.91
"DW for mild diarrhea"	~ Uniform (0.04; 0.09)
"Duration of EPEC induced diarrhea"	0.01
"Probability of EPEC induced moderate diarrhea"	0.085
"DW for moderate diarrhea"	~ Uniform (0.13; 0.30)
"Probability of EPEC induced severe diarrhea"	0.005
"DW for severe diarrhea"	~ Uniform (0.18; 0.40)