LWT - Food Science and Technology 174 (2023) 114450

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Burden of disease estimation based on *Escherichia coli* quantification in ready-to-eat meals served in Portuguese institutional canteens

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ARTICLE INFO

Keywords:
Burden of disease
Escherichia coli
Disability adjusted life years
Ready-to-eat foods
Quantitative microbial risk assessment

ABSTRACT

Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC) are associated with the onset of gastroenteritis with different severities, and STEC has been associated with other sequelae, such as hemolytic uremic syndrome and end stage renal disease. The main goal of this study was to estimate the annual foodborne burden of disease associated with STEC, ETEC and EPEC infection based on *E. coli* quantification results obtained in the routine analysis of ready-to-eat meals served in institutional canteens from 2018 to 2019. A stochastic Quantitative Microbial Risk Assessment (QMRA) model was used to estimate the expected number of cases per health outcome and Disability Adjusted Life Years (DALYs). Assuming a daily consumption of a whole meal portion (450 g), the estimated burden was of 4.2 x 10^{-3} DALYs/person/year for STEC infection, 2.82 x 10^{-4} DALYs/person/year for ETEC infection and 7.91 x 10^{-6} DALYs/person/year for EPEC infection. Additionally, using the Sobol method, the sensitivity analysis revealed that the factors with higher influence on the final output (DALYs) were the pathotype's prevalence for the STEC model, the number of people exposed to the hazard for the ETEC model and *E. coli* concentrations for the EPEC model.

1. Introduction

E. coli is a gram negative, facultative anaerobe, non-sporulating rod that belongs to the Enterobacteriaceae family (Feng, 2013). This bacterium mainly inhabits the lower intestinal tract of warm-blooded animals, and is also present in the environment (Jang et al., 2017). 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 & García, 2018). This particular group of pathogenic *E. coli* is classically sub-divided into categories that are 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) (Cabrera-Sosa & Ochoa, 2020; Croxen et al., 2013; Heredia & García, 2018). Enterohemorrhagic *E. coli* (EHEC) is considered a subset of pathogenic STEC strains (Feng, 2013).

The EPEC pathotype 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 & Ochoa, 2020). Transmission occurs via the fecal-oral route through contaminated food, water or fomites (Donnenberg, 2013). To date, humans and domestic animals are considered the main hosts (Denamur et al., 2021).

The ETEC pathotype causes diarrhea in all ages and is a common cause of traveler's diarrhea; infections are linked to the consumption of contaminated food and 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 (Alerasol et al., 2014; Mirhoseini et al., 2018; Nazarian et al., 2012). Known hosts of the strain include humans, pigs and cattle (Denamur et al., 2021). *E. 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). Clinical manifestations can range from asymptomatic non-bloody diarrhea, to hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Gyles, 2007). Consumption of contaminated food remains the main cause of infection, although contact with manure, animals and infected people are also responsible for the

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https://doi.org/10.1016/j.lwt.2023.114450
Received 20 May 2022; Received in revised form 20 December 2022; Accepted 6 January 2023
Available online 8 January 2023

appearance of cases, but at a much lower frequency (Ferens & Hovde, 2011; Vidovic & 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 & Doyle, 2007). Globally, the most common serotype associated to HUS is O157:H7 (Alconcher et al., 2021; Davis et al., 2014). Moreover, STEC strains, including 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). 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, as a last resource, may need kidney transplantation (Palermo et al., 2009).

Recognizing the need to measure the burden and distribution of foodborne diseases and to enable policy-makers to set public health priorities, allocate resources and intervention efforts, in 2015 the World Health Organization (WHO) released the WHO Estimates of the Global Burden of Foodborne Diseases (Pires et al., 2021). This initiative included diseases caused by microbial, parasitic, and chemical hazards transmitted by food, water, soil, air or contact with infected animals and humans, such as *Brucella* spp., *Mycobacterium bovis*, Shiga toxin-producing *E. coli*, Norovirus, invasive non-typhoidal *Salmonella enterica*, *Listeria monocytogenes*, *Taenia solium*, *Trichinella* spp., *Toxoplasma gondii*, aflatoxins and dioxins, among others. According to the most recent estimate, in 2010, STEC was responsible for 12,953 Disability Adjusted Life Years (DALYs), ETEC for 2,084,229 DALYs and EPEC for 2,938,407 DALYs (WHO, 2015). According to the European Union One Health Zoonoses Report, in 2019, infection from STEC, was the third most reported zoonosis in the European Union ("The European Union One Health 2019 Zoonoses Report," 2021). It is estimated that, annually, STEC is responsible for 2,801,000 infections worldwide, 3890 HUS cases, 270 ESRD cases and 230 deaths (Majowicz et al., 2014).

Regarding laboratorial detection of the pathogen in food, bacterial culture-based methods are used as the gold standard for *E. coli* O157:H7, despite the relatively low sensitivity when compared to other methods (Rani et al., 2021). Polymerase chain reaction (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). 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). 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 of new O157 phenotypes that are β-glucuronidase-positive (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.

Quantitative Microbial Risk Assessment (QMRA) is a scientifically based process, that allows to estimate the adverse health effects from exposure to microorganisms (Boone et al., 2010). Human dose-response models and predictive microbiology are two main components of QMRA, that also incorporates variability and uncertainty, crucial when modeling biological phenomena (Havelaar et al., 2008).

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., 2014). 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., 2014). While years of life lost (YLL) expresses the years lost due to a specific cause of death, 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). DALY can therefore be obtained by summing the YLLs and YLDs for each health state in the disease model.

The main goal of this study was to estimate the risk of *E. coli* infection related to the consumption of institutional canteen meals using a stochastic Quantitative Microbial Risk Assessment (QMRA) model, considering STEC, ETEC and EPEC pathotypes. Additionally, a projection of the annual number of cases per health outcome was made, and the annual burden of disease (DALYs) was calculated considering the assessed institutional canteens consumers population.

2. Materials and methods

This study was based on *E. coli* quantification results gathered by the bromatology laboratory in charge of institutional foodservice monitoring. The burden of disease associated with STEC, ETEC and EPEC infection due to food consumption by the institution's personnel was estimated using a risk assessment approach. Data, assumptions, and methodology are further detailed below.

2.1. Data source and assumptions

E. coli quantification results of routine microbiological testing of ready-to-eat meals (n = 473) served to healthy adults in institutional canteens (N = 30) from February 2018 to December 2019 were used. The canteens, located in Portugal (mainland and islands), belonged to the same institution, and served meals on a daily basis to regular consumers aged from 18 to 65 years. The analyzed food samples consisted of the main course of these meals, which is composed of meat or fish, vegetables or salad and a carbohydrate source (rice, potatoes, pasta). 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 <10 CFU/g were considered satisfactory, so all values ≥ 10 CFU/g were considered unsatisfactory, and therefore, were an input to the model. These results were gathered in a database using MS Excel software (Microsoft Corporation, Redmond, WA, USA).

In this study, due to the lack of available data, several assumptions were made. The population exposed to the hazard was composed of the institution's personnel, that was assumed to be of 150 people per day, with the 25-29 year-old group being the most frequent one. As for the ingested portion of food, 450 g were considered to be the representative amount of a whole main course ingestion.

Because *E. coli* presents genetic and phenotypic diversity, with different *E. coli* pathotypes determining distinct clinical outcomes in the human host, and due to the fact that no *E. coli* pathotype identification was performed in the food microbiology laboratory, two scenarios were built to better represent this reality. While the "Worst Case" scenario considered that all CFU belonged to the same *E. coli* pathotype, the "Adjusted" scenario added the prevalence of each pathotype within total *E. coli* counts based on literature review.

For risk estimation, dose-response models parameters 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 the meta analysis conducted by WHO (WHO, 2015). All QMRA and Disability Adjusted Life Models were performed using R software version 4.0.3 (R core team, 2016) 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 built considering that 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 formula $p = Beta\ (s + 1,\ n - s + 1)$ (Vose, 2008).

2.2. Methods

2.2.1. Quantitative microbial risk assessment (QMRA)

2.2.1.1. Exposure assessment. 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 (Delignette-Muller & Dutang, 2015). A lognormal distribution was chosen as data's best fit: lognormal (0.74, 0.46). A final distribution representing *E. coli* concentrations of the assessed two-year period was obtained (Figure A1). For the dose-response application, the total number of ingested CFU is needed, constituting the input for the dose-response model. Therefore, since the laboratory results were in CFU per gram of food sample, a multiplication of these results by the total of grams ingested (450g) by the people exposed gives the number of total CFU ingested. The obtained median dose (95% CI) representative of the ingested dose was 4.75 (3.50; 7.81) of *E. coli* log CFU.

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 was described by a beta distribution ~ Beta (31,444).

To assess the prevalence of each pathotype group within general *E. coli* counts, a literature review was made considering molecular identification of *E. coli* recovered from food samples, in order to allow for the estimation of prevalence of each pathotype. Articles that performed PCR analysis of positive samples' culture isolates were chosen for *E. coli* pathotype prevalence assessment. A summary of the assessed literature with number of samples, prevalence of each *E. coli* group and related data is presented in Table A1.

2.2.1.2. Hazard characterization: dose-response models. For risk calculation, Beta-Poisson models were used, with equation (1) used for STEC and EPEC and equation (2) used for ETEC.

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

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

All of the necessary parameter values for the application of the approximated Beta-Poisson dose-response models (equations (1) and (2)) were obtained through literature review (Table 1).

2.2.1.3. Risk characterization. Determination of the expected number of cases per year was obtained through multiplication of the median risk of illness by the total consumption occasions for that specific population. Based on the model developed for *Listeria monocytogenes* by Pérez-Rodríguez et al. (2017), the following equation (3) was developed to determine the expected number of cases of *E. coli* infections, where *R* is the marginal risk, *Pcp* the prevalence of contaminated portions, *Pg* the prevalence of each pathotype of *E. coli*, *N* the number of eating occasions and *PE* the number of exposed people. Equation (3) input parameters are presented in Table 2.

$$Number\ of\ cases = R\ x\ P_{cp}\ x\ P_g\ x\ N\ x\ PE \quad (3)$$

Table 1
Dose-response models parameters used in this study.

Parameter	STEC	ETEC	EPEC
α	0.0571	7.54×10^{-2}	0.221
β	2.2183	–	3.11×10^{6}
N_{50}	–	1.7×10^{6}	–
Reference	Strachan et al. (2005)	Enger (2015)	Strachan et al. (2005)

2.2.2. Burden of disease estimation

Burden of disease estimation included the calculation of years lost due to disability (YLDs, equation (4)), years of life lost (YLLs, equation (5)) and disability-adjusted life years (DALYs, equation (6)) for each health outcome, as well as total DALYs and DALYs/person/year.

$$YLD = Number\ of\ cases\ x\ Duration\ till\ remission\ or\ death\ x\ Disability\ weight \quad (4)$$

$$YLL = Number\ of\ deaths\ x\ Life\ expectancy\ at\ the\ age\ of\ death \quad (5)$$

$$DALY = YLD + YLL \quad (6)$$

Health outcome trees were designed for each *E. coli* pathotype (Figs. 1 and 2) following literature review and disease models described by WHO (WHO, 2015). However, some of the possible outcomes were not included in the model due to absence of available data (Figs. 1 and 2).

The probabilities of developing each specific health outcome, disability weights, duration of health outcomes and case fatality ratios (Table A2; Table A3) were retrieved from *WHO's Estimates of the Global Burden of Foodborne Disease* (WHO, 2015). Case fatality ratios were only considered for Hemolytic Uremic Syndrome (HUS) and End Stage Renal Disease (ESRD) outcomes of STEC infection, and no deaths were considered for ETEC and EPEC infections, because Portugal belongs to WHO's EUR A subgroup for which no deaths were estimated in *WHO's estimates of the global burden of foodborne diseases* (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 *i.e.,* 0.8% for serogroup O157 and 0.03% for non-O157 (WHO, 2015). The first probability was chosen considering that the dose-response model used for STEC was built with data from *E. coli* O157 outbreaks. The life expectancy table from WHO was used for the calculation of YLL (Table A4). 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.

2.2.3. Sensitivity analysis

The Sobol method was followed to determine the factors whose variability contributed the most to final DALYs variability. 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.,

Table 2
Factor description and input parameters for annual number of cases determination.

Factor Description	Input
R (Marginal Risk)	Median risk value resulting from the dose-response model application
P_{cp} (Prevalence of 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 (Number of people exposed)	150

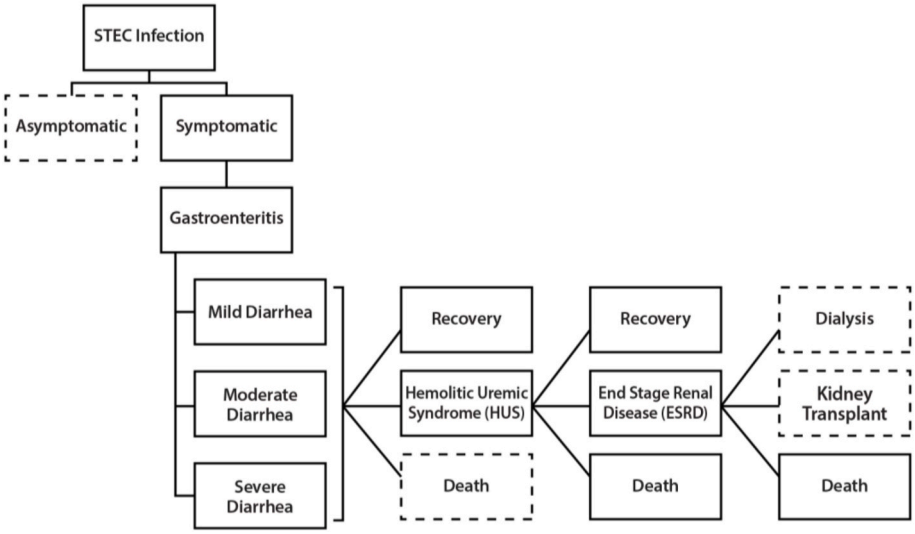

Fig. 1. Outcome tree for STEC infection-adapted from WHO (2015) and Monteiro Pires et al. (2020). Clinical outcomes included in the model are presented with a solid line, and those excluded are presented with a dashed line.

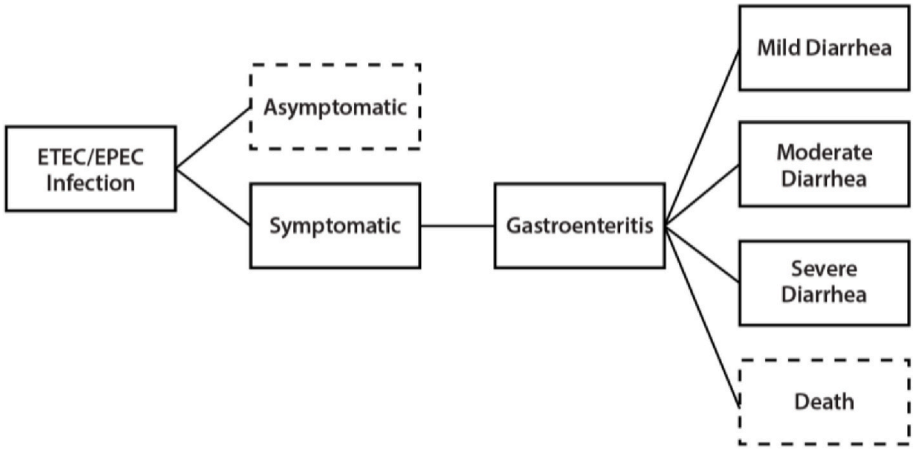

Fig. 2. Outcome tree for ETEC/EPEC infection-adapted from WHO (2015). Clinical outcomes included in the model are presented with a solid line, and those excluded are presented with a dashed line.

Table 3
Median values of the estimated risk for the STEC, ETEC and EPEC scenarios due to the consumption of contaminated meal portions.

Model	Median (95% CI) of Risk
STEC	0.44 (0.34; 0.63)
ETEC	0.35 (0.20; 0.62)
EPEC	3.91×10^{-3} (2.26×10^{-4}; 0.49)

2015). All the information and respective input distributions are described in detail and summarized in Tables A5, A.6, and A.7. The analysis was performed using "sensitivity" package (Iooss et al., 2021).

3. Results

3.1. QMRA

The dose-response models were applied to the ingested doses and the output can be defined as the risk or the probability of illness (Table 3).

The total number of cases obtained for the "Worst Case" scenario was 1555.21 (1079.04; 2145.61), 1250.28 (867.47; 1724.92) and 13.86 (9.61; 19.11) for STEC, ETEC and EPEC infection, respectively. Considering the "Adjusted" scenario, the total number of cases obtained was 21.24 (7.91; 47.38), 74.42 (46.06; 116.23) and 2.09 (1.38; 3.04) for STEC, ETEC and EPEC infection, respectively.

The projected total number of cases discriminated per health outcome for STEC infection (Table A.8), ETEC infection (Table A.9) and EPEC infection (Table A.10) were calculated.

3.2. Burden of disease

Although YLL for all gastroenteritis outcome models and scenarios was 0, YLD presented variability in the considered models and scenarios (Tables 4–6). YLL was considered 0 for all diarrhea models and scenarios, due to the lack of available data on possible foodborne outbreaks regarding these ready-to-eat meals, which is in line with data from WHO (2015), in which no deaths for STEC, ETEC and EPEC diarrhea's outcome were estimated for Portugal.

In the "Worst Case" scenario, STEC infection is expected to have a burden of 45.66 (31.68; 62.99) DALYs, ETEC infection of 0.71 (0.49; 0.98) DALYs, and EPEC infection of 7.87×10^{-3} (5.46×10^{-3}; 0.01) DALYs (Tables 4–6).

Considering the "Adjusted" scenario (Tables 4–6), STEC infection is expected to have a burden of 0.63 (0.23; 1.38) DALYs, ETEC infection of

Table 4
YLDs, YLLs and DALYs of STEC infection per health outcome.

Burden of Disease of STEC infection (Median [95%])				
Health Outcome			Scenario	
			"Worst Case"	"Adjusted"
Gastroenteritis	Mild Diarrhea	YLD	1.46 [1.01; 2.01]	0.02 [7.41 x 10^{-3}; 0.04]
		YLL	0	0
		DALY	1.46 [1.01; 2.01]	0.02 [7.41 x 10^{-3}; 0.04]
	Moderate Diarrhea	YLD	1.08 [0.75; 1.50]	0.01 [0.01; 0.03]
		YLL	0	0
		DALY	1.08 [0.75; 1.50]	0.01 [0.01; 0.03]
	Severe Diarrhea	YLD	0.17 [0.12; 0.23]	2.29 x 10^{-3} [8.53 x 10^{-4}; 5.11 x 10^{-3}]
		YLL	0	0
		DALY	0.17 [0.12; 0.23]	2.29 x 10^{-3} [8.53 x 10^{-4}; 5.11 x 10^{-3}]
Hemolytic Uremic Syndrome (HUS)	YLD		0.20 [0.14; 0.28]	2.74 x 10^{-3} [1.02 x 10^{-3}; 6.11 x 10^{-3}]
	YLL		26.28 [18.23; 36.25]	0.36 [0.13; 0.80]
	DALY		26.48 [18.37; 36.53]	0.36 [0.13; 0.81]
End Stage Renal Disease (ESRD)	YLD		12.21 [8.47; 16.84]	0.17 [0.06; 0.37]
	YLL		4.26 [2.96; 5.88]	0.06 [0.02; 0.13]
	DALY		16.47 [11.43; 22.72]	0.23 [0.08; 0.50]
Total DALYs			45.66 [31.68; 62.99]	0.63 [0.23; 1.38]
DALYs/person/year (Median [95% CI]) (Total DALYs/*PE*)			0.30 [0.21; 0.42]	4.2 x 10^{-3} [1.53 x 10^{-3}; 9.23 x 10^{-3}]

Table 5
YLDs, YLLs and DALYs of ETEC infection per health outcome.

Burden of Disease of ETEC infection (Median [95%])				
Health Outcome			Scenario	
			"Worst Case"	"Adjusted"
Gastroenteritis	Mild Diarrhea	YLD	0.53 [0.37; 0.73]	0.03 [0.02; 0.05]
		YLL	0	0
		DALY	0.53 [0.37; 0.73]	0.03 [0.02; 0.05]
	Moderate Diarrhea	YLD	0.16 [0.11; 0.23]	0.01 [0.01; 0.02]
		YLL	0	0
		DALY	0.16 [0.11; 0.23]	0.01 [0.01; 0.02]
	Severe Diarrhea	YLD	0.01 [0.01; 0.02]	8.02 x 10^{-4} [4.96 x $10^{-4;}$ 1.25 x 10^{-3}]
		YLL	0	0
		DALY	0.01 [0.01; 0.02]	8.02 x 10^{-4} [4.96 x 10^{-4}; 1.25 x 10^{-3}]
Total DALYs			0.71 [0.49; 0.98]	0.04 [0.03; 0.07]
DALYs/person/year (Median [95% CI]) (Total DALYs/*PE*)			4.74 x 10^{-3} (3.29 x 10^{-3}; 6.54 x 10^{-3})	2.82 x 10^{-4} (1.74 x 10^{-4}; 4.40 x 10^{-4})

Table 6
YLDs, YLLs and DALYs of EPEC infection per health outcome.

Burden of Disease of EPEC infection (Median [95%])				
Health Outcome			Scenario	
			"Worst Case"	"Adjusted"
Gastroenteritis	Mild Diarrhea	YLD	0.01 [4.09 x 10^{-3}; 0.01]	8.89 x 10^{-4} [5.86 x 10^{-4}; 1.30 x 10^{-3}]
		YLL	0	0
		DALY	0.01 [4.09 x 10^{-3}; 0.01]	8.89 x 10^{-4} [5.86 x 10^{-4}; 1.30 x 10^{-3}]
	Moderate Diarrhea	YLD	1.82 x 10^{-3} [1.27 x 10^{-3}; 2.25 x 10^{-3}]	2.75 x 10^{-4} [1.81 x 10^{-4}; 4.01 x 10^{-4}]
		YLL	0	0
		DALY	1.82 x 10^{-3} [1.27 x 10^{-3}; 2.25 x 10^{-3}]	2.75 x 10^{-4} [1.81 x 10^{-4}; 4.01 x 10^{-4}]
	Severe Diarrhea	YLD	1.49 x 10^{-4} [1.04 x 10^{-4}; 2.06 x 10^{-4}]	2.25 x 10^{-5} [1.48 x 10^{-5}; 3.28 x 10^{-5}]
		YLL	0	0
		DALY	1.49 x 10^{-4} [1.04 x 10^{-4}; 2.06 x 10^{-4}]	2.25 x 10^{-5} [1.48 x 10^{-5}; 3.28 x 10^{-5}]
Total DALYs			7.87 x 10^{-3} [5.46 x 10^{-3}; 0.01]	1.19 x 10^{-3} [7.83 x 10^{-4}; 1.73 x 10^{-3}]
DALYs/person/year (Median [95% CI]) (Total DALYs/*PE*)			5.25 x 10^{-5} (3.64 x 10^{-5}; 7.24 x 10^{-5})	7.91 x 10^{-6} (5.22 x 10^{-6}; 1.15 x 10^{-5})

0.04 (0.03; 0.07) DALYs and EPEC infection of 1.19 x 10^{-3} (7.83 x 10^{-4}; 1.73 x 10^{-3}).

In general, results revealed considerable DALYs values, with the highest overall burden of *E. coli* infection obtained for STEC, even when the proportion is adjusted, given its virulence and pathogenic effects. The median DALYs per person per year (pppy) for *E. coli* ranged from 10^{-6} to 10^{-3} (Tables 4–6) considering the "Adjusted" scenario.

Different health outcomes had different contributions to the total burden of disease. The major contributions to DALYs of STEC were HUS and ESRD. Although these two clinical outcomes presented the lowest number of cases in the "Worst Case" scenario, and virtually none in the "Adjusted" scenario, the severity and consequences associated with its occurrence are considerable, with higher values of disability weights and case-fatality ratios, as this is a probability-based approach.

For the ETEC/EPEC infection, mild diarrhea was the major cause of the associated burden.

3.3. Sensitivity analysis

Sensitivity analysis of the STEC model (Fig. 3) indicated that the main contributors to risk variability were the prevalence of the *E. coli* group and the number of people exposed to the hazard while the other considered parameters had no significant contribution in terms of main and total effect.

Sensitivity analysis of the ETEC model (Fig. 4) revealed that the number of people exposed to the hazard and the *E. coli* concentrations were the main contributors to risk variability, while the other considered parameters had no significant contribution in terms of main and total effect.

In the EPEC model (Fig. 5), sensitivity analysis revealed as main contributor to risk variability the *E. coli* concentration, while the other considered parameters had no significant contribution in terms of main effect; still, the number of people exposed to the hazard contributed slightly to the total effect.

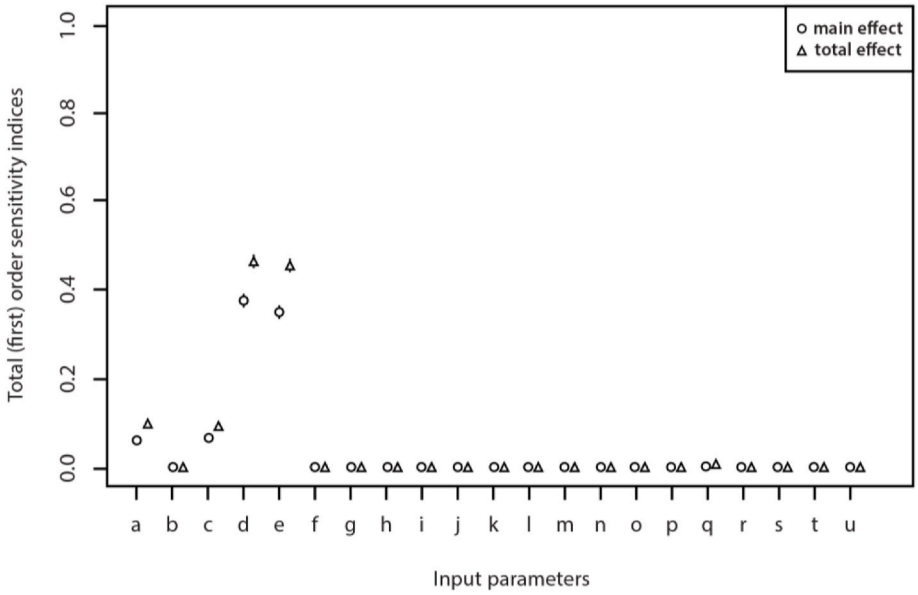

Fig. 3. Sensitivity analysis for the STEC model. Input parameters as follows: a- *E. coli* concentrations; b-meal portion; c-prevalence of contaminated portions; d-prevalence of *E. coli* group; e−number of people exposed to the hazard; f-duration of STEC induced diarrhea; g-probability of developing 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.

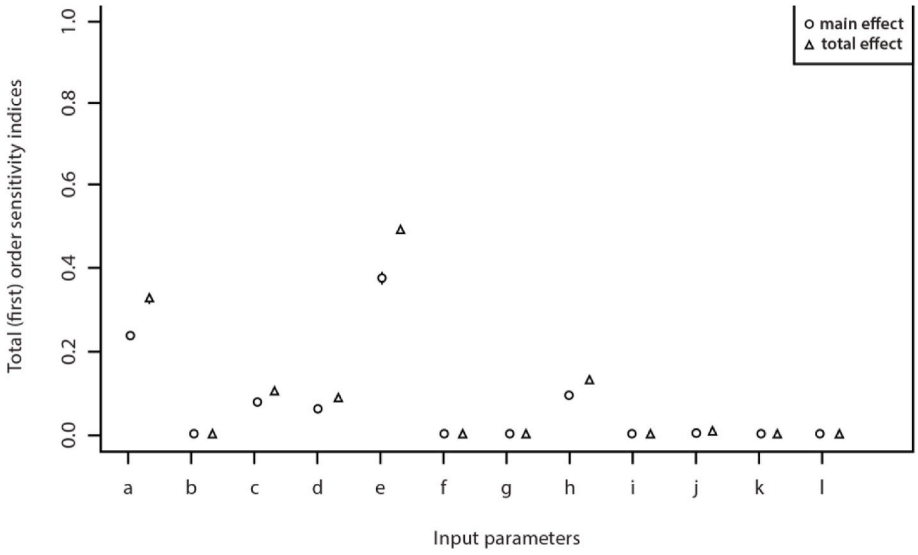

Fig. 4. Sensitivity analysis for the ETEC model. Input parameters as follows: a- *E. coli* concentrations; b-meal portion; c-prevalence of contaminated portions; d-prevalence of *E. coli* group; e−number of people exposed to the hazard; f-duration of ETEC induced diarrhea; g-probability of developing 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.

4. Discussion

A scenario-based approach was used in this study 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 during a two-year period. 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 molecular-based identification of isolates from food samples was not performed by the food microbiology laboratory. In this type of dose-response model, different age-groups responses can be included. Still, when age-groups are not coincident with the ones observed in the exposed population, limitations occur, as children and adults response may differ, and the clinical outcome may be dependent on the demographic of the affected population (Sperandio & Hovde, 2015).

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. Additionally, most of the available dose-response models 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; Nauta et al., 2007). Moreover, the presence of genes by itself does not translate into *in vivo* pathogenicity, since there is a variety of environmental factors that influence gene expression. Considering ETEC, factors such as bile, pH, bicarbonate, osmolarity, glucose and intestinal oxygen availability modulate gene regulation (Crofts et al., 2018). Regarding STEC, lactic acid, butyric acid, formic acid, probiotic bacteria, colicins, microcins and vitamin B12 have been proposed as factors that regulate *stx* expression (Nawrocki et al., 2020). The diversity of these factors within the host, the overall health condition and the uncertainty associated with QMRA methods contribute to possible variations of the calculated

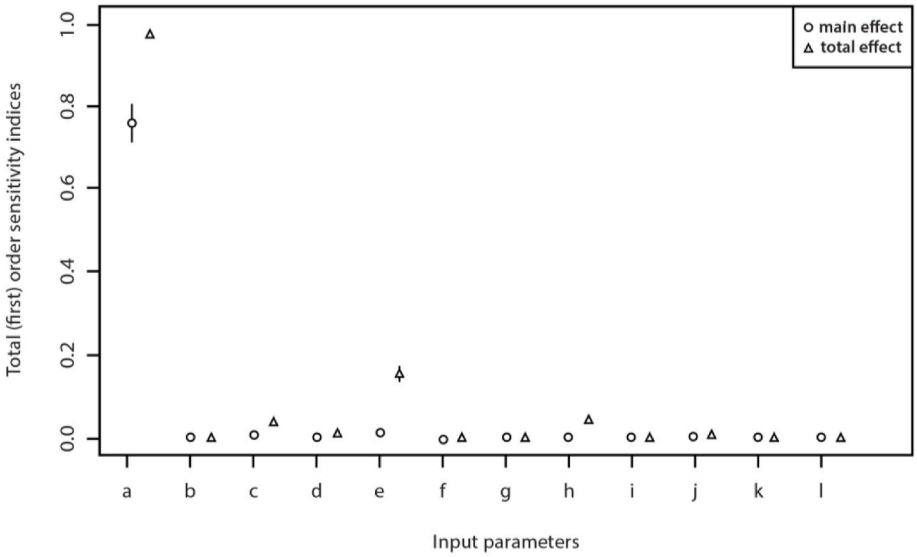

Fig. 5. Sensitivity analysis result for the EPEC model. Input parameters as follows: a- *E. coli* concentrations; b-meal portion; c-prevalence of contaminated portions; d-prevalence of *E. coli* group; e–number of people exposed to the hazard; f-duration of EPEC induced diarrhea; g-probability of developing 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.

risk, number of cases and respective clinical outcome.

In this study, some uncertainties were considered, namely the fact that the used *E. coli* quantification method did not allow to discriminate EHEC, and the fact that the available database did not discriminate countings <10 CFU. Because of this, some solutions have been proposed, such as to replace the non-detects with zero, log-linear extrapolation or substituting the non-detections with the limit of detection, although there is still a lack of agreement on how results below the limit of detection should be treated (Owens et al., 2020). These uncertainties enhance the need for interaction between epidemiology and QMRA, and the epidemiological approach should be country specific, in order to increase the chances of better adjustment of projected values to reality.

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 $1.46 \times 10^{-9} - 1.88 \times 10^{-2}$ 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 and 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 used and analyzed bacterial pathogen.

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 include the prevalence of each *E. coli* pathotype considered in this study, since no molecular identification was performed in food isolates. Therefore, the final output values of DALYs for the built "Adjusted" scenario could be considered as the ones closer to reality, by including each pathotype's prevalence and representing the expected burden for the three considered pathotypes. However, as no values for the acceptable risk of food-related infections are available, comparisons are difficult to perform. It would be most useful if acceptable risk for foodborne infections was established, but these metrics are troublesome to set from a social, economic, and political standpoint. When explicitly stating a level of safety, a level of unsafety is also implicitly set. Consumers, food business operators, and politicians will have to deal with a certain level of unsafety, which will be challenging to accept. To overcome this problem, a new and integrated approach in risk assessment, risk management, and risk communication is required (Manfreda & De Cesare, 2014). The WHO has set a maximum acceptable level of risk of 10^{-6} DALYs per person per year (pppy) for all water-related illnesses, translating into a value of tolerable burden of disease (WHO, 2011). Considering this study's median DALYs pppy for *E. coli* infection in the "Adjusted" scenario - ranging from 10^{-6} to 10^{-3} (Tables 4–6), these are above the limit stipulated by WHO (2011) for water-related illnesses, although, as previously mentioned, a limit value for food matrixes is still lacking and differences may exist. Furthermore, studies describing to have performed molecular analysis for *E. coli* pathotype classification have been performed in countries outside the European Union, and the proportion of each pathotype in different geographical locations may change. These factors can contribute to an over or underestimate of the expected number of cases and health metrics estimates attributable to each pathotype. This reveals the need of further molecular identification of *E. coli* food isolates beyond culture methods, to increase the knowledge of each pathotype's real prevalence.

Regarding the sensitivity analysis, results revealed that the factor whose variability contributed the most to the final variability of the STEC model output (DALYs) was the prevalence of the *E. coli* pathotype (Fig. 3), which can be associated to STEC infectious dose, considered to be < 100 CFU for O157:H7 (Smith et al., 2014). Therefore, *E. coli* concentrations do not contribute considerably to variability, because infection or illness is estimated to occur with a relatively low number of ingested microorganisms. For the ETEC model, the factor contributing the most to variability was the number of people exposed to the hazard (Fig. 4), and for the EPEC, the factor that contributed the most to variability was *E. coli* concentrations (Fig. 5). In the ETEC model, variability in disability weight for mild diarrhea also had a contribution to the overall variability of the model, in contrast with the STEC model, for which none of the parameters from the YLD equation had a significant impact. Results for the STEC models differ from the ones obtained for the EPEC model, in which *E. coli* concentration was the factor with the highest impact, probably due to the number of organisms necessary to initiate an infection or illness. For EPEC induced infection or illness, it is postulated that a large inoculum, of approximately 10^{8}-10^{10} bacteria, is needed to cause infection in adults (Landraud & Brisse, 2010; Mellies et al., 2007), 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. In none of the models the variation of the ingested portion considering a full dose (450 g) or half a dose (225 g) contributed to the models final output variability.

5. Conclusions

In this work, 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. A stochastic QMRA model, considering STEC, ETEC, and EPEC pathotypes was used. The burden of disease was also calculated, revealing considerable DALY values in any of the built scenarios, providing important initial information for a better characterization of the health impacts of *E. coli* infection in institutional canteens. Mild diarrhea was the most expected clinical outcome of infection with the considered *E. coli* groups, and no cases of HUS or ESRD were expected to arise from the consumption of this study's meals. Regarding sensitivity analysis, different input variables have different contributions for each of the developed models.

This study draws attention to an innovative approach combining quantitative microbial risk assessment and health metrics estimates. Although a long way has yet to be wandered, this scenario-based approach can be useful for future studies involving burden of disease estimation, while contributing to a better understanding of *E. coli* in ready-to-eat foods, its potential consequences and impact in consumer's health.

Funding

This work was supported by the Portuguese Foundation for Science and Technology (FCT), throughs grants UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4AnimalS).

CRediT authorship contribution statement

Leonor Antunes: Methodology, Software, Formal analysis, Investigation, Writing – original draft, preparation, All authors have read and agreed to the published version of the manuscript. **António Lopes João:** Methodology, Investigation, Resources, All authors have read and agreed to the published version of the manuscript. **Telmo Nunes:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **Ana Rita Henriques:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Supervision, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A

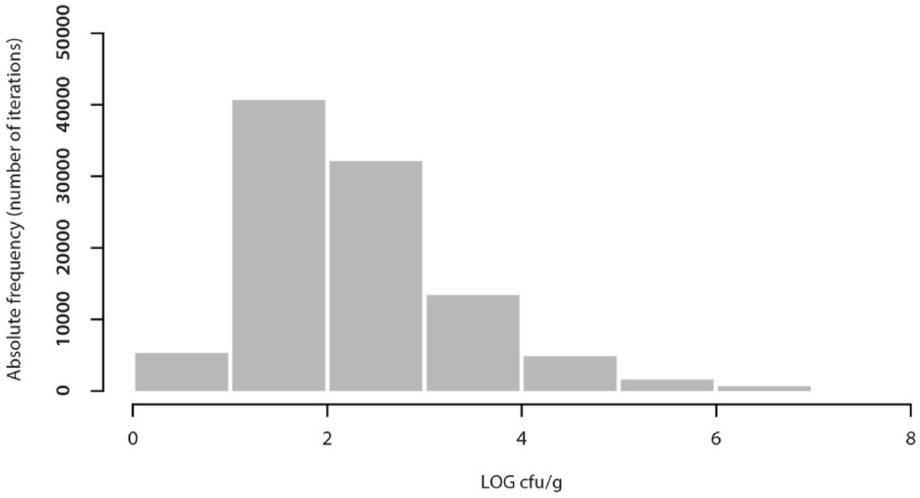

Fig. A1. Histogram of *E. coli* concentrations (log CFU/g) in meals during the assessed two-year period.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114450.

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