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# Microbial risk assessment of *Escherichia coli* shiga-toxin producers (STEC) in raw sheep's milk cheeses in Italy

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ABSTRACT

Shiga toxin-producing *E. coli* (STEC), widespread pathogens associated with severe foodborne disease, can contaminate milk during the milking process through faecal matter and survive or grow during cheese making if a pasteurization treatment has not been applied. Thus, a stochastic "farm-to-fork" model was developed to assess the risk of human infection by O157 STEC, one of the main pathogenic serotypes, associated with the consumption of a portion of raw sheep's milk cheese produced in a farmhouse dairy in Italy. The average risk of illness after the consumption of a portion of brief-, medium- and long-ripened cheese ranged between $1.64 \times 10^{-4}$ and $4.03 \times 10^{-4}$ for adults. Considering only a difference in serving size, the risk for children varied from $1.35 \times 10^{-4}$ to $3.34 \times 10^{-4}$. Among the several intervention strategies simulated to mitigate the risk, administration of bacteriophages was, by far, the most effective measure with an average risk reduction of 34 times followed by use of probiotics and antimicrobials, which lowered the risk about 12 times. The sensitivity analysis showed that the probability that a shedder is present in the herd, the occurrence of the milk contamination with faeces and the within-herd prevalence of the pathogen were the parameters that most affected the risk. While further data is necessary to confirm the conclusion of this study, the model results might be able to assist producers and policymakers to manage the risk of STEC infection linked to such products.

## 1. Introduction

*Escherichia coli* are well-known gram-negative bacteria of the normal gastrointestinal flora of a wide range of warm-blooded animals (EFSA, 2020). Although they are considered non-pathogenic, some strains can exhibit virulence factors that can lead to human illness, such as Shiga toxin-producing *E. coli* (STEC), widespread pathogens associated with severe foodborne disease. These organisms produce shiga toxin types 1 and 2 (encoded by the *stx* virulence genes) able to cause a variety of illnesses in humans, from mild diarrhoea to haemorrhagic colitis (HC). In the most severe cases, thrombocytopenia can occur as well as haemorrhagic uremic syndrome (HUS), particularly in young children, which is the leading cause of renal failure. STEC can also carry the intimin-encoding gene (*eae*) which enables these strains to cause attaching and effacing (A/E) lesions in infected cells thus exacerbating the clinical signs (EFSA, 2020).

Human infections are mainly foodborne (e.g., from undercooked raw meats, dairy products, vegetables and drinking water), although environmental and direct person-to-person or animal-to-person infections are also confirmed. Livestock, mainly cattle and small ruminants, can be healthy carriers of these bacteria and represent a major reservoir of STEC for humans (Henry et al., 2017); raw ingredients and food can be contaminated with STEC through faecal contamination of fields (for vegetables) or during the slaughter process (from fleece to carcases). In addition, STEC can contaminate milk, through faecal matter during milking, and survive or grow during cheese-making in some processing technologies, particularly in unpasteurized (raw) milk cheeses.

Although sheep are a potential source of human infection through faecal shedding, few studies about STEC prevalence are available for this species. Most of those studies focused only on serotype O157, while ignoring other serotypes that are frequently responsible for human infection (EFSA & ECDC, 2019). For example, herd prevalence of O157 STEC in sheep was found to be around 8.5% in Spain and Greece using culture methods (Oporto, Esteban, Aduriz, Juste, & Hurtado, 2008; Pinaka et al., 2013), while a year-long survey revealed that O157:H7

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STEC was isolated in the faeces of 38 out of 533 adult sheep (7.3%) slaughtered in an Italian abattoir (Franco et al., 2008). Data on STEC prevalence in sheep's milk are scarce, but the presence of these bacteria in milk is confirmed. In Spain, Rey et al. (2006) isolated four different non-O157 STEC strains from 287 samples (1.4%) taken from the bulk tank of 64 dairy farms, while Otero et al. (2017) found a much higher prevalence, also using a culture method (8.8%, 34/388). A study conducted in Greece estimated a 0.84% O157 STEC occurrence (i.e. isolated strains) in bulk milk tanks on farms (Solomakos et al., 2009). The few investigations concerning the occurrence of viable STEC in sheep's cheese performed using culture methods show rather uneven results (Farrokh et al., 2013; Marozzi et al., 2016). No STEC positive sheep's cheese samples were found in a large sampling of different food matrices in Scotland (Coia, Johnston, Steers, & Hanson, 2001) and no O157 STEC positive samples were isolated from raw sheep's milk cheese collected at retail in Italy (Marozzi et al., 2016). In contrast, researchers from Spain and Switzerland reported a STEC prevalence in sheep's milk cheese samples of 3.6% and 9.1%, respectively (Caro & Garcia Armesto, 2007; Stephan et al., 2008).

Human outbreaks due to the consumption of raw milk cheeses have been reported worldwide but the majority were specifically related to cow's milk cheeses (Currie et al., 2018; Honish et al., 2005; McCollum et al., 2012). To our knowledge, no outbreaks of O157 STEC from sheep's cheese in Europe have been reported in the scientific literature, although at least one outbreak linked to the consumption of cheese made with small ruminant's milk has been described by Espié et al., 2006 (fresh unpasteurized goat's cheese). However, the above-mentioned data referring to sheep species, along with several notifications reported by the European Union's (EU) Rapid Alert System for Food and Feed (RASFF) about the presence of STEC in untreated sheep's milk cheese, pose important concerns regarding public health, in particular for those countries where these products are commonly consumed.

In Europe, the sheep's milk sector has a significant economic impact and represents an important resource for many farmers. Sheep's milk production is concentrated mainly in the Southern European countries, such as Greece, Spain, and Italy, but also in Bulgaria, France, and Romania. In the EU, ewe's milk production is around 2.8 million tonnes per year (EUROSTAT, 2018), 17% of which is produced by Italy alone (around 463 thousand tons). Sheep's milk in Italy is completely reserved for cheese-making, approximately 75.8 thousand tons per year (ISMEA, 2020). This production is concentrated in a patchwork of regions linked to high-quality traditional products, namely Sardinia, Sicily, Lazio and Tuscany, and is often conducted in small local dairies that do not pasteurize the milk to preserve the characteristics of the cheese-like flavour and aroma, as well as to protect the authenticity of the product together with its traditional recipe. Hence, human exposure to STEC through the consumption of raw sheep's milk cheeses is possible and preventive measures should be adopted by risk managers if an unacceptable risk for consumers is assessed.

In this context, quantitative microbial risk assessments (QMRAs) are considered an effective tool to evaluate food-related health risks associated with foodborne pathogens. Different QMRAs have been developed to determine the risk of *E. coli* O157 infection in several foods, including beef meat (Cassin, Lammerding, Todd, Ross, & McColl, 1998; Delignette-Muller & Cornu, 2008; Smith, Fazil, & Lammerding, 2013), vegetables (Kundu, Wuertz, & Smith, 2018; Pang, Lambertini, Buchanan, Schaffner, & Pradhan, 2017) and milk (Giacometti et al., 2012; Ntuli, Njage, Bonilauri, Serraino, & Buys, 2018). In 2015, Perrin et al. published the first and, so far, unique quantitative assessment of the risk of developing HUS linked to the consumption of cow's milk cheeses contaminated with O157 and non-O157 STEC serotypes (Perrin et al., 2015). However, no QMRAs have been developed so far for sheep's cheeses.

The aim of this study is to assess the risk of infection by O157 STEC associated with the consumption of cheeses made with raw sheep's milk in Italy and the reduction in this risk by applying intervention strategies along the food chain. The model was built using data referring only to the O157 serotype because of the relative abundance of data compared to other serotypes but it can be adapted to non-O157 STEC.

## 2. Materials and methods

### 2.1. Model overview

On the basis of *Codex Alimentarius* guidelines, a stochastic "farm-to-fork" model is developed to estimate the risk of human infection by O157 STEC associated with the consumption of a portion of raw sheep's milk cheese produced in a farmhouse dairy in Italy. The simulation considers a situation where milk is collected from only one farm annexed to the dairy and it is entirely used for the production of a single batch of cheese. This could be considered a worst-case scenario as the bulk tank milk is not diluted with milk from other farms (Condoleo et al., 2017; FDA, & Health Canada, 2015). Regarding the investigated hazard, we defined STEC as the only *E. coli* that possess the *stx* and *eae* gene since epidemiological data seems to demonstrate that human cases are caused almost totally by STEC with this biomolecular profile (EFSA et al., 2020).

The variation in prevalence and concentration of the microorganism along the exposure pathway is described through three modules: (i) contamination during milk collection at farm level, (ii) the cheese-making process, (iii) home consumption (consumer intake) (see Fig. 1).

The outcome of the first module is an estimate of the concentration of

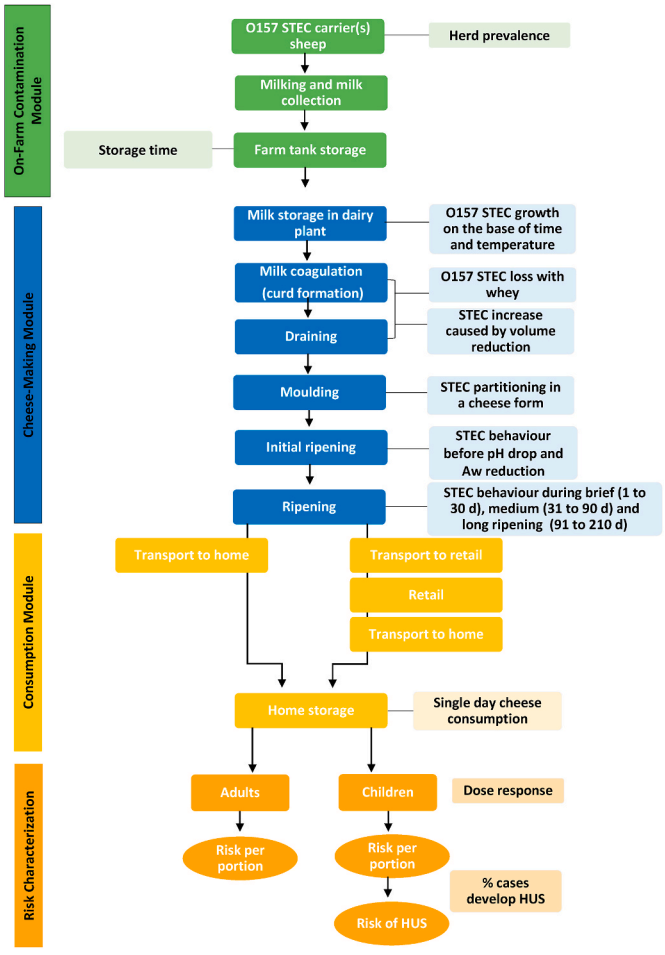


**Fig. 1.** Flow chart of the quantitative risk assessment model for O157 STEC in three types of raw sheep's milk cheese (brief (1–30 days), medium (31–90 days) and long ripening (91–210 days)). Central boxes are the steps of the exposure pathway, lateral boxes the main parameters, and in circles the final outputs.

O157 STEC in milk after the collection on farm. Thus, indirect data about the amount of faecal contamination in the bulk tank milk were used to estimate milk contamination by STEC, together with data on herd and within-herd STEC prevalence. In addition, collected milk is assumed to be transported immediately to the dairy after the last milking session in accordance with common practices of small artisanal dairies. The second module simulates the process of cheese-making and the concentration of O157 STEC during the manufacturing process. The risk is differentiated for three types of cheese in relation to the length of the ripening period: brief, medium and long-ripened cheese. The third module assesses the probability and the level of contamination at the time of consumption considering the change in concentration of O157 STEC in cheese after the purchase and the size of the ingested portion.

Finally, an additional fourth module ("risk characterization") combines the estimated pathogen concentration in a portion with a dose response function to compute the final outputs, which are the risk estimates of STEC infection per single day consumption of, (i) a random portion of traditional Italian cheese made from raw sheep's milk, and (ii) a portion made with milk from a farm with infected animals, as well as (iii) the risk for children to develop HUS based on the type of cheese consumed.

In addition, alternative scenarios are evaluated in order to explore the impact of mitigation actions occurring before or after milking on the final risk. Moreover, a sensitivity analysis is performed to detect which parameters of the framework have most influence on the risk of infection. The model is developed using a Microsoft ® Excel spreadsheet and the simulations were run (250,000 iterations) with @Risk software (Palisade Corporation, Ithaca, NY, USA, v. 6.2).

### 2.2. On-farm contamination module

The first step in the pathway in Fig. 1 is whether there is at least one O157 STEC carrier present in the single farm that is providing the milk for the production of cheese. Presence of at least one O157 STEC carrier on the single farm is represented by the indicator variable $N_{posherd}$, which is determined by the probability, $P_{herd}$, i.e. $N_{posherd} \sim Bern(P_{herd})$. This probability is estimated using the results from an extensive survey which studied the occurrence of STEC in faeces (Oporto et al., 2008). The next step is to determine the amount of STEC eventually present in the bulk milk, if the farm is contaminated. At present, existing data are insufficient to determine the probability of contamination of the collected bulk milk, as well as the extent of such contamination, based upon the presence of O157 shedders on a sheep farm. Therefore, similar to a previous QMRA (Perrin et al., 2015), we assumed that STEC have the same dynamic as non-pathogenic *E. coli* – infected animals excrete STEC in their faeces and milk can be contaminated during the milking process. Thus, both the occurrence and concentration of O157 in a bulk tank on a positive farm are determined by considering faeces as a unique source of contamination and, consequentially, are dependent on the eventual presence and quantity of faeces in raw milk. However, it is impossible to quantify and determine the presence of faecal matter in bulk milk through specific laboratory analysis. Therefore, the model assesses the occurrence and concentration of faeces (g per ml) in bulk milk using an indirect approach based on the presence of *E. coli*, a reliable indicator of the presence of faeces (Perrin et al., 2015; Ribeiro Júnior et al., 2019).

The final concentration of O157 *E. coli* in bulk milk, $S_{bulk}$ (CFU/ml), that will be used by the farmhouse dairy is calculated by multiplying together the number of O157-positive sheep that contaminate the milk with faeces ($N_{fcO157}$), the faecal concentration in milk from a single sheep ($F_{sheep}$), and the average O157 STEC concentration in faeces for these infected sheep, $S_{milk}$ (Fig. 2). That is,

$$S_{bulk} = N_{fcO157} * F_{sheep} * S_{milk} \quad (1)$$

To calculate the first term, namely the number of O157-positive sheep that contaminate the milk with faeces, $N_{fcO157}$, the number of sheep on farm that contaminate the bulk milk with faeces, $N_{fc}$, is used within a Binomial alongside the within-herd prevalence of O157 STEC, $P_{wherd}$ (Oporto et al., 2008) i.e,., $N_{fcO157} \sim Bin(N_{fc}, p_{wherd})$. However, there are no studies that have investigated the proportion of milked animals that contaminate the bulk milk after each milk collection. Therefore, to calculate $N_{fc}$, it was assumed that each milked animal $N_l$ has the same probability, 50%, to contribute to the faecal contamination, i.e., $N_{fc} \sim Bin(N_l, 0.5)$.

The faecal concentration in raw milk deriving from a single milked sheep, $F_{sheep}$, requires a few calculation steps. Firstly, whether raw milk collected on a single dairy farm is contaminated, $N_{EC}$, is calculated under the assumption that *E. coli* cells in a farm bulk tank exclusively derive from direct and/or indirect contamination with faecal material during the milking phase (Perrin et al., 2015). Therefore, it is calculated using the probability of *E. coli* occurrence in bulk milk, $P_{milk}$, reported by Condoleo et al. (2020) i.e., $N_{EC} \sim Bern(P_{milk})$. When a faecal contamination has been predicted ($N_{EC} = 1$), the faecal concentration in raw milk, $F_{milk}$ (g of faeces/ml), is estimated by dividing the concentration of *E. coli* (CFU/ml) in raw milk from a sheep farm in Italy, $EC_{milk}$ (Condoleo et al., 2020), by the concentration of *E. coli* that is normally present in sheep faeces (CFU/g), $EC_{faeces}$ (Moriarty et al., 2011). That is $F_{milk} = EC_{milk}/EC_{faeces}$. This produces a concentration based on bulk milk; in order to compute the faecal concentration in raw milk deriving from a single milked sheep ($F_{sheep}$), $F_{milk}$ is divided by the number of sheep that contaminate the bulk milk $N_{fc}$, which was computed above, based on the

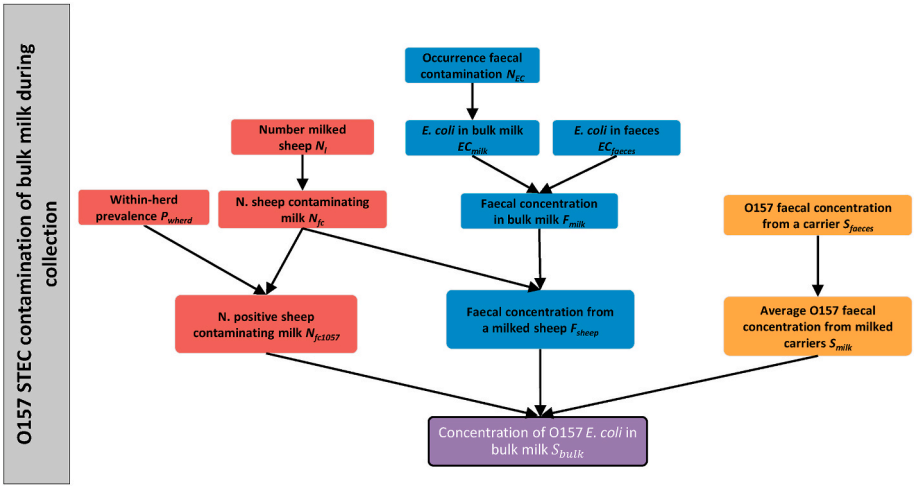


**Fig. 2.** Details of the model flow chart for the On-Farm contamination Module.

assumption that each animal contributes an equal amount of faeces.

Lastly, we need to calculate the mean concentration of O157 *E. coli* cells per gram of faeces eliminated by a shedder animal, $S_{milk}$. This is calculated using the Risk Compound function in @Risk (Palisade, 2021) so that for each of the involved shedders of faeces into the milk $N_{fcO157}$, a separate value for the concentration in faeces from an infected sheep $S_{faeces}$ (CFU/g of faeces), is drawn from a distribution and summed; the average is then calculated by dividing by the number of shedders, $N_{fcO157}$. This function is used in order to incorporate the variability amongst the shedders. The distribution for $S_{faeces}$ was modelled using data from an investigation in adult sheep at slaughter from Italy (Franco et al., 2008) and takes this variability into account.

In this module, it is assumed that faecal material, and consequently *E. coli* cells, are uniformly distributed in the mass of milk collected into the farm tank and the storage time of bulk milk in a farm tank is null. All parameters of this module are listed in Table 1.

**Table 1**
Parameters of on-farm contamination module.

| Description | Variable | Unit | Value/ Distribution | Source |
|---|---|---|---|---|
| Probability that there is present at least one O157 carrier | $P_{herd}$ | Proportion | 0.087 | Oporto et al. (2008) |
| Probability that each ovine is a O157 carrier within a positive herd | $P_{wherd}$ | Proportion | 0.073 | Oporto et al. (2008) |
| Probability that bulk tank milk is contaminated with *E. coli* after the milking of the animals | $P_{milk}$ | Proportion | 0.61 | Condoleo et al. (2020) |
| *E. coli* concentration in contaminated raw bulk milk from an ovine farm | $EC_{milk}$ | Log CFU/ ml | $10^X$ where X = Cumulative Distribution(0; 4.11;{0; 0.30; 0.47; 0.65; 0.93; 1.18; 1.34; 1.64; 2.10; 2.65; 3.41};{0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9; 0.95) | Condoleo et al. (2020) |
| *E. coli* concentration in ovine faeces | $EC_{faeces}$ | Log CFU/g | $10^Y$ where Y = Triangular (5.98; 7.48; 8.97) | Moriarty et al. (2011) |
| Amount of O157 STEC in faeces excreted by an ovine that harbours such bacteria at intestinal level | $S_{faeces}$ | CFU/g | Cumulative Distribution (0.04, 1500000; {99; 999; 9999; 99999; 999999}; {0.631; 0.71; 0.921; 0.947; 0.973})) | Franco et al. (2008) (provided us with specific data) |
| Number of sheep per herd | $N_l$ | Number | Cumulative Distribution(9; 2500;{0.05; 0.1; 0.15; 0.2; 0.25; 0.3; 0.35; 0.4; 0.45; 0.5; 0.55; 0.6; 0.65; 0.7; 0.75; 0.8; 0.85; 0.9; 0.95}; {9; 28.5; 30; 40; 50; 60; 70; 80; 100; 100; 120; 130; 144; 150; 168; 200; 200; 229; 288; 315; 1090}) | Mezher, Titarenko, Morena, Giangolini, and Condoleo (2022) |

### 2.3. Cheese-making module

During the cheese-making phase, raw milk undergoes a sequence of treatments finalized in the production of cheese forms. The number, typology and process parameters of such treatments are specific to each type of cheese because they determine the organoleptic characteristics of the desired final product. Consequently, it is not possible to outline a cheese-making framework that is valid for all products, considering the numerous existing types of sheep's milk cheese. Fig. 1 illustrates the steps that are simulated in this QMRA; they were selected because they are essential for producing cheese and, based on an investigation of the literature, can potentially impact on the prevalence and concentration of STEC.

After harvest, bulk milk is immediately moved from the farm to the dairy facilities and, before processing, it is stored for a maximum of 24 h ($Time_{milk}$). During this time, O157 STEC is assumed to grow in milk if the temperature is above 4 °C ($T_{min}$; de Garnica, Santos, and Gonzalo (2011)) at a specific Exponential Growth Rate (*EGR)* (Log CFU/h). To estimate this parameter, we extracted 78 growth curves from Combase database (ComBase, 2021) concerning the behaviour of *E. coli* on both whole and skimmed raw milk at temperatures ranging from 4 to 40 °C. A simple linear approach, assuming absence of a lag phase, was adopted as the primary model to estimate the EGR for each curve (*EGR(T)*) at the corresponding experimental temperature (*T)* (FDA, & Health Canada, 2015). Then, a secondary model based on a linear relationship between temperature and square root of bacterial growth (Ratkowsky, Olley, McMeekin, & Ball, 1982) was used to standardize any *EGR(T)* at the reference temperature $T_{ref}$ of 5 °C:

$$EGR\left(T_{ref}\right) = EGR(T)*\left(\frac{T_{ref} - T_{min}}{T - T_{min}}\right)^2 \quad (2)$$

We used all $EGR(T_{ref})$ to calculate the average value, which we denote $EGR(5)_{milk}$ (0.00054 Log CFU/h).

Therefore, now we have the reference growth rate, we can compute the growth rate at each model's iteration, using the temperatures and time involved in the storage process. The square root equation is extended in the following manner:

$$G_{storage} = Time_{milk}*EGR(5)_{milk}*\left(\frac{T_{milk} - T_{min}}{5 - T_{min}}\right)^2 \quad (3)$$

That is the overall O157 *E. coli* increase over the storage phase, $G_{storage}$, is given by multiplying $Time_{milk}$, the time during which the milk is stored, by the calculated rate of increase of O157 *E. coli* in milk stored in a dairy (per hour). The temperature of milk during the storage, $T_{milk}$, drawn from a logistic distribution based on data from 115 farmhouse dairies (Mezher et al., 2022). The final O157 STEC concentration after the storage, $S_{storage}$, is given by the sum of $S_{bulk}$ and $G_{storage}$.

The subsequent treatments, namely milk coagulation through the addition of rennet and draining of the curd, determine the transformation of milk (liquid matrix) into curd and then fresh cheese (solid matrix). The concentration of O157 STEC $S_{fcheese}$ after these steps is mainly given by the consequence of two events: 1) the loss of a portion of pathogen cells originally present in milk through whey and 2) the volume reduction of the mass of milk, due to the curdling, which causes a physical concentration of the remaining bacteria. Therefore, first, the model subtracts a proportion of cells $P_{whey}$ from $S_{storage}$ to compute $S_{curd}$, the bacterial concentration of the remaining STEC cells trapped in the curd, i.e., $S_{curd} = S_{storage}*(1\text{-}P_{whey})$. Data regarding the amount of STEC cells lost in whey was provided by two contamination studies that investigate the STEC behaviour during cheese making (D'Amico, Druart, & Donnelly, 2010; Reitsma & Henning, 1996). The STEC concentration in fresh cheese following the volume reduction, $S_{fcheese}$ (CFU/g), is

estimated by converting the original volume of milk (in ml) by the derived amount of cheese (in g):

$$S_{fcheese} = \frac{S_{curd}}{P_{yield}} \quad (4)$$

where $P_{yield}$ is the cheese yield, a ratio between the weight of a certain amount of cheese and the corresponding volume of milk the cheese-maker used for its production. For each iteration, the model draws from a distribution modelled using the minimum and maximum cheese yield reported by several studies. These examined the cheese-making process of different types of cheese made with sheep's milk (Addis et al., 2018; Aldalur, Bustamante, & Barron, 2019; Jaeggi, Wendorff, Romero, Berger, & Johnson, 2005; Vannini et al., 2008).

After the cheese is reduced in volume, the obtained mass of fresh cheese is split up by the cheesemaker and placed in moulds to shape the cheese forms, whose size depends on the type of cheese and commercial preference of the producer. As done by previous QMRAs (Condoleo et al., 2017; FDA, & Health Canada, 2015), the model distributes the O157 STEC cells from a single unit (curd) to a variable number of sub-units (forms) assuming a Poisson portioning process:

$$S_{form} = Poisson\left(S_{fcheese} * C_{weight}\right) \quad (5)$$

where $C_{weight}$ is the weight of a form of cheese produced in farmhouse dairies in Italy as defined by a cumulative distribution that was modelled using data from 110 producers (Mezher et al., 2022). Therefore, $S_{form}$ represents the amount of O157 STEC cells in a single form of fresh cheese after moulding while $S_{cform}$ is the expected concentration (CFU/g) obtained dividing it by the form weight.

During the ripening phase, the behaviour of STEC in cheese is mainly influenced by the decrease of pH and water activity ($aW$), which cause an adverse environment for the pathogen leading to a progressive reduction of *E. coli* concentration over time. Although the drop of both pH and $aW$ starts immediately after the curd formation, data from several studies suggest that the critical conditions affecting the survival of STEC only occur 24 h later (after the initial ripening phase). Therefore, during the initial ripening phase, the pathogen may still be able to grow. The potential growth (or decrease) of the pathogen $G_{irip}$ is estimated using data reported by nine studies (see Appendix for details) resulting in a normal distribution with parameters $Normal(0.56, 1.07)$ min = -0.78, max = 2.89)(Log CFU/g). The concentration after initial ripening, $S_{irip}$, is calculated by adding $G_{irip}$ to $S_{cform}$. We defined a maximum concentration in cheese, *MDP,* that O157 STEC can achieve.

During the rest of the ripening period (secondary ripening), the model assumes that the O157 STEC concentration declines in cheese at 20 °C with a rate (Log CFU/g/day) following a cumulative distribution, $EGR(20)_{cheese}$. This distribution was built adopting the same method previously described to estimate the behaviour of the microorganism in milk but see the Appendix for further details of this model to describe the decrease in STEC during secondary ripening. For each iteration, the model draws a different $EGR(20)_{cheese}$ and uses equation (3) to estimate the overall reduction in STEC concentration during the ripening phase, $S_{srip}$, replacing $EGR(5)_{cheese}$ and the input of 5 with the equivalent for 20 °C, and using the time and temperature values during the cheese ripening in the farmhouse dairy, $Time_{srip}$ and $T_{srip}$, respectively.

The ripening time was set to consider three possible scenarios: brief (1–30 days), medium (31–90 days) and long ripening (91–210 days). Combining the changes to STEC growth during both initial and secondary ripening to the level of STEC prior, $S_{cform}$, results in the final level of the pathogen in the cheese form at the end of the ripening, $S_{srip}$. All parameters of this module are listed in Table 2.

### 2.4. Consumption module

After the end of the ripening period, cheese can be purchased by consumers directly from the cheesemaker or from local retailers, where

**Table 2**
Parameters of cheese-making module.

| Description | Variable | Unit | Value/ Distribution | Source |
|---|---|---|---|---|
| Maximum growth of *E. coli* in raw milk at 5° estimated using a linear approach | $EGR(5)_{milk}$ | Log/h | 0.00054 | ComBase (2021) |
| Minimum growth temperature of *E. coli* in milk and cheese | $T_{min}$ | °C | 4 | de Garnica et al. (2011) |
| Time before cheese-making during which the milk is stored | $Time_{milk}$ | h | Uniform(0; 24) | Mezher et al. (2022) |
| Temperature during the storage period before cheese-making. | $T_{milk}$ | °C | Logistic (3.933; 0.559) | Mezher et al. (2022) |
| Proportion of O157 cells lost during the whey loss | $P_{whey}$ | Proportion | Uniform(0.07; 0.13) | D'Amico et al. (2010); Reitsma and Henning (1996) |
| Ratio between the weight of a certain amount of cheese and the volume of milk originally used for its production | $P_{yield}$ | Number | Uniform(0.18; 0.25) | Addis et al. (2018); Aldalur et al. (2019); Jaeggi et al. (2005); Vannini et al. (2008); |
| Final weight of the form of cheese | $C_{weight}$ | g | Cumulative distribution (150,4500; {0.05; 0.1; 0.15; 0.2; 0.25; 0.3; 0.35; 0.4; 0.45; 0.5; 0.55; 0.6; 0.65; 0.7; 0.75; 0.8; 0.85; 0.9; 0.95,0.99}; {200; 400; 600; 750; 1000; 1000; 2000; 2410; 4137.5}) | Mezher et al. (2022) |
| O157 STEC growth during initial ripening | $G_{irip}$ | Log CFU/g | Normal(0.57; 1.07; min = -0.78, max = 2.89) | Estimated using a model (see Appendix) |
| Maximum concentration that O157 STEC can achieve in cheese | $MPD$ | Log CFU/g | 9 | Assumption |
| Exponential Growth Rate of O157 STEC at 20 °C in cheese | $EGR(20)_{cheese}$ | Log CFU/g/day | Cumulative distribution (−1.8; 0; {-1.42;-0.80;-0.64;-0.60;-0.30;-0.28;-0.24;-0.22;-0.21;-0.16;-0.12;-0.11;- | Estimated using several contamination studies (see Appendix) |

(*continued on next page*)

**Table 2** (*continued*)

| Description | Variable | Unit | Value/ Distribution | Source |
|---|---|---|---|---|
| | | | 0.10;-0.08;-0.08;-0.08;-0.07;-0.04;-0.02;-0.001}; {0.05; 0.1; 0.15; 0.2; 0.25; 0.3; 0.35; 0.4; 0.45; 0.5; 0.55; 0.6; 0.65; 0.7; 0.75; 0.8; 0.85; 0.9; 0.95; 0.99}) | |
| Temperature during the cheese ripening in the farmhouse dairy | $T_{srip}$ | °C | Cumulative distribution (4; 25;{4; 5;7; 8;9.3; 10; 12; 13.1; 16; 17; 20}; {0.05; 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9; 0.95}) | Mezher et al. (2022) |
| Length of the cheese ripening in the farmhouse dairy | $Time_{srip}$ | Day | Brief-ripening: Uniform(1; 30) Medium-ripening: Uniform(31; 90); Long-ripening: Uniform(91; 270)) | Assumption |

it is transported home and consumed before the expiry date. During this phase O157 STEC concentration continues to decrease at the estimated daily rate $EGR(T_{ref})_{cheese}$, which is calculated through equation (3) and is dependent on the temperature values occurring in each step of the consumption process. The overall variation in concentration attributed to each step is obtained by multiplying the corresponding value of time, and then subtracting it from the O157 STEC concentration of the previous step to obtain the new level of the pathogen in cheese.

According to the information provided by Mezher et al. (2022), many of the farmhouse dairies sell their products to consumers at the place of production only (40.5%) while a few producers commercialize them exclusively through local or provincial retailers (6.5%). The remaining dairies (53%) take advantage of both distribution channels. Since we did not have specific trade data, we assumed that half of the product on such dairies is commercialized on farm. Hence, the model simulates that cheese manufactured in an Italian farmhouse dairy has a probability, $P_{dirpurchase} = 0.67$, of being purchased directly from the producer and $P_{indpurchase} = 1\text{-}P_{dirpurchase}$ of being acquired through local retailers. In the first case, we assume that cheese is sold no later than 96 h after being produced ($Time_{purchase}$) and kept at the same temperature adopted during ripening. In the second case, cheese is transported to a local retailer ($Time_{rtransport}$) at refrigeration temperature ($T_{rtransport}$) where is sold within four days ($Time_{purchase}$). After purchase, cheese is transported home by the consumer ($Time_{transport}$) at room temperature ($T_{transport}$), and kept at fridge temperatures ($T_{home}$), until the time of consumption, $Time_{home}$, which we assume occurs before the expiry date. We set a different shelf life $Time_{shelf}$ (days) for brief-, medium- and long-ripened cheese on the basis of the information collected by Mezher et al. (2022). The time of consumption $Time_{home}$ is calculated drawing a random value from such distributions and subtracting the time during which cheese has been kept on sale and transported. For all of the above, the reduction in concentration of cheese during these times, with the corresponding temperatures, is again calculated using Equation (3).

The size of a typical portion ingested by adult individuals ($N_{por}$) is estimated through a cumulative distribution based on a National Food Consumption Survey (Condoleo et al., 2017; Leclercq et al., 2009). We assumed that single day consumption corresponds to a single serving; the curve was built excluding the consumption of cheese as an ingredient in cooked food since STEC would be easily killed by the cooking treatment. We did not have detailed data for children's portion sizes $N_{porch}$, compared to adults and so we reduce the size of adults' portion by a factor $P_{por}$ which reflects the percentile variation in daily consumption between the two populations for cheese (CREA, 2005).

Finally, the amount of STEC cells ingested by adults ($S_{por}$) or children ($S_{porch}$) for a single day consumption is calculated as:

$$S_{por/porch} = Poisson\left(S_{home} * N_{por/porch}\right) \quad (6)$$

where $S_{home}$ is the final O157 STEC concentration in a portion before consumption. All parameters of this module are listed in Table 3.

### 2.5. Risk characterization and risk output

The risk of developing illness after the ingestion of O157 STEC cells is computed using a *Beta-Poisson* dose response relationship (Strachan, Doyle, Kasuga, Rotariu, & Ogden, 2005):

$$R = 1 - \left[1 + \frac{S_{por/porch}}{\beta}\right]^{-\alpha} \quad (7)$$

where $\alpha = 0.0565$, $\beta = 2.5487$ and $S_{por/porch}$ is the previously estimated ingested dose for an adult or child's portion, respectively.

The main output of the simulation is the risk of getting ill after the ingestion of a random portion of raw sheep's milk cheese produced in a generic farmhouse dairy in Italy, for both adults ($R_A$) and children ($R_C$). The model also estimates the risk $R_{posherd}$ due to the consumption of cheese manufactured exclusively in farmhouse dairies where infected animals are present (the simulation is run fixing the parameter $N_{posherd} = 1$). A third output, $R_{HUS}$, represents the risk of children developing HUS, a serious health complication that can emerge after STEC infection in this subpopulation. It is calculated by multiplying $R_c$ by $P_{HUS}$ (*Uniform (0.075;0.133)*), the probability that a child develops HUS after becoming infected (Bell et al., 1997; Cassin et al., 1998; Gould et al., 2009), i.e., $R_{HUS} = R_C{*}P_{HUS}$.

### 2.6. Alternative scenarios (control measures)

Pre- and post-harvest preventive measures are simulated to calculate the variation in risk for adults $R_A$ and to compare the output to the baseline results. Regarding pre-harvest measures, the model reduces the O157 STEC concentration in faeces from positive animals ($S_{faeces}$) on the basis of the efficacy of five different control interventions, namely, administration of vaccines, probiotics, antimicrobials (lactoferrin), sodium chlorate and bacteriophages (Table S1). Currently, all these preventive measures are not commercially available, but they have been tested on sheep and data are published in literature (Callaway et al., 2003; Lema, Williams, & Rao, 2001; Raya et al., 2011; Yekta, Cox, Goddeeris, & Vanrompay, 2011; Yekta, Goddeeris, Vanrompay, & Cox, 2011).

Post-harvest measures are preventive interventions applied after the milk collection on farm and, in this study, consist of testing raw milk or cheese to detect the presence of O157 STEC cells and, in case of contamination, avoiding the distribution of the cheese batch in order to reduce the consumers' exposure to the pathogen. The model simulates three different scenarios during which each batch of milk or cheese is tested at a different sampling point along the production chain: just after the milk storage (raw milk test scenario), after the initial ripening (unripened cheese test scenario) and at the end of the ripening (final product test scenario). Further details of how the pre- and post-harvest

**Table 3**
Parameters of the consumption module.

| Description | Variable | Unit | Value/Distribution | Source |
|---|---|---|---|---|
| Probability that indicates if the product is sold directly in the farmhouse dairy | $P_{dirpurchase}$ | Proportion | 0.67 | Mezher et al. (2022) |
| Probability that indicates if the product is sold through local or provincial retailers | $P_{indpurchase}$ | Proportion | 1-$P_{dirpurchase}$ | Mezher et al. (2022) |
| Time during which the cheese is on sale before the purchase | $Time_{purchase}$ | h | Uniform(0; 96) | Assumption |
| Temperature during sale at farmhouse dairy | $T_{fspurchase}$ | °C | $T_{srip}$ | Mezher et al. (2022) |
| Time for transport to the local retailer | $Time_{rtransport}$ | h | Uniform(0.25; 24) | Assumption |
| Temperature during transport to the local retailer | $T_{rtransport}$ | °C | Normal(4.98; 2.90; min = 0; max = 11.7) | Koutsoumanis, Pavlis, Nychas, and Xanthiakos (2010) |
| Temperature during commercialization of cheese | $T_{purchase}$ | °C | Normal(4.98; 2.90; min = 0; max = 11.7) | Koutsoumanis et al. (2010) |
| Time during which the cheese is transported to home | $Time_{transport}$ | h | Triangular(0.25; 2;24) | Assumption |
| Temperature of the cheese during the transport to home | $T_{transport}$ | °C | Normal(18.2; 7,1; truncate(-2.6; 42) | ISPRA (2014) |
| Number of days before the expire date | $Time_{shelf}$ | day | Brief-ripening: Uniform(2; 60)<br>Medium-ripening: Uniform(7; 260);<br>Long-ripening: Uniform(60; 700)) | Mezher et al. (2022) |
| Time before the consumption of a portion of cheese | $Time_{home}$ | h | Uniform(0; $Time_{shelf}$ - $Time_{purchase}$ - $Time_{transport}$) | Calculated |
| Temperature in a fridge in Italy | $T_{home}$ | °C | Logistic(7.18; 1.12) | Roccato, Uyttendaele, and Membré (2017) |
| Define the size of the portion ingested by adults in grams | $N_{por}$ | g | Cumulative(4.4; 300;{17.5; 22.5; 28.1; 30; 41.7; 49.2; 55; 66.7; 85; 110; 171.7};{0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9; 0.95; 0.99} | Condoleo et al. (2017); Leclercq et al. (2009) |
| Difference in portion size of cheese between adults and children | $P_{por}$ | Proportion | Uniform(0.42; 0.80) | CREA (2005) |

measures affect the model parameters, and the parameters used for each measure, can be found in Appendix.

### 2.7. Sensitivity analysis

A sensitivity analysis is performed to identify which of the model's parameters have a higher impact on the risk for adults, $R_A$. After selecting a list of variables of major interest, we run a number of simulations increasing or decreasing one parameter at a time, respectively by 75%, 50% and 25%, and keeping unchanged other values. The new estimates of the risk per random portion $R_{sens}$ are compared with those from the baseline scenario using the following formula (Møller et al., 2015):

$$R_{rel} = Log\left(\frac{R_{sens}}{R_A}\right) \quad (8)$$

which is the relative risk transformed in logarithm scale to better appreciate the risk variation. An $R_{rel}$ value close to zero indicates that the difference in risk with the base scenario is small.

## 3. Results

The model estimated that O157 STEC contamination occurs in 4.8% of raw milk batches collected on sheep farms and the mean level of the pathogen in a contaminated bulk tank was 0.16 CFU/ml with a maximum of 199.9 CFU/ml (95% CI $1.7 \times 10^{-7}$ – 0.12 CFU/ml). Only 8.4% of the farm tanks with contaminated milk presented an O157 STEC concentration above 0.04 CFU/ml. Fig. 3 shows the change in O157 STEC levels along the food supply chain, from harvest of contaminated milk on farm to the moment of consumption of a brief-ripened cheese at home. The most remarkable variations of the median concentration are observed after the formation of fresh cheese and after the ripening phase (both initial and secondary ripening) while only minimal changes after the other steps are denoted.

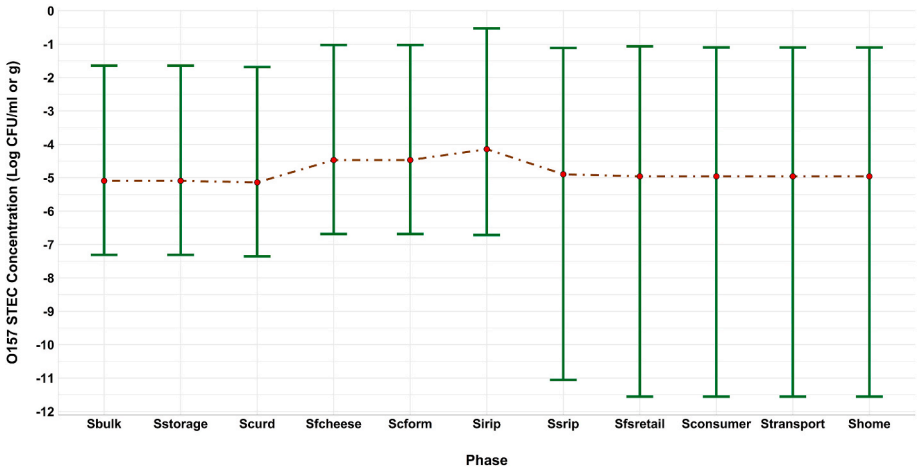


**Fig. 3.** Change in O157 STEC concentration (Log CFU/ml or g) from milk harvest to home consumption (only brief-ripened cheese) when a milk contamination occurs. The red dotted line represents the median values while green bars define the 5th and 95th percentiles.

Overall, the model predicted that the proportion of contaminated portions (presence of at least 1 pathogen cell) ingested by adults was 0.34%, 0.28% and 0.24% for brief, medium and long-ripened cheese with an expected mean number of O157 STEC cells of, respectively, 2173, 1855 and 1304 (median contamination for all types of cheese = 6).

The simulation estimated that the average risk of illness after the ingestion of a portion of raw sheep's milk cheese from a farmhouse dairy ranged between $1.61 \times 10^{-4}$ and $4.03 \times 10^{-4}$ for adults and between $1.35 \times 10^{-4}$ and $3.34 \times 10^{-4}$ for children (Fig. 4, Table S2), dependent on the type of cheese. For both adults and children, the risk associated with consumption of brief-ripened cheese was higher than for medium (+61%) and long-ripened cheese (+151%). When cheese is produced using milk from a farm where a O157 STEC strain is circulating, the mean risk increases approximately 11 times for all types of cheese reaching the value of $4.42 \times 10^{-3}$ (one case of human infection every 226 ingested portions)(Table S2). The mean risk for a child to develop a HUS after eating a portion of cheese ($R_{HUS}$) varied between $1.40 \times 10^{-5}$ (long-ripened cheese) and $3.47 \times 10^{-5}$ (brief-ripened cheese).

The simulated pre-harvest control measures decreased the mean risk per random portion from a minimum of 5 to a maximum of 36 times. Considering all types of cheeses and both exposed subpopulations, administration of bacteriophages was, by far, the most effective measure with an average reduction in risk of 34 times. This was followed by the use of probiotics and lactoferrin administration (about 12 times), sodium chlorate administration (10) and vaccination (5). Regarding post-harvest measures, the most advantageous control measure was to reject positive forms of cheese after testing the product at the end of the initial ripening phase. This resulted in a 19-times average risk reduction, whereas in comparison, testing collected raw milk or the final product would reduce the risk on average by 2.5 and 11 times respectively.

The median risk calculated by only considering the contaminated portions varied between 0.018 (unripened cheese test scenario) and 0.072 (all types of cheese) (Fig. 5). However, note that only considering contaminated portions to calculate the median risk excludes understanding of how the control measures impact on the prevalence of contaminated portions.

Performing the sensitivity analysis highlighted that changes in O157 STEC prevalence in sheep farms and the probability of *E. coli* contamination of bulk milk had the highest impact on the risk of getting ill after consuming a portion of raw sheep's milk cheese (Fig. 6).

## 4. Discussion

In this study, the risk of STEC infection associated with the consumption of raw sheep's milk cheese was assessed through a stochastic model, to our knowledge for the first time. Our estimates, despite the limitations of the model and data gaps, indicated that the risk for consumers may be important to consider. This finding is consistent with other studies (Adams et al., 2019) and the opinion of Food Safety Agencies, like the European Food Safety Authority (EFSA et al., 2020), that deemed raw milk cheeses a relevant source of STEC infection for humans.

On the basis of our simulation's results, the expected prevalence of O157 STEC in bulk milk at farm level can be considered important (approximately 5%) although the level of the pathogen was overall rather low, with 98.4% of the contaminated bulk tanks, having a level below 1 CFU per ml. Indeed, if we assume the usage of a laboratory test with a limit of detection of 1 cell per 25 ml (ISS, 2020), the apparent prevalence would be around 0.04%. This estimate is in accordance with field studies that reported a prevalence of O157 STEC in sheep's milk ranging between 0 and 8.8% (Otero et al., 2017; Rey et al., 2006; Solomakos et al., 2009). Also the estimated proportion of contaminated portions (<0.4% for all types of cheese) is in line with the available surveys that reported O157 STEC prevalence in sheep's cheese between 0 and 9.1% (Coia et al., 2001; Stephan et al., 2008).

In contrast, we did not find outbreaks associated with consumption of cheese made with sheep's milk cheese although outbreaks caused by dairy products made with cow's milk have been reported (Farrokh et al., 2013) and our estimated risk should lead to similar circumstances. Whilst the reasons for the lack of outbreaks are not clear, we can hypothesise that the absence of notifications are because of a lower consumption of this type of products or difficulties in demonstrating the cause of illness in case of outbreak (Boxrud, Monson, Stiles, & Besser, 2010). Furthermore, our conservative (although robust) dose-response curve may result in an over-estimate, and we have simulated a worst-case situation in Italy. However, given our results are in line with previous studies, and there is no biological reason to assume sheep's cheese is less of a risk than cow's cheese, there remains the possibility of outbreaks due to sheep's cheese in the future.

We observed that the bacterial load remained substantially unchanged after the milk storage and the loss of the whey. This is likely due to the fact the brief milk storage at low temperatures (below 5.6 °C in 99% of the iterations) does not allow a substantial microbial growth and only a minority of the cells is lost through the whey (D'Amico et al., 2010; Reitsma & Henning, 1996). On the contrary, variation in STEC concentration was significant during cheese formation and the first part of the ripening. The first treatment does not originate from a real bacterial growth but is the unavoidable result of the curdling process, which is responsible for an approximately 10-fold physical concentration of O157 STEC cells (Schvartzman et al., 2011). Regarding the initial ripening, STEC, like other pathogens such as *Listeria monocytogenes*, can

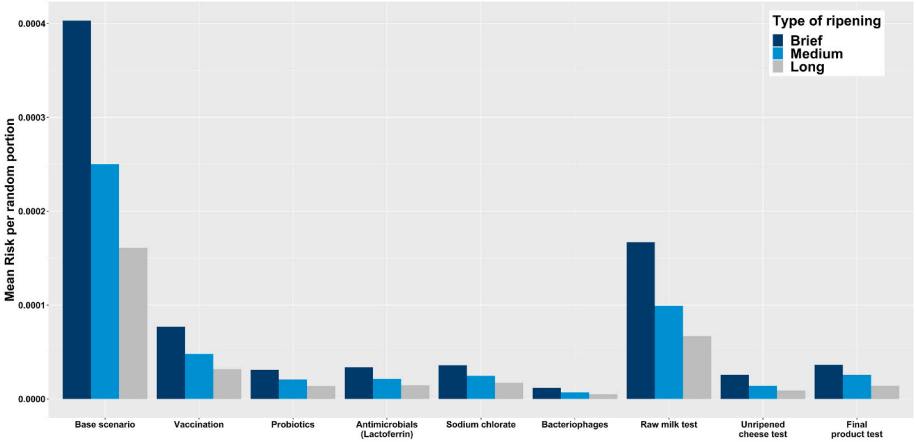


**Fig. 4.** The risk of O157 STEC infection after eating raw sheep's milk cheese manufactured in an Italian farmhouse dairy by type of cheese for adults ($R_A$). Mean risk is reported for a random portion of brief-, medium- and long-ripened cheese.

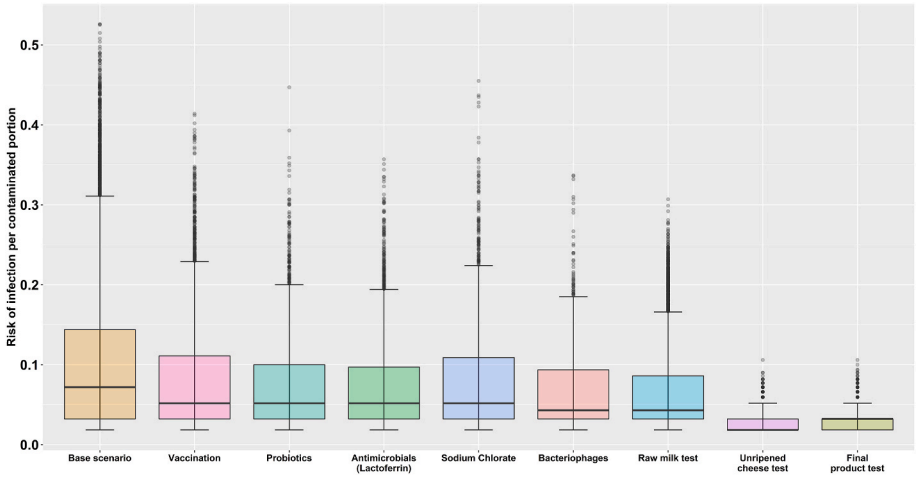


**Fig. 5.** Box-plot of the risk per contaminated portion of brief-ripened cheese and after adopting pre- and post-harvest control measures. Lower and upper box boundaries are 25th and 75th percentiles, respectively; the line inside each box is the median. Lower error line limits the values from the 25th percentiles to the smallest within 1.5 times interquartile range below it. Upper error line limits the values from the 75th percentiles the largest within 1.5 times interquartile range above it. Circles are data falling outside these ranges.

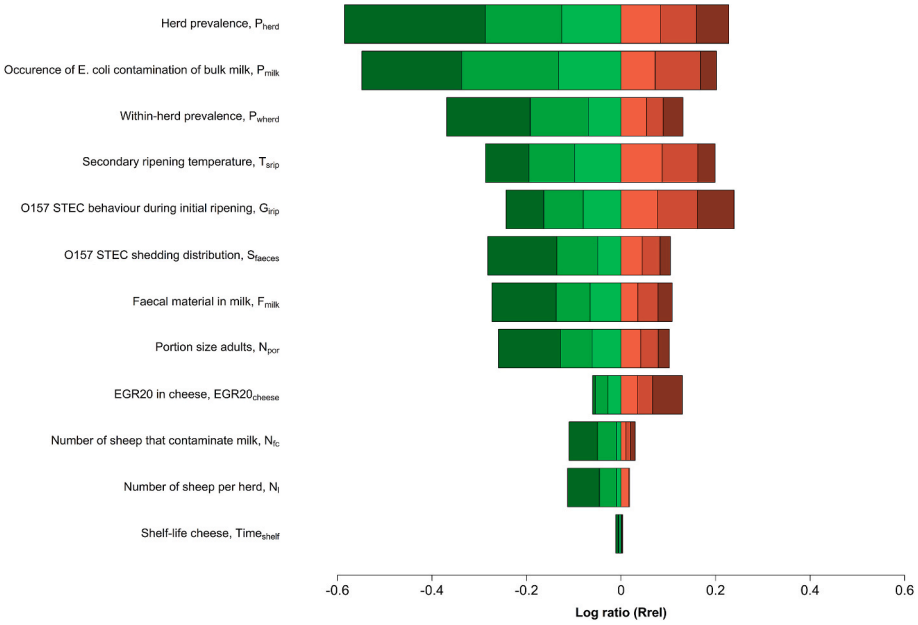


**Fig. 6.** A sensitivity analysis of the main parameters involved in the model. Each change of colour represents the change in mean risk ($R_A$) when the value of a selected parameter is increased (orange) or decreased (green) by 25%, 50% and 75%.

grow or start their decline in fresh cheese. The reason for this effect is unclear but is probably the result of a combination of several factors such as the parameters and procedures adopted during the manufacturing, the type of starters and autochthonous flora, etc.. which may cause a slow drop of *aW* and pH and, as a consequence, make the environment still suitable for the growth/survival of certain pathogens. In contrast, we found that O157 STEC seems unable to survive during the second part of cheese ripening although the expected decrease rate can be very low in most cases and there is no guarantee that all pathogen cells die, even in the case of long ripening periods. The possibility that detectable concentrations of *E. coli* are found after ripening is confirmed by other studies that reported the presence of STEC in hard/long-ripened cheese (Currie et al., 2018; McCollum et al., 2012; Stephan et al., 2008). Nevertheless, additional contamination studies specifically concerning sheep's cheese should be conducted to refine this parameter.

It is difficult to develop an accurate and comprehensive STEC dose-response relationship considering the absence of animal models that reliably mirror the human pathogenesis and the impossibility of recruiting human volunteers due to the serious health consequences caused by the pathogen (EFSA et al., 2020). The available dose-response models can significantly differ regarding their estimates, as they use different data sources, and they have limitations in their predictions in relation to important factors such as the type of food that vehicles the bacteria, which may protect the pathogen during the passage through the gastrointestinal tract (EFSA et al., 2020), or the difference in virulence within and between the STEC serogroups (Cassin et al., 1998; Delignette-Muller & Cornu, 2008; Giacometti et al., 2012; Kundu et al., 2018; Pang et al., 2017; Powell, Ebel, Schlosser, Walderhaug, & Kause, 2000; Strachan et al., 2005; Teunis, Takumi, & Shinagawa, 2004). Regardless, most studies tend to associate a significant probability of clinical manifestations with lower doses compared to other microbial pathogens. In this context, we decided to use the Strachan at al.'s *Beta-Poisson* model (Strachan et al., 2005) for our simulation because it is the most conservative for low levels of contamination; this is the most frequent situation in this simulation and, unlike others, it is based on data from O157 STEC outbreaks caused by a wide variety of foods (including raw milk cheese) and different strains. However, the probability of sickness does not take into account the possible different

susceptibilities of certain groups of individuals, such as children or immune-compromised adults. This explains why the mean risk of illness for adults was only slightly higher than the one for children; this difference only occurs due to the larger portion size consumed by adults compared to children (De Rauw, Buyl, Jacquinet, & Pierard, 2018).

As previously stated, our results suggest that the risk for consumers linked to raw sheep's milk cheese could merit concern. There are no other studies similar to ours to make a comparison but a previous QMRA regarding the risk of listeriosis associated with the consumption of similar products (semisoft raw sheep's milk cheese) reported a far lower probability of acquiring the disease ($8.02 \times 10^{-12}$, 1 case out of roughly 124 billion of eaten portions) (Condoleo et al., 2017). Although there are differences in model building and data between the two assessments, the fact that low doses of STEC can cause human illness (contrarily to *L. monocytogenes*) might be the most important reason for this difference in risk. This assessment covers only one serogroup among several commonly associated with human cases in Europe (EFSA & ECDC, 2019) therefore the risk may be higher when considering multiple serogroups. Furthermore, the mean risk is expected to further increase when cheese is produced with milk collected in a farm where O157 STEC is spreading.

On the other hand, there remain many unknowns regarding the pathogenicity of the different STEC strains; we are not able to predict with a high confidence that ingestion of STEC with a certain virulence gene profile, such as those considered in this study, can be definitely responsible for illness nor whether the presence of *stx*-gene is necessarily correlated to toxin expression (EFSA et al., 2020; Gardette et al., 2019). It is important to highlight that our results specifically refer to consumption of cheese produced by a farmhouse dairy, a worst-case situation compared to the conventional Italian dairies because milk is provided by only one herd (Condoleo et al., 2017; FDA, & Health Canada, 2015). Indeed, the computed risk will decrease when milk is collected from more farms since batches of milk with the presence of viable O157 STEC will be diluted with uncontaminated milk, leading to a lower pathogen concentration. Moreover, as suggested by the lack of known O157 STEC outbreaks associated with the consumption of sheep cheese, the risk might be overestimated, likely due to the fact that some model parameters were calculated through a limited number of studies or small investigations and adopting a conservative approach. Collecting additional data from monitoring or performing further extensive studies is highly recommended to confirm our assumptions and/or to update the present model.

In respect to the risk of developing HUS, our estimates are around 8 times higher than those reported by Perrin et al. (2015) who assessed the risk for children of acquiring the disease after the ingestion of a 25 g serving of raw cow's milk cheese ($4.2 \times 10^{-6}$ compared to $3.47 \times 10^{-5}$). This result may appear surprising especially considering that the Authors considered the five main pathogenic serogroups of STEC and assumed that detection of *stx* gene in milk was equivalent to the presence of viable STEC. However, their model simulated the production of cheese using milk collected from 31 dairy herds which, as mentioned before, entails a drop in STEC level. Moreover, the researchers assumed that the serving size consumed by children was 25 g whereas we adopted a larger portion size.

All pre-harvest control measures we included are experimental, so their sustainability in the sheep husbandry sector and their efficacy on-field have not yet been demonstrated. While different, all treatments appeared effective and the administration of bacteriophages, in particular, seems particularly promising to mitigate the risk. A widespread diffusion of pre-harvest interventions among sheep farmers represents one of the main ways to limit the farm-to-farm transmission of STEC. As a consequence, it would result in a progressive reduction of prevalence at herd level which is the factor that most impacted on the risk of illness (Fig. 6) (EFSA et al., 2020). However, more studies should be performed to test such measures in order to fill the numerous information gaps regarding these control measures, such as the real efficacy against the different STEC wild strains, the length of the protective effect for animals, eventual onset of side effects and development of microbial resistances.

As expected, the implementation of post-harvest measures decreased the risk, although testing milk before cheese-making had a limited impact on the mean risk since, in the case of contamination, the STEC concentration at this stage is frequently too low for the test to be able to detect it. Sampling unripened cheese was the best option because the highest STEC concentration occurs during this production phase due to the volume reduction caused by milk coagulation and the possible bacterial growth during the initial part of ripening. The impact on the risk for consumer health induced by post-harvest interventions was comparable or lower than the one caused by pre-harvest interventions although we simulated a strict control plan that consisted of a systematic control of each batch of milk/cheese. Such a solution is very resource-demanding and may not be appropriate for small business operators like farmhouse dairies. Unfortunately, reducing test frequency, for instance to one sampling per week, would not have reduced the risk compared to the base scenario (data not shown).

These findings suggest that the adoption of mitigation measures at farm level to reduce the mean risk linked to raw milk cheese might be a more convenient strategy than carrying out interventions/controls after processing or distribution phase.

Similarly, our sensitivity analysis highlighted that the parameters that most impact on the mean risk are within the on-farm module. Therefore, controlling the hazard would be better achieved if control measures are applied during this phase of the food supply chain, such as measures to reduce the prevalence of positive sheep farms. Apart from the measures we simulated through the alternative scenarios, introduction of STEC strains on the farm can be limited by reducing the exposure of animals through a rigorous control of water quality, feed hygiene and contact with other flocks and wildlife (EFSA, 2015). Also, frequency of bulk milk contamination with faecal matter seems to significantly affect the risk. Therefore, a meticulous attention to good milking practices and hygiene standards by farmers may produce a positive effect on risk for consumers. The sensitivity analysis also highlighted the importance of reducing the within-herd prevalence; in other words, to limit the animal-to-animal spread of O157 STEC on farm. Implementing a low animal density on farm or administrating treatments to decrease the pathogen excretion from carriers (when they will be commercially available) represent possible options for farmers to achieve such a goal (EFSA, 2015). Two model parameters included in the cheese-making module, namely the temperature of cheese ripening and O157 STEC behaviour after initial ripening showed a relevant influence on the outcome as well.

Some limitations of our model have been already described above; most are related to the scarcity of information regarding the epidemiology and diffusion of O157 and non-O517 STEC in sheep farms and derived milk products. For this reason, we limited our investigation to one STEC serogroup (the most studied) and we used data from a Spanish study because it was impossible to retrieve data from a similarly robust survey conducted on Italian sheep farms that described both between and within herd prevalence. Although sensitivity analysis indicated that this latter information is particularly important to obtain accurate risk estimates, we believe that our assumption is acceptable considering that the adopted values are close to those found in other Mediterranean countries (Franco et al., 2008; Pinaka et al., 2013). In addition, quantification of the pathogen levels in bulk milk was performed using an indirect approach since no data regarding O157 STEC concentration or the extent of faecal contamination was available. However, despite the mentioned limitations, the model produced realistic estimates of O157 STEC prevalence in milk and cheese, as confirmed by our validation performed through a comparison with real data.

## 5. Conclusions

In conclusion, our study suggested that raw sheep's milk cheese, in

particular those produced by small dairies, might be of concern regarding the risk of STEC infection and might represent a potential source of illness for consumers. Nevertheless several gaps in knowledge remain and risk estimates should not be interpreted in an absolute manner. The model results may support producers and food regulators to manage the risk associated with the consumption of this product, such as prioritising the adoption of measures at farm level, in order to assure that their food safety standards are met. Further studies should be conducted to better identify the hazard and collect information about the diffusion and epidemiology of STEC, especially non O157 STEC, along the food chain.

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## CRediT authorship contribution statement

**Roberto Condoleo:** Conceptualization, Methodology, Data curation, Visualization, Software, Writing – original draft. **Roberta Palumbo:** Data curation, Writing – review & editing. **Ziad Mezher:** Data curation, Writing – review & editing. **Luca Bucchini:** Writing – review & editing. **Rachel A. Taylor:** Visualization, Supervision, Writing – review & editing.

## Declaration of competing interest

X All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
X This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
X The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.108951.

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