



Quantifying current and future raw milk losses due to bovine mastitis on European dairy farms under climate change scenarios

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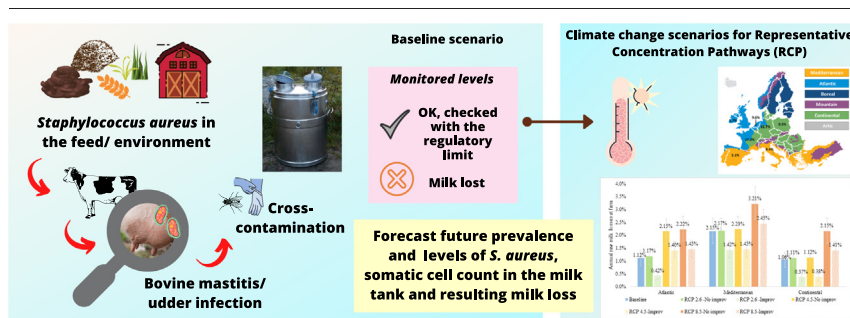
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HIGHLIGHTS

- Current and future raw milk losses due to bovine mastitis were quantified.
- An exposure assessment and stepwise probabilistic model were implemented.
- Bedding material is found to be the main source of *S. aureus* exposure.
- Higher raw milk losses are estimated for the Mediterranean regions of Europe.
- Annual milk losses ranged from 0.37% (RCP2.6) to 3.21% (RCP8.5).

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Huu Hao Ngo

Keywords:

Food losses
Risk assessment
Pathogen infection
Predictive modelling
Stepwise probabilistic model
Staphylococcus aureus

ABSTRACT

Bovine mastitis is an infectious disease that causes udder inflammation and is responsible for raw milk losses across European dairy farms. It is associated with reduced cow milk yield and contributes to elevated Somatic Cell Count (SCC) in raw milk. *Staphylococcus aureus* is one of the most prevalent mastitis pathogens that cause subclinical and clinical mastitis and can be present as a coloniser bacterium in cows. Climate change and geographical variability may influence the prevalence of this pathogen. Thus, this research aimed to predict the raw milk losses in three major dairy-producing regions across Europe (i.e. Mediterranean, Atlantic and Continental) under climate change scenarios. An exposure assessment model and a stepwise probabilistic model were developed to predict potential cow milk yield reduction, *S. aureus* and SCC concentrations in the bulk tank milk at dairy farms. Baseline (i.e. present) and future climate change scenarios were defined, and the resultant concentrations of SCC and *S. aureus* were compared to the actual European regulatory limits. Across the three regions, raw milk losses ranged from 1.06% to 2.15% in the baseline. However, they increased up to 3.21% in the climate change scenarios when no on-farm improvements were considered. Regarding geographical variation, the highest potential milk losses were reported for the Mediterranean and the lowest for the Continental region. Concerning the fulfilment of the regulatory limits, the mean of *S. aureus* and SCC levels in milk did not exceed them either in any region or scenario. Nevertheless, when looking at percentiles, the 10th percentile remained above the limits of *S. aureus* in Atlantic and Mediterranean, but not in the Continental region. The findings provide a snapshot of climate change impacts on raw milk losses due to mastitis. They will allow farmers to detect weaknesses and prepare them to develop adaptation plans to climate change.

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<http://dx.doi.org/10.1016/j.scitotenv.2022.155149>

Received 4 February 2022; Received in revised form 18 March 2022; Accepted 6 April 2022

Available online 11 April 2022

1. Introduction

The dairy sector is a leader in the agricultural economy of the European Union (EU). In 2020, milk farmers across the EU produced 154 million tonnes of cow's milk (Eurostat, 2021), which is expected to increase (Bórawski et al., 2020). Even though milk is produced across all the EU member states, approximately 77% (119 million tonnes per year) of the total raw milk produced in the EU is located in seven countries (Eurostat, 2021). Bovine mastitis causes bacterial intramammary infections, and it is one of the most widespread global diseases that impact European dairy farms (Nalon and Stevenson, 2019). On dairy farms, the total raw milk losses due to mastitis can occur in two ways. On the one hand, this infection leads to a significant decrease in the cows' milk yield, being more pronounced in cows with clinical mastitis (where milk is typically discarded) than in cows with subclinical infection (Ingalls, 2001); on the other, this infection alters the raw milk quality, becoming unsuitable for human consumption and further processing (Gonçalves et al., 2018). The somatic cell count (SCC) is an indicator used in the dairy industry to determine the raw milk quality (Pantoja et al., 2009). Another relevant indicator is the total bacteria count (TBC), which determines hygienic on-farm conditions during milk production (Robles et al., 2020). Many pathogens can induce bovine mastitis such as *Escherichia coli*, *Streptococcus* spp., and *Staphylococcus* spp. Across European dairy farms, *Staphylococcus aureus* has been the most frequent isolated mastitis pathogen from raw milk samples (Mekonnen et al., 2018; More et al., 2013; Tegegne and Tesfaye, 2017). It can appear as a coloniser on cows (coM) and remain unnoticed without inflammatory symptoms. However, it can also transmit from cow to cow, turning into subclinical bovine mastitis (sM) or evolving into clinical mastitis (cM), where symptoms are visible in the latter (Wald et al., 2019; Wellnitz and Bruckmaier, 2012). Besides being a bacteria found in the cows' udders, *S. aureus* has also been found at dairy facilities such as on milking systems, bedding material, faeces and feed (Zadoks and Fitzpatrick, 2009).

The proliferation of pathogens is expected to benefit from the changes in climatic conditions (European Food Safety Authority, 2020) and, therefore, increase the microbial load in raw milk (Misiou and Koutsoumanis, 2021). In particular, the rise of average temperatures as a consequence of climate change around the globe (IPCC, 2014) is expected to increase the prevalence of bovine mastitis among dairy herds and, consequently, contribute to higher raw milk losses (Jingar et al., 2014).

Projected climate change effects are not expected to occur uniformly worldwide. Across Europe, six biogeographical regions have been defined by the European Environment Agency (2017) depending on the climate change impacts and vulnerabilities, being the Mediterranean (i.e. southern Europe) one of the most vulnerable regions. This region is experiencing and is projected to continue experiencing temperature rises and a reduction of rainfall, mainly in summer, leading to droughts (European Environment Agency, 2017, 2019; Giorgi and Lionello, 2008). Similarly, the Continental region (i.e. central and eastern Europe) will suffer from an increment of heat extremes and rainfall reduction during the summer, increasing the risk of drought. On the contrary, an increment of extreme precipitation events is projected to occur in the Atlantic regions (i.e. north-western Europe) (European Environment Agency, 2017).

Quality assurance of dairy products commences on the dairy farms, and thus, regulatory limits for SCC and *S. aureus* in raw milk have been set in Europe (European Commission, 2003, 2004). Both SCC and *S. aureus* load in raw milk have been widely studied previously (Jayarao et al., 2004; Kateřina et al., 2016; Malek dos Reis et al., 2013; Mekonnen et al., 2018; Naito et al., 2013; Wang et al., 2018), but none of these studies analysed the contamination pathway of *S. aureus* from the dairy farm to the farm bulk tank milk (BTM), which includes the intramammary and environmental *S. aureus*. Also, to the best of the authors' knowledge, even though the prevalence of bovine mastitis is affected by climatic conditions, all the available studies only estimate raw milk losses from bovine mastitis under current conditions without considering the future effects of climate change, which are expected to modify climatic variables.

Quantitative Microbial Risk Assessment (QMRA) is a tool that aims to develop knowledge of the spread and control of infectious diseases (Mitchel et al., 2021). QMRA is particularly useful in developing control strategies and evidence-based policy decisions through the lens of health risk. QMRA is not limited to human health risks; it can also be developed for animal and plant pathogens (Mitchel et al., 2021).

The overall hypothesis of this research is “*Bovine mastitis may pose a threat to European dairy farms causing milk losses, which may change under projected climate change scenarios*”. Hence, under climate change scenarios, this study aimed to predict the annual raw milk losses due to bovine mastitis across the main European milk producer regions. Climate change scenarios were considered to investigate the influence of temperature increment on the annual raw milk losses at dairy farms located in the Mediterranean, Atlantic and Continental. Herds were assumed to be comprised of 100 lactating cows in an indoor dairy housing system. Moreover, given the lack of studies that includes the complete contamination pathway of *S. aureus*, this research includes both the environmental and intramammary contamination of this pathogen from the dairy cow to the BTM.

2. Material and methods

A model framework was developed, composed of an exposure assessment and a stepwise probabilistic model, to predict the annual raw milk losses based on the SCC and *S. aureus* concentrations at BTM under climate change scenarios (Fig. 1). The total raw milk losses include losses due to (i) exceeding the *S. aureus* and SCC limits in BTM, (ii) losses due to a cow's milk yield reduction, and (iii) raw milk discarded due to cow with clinical mastitis. The model inputs, computations and simulated outputs used in the model framework are detailed in Table 1. Specific parameters affected by temperature increase due to climate change are marked with an asterisk in the same table. The framework begins by defining the baseline scenario and climate change scenarios by 2050 (step 1). Then, an exposure assessment adapted from Vissers et al. (2006) was performed to estimate the amount of *S. aureus* in the environment to which cows are exposed. In the exposure assessment section, the different production stages¹ involved at a dairy farm are included (steps 2 to 8). Later, a stepwise probabilistic model was necessary to estimate the annual mastitis prevalence (step 9) and predict the cows' milk yield reduction and SCC and *S. aureus* concentrations at BTM (step 10). Finally, to estimate the raw milk losses associated with not meeting the standards, the *S. aureus* and SCC concentrations in the different scenarios were compared to the regulatory limits set in the EU (step 11). A sensitivity analysis was performed to identify the most influential model's inputs and observe its influence on the predicted raw milk losses.

2.1. Definition of baseline and climate change scenarios (step 1)

The baseline scenario was built on current average annual temperatures (T) representative of the regions (i.e. Atlantic, Mediterranean, and Continental) that deviated from the World Bank Group (2021). The climate change scenarios were constructed based on the Representative Concentration Pathways (RCP) developed by the Intergovernmental Panel on Climate Change (i.e. RCP 2.6, 4.5 and 8.5) (IPCC, 2014). Unlike other emission scenarios, the RCPs project totals radiative forcing, considering the effect of efforts (e.g. international policies and agreements) to reduce greenhouse gas (GHG) emissions and mitigate climate change. Therefore, the RCP2.6 is considered a scenario with great mitigation efforts. RCP4.5 refers to an intermediate stabilised scenario, and RCP8.5 refers to a scenario where no efforts to reduce GHG emissions are made (IPCC, 2014). Each of the mentioned RCP scenarios was split into two sub-scenarios (Table 2), assuming ‘on-farm improvements’ and ‘no on-farm improvements’.

‘On-farm improvements’ scenarios consider an increase in the annual average milk yield (AAMY) and a decrease in annual mastitis prevalence.

¹ Production stages refer to the sources and route of transmission of *S. aureus* at dairy farms. Six production stages are identified throughout the exposure assessment model.

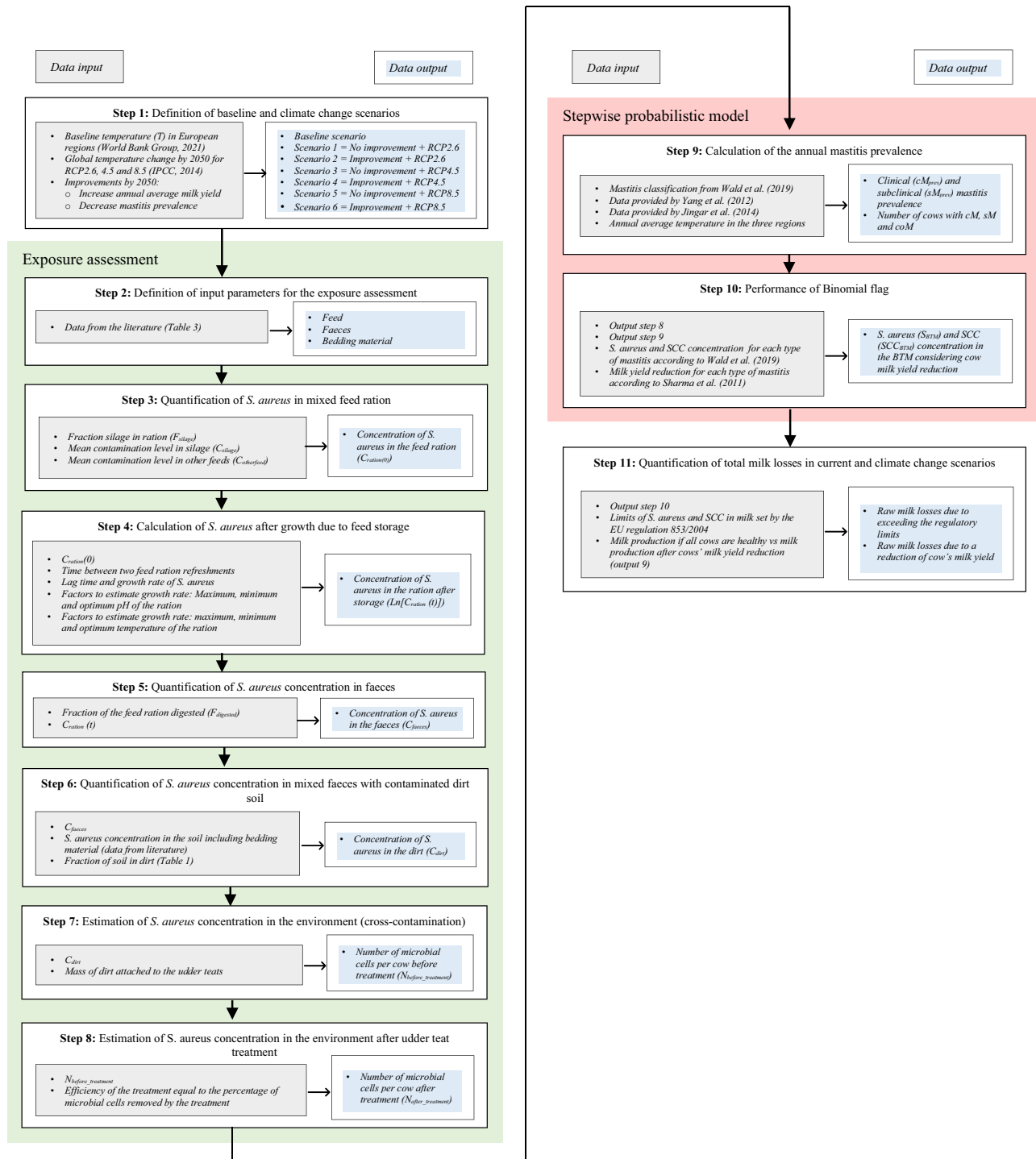


Fig. 1. Schematic of the model framework for estimating bovine milk losses. The exposure assessment model (i.e., steps 2 to 8) was adapted from Vissers et al. (2006).

The former results from the continuous efforts to increase the AAMY through feeding strategies and genetic enhancement, which are expected to keep increasing in the near future (Bórawski et al., 2020). According to Reijs et al. (2013), there is a constant increment in the milk yield per dairy cow in different countries. By applying a linear model on the data on milk yield over the years, an increment of 50% by 2050 was projected compared to the AAMY in 2019, obtaining an R^2 of 0.9. Thus, a projected AAMY of 18,800 (SD 1979) L year⁻¹ by 2050 was calculated. The latter results from farm enhancements such as improvement in udder health and novel mastitis treatments (IDF, 2018). These improvements reduced the rate of mastitis prevalence over the past years, and it is expected to

continuously improve in the near term. However, data on the potential prevalence of mastitis by 2050 and historical records to predict it were not found. Thus, this study assumes a 10% reduction of annual prevalence by 2050, compared to the present prevalence.

Conversely, in the 'no on-farm improvements' scenarios, upgrades are not considered. The AAMY remains stable in 12,500 year⁻¹, and the annual mastitis prevalence increases by 10% due to a rise in average global temperatures, leading to an increase of bovine mastitis (Jingar et al., 2014). The T change by 2050 is expected to vary among RCPs, so, Table 2 shows the corresponding increments for each scenario.

Table 1

Description and distributions of the inputs for the stepwise probabilistic model to predict the concentration of *S. aureus* in the farm bulk tank milk (S_{BTM}), adapted from [Vissers et al. \(2006\)](#). Inputs and computations affected by the climate change scenarios are pointed out with an asterisk (*).

Symbols	Description and references	Model/distribution baseline	Units
Model inputs			
F_{silage}^b	Fraction silage in the ration (Driehuis et al., 2008 ; based upon expert opinion, CLUN)	Uniform (min 0.6, max 0.84)	%
C_{silage}	Mean contamination level in silage	Table 3	$\text{Log}_{10} \text{CFU g}^{-1}$
$C_{concentrated}$	Mean contamination in concentrated feed	Table 3	$\text{Log}_{10} \text{CFU g}^{-1}$
C_{∞}	Maximum attainable contamination level in the feed ration Vissers et al. (2006) .	8	$\text{Log}_{10} \text{CFU g}^{-1}$
t^b	The time between two feed ration refreshments (based upon expert opinion, CLUN)	Uniform (min 12, max 24)	Hours
λ	Lag time (Zwietering et al., 1996)	1	Hours
μ_{opt}	Growth rate under optimal conditions (Vissers et al., 2006)	0.12	Hours
T_{ration}^*	The temperature of the ration, which was assumed to be the same as the current shade temperature and varies depending on the region under study (World Bank Group, 2021)	Atlantic: Uniform (min 3.3, max 18.3) Mediterranean: Uniform (min 4.2, max 21.9) Continental: Uniform (min 0.5, max 17.8)	°C
T_{min}	Minimum growth temperature (Medveov and Valk, 2012)	7	°C
T_{opt}	Optimal growth temperature (Medveov and Valk, 2012)	Uniform (min 37, max 40)	°C
pH_{ration}	The pH of the feed ration (Borreani and Tabacco, 2010)	4.1	
pH_{min}	Minimum growth pH (Medveov and Valk, 2012)	4	
pH_{max}	Maximum growth pH (Medveov and Valk, 2012)	9.8	
pH_{opt}	Optimal growth pH (Medveov and Valk, 2012)	Uniform (min 6, max 7)	
$F_{digested}$	Fraction of the feed ration digested (Lassey, 2007)	75	%
$F_{bedding}^a$	Fraction of bedding material in the dirt (Vissers et al., 2006)	Uniform (min 0, max 20)	%
$C_{bedding}$	Bedding material contamination level	Table 3	$\text{Log}_{10} \text{CFU g}^{-1}$
M_{dirt}	Mass of dirt attached to the udder teats (Vissers et al., 2006)	1	G
$PT_{efficiency}^a$	% of spores removed without pre-treatment (Vissers et al., 2006)	Pert (30, 75, 90)	%
$AAMY^{b*}$	Data on annual average milk yield from a healthy cow (records provided by CLUN)	Lognormal (mean 12,500, SD 1318.99)	L year ⁻¹
SCC_{Wald}	Data on Somatic Cell Count from Wald et al. (2019) required in the binomial flag	Table 4	Cells mL ⁻¹
S_{Wald}	Data on <i>S. aureus</i> concentration from Wald et al. (2019) required in the binomial flag	Table 4	CFU mL ⁻¹
$MP_{aft,red}$	Annual raw milk production by the herd considering milk yield reduction. Data on milk yield reduction per type of mastitis was retrieved from Sharma et al. (2011) and required in the binomial flag	Table 5	L year ⁻¹
Model outputs			
$C_{ration(0)}$	The concentration of <i>S. aureus</i> in the ration after mixing feed components (adapted from Vissers et al., 2006)	Eq. (1)	CFU g ⁻¹
$C_{ration(t)}$	The concentration of <i>S. aureus</i> in the ration considering the effect of feed storage (adapted from Vissers et al., 2006)	Eq. (2)	CFU g ⁻¹
C_{faeces}	The concentration of <i>S. aureus</i> in the faeces (adapted from Vissers et al., 2006)	Eq. (8)	CFU g ⁻¹
C_{dirt}	The concentration of <i>S. aureus</i> in the dirt because of a mix of faeces and bedding material (adapted from Vissers et al., 2006)	Eq. (9)	CFU g ⁻¹
$N_{before,treatment}$	Most probable number of <i>S. aureus</i> in cow's udders due to cross-contamination from dirt before treatment (adapted from Vissers et al., 2006)	Eq. (10)	CFU
$N_{after,treatment}$	Most probable number of <i>S. aureus</i> in cow's udders after treatment (adapted from Vissers et al., 2006)	Eq. (11)	CFU
sM_{prev}^*	Prevalence of subclinical mastitis based on the temperature from each region	Eq. (12)	%
cM_{prev}^*	Prevalence of clinical mastitis based on the temperature from each region	Eq. (13)	%
SCC_{BTM}	Somatic Cell Count concentration in the bulk tank milk	Eq. (14)	Cells mL ⁻¹
$M_{yield,after}$	Actual annual milk yield after considering cow milk yield reduction. Data from Sharma et al. (2011) was required	Eq. (15)	L year ⁻¹
S_{BTM}	<i>S. aureus</i> concentration in the bulk tank milk, considering the load in the environment and the raw milk due to the intramammary infection	Eq. (16)	CFU mL ⁻¹

^a In the sensitivity analysis, $F_{bedding}$ and $PT_{efficiency}$ were modified by the lowest and highest value presented in this table.

^b Data related to on-dairy farms was retrieved from expert opinion (i.e. dairy farmers and veterinaries) ([CLUN, 2021](#)).

2.2. Input parameters definition for the exposure assessment model (step 2)

This step includes collating information for the input parameters in the exposure assessment model for *S. aureus* concentration in the dairy facilities environment. Three sources of contamination of *S. aureus* were identified (bedding material, silage, and the concentrated feed), and the number of *S. aureus* on each of these sources was retrieved from the literature

Table 2

Description of the six climate change scenarios applied in this research.

	Temperature change by 2050 (°C)		
	RCP2.6 (+0.3 to 1.7) ^a	RCP4.5 (+1.1 to 2.6) ^a	RCP8.5 (+2.6 to 4.8) ^a
No Improvement	Scenario 1	Scenario 3	Scenario 5
Improvement	Scenario 2	Scenario 4	Scenario 6

^a Data retrieved from [IPCC \(2014\)](#).

([Table 3](#)). Silage includes grass and maize ensilage. Also, the material used for the cows' bed is assumed to be straw and sawdust since both are the materials most used by dairy farmers ([Leso et al., 2020](#)).

Table 3

Data collation on the number of *S. aureus* for each input parameter of the model.

Input	Symbol	Raw data (CFU g ⁻¹)	Reference
Bedding material	$C_{bedding}$	3.1×10^8	(Rendos et al., 1975)
Bedding material	$C_{bedding}$	4.9×10^7	(Rendos et al., 1975)
Bedding material	$C_{bedding}$	2.2×10^9	(Rendos et al., 1975)
Bedding material	$C_{bedding}$	$10-8 \times 10^6$	(Bradley et al., 2018)
Bedding material	$C_{bedding}$	$1.4 (\pm \text{SD } 2.5) 10^8$	(Black et al., 2014)
Bedding material	$C_{bedding}$	$6.9 \log_{10}$	(Hogan et al., 1990)
Bedding material	$C_{bedding}$	$5.5 \log_{10}$	(Hogan et al., 1990)
Bedding material	$C_{bedding}$	$7.7 \log_{10}$	(Hogan et al., 1990)
Silage	C_{silage}	<10	(CESFAC, 2007 ; CLUN, 2021)
Concentrated feed	$C_{concentrated}$	<10	(CESFAC, 2007 ; CLUN, 2021)

Note: For bedding material, a distribution is fitted as 'Expon (345172279)' CFU g⁻¹.

2.3. Quantification of *S. aureus* when feed components are mixed (step 3)

Step 2 addresses the first production stage at dairy farms, consisting of the potential contamination of *S. aureus* in the cows' feed ration. The feed ration is a mix between concentrated feed and silage. However, the process of mixing potentially results in a contaminated feed ration with pathogens (Hope et al., 2009). Eq. (1) was applied to quantify the initial *S. aureus* concentration in the feed ration ($C_{ration(0)}$) after mixing the silage and concentrated feed, in which the obtained unit is CFU g⁻¹.

$$C_{ration(0)} = F_{silage} \times C_{silage} + (1 - F_{silage}) \times C_{concentrated} \quad (1)$$

where F_{silage} is translated as the fraction of silage in the cows' diet ration, which varies depending on dairy farmers' practices and dairy production systems (i.e. confined indoor and grazing outdoor). The value for the F_{silage} for an indoor system was obtained from the farmers' opinion (Table 1). Regarding C_{silage} and $C_{concentrated}$, they refer to the most probable number of *S. aureus* in silage and concentrated feed, respectively. Respective data was deviated from the allowable limits of *S. aureus* in ruminant feed production (Table 3) according to the Guide of Feed Sanitization Standards (CESFAC, 2007). This guide is commonly used among farmers from the cooperative to reference quality controls at dairy farms.

2.4. Calculate the effect of pathogen growth due to feed storage (step 4)

During feed storage, the growth of *S. aureus* can occur, and it can be influenced by environmental conditions such as temperature, and consequently, affected by the climate change scenarios. Thus, to calculate the pathogen concentration in the ration after storage ($\ln[C_{ration}(t)]$), Eq. (2) was retrieved from Vissers et al., 2006. The obtained unit is ln CFU g⁻¹.

$$\ln [C_{ration}(t)] = \ln [C_{ration}(0)] + \mu \times A_n(t) - \ln \left(1 + \frac{e^{\mu \times A_n(t)} \times t}{e^{\ln(C_{\infty}/C_{ration(0)})}} \right) \quad (2)$$

where $C_{ration(0)}$ is the initial concentration previously calculated in Eq. (1); μ is the microbial growth in the feed ration, and it is calculated based on the Baranyi and Roberts model (Baranyi and Roberts, 1994); A_n is equal to the time between two feed ration refreshments (t), which are obtained in hours; e is a mathematical constant equal to 2718; C_{∞} refers to the maximum level of contamination achievable in the feed ration, which according to Vissers et al. (2006), it is assumed equal to 8 log₁₀ pathogen g⁻¹.

Also, Eq. (3) was needed to solve μ in Eq. (2). It followed a gamma concept model since temperature (Eq. (4)) and pH (Eq. (5)) effects are considered separately. *S. aureus* prefers an optimum temperature (T_{opt}) that ranges from 37 °C to 40 °C and a minimum temperature (T_{min}) of 7 °C. The T_{ration} was considered equal to the environment temperature. Thus, the average temperature was used from each biogeographical region for the baseline and different climate change scenarios (Step 1). Concerning the pH ($\gamma(pH)$), this mastitis pathogen prefers an optimum pH (pH_{opt}) that ranges from 6 to 7. It tolerates a minimum and maximum pH of 4 and 9.8, respectively (Medveov and Valk, 2012). The pH from the ration (pH_{ration}) was 4.1 (Borreani and Tabacco, 2010).

$$\mu = \gamma(T) \times \gamma(pH) \times \mu_{opt} \quad (3)$$

$$\gamma(T) = \left(\frac{T_{ration} - T_{min}}{T_{opt} - T_{min}} \right)^2 \quad (4)$$

$$\gamma(pH) = \left[\frac{(pH_{ration} - pH_{min}) \times (pH_{max} - pH_{ration})}{(pH_{opt} - pH_{min}) \times (pH_{max} - pH_{opt})} \right] \quad (5)$$

Lastly, Eq. (6) (Vissers et al. (2006)) solves $A_n(t)$ in Eq. (2), in which the obtained unit is hours. λ refers to the lag time, which is equal to 1, and t (in h) refers to the time between one feed ration and the next refreshment,

which depends on dairy farmer's practices, so data from CLUN was used (Table 1).

$$A_n(t) = t + \frac{1}{\mu} \ln \left(\frac{e^{-\mu t} + q_0}{1 + q_0} \right) \quad (6)$$

$$q_0 = \frac{1}{e^{\lambda \cdot \mu} - 1} \quad (7)$$

2.5. Effect of animal digestion in the *S. aureus* concentration of faeces (step 5)

The next production stage is associated with the *S. aureus* concentration in cows' faeces. Microorganisms can survive the ruminant digestion process and return to the environment throughout the animals' excretion. Eq. (8) quantifies the *S. aureus* concentration in the faeces (C_{faeces}) in CFU g⁻¹. To do so, the fraction of the feed ration that is digested ($F_{digested}$) is retrieved from Lassey (2007), which is equal to 75%.

$$C_{faeces(t)} = \left(\frac{1}{1 - F_{digested}} \right) \times C_{ration}(t) \quad (8)$$

2.6. Effect of mixing the faeces with soil resulting in contaminated dirt (step 6)

Another production stage is linked to a mix between faeces and bedding material, which are commonly mixed due to the natural movement and displacement of the cows resulting in contaminated dirt. Following Vissers et al. (2006), Eq. (9) estimates the *S. aureus* concentration in the contaminated dirt (C_{dirt}) in CFU g⁻¹.

$$C_{dirt} = F_{bedding} \times C_{bedding} + (1 - F_{bedding}) \times C_{faeces} \quad (9)$$

where $F_{bedding}$ refers to the fraction of bedding material in the dirt, as shown in Table 1. The pathogen concentration in bedding material ($C_{bedding}$) was calculated based on the values extracted from the literature, as shown in Table 3, and C_{faeces} was obtained previously from Eq. (8).

2.7. Evaluation of the cross-contamination from dirt to udder teats (step 7)

This production stage refers to the *S. aureus* contamination resulting in cross-contamination from the dirt to the dairy cows' teats. Thus, Eq. (10) quantifies the number of microbial cells per cow (stated in CFU) before a common treatment that dairy farmers carry out to rinse off as much as possible the dirt on teats:

$$N_{before_treatment} = M_{dirt} \times C_{dirt} \quad (10)$$

where M_{dirt} is the mass of dirt stuck to the cows' teats (in g) and obtained from Vissers et al. (2006), as shown in Table 1, while C_{dirt} was previously calculated in Eq. (9).

2.8. Effect of the treatment process of udder teats (step 8)

In this step, the final concentration of *S. aureus* to which cows are exposed environmentally was calculated. During the production stage, teats are rinsed off, and microorganisms are removed on the cows' teats. Eq. (11) estimate the number of microbial cells per cow ($N_{after_treatment}$) after the treatment process, obtaining a value in CFU.

$$N_{after_treatment} = PT_{efficiency} \times N_{before} \quad (11)$$

where $PT_{efficiency}$ refers to the treatment efficiency based on the percentage of microorganisms removed. The $PT_{efficiency}$ was assumed 75%, as reported by Vissers et al. (2006) (Table 1).

2.9. Annual mastitis prevalence (step 9)

An estimation of the annual mastitis prevalence was required to calculate the concentration of intramammary *S. aureus*. Since the study looks at the effect of climate change on raw milk losses due to mastitis, the prevalence of each mastitis was calculated in function of temperature. To do so, the sM prevalence (sM_{prev}) was estimated with a linear model, using the reported data on temperature and subclinical prevalence by Yang et al. (2012) and obtaining the result in percentage. The following equation (Eq. (12)) was obtained with an R^2 equal to 0.6:

$$sM_{prev} = 7.734(T)^{0.4262} \quad (12)$$

Similarly, the cM prevalence (cM_{prev}) was calculated with a linear model, implementing data on temperature and clinical prevalence reported by Jingar et al. (2014) and obtaining the result in percentage. The following equation (Eq. (13)) was obtained with an R^2 equal to 0.9:

$$cM_{prev} = 0.0026(T) - 0.0263 \quad (13)$$

Later, T (Eqs. (12) and (13)) was substituted by the average annual temperature representative of each region (Atlantic, Mediterranean, and Continental) in the baseline and climate change scenarios.

Lastly, due to a lack of data on the prevalence of mastitis provoked by coloniser *S. aureus*, the prevalence was estimated with two conversion factors. These factors were obtained from the subclinical-to-coloniser (70:12) and clinical-to-coloniser (18:12) ratios provided by Wald et al. (2019). Then, a uniform distribution was applied by using both conversion factors.

2.10. Binomial flag (step 10)

A binomial flagging method (Vose, 2000) was used to estimate the *S. aureus* and SCC concentration in the BTM. This approach is built on the binomial distribution, and it can be useful to resolve problems and estimate the probability of success of a trial, such as done by Coffey et al. (2009). They implemented the binomial flagging approach to estimate the presence of Aflatoxin B1 in maize, finding 51 samples positive out of 139. Translating it to the present research, the mastitis prevalence previously calculated together with the binomial flag allowed the calculation of the number of infected cows out of a herd of 100. A second binomial flag was applied only for the infected cows to determine the number of cows with cM (labelled as category 3), sM (labelled as category 2) and/or coM (labelled as category 1) (Table 4), while healthy cows were labelled as category 0. Once the number of cows within each category was estimated, data on the concentration of SCC and *S. aureus* per cow was derived using data from Wald et al. (2019) (Table 4). It allowed the calculation of the SCC (SCC_{BTM}) and *S. aureus* (S_{BTM}) concentrations per mL in the BTM. Also, the cows' milk yield reduction was estimated since these concentrations depend on how much each cow contributes to the BTM (Table 5).

Table 4

S. aureus and SCC concentration in the milk corresponding to each type of bovine mastitis. Adapted from Wald et al. (2019). Data on the column of SCC concentration (SCC_{wald}) is used in Eq. (14) and data on the column of *S. aureus* concentration (S_{wald}) is used in Eq. (18).

Flag label	SCC concentration (cells mL ⁻¹)				<i>S. aureus</i> concentration (CFU mL ⁻¹)		
	Median	Mean	SD	Distribution	Min	Max	Distribution
3 cM	>10 ⁶	2.18 × 10 ⁶	1.58 × 10 ⁶	Lognormal(2.18 × 10 ⁶ , 1.58 × 10 ⁶)	10 ⁵	10 ⁶	Uniform(10 ⁵ , 10 ⁶)
2 sM	200,000–500,000	1.00 × 10 ⁶	1.21 × 10 ⁶	Lognormal(1.00 × 10 ⁶ , 1.21 × 10 ⁶)	10 ³	10 ⁴	Uniform(10 ³ , 10 ⁴)
1 coM	≤100,000	4.50 × 10 ⁴	3.30 × 10 ⁴	Lognormal(4.50 × 10 ⁴ , 3.30 × 10 ⁴)	10 ³	10 ⁴	Uniform(10 ³ , 10 ⁴)

The SCC_{BTM} was estimated by Eq. (14) and stated in cells mL⁻¹. Cows with clinical mastitis were excluded since they are assumed to be separated from the rest of the herd, and their milk does not enter the BTM.

$$SCC_{BTM} = \frac{[(Cow_{coM} \times SCC_{wald}) \times M_{yield_after}] + [(Cow_{sM} \times SCC_{wald}) \times M_{yield_after}] \times 1000}{MP_{aft_red} \times 1000} \quad (14)$$

where the total number of cows with coloniser (Cow_{coM}) and subclinical mastitis (Cow_{sM}) in the herd were multiplied by the respective SCC concentration stated by Wald et al. (2019) (SCC_{wald}) in Table 4. Later, this contribution was multiplied by the actual annual milk yield after considering cow milk yield reduction (M_{yield_after}) (L year⁻¹) using Eq. (15):

$$M_{yield_after} = AAMY - MYR_{mastitis} \quad (15)$$

Where $AAMY$ refers to the annual average milk yield (L year⁻¹) from healthy cows considering data provided by CLUN (mean 12,545, SD 1319). $MYR_{mastitis}$ refers to the milk yield reduction according to the type of bovine mastitis (sM or coM), calculated from Sharma et al. (2011) (Table 5), who provided the respective milk yield reductions based on SCC concentration in the raw milk per cow.

Finally, the total concentration of SCC (cells year⁻¹) from sM and coM was divided by the annual raw milk production in L year⁻¹ (MP_{aft_red}), in which a reduction of the cows' milk yield due to bovine mastitis in the herd was also considered.

In order to cover the whole contamination pathway, Eq. (16) estimates the S_{BTM} (stated in CFU mL⁻¹) by adding up the *S. aureus* load to which cows are exposed in the facilities environment (S_{env}) and the *S. aureus* concentration in the milk (S_{sa}) coming from the intramammary infection. Cows with clinical signs were also excluded here.

$$S_{BTM} = S_{env} + S_{sa} \quad (16)$$

The first summand is given by Eq. (17) and stated in CFU mL⁻¹, where Cow_{coM} and Cow_{sM} refer to the number of cows with either coloniser or subclinical mastitis in the herd, $N_{after_treatment}$ to the number of microbial cells a cow is exposed to after treatment and is retrieved from step 7 (Eq. (11) in CFU) and MP_{aft_red} to the total milk produced in l day⁻¹ after milk yield reduction, as mentioned in Table 5.

$$S_{env} = \frac{(Cow_{coM} + Cow_{sM}) \times N_{after_treatment}}{MP_{aft_red}} \quad (17)$$

The second summand is given by Eq. (18) and is also stated in CFU mL⁻¹. S_{sa} the calculation is similar to SCC_{BTM} , but considering data on *S. aureus* from Table 4, where the total number of cows either with coloniser (Cow_{coM}) or subclinical mastitis (Cow_{sM}) were multiplied by the respective *S. aureus* concentration estimated by Wald et al. (2019) (CFU mL⁻¹). The contribution of *S. aureus* per mL of raw milk was multiplied by the actual

Table 5

Milk yield reduction per cow depending on its SCC concentration in the raw milk per cow. Adapted from Sharma et al. (2011).

SCC (cells mL ⁻¹)			Flag label of the type of mastitis	Milk yield reduction (1 305-days ⁻¹) ^a
Minimum	Mid-point	Maximum		
0	1250	17,000	0	0
18,000	25,000	34,000	0	0
25,000	50,000	70,000	1	0
566,000	800,000	1,130,000	2	726
1,131,000	1,600,000	2,263,000	3	907

Note: a) Lactation period in a year = 305 days.

annual milk yield once cow milk yield reduction was considered (M_{yield_after}).

$$S_{Sa} = \frac{[(C_{ow_{coM}} \times S_{Wald}) \times M_{yield_after}] + [(C_{ow_{sM}} \times S_{Wald}) \times M_{yield_after}] \times 1000}{MP_{aff_red} \times 1000} \quad (18)$$

2.11. Total raw milk losses under current and climate change scenarios (step 11)

To obtain the raw milk losses related to not meeting the quality standards, the S_{BTM} and the SCC_{BTM} were compared to the regulatory limits for raw milk intended for processing in the EU: i.e. 400,000 cells mL⁻¹ of SCC (European Commission, 2004) and 2000 CFU mL⁻¹ of *S. aureus* (European Commission, 2003). The raw milk losses due to milk yield decrease were calculated from the difference between the total milk production assuming all cows are healthy and the total milk production with reduced milk yield due to bovine mastitis, divided by the total milk production to obtain a percentual value. The milk yield reduction from clinical cows was excluded since their milk production is directly considered a loss.

3. Results and discussion

3.1. Current raw milk losses due to bovine mastitis

As a result of step 9, the highest annual prevalence of *S. aureus* mastitis was estimated for the Mediterranean. For this region, the proportions of

each mastitis category were 22.7% for sM, 0.6% for cM, and 2.5% for coM (detail values per biogeographical region and mastitis category in Supplementary Fig. S1). The prevalence of mastitis varies across Europe and precise percentages on the bovine mastitis prevalence are not available since national recording systems on cows' welfare and mastitis rates are unavailable in most European countries. However, according to the literature, the annual mastitis prevalence in Europe ranges from 8 to 48% per herd (European Food Safety Authority, 2009; IDF, 2018), matching the annual prevalence calculated in the present research. Similarly, step 10 showed the highest annual concentration of *S. aureus* and SCC throughout the whole contamination pathway for the Mediterranean region, 1.5×10^3 CFU mL⁻¹ and 2.0×10^5 cells mL⁻¹, respectively (Fig. S2 and Fig. S3).

The results of the annual cumulated raw milk losses are shown in detail in Fig. 2. In a baseline scenario and across the three regions, the average annual raw milk loss due to bovine mastitis caused by *S. aureus* was 1.44%. In order to validate the calculated raw milk losses, the results of this research were compared to reported values. Houben et al. (1993) estimated the cumulated milk yield losses due to bovine mastitis, ranging from 0.5 to 2.0% when the cows were during their first lactation. The prevalence of mastitis used in that study was 13.7% (SD 10.3%). Another study found a similar percentage (i.e. 0.5%) when the prevalence of mastitis ranged from 18.1 to 27.4% Myllys and Rautala (1995). The percentages of mastitis prevalence calculated in the present study align with the values used in the present research, which vary from 21.7 to 30.5%. In a more recent study, Heikkilä et al. (2018) quantified the raw milk losses due to bovine mastitis caused by *S. aureus*. They estimated a value of approximately 4.3% across Finnish dairy farms. The slight difference between the outcome found in that research and this research is attributed to the different annual mastitis prevalence selected by the authors, ranging from 72% for subclinical mastitis to 28% for clinical mastitis. They assumed higher values than the used in the present research. In addition, considering that *S. aureus* is not the only pathogen that induces bovine mastitis, the obtained results are compared to reported values from different microorganisms. In the same study, Heikkilä et al. (2018) reported an annual raw milk loss of roughly 5% from *Streptococcus* spp. and up to 10.6% from *Escherichia coli*. Also, Feliciano et al. (2021) estimated the raw milk losses from *E. coli* with a probabilistic exposure assessment model, reporting raw milk losses of 10% in dairy farms located in hot weather. Even though *E. coli* presents the largest percentage, it is important to mention that this microorganism

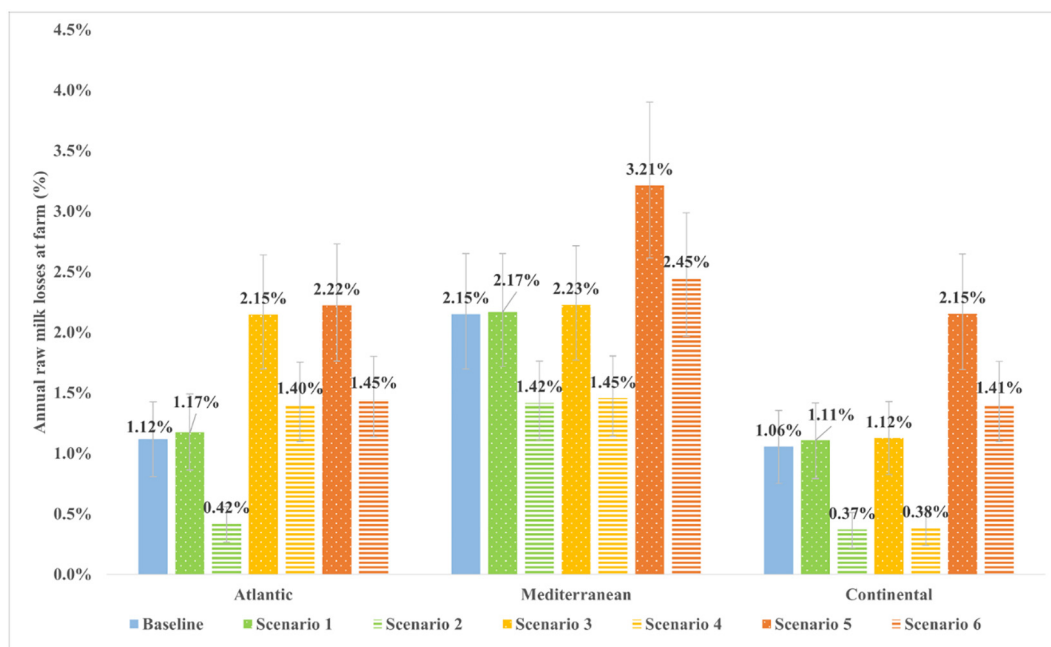


Fig. 2. Predicted percentage of raw milk losses from the cumulated annual milk yield reduction due to bovine mastitis. Scenarios 1, 3 and 5 refers to no improvement scenarios, while scenario 2, 4, 6 refers to improvement scenarios, respectively RCP2.6, RCP4.5 and RCP8.5.

is not considered a major issue in dairy farms since its visual symptoms make its detection unchallenging. Finally, the obtained results are compared with other contagious pathogens similar to *S. aureus*; so, [Gonçalves et al. \(2018\)](#) reported 3% of raw milk losses from bovine mastitis caused by *Streptococcus agalactiae* in warm weather conditions, which is similar to the obtained here for the Mediterranean region in a baseline scenario (i.e. 2.15%).

3.2. Future raw milk losses due to bovine mastitis under climate change scenarios

Mastitis prevalence was one of the computations influenced by the modification of temperatures according to each RCP (i.e. 2.6, 4.5 and 8.5). As a result of step 9, the highest annual prevalence of mastitis was also observed for the Mediterranean in all the climate change scenarios since temperatures in this region are higher than in the Atlantic and Continental regions. As a result, the proportions for each mastitis category increased for this region and different scenarios. In a pessimistic climate change scenario without farm improvements, the proportions increased up to 25.4% for sM, 1.6% for cM and 3.5% for coM due to a rise of temperature by 2050 (detail values per biogeographical region and mastitis category in Supplementary Fig. S1). On the contrary, they dropped to 15.4%, 1.6% and 2.5%, respectively, as an effect of the on-farm improvements. Similarly, the highest annual *S. aureus* and SCC concentrations were also presented in the Mediterranean region, increasing up to 1.8×10^3 CFU mL⁻¹ and 2.3×10^5 cells mL⁻¹, respectively, in scenario 5. On-farm improvements have a significant positive effect on the *S. aureus* and SCC concentration, dropping to 1.0×10^3 CFU mL⁻¹ and 1.4×10^5 cells mL⁻¹, respectively, when scenario 6 is considered (Figs. S2 and S3). As a consequence, in the same scenario, annual raw milk losses reached only 2.45% in dairy farms located in the Mediterranean. However, when no on-farm improvements are considered, raw milk losses increased by 3.21% (Fig. 2).

Due to the lack of similar studies, the comparison of the predicted raw milk losses for future scenarios obtained in this section was not possible.

3.3. Sensitivity analysis

A sensitivity analysis was conducted to investigate which input parameters had the largest influence on the predictive model, which quantified the *S. aureus* from the environment and cross-contamination. According to Fig. 3, $C_{bedding}$ resulted as the most influential parameter on the concentration of *S. aureus* in the BTM, showing a positive correlation coefficient of 0.96. The value used for the *S. aureus* concentration in the bedding material contributes significantly to the pathogen concentration due to environment and cross-contamination. It explains that the concentration of *S. aureus* from the environment obtained in all the scenarios remains similar. Another input parameter of importance is $F_{bedding}$, reaching a positive correlation coefficient of 0.20. Besides, temperature and pH were also part of the sensitivity analysis; however, both showed an insignificant influence, 0.01 and 0, respectively. In other words, the bedding material is an

important source of bacteria load, and the material used at dairy facilities influences the *S. aureus* load on cows' udder and teats. Later, a negative correlation coefficient of 0.13 was found for $PT_{efficiency}$, which means this parameter can reduce the *S. aureus* contamination on cows' udders. For the sensitivity analysis, the values of these three input parameters were changed towards a worst and optimal scenario by selecting the lowest and the highest values. In an optimal scenario, the value of *S. aureus* concentration after treatment was reduced to 7.9×10^{-2} CFU lactating cow⁻¹ year⁻¹, compared to the value obtained in the baseline scenario 3.02×10^7 CFU lactating cow⁻¹ year⁻¹. Conversely, in the worst scenario, the *S. aureus* concentration value increased to 3.08×10^8 CFU lactating cow⁻¹ year⁻¹ compared to the baseline scenario.

4. Recommendation

This research aimed to predict the future influence of climate change on milk losses due to bovine mastitis caused by *S. aureus*. The results make it possible to establish the magnitude and direction of the expected consequences on milk production under the future effects of climate change. If unchecked, a potential increase in the prevalence of mastitis is anticipated due to global warming, resulting in milk loss increases, which are anticipated to be more pronounced in the Mediterranean and Atlantic regions of Europe. The predictive model and the results of this research will serve as a framework for farmers to design corrective measures to reduce milk losses due to mastitis. The model may also help farmers and policymakers make the right decision for future adaptation plans to counter climate change scenarios.

Global warming is expected in the near future by 2050 (IPCC, 2021); however, dairy farmers can modify on-farm parameters to cope with this potential increase in the prevalence of mastitis. This study demonstrates the positive effect of farm improvements in reducing bovine mastitis. According to the (IDF, 2018), one way to reduce mastitis will be to develop new mastitis treatments. However, medicaments to prevent and treat mastitis can enter in the environment (Guo et al., 2021) and potentially lead to an environmental impact (Guzmán-Luna et al., 2021). Other alternatives to reduce the prevalence of mastitis on farms in the future also need to be considered. Based on the sensitivity analysis of this study, bedding material significantly influences the concentration of *S. aureus* in raw milk. The reason is that bedding material carries mastitis pathogens to which the udders are exposed, causing bovine mastitis. In this context, the use of low moisture and clean bedding materials could become more important and be an effective strategy to help control potential contamination on cows under climate change conditions. In addition, periodic and more frequent cleaning of animal waste and hygiene practices on the farm are recommended. Bovine mastitis will mean lost profitability on farms due to the loss of raw milk due to the inefficient use of resources used for its production (Halasa et al., 2007).

Mastitis in dairy farms has been shown to have a significant environmental impact. For instance, the prevalence of this disease leads to

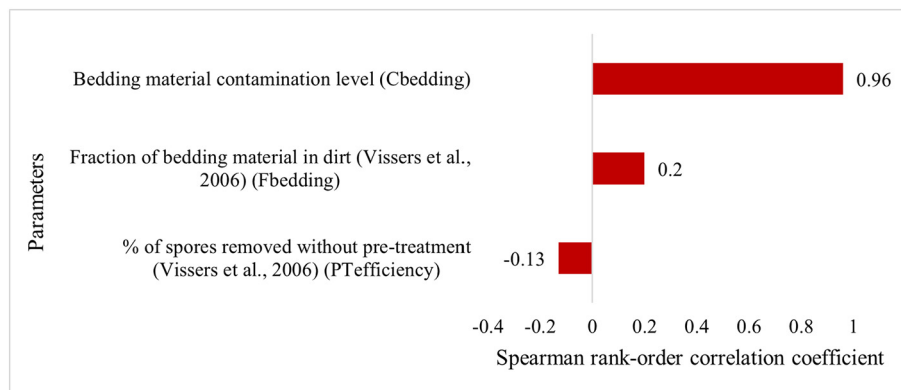


Fig. 3. Effect of input parameters in the exposure assessment model used to calculate the environmental *S. aureus* at dairy facilities. (Spearman Rank correlation coefficient).

increased greenhouse gas emissions per unit of product (Hospido and Sonesson, 2005; Mostert et al., 2019; Vida and Tedesco, 2017). In addition, the dairy sector looks at adapting to the future effects of climate change, but another challenge is to reduce its emissions. This sector is part of climate action and is committed to reducing GHG emissions from the European Union by 2050 (European Dairy Association, 2019). Therefore, in addition to designing adaptation plans to climate change that guarantee animal welfare and food quality, the environmental consequences must also be considered.

5. Conclusions

The present research aimed to predict the annual raw milk losses due to bovine mastitis considering several climate change scenarios using a risk assessment approach. Therefore, a stepwise probabilistic model was developed and proposed. To the best of our knowledge, this is the first time that the complete contamination pathway of *S. aureus* is covered by including all the on-farm production stages and the different sources of *S. aureus* exposure. Also, this is the first probabilistic model that can explain the influence of each input parameter of the process on the overall outcome. One of the challenges in developing the present model was the lack of studies that partially cover the contamination pathway of *S. aureus* from the farm to the BTM. Instead, they only tracked the *S. aureus* concentration of different on-farm spots and unit operations without covering all of them in the same study.

The predicted annual raw milk losses varied across regions and scenarios, being the Mediterranean the region that experiences the largest percentage loss in the baseline and climate change scenarios. For the baseline scenario, the annual losses ranged from 1.06% to 2.15% across all regions. For the future climate change scenarios, the annual raw milk losses varied from 1.17% to 3.21% across all regions when no on-farm improvements were assumed, whereas the losses dropped from 0.37% to 2.45% when on-farm improvements were considered. Bedding material contributes significantly to the concentration of *S. aureus* on-farm, and it is an important parameter to focus on for mastitis control programmes. The main cause of raw milk losses was not attributed to losses associated with exceeding quality standards but a reduction in the subclinical and coloniser cows' milk yield. The largest milk yield reduction was found in cows with clinical mastitis. Cows with coloniser and subclinical mastitis present lower milk yield reduction. However, unlike cows clinically infected, these cows are commonly milked, and their milk may enter the BTM due to the lack of infection physical symptoms. Thus, cows with subclinical and coloniser mastitis contribute to the two types of raw milk losses found on the farm. The outcome of the present study will allow farmers to assess and predict potential raw milk losses under climate change scenarios and help identify adaptation plans to reduce the impact of climate change on milk yield loss.

CRedit authorship contribution statement

Paola Guzmán-Luna: Conceptualization, Methodology, Data curation, Data analysis, Visualization, Investigation, Writing-original draft. **Rajat Nag:** Conceptualization, Supervision, Writing-review & editing, Visualization. **Ismael Martínez:** Supervision and data provision. **Miguel Mauricio-Iglesias:** Conceptualization, Writing-review & editing. **Almudena Hospido:** Conceptualization, Writing-review & editing. **Enda Cummins:** Conceptualization, Supervision, Writing-review & editing.

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.

Acknowledgements

We gratefully acknowledge the help of the Research and Development Department of CLUN for his contribution of data representative of the selected regions. This project is part of the PROTECT ITN (<http://www.>

[protect-itn.eu/](http://www.protect-itn.eu/)), funded under the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 813329. P. Guzmán-Luna, M. Mauricio-Iglesias and A. Hospido belong to a Galician Competitive Research Group (GRC), a programme co-funded by the FEDER (EU).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.155149>.

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