Quantitative risk assessment for shigatoxin producing *E. coli* in bulk milk sold directly from producer to consumer.

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Abstract

We recently reported presence of Shigatoxin producing *E. coli* (STEC) in raw and pasteurised producer-distributor bulk milk (PDBM) in South Africa. Quantitative microbiological risk assessment and predictive modelling are important scientific tool which provides evidence-based and transparent estimation of the risk of foodborne illnesses. This study was envisaged to estimate the haemolytic uraemia syndrome (HUS) risk associated with consumption of STEC contaminated PDBM and estimate the resulting burden of illness that may be associated with consumption of PDBM in South Africa. Data was obtained from recently completed studies in South Africa taking into account prior collected prevalence data of STEC in raw and pasteurised PDBM, and survey information from producer-distributor (PD) outlets and households. Inputs for the models were complemented with data from published and unpublished literature. A probabilistic exposure model was developed with Monte Carlo simulation in excel add in software using @Risk software. Hazard characterisation was based on an exponential dose-response model to calculate the probability of illness from STEC in age groups below and above 5 years. Mean estimated STEC concentration was 0.12 Colony Forming Units (CFU)/ml (95% CI: 0 – 1.2; $\sigma = 0.34$), cfu/ml for raw PDBM and 0.08 cfu/ml (95% CI: 0 – 1; $\sigma = 0.27$), cfu/ml for pasteurised PDBM. A higher risk of HUS cases per year was recorded in raw than pasteurised PDBM and also in age groups above than below 5 years. For every 10 million PDBM portions consumed, the expected number of HUS cases per-year were 154 and 28 for age groups under 5 years and above 5 years in raw PDBM (median risk based estimation). The cases per-year attributable to pasteurised PDBM were 102 and 19 for age groups under 5 years and above 5 years, respectively. Sensitivity analysis revealed serving volume and time taken to sell PDBM at PD outlets as factors with the greatest impact on probability of illness. Results from this study can be useful in formulating risk-based mitigation strategies and policies. Additionally, the models developed in this study are an example of risk assessments for milk produced and marketed from similar scenarios across the globe.

Key words: Bulk-milk, Shigatoxin, *E. coli*, Risk-Assessment, haemolytic-uraemia-syndrome (HUS)

1 Introduction

Over the years, shiga toxin producing *Escherichia. coli* (STEC) have globally evolved from clinical novelty to primary food safety and public health concern (Khan et al., 2002). Long-term sequelae of STEC infections range from mild diarrhoea and intestinal discomfort to serious complications such as haemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). STEC O157:H7 is still recognized as epidemiologically significant world-wide, however, in some geographical regions, non-O157 STEC (O26, O103, O111 and O145) are becoming prominent as important *E. coli* pathotypes (Khan et al., 2002; Delignette-Muller et al., 2008). Ntuli et al. (2017) documented emerging non-O157 STEC O2, O9, O20, O43, O64, O68, O83, O112, O155 and O157 in PDBM in South Africa (SA).

Documented milkborne disease outbreaks have been linked to consumption of both raw (CDC, 2007; Denny et al., 2008; Claeys et al., 2013) and pasteurised (Goh et al., 2002) bulk milk contaminated with shigatoxin producing *E. coli* (STEC), in particular O157. EFSA (2015) documented 27 foodborne outbreaks in Europe attributed to STEC in bulk milk. During the period 2007-2012, thirteen outbreaks associated with STEC in bulk milk were recorded in 26 states in the US (Mungai et al., 2015). Epidemiological statistics on STEC in food in Africa are imprecise and the studies are few, although a recent review linked several outbreaks of STEC to food in the region (Raji et al., 2006). In SA, there are no official data existing on the prevalence of STEC linked to contaminated food. However, studies have indicated prevalence of STEC isolated from humans and livestock faeces, water and food, ranging from 15% to 42.8% (Aijuka et al., 2014; Iweriebor et al., 2015; Ndlovu et al., 2015; Ntuli et al., 2017). A survey on producer-distributor

bulk milk (PDBM) in SA revealed high levels of *E. coli* (Ntuli et al., 2016; 2017), above stipulated limit (SA, 2001 Act (54), (1972)). Ntuli et al. (2016; 2017) also reported a diversity of EHEC seropathotypes, (with different shigatoxin virulence factors, multi drug resistant and extended-spectrum β-lactamases (ESBLs) producing capacity in the PDBM. Other research studies carried out on *E. coli* isolated from bulk milk in SA by Caine et al. (2014) and Msolo (2016), documented diarrheagenic *E. coli* belonging to enteroaggregative *E. coli* (EAEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and uropathogenic *E. coli* (UPEC)

Producer-distributor bulk milk in SA is typically (i) raw milk for human consumption, (ii) raw milk received with the intention to process and sell as pasteurised milk, and (iii) pasteurised milk received to be sold, that has been pasteurised elsewhere at an approved facility. The milk constitute 2% of all the milk produced and processed in SA. Only state certified producer-distributors (PDs) are permitted to sell raw milk directly to consumers in SA (SA, 2001 Act (54), (1972), however unregistored/authorised producer also find their way in PDBM value chain. The sale of PDBM directly to consumers is a common practice in SA and around the world. In SA, no attempts have been made to quantify the risk posed on human health by pathogens in milk.

To gain an insight on the accurate estimates of the actual risk posed by consumption of PDBM contaminated by pathogenic *E. coli*, a quantitative microbial risk assessment modeling is one of the practice to evaluate food health risks and control (CAC, 1998; FAO/WHO, 2003). Several risk assessment studies have been conducted in US, Europe and Africa in an attempt to quantify disease cases as a result of milk borne pathogens, (Grace et al., 2008; Clough et al., 2009; Giacometti et al., 2015)..

Owing to the lack of epidemiological data, the burden of pathogenic *E. coli* linked to consumption of PDBM in SA has not been assessed. In this study we conducted a quantitative risk assessment of STEC in PDBM under the current production and marketing conditions in SA. The study was envisaged to estimate the HUS risk associated with consumption of STEC contaminated PDBM. This will enable assessment of factors that would have the greatest impact on public health and safety along the PDBM supply chain as well as formulating hypothetical mitigation strategies. Furthermore, this risk analysis facilitated the identification of data sparsity, which needs to be addressed for future quantitative risk assessments on PDBM. The models developed in this study are an example for other risk assessments in milk produced and marketed from similar scenarios across the globe.

2 Materials and methods

2.1 Hazard identification

Recent reports on raw and pasteurised PDBM in SA indicated prevalence of STEC O157 and non-O157 ranging from 10 – 54% (Caine et al., 2014; Msolo, 2016; Ntuli et al., 2016; Ntuli et al., 2017). Lately, milkborne disease outbreaks were incriminated to consumption of raw milk contaminated with STEC in the EU and US, especially raw milk sold directly from producer to the public (CDC, 2007; Denny et al., 2008; EFSA, 2015). A review by Claeys et al. (2013) reported 13 *E. coli* outbreaks in Europe and 28 worldwide associated with consumption of raw milk, between 1970 and 2010. The same authors documented an increased incident consisting of 27 STEC outbreaks in Europe between 2007 and 2012 as a result of raw milk consumption. However, few outbreaks were reported for pasteurised milk during that period (Clough et al., 2009). Farrokh et al. (2013) documented STEC outbreaks from 1986 - 2010 that have been linked to milk and

dairy products in Europe, US and Canada. Most of these outbreaks, reviewed by Farrokh et al. (2013), were associated with STEC O157, although other serotypes or serogroups, including O22:H8, O110:H^{-}, O80:H^{-}, and O145 have also been identified as causative agents. *E. coli* can grow at temperature range of 7 to 46 °C with an optimum of 37 °C, however, studies have shown that depending on different food matrix, the organisms can resist pasteurisation temperatures of up to 72 °C (Mercer et al., 2015). Faecal-contaminated foods including, raw vegetables, under-cooked beef burgers, milk and milk products, are the most common vehicles for transmission of STEC from animals to humans.

2.2 Hazard characterisation

Virulence properties, mechanisms of pathogenicity, clinical symptoms and distinct serogroups are used to distinguish different *E. coli* strains that cause diarrheal diseases. Albeit, effects of *E. coli* pathogens being dependent on host susceptibility (immune status and immunity imparted by previous exposure) and dose ingested, the most vulnerable members from the diseases are children under 5 years, the elderly and immune-compromised individuals. However, some STEC strains (O104 and O157 serotypes) have proven to cause severe illnesses even in healthy adults (Mellmann et al., 2011). *E. coli* pathotypes exhibit different clinical syndrome with distinctive pathological and epidemiological characteristics of disease (Robins-Browne, 2004). For example, Enterotoxigenic *E. coli* (ETEC) causes watery/cholera-like diarrhea in children in developing countries, whereas, Enteropathogenic *E. coli* (EPEC) cause gastroenteritis in infants. Enteroinvasive *E. coli* (EIEC) has been associated with watery dysentery diarrhea in all age groups and is common in developing countries, while, Enterohaemorrhagic *E. coli* EHEC are implicated with diarrhea in infants, haemorrhagic colitis and haemolytic uremic syndrome (Nataro and Kaper, 1998). Studies have shown that a few EPEC cells are necessary to cause illness in children,

10^8 – 10^{10} cells of ETEC and EAEC are necessary for illness in adults although the infective dose is probably less for infants and children, 10^8 EIEC cells are necessary to cause illness in adults, and ingestion of 10^2 of EHEC can cause fatal illness to humans (Nguyen and Sperandio, 2012; Dean et al., 2013).

2.3 Exposure assessment

2.3.1 Field survey

A survey was conducted in urban and peri-urban parts of Pretoria in SA (one of the PDBM sampling areas) with the aim of getting an insight on the typical flow of PDBM from outlets to consumer and PDBM consumption patterns. A questionnaire was developed to capture the following information; (i) PDBM handling practices and storage conditions at outlets, (ii) PDBM handling practices during transportation to home, (iii) consumer handling practices and storage conditions, and also consumption patterns. A total of 15 PDBM outlets and 80 consumers were interviewed and the information was used as input for the models.

2.3.2 Overview of PDBM pathway to consumer and exposure model

Consumers of PDBM obtain their milk from different sources either raw or pasteurised. Stages prior PD outlets were not included in this model. The model was developed starting from PD outlets to household level for PDBM which was sold either as raw or pasteurised. The conceptual model upon which the mathematical model was based to estimate the exposure of STEC to consumers is depicted in Fig 1. A “modular process risk” framework (Nauta, 2002) was adopted to simulate the scenario which the milk undergoes from the PD outlets to consumption. The same scenario was used for either raw or pasteurised PDBM, however, what differed was the STEC prevalence and concentration in raw and pasteurised PDBM at the time of sale at PD outlets (Ntuli

et al., 2016). Consumers either brought containers which were filled directly from the bulk tank or they bought small plastic containers (1 to 5 L) prefilled with bulk milk at the outlets. We modeled changes in prevalence and concentration of STEC in PDBM from outlets to consumption after storage at home. At each step, basic microbial and milk handling processes, such as growth and partitioning were identified and applied. We divided the model into the following steps: (i) PD storage (ii) transport time and temperature from PD to home and consumer handling (iii) consumption habits at home and exposure to STEC. Each step, in sequence, produced one or more output distributions that served either as inputs to the next step or as final outputs of the estimation of the probability and concentration in a single serving at consumption. The model was developed from input data derived from the field survey (section 2.3.1), a completed study on PDBM by Ntuli et al. (2016), other published literature and expert opinion whenever possible. Input parameter variables, their description and associated equations or distributions, for PDBM production model are presented in Table 1. The same model was used for both raw and pasteurised PDBM.

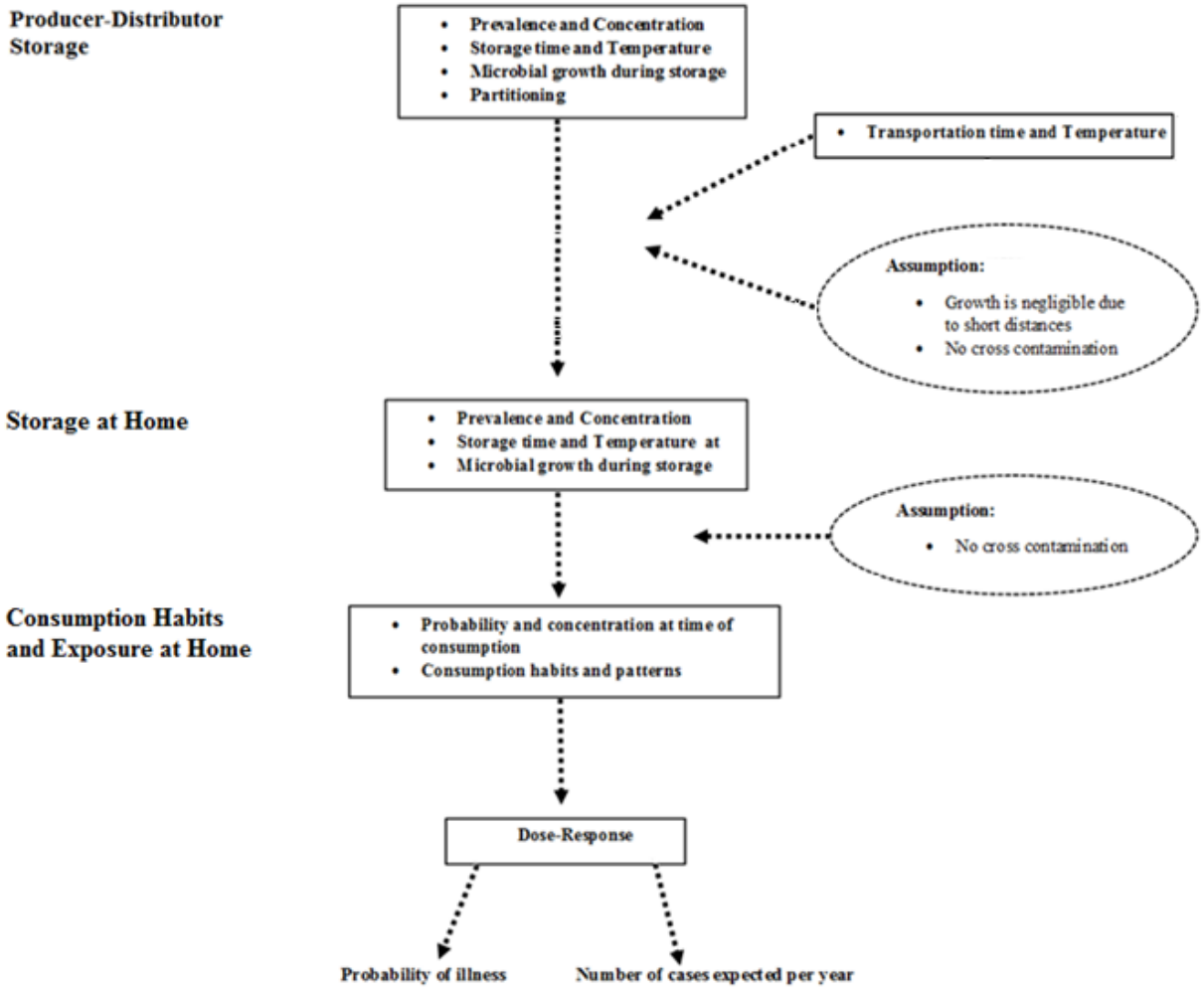

Figure 1: Schematic overview of the quantitative risk assessment model for shigatoxin producing *E. coli* in producer-distributor bulk milk.

2.3.3 Estimation of STEC concentration in PDBM

Data on the prevalence of STEC in raw and pasteurised PDBM samples reported in previous studies by (Ntuli et al., 2016; 2017) were considered in the estimation of STEC concentration. The data reported positive for STEC in 17 (n=154) and 8 (n=104) raw and pasteurised PDBM samples, respectively. A direct plate count method using 3M petrifilm plates and molecular techniques was adopted by the authors, Colony Forming Units (CFU)/ml of STEC in the positive samples ranged

from 1 to 3. We used a Poisson distribution to calculate the mean concentration of STEC in both raw and pasteurised PDBM (Sanaa et al., 2004). To test that STEC in PDBM follow a poison distribution we carried out a chi-square goodness of fit test.

2.3.4 Producer-distributor storage

The distribution of PDBM volumes produced or received per day at outlets were computed from a study by Caine et al. (2014) and from oursurvey (Table 1). Milk is stored as either raw or pasteurised and the volumes of PDBM was in-cooperated in the model as a distribution representing variability in milk volumes at each outlet. As a result of limited data from the survey, we used uniform distributions to simulate variability in storage temperatures and time taken to sell all the milk from one batch and this was used in the growth model (Table 1). Cross contamination at PD storage is uncertain due to lack of data or published data, therefore, we assumed no cross contamination during modelling. Maximum population density (MPD) and maximum growth rate (μmax) of STEC at 9.5 ºC in milk used in the growth model was obtained from a study by Kauppi et al. (1996). Concentration after growth occurs during storage was computed taking into account, MPD, μmax , time all the milk is sold and the initial concentration (Table 1). Prevalence after growth occurs was considered unaffected.

Consumers buy PDBM in plastic containers or the milk is packaged into retail units (0.5 to 5 L) at PD outlets. The model used to calculate STEC prevalence and concentration in smaller units assumed that STEC is randomly distributed in the milk. The new prevalence is the probability that at least one STEC cell is present in the new smaller units, given that the bulk milk where it is drawn was previously contaminated with STEC, was considered equal to the fraction of bulk milk that the small unit represents. Therefore, STEC prevalence in the containers is adjusted by the probability that one or more STEC cells will end up in a random smaller unit. Concentration of

STEC after packaging into smaller volumes was calculated by binomial sampling of the number of STEC cells that are in the small units. Therefore, the new STEC concentration in randomly generated contamination count divided by the small unit volume (Table 1) (Njage and Buys, 2016). A uniform distribution was used to model the distribution of milk volumes sold per day and this was used to calculate number of servings consumed per day.

2.3.5 Transport from PD to home and consumer handling

Based on the survey, we assumed that there was negligible or no STEC growth during transportation of PDBM, given short distance and time from outlets to home, even though the milk was transported at abused temperatures (Table 1). PDBM has a shelf-life of 3 days (Giacometti et al., 2012b), but on the basis of interview answers, milk was consumed up to 5 days. Hudson and Hartwell (2002) and Marklinder et al. (2004) noted that there is variability in refrigeration temperatures in homes that can allow *E. coli* growth in food. The authors observed that 39% of households refrigerated their food at 6-7°C, 4% at 7 – 8àC, 4% at 9 – 10°C and 1% at 12ºC. We used a cumulative distribution of the different refrigeration temperatures from different proportions of consumer as an input for the growth model at home storage. Growth was further computed taking into account; STEC concentration after packaging into smaller units, maximum growth rate, distributions representing variability of time taken to transport the milk from PD to home and storage at home, and MPD of STEC in milk at refrigeration temperatures (Kauppi et al., 1996). Maximum growth rate (μmax) of STEC at refrigeration temperatures was derived from a cumulative distribution of the different μmax values at 6-7, 7 – 8, 9 – 10, 12 ºC and proportions of consumers storing milk under the respective different refrigeration temperatures (Hudson and Hartwell, 2002; Marklinder et al., 2004). Prevalence after growth during storage at home in refrigerators was considered unaffected (Table 1).

2.3.6 Consumption habits at home and exposure to STEC

According to the interviews carried out in the survey, 67% of the consumers boiled PDBM before consumption. Giacometti et al. (2012b) observed that boiling milk completely eliminates viable *E. coli* cells. However, the remaining 33% used methods such as microwave, mixing with hot tea or porridge, which we consider as inadequate/insufficient heat treatment of milk. Log reduction for the insufficient heat treatment of milk was modeled using the a triangular distribution (Giacometti et al., 2012b) (Table 1). Consumer habits was used in the final exposure model. The distribution of PDBM serving size was characterised by values from the survey (Table 1). Final exposure (concentration) of STEC per serving was calculated as an output using the model in Table 1.

2.4 Dose response

We adopted the dose response model of STEC in food used by Delignette-Muller et al. (2008). The authors directly modeled the probability of HUS as a function of ingested dose. Probability of illness from STEC infections is dependent on age and other factors as reported by Nauta et al. (2001). In the current study, we used two dose-response models for two age groups, 0 to 5 and > 5 years (Table 2). Children under 5 years are more susceptible to STEC as documented from an epidemiological data (Loirat et al., 2008). The values for r used in the model were estimated by Delignette-Muller et al. (2008) for each age group (Table 2). Probability of HUS per-serving was computed by combining the dose estimate and contamination prevalence (Ross et al., 2009). Number of cases per year was calculated by multiplying the probability of HUS per-serving and the number of serving per year for each target age group (Table 2).

2.5 Simulation and analysis

Stochastic modelling of the exposure with STEC for all scenarios were implemented with the Monte Carlo simulation technique by using the risk analysis software @Risk 7.5 (Palisade Corporation, Ithaca, USA). All models were simulated for 100 000 iteration as carried out by Latorre et al. (2011) and Giacometti et al. (2012a). The outputs of the model was the median risk of HUS per services in each class of consumers ages. The total amount of HUS cases predicted by the model was obtained directly moltipling the median HUS risk by the number of portion consumed per year by yang (less than 5 years) and normal consumers (>5 years). Sensitivity analysis for each scenario was performed to identify important parameters from their corresponding distributions. Spearman's correlation coefficient was used to estimate the impact of PDBM value chain practices on the variability in exposure with STEC in PDBM per year. We introduced possible PDBM handling scenarios to test the associated effects in reducing exposure per-serving to shigatoxin producing *E. coli* to consumers who insufficiently heat the milk. The scenarios include: storage and handling of PDBM at 4°C throughout the whole chain, time taken to sell the milk at PD outlets, time taken to consume all the milk at home and a combination of, some of the, PDBM handling practices (Table 5). Storage and handling of PDBM at 4°C throughout the whole chain was broken down into points where this storage effect was modeled (PD outlets, transportation to home and home refrigeration). We used 5, 6 and 7 hr as time taken to sell the milk at PD outlets. This is the possible and realistic time PDs can acquire milk and sell within the same day. Maximum recommended time for raw milk storage at house hold is 3 days (Giacometti et al., 2012a), therefore, we used half, one and two days as possible and realistic time taken to consume all the milk at home. A combination of scenarios (storage at 4°C throughout the

whole chain + time taken to sell the milk (5 hr) + time taken to consume the milk (half a day)) was also evaluated.

Table 1: Input parameters for exposure model of STEC in raw and pasteurised producer-distributor bulk milk: Description, equations or distribution, values and units of the input parameters and data sources

Steps	Parameter	Description	Distribution/ Equation/values	Units	Data Source/reference
Producer-distributor storage	IP_{PDS}	Initial prevalence of STEC positive PDBM	11 raw milk 8 pasteurised milk	% %	(Ntuli et al., 2016)
	IC_{PDS}	Initial concentration of STEC in PDBM samples	0.12 raw milk 0.08 pasteurised milk	cfu/ml	
	V_{outlet}	Volume produced or received at outlets.	RiskPert(500000, 1000000,5000000)	ml	(Caine et al., 2014) Survey
	$Temp_{PDS}$	PDBM storage temperature	RiskUniform(8,11)	°C	This study
	$Time_{PDS}$	Time taken to sellPDBM	RiskUniform(24,48)	hr	This study
	$Milk_{sale/day}$	Average PDBM sale per-day	RiskUniform(500000, 5000000)	ml	This study
	G	Growth of STEC during PDBM storage	RiskPoisson($Time_{PDS}$ in hr x μmax) Where: μmax = 0.036	log cfu log cfu/h	(Kauppi et al., 1996)
	$Conc_{PDS}$	Concentration of STEC after growth occurs during storage of PDBM	IF($IC_{PDS} \times 10^{G}$)>MPD,MPD,($IC_{PDS} \times 10^{G}$) Where: MPD = 31622777	cfu/ml cfu/ml	
	$Size_{PDS}$	Volume of milk sold to consumer and size of containers	RiskPert(500,1000,5000)	ml	This study
	$Prev_{consumer}$	New PDBM sample prevalence with STEC after packaging into smaller units	IP_{PDS} x $Psmall_{consu}$ Where: $Psmall_{consu} = (1-(1-(Size_{PDS}/V_{outlet}))^{Nconsu}$		

			N_{consu} = round($Conc_{PDS}$ x V_{outlet})		
	$C_{consumer}$	New PDBM STEC concentration after packaging into smaller units	RiskBinomial($N_{consu,}$ $Size_{PDS}$ /V_{outlet}) / $Size_{PDS}$	cfu/ml	
Transport time and temperature from producer-distributor to home and Consumer handling	$Trans_{Temp}$	Transportation temperature	RiskPert(14.3, 26, 38)	°C	This study
	$Trans_{Time}$	Transportation time	RiskUniform(0.5,3)	hr	This study
	$Time_{shelflife}$	Time until all PDBM is consumed at home	RiskPert(1,2,5)	days	This study
	$Temp_{fridge}$	Temperature refrigeration at home	RiskCumulD(5.5,12,{5.5,7.5,9.5,12}, {0.39,0.04,0.04,0.01})	°C	(Hudson and Hartwell, 2002; Marklinder et al., 2004)
	G1	Growth of STEC in PDBM during refrigeration storage at home	RiskPoisson($Time_{shelflife}$ in hr x µmax) Where: µmax = RiskCumulD(0.019,0.041,{0.019,0.028,0.036, 0.041}, {0.39,0.04,0.04,0.01})	log cfu log cfu/h	
	C_{HST}	Concentration STEC in PDBM after growth occurs during refrigeration storage at home	IF($C_{consumer}$ x 10^{G1})>MPD,MPD,($C_{consumer}$x 10^{G1})		
Consumption habits at home and exposure to STEC	$Serving_{size}$	Milk serving size at consumer level	RiskPert(150,500,1000)	ml	This study
	D_{EX}	Log reduction of those who partially boiling by microwaving and other	RiskTriang(2,4,6)	log cfu/ml	(Giacometti et al., 2012a)

	insufficient heating at consumer level			
B_{EX}	Consumer habits (milk boiling before consumption at home)	RiskBernoulli(0.5) x D_{EX}		
$Time_{GT\text{-}EX}$	Generation time of STEC under refrigeration conditions	RiskTriangle(34.2, 45.1, 56)	hr	(Giacometti et al., 2012b)
$Dose_{per\text{-}serving}$	Dose of STEC per serving	$10^{\wedge}(\log[10^{CHST} \times 2^{\wedge(DEX / TimeGT\text{-}EX)}] - B_{EX}) \times Serving_{size}$	cfu	(Giacometti et al., 2012a)

Table 2: Dose response assessment for STEC in raw and pasteurised producer-distributor bulk milk: Description, equations or distribution, values and units of the input parameters and data sources

Parameter	Description	Distribution/ Equation/values	Units	Data Source/reference
P_{HUS}	Probability of haemolytic uremic syndrome	$P_{HUS} = 1 - (1 - r)^D$ Where: *r* is the dose response parameter per organism: r = 1.28×10^{-3} (0 to 5 years) r = 2.4×10^{-4} (> 5 years) *D* is dose per-serving ($Dose_{per\text{-}serving}$)	cfu	(Delignette-Muller et al., 2008)
$P_{Serving}$	Probability of illness per-serving	$P_{Serving} = P_{HUS} \times Prev_{consumer}$		(Latorre et al., 2011)
N_{case}	Number of haemolytic uremic syndrome cases per year	$P_{Serving}$ x Number of servings per year Where: Number of servings per year = proportion per each age group x portions consumer with insufficient heat treatment.		(Latorre et al., 2011)
		Proportion per each age group: (0 to 5) = 6311000/55910000		(Statistics South Africa, 2016)
		Portions with insufficient heat treatment = 0.33 x portions consumed per year Where: 0.33 is proportion of consumers who insufficiently heat the milk		This study
		Portions consumed per year = $((Milk_{sale/day} \times 178)/ Size_{PDS}) \times 365$ days Where: 178 is number of PDs in SA		This study (MPO, 2016)

3. Results

3.1 Concentration of STEC in raw and pasteurised PDBM

Number of samples contaminated with n=0, 1, 2, or 3 cfu/ml STEC in raw and pasteurised producer-distributor bulk milk was 17 (n=154) and 8 (n=104), respectively. From the prevalence data on STEC positive samples, estimated number of STEC in raw (0.12 cfu/ml) and pasteurised (0.08 cfu/ml) PDBM was by done by fitting a Poisson distribution. Chi-square goodness of fit test was used to test the fit of the Poisson distribution. Pearson χ^2 at 1.79, degrees of freedom was 2 and *p*-value based on χ^2 distribution was 0.20 for raw PDBM. For pasteurised PDBM, Pearson χ^2 at 1.90 degrees of freedom was 1 and *p*-value based on χ^2 distribution was 0.11. Therefore, the Poisson distribution adequately predicted the estimated number of STEC in both PDBM types. The model gave a mean STEC estimate concentration of 0.12 cfu/ml (95% CI: 0 – 1.2; σ = 0.34 cfu/ml), for raw PDBM and 0.08 cfu/ml (95% CI: 0 – 1; σ = 0.27), cfu/ml for pasteurised PDBM.

3.2 Exposure assessment

The estimated levels of STEC per-serving, after boiling the milk, in both raw and pasteurised PDBM are depicted in Table 3. The quantity of STEC that a consumer was exposed to in a single serving of milk was a function of the initial concentration of STEC in PDBM at PD outlets, and the subsequent effects of handling and storage along the milk chain. STEC levels increased during storage at PD outlets and home refrigeration, reaching microbial loads of 42 (95% CI: 15 – 569) cfu/per-serving in raw and 28 (95% CI: 10 – 385) cfu/per-serving in pasteurised PDBM, prior heat treatment. Considering the 33% of consumers who insufficiently heat the milk before consumption, the STEC concentration per-serving resulted between 4.06×10^{-4} and 6.09×10^{3}

cfu/per-serving for raw PDBM and 2.95 x 10^{-4} to 6.42 x 10^{3} cfu/per-serving for pasteurised PDBM (Table 3). The median STEC concentration per-serving resulted 0.42 cfu/per-serving and 0.37 cfu/per-serving in raw and pasteurised PDBM, respectively, for the 33% of consumers who insufficiently heat the milk before consumption. The model predicted prevalence of PDBM contaminated with STEC to be 11% and 8 % for both raw and pasteurised PDBM at the time of consumption.

3.3 Risk characterisation

To assess the risk posed to consumer from consuming STEC contaminated PDBM we used the exposure assessment model and each iteration predicted a probability of illness and consequently the number of HUS cases per-year (Table 4). In simulations where all consumers boil milk before consumption, no risk was calculated for both raw and pasteurised PDBM. When we considered consumers who insufficiently heat the milk before consumption, the highest median probability of illness per-serving was noted in children under 5 years for raw PDBM (5.96 x 10^{-5}). The highest recorded median number of HUS cases per-year (48.7) was observed in consumers above 5 years who consume raw PDBM (Table 4). We observed lower median probability of illness per-serving (<5 years = 3.85 x 10^{-5} and >5 years = 7.22 x 10^{-6}) and lower median number of HUS cases (<5 years = 19.5 and >5 years = 28.7) per-year in pasteurised than raw PDBM scenarios. For every 10 million PDBM portions consumed, the expected number of HUS cases per-year were 154 and 28 for age groups under 5 years and above 5 years in raw PDBM. The cases per-year attributable to pasteurised PDBM were 102 and 19 for age groups under 5 years and above 5 years, respectively. We observed lower probability of illness and number of HUS cases in consumers of both raw and pasteurised PDBM who were under 5 years than age group above 5 years (Table 4).

Table 3: Estimation of shigatoxin producing *E. coli* concentration per-serving in raw and pasteurised producer-distributor bulk milk.

Parameter / percentile	**Estimated level of STEC (cfu/per-serving)**	
	Raw PDBM	**Pasteurised PDBM**
Minimum	4.06×10^{-4}	2.95×10^{-4}
Mean	4.06×10^{2}	3.66×10^{2}
Maximum	6.09×10^{3}	6.42×10^{3}
5^{th}	5.84×10^{-3}	5.12×10^{-3}
50^{th}	0.42	0.37
95^{th}	1.88×10^{3}	1.69×10^{3}

Table 4: Probability of illness per serving and number of haemolytic-uremic syndrome cases per-year with consumption of raw and pasteurised producer-distributor bulk milk.

Milk category	Population	Probability of illness per-serving Median (5^{th}, 95^{th}) percentiles	Number of cases per-year Median (5^{th}, 95^{th}) percentiles
Raw PDBM	Under 5 years	5.96×10^{-5} (8.37×10^{-7}, 0.10)	330 (2.85, 7.26×10^{5})
	Above 5 year	1.12×10^{-5} (1.57×10^{-7}, 4.02×10^{-2})	487 (4.20, 2.37×10^{6})
Pasteurised PDBM	Under 5 years	3.85×10^{-5} (5.34×10^{-7}, 7.06×10^{-2})	195 (1.87, 5.12×10^{5})
	Above 5 year	7.22×10^{-6} (1.00×10^{-7}, 2.64×10^{-2})	287 (2.76, 1.54×10^{6})

*Values are the median, 5^{th} and 95^{th} percentile obtained after 100 000 iteration, using @ risk 7.5 in both raw and pasteurised PDBM

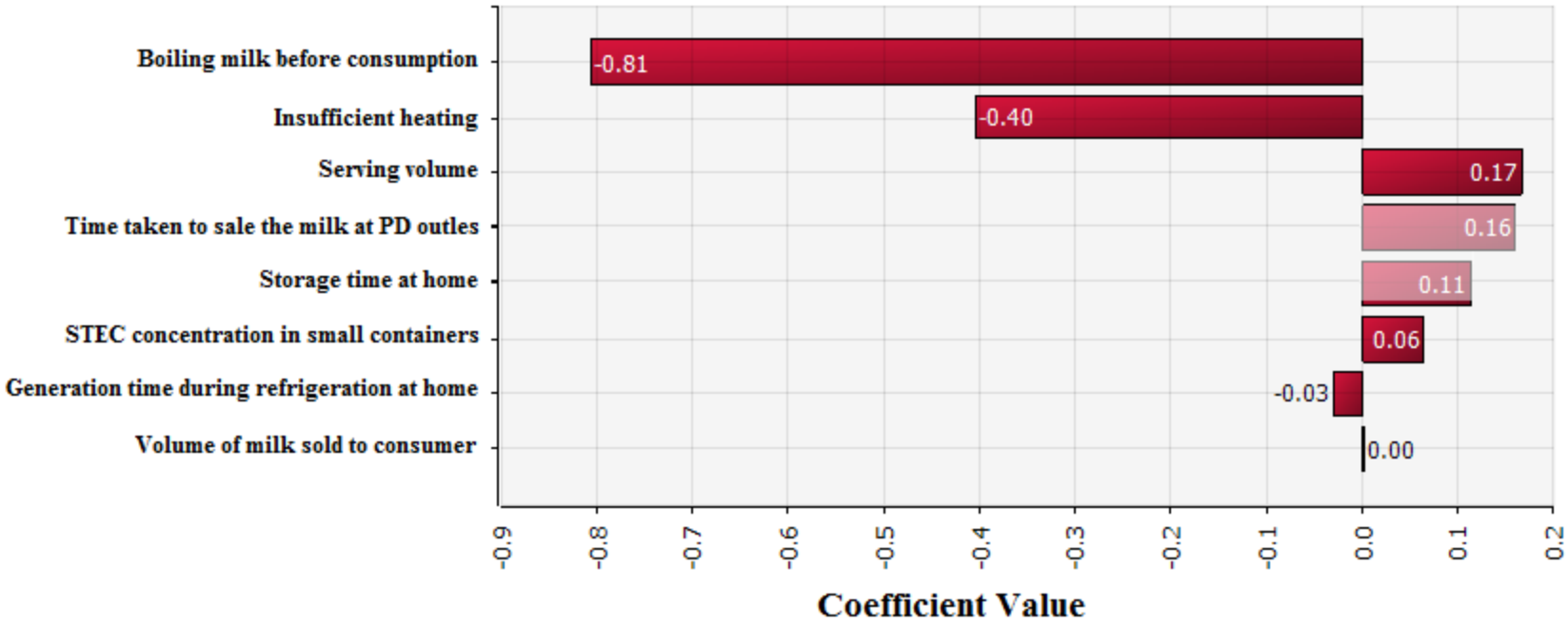

Figure 2: Sensitivity analysis between estimated probability of illness after one serving of producer-distributor bulk milk and important predictive factors along the value chain.

3.4 Effect of model parameters on the risk of HUS

In the current study, sensitivity analysis on the models indicated that serving volumes (Spearman's correlation coefficient (ρ) = 0.17) had the greatest effect on the probability of HUS and the annual number of cases (Fig 2). Time taken to sell the milk at PD outlets (ρ = 0.16) and PDBM storage time at home (ρ = 0.11) were also important factors that influenced probability of HUS and the annual number of cases. After packaging bulk milk into small containers for the consumer, the new modeled concentration of STEC (ρ = 0.06) also affected probability of illness. Insufficient heat treatment of PDBM before consumption, greatly reduced the level of STEC and the subsequent risk of HUS in both raw and pasteurised PDBM (ρ = -0.40) (Fig 2). Generation time of STEC during refrigeration at home, which is achieved by proper refrigeration, also reduced the risk of HUS (ρ = - 0.03)

3.5 Possible PDBM handling scenarios

Storing PDBM at 4°C throughout the whole chain revealed that it was most effective when applied at PD outlets in both raw (23.1% reduction) and pasteurised (19.6% reduction of STEC concentration) PDBM. We observed that, as time to sell PDBM and time to consume the milk after arriving at home reduced, the concentration of STEC per-serving also reduced significantly (Table 5). Reducing time taken to taken to consume all the milk after arriving to half a day, was the most effective single handling practice, with 55.8 and 57.1% reduction in STEC concentration per-serving in raw and pasteurised PDBM, respectively. Considering time taken to sell the milk at PD outlets, the highest reduction in consumer exposure to STEC was observed when milk is received and sold to consumers within 5 hr per-batch (54.2 and 56.0% reduction in both raw and pasteurised PDBM respectively). Combining possible handling scenarios (storage at 4°C throughout the whole

chain + time taken to sell the milk (5 hr) + time taken to consume the milk (half a day)) was the most effective practice in reducing consumer exposure to STEC for both raw (83.2% reduction) and pasteurised (88.5% reduction of STEC concentration) PDBM.

Table 5: Possible handling scenarios and their associated effects in reducing exposure per-serving to shigatoxin producing *E. coli* to consumers who do not boil producer-distributor bulk milk.

Handling procedures	**Reduction in concentration of STEC per-serving (%)**	
	Raw PDBM	Pasteurised PDBM
[a]Storage and handling at 4°C:		
PD outlets	23.1	19.6
Transportation home	8.0	9.7
Home refrigeration	13.3	11.9
[b]Time taken to sell the milk:		
5 hr	54.2	56.0
6 hr	51.8	54.3
7 hr	44.0	45.1
[c]Time taken to consume all the milk at home:		
½ a day	55.8	57.1
1 day	43.5	46.4
2days	34.9	37.2
a (4°C)+b (5hr)+c (½ a day)	83.2	88.5

Discussion

We carried out a stochastic quantitative microbial risk assessment, from PD outlets to consumption, of HUS associated with the consumption of STEC contaminated PDBM based on results from our study, Ntuli et al. (2016; 2017) and also from a survey carried out in one of the sampling areas in SA. This provided an estimate of the nationwide PDBM scenario of HUS cases that may be linked to the consumption of STEC contaminated PDBM.

STEC concentration was lower in pasteurised (0.08 cfu/ml) than raw (0.12 cfu/ml) PDBM. Under ideal conditions, no STEC cell survive pasteurisation temperatures (Goh et al., 2002). However, in our previous study, we recorded presence of alkaline phosphatase in 21% (n=104) of PDBM samples (Ntuli et al., 2016). We could not establish the possible source and pathway of STEC in PDBM, although this was explained as either inadequate pasteurisation process or contamination/cross-contamination of a batch of PDBM after a successful pasteurisation (Ntuli et al., 2016). Depending on the food matrix, recent studies have shown that *E. coli* can resist pasteurisation temperatures (Mercer et al., 2015). Using a modelling approach, Clough et al. (2009) highlighted area of uncertainty and critical control points (CCPs) within the production chain of pasteurised milk; the authors estimated that STEC contamination in milk occurs **ei**ther for inadequate pasteurisation or post-pasteurisation contamination. In their study, they reported that, though inadequate pasteurization may result in survival of STEC, subsequent dilution effects lowers the health risk associated with STEC to very low levels occur in packed milk.

Our model also assessed the risk introduced during consumer handling. These consist of steps after consumer purchase and the subsequent handling at household level. At these stages, PDBM is no longer controlled by professionals (Nauta and Christensen, 2011; Crotta et al., 2016). We treated temperature and time of milk handling and storage as independent parameters. This may have overestimate the risk of HUS since an implicit assumption, underlying the model, that all the milk will be consumed whatever the time-temperature combination. Practically some milk can end up not being consumed due to spoilage at certain time-temperature combination thereby reducing the risk. A study by Crotta et al. (2016) developed a model which captured the dependencies between time and temperature to express the likelihood for milk serving to be actually consumed for any

computed storage time-temperature combination and extent to which the dependency would affect the output. However, the scenarios they used may not apply for PDBM scenario in SA.

In the current study, 11.3 and 88.7% of the consumers were children under 5 years and population above 5 years, respectively. A total of 33% of consumers insufficiently heat treat PDBM before consumption and according to survey estimations, the population under 5 years consumes 4203352 portions of milk per-year and the age group above 5 years consumes 33034716 portions per-year. Exposure concentration of STEC in PDBM per-serving was dependent of the estimated concentration of STEC at PD outlets. Raw PDBM had higher concentration of STEC per-severing than pasteurised PDBM. Based on our survey, the frequency of consumption was 2 times high in children under 5 years than population above 5 years (data not shown) and ideally this pose greater risk to children under 5 years. However, considering the consumed portions by both the age groups, the probability of illness per-serving and number of HUS cases per-year was lower in children under 5 years than population above 5 years. This can be explained by the fact that children 0 – 5 years consume smaller portions/volumes. This same situation was also observed by Delignette-Muller et al. (2008) who conducted a risk assessment for STEC in frozen ground beef patties consumed in France. This is in disagreement with the fact that children under 5 years have an increased probability of sever outcomes such as HUS and death following infection (Signorini and Tarabla, 2009). The probability of illness for both the age groups consuming PDBM were extremely small (far less than 1) but this is difficult to validate given the uncertainty which underlie in the number of PDBM milk consumers. There are no official reports on HUS cases in SA to benchmark our model outputs. However, in Italy, STEC risk assessment for milk reported similar cases of HUS as reported by the Health Ministry (Giacometti et al., 2012a; Giacometti et al., 2016). Latorre et al. (2011) in the US conducted a risk assessment of listeriosis due to consumption of

raw milk and, also reported number of listeriosis cases which were in line with reports from the CDC. In our study, for 10 million PDBM portions per-year, the expected number of HUS cases per-year were 154 and 28 for age groups under 5 years and above 5 years, respectively, in raw PDBM and the expected number of HUS cases per-year in pasteurised PDBM were 102 and 19 for age groups under 5 years and above 5 years, respectively. Our results differ considerably with those of Grace et al. (2008), who reported a higher estimate of 2.40 to 2.83 cases of STEC infections per 10,000 servings portions of raw milk. Giacometti et al. (2012a) also predicted that cases of HUS per-year for 5.25 million portions of milk were 0.09 and 0.5 for children under 5 years and age group above 5 years per, respectively. Latorre et al. (2011) reported that disparities in model output can be as a result of the risk model and the data used in each model, for example temperature distributions, time distributions as well as prevalence of the pathogen in context. Median probability of illness per-serving for STEC in PDBM varied from 7.22×10^{-6} to 1.12×10^{-5} for all age groups. In Europe the reported median probability of illness per-serving for STEC in milk ranged from 9.36×10^{-11} to 2.56×10^{-3} (EFSA, 2015).

In the current study, the risk of infection and the subsequent development of HUS was most influenced by serving volumes followed by time taken to sell the milk at PD outlets. The parameters were the most important in increasing the risk of HUS in both age groups who consume either raw or pasteurised PDBM. Latorre et al. (2011) also reported serving volume as a parameter with great influence in the risk of listeriosis in raw milk (correlation coefficient varied from 0.19 to 0.30 for all the scenarios they studied). In our study, the STEC exposure per-serving was very high for both raw and pasteurised PDBM compared to results in a report by FDA (2003). This could explain why sensitivity analysis picked serving volume as the most important parameter. Partitioning of milk into smaller containers also had an influence in the risk and probability of

illness in this current study. During partitioning, aerial contamination can take place as inflation clusters drop to the floor and pick up microorganisms that can be drawn into the milk (Ledenbach and Marshall, 2010). Therefore, extreme caution needs to be taken during partitioning. Insufficient heat treatment of milk greatly reduced the risk of HUS associated with consumption of STEC contaminated milk. Using a linear regression model, Giacometti et al. (2016) noted that the number of predicted HUS cases is directly influenced by the probability of heat treatment of milk before consumption and again that consumer behavior is a variable and operational reference point useful to obtain appropriate mitigation measures. Grace et al. (2008), Giacometti et al. (2012a) and Clough et al. (2009) reported a zero risk of acquiring HUS in consumers who boil milk before consumption. *E. coli* is destroyed by temperatures above 63 °C in fluid milk (D'Aoust et al., 1988). Pasteurisation of milk effectively eliminates STEC and other common milk borne pathogens (*Listeria monocytogenes*, *Campylobacter* and *Salmonella*) that could cause severe disease, without causing significant change to nutritional properties in milk (Angulo et al., 2009). However, depending food matrix, *E. coli* may resist pasteurisation temperatures of up to 72 °C (Mercer et al., 2015).

A simulated scenario in this study where milk was stored at 4 °C throughout the whole PDBM chain, clearly indicated a reduction of HUS risk to consumers by more than 50%. PDBM food chain should enforce handling, transportation and storage between 0 and 4 °C. These temperatures have been known to prevent microbial growth and subsequent risk of high pathogen level at consumption (Signorini and Tarabla, 2009). In their risk assessment, Giacometti et al. (2012a) observed that when farmers did not maintain correct temperatures throughout the supply chain and also due to thermal abuse practices during home transportation and storage, the annual expected cases of HUS infections were higher. The same authors also reported that effective

maintenance of the cold chain also reduces the risk of HUS associated with consumption of raw milk. We also noted that reduction of time taken to sell milk and consume all the milk at home, significantly reduced the risk of STEC in PDBM. Factors affecting risk of infection by pathogen in milk sold directly to the public include time taken to sell the milk per-day and time taken to consume all the milk at household level (Latorre et al., 2011; Giacometti et al., 2015) which is also in accordance with our study. Latorre et al. (2011) reported that additional time in milk storage along the food chain increase growth of the pathogen and the subsequent exposure per-serving and risk of illnesses per-serving. We therefore recommend consumption of milk within the shortest possible time just after purchasing, to reduce bacterial growth during inadequate refrigeration which has subsequent consequences of increasing the risk of infection. Studies have proven that *E. coli* cells can grow even at refrigeration temperatures (Kauppi et al., 1996). Combination of PDBM handling practices (storage at 4°C throughout the whole chain + time taken to sell the milk (5 hr) + time taken to consume the milk (half a day)) along the product chain had more impact in reducing the risk of infection and probability of illness. A study by Njage and Buys (2016) on quantitative assessment of human exposure to extended spectrum and AmpC ß-lactamases bearing *E. coli* in lettuce attributable to irrigation water and subsequent horizontal gene transfer, revealed that combination of mitigatory interventions, was effective in reducing the exposure with the *E. coli* by up to 99.4%. Most WHO guidelines recommend combination of different mitigatory measures in food value chain to increase food safety (Wilcock et al., 2004).

During our analysis, certain model inputs introduce uncertainties. We identified one study (Caine et al., 2014), apart from our reports (Ntuli et al., 2016; 2017), which provides information regarding the prevalence of STEC in PDBM in SA or in the region. Furthermore, there is no information pertaining quantitative data of STEC and the inherent variability in this parameter in

PDBM. In the current study one of the main source of uncertainty was the estimated concentration of STEC in both raw and pasteurised PDBM. Very few studies have quantified pathogen levels in bulk milk (Marshall et al., 2016). Most studies have used the Bayes' theorem techniques to quantify pathogen levels based on prevalence (qualitative) data in milk (Giacometti et al., 2012a; Giacometti et al., 2015). We estimated the level of STEC in PDBM based on the method of isolation and quantification that we used in our previous study (Ntuli et al., 2016; Ntuli et al., 2017). One of the main disadvantage in the method was that, *E. coli* (STEC) cells are known to enter a dormancy state in the milk, i.e, they are still viable but non-cultrable (Dinu and Bach, 2011). Therefore, this may have underestimate the quantities of STEC in PDBM, although the cells may still be viable and retain pathogenicity. The most sensitive method for STEC isolation and quantification in food, including milk, is the immunomagnetic separation following selective enrichment, and subsequent spread-plating of the concentrated target cells onto STEC chromagar (Boer and Heuvelink, 2000). Obtaining quantitative data on STEC concentration in PDBM or milk produced and marketed in the same scenario, would enable a more realistic modelling at this PDBM value chain stage. In other studies, estimated concentrations of STEC in bulk milk ranged from -4.00 logcfu/ml to -3.5 logcfu/ml (Giacometti et al., 2012a; Perrin et al., 2015). However, these were much lower than what we estimated in raw and pasteurised PDBM despite the underestimations.

Storage temperatures at house hold refrigeration was modeled using data obtained from Europe and other western countries, and this might not be a representative of home refrigeration temperatures in SA. Another source of uncertainty and variability in the model was the lack of data available regarding (i) average volumes of PDBM produced or received at outlets per-day (ii) average volumes of PDBM sold per-day (iii) serving volumes (iv) percentage of consumers who

boil milk before consumption (v) frequency of PDBM consumption (vi) the actual population (both children and adults) that consume PDBM in SA. We believe future risk assessment will model this source of variability and uncertainty if appropriate data could be identified. Furthermore, sampling was done in a similar region where we collected PDBM sample in our previous study, (Ntuli et al., 2016), as a representative of PDBM scenario in urban and pre-urban SA. This might have underrepresented the PDBM situation in SA since the socio-economic status in the country include a vast rural population who cannot access refrigeration and whose commute consists of walking for long distances. The milk may therefore be subject to more prevalence and levels of abuse temperatures between PD outlets and domestic levels. We used a triangular distribution to represent log reduction counts to represent insufficient heating (33% of the consumers) and this was adopted from (Giacometti et al., 2012a). The authors reported this as a source of uncertainty in their model as the experimental data on the reduction of STEC counts achieved by insufficient boiling may not be reproduced in home setup, thus, they assumed a triangular distribution. Regarding the set of data we had it was not possible to estimate precisely the absolute risk of HUS in SA in our model. The actual number of children and adults who consume PDBM was estimated from SA population statistic. We recommend future risk assessment work to include other vulnerable members of the population, for example the perinatal and the immune compromised.

Conclusion

A higher risk of HUS cases per-year was estimated in raw than pasteurised PDBM. We also observed a higher risk of STEC infections in age group above 5 years in comparison to children below 5 years. The model estimates show that the public health significance of HUS cases due to STEC contaminated PDBM depends on the current variability surrounding the risk profile of the milk and is explicitly influenced by consumer behavior. Serving volumes, time taken to sell the

milk at PD outlets and PDBM storage time at home had the greatest effect on the probability of HUS and the annual number of cases. A combination of PDBM handling practices (storage at 4°C throughout the whole chain, time taken to sell the milk (5 hr) and time taken to consume the milk (half a day)) along the product value chain had more impact in reducing the risk of infection and probability of illness. Given that partitioning of milk also contributes to the risk of HUS, extreme caution needs to be taken during partitioning.

This study recommends strict enforcement of and adherence to SA Standard Code of Practice Food Hygiene Management (SABS 049), which regulates food hygiene in the dairy industry, especially for PDs. We also recommend the inclusion of, within the SABS 049, a specific guideline that regulates the production, processing and supply of PDBM. Furthermore, the training on dairy technology and safety for producers and suppliers of PDBM by the Department of Health, in collaboration with environmental health officers (in the different municipalities across SA) and non-governmental organisations, such as the Dairy Standard Agency needs to be strengthened to improve public health and safety. The raising of awareness on the health risks associated with the consumption of raw milk for, particularly, consumers of raw PDBM, also needs to be scaled-up for them to make informed decisions when buying milk. The awareness will indirectly encourage consumers to buy certified raw milk. Figure 3 presents a collaborative effort on how academia, industry, non-Governmental Organisation and the Government, can improve public health and safety associated with STEC in PDBM. Results from this study can be useful in formulating risk-based mitigation strategies and policies. Additionally, the models developed in this study are an example of risk assessments for milk produced and marketed from similar scenarios.

A survey on producer-distributor bulk milk (PDBM) in SA revealed high levels of *E. coli* (Ntuli et al., 2016; 2017), above stipulated limit (SA, 2001 Act (54), (1972)). Ntuli et al. (2016; 2017) also reported a diversity of EHEC seropathotypes, (with different shigatoxin virulence factors, multi drug resistant and extended-spectrum β-lactamases (ESBLs) producing capacity in the PDBM.

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University communicates to MilkSA

ASSESSMENT

MILK SOUTH AFRICA

Milk SA engages the DoH for interventions

The University conducted a risk assessment in an effort to quantify the risk to the public from consumption of STEC contaminated PDBM

Finding:

- *For every 10 million PDBM portions consumed,*
  - *the expected number of HUS cases per-year = 154 and 28 for age groups under 5 years and above 5 years, respectively, who consume raw PDBM.*
- *Consumers of raw PDBM were at higher risk of HUS than those who consumed pasteurised PDBM.*

health
Department:
Health
REPUBLIC OF SOUTH AFRICA

Intervention by the DoH include:

1. *Strict enforcement of and adherence to SA Standard Code of Practice Food Hygiene Management (SABS 049).*
2. *Inclusion of, within the SABS 049, a specific guideline that regulates the production, processing and supply of PDBM.*
3. *The training on dairy technology and safety for producers and suppliers of PDBM*

STANDARD DAIRY AGENCY (DSA)

Environmental health officer (EHO)

Education and Training of PDs By DSA and EHOs

Awareness on the health risks associated with the consumption of raw milk

PDs – Producer Distributors
BM – Bulk Milk
DoH – Department of Health
MilkSA – Milk South Africa is a board which comprises of the milk industry (milk producers and Processors)
DSA – Dairy Standard Agency's primary objective is to promote the compliance of milk and other dairy products with product composition, food safety and metrology standards

Dashed lines: Red represent the problem; Amber represent the solution and green represent interventions

FOOD SECURITY:

Consumer food safety and public health protection

Figure 3: A collaborative effort of academia, industry, non-Governmental Organisation and the Government to improve public health and safety associated with Shigatoxin producing *E. coli* in Producer -Distributor Bulk Milk.

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