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Risk assessment of staphylococcal poisoning due to consumption of informally-marketed milk and home-made yoghurt in Debre Zeit, Ethiopia

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ABSTRACT

The objectives of the study were twofold: to prove that participatory risk assessment can be applied to informally-marketed foods, and to assess the risk of staphylococcal poisoning through consumption of raw milk and home-made yoghurt in Debre Zeit, Ethiopia. Rapid urban appraisals were combined with conventional interviews to identify and quantify formal and informal milk value chains and to collect information on consumers' food preparation and consumption behavior. Milk was sampled in 170 dairy farms and 5 milk collection centers and microbiological tests were conducted. Published data on milk fermentation in Ethiopia was combined with a growth model of *Staphylococcus aureus* to develop a stochastic risk model. The annual incidence rate of staphylococcal poisoning was estimated to be 20.0 (90% CI: 13.9–26.9) per 1000 people. When the effect of fermentation was removed from the model, the annual incidence rate increased to 315.8 (90% CI: 224.3–422.9) per 1000 people, showing the importance of traditional food preparation methods in risk mitigation; traditional milk fermentation reduced the risk by 93.7%. Improving the safety of milk and dairy products could be achieved through supporting appropriate traditional food preparation and consumption where an industrial risk mitigation system is not feasible. Participatory risk assessment was shown to be applicable to informal food value chain.

1. Introduction

Staphylococcal food poisoning is one of the most common foodborne diseases in the world (Holmberg and Blake, 1984; Wieneke et al., 1993; Asao et al., 2003); it is caused by ingestion of staphylococcal enterotoxin (SE) produced in food by certain strains of *Staphylococcus aureus*. The intoxication is characterized by the sudden onset of nausea, vomiting, abdominal cramps and diarrhea which usually appear 1 to 6 h after the ingestion of SE (Asao et al., 2003); the symptoms generally last from 24 to 48 h and the mortality rate is very low or nil (Jay, 2000).

SEs were previously divided into five serological types (SEA to SEE, alphabetically) based on their antigenicity (Bergdoll, 1989); more recently, many new types of SEs have been described. Only three of the novel SEs (SEG, SEH and SEI) have been shown to cause vomiting after oral administration of them to a primate as is the case for SEA to SEE (Kérouanton et al., 2007). Other toxins which either lack emetic property or have not been tested, have the proposed designations of staphylococcal enterotoxin-like (SEl) superantigens: SElJ, SElK, SElL, SElM, SElN, SElO, SElP, SElQ, SElR, and SElU (Omoe et al., 2005). In addition to food poisoning SEs and the SE-related toxin, toxic shock syndrome toxin-1 (TSST-1), are also members of the superantigenic toxin family and have the ability to cause life-threatening toxic shock syndrome (McCormic et al., 2001; Omoe et al., 2005; Uchiyama et al., 1994). A small amount of SE can cause illness: 100–200 ng of SEA in 2% chocolate milk has been reported to have caused intoxication among students in the United States (Evenson et al., 1988). In a large scale outbreak caused by contaminated low-fat milk in Japan, the total individual intake of SEA was estimated to be approximately 20–100 ng (Asao et al., 2003).

S. aureus starts to produce SE when the population density in milk reaches about $10^{6.5}$ cfu/ml and thereafter the amount of SE increase linearly with time (Fujikawa and Morozumi, 2006). Generally, high oxygen tension favors both growth and toxin production (Barber and Deibel, 1972). The staphylococci grow in the temperature range between 7 and 48 °C and produce SE between 10 and 48 °C, with optimum SE producing temperature of 40 to 45 °C (ICMSF, 1996; Aycicek et al., 2005). The optimal pH for *S. aureus* growth is 7 (Su and Wong, 1998) and the minimum pH is reported to be 4.9 (Barber and Deibel, 1972). The optimum pH for toxin production is between 6.5 and 7.3 (Jarvis et al., 1973) and the minimum pH that staphylococcal strains produce detectable SE is reported to be 5.1 (Barber and Deibel, 1972). However once SEs are produced, they are

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resistant to low pH condition that easily destroys the bacteria that produced them, and retain the activity in the digestive tract after ingestion (Argudín et al., 2010).

It is well known that the most frequent source of contamination of food in staphylococcal food poisoning is food handlers (Asao et al., 2003). Staphylococci may be present in the nasal passages, throat, hair and skin of healthy people, and are abundant in cuts, pustules, and abscesses (Bergdoll, 1989). Approximately 20% of the adult population carry *S. aureus* in their nose persistently, another 30% intermittently, whereas 50% are non-carriers (Wertheim et al., 2005, 2008). In addition to above mentioned dairy products, fermented sausage (Barber and Deibel, 1972) and ready-to-eat meals such as scrambled eggs in lunch boxes (Miwa et al., 2001) have been reported to be the sources of staphylococcal food poisoning.

In addition to humans, most domesticated animals harbor *S. aureus* (Jay, 2000). *S. aureus* is an important cause of contagious bovine mastitis (Hata et al., 2010), although many strains of the organism which cause bovine mastitis are of human origin (Jay, 2000). Mastitic cow's milk can be harmful if it is served for consumption; however, such milk is usually excluded from a formal value chain and only healthy cow's milk is consumed. It must be noted that SE cannot be inactivated by heat treatment as it is thermostable (Varadaraj and Nambudripad, 1986). Otherwise, contamination of milk with a toxic dose of SE may occur during manufacturing: when processed milk is stored at a temperature of 35 °C for 8 h or at 25 °C for 24 h during a power failure, milk can contain toxic dose of SE (Soejima et al., 2007). This is the case of industrial milk processing, but milk is still commonly sold raw and at ambient temperature in Ethiopia. Ethiopia has a tradition of fermenting milk from raw milk (Ashenafi, 1990; Gonfa et al., 1999). The fermented milk (yoghurt) is called ergo and is usually served with mashed green pepper, onion and salt (Gonfa et al., 1999). Ayib is a traditional Ethiopian cottage cheese and the poor hygienic status of the products sold in markets has been described (Ashenafi, 1990; Addis et al., 2011). Raw milk is often consumed under unsatisfactory hygiene conditions (Wubete, 2004).

Microbiological risk analysis in foods (CAC/GL 63, 2007) has greatly contributed to improve food safety in developed countries but it has not been applied much in developing countries due to insufficient public data and human resources and dominance of informal marketing system. Participatory methods have been used in development studies to collect the communities' needs with a bottom-up approach (Chambers, 1997). It has been recently argued that participatory methods can be applied to food safety risk assessment in data collection and sustainable food hygiene control in developing countries (Grace et al., 2008). The present study was conducted to prove the concept of the participatory risk assessment, and at the same time, to assess the risk for staphylococcal poisoning due to consumption of informally marketed raw milk and homemade yoghurt (ergo) in Debre-Zeit.

2. Materials and methods

2.1. Study sites

The study was conducted in and around Debre-Zeit town. Debre-Zeit is located at 9°N and 40°E, in Oromia National Regional State about 47 km southeast of the capital city of Ethiopia, Addis Ababa. The altitude is about 1850 m above sea level. It has a bimodal pattern of rainfall with the main rainy season extending from June to September and a short rainy season from March to May with an average annual rainfall of 800 mm. The mean annual minimum and maximum temperatures are 12.3 °C and 27.7 °C, respectively, with an overall average of 18.7 °C. The highest temperatures are recorded in May and the mean relative humidity is 61.3% (Central Statistical Authority, 2006).

2.2. Study design

Participatory risk assessment (Grace et al., 2008) was used for the present study following the Codex Alimentarius Commission system framework (CAC/GL 63, 2007). Participatory methods were used in the data collections.

2.3. Identification of milk value chains

A rapid urban appraisal was conducted with the representatives of Ada Dairy Cooperative to identify both formal and informal milk value chains in Debre Zeit. Formal value chains refer to the chain regulated by the government. The final product in this chain is pasteurized and packaged milk sold to consumers. The informal value chain on the other hand sells raw liquid milk and escapes formal inspection. In the appraisal, participants described the flow of milk distribution in both urban and peri-urban areas. Additional rapid urban appraisals were conducted at milk collection centers, cafes and a dairy processing plant to understand both value chains into details.

2.4. Dairy farm survey

Stratified random sampling was used to select farmers. Strata were 14 milk collection centers assigned to the farmers and the sampling units were farmers. The sample size of farmers was calculated using an expected prevalence of 29.1% (the prevalence of *S. aureus* in milk from dairy farms in Debre Zeit in a previous study (Tesfaye, 2008)), level of confidence 95% and desired level of precision 5%. As the number of dairy farmers was small (368), the sample size was adjusted for the finite population (Thrustfield, 2005) and calculated as 170. Proportional allocation (Scheaffer et al., 1996) was applied to decide the numbers of farms sampled within each sub-group selling to a milk collection center.

Due to the limitation of time and resources, convenience sampling of farm bulk milk was conducted at all the 14 milk collection centers (strata) to fulfill the sample sizes allocated within the strata, waiting farmers to come at the milk collection centers for milk sales. The purpose of the study was explained to the 170 farmers participating in the study prior to the survey and verbal consents were obtained. Bulk tank milk was sampled aseptically and transferred to an Eppendorf tube and a structured questionnaire was administered to obtain information on the quantity and destination of sales, boiling practice, storage time limit of milk for consumption, temperature (ambient or refrigerated temperature) and quantity of home consumption. The pH of milk was not measured. The milk samples were carried in a cool box to the Microbiology Laboratory in the Faculty of Veterinary Medicine, Addis Ababa University each day of the sampling.

2.5. Milk collection center survey

Five out of fourteen milk collection centers sold raw liquid milk to consumers and therefore milk was sampled from these centers in order to determine the prevalence of *S. aureus* in milk. Verbal consent was obtained prior to survey, and five milk samples were collected aseptically at each collection center. The milk samples were collected and transported to the laboratory in a same manner with the sampling at farms.

2.6. Isolation and identification of S. aureus

Isolation and identification of *S. aureus* were conducted in the Microbiology Laboratory of the Faculty of Veterinary Medicine of Addis Ababa University. The bacteriological culture was performed following the standard microbiological technique recommended by Quinn et al. (1999).

2.7. Consumer survey

Interviews with consumers on boiling practice before consumption were conducted at the milk collection centers. Verbal consent was obtained prior to interviews with customers. In addition to the interviews, a rapid urban appraisal with consumers was conducted to obtain information regarding quantity of average milk consumption per person and storage time limit of milk for consumption. The storage limit was asked in order to check that this behavior in non-dairy farming households is not different from dairy farming households using triangulation technique (Mariner and Paskin, 2000).

2.8. Risk assessment

Microbiological food safety risk assessment involves hazard identification, hazard characterization, exposure assessment and risk characterization (CAC/GL 63, 2007). Hazard identification – identification of agent which can cause the adverse health effects to humans – and characterization – the qualitative and/or quantitative evaluation of the adverse health effects associated with the hazard, SE – were described in the introduction section. Dose–response assessment was based on literature review (Fujikawa and Morozumi, 2006).

Risk characterization was conducted by combining the exposure assessment and dose–response assessment by developing a risk model. The risk model consisted of three parts: (1) quantitative value chain, (2) bacteria growth and (3) inhibition of SE production by the decrease of pH. (1) The quantitative value chain was developed using information obtained from rapid urban appraisals with the representatives of Ada Dairy Cooperatives, milk collection centers, cafes, a processing plant and consumers, and interviews with farmers and consumers. The value chain model included prevalence of *S. aureus* in milk and boiling practice. (2) The *S. aureus* growth model was developed based on data in literatures (Table 1). The present study used *S. aureus* growth rate reported in Fujikawa and Morozumi (2006).

$$dN/dt = rN(1-N/N\max)\{1-(N\min/N)^c\}.$$

Where N is the population of *S. aureus* (colony forming unit/ml) at time t, $N\min$ is the minimum population, $N\max$ is the maximum population at stationary phase, c is the adjustment parameter and r is the temperature dependant rate constant.

These parameters are listed in Table 1 with references. $N\min$ was modeled as very slightly smaller population than N_0 which is the bacteria population at the time of production, as expressed in Fujikawa and Morozumi (2006). Milk temperature was modeled using annual average and mean minimum and maximum temperature of Debre Zeit available in the statistical abstract of Ethiopian Central Statistical Authority (2006), as milk temperature was not taken during the survey. The Normal distribution was used for modeling temperature. According to Middleton et al. (2004), following an artificial intramammary staphylococcal infection, shed milk contained 10^6 cfu/ml of *S. aureus*. We thus modeled the upper limit of N_0, the cfu/ml of *S. aureus* at time 0, dividing 10^6 cfu/ml by the number of teats of a cow, four, and the lower limit 1, which made the range in $\log_{10}$ scale, 0 to 5.4. The upper limit of N_0 in farm bulk milk could be modeled to be 10^6 cfu/ml, in the case that a farm keeps only one cow and the cow has mastitis, and that all the four teats of the cow shed the maximum numbers of *S. aureus*. However in such an extreme event, milk would not be bought at milk collection centers or not be consumed raw at home. Here, there is a clear limitation in our study that milk spoilage is not modeled; unknown quantity of milk spoiled might influence the incidence rate estimated.

In Ethiopia, milk is fermented from raw milk using traditional techniques (Gonfa et al., 1999). Gonfa et al. (1999) recorded the change of pH over time every four hours from the time of milking for 24 h. To model (3) the inhibition of SE production by the decrease of pH, these data were used. In an acidification process of dairy products, three phases are observed: slow decrease of pH at the beginning, sharp acidification, and slow and continuous acidification again (Briggiler-Marcó et al., 2007). Gonfa's data followed this acidification process and to model the decrease of pH, the first two processes were modeled linearly, and the final phase was modeled using the transformed data from 8 to 24 h into exp(1/pH) with linear regression, so that the pH does not decrease below zero. As the lowest pH at which *S. aureus* produces SEs, 5.1 is higher than the limit at which *S. aureus* can grow (4.9, Barber and Deibel, 1972), pH 5.1 might be a conservative threshold for the judgment of SE production. However as the growth model was used in the present analysis, pH 4.9 was used for the judgment: whether the population of *S. aureus* reached enough bacteria concentration to produce SE by the time. The bacteria growth was modeled to stop at the time when the pH of milk reaches at 4.9.

Storage time of milk determines the population of *S. aureus* in milk. Storage time was modeled with Dirichlet distribution (Vose, 2000), which is a multinomial distribution- for a selection from more than three choices, using data collected in the interviews with farmers who consume un-boiled milk (Table 1).

Quantity of milk contaminated with *S. aureus* at each pathway of milk value chains was modeled by multiplying the quantity of milk sold with the contamination rate and the probability of not boiling milk before sales or consumption, and these quantities at the end of all pathways were added together. This total quantity of milk contaminated with *S. aureus* was multiplied by the probability of *S. aureus* having SE producing genes (Arcuri et al., 2010) to calculate the quantity of milk with *S. aureus*, which potentially can produce SEs. This amount of contaminated milk was randomly sampled to be consumed at different hours after purchase using the above mentioned distributions. The daily cases of illness were estimated by summing the quantities of milk containing toxic dose of SEs and dividing it by the quantity of average daily milk consumption. Finally, as a risk characterization, annual incidence was calculated by dividing annual cases – which was calculated by multiplying estimated daily cases and 365 days – by the human population in Debre Zeit. The model was developed in @Risk (Palisade Corporation, USA) and Monte Carlo simulation was run for 10,000 iterations. Sensitivity analysis was done selecting all the uncertainty parameters and run 84 times each for 1000 iterations. The present model contained only one variability parameter: adjustment parameter c; we regarded temperature as uncertainty as it is a temperature of milk. To separate the variability from uncertainty, the parameter c was changed to point estimate and the rest of uncertainty was estimated under different values of c (every 0.2 different values from 3.6 to 5.8, which c took in the stochastic simulation).

In addition to the risk assessment, effect of traditional fermentation was assessed by making bacteria growth continue without being stopped by the acidification. Also, the model stopping SE production at pH 5.1 (Barber and Deibel, 1972) was run for 10,000 iterations to compare with the model stopping at pH 4.9.

3. Results

3.1. Quantitative value chain

Integrating a rapid urban appraisal and interviews with farmers showed the quantitative structure of the daily milk value chain in Debre Zeit (Fig. 1, Table 2). Among the dairy farms (n = 170) included in the study, 131 (77.1%) were urban dairy farms and 39 (22.9%) were peri-urban dairy farms. Most of the urban farms were smallholder farms with two or three cows and (92.4%, 121/131) kept cross-breed and the rest (7.6%, 10/131) indigenous breed dairy cattle. In peri-urban areas, all the farms (n = 39) kept indigenous cattle and most of them were smallholder. Most liquid raw milk (2940 L) was

Table 1
Parameters and distributions used for the risk modeling.

Parameters	Statistics (90% CI)	Distributions used	Description
1. Bacteria growth and toxin production			
Log of N_0, (N cfu/ml at time 0)	2.70 (0.27–5.13)	Uniform (0, 5.4)	10^6 cfu/ml was shed from a teat in an experiment. Log (10^6/4) = 5.4. Middleton et al. (2004).
Log of Nmin	2.70 (0.27–5.13)	Dependant of N_0	$N\text{min} = (1 - 1/10^6)^* N_0$
Log of Nmax	8.15	Point estimate	Fujikawa and Morozumi (2006)
Adjustment parameter: c	4.7 (3.71–5.69)	Uniform (3.6, 5.8)	$c = 4.7 \pm 1.1$, Fujikawa and Morozumi (2006)
Temperature dependant rate constant: r	NA	Dependant of temperature	$r = (0.0442\,T - 0.239)^2$, Fujikawa and Morozumi (2006)
Temperature (°C)	18.7 (14.3–23.1)	Normal (18.7, 2.7)	Annual average 18.7, minimum 12.3, maximum 27.7. Central Statistical Authority (2006).
Temperature in refrigerator (°C)	4 (3.5–4.5)	Trigen with most likely, 5th and 95th percentile	An estimate for a household refrigerator. Percentiles were purposively determined.
Log_{10} of N_i	NA	Dependant of N_{i-1}	$\text{Log}_{10}\left(N_{i-1} + \int_{i-1}^{i} {}^{d}N/_{d}t(x)\right)$
Cfu/ml when SEs reach 0.2 ng/ml	$10^{7.2}$	Point estimate	From Fig. 1 (B) of Fujikawa and Morozumi (2006) A person drinks 500 ml (present study). 100 ng of SE causes illness. Evenson et al. (1988), Asao et al. (2003).
2. Ingestion of contaminated milk with staphylococcal enterotoxin			
Storage time	Shown in Table 3	Dirichlet distribution	Interviews with dairy farmers.
Choice of the time of milk consumption in a day	NA	Bootstrap	1 to 24 h
Quantities of milk sold in value chains	Shown in Table 2	Point estimates	Calculation based on rapid rural appraisals
Milk contamination rates with *S. aureus*	Shown in Table 2	Beta distribution	Microbiological test results 74/170 at farm; 18/25 at collection centers.
Probability of boiling milk	Shown in Table 2	Beta distribution	Interviews with dairy farmers and consumers 116/170 farmers boil; 16/25 consumers boil.
Probability of *S. aureus* having SE genes	0.375 (0.330–0.422)	Beta distribution	109 out of 291 *S. aureus* had SE genes in a study by Arcuri et al. (2010).
Quantity of daily milk consumption by a consumer	500 ml	Point estimate	A rapid rural appraisal with consumers
Population of Debre Zeit	95,000	Point estimate	2006 census

sold to restaurants and cafeterias. Farm gate sales of raw milk to consumers amounted to 1960 L and the same volume of milk was consumed by farmers at home. Liquid raw milk was purchased at milk collection centers by consumers (400 L) and cafeterias (75 L). Some cafeterias bought raw milk before processing at a milk processing plant (accounting for 50 L).

3.2. Microbiological test results

Out of 170 bulk milk samples collected at dairy farms, *S. aureus* was recovered from 74 samples (43.5%, Table 1). From five milk collection centers, 25 samples were collected and *S. aureus* was recovered from 18 samples (72%). The contamination rate in milk at milk collection

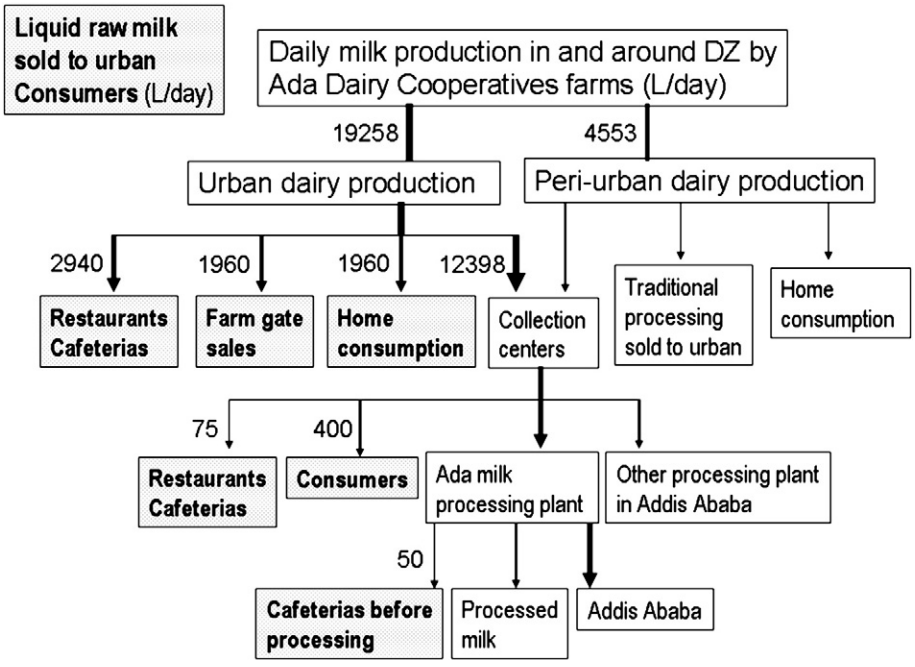

Fig. 1. A quantitative dairy value chain in Debre Zeit. Gray boxes show the distribution of raw liquid milk to urban consumers. The numbers show quantities in Liters per day.

Table 2
The sources of informally-marketed milk consumed by Debre Zeit population and the proportions of risk distributed with 90% confidence intervals.

Sources	Quantity of milk (L/day)	Contamination rate with SA at purchase	Probability of boiling (%)	Milk contaminated at consumption (L/day)	Proportion of risk distributed (%) (column total 1)
Sales to cafeterias from urban farms	2940	0	100 (boiled in cafeteria)	0	0
Home consumption by urban farmers	1960	0.436 (0.374–0.499)	68.0 (62.1–73.7)	273 (213–340)	39.9 (30.4–50.5)
Farm gate sales to neighbors	1960	0.436 (0.374–0.499)	63.0 (47.4–77.4)	317 (187–460)	45.0 (32.6–55.6)
Cafeterias who purchase milk at collection centers	75	0	100 (boiled in cafeteria)	0	0
Consumers purchase at collection centers	400	0.704 (0.553–0.837)	63.0 (47.4–77.4)	104 (60–155)	15.1 (8.8–22.4)
Cafeterias purchase raw milk at processing plant	50	0	100 (boiled in cafeteria)	0	0

centers (72%) was significantly higher than that of farms (43.5%, $x^2 = 5.99$, df = 1, p = 0.014).

3.3. Boiling and fermentation of milk

According to the interviews with dairy farmers, 116 of 170 (68.2%) farmers boiled milk at home before consumption (Tables 1 and 2). In other words, 31.8% of farmers consumed raw and/or fermented milk. None of them boiled milk for sale. Interviews with consumers also revealed that raw milk consumption (9/25: 36%, Tables 1 and 2). There was no significant difference in probability of boiling between farmers (68.2%) and consumers (64.0%, 16/25, $x^2 = 0.038$, df = 1, p = 0.85).

Rapid urban appraisals with consumers confirmed that milk storage time at their households were not different from data collected from farming households, and milk was traditionally fermented from raw milk without boiling. Storage of milk at room temperature for more than a day was a common practice among those who do not boil milk for consumption in Debre Zeit (41/50: 82.0%, Table 3). Among farmers interviewed, boiling was less common among those who store milk for more than a day (75/120, 62.5%) than those who consume milk in the day of production (41/50, 82.0%, $x^2 = 5.3$, df = 1, $p = 0.02$, data not shown in tables); raw milk is stored for traditional fermentation.

3.4. Decrease of pH in traditional milk fermentation

Traditional milk fermentation in Ethiopia is reported to inhibit bacteria growth (Gonfa et al., 1999). The point estimates of pH in this literature showed that pH decreased slowly from 0 to 4 h (slope = −0.02), then sharply (slope = −0.29) and again slowly (Fig. 2). The exponential of the reciprocal of pH: exp (1/pH) was found to increase linearly over time from 8 to 24 h (slope = 0.002, intercept = 1.187, df = 3, $r^2 = 0.90$, $p = 0.009$). Fig. 2 shows the decrease of pH over time with the fitted line (the two latter lines met at 7.6 h). The pH decreased below 4.9 at 21.5 h.

Table 3
The numbers of respondents and the probabilities of storing raw milk for consumption until given days among households that do not boil milk.

Storage time	Room temperature	Refrigerator
Within a day	9	0
	16.1 (9.2–24.3)	1.6 (0.1–4.8)
1–2 days	8	4
	14.5 (7.9–22.3)	8.1 (3.3–14.4)
3–4 days	29	0
	48.4 (38.0–58.6)	1.6 (0.1–4.8)
>4 days	4	0
	8.1 (3.3–14.5)	1.6 (0.1–4.8)
Total	50	4

Dirichlet distribution was used for modeling the probabilities. Numbers between brackets show 90% confidence intervals.

3.5. Risk assessment

3.5.1. Exposure assessment

A rapid urban appraisal with consumers found that an Ethiopian individual consumes 500 ml of milk in average in a day (Table 1). Consulting literature, we modeled that 100 ng of SE causes illness. To make 100 ng of SEs, 500 ml of milk with 0.2 ng/ml of SE can cause illness. According to the published Fig. 1 (B) in Fujikawa and Morozumi (2006), 0.2 ng/ml of SE can be produced in milk when the concentration of *S. aureus* reaches at $10^{7.2}$ and this was used as the threshold level of causing illness (Table 1). A Monte Carlo simulation showed that 0.011% (90% CI: 0.008–0.015) of informally marketed milk contains toxic dose of SEs (more than 0.2 ng/ml) at consumption (Table 4).

3.5.2. Risk characterisation

Daily incidence in Debre Zeit was estimated to be 5.2 cases (90% CI: 3.6–7.0, Table 4). Annual incidence rate was estimated to be 20.0 (90% CI: 13.9–26.9) per 1000 people.

3.5.3. Effect of traditional fermentation

We assessed the effect of traditional fermentation by modeling continued bacteria growth after 21.5 h without inhibition by the decrease

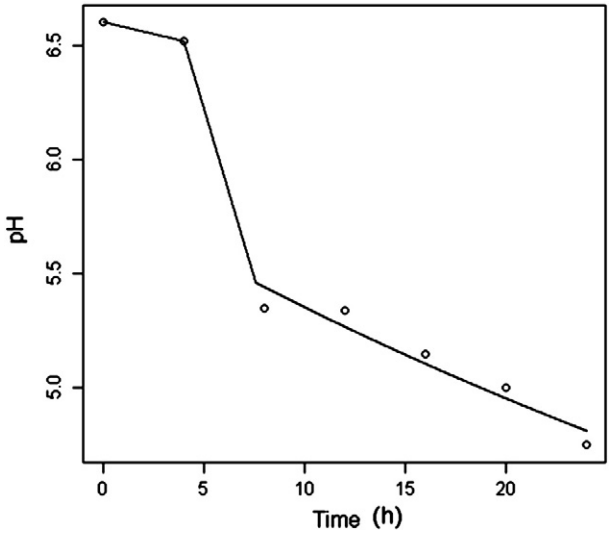

Fig. 2. Decrease of pH of milk over time. Dots show the data reported by Gonfa et al. (1999). The straight line from 0 to 4 h is pH = −0.02 t (h) + 6.6 and from 4 to 7.6 h, pH = −0.29 t (h) + 7.69. The line from 7.6 to 24 h is the back-transformed fitted line of 1/pH = 0.002 t (h) + 1.187 (df = 3, $r^2 = 0.90$, $p = 0.009$).

Table 4
Comparison of contamination rate of informally marketed milk with toxic dose of Staphylococcal enterotoxin and annual incidence rate of staphylococcal poisoning through consumption of raw and fermented milk between the estimates with and without milk fermentation.

	With fermentation	Without fermentation
Quantity of contaminated milk with toxic dose of SEs (L/day)	2.6 (1.8–3.5)	41.1 (29.2–5.0)
Contamination rate of informally marketed milk with toxic dose of SEs at consumption (%)	0.011 (0.008–0.015)	0.176 (0.12–0.232)
Daily incidence of staphylococcal poisoning	5.2 (3.6–7.0)	82.2 (58.3–110.1)
Annual incidence rate per 1000 people	20.0 (13.9–26.9)	315.8 (224.3–422.9)

and annual incidence rate was 315.8 (90% CI: 224.3–422.9) per 1000 people. Traditional milk fermentation was found to protect 295.8 cases per 1000 people annually, which is a reduction of potential incidence by 93.7%. When the production of SEs was modeled to stop at pH 5.1 (at 16.2 h after milking in a farm), the annual incidence was estimated to be 5.4 cases (90% CI: 1.9–17.1) per 10,000 people and in this scenario, staphylococcal food poisoning was much rarer event.

3.5.4. Sensitivity analysis

Sensitivity analysis found two very sensitive parameters: initial population of *S. aureus* (N_0) and temperature of milk (Table 5). The other factors did not differ very much from the estimated annual incidence of staphylococcal poisoning. By the separation of variability, the adjustment parameter *c*, from total uncertainty, the pure uncertainty of annual incidence rate varied from 8.6 (90% CI: 2.9–12.2) per 1000 people at *c*: 3.6 to 33.4 (90% CI: 23.1–45.4) per 1000 people at *c*: 5.8 (Fig. 3).

4. Discussion

Ethiopia is reported to have the world's fifth largest number of child death each year due to diarrhea (UNICEF/WHO, 2009). The proportion of diarrheal cases due to unsafe foods in Ethiopia is not known; however, considering the common practice of consumption of raw milk and traditional dairy products prepared from raw milk in unhygienic conditions (Wubete, 2004) and the suitability of milk for bacteria growth, informally-marketed milk might be a significant source of foodborne illness. The present study assessed the stochastic risk for staphylococcal food poisoning through consumption of informally marketed liquid raw milk in Ethiopia for the first time. Despite initial expectation of high risk, and notwithstanding high prevalence of *S. aureus* in bulk milk both at farms (43.5%, 74/170) and collection centers (72.0%, 18/25), the factors did not influence much the estimated annual incidence of staphylococcal poisoning (20.0 per 1000 people). In the scenario that the SE production stops at pH 5.1, the annual incidence was much lower (5.4 per 10,000 people). This shows the efficacy of traditional risk mitigation strategy.

From technical aspects, the present study also proved the usefulness of participatory techniques in a food safety risk assessment in developing countries where rapid and low-cost assessments are desirable and public data are scarce. Specifically for a risk assessment in informal value chains, these techniques can be used in understanding of perception of risks and incentives to improve hygiene-related practice among actors in a milk value chain and in participatory learning for corrective action in communities as well.

We observed a significant increase of milk contamination with *S. aureus* from farms (43.5%) to milk collection centers (72.0%); this might be due to mixing of milk from mastitic cows and/or poor handling. The sensitivity analysis results showed that temperature of milk and initial bacteria population influenced the risk assessment result the most. Although information on these variables was not collected, they should be included in future studies. The variation of pure uncertainty according to the different values of the adjustment parameter *c* showed the variation of biological ability of growth of *S. aureus* strains. The annual incidence rate by the highest growth ability of *S. aureus* strain was estimated to be 33.1 (90% CI: 22.9–46.3) per 1000 people, and it was just 1.7 times higher than the mean estimate of annual incidence rate.

From a socio-economical perspective, the present study clearly showed the importance of the traditional milk fermentation from raw milk. Informal marketing of raw liquid milk was practiced by those who participate in formal marketing as well (all the farmers were members of a cooperative). They were smallholder farmers and informal sales helped their livelihood. Their participation in the formal value chain as well suggested that the farmers and collection centers may be willing to shift more towards the formal system if the demand from consumers for pasteurized milk becomes high.

Table 5
Sensitivity analysis results showing 50th, 1st and 99th percentile values and the mean annual incidence rate per 1000 people with 90% confidence interval at the 50th percentile value of each parameter in order of the sensitivity for the risk output: annual incidence rate of staphylococcal poisoning in Debre Zeit.

Order	Parameters	Values with 50th, 1st and 99th percentiles	Mean annual incidence rate per 1000 people*
1	Ambient temperature (°C)	28.1 (19.6–36.5)	432 (313–572)
2	Log_{10} of N_0	4.8 (3.4–6.3)	104 (66–163)
3	Probability of *S. aureus* having SE genes	0.56 (0.39–0.73)	41 (28–59)
4	Probability of boiling milk among farmers	1.0 (0.72–1.0)	16 (9–25)
5	Probability of boiling milk among consumers	0.94 (0.66–1.0)	17 (11–24)
6	Probability of storing milk for 3–4 days	0.44 (0.31–0.57)	34 (24–49)
7	Contamination rate at farms	0.65 (0.46–0.85)	33 (22–49)
8	Contamination rate at milk collection centers	1.0 (0.74–1.0)	29 (19–43)
9	Probability of consuming milk on the day of production	0.41 (0.28–0.53)	22 (15–33)
10	Probability of storing milk for 1–2 days	0.15 (0.10–0.19)	28 (18–40)
11	Temperature in a refrigerator (°C)	4.0 (4.0–4.0)	27 (18–40)

* The large mean annual incidence rates represent highly sensitive variables.

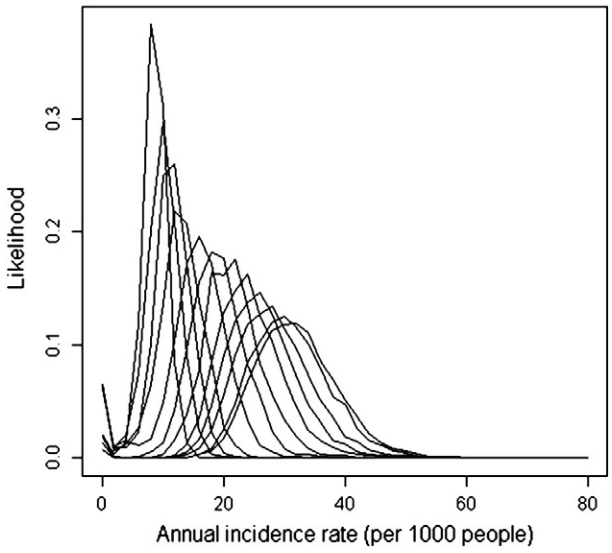

Fig. 3. Separation of variability from uncertainty. The lines show uncertainty at different values of adjustment parameter *c* every 0.2 from 3.6 to 5.8. The most left distribution with the highest mode is at *c* of 3.6 (mean 8.6 (90% CI: 2.9–12.2) per 1000 people). The most right distribution is at *c* of 5.8 (mean 33.4 (90% CI: 23.1–45.4) per 1000 people).

There are two main messages to policy makers. The first message is that where cooling systems and pasteurization is not economically feasible, traditional food preparation should be promoted to reduce the risk of milk-borne disease, preferably complemented by hygiene education for both milking and handling milk. The second message is, at the same time, that caution is advised in the consumption of raw and fermented milk. In the present study, we assessed only the risk for staphylococcal food poisoning. However cow's raw milk is known to contain many types of pathogenic organisms such as *Escherichia coli* O157:H7 (Tsegaye and Ashenafi, 2005) and *Brucella abortus* (Tesfaye et al., 2011). An experiment of fermenting ergo showed that *E. coli* O157:H7 survived even at 72 h when pH dropped to 3.8–3.9 (Tsegaye and Ashenafi, 2005). *Brucella* dies off fairly quickly when the acidity drops below pH 4, and very rapidly below pH 3.5 (WHO, 2006); however as ergo is preferably consumed soon after fermentation (24 h) (Tsegaye and Ashenafi, 2005), the risk of brucellosis cannot be eliminated. In addition, previous studies showed that *Salmonella* spp. and *Listeria monocytogenes* could survive during the souring of ergo for 24 to 48 h (Ashenafi, 1993, 1994). Therefore, the risk of fermented milk cannot be determined unless thorough studies on these bacteria in sold milk are conducted. Further risk assessments for other pathogens in milk and also for other foods are required.

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