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

Kohei Makita [a,b,*], Fanta Desissa [c], Akafete Teklu [c], Girma Zewde [c], Delia Grace [a]

[a] Improving Market Opportunities Theme, International Livestock Research Institute (ILRI), PO Box 30709, Nairobi, Kenya
[b] School of Veterinary Medicine, Rakuno Gakuen University, 069-8501, Ebetsu, Japan
[c] Faculty of Veterinary Medicine, Addis Ababa University, PO Box 34, 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

* Corresponding author at: School of Veterinary Medicine, Rakuno Gakuen University, 069-8501, Ebetsu, Japan. Tel.: +81 11 386 4761; fax: +81 11 387 5890.
E-mail addresses: kmakita@rakuno.ac.jp (K. Makita), fntdesi@yahoo.com (F. Desissa), girmazewde@gmail.com (G. Zewde), d.grace@cgiar.org (D. Grace).

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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).