Salmonella Enterica Incidences In Dairy Products From Ethiopia's Central Highlands

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Abstract: Animal-sourced food is a major source of S. enterica infections and a serious public health concern around the world, particularly in developing countries. More recent review studies on the prevalence of S. enterica in dairy products show a median of 6% in raw milk and dairy products. However, almost all previous work in this area has been limited to biochemical confirmation of suspected S. enterica. The prevalence report from dairy products had high uncertainty. As a result, almost all reports of the prevalence of S. enterica in dairy products and raw milk were highly variable. Furthermore, almost all previous reports on the prevalence of S. enterica in dairy products in Ethiopia do not take the dairy value chain into account when determining the major point of contamination of the product. To overcome the limitations of previous studies, molecular techniques, as well as the milk and dairy value chain in the country were used to confirm the presence of S. enterica in each value chain. From December to March 2020, a cross-sectional study was conducted on milk and dairy products in the Welmera, Bishoftu, Asella, and Fiche milk shades of Ethiopia's Oromia region to determine the incidence of S. enterica. A total of 480 dairy product samples were collected using simple random techniques from producers, collectors, processors, and retailer value chains Isolated S. enterica was confirmed using the latex agglutination test and the presence of a highly conserved region of the invA gene. The overall prevalence was 14.79 percent (71/480). From a total of 480 tested samples 21.35 percent raw milk, 12.5 percent pasteurized milk, and 6.5 percent cottage cheese are positive for S. enterica. According to this finding, dairy products in the area are sub standards of east African standards. As a result, strict hygienic approaches and quality control measures should be implemented to improve product safety in the area.

Keywords: Central highland, dairy product, Incidence, Salmonella enterica.

1. Introduction

Foodborne diseases cause significant public health problems as well as massive economic losses in both developed and developing countries. According to an annual World Health Organization report, 30 percent of the population in developed countries suffers from a foodborne disease, with up to 2 million deaths occurring in developing countries [1], [2]. Most foodborne disease outbreaks in developing countries go unreported or are underreported [3]. The safety of foodborne diseases in dairy products is a major global concern, particularly in developing countries where milk and dairy products are produced using poor hygienic, sanitary, and agricultural practices [4]. The problem is exacerbated in countries such as Ethiopia, where there are no food safety measures in place and no reliable statistics on foodborne diseases due to poor reporting systems [5], [2]. Milk has been regarded as the perfect food due to its essential nutrient content, which the body requires in appropriate proportions. As a result, it is the best medium for pathogenic microorganisms and a potential vehicle for pathogen transmission to humans. In Ethiopia, more than 3.3 billion liters of milk were produced from dairy cows in rural, urban, and pre-urban areas each year, with rural dairy systems accounting for 98 percent of production and pre-urban and urban areas accounting for 2 percent [6]. Around 92 percent of Ethiopia's annual milk production was produced in four regions; Oromia 44.4 percent, SSNNP 21.7 percent, Amhara 19.4 percent, and Tigrai 6.3 percent [7]. S. enterica is the most prevalent pathogen among the most common foodborne pathogens worldwide (Sanchez et al., 2007). It is the most commonly isolated foodborne pathogen and is found primarily in poultry, milk, and other dairy products [8]. S. enterica is a worldwide public health concern, causing more than 93.8 million foodborne illnesses and 155,000 deaths each year [9], [10]. They have a substantial economic impact on humans and animals, primarily in developing countries [11]. S. enterica is a gram-negative, rod-shaped bacteria that is a facultative anaerobe and a flagellated bacterium in the Enterobacteriaceae family [8],[12]. Currently, approximately 2610 S. enterica serotypes have been identified, with over 1540 belonging to S. enterica, which accounts for the vast majority of S. enterica infections in humans [13]. Milk and dairy products are major sources of S. enterica infection, especially among dairy-consuming consumers. S. enterica contamination of milk and other products can occur at any point along the value chain, from production to consumption[14],[15]. S. enterica infections are caused by a variety of factors, including poor farm hygiene, improper food storage, poor personal hygiene practices, insufficient cooling, and reheating of food items [11], [4]. Food processing can improve food safety for consumers [16], however, consumers' attitudes toward the consumption of unprocessed or minimally processed foods, such as raw milk and dairy products made from raw milk, are changing [17]. In Ethiopia, there is not enough evidence on the incidence of salmonellosis caused by dairy products. However, there is a widespread raw animal product consumption habit in a significant segment of the population, which suggests that there is a risk of acquiring S. enterica from animal products. As a result, quantitative estimates of S. enterica in raw dairy products could help us understand the level of contamination as well as the relative importance of dairy products as potential sources of S. enterica infections in humans in the area. Many studies have been conducted in Ethiopia to determine the prevalence of S. enterica. in dairy products. However, almost all previous research in this area has been limited to biochemical confirmation of suspected S. enterica isolated from dairy products, which had high uncertainty on the output information. As a result, nearly every previous report on the prevalence of S. enterica in dairy products and raw milk was highly variable. almost all previous reports on the incidence of S. enterica. in dairy products in Ethiopia do

not take the dairy value chain into account when determining the major point of contamination of the product. To overcome the limitations of previous studies, molecular techniques, as well as dairy value chains in the country were used to confirm the presence of S. enterica along the value chains. As a result, this study is required to estimate the incidence of S. enterica in milk and dairy product sources at different value chains using molecular detections of the isolates to intervene and improve the safety of milk and other dairy products in the country.

2. Materials and Methods

2.1. Study area

The study was conducted in four towns in Ethiopia's central highlands: Welmera, which is located 49 kilometers west of Addis Abeba, Fiche, which is located 109 kilometers northwest of Addis Abeba, Bishoftu, which is located 49 kilometers east of Addis Abeba, and Asella, which is located 120 kilometers southeast of Addis Abeba. These towns were chosen based on their milk production potential in the area.

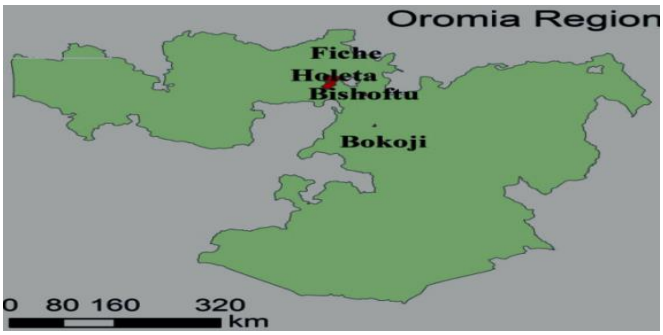

Figure 1: *Study area*

2.2. Sampling and Design of the Study

A cross-sectional study was conducted to assess the prevalence of S. enterica in raw milk and other dairy products between December 2020 and March 2020. Milk producers (farmers), collectors, and retailers were all involved in this study's milk value chains. A total of 192 raw milk samples were collected from a producer (n=96), collectors (n=96), and retailers (n=96), and a total of 192 pasteurized milk samples were collected from a processor (n=96) and retailers (n=96), while 92 cottage cheese samples were collected from two value chain producers (n=48) and retailers (n=48) using simple random sampling techniques. Approximately 250 mL of raw and pasteurized milk was aseptically sampled into a sterile polyethylene bottle, and 500gm of cottage cheese was collected in a sterile polyethylene zip bag and stored in a jet cooler less than 4 °C before being transported to the Holeta National Agricultural Biotechnology, Microbial Biotechnology Research Laboratory and stored at 4^{o}C until analyzed. Samples were analyzed within 6-12 hours of being delivered to the laboratory.

2.3. Microbiological analysis

S. enterica isolation and identification were carried out following [18]. 25 mL/gm of the samples were transferred to 225 mL sterile buffered peptone water (Oxoid, CM 0509), mixed well in a stomacher bag, and incubated at 35 ^{o}C for 18 hours before being aseptically inoculated into 10 mL of sterile Rappaport Vassiliadis (RV) broth (HIMIDIA) and Muller Kaufmann Tetrathionate broth (HIMI). The culture in RVS broth was incubated for 24 hours at 41 0C. These were incubated in Muller Kaufmann Tetrathionate broth for 24 hours at 37 ^{o}C. A 10µl loop of cultured RV broth and Muller Kaufmann Tetrathionate broth was streaked onto Xylose Lysine Deoxycholate (XLD) Agar (HIMIDIA) and Hektoen enteric agar (HIMIDIA), and then incubated aerobically at 37 ^{o}C for 24 hours. The suspected colonies (pink colonies or red colonies with or without black centers on XLD and blue-green to blue colonies with or without black centers on Hektoen enteric agar) were picked up and sub-cultured on BHI agar (BBLTM) and incubated for 24 ± 2.0 hours at 35± 2.0° C for molecular identification.

2.4. Test for latex agglutination

Perceptive S. enterica colonies were confirmed using the latex agglutination test. One drop of saline was placed in a reaction card well, and a typical suspected colony was emulsified in the drop of saline. One drop of test latex (Oxoid, R6248pw, UK) was added and mixed for two minutes with a sterile mixing stick, and agglutination was examined. These forming agglutinations indicated the presence of S. enterica [19],[20].

2.5. Molecular identification

These culturally suspected S. enterica isolates were confirmed by PCR based on the presence of the invA gene. The extraction of DNA of suspected isolates was performed by thermal cell lysis [20]**.** The DNA was used as a template for the amplification of the highly conserved region of the invA gene using the primers Salm3 (5´-GCTGCGCGCGAACGGCGAAG-3') and Salm4 (5´-TCCCGGCAGAGTTCCCATT-3´) which amplify a 389 bp fragment of the conserved invA gene sequence of S. enterica. Cycling conditions were optimized by performing an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of amplification (denaturation at 95°C for 90 s, annealing at 60°C for 60 s, and extension at 72°C for 90 s) on a thermocycler (BIO-RAD T100TM Thermal cycler 621BR43010, Singapore), and finished with a final extension at 72°C for 7 minutes. The PCR products were electrophoresed in a 1.5 percent agarose gel stained with a 10% gel loading dye (B7025S, New England) using a 100 bp DNA ladder (Product no. N32315). The gel documentation system was used to visualize the DNA band [20].

2.6. Statistical analysis

Data obtained from the Laboratory test were analyzed using descriptive statistics of frequency distribution and percentage using SPSS version 25.0 Software SPSS, 2017.

3. Result and Discussion

S. enterica is one of the most common foodborne pathogens and important zoonotic microorganisms in humans, particularly in developing countries [11]. In this study, 14.79 percent (71/480) of the 480 samples 192 raw milk, 192 pasteurized milk, and 96 Cottage cheese) tested positive for S. enterica. This is comparable to the 14.3 percent and 15 percent S. enterica incidence rates in dairy products reported by [21],[20]. The incidence of raw milk, pasteurized milk, and cottage cheese was 21.35 percent (41/192), 12.5 percent (24/192), and 6.25 percent (6/96) of the overall dairy product incidence of 14.79 percent (71/480), respectively. Raw milk had the highest incidence of S. enterica among these dairy

products, followed by pasteurized milk (Figure 1). This increased S. enterica incidence could be attributed to a variety of factors such as hygiene, storage, farm management, farm size, environmental factors, and season. The prevalence of S. enterica detected in raw milk in this study agreed with the findings of [22], who found S. enterica in raw milk at a rate of 21% in Egypt. [23],[24],[25], reported higher S. enterica incidence of 35.71 percent, 28.6 percent in Ethiopia, and 44.44 percent in Egyptian dairy products, respectively. In Ethiopia, there is also a low incidence of 3.1 percent, 6 percent, and 10.76 percent in raw milk, as reported by [26], [11], [27].

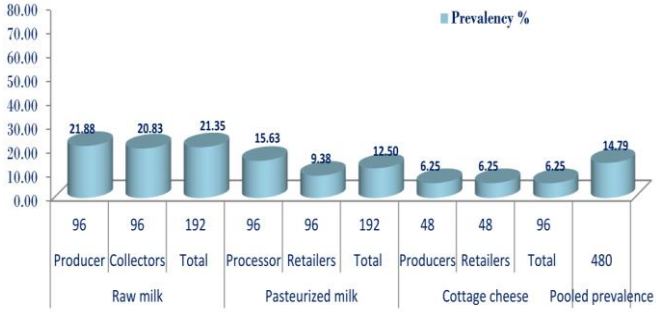

Figure 1: *Incidence of S. enterica across milk value chain*

This variation in the incidence of S. enterica in raw milk among these studies could be attributed to a variety of factors such as hygienic practices, farm management practices, sample size, sampling season, farm size, and detection test methods. Cottage cheese was another dairy product included in this study, with a prevalence of 6.5 percent across a value chain of producers and retailers. The prevalence of S. enterica detected in Cottage cheese in this study was higher than the 2.1 percent reported in Ethiopia by [28],[26]. This could be due to post-contamination from processing water, hygiene of storage equipment, and personal hygiene and storage conditions [4],[11].

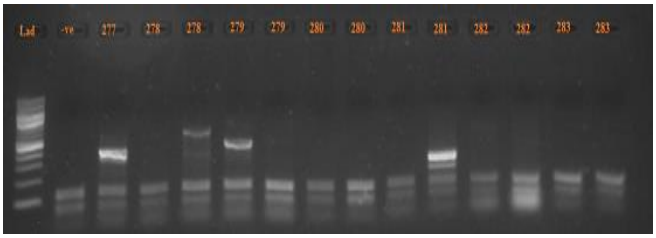

Fig 2: *Molecular Identification of Salmonella spp.*

This finding contradicts the findings of [11],[25], who found no S. enterica in Ethiopian cottage cheese. The processing effect of Cottage cheese preparation (smoking of fermentation equipment, low pH during fermentation, and heat treatment) can destroy or inactivate S. enterica [29]. Cottage cheese was made in Ethiopia by spontaneously fermenting raw milk for 2-3 days in a container well smoked with olive-wood sticks at room temperature, lowering the pH to 4.0 to 4.2 [30]. By churning the fermented milk, butter was extracted, and the buttermilk was heated at 40 – 70oC until a distinct curd mass (Cottage cheese) formed. Smoking of fermentation equipment, low pH during fermentation, heat treatment, and the production of antimicrobial substances by fermenting microbes during milk fermentation can all help to reduce foodborne pathogens like S. enterica spp. in cottage cheese [31], [30], [32]. S. enterica cannot withstand proper pasteurization of time-temperature combinations, according to various reports. This is supported by the findings of [11], who found no S. enterica in pasteurized milk from Gondar, Ethiopia. However, an unexpectedly high S. enterica incidence of 12.5 percent was observed in this study. This could be the result of insufficient pasteurization or post-contamination during packing, storage, and transportation. The prevalence of S. enterica was shown in Table 1 across the dairy value chain and study area. S. enterica was found in 6.6 percent, 23.33 percent, 11.67 percent, and 17.5 percent in Welmera, Bishoftu, Asella, and Fiche town respectively.

Table 1: *Incidence of S. enterica across value chain*

Location	Product type	Value chain	Total samples	Positive samples	Prevalence %
Welmera	Raw milk	Producer	24	4	16.67
		Collector	24	3	12.5
		Processor	24	0	0
	Pasteurized milk	Retailer	24	0	0
	Cottage cheese	Producer	12	1	8.33
		Retailer	12	0	0
Bishoftu	Raw milk	Producer	24	15	62.5
		Collector	24	11	45.83
		Processor	24	2	28.33
	Pasteurized milk	Retailer	24	0	0
	Cottage cheese	Producer	12	0	0
		Retailer	12	0	0
Asella	Raw milk	Producer	24	2	8.33
		Collector	24	6	25
		Processor	24	0	0
	Pasteurized milk	Retailer	24	4	16.67
	Cottage cheese	Producer	12	2	16.67
		Retailer	12	0	0
Fiche	Raw milk	Producer	24	0	0
		Collector	24	0	0
		Processor	24	13	54.17
	Pasteurized milk	Retailer	24	5	20.83
	Cottage cheese	Producer	12	0	0
		Retailer	12	3	25

The variation among study areas could be attributed to differences in hygienic practices, geography, farm management, and the area's quality control system. Among these study areas, raw milk from Bishoftu had the highest incidence of S. enterica 54.17 percent, followed by 14.58 percent from Welmera town. S. enterica was most likely not found in raw milk samples collected from Fiche town. Pasteurized milk samples from Welmera, on the other hand, had no S. enterica contamination. Pasteurized milk samples collected from Fiche and Asella town, on the other hand, had a high incidence of 37.5 percent and 8.33 percent, respectively. [23],[26],[24],[21], reported S. enterica incidences of 18.5 30 percent and 35.71 percent in Jimma Zone, 2.1 percent, and 28.6 percent in Addis Ababa, and 14.3 percent in Asella town, respectively. This suggests that the prevalence of S. enterica varies by location, dairy product, dairy farm size, duration, and season.

4. Conclusions

According to the findings of this study, the safety of milk and other dairy products in the region fell short of East African standards. To ensure the quality and safety of raw milk and milk products, training and awareness creation for all actors involved in milk and dairy production (producers, collectors, processing factories, retailers/supermarkets) and public education about hygienic practices, safety, and risks of raw or dairy product consumption are important lines of

defense against S. enterica infection and other food-borne pathogens transmitted through dairy products in the region.

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