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Total Bacterial Counts of Raw Milk in Selected Dairy Farms in Nekemte Town, Ethiopia

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Abstract: A cross-sectional study was conducted in Nekemte town in selected dairy farms from November 2011 to May 2012 to evaluate the quality of raw milk measured by total bacterial count (TBC) based on cultural examination. The raw milk for total bacterial count was collected from ten randomly selected dairy farms and was carried out in Wollega University Veterinary microbiology laboratory. Out of 389 raw milk samples examined for TBC the overall mean total count was 9.07logcfu/ml. In addition, the mean total aerobic plate count analyzed were 7.42 log cfu/ml (udder) 9.07logcfu/ml (bucket), 9.87logcfu/ml sample from transporting materials with car or cart to customer/hotels and 9.94logcfu/ml (kiosk/shop).The result of mean TBC at farm was 7.42 log cfu/ml and it was found higher than that of international standard 5 log cfu/ml ($1x10^5$cfu/ml). For this study the TBC at farm level was none significant (p=0.08) where as TBC at other critical point was differed significantly (p=0.004). The result obtained from this study indicated that the current situation needs real improvement from production point to consumption. The actual bacterial counts indicated that the quality of milk produced by dairy farm in the study area was poor and this call for scrupulous hygienic measures during production and handling of milk. Based on the result obtained, recommendations were forwarded to buildup awareness among dairy caw owners to improve the milk hygienic quality and to establish standards and use of effective enforcement.

Key words: Quality Raw Milk · Hygienic Measures · Total Bacterial Count · Nekemte

INTRODUCTION

Total bacterial count (TBC) is the bacterial growth per ml of milk over a fixed period of time [1]. Milk is the lacteal secretion of the mammary glands of a mammal. As it is well known, milk is the first natural food of all young mammals during the period immediately after birth [2]. Man has consumed milk and milk products even before the dawn of civilization. Because of its high nutritive value, milk is considered as one of the most important diet items of many people [3]. Nutritionally, milk has been defined as "the most nearly perfect food". It provides more essential nutrients in significant amounts than any other single food. Milk is an outstanding source of calcium and phosphorus for bones and teeth and contains riboflavin, vitamin B_6, A and B_1 in significant amounts. It also contains vitamin B_{12}, the anti pernicious anemia vitamin [4]. As milk, milk products play an important role in human nutrition throughout the world. Consequently, the products must be of high hygienic quality. In developing countries and especially in hot tropics high quality of safe product is most important but

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not easily accomplished [5]. This is required since milk is also a suitable substrate for microbial growth and development.

The fluid or semi-fluid nature of milk and its chemical composition (containing the essential nutrients) renders it one of the ideal culture media for microbial growth and multiplication [6-8]. Mainly because of this reason, milk and milk products are more prone to the harboring and proliferation of microorganisms. Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources i.e., within the udder, the exterior of the udder and the surface of the milk handling and storage equipment [9, 10]. Microorganisms may contaminate milk at various stages of milk procurement, processing and distribution. The health of the cow and its environment, improperly cleaned and sanitized milk handling equipment and workers who milk cows and come in contact with milk due to a number of reasons could serve as sources of microbial contamination of milk. Use of non potable water may also cause entry of pathogens into milk. It is known that tropical conditions which have a hot, humid climate for much of the year are ideal for quick milk deterioration so pose particular problems because the temperature is ideal for growth and multiplication of many bacteria [11] but milk quality is required to be within certain threshold according to European law [12] total bacterial count must not exceed an average of 100,000 cfu/ml of milk.

Although milk is known to possess antimicrobial systems, bacterial numbers will double in less than 3 hours in un-chilled milk. The rate of microbial growth will depend on initial numbers and the temperature at which milk is held after milking and thereafter [13]. The increase in urban populations during the present century and improvements in methods of milk preservation have led to large scale transportation of milk from the producer to the consumer areas [14]. Raw milk collection and its transportation to the processing centers present a number of technical, economical and organizational problems in most developing countries in tropical regions. These have inevitably increased the risk of infection of many people from a common source. Lack of refrigeration facilities at the farm and household level, with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation [13]. The risk can be reduced by suitable precaution. Methods employed for improving the keeping quality of milk are often adequate to render safe for consumption [15]. Diseases that commonly spread from the milk to human beings are tuberculosis, brucellosis, salmonellosis, listeriosis, campylobacteriosis, yersinoses and Q-fever. Other bacterial pathogens transmitted to humans include *Streptococcus agalactae*, *Staphylococcus aureus* and *Escherchia coli* [16]. Milk may contain both pathogenic and nonpathogenic organisms. Pathogenic organisms, which may come directly from the cow's udder, are species of *Staphylococcus*, *Streptococcus*, *Mycobacterium, Brucella, Escherchia*, *Corynebacterium*, etc. Various other pathogenic causing diseases like cholera and typhoid may find access in the milk from various other sources, which may include water and the persons handling the milk. Nonpathogenic microflora may come directly from the udder and may also enter in the milk from milker's hands, utensils, cow barn, water, etc. [16].

In Ethiopia the average lactation milk yields per cow in indigenous cattle is estimated to be 213Kg of milk. At present, the per capita consumption of milk estimated is to be 10 Kg, which is lower than other countries in the region [17]. Total annual milk production is estimated to be 830,000 tons of raw milk equivalents of the total national production; 85-89% is obtained from cattle [17]. Milk in most places in Ethiopia is consumed raw. Milk products such as yoghurt, butter and buttermilk are also produced using raw milk as a starting material. Hence there exists the possibility of consuming milk which has been contaminated with disease causing organisms [3].

The actual methods of storage on the farm and mode of transportation to the processing plant don't prevent microbial activity in the raw milk in the country. Due to post pasteurization contamination, the shelf life of pasteurized milk at ambient temperature or refrigeration temperature decreases through time. Hygienic quality control of milk and milk products in Ethiopia is not usually conducted on routine basis. Apart from this, door-to-door raw milk delivery in the urban and peri-urban areas is commonly practiced with virtually no quality control at all levels [10].

In countries with poor milk production and marketing practices, one can expect high initial viable bacterial counts posing a health hazard as well as spoilage of large quantities milk [18]. The number of bacteria may increase considerably if samples taken at the time of milking from the cows and distribution to the consumers are tested for

total bacteriological counts. Information on the bacterial content of a milk sample may reflect on the state of health of the cow, the contributions under which the milk is stored and distributed and its public health significance [19].

Therefore, the present study was carried out, with the following objectives: To determine some of the critical control points in milk handling, to estimate the total bacterial count of milk at each critical control point and evaluate the quality of raw milk as influenced due to milk handling procedures on total bacterial count (TBC) as the milk quality indicator.

MATERIALS AND METHODS

Study Area: The study was conducted from November to May in Nekemte, East Wollega Zone, Oromia, Ethiopia. Nekemte is a market town in Western Ethiopia, located in Eastern Wollega Zone of Oromia Region. It is found in the west of capital city Addis Ababa at the distance of 331km with its administrative center at Nekemte having 21 districts. It is characterized by crop livestock mixed farming system. Teff, wheat, barley, maize, sorghum, peans, beans, chickpea, linseeds, nug and rapeseed are major annual crops grown in the area. The estimate animal population of the area is 78,178 cattle, 9894 sheep, 6477 goats, 3287 donkeys, 1598 horses, 665 mules and 2428 cats and 4572 dogs. The total land area of the region is about 769,725 hectares of which is 336,220 hectares are used for crop production, 182,412 hectares for animal grazing, 256,901 hectares forest covered and 20,492 hectares for other activities. The town is located at latitude and longitude of 9°5' N36°33' E and 9.083°N 36.55°E, respectively and with elevation of 2,088 meters. The zone receives the maximum rain fall of 1500mm with the average rain fall of 1850mm. The minimum and maximum temperature for the zone is to be 14° to 26°C respectively.

Study Populationl: In Nekemte there are a number of small dairy farms with small numbers of cows and one with large numbers of animal with 16 lactating cows. Each of the dairy cow owners has five or more Holstein-Friesian, cross-breed, jersey cow. The estimated average milk yield is 7.5 liters/day/cow. The number of lactating cows present at the farms ranged from 8 to 16. All animals were identified by name. After milking their cows, the dairy owners delivered the milk to their nearest milk consumers and hotels.

Methods

Study Type: A cross-sectional study was conducted from November 2011 to May 2012. Sampling was carried out repeatedly from four critical control points (udder milk, Bucket milk, from transporting materials to customer and from distribution shop/kiosk).

Sampling Technique: A total number of samples required for the study were calculated based on the formula given by Thrusfield [20]. The lists of all dairy members were taken from Nekemte town and then using simple random sampling technique ten study dairy farms were identified. The study involved 384 raw milk of which 150 udder milk and 150 bucket milk from the selected dairy farms and 79 pooled farm milk from transporting materials (during distribution of milk for customers) and 105 pooled farms milk from the container (where they distribute milk for consumers) and 10 from distribution kiosks/shop.

Data Collection

Questionnaire Survey: In the study farms, basic farm data and data on milking technique, udder preparation, milk handling and hygiene were collected through questionnaire.

Collection of Raw Milk Sample at Critical Control Points: Raw milk samples were collected from critical control points that are considered to be associated with the hazard, when a measurement can be conducted and when control measures can be taken in order to reduce the hazard to an acceptable level and total aerobic plate count (TAPC) on pooled milk samples collected at the following critical control points:

1) Directly from the cows' udder at farm level, 2) From the milking bucket at farm level, 3) From transporting materials and 4) From distribution shop/kiosk. Pooled raw milk samples were collected from each critical point aseptically for the study period in the afternoon time. Accordingly 389 raw milk samples were collected separately and aseptically from which 150 samples were directly from the udder, 150 samples were from milking bucket and 79 were from transporting material to customer with cart/car and 10 from kiosk/shop. During sampling of raw milk from the udder, the surface of the teat end was cleaned by wiping it with clean cotton dipped in 70% alcohol. Before sampling from milking bucket, storage container, the milk was thoroughly mixed after which 15 ml of milk is transferred into sterile sampling, the sampling bottles were capped, labeled with permanent marker and stored in an ice packed cool box and transported to the

school of veterinary microbiology laboratory, Wollega University.

Bacterial Counts: Decimal dilutions of milk samples were placed on plate count agar for total aerobic plate following the standard procedures recommended by American Public Health Association [21]. Each plate was marked with sample number, before shaking samples and making dilutions.

Test Procedure for Total Aerobic Plate Count of Milk Samples: The dilution distilled water was labeled with sample number and dilution number, One ml of the milk sample was added to dilution blanks and serially diluted by taking one ml to the next dilution bottle after mixing. After thorough mixing, 1 ml of diluted milk was taken from each dilution starting from the highest dilution and put on sterile labeled petridishes using sterile pipettes. The covers of the Petri-dishes were lifted just high enough to insert pipettes. Two plates were inoculated per dilutions. About 10-12 ml of the melted (44°C to 46°C) plate count nutrient agar for TAPC were poured into each plate by lifting gently the cover of the Petri- dish just high enough to pour the medium, The medium was gently mixed with the test portions in the Petri-dish by rotating and tilting, After the mixture was evenly spread over the bottom of the plate, it was allowed to solidify on a level surface, The plates were then inverted and placed in an incubator at 37ºC for 48 hours, Incubating control plates for each sterilization lot of dilution blanks and medium were used to check sterility of the dilution water and medium [22].

Reading and Interpretation Results: Total aerobic plate count (TAPC): Total aerobic plate count from milk was determined following aseptic sampling techniques. After incubation at 37°C for 48 hours, all colonies are counted on selected plates. Results from plates, which contained 25 to 250 colonies per plate were recorded. If plates from two consultative decimal dilutions yield colony counts of 25 to 250, the counts for each dilution were computed by the following formula [22].

$$N = \frac{\sum \text{colonies}}{[(1 \times n_1) + (0.1 \times n_2)\, d]}$$

where

N = Number of colonies per milliliter of milk,
ΣC = Sum of colonies on plates counted,
n_1 = Number of plates on lower dilution counted,
n_2 = Number of plates in next higher dilution counted and
d = Dilution from which the first counts are obtained.

When plates contained less than 25 colonies, the results were read as less than 2.5×10^2 (in1:10 dilutions). If more than 250 colonies developed on the highest dilution plate, the count was recorded as more than 250 times the reciprocal of dilution [22]. In case of crowded plates (more than 250 colonies); if there were fewer than 10 colonies per square centimeter, colonies in 12 squares; select representative, 6 consecutive squares horizontally across the plate and 6 consecutive squares at right angles were counted being careful not to count a square more than once. When there were more than 10 colonies per square centimeter, colonies in 4 successive representative portions were counted. In both instances, the average numbers of colonies found per square centimeter were multiplied by the area of the plate used to determine the estimated number of colonies per plate. When computing the total aerobic plate counts (TAPC) only the first two significant digits were reported to avoid creating a factious impression of precision and accuracy. When making the conversion, the second digit was rounded off to the next highest number when the third digit was > 6 and rounded down when the third digit was < 4. When the third digit is 5, the second digit was rounded up if the second digit was odd and rounded down when the second digit was even the bacterial count result in this study was compared with standard viable bacterial count in specially collected milk from clean healthy cow generally has an TBC of less or equal to 1×10^5cfu/ml which is considered to be grade A Milk [23].

Data Management and Analysis: Microsoft excel was employed for raw data entry, computation of descriptive statistic were analyzed using SPSS virgin 16. Descriptive statistics such as mean, standard error and percentage were used to compute some of the data. Log10 transformation was done before the analysis of bacterial count.

RESULTS

General Data

Description of Dairy Production: All the farms encountered in this study were small holder dairy farms having 8 to 16 cross-breed (Holstein-Friesian x indigenous) lactating dairy caws. Hay, straw (teff, barley

Table 1: The mean (±standard error) of the total aerobic plate count (TBC) of pooled milk sample (log10cfu/ml) in Nekemte selected dairy farms, 2004 EC

	Critical control points of sampling			
Collection farms	Udder	Bucket	Transporting materials	Kiosks/shop
1	7.44 (7.11)	9.47(9.22)	9.47 (9.38)	9.50 (8.7)
2	7.37 (7.01)	8.55 (8.27)	9.55 (9.13)	10.35 (9.89)
3	7.53 (7.21)	9.55 (9.5)	9.47 (9.43)	9.52 (9.06)
4	7.42 (7.31)	8.46 (8.15)	8.47 (8.4)	9.36 (8.81)
5	7.36 (7.05)	9.42 (9.13)	9.44 (9.1)	9.35 (8.73)
6	7.40 (6.88)	9.45 (8.55)	9.46 (9.41)	9.51 (9.2)
7	7.45 (7.11)	9.47 (8.76)	8.52 (8.44)	10.35 (10.09)
8	7.42 (7.05)	9.45 (9.19)	8.54 (8.29)	10.49 (10.15)
9	7.37 (6.91)	8.44 (7.68)	9.45 (9.09)	10.51 (10.08)
10	7.44 (7.11)	8.47 (8.04)	8.51 (8.07)	10.41 (10.12)
Total	7.42 (7.08)	9.07 (8.65)	9.08 (8.87)	9.93 (9.48)

and sorghum), nug cake, linseed cake, wheat bran and wheat middling were some of the feed items in the farms. The barn was cleaned daily only by removing the feces. Cleaning with water was done on average two weeks.

Udder Preparation and Hygiene: All dairy owners used pipe line water to clean milk equipment and to wet the cows and clean them from soil and dirt. Most farms (70%) had not have towels for cleaning purposes of cows udder. Those farms that had towels, they reused it for cleaning and sanitizing of other materials. This may result in recontamination of the udder. In addition to that a single towel was used common to all cows, which may result in cross contamination of the udder. No udder was properly dried. In general the udder was not prepared properly and furthermore, in some of the farms (20%), the milkers were inserted their finger into the milked milk to moisten the teat whenever it got dry while milking. Milkers were not seen washing of their hands between cows. The use of detergents for cleaning of milk equipment was not observed in the majority of the farms (60%). Hot water which is indispensable for cleaning milking equipment was not available in the farms. Most of the milkers were illiterate and they did not worry about the hygiene of milk.

Milking Technique and Milking Containers: All dairy cow owners milk their cows by hand and all did not cool the milk. They simply supply it to the nearest milk consumer or their customer two times a day (morning and evening). 90% the farms included in this study had plastic containers for milking and transporting the milk to milk consumers or customer. At the level of transporting milk to consumers or to kiosk, soft plastic materials which were not washed properly and improperly handled were used to tighten the lid covers of the milk collection containers.

Bacterial Count of Raw Milk: On cultural examination three hundred eighty nine (389) raw milk samples were collected from selected dairy farms, the recovery rate was (379/389 (97.4%)). However, the rest samples were with no considerable observation of bacterial growth. In the present study there was an increasing trend of mean of total aerobic plate count as the milk passed through the udder (7.42 log cfu/ml), milking bucket (9.07 log cfu/ml), transporting to customer (9.87 log cfu/ml), or to kiosk/shop (9.94 log cfu/ml). The result of analysis indicated that there were significant differences in total bacterial count ($p<0.01$) between the critical points other than udder. The mean ± standard error for total aerobic plate count (expressed in log10 cfu/ml) of raw milk sampled at four critical points are shown in the below Table 1.

It has been found out that 80% of the raw milk samples from udder, 86% of raw milk samples from milking bucket and 93% of raw milk samples from transportation materials and kiosk/shop had total bacterial count $>1x10^5$cfu/ml which is higher than the given international standard set for minimum acceptable level of bacterial count in milk [22]. The overall mean of total bacterial count of cows' milk produced in the study area was 9.07 log cfu.

DISCUSSION

The result of this study showed that some (30%) of the dairy cow owners used detergent and water for cleaning milk equipments. In contrary, most of the dairy cow owners cleaned milk equipments without detergents after each usage, which may lead to insufficient cleaning and hence it could serve as a major cause of milk contamination. In addition, the entire dairy owners in the present study cleaned their cows' udder only with cold

water and did not perform the cleaning sufficiently and properly since the time elapsed for cleaning of the udder should be until the washed water become clean but this was not observed in this study. It was reported that pre-milking udder preparation plays an important part in the contamination of milk during milking [24].

Most of the dairy owners did not use towel and a few dairy owners used a single towel for all cows commonly to dry the udders. The reuse of towel for cleaning and sanitizing may result in recontamination of the udder. Since drying was not or insufficiently practiced, contamination level of milk was becoming higher. Furthermore, milkers wash their hands at the beginning of milking but did not dry their hands and not repeat washing between milkings and some of the milkers used milk to moisten the teats when they become dry in between milking, which could be additional source of contamination for milk. Handling of small quality of milk with a big container is subjected to high rate of contamination with small milk volume to container ratio [25]. This also contributes to the high level of contamination of milk. Milk handling equipment can become contaminated due to milk hygiene and cleaning, the bacteria in turn can pass in to the milk line, thus increasing TBC [26]. In most countries where the risk of contamination during milking, transporting, storage etc is low, the total bacterial count (TBC) of raw milk is also low [27]. But possible reason for high count in study could be due to dairy udders of the cow, unhygienic milking procedures or equipment and /or inferior microbiological quality of water used for cleaning utensils and animals as well as milk delivering conditions. The milking process, especially the equipment associated with it, introduces the greatest proportion of microorganisms in raw milk [28]. On cultural examination the recovery rate was 379/389 (97.42%), the rest sample with no considerable observation of bacterial growth. This indicate that the dairy farm needs quality improvement at the time of milking, cleaning the equipment and during selling at kiosk specially, no proper cleaning found and no basement on which the milk was placed. During milking most of the milkers leave the milk open mouth until they finish milking process this may cause recontamination of milk from flies. In contrast, Zellem [29] reported a culture recovery rate of 76.8%.

In this study, 73.3% of the milk samples from udder, 80% from milking bucket and 87.8% from transporting materials and kiosk/shop had TBC greater than $4x10^5$ cfu/ml. the higher value found in this study for TBC count as compared to the EU and United state of America standard were attributed to the cumulative result of milk contamination at different levels while milk was passing through different moments. Factors that could contribute a lot to the contamination udder of milk in this study include insufficient pre milking udder preparation, insufficient cleaning of milkers' hands and milking utensils, use of poor quality and non boiled water for cleaning of udder, milk equipment and plastic containers; additional handling of milk in to different plastic container and sieves may cause the contamination of milk higher, since as the number plastic containers have characteristics that make them unsuitable for milk handling. Since plastic containers scratch easily and provide hiding place for bacteria during cleaning and sanitization and plastic container are poor conductor of heat and hence will hinder effective sanitization by heat [22]. It was indicated that the TBC in milk in developed countries fall between $7.5x10^5$ to $2x10^6$fu/ml [27] According to the European and American community member state, the acceptable limit for TBC counts for raw milk are between $2x10^5$ and $4x10^5$cfu/ml [24].

In this study, udder milk had a better (acceptable) bacterial load than other because it was not subjected to further contamination after milking. The continuous and progressive contamination of raw milk as the milk passed through the critical comments evidenced by the highly significant increment of TBC count from the initial point of milk production to fork or consumption. The first contamination occurred up on milking due to insufficient udder preparation, followed by contamination from milking bucket at milkers' hand at farm level. In addition, contamination of milk occurred as the milk was transported to the customer due to lack of cold chain, use of milk containers lacking tight sealing, handling of milk with unclean hands and utensils.

The total bacterial count obtained in this study is generally high as compared to the acceptable level of $1x10^5$ bacterial per ml of raw milk. Aseptically collected milk from clean healthy cow generally has an TBC of less or equal to $1x0^5$cfu/ml which is considered to be grade A milk while counts of less than $4x10^5$cfu/ml is possible by most farms. According to the European and American community member state, the acceptable limit for TBC counts for raw milk are between $2x10^5$ and $4x10^5$cfu/ml standard plate count for America is no more than $3x10^5$cfu/ml, while the standard for Kenya is no more than $2x10^6$ cfu/ml [30]. In Sweden, the accepted limit for the total number of bacteria and somatic cell count in raw milk was found $1x10^5$cfu/ml, but in this study the result was higher than standard. Bacterial growth could be considered as the possible cause of increment in TBC counts at the critical point of milking bucket at the farm;

since a relatively short time elapsed between milking and distributing to consumer these moments and because of existence of bacteriostatic compounds in fresh raw milk that would counter act bacterial growth for 3 hours [31]. Of course with slight effort in udder and milk equipment hygiene and by increasing the awareness of milk handle about milk hygiene, the TBC count could be reduced at various points of sampling significantly. The bacterial count and type of bacteria determines the keeping quality of milk, resulting milk products after processing. Contamination of raw milk in the order of 10^2 to 10^3cfu/ml is practically in avoidable.

CONCLUSION AND RECOMMENDATIONS

Milk intended for human consumption must be free from high bacterial load and must, if condition permit, no or few bacteria. Clean milk could only be obtained if effective sanitary measure is taken starting from the point of milk withdrawn from the cow until it reaches the consumers. In line with this fact, the quality of milk produced area was substandard and the health of dairy herd, hygiene condition of milking and storage process, transferring of milk into different containers and sieves, unclean milk equipment and the use of contaminated water were basic determination of milk quality. Result from sampling at critical control point showed that sever contamination started from the firs moment the milk left the udder. The different between total aerobic plate count (TAPC) counts of milk at different critical control points clearly demonstrated exogenous source of milk bacterial contamination. This occurred primary at very initial phase the milking procedure. Majority of raw milk samples from the bucket and all the samples from transporting materials had higher APC counts, which was higher than the international acceptable limit. The result obtained in this study showed that the raw milk available to the consumers has high bacterial level of contamination. Based on the high level of bacterial count found in the milk, one may suppose that the raw milk may pose a public health risk and this suggests the need for more strict preventive measures.

Therefore, based on the findings of the present study the following recommendations were forwarded:

- Awareness should be created among dairy cows owners as to the importance of adequate udder preparation, hygienic milking technique, use of clean dairy equipment, washing of utensils and milkers' hand using properly treated water to improve the milk hygienic quality and shelf life.
- Raw milk intended for consumption should be subjected to heat treatment at least equivalent to pasteurization temperature.
- If possible, potable water should be available for effective cleaning and sanitizing of milk equipment and udder preparation; otherwise, very well heat treated water should be used for such purpose.
- Regulation of the dairy development enterprise, establishment of standard and the use of effective enforcement are essential in order to fulfill the consumers' expectation of safe and wholesome milk and milk product.

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