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Quality and safety assessment of raw bovine milk in Herat province, Afghanistan

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ABSTRACT

Milk and various value-added dairy products are popular throughout Afghanistan. Over the past 10 years, several aid and governmental agencies have made concerted efforts to increase Afghan dairy production. At the same time, there has been visible growth in small milk shops in urban and peri-urban areas. These shops provide fluid milk and dairy products to consumers in the local economy. The quality and consistency of value-added dairy products are dependent on the quality of milk used as raw material. Here, we conducted a quality assessment of raw milk sold in open markets (bazars) as well as through small milk shops. Each sample (n = 100) was analyzed for fat, protein, and carbohydrate percentages, total bacterial concentrations, coliform concentrations, and various adulterations including added water, starch, and antibiotic residues. Only 34.4% of all samples contained < 10^5 colony forming units (CFU)/ml total bacteria, a standard acceptable bacterial concentration for raw milk in many countries. Similarly, the majority of milk samples (92.0%) contained > 100 CFU coliforms/ml. Milk purchased from milk shops was statistically more expensive, but milk samples from bazars more frequently contained irregularities such as added starch, low-fat percentages, or high water percentages. Many of the quality issues, we found were likely related to hygiene and sanitation practices at different points along the entire production chain. Decreasing overall bacterial concentrations could extend shelf life, improve consistency of fermented products and reduce waste due to unmarketable and unusable fluid milk.

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1. Introduction

Afghanistan has a largely agrarian economy with over 50% of all household incomes dependent on agriculture on some level, while the country remains among the most food insecure nations in the world. Per capita, dairy consumption in Afghanistan is estimated at 66 K/years (whole milk equivalent; 4) compared to worldwide and industrial countries per capita averages of 108 K/years and 217 k/years, respectively (1). While Afghanistan currently produces over 1.5 million tons of milk annually (2), it imports an estimated 70% of the dairy products consumed annually across the country (3,4,5).

In the past 10 years, numerous international and governmental programs have focused on increasing milk production and identified dairy production and processing as having substantial potential for development and growth (6,7). In turn, there are growing dairy segments in several parts of Afghanistan (8,9) including Herat Province (5). While much of this growth is in larger milk cooperatives or processing factories (8), there has also been visible growth in small milk shops, which serve as an important link in supplying dairy products to customers in the local economy (personal observation). These shops sell fluid milk and often

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produce value-added dairy products on site including yoghurt, cheese, and other fermented dairy products (e.g., dough). While these small businesses provide direct employment, they also provide market opportunities for small-scale, peri-urban dairy producers. They also serve as a source of raw milk for consumers who commonly produce various fermented dairy products in the home (5).

The quality of value-added dairy products is dependent on the quality of raw milk. Through extension workshops conducted by the authors, milk shop owners reported shelf life challenges as well as difficulties in producing consistent fermented products (unpublished data). While similar studies exist examining quality of milk available through various retail outlets in neighboring countries (10,11,12,13), to our knowledge, no quality assessments of raw milk available to Afghans through bazars and milk shops have been reported. The results will serve as primary data for university-led outreach/extension programs focused on this growing segment of Afghan food production.

2. Materials and methods

2.1. Sample collection

Milk samples (500 g) were collected in aseptic bags from milk shops (n = 45) and bazars (n = 55) through Herat city, Afghanistan, over 4 days in October 2015. The samples were then transported in coolers to the Herat University Food Technology Laboratory for further processing. All samples were cataloged based on the name of the collector, time of collection, location of shop or bazar, price of milk (per kg), age of milk, and original source of milk (e.g., contracted farmer, and bazar). All milk samples underwent a preliminary examination of color, odor, and any obvious signs of spoilage before laboratory analysis.

2.2. Bacteriology

Concentrations of total aerobic bacteria were measured using an aerobic plate count method with slight modifications (14). Briefly, milk samples were serially diluted in phosphate buffered saline (PBS) (140 mM NaCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, and pH 7.5) and 100 µL of each dilution was transferred to plates containing Plate Count Agar (Himedia Laboratories, Mumbai, India). Samples were then incubated overnight at 37° C and colonies were enumerated the following morning. Total coliforms were measured by serially diluting milk samples in PBS and transferring 100 µL of each dilution to plates containing violet red bile agar (hardy diagnostics, Santa Maria, CA). Samples were then incubated overnight at 37 °C and colonies were enumerated the following morning. Colonies with pink centers and surrounding red zones were considered presumptive lactose-fermenting coliforms.

2.3. Milk composition

Milk composition was measured by ultrasound with the Lacto scan Ultrasonic Milk Analyzer (Milkotronic, Nova Zagora, Bulgaria) according to the directions of the manufacturer. Briefly, milk samples (~2 ml) were warmed to room temperature before analysis and percentages of fat, protein, lactose, and salts, pH, freezing point, and presence of added water were estimated by ultrasound. In cases, where ultrasonic analysis indicated water irregularities (e.g., high percentages and irregular freezing point), milk density was measured by lactometer (hydrometric analysis). Samples with irregular ultrasounds and specific densities lower than 1.027 were considered to contain water irregularities.

2.4. Presence of adulterants

Milk samples were screened for the presence of antibiotic and sulfonamide residues using ROSA lateral flow antibiotic strips (Charm Sciences, Inc., Lawrence, MA) according to the manufacturer's instructions with slight modifications. To test for the presence of beta-lactams, 300 µl of room temperature milk sample was applied to the Charm 3 SL3 Strip (Charm Sciences), and samples were incubated at 58 °C in a standard incubator for 3 minutes. Similarly, to test for the presence of sulfonamides, 300 µl of room temperature milk sample was applied to the ROSA SULF Strip (Charm Sciences), and samples were incubated at 58 °C in a standard incubator for 3 minutes. Finally, to test for the presence of tetracycline, 300 µl of room temperature milk sample was applied to the ROSA TET (Charm Sciences), strip and samples were incubated at 58 °C in a standard incubator for 8 minutes. After incubation, the band patterns of each strip were analyzed visually and compared to standards provided by the manufacturer to determine positive or negative samples.

The presence of starch was determined by adding two drops of iodine solution (0.1 N) to 5 ml of milk sample at room temperature and observing any color changes. Milk samples that became dark-blue/black were considered positive for added starch. Sodium carbonate was detected by adding two drops of Rosalic acid (1.0%) to 5 ml of milk sample and observing any color changes. Milk samples that became pink to red were considered positive for added calcium carbonate.

2.5. Statistical analysis

Statistical analysis of binary comparisons was made using a t-test. When necessary (e.g., bacterial concentrations), data were compared statistically using a one-way ANOVA with *post hoc* Tukey analysis. Comparisons were considered statistically different at P < 0.050.

3. Results

3.1. Sample characteristics

A total of 100 milk samples from bazars (n = 55) and milk shops (n = 45) were collected and processed over 5 days in October 2015 (Table 1). Sellers were questioned as to the source of the milk as well as time of milk of collection. Based on their answers, the age of the milk samples did not differ between groups. Across both groups, most milk samples (53.0%) were collected from their original source (e.g., farmer and another market) < 6 hours before sale. 44% of samples were estimated to be between 6 and 12 hours old. A small number of samples (2.0%) were estimated to be > 24 hours old, and one sample was "mixed," containing older milk combined with freshly collected milk.

Table 1. Age and price of milk samples used in this study

Source	Age (% samples)			Mixed	Price*
	< 6 hours	12-24 hours	> 24		AF
Overall (n = 100)	53.0	44.0	2.0	1.0	28.7
Bazar (n = 55)	52.7	41.8	3.6	1.8	26.2[b]
Milk shop (n = 45)	53.3	46.7	0.0	0.0	31.1[a]

Numbers with different superscripts are significantly different at P < 0.050. Comparisons are between sampling groups (bazars vs. milk shops) and within column. *At time of collection

3.2. Bacteriology

Only 34.4% of all samples contained < 10^5 colony forming units (CFU) total aerobic bacteria CFU/ml (Figure 1).

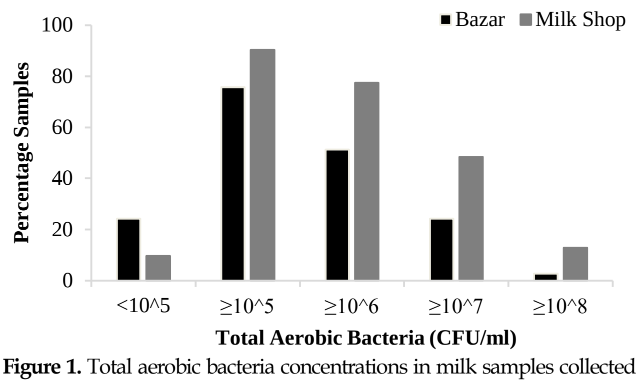

Figure 1. Total aerobic bacteria concentrations in milk samples collected from bazars and milk shops throughout Herat city, Afghanistan. There were no statistical differences between the two sampling groups

There was a tendency for a higher number of samples collected from bazars (25.0%; P = 0.092) to have < 10^5 CFU/ml of total aerobic bacteria compared to samples collected from milk shops (9.4%). There were no significant differences in the number of samples with anaerobic bacteria plate counts ≥ 10^5, ≥ 10^6, ≥ 10^7, or ≥ 10^8 between the two groups. Similarly, only 15.8% of all samples contained < 100 CFU/ml coliforms (Figure 2). There were no differences in the number of samples containing ≥ 100, ≥ 10^3, ≥ 10^4, or ≥ 10^5 CFU/ml coliforms between the two groups.

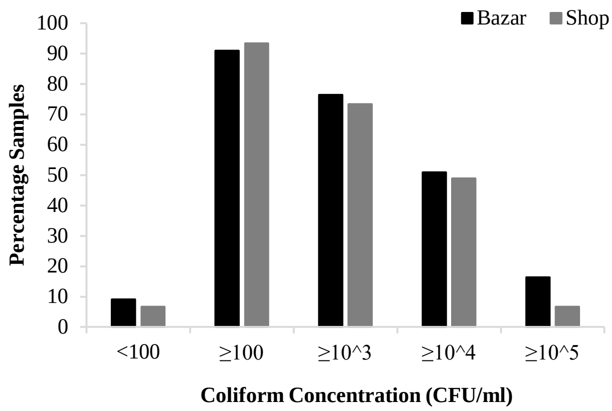

Figure 2. Coliform concentrations in milk samples collected from milk shops and bazars throughout Herat city. There were no significant differences between the sampling groups

3.3. Composition

The composition (fat, protein, lactose, solids-non-fat [SNF], and salt percentages) of each milk sample was estimated by ultrasound (Table 2). Overall, milks samples contained an estimated 3.25% fat, 8.45% SNF, 3.33% protein, 4.76% lactose, and 0.57% salts (Table 2). The fat percentage of milk samples collected from bazars (3.0%) was significantly lower than milk samples collected from milk shops (3.5%; P < 0.050). Similarly, the SNF percentage of milk samples collected in bazars (8.10%) was significantly lower than milk samples collected from milk shops (8.80%; P < 0.050). While numerically lower in each case, there were no significant differences in protein, lactose, or salt percentages between milk samples collected from bazars (3.29%, 4.70%, and 0.56%, respectively) and milk samples collected from milk shops (3.38%, 4.82%, and 0.58%, respectively).

Table 2. Composition of milk samples collected from bazars and milk shops in Herat city, Afghanistan

Source	Fat (%)	SNF (%)	Protein (%)	Lactose (%)	Salts (%)
Overall (n = 100)	3.25	8.45	3.33	4.76	0.57
Bazars (n = 55)	3.0[b]	8.10[b]	3.29	4.70	0.56
Milk Shops (n = 45)	3.5[a]	8.80[a]	3.38	4.82	0.58

Numbers with different superscripts are significantly different at P < 0.050; comparisons are between sampling groups (bazars vs. milk shops) and within columns. SNF: Solids-non-fat

Table 3. Percentage of milk samples with irregularities (fat or water) or adulterants (starch or antibiotic residues)

Source	Fat	Water	Starch	Antibiotic residues		
				Beta-lactams	Tetracyclines	Sulfonamides
Bazaar	34.5^a	32.7^a	25.5^a	4.0^b	2.0	0.0
Milk shop	11.1^b	15.6^b	2.2^b	19.0^a	4.8	0.0

Numbers with different superscripts are significantly different at P < 0.050. Comparisons are within column

3.4. Irregularities and adulterants

Composition was measured by ultrasound. Ultrasound is generally regarded as less accurate than infrared estimation. As such, for the purposes of this study, we considered fat concentrations < 2.5% as measured by ultrasound as irregular. Using this threshold, 24.0% of all samples contained fat irregularities overall (Table 3). Milk samples collected from bazars were statistically more likely to contain fat irregularities (34.5%; P < 0.050) than samples collected from milk shops (11.1%).

Antibiotic residues were detected in 14.1% of total samples (Table 3). Antibiotics belonging to the beta-lactam family were the most frequently detected antibiotics in both sample groups (12.8%) with samples collected from milk shops statistically more likely to contain beta-lactam antibiotic residues (21.0%; P < 0.050) compared to samples collected from bazars (5.2%). Tetracycline and sulfonamide drugs were only detected in 2.0% of all samples, and there were no differences in the frequency of tetracycline or sulfonamide detection between milk samples collected from bazars versus those collected from shops.

About 25% of all milk samples contained water irregularities (Table 3). Milk samples collected from bazars were statistically more likely (32.7%; P < 0.050) to contain water irregularities compared to samples collected from milk shops (15.6%). In all but one case, water irregularities were the result of abnormally high water percentages as first detected by ultrasound (water % and freezing point) and confirmed by lactometer (specific density < 1.027). The outlier was a milk sample purchased from a bazaar where the water percentage could not be measured by ultrasound (unreadable outside of range) but had a specific density of 1.052 when measured by lactometer. Similarly, milk samples collected from bazars were statistically more likely (25.5%; P < 0.050) to contain starch compared to samples collected from milk shops (2.2%). In contrast, calcium carbonate was not detected in any of the milk samples regardless of where the samples were collected.

Across all parameters measured, 46% of all samples contained at least one irregularity (water, fat, starch, or antibiotic residues - Figure 3). Milk samples collected from bazars, however, were statistically more likely to contain at least one irregularity (58.2%; P < 0.050) compared to milk samples collected from milk shops (31.1%). There were no differences in the number of samples containing two or more irregularities (24.0% of all samples) between milk collected from bazars (29.1%) compared with milk collected from milk shops (17.8%).

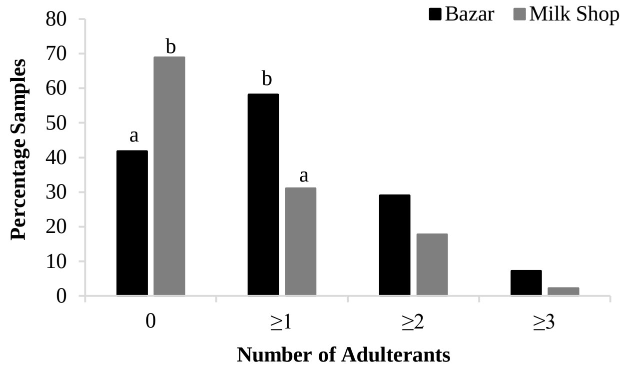

Figure 3. Percentage of milk samples with 0, ≥ 1, ≥ 2, or ≥ 3 irregularities or adulterants. Numbers with different subscripts are significantly different at P < 0.050. Comparisons are within groups (e.g., "0 adulterants")

4. Discussion

Throughout the world, milk quality is assessed based on a core set of parameters: While recent studies have examined Brucellosis and Coxiellosis prevalence in ruminant populations in Herat Province (15), published reports on milk quality in Afghanistan are scarce. To our knowledge, this is the first published assessment on the overall quality of milk available through bazars and milks shops in Afghanistan using a core set of universal milk quality parameters: composition (fat, protein, and carbohydrate percentages), total bacteria and coliform bacteria concentrations and presence of various adulterants. It should be noted, however, that we did not measure somatic cell counts due to limited resources and equipment. While the California Mastitis Test was available, it is not recommended for pooled milk samples such as we tested here.

The production of high-quality value-added dairy products is directly dependent on the quality of raw fluid milk. High initial bacterial concentrations rapidly spoil and limit the shelf life of fluid milk. As spoilage organisms replicate, off colors or flavors can be produced. Overproduction of acid denatures proteins reducing the milk's capacity to withstand different processes such as heating and fermentation (16). Only 34.4% of all samples evaluated here, however, contained < 10^5 CFU/ml, a common international standard for acceptable total bacteria in raw milk (17). Likewise, the majority of both milk samples collected from bazars (90.1%) and milk samples collected from milk shops (93.3%) contained > 100 CFU/mL coliforms. While comparisons of these levels to levels found in different areas of Afghanistan is not possible, studies in neighboring Pakistan, which has similar milk markets, have produced similar results. Younus et al. (10) examined the microbial composition of dahi (yogurt from undefined cultures) available to consumers and found total average bacterial concentrations of 7.34×10^7 CFU/ml, and total coliform concentrations of 4.34×10^3 CFU/ml. Likewise, Soomro et al. (11) identified *Escherichia coli* in over half of milk samples collected from milk shops and homes in urban Pakistan. In each case, high levels of bacteria and coliform bacteria in particular, of great concern for milk safety as many foodborne illnesses and outbreaks are associated with contaminated milk and dairy products (18). These issues could be remedied by addressing collection and sanitation practices from farm to the consumer.

Perhaps more problematic for both milk processors and consumers was the high percentage of milk samples that contained one or more irregularity or adulteration. Adulteration, either intentional or unintentional, can also significantly reduce fluid milk quality, decreasing the ability to add value to milk by interfering with processes that rely on standard concentrations of fat or protein for consistency (19). Similarly, the presence of antibiotics, active antibiotic metabolites, or preservatives can limit or interfere with bacterial growth during fermentation (20). Again, while comparable studies in Afghanistan are not available, adulteration of raw milk is not uncommon in neighboring countries (12).

For the purpose of this study, we considered the following as irregularities: fat percentages lower than 2.5% as tested by ultrasound, abnormal water percentage as measured by ultrasound and confirmed by lactometer, presence of starch, presence of sodium bicarbonate, or presence of antibiotic residues. While nearly half of all samples contained irregularities, milk collected from bazars was significantly more likely to contain one or more irregularities compared to milk collected from milk shops. When antibiotic residues were not considered, however, far fewer milk shop samples contained irregularities. The presence of antibiotic residues is most often a management error at the farm level and any future extension programs focused on improving milk quality should include the proper use of the drugs and proper withdrawal times.

Milks samples from bazars were also more likely (32.7%) to contain water irregularities compared to milk samples from milk shops (15.6%). 86% of the samples from bazars with water irregularities also contained fat irregularities. Thus, while it may be that water was added somewhere along the value chain to increase volume, an alternative explanation may be that some milk samples were actually reconstituted powder milk or, more likely, a mixture of raw and reconstituted powder milk. Powder milk is readily available and often less expensive than raw fluid milk (personal observations). If powder milk was not reconstituted with the proper volume of water, the resulting milk product would have higher than normal water percentages and lower than normal fat percentages. The irregularities would be exacerbated if skim milk powder was used and improperly reconstituted.

Surprisingly, over 25% of samples collected reacted to iodine. Reaction to iodine in milk is most often caused by the addition of starch. Starch is a polysaccharide produced by plants, and the amylose portion reacts with iodine to generate a colorimetric reaction (dark blue to black). Starch is not a natural component of milk but is regularly detected (13) in milk sold in similar Pakistani bazars. Its addition could be used to mask added water or skimmed fat as it acts to increase total solids. In most cases, the milk samples, we collected changed hands many times before our sampling, so it is not clear where along the value chain this potential adulteration took place.

5. Conclusion

These results will serve as primary data for extension programming aimed at improving milk quality in the region. Our results indicate that a large part of these efforts should focus on milk hygiene and sanitation with high coliform concentrations being addressed specifically. To be effective, these programs will have to utilize a multifaceted farm-to-consumer approach. At the same time, future extension programming must evaluate and introduce appropriate technologies while genuinely considering the realities and constraints faced by milk producers and sellers, such as lack of refrigeration and intermittent power outages. In the meantime, milk shop owners could reduce wasted or lower quality products by avoiding unfamiliar sources for raw milk (e.g., bazars) and working more closely with their established suppliers to better ensure that milk collection and preparation is done to appropriate and necessary sanitation standards.

Conflict of Interests

Authors have no conflict of interest.

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