www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

A survey of microbial total count and prevalence of *Escherichia coli* in raw milk in Markazi Province, Iran

Mohammad Rezaei[1], Fereshteh Karimi[1], Mohammad Yahyaei*[2], Hossein Javdani[3], Arman Shahabi[3] and Ali Farahi[3]

[1]Department of Food Safety and Hygiene, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of New Sciences and Technologies, Life Science Engineering Group, University of Tehran; [3]Department of Veterinary, School of Veterinary, Shahid Bahonar University of Kerman, Kerman, Iran

Abstract

Milk is very susceptible to growth and development of many microorganisms because it is rich in nutrients and has suitable condition for the growth of microbes. The objective of the present investigation was to determine the total bacterial count and prevalence of *Escherichia coli* in raw milk from milk collection centers in Markazi Province. A total 248 milk samples were collected from 31 centers in November 2012 and were analyzed according to standard methods to find the prevalence of *E. coli*. None of the raw milk samples under investigation, had superior or grade 1 quality. Only 9.09% of the samples were classified with grade 3 quality and 90.9% were non-standard. The mean of microbial total count in logarithm base were 6.8±.44 and average prevalence of *E. coli* in the samples was 29.14%. The cities of Arak and Shazand have the lowest and the highest microbial total count, respectively. In general, the result of this study showed unsatisfactory conditions of the milk in milk collection centers of the Markazi province.

Keywords: Raw milk; food safety; *Escherichia coli*; microbial total count

To cite this article: Rezaei M, F Karimi, M Yahyaei, H Javdani, A Shahabi and A Farahi, 2013. The survey of microbial total count and prevalence of *Escherichia coli* in raw milk in Markazi Province, Iran. Res. Opin. Anim. Vet. Sci., 3(12), 474-477.

Introduction

Milk is part of human food and rich in calcium, phosphorus, vitamins and proteins. The surveys by FAO and WHO showed that animal source protein must constitute 50% of daily protein intake of man (Yarahmadi et al., 2006). The quality of the milk used by one is one of the main factors under consideration. Quality of raw milk is a function of nutrition and health of the animal, chemical combination, and its microbial activities. Thus, the time before delivery to the consumer and condition of keeping the product are two dominant factors of the quality (Yarahmadi et al., 2008). The most commonly accepted method for raw milk quality analysis is determining total microbial load in the milk (Kamal zade et al., 2010). Necessity of obtaining total number of bacteria not only lies in evaluation of the quality but in spotting harmful and disease causing bacteria. Among many diseases causing bacteria, coliforms are important. The presence of some coliforms such as *E. coli* indicates contamination with excreta (Aleksieva and Krushev, 1981). Dairy factories usually receive the milk directly from the producer or from mobile and fixed milk collection centers. This process may contribute to increase in the microbial load (Imami meybodi et al., 2010).

Markazi Province with milk production of 413.32 thousands kilograms is a dominant player in national milk industry (Imami meybodi et al., 2010). Since village-based producers deliver their milk to milk gathering centers before it is sent to the factories, a survey of bacteriology quality of milk at collection centers seems to be essential to find the major contamination sites in the province. Further, the aim of

***Corresponding author:** Mohammad Yahyaei, Department of New Sciences and Technologies, Life Science Engineering Group, University of Tehran. M.yahyaei@ut.ac.ir

this study was to find total microbial load and prevalence of *E. coli* contamination in the milk at the milk collection centers in Markazi province of Iran.

Materials and Methods

Milk sampling was conducted at the licensed stations with health supervisor at the site in the province (Table 1). Eight samples were collected from each station in November 2012 (two samples per week). A total of 248 samples were collected in sterile condition and transferred to microbiology laboratory under cold chain process. Samples at different concentrations were prepared. Total microorganism counting and *Escherichia coli* number determining tests were conducted on every sample in compliance with Iran National Standard (ISIRI, 2008).

Total bacterial counts

Total bacterial counts (TBCs) were determined using plate count agar (PCA) with 1 g/L skim milk powder and incubated at 30°C for 72h (ISIRI, 2000).

Coliform bacteria (*E. coli*)

The coliform test was done by plating one ml sample onto MacConkey agar media. The plates were incubated at 37ºC for 48 h and the counts were presented as colony forming unites per gram (cfu/g). Plates showing positive coliform were subjected to the confirmatory test using Brilliant green bile lactose broth in test tubes with inverted Durham tubes and incubated at 44ºC for 48 h. Each positive tube was sub-cultured into E.C. broth medium and then incubated at 44.5ºC for 24 h. Tubes showing gas productions were considered *E. coli* positive. All the samples positive for *E. coli* contamination were confirmed using Gram's staining, cultural and biochemical tests. For the isolation and identification of *E. coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37ºC for 24 h. Morphologically, typical colonies (at least 4/plate) producing metallic sheen were taken into nutrient broth for further identification. Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, Urease production, Simmon's citrate agar and various sugar fermentation tests.

Results and Discussion

Average total microbial load and number of samples with *E. coli* at different cities is given in Table 2. In this regard, Arak and Shazand cities were at top and bottom of the list (Khosravi and Ghaznavi, 2008; Imami meybodi et al., 2010). However, regarding determination of *E. coli* in the samples, Arak (19%) and Khondab (41%) recorded the lowest and highest percentage of contaminated samples. About 90.09% of the samples had higher contamination than the standard microbial load at milk collection sites (Table 3).

Surveys of quality of raw milk at milk collection centers is very important step in improving quality of raw milk delivered to dairy factories. In lorestan province average logarithm of microbial load at milk collection centers with different capacity was reported to be 6.43 in 2003 and quantity for *E. coli* was 3.13 % (Yarahmadi et al., 2008). In comparison with this study, the raw milk at the collection centers of the Lorestan province had lower microbial load. Average logarithm of microbial load of raw milk from traditional farm at collection centers and in pool at factory site were 6.9 and 6.8%, respectively, in Kashmar city (Khosravi and Ghaznavi, 2008). In a study over 54 raw milk samples from collection centers in Khozestan Province, Kamal zade (2010) reported that none of the samples passed the standard criteria. Similar results were obtained in the present study. Moreover, average logarithm of microbial load in milk collection centers was 7.1 cfu/gm which suggested better quality of milk at collection centers of Markazi Province. Average logarithm of microbial load from collection centers in Fars Province was 6.9 which was almost equal to the results obtained in this study (Hashemi and Shekarforoush, 2008). The count for Yazd Province was 15.96 CFU/gm which was too high compared with our results (Imami meybodi et al., 2010). Ebrahimi (2011) reported 8.5 as microbial load in milk collection centers in Mahabad city (Ebrahimi, 2011). In another study in 2560 records from 1999 to 2001 provided by Pegah Dairy Laboratory in Lorestan Province, an average microbial load of 6.8 CFU/ml was reported (Yarahmadi et al., 2006). In India, the microbial load has been reported in the range of 2.2 to 2.6 (Desai and Natarajan, 1981), which is lower than our results. Holm et al. (2004) studied microbial level in 75 raw milk samples and obtained an average logarithm microbial load of 2.4 CFU/ml In Chili, the survey of microbial load of raw milk in 42 gathering sites was 3.8 (Van schaik et al., 2005). The load was reported 2.1 in Ethiopia (Godefay and Molla, 2000). Majority of the works reported traces of *E. coli* contamination. Ombui et al. (1994) reported that about 50% of the samples collected from milk collection centers in Kenya were contaminated with about 2.7 coliform per ml.

Table 1: Number of station and number of samples from each station

City	Shazand	Saveh	Tafresh	Mahalat	Komijan	Khondab	Khomain	Farahan	Delijan	Ashtian	Arak
Station	4	2	1	2	2	4	6	5	1	1	3
Sample	32	16	8	16	16	32	48	40	8	8	24

Table 2: Average logarithmic microbial load and prevalence of *Escherichia Coli* in each city

City	*Escherichia coli* (%)	Average logarithmic microbial load (CFU/ml)
Arak	19	6
Ashtian	24	6.6
Delijan	26	6.5
Farahan	39	7.4
Khomain	38	6.9
Khondab	41	7.3
Komijan	23	6.6
Mahalat	36	6.9
Tafresh	32	6.6
Saveh	25	6.8
Shazand	27	7.5
Average	29.14	6.8

Table 3: Microbial load in milk samples collected from eleven Markazi Province, Iran

Percent	Milk ranking	Number of cities	CFU/ml
0	Excellent	0	<30000
0	First-rate	0	30000 to 100000
0	Second-rate	0	100000 to 500000
9.9	Third-rate	1	500000 to 1000000
90.9	Non-standard	10	>1000000

microbial load of 6.8 CFU/ml was reported (Yarahmadi et al., 2006). In India, the microbial load has been reported in the range of 2.2 to 2.6 (Desai and Natarajan, 1981), which is lower than our results. Holm et al. (2004) studied microbial level in 75 raw milk samples and obtained an average logarithm microbial load of 2.4 CFU/ml In Chili, the survey of microbial load of raw milk in 42 gathering sites was 3.8 (Van schaik et al., 2005). The load was reported 2.1 in Ethiopia (Godefay and Molla, 2000). Majority of the works reported traces of *E. coli* contamination. Ombui et al. (1994) reported that about 50% of the samples collected from milk collection centers in Kenya were contaminated with about 2.7 coliform per ml.

Part of the differences between different reports (microbial load and *Escherichia coli* contamination) and the results here might be due to different specification of collection centers, while the differences in production process or delivery time may also affect the results. Contamination of very high number of milk samples could be due to unhygienic farms, failing to wash the udders before milking, no mastitis checks, unhygienic milking pots and milk containers or tanks, lengthy delivery time, and poor staff's hygiene. There are plenty of studies emphasizing significant effects of these factors on quality of milk (Van Schaik et al., 2005; Arab ameri et al., 2008; Khosravi and Ghaznavi, 2008; Kamal zade et al., 2010; Imami meybodi et al., 2010). In addition, the season of milking and age of the animal play a role in the difference of results (Yarahmadi et al., 2006; Yarahmadi et al., 2008; Khosravi and Ghaznavi, 2008; Imami meybodi et al., 2010).

Conclusion

In general, the results showed an undesirable condition at milk collection sites in Markazi Province. Therefore, is it essential to initiate infrastructural measures to improve animal hygiene, train farmers, their families, and staff of handling milk at collection sites.

References

Aleksieva, V. and Krushev, B. 1981. [Quality of raw cow's milk]. *Veterinarno-meditsinski nauki*, 18: 65.

Arab ameri, M., Zolfaghari, M., Nazarian, A., Norian, A. and Jalali, A. 2008. Study of bacterial contamination of raw and pasteurized milk in the shahrod city. The twelfth National Conference of Environmental Healt.Tehran,Shahid Beheshti University of Medical Sciences.

Desai, P. and Natarajan, A. 1981. Bacteriological Quality of Raw Milk Collected From Societies for Transporta- tion to Chilling Centers. *Cheiron*, 10: 146-150.

Ebrahimi, M. 2011. Evaluate the microbial quality of raw milk in dairy cattle units and milk collection centers in Mahabad city. *The first National Congress of dairy and related industries. Qom*,137.

Godefay, B. and Molla, B. 2000. Bacteriological quality of raw cow's milk from four dairy farms and a milk collection centre in and around Addis Ababa. *Berliner und Münchener Tierärztliche Wochenschrift*, 113: 276-278.

Hashemi, M. and Shekarforosh, S.S. 2008. Microbial and chemical changes of raw milk at various stages of production in industrial dairy farms of Fars province during various seasons. *Journal of Faculty of Veterinary Medicine University of Tehran*: 63 221-227.

Holm, C., Mathiasen, T. and Jespersen, L. 2004. A flow cytometric technique for quantification and differentiation of bacteria in bulk tank milk. *Journal of applied microbiology*, 97: 935-941.

Imami meybodi, M.A., Mahdavi adali, H.R., Shokohmand, M., Karimi, A. and Mophidi, M.R. 2010. The survey of microbial load and raw milk additives in Yazd province. *The fourth Congress on animal science. Iran.*

ISIRI 2000. Milk and milk products - Enumeration of colony-forming units of micro-organisms - Colony-count technique at 30 degrees C. Institute of Standards & Industrial Research of Iran, 5484.

ISIRI 2008. Microbiology of milk and its products. *Institute of Standards and Industrial Research of Iran*, 2406.

Kamal zade, L., Kamal zade, A. and Heydar nejad, A. 2010. Total bacterial, coliforms and the *staphylococcus aureus* bacteria count of raw milk (from farms to the processing factory), and pasteurized milk in khozestan province. *The fourth Congress on Animal Science, Iran.*

Khosravi, M. and Ghaznavi, A. 2008. The survey of microbial load and PH traditional animal husbandry milk collected from kashmar city in different seasons. *Journal of Modern Agriculture,* 4: 34-7.

Ombui, J.N., Kaburia, H.F., Macharia, J.K. and Nduhiu, G. 1994. Coliform counts and *Escherichia coli* in raw commercial milk from dairy farmers in Kiambu District, Kenya. *East African Medical Journal,* 71: 635-639.

Van Schaik, G., Green, L., Guzman, D., Esparza, H. and Tadich, N. 2005. Risk factors for bulk milk somatic cell counts and total bacterial counts in small holder dairy farms in the 10th region of Chile. *Preventive Veterinary Medicine,* 67: 1-17.

Yarahmadi, B., Eslami, M., Mahdavi, H.R. and Moayedi nejad, A. 2006. The survey of bacteriological quality of raw milk and seasonal affecting factors in lorestan province. Pajouhesh and sazandag, 18: 28-33.

Yarahmadi, B., Mahdavi, H.R. and Moayedi nejad, A. 2008. Study of total count, *E. coli* and coliforms contamination of raw milk from letdown stage till delivery to factory in Lorestan province. *Yafteh,* 10: 67-78.