In the name of God

Shiraz E-Medical Journal
Vol. 12, No. 1, January 2011

http://semj.sums.ac.ir/vol12/jan2011/88047.htm

The Spatial Distribution of Bacteria Pathogens in Raw Milk Consumption on Malayer City, Iran.

Pourhassan M*, Taravat Najafabadi AR**

* Institute of Human Nutrition and Food Science, Christian-Albrechts-University, Kiel, Germany, ** Earth Observation Lab, Department of Computer Science, Systems and Production, University of Tor Vergata, Rome, Italy.

Correspondence: Alireza Taravat, Earth Observation Lab, Dept of Computer Science, Systems and Production, University of Tor Vergata, Telephone: +39(6) 3899350787, Fax: +39(6) 3899350787, Email: art23130@gmail.com

Received for Publication: January 10, 2010, Accepted for Publication: December 4, 2010.

Abstract:

Milk has an outstanding nutritional quality but is also an excellent medium for bacterial growth and an important source of bacterial infection when consumed without pasteurization. The present paper reports the results of a cross-sectional survey aimed at obtaining information on the agents of milk-borne bacterial infections and the prevalence rates in raw milk, and the spatial distribution of the prevalence of foodborne pathogens in Raw Milk in Malayer city in Iran. Different geographical information system tools were used to plan the sampling procedures, to display the results as maps and to detect spatial clusters of bacteria in raw milk in the study area. A total of 100 raw milk samples collected in May 2009 from the cow sheds and milk centers were cultured and the isolated organisms identified by standard bacteriological methods.

Overall, the organisms identified and their prevalence rates were Escherichia Coli, (75%), Staphylococcus aureus, (52%), Enterobacter, (42%), Klebsiella, (36%), Pseudomonas pyocyaneus, (25%), Proteus, (4%).The result has shown the spatial distribution and the isolation of six potential and opportunistic pathogens from the two different sources of collected raw milk samples in Malayer City. Milk suggested contamination from various sources, which may include animal, human, environment, and utensils in their presence.

Keyword: raw milk, pathogens microorganisms, geographical information systems (GIS)

Introduction:

Although milk and dairy products are important components of a healthy diet, if consumed unpasteurized, they also can present a health hazard due to possible contamination with pathogenic bacteria. Milk and other dairy products, primarily from cows but also less frequently from goats and sheep, are important components of diet. The US Department of Agriculture recommends that people consume 2–3 servings of dairy products daily. Inclusion of these products in the diet aids in the prevention of certain diseases, such as obesity, hypertension, and diabetes, and they are a source of calcium (important for growing bones and the prevention of osteoporosis).[1] In addition, dairy products also provide dietary sources of protein, vitamins, and other minerals.[2] But raw milk has been known as vehicle for pathogens for more than 100 years.[3-5]

There are 2 primary factors that contribute to the microbiological quality of milk: the inclusion of organisms in excreted milk (pre harvest) and the contamination of milk at the time of collection, processing, distribution, and storage (post harvest).[6]Therefore, milk is susceptible to contamination by many pathogenic microorganisms, which result in infection and threat to consumer's health. Microorganisms in milk are capable of rapid multiplication when exposed to high ambient temperatures and milk-borne infections and intoxications in consumers are well documented in the literature. A number of bacteria including Staphylococcus aureus, Escherichia coli and Salmonella have been recovered from raw milk [7] and some of these have been determined to be pathogenic and toxigenic, and implicated in milk-borne gastroenteritis.[8] Studies have reported that the most prevalent consumers of raw milk are dairy farm families and dairy farm employees.[9] Among the nonfarming population, a growing number of consumers are claiming that raw milk is healthier and are choosing raw milk over pasteurized milk.[3]

Several approaches have been used to minimize the possibility that milk contaminated with pathogenic organisms will reach the consumer. These include enhanced animal health, improved milking hygiene, and pasteurization.[10]

The present paper reports the results of a cross-sectional survey aimed at obtaining information on:

1) The agents of milk-borne bacterial infections and the prevalence rates in raw milk, and2) The spatial distribution of the prevalence of foodborne pathogens in Raw Milk in Malayer city in Iran .Different geographical information system (GIS) tools were used to plan the sampling procedures, to display the results as maps and to detect spatial clusters of bacteria in raw milk in the study area.

Materials and Methods:

Source and collection of milk samples:

This is a cross-sectional study was carried out in May 2009 in Malayer City, located in the Hamadan's provinces. This area is mainly flat, though in small parts both hilly and mountainous, and extends from 1709 to 2015 m above sea level. A total of 100 raw milk samples from 6 cowsheds (60 samples) and 40 milk cen-

ters were included into study. Malayer City contains 17 blocks, amongst these blocks, 6 blocks were chosen at purposely because only these blocks have cow-sheds, and from each block 1 cow-shed randomly were selected. The samples were collected in sterile containers after thorough mixing of the milk under aseptic conditions, immediately kept in cooler boxes, at approximately 2 °C and transported to the laboratory.

Laboratory Analysis:

For isolation of Mycobacterium, the milk samples were centrifuged at 3000 pm for 15 minutes and appropriate sediment were placed on Blood Agar (BA) and Eosin Methylene Blue (EMB) media and incubated at 37°C for 24 hours. After incubation, about 100µl of the inoculated plates were sub-cultured onto plates of TSI (Triple Sugar Iron), SIM (Sulfur reduction, Indole production, Motility) and Simmons' Citrate Agar, and further incubated at 37°C for 24 hours. Bacterial growth produced on the various culture media were stained by Gram staining and examined microscopically.

Statistical analysis:

Frequency distribution was analyzed by chi-squared test (e.g. distribution of positive bacteriological findings in raw milk). All statistical analyses were performed with the SPSS 16 statistics package (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered significant.

GIS data layers:

A GIS was constructed utilizing as data-layers the digital aerial photographs (at 1.0-m resolution; source: Cartographic Office of the Hamedan region) of the study area. Before conducting the raw milk sample survey, all the milk centers and cow-sheds located in the study area were georeferenced on aerial photographs within the collected points manually with a global positioning system (GPS). All GIS databases were developed using Arc-GIS 9.2 GIS software (ESRI, USA).[11]

Results:

Some potential pathogenic bacteria were isolated from raw milk samples in Malayer City. The isolates were as follows: E.coli, (75%), Staphylococcus aureus, (52%), Enterobacter, (42%), Klebsiella, (36%), Pseudomonas pyocyaneus, (25%), Proteus, (4%).

The overall prevalence rates of the organisms were 4-75%. The prevalence rates of the various bacteria identified in milk for cow-sheds and milk centers were 6-83.3-% and 12.5-62.5% respectively. The most prevalent organism overall was E.coli while the least prevalent was Proteus (table1).

The Escherichia Coli bacteria were detected more frequently in cow-sheds (50/60, 83.3%) than in milk centers (25/40, 62.5%, $p<0.018$). The same was true, when Enterobacter and Klebsiella were evaluated (34/60, 56% vs. 8/40, 20%, $p < 0.0001$) and (31/60, 51% vs. 5/40, 12.5%, $p < 0.0001$) respectively. The spatial distribution of each bacteria at the milk centers in the study area shows in the point distribution maps (Figs 1-3).

The results of the spatial correlations between Escherichia Coli in 20 milk centers and 2cow-sheds in south of Malayer City (Fig 4) showed one cluster of positive

Escherichia Coli (17/20, 85% vs. 11/20, 55%P< 0.038) (table 2). No clusters of positive bacteria were found in any of the other location in MC (P >0.05).

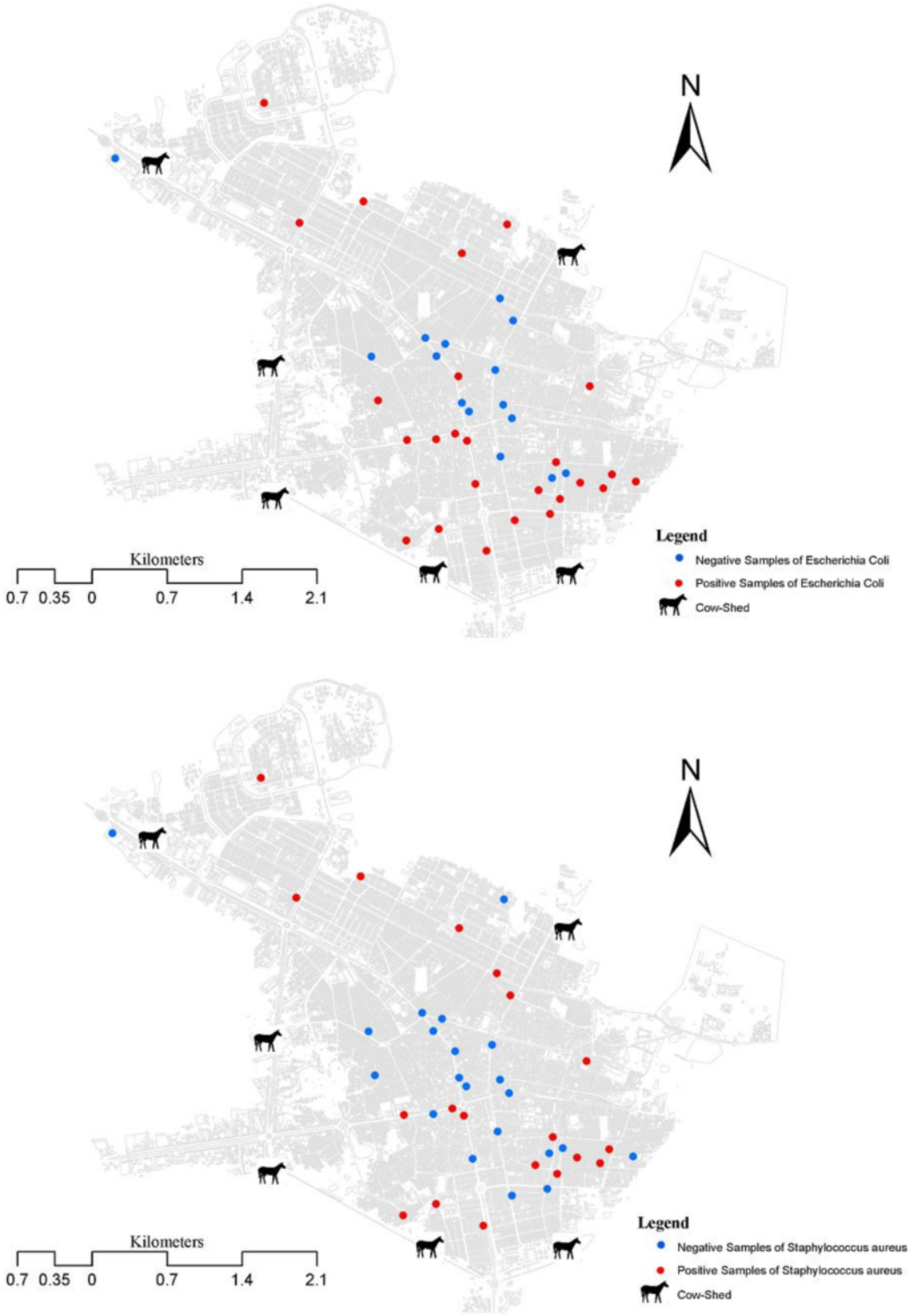
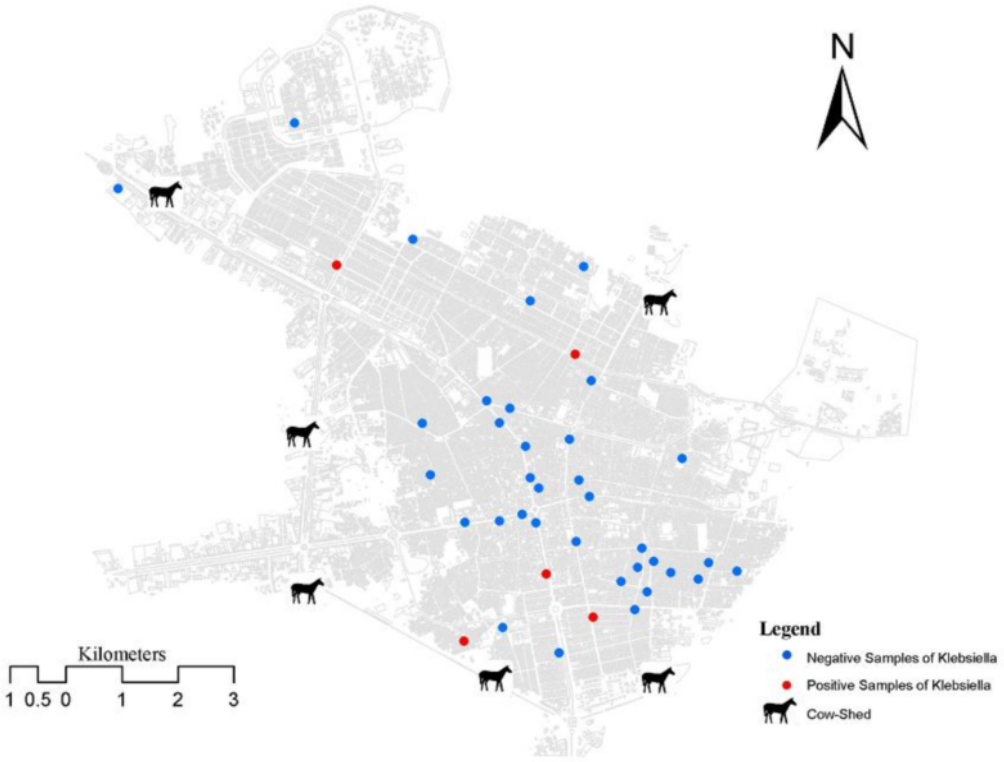


Figure 1

Figure 2



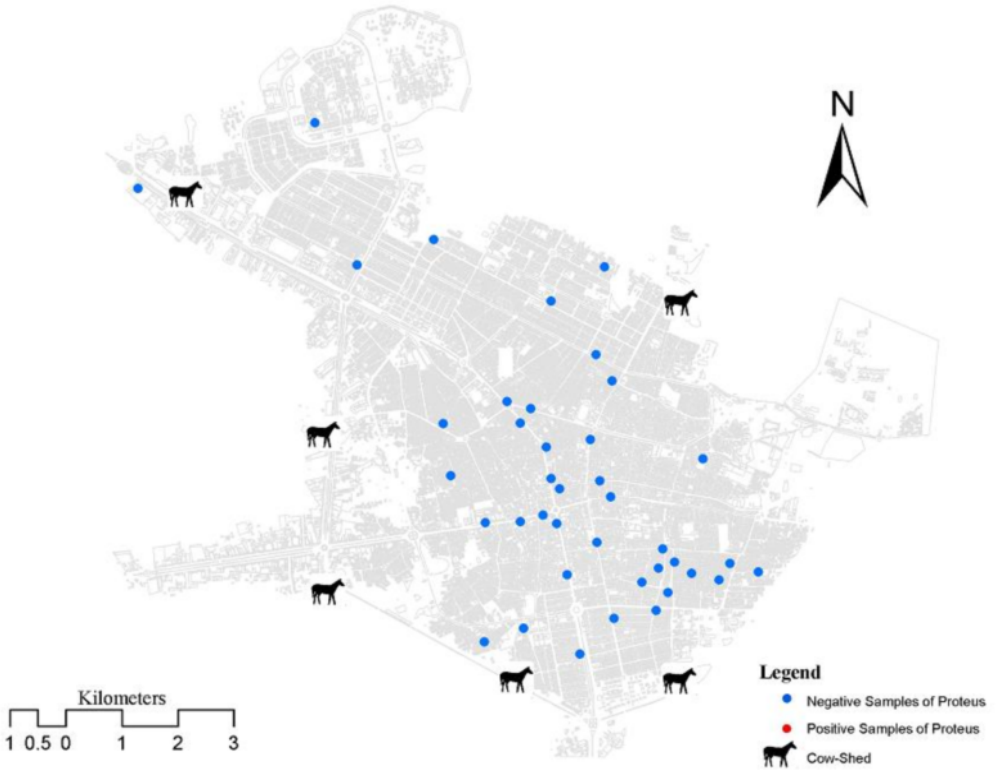


Figure 3

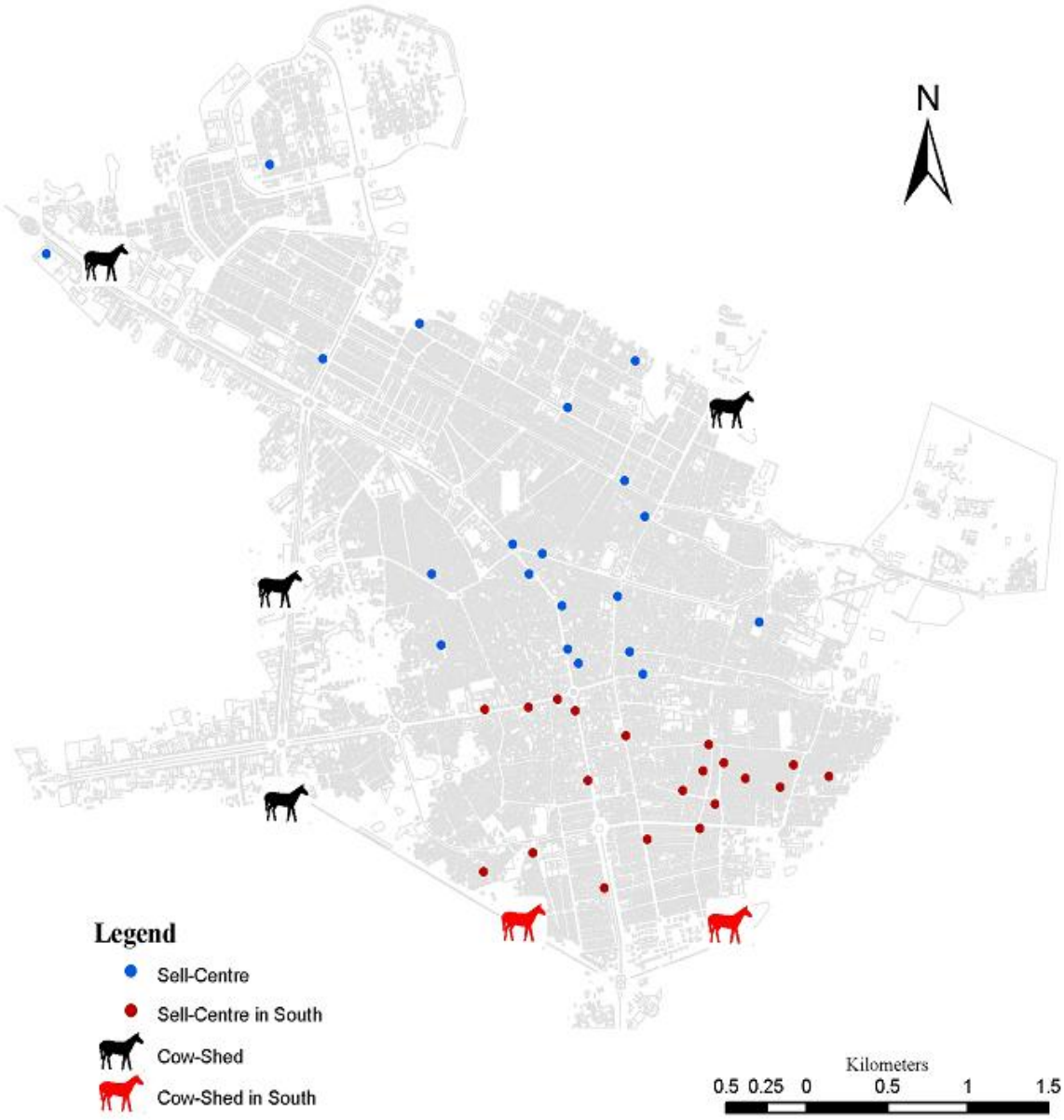


Figure 4

Table 1. bacteria identified in 100 row samples from cow- sheds and milk – centers at MC , Iran .

Bacteria	Cow- shed		Sell Centre		Total	
	N	%	N	%	N	%
Escherichia Coli	50	83.3	25	62.5	75	75
Staphylococcus aureus	33	55	19	47.5	52	52
Enterobacter	34	56	8	20	42	42
Klebsiella	31	51	5	12.5	36	36
Pseudomonas pyocyaneus	17	28	8	20	25	25
Proteus	4	6	0	0	4	4

Sample sizes from Cow- sheds and Milk – centers where 60 and 40 respectively

Table 2 .bacteria identified in raw milk samples from two cow- sheds (20 samples (and 20 milk – centers (20 samples)at south of Malayer City, Iran

Bacteria	Cow- shed			Sell Centre			Total	
	N	*%	%**	N	*%	%**	N	%
Escherichia Coli	11	55	22	17	85	68	28	70
Staphylococcus aureus	10	50	30.3	12	60	63.15	22	55
Enterobacter	7	35	20.6	3	15	37.5	10	25
Klebsiella	14	70	45.2	3	15	60	17	42.5
Pseudomonas pyocyaneus	5	25	29.4	6	30	75	11	27.5
Proteus	2	10	50	0	0	0	2	5

The percentage of each bacteria in south MC

**The percentage of each bacteria in south region according to all positive samples in whole in whole study area

Discussion:

The present study has shown the spatial distribution and the isolation of six potential and opportunistic pathogens from the two different sources of collected raw milk samples in Malayer City. Their presence in milk suggested contamination from various sources, which may include animal, human, environment, utensils and others.[8],[6] Several animal pathogens can cause human disease and they are well known to be transmitted to human by consumption of raw milk.[12] The high numbers of the isolated microorganisms in milk during the present study indicated that those microorganisms not only contaminate milk but also multiply and grow on it. This might be due to the fact that the milk is a good nutritive medium for the growth of the microorganisms, especially with poor sanitary procedures [8] and the lack of the cooling facilities.[13] Moreover, the tropical hot climate aggravates the conditions.[14]

The members of E. coli bacteria constituted 75% of the isolates; Staphylococcus aureus, Enterobacter, Klebsiella and Pseudomonas pyocyaneus accounted for 52%, 42%, 36 % and 25% of the isolates, respectively. The incidence of isolated bacteria was found to be higher in the cow-sheds compared with milk centers. This may indicate extra sources of contamination like milk utensils, equipment.[8]

The higher count of E. coil followed by S.aureus in both sources might refer to improper public health measurement and sanitary and poor cleaning of people concerned with milk marketing in addition to the primitive system of transportation and marketing practiced in Malayer City. Staphylococcus aureus may originate from mastitic animals [8] or human sources.[15] Moreover, S. aureus was found to be involved in food poisoning.[8] In the present study, the use of GIS tools were instrumental in order to plan the sampling procedures, to show the results with point maps and to detect clusters of distribution of each bacteria detected.

The results of the spatial correlations between Escherichia Coli in milk centers and cow-sheds in south region showed the members of Escherichia coli bacteria constituted 68% and 22% of the total isolates detected respectively (table 2).The higher count of E. coil in south Malayer City might refer to poor cleaning of people concerned with milk marketing and the close distance to industrial area. The high incidence and counts of potential pathogens might create health hazards for consumers and handlers of such milk particularly because the transfer of the resistance via food chain was very well documented. Hence educational and extension programs for consumers, producers and users about the risks of consumption of raw milk are needed in order to evaluate and correct this situation.

References:

1. MCCARRON, D.A., HEANEY R P., Estimated healthcare savings associated with adequate dairy food intake. American journal of hypertension 2004. 17 (1): p. 88-97.

2. Huth, P.J., D.B. DiRienzo, and G.D. Miller, Major scientific advances with dairy foods in nutrition and health. J Dairy Sci, 2006. 89 (4): p. 1207-21.

3. Potter, M.E., et al., Unpasteurized milk. The hazards of a health fetish. JAMA, 1984. 252 (15): p. 2048-52.

4. Gillespie, I.A., et al., Milkborne general outbreaks of infectious intestinal disease, England and Wales, 1992-2000. Epidemiol Infect, 2003. 130 (3): p. 461-8.

5. Jayarao, B.M., et al., A Survey of Foodborne Pathogens in Bulk Tank Milk and Raw Milk Consumption Among Farm Families in Pennsylvania. J. Dairy Sci., 2006. 89 (7): p. 2451-2458.

6. Morgan, F., et al., Survival of Listeria monocytogenes during manufacture, ripening and storage of soft lactic cheese made from raw goat milk. Int J Food Microbiol, 2001. 64 (1-2): p. 217-21.

7. De Buyser, M.L., et al., Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. Int J Food Microbiol, 2001. 67 (1-2): p. 1-17.

8. A.A. Adesiyun, S., Stoute, B. David, Preprocessed bovine milk quality in Trinidad: Prevalence and characteristics of bacterial pathogens and occurrence of antimicrobial residues in milk from collection centres. food control, 2007. 18 (4):
p. 312-320.

9. Goff, H.D. and M.W. Griffiths, Major Advances in Fresh Milk and Milk Products: Fluid Milk Products and Frozen Desserts. J. Dairy Sci., 2006. 89 (4): p. 1163-1173.

10. LeJeune, Jeffrey T. and Päivi J. Rajala Schultz, Food Safety: Unpasteurized Milk: A Continued Public Health Threat. Clinical Infectious Diseases, 2009. 48 (1): p. 93-100.

11. Najafabadi, A.T., Applications of GIS in Health Sciences. Shiraz E-Medical Journal 2009. 10(4): p. 221-230.

12. Adak, G.K., S.M. Long, and S.J. O'Brien, Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. Gut, 2002. 51 (6): p. 832-41.

13. Baumgartner, A., et al., Frequency of Cryptosporidium spp. as cause of human gastrointestinal disease in Switzerland and possible sources of infection. Schweiz Med Wochenschr, 2000. 130 (36): p. 1252-8.

14. Wood, L.V., et al., Incidence of bacterial enteropathogens in foods from Mexico. Appl. Environ. Microbiol., 1983. 46(2): p. 328-332.

15. Chambers, H., Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev., 1997. 10 (4): p. 781-791.