J. Agric. Sci. Mansoura Univ., 28 (9): 6775 - 6784, 2003

HEALTH HAZARDS ASSOCIATED WITH RAW MILK IN EGYPTIAN MARKETS

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ABSTRACT

Bacteriological quality of 160 raw cow's milk collected from 6 different governorates in Egypt was evaluated. Milk samples were obtained from retail markets in winter and summer seasons. Total Bacterial Count, Total Coliform Count, Faecal Coliform Count and *Staphylococcus aureus* count were estimated in the examined samples. 100% of the examined samples failed the legal standards for Total Bacterial count, Total Coliform Count and *Staphylococcus aureus* count which are 10^4, $10^2$and 10^2*cfu*/ml respectively. *Bacillus cereus, Salmonella sp., E.coli O157:H7* and *Listeria monocytogenes* could be detected in the examined samples with ratios: 26.25%, 0%, 5% and 5% in winter season samples and 32.50%, 3.75%, 6.25% and 6.25% in summer season samples respectively. The findings of this study raised the need for educational programs for dairy producers and consumers about the risk associated with the consumption of raw milk.

INTRODUCTION

The public health problems associated with the consumption of raw milk have been well-documented (Djuretic *et al.*, 1997; Little and Louvois, 1999). Pathogenic microorganisms can gain access to milk through many ways such as faecal contamination particularly around the teats, direct excretion from the udder into the milk, from contaminated water used to clean milking system and from bacteria that present in the milking system itself (Thomas and Thomas, 1973; Barmley and McKinnon, 1990; Little and Louvois, 1999).

Of the various measures of raw milk quality, the total bacterial count (TBC) is of particular interest to the dairy farmer and processor (Barmley and McKinnon, 1990; Hayes *et al.*, 2001). The TBC frequently affects the prices that farmers receive for their milk, as many raw milk purchasers establish price incentives for milk with a low TBC. Furthermore, in the United States, milk with a TBC value greater than 100,000 cfu/ml may not be sold as Grade A milk (Pasteurized milk ordinance (PMO) 1995; Hayes *et al.*, 2001). From a different perspective, the TBC serves as a preliminary indicator of herd health, farm sanitation efficacy, and proper milk handling and storage temperatures.

Salmonella sp., *Listeria monocytogenes, Staphylococus aureus, E.coli O157:H7* and *Bacillus cereus* are food poisoning pathogens which could be transferred to Human through consumption of raw milk (Little and Louvois, 1999; Jayarao and Wang, 1999; Jayarao and Henning, 2001).

Gram negative bacteria concerned in raw milk include organisms that are pathogenic to human and animals (*Salmonella* and *E.coli*) and those that lower the quality of milk. The latest group can be classified into coliforms (like *Citrobacter* sp., *Enterobacter* sp. and *Klebsiella* sp.) and non coliforms (like *Aeromaonas, Flavobacterium* and *Moraxella*) which are responsible for

lowering the milk quality and cause defects in milk and its products' processing. (Suhren, 1989; Barmley and McKinnon, 1990).

The source of *Staphylococci* in raw milk may be due to many reasons such as from a clinically ill or apparently healthy cows or due to bad sanitary practices during milk handling as these types of bacteria originate also from ill or apparently healthy handlers (Adesiyun *et al.*, 1998).

The prevalence rate of these pathogens vary considerably among surveys, and could be influenced by several factors such as geographical area, season, farm size, number of animals in farms, hygiene and farm management practices (Roherbach *et al.*, 1992; Jayarao and Henning, 2001).

Consumption of raw or inadequately pasteurized milk has been associated with several outbreaks of enteric infections associated with pathogens like *E.coli* (Martin *et al.*, 1986; Borczyk *et al.*,; 1987; Keene *et al.*, 1997; Jayarao and Henning, 2001) , *Salmonella* sp. (D'Aoust, *et al.*, 1985; Ryan *et al.*, 1987; Spake *et al.*, 1997; Jayarao and Henning, 2001), *Listeria monocytogenes* (Fleming *et al.*, 1985; Linnan *et al.*, 1988; Jayarao and Henning, 2001), *Staphylococci* (Bergdoll, 1979; Adesiyun *et al.*, 1998 ; Jayarao and Henning, 2001) and *Bacillus cereus* which is considered as a well-known organism of food poisoning, can survive milk pasteurization and can produce one or several enterotoxins (Adesiyun *et al.*, 1998).

Roherbach *et al.* (1992) reported that 34.9% of dairy producers in eastern Tennessee and southwest Virginia consumed raw milk. In California, about 3.2% of respondents to a survey on raw milk consumption practices consumed raw milk (Headrick *et al.*, 1997). A questionnaire-based survey conducted by Jayarao and Cassel (1999) showed that nearly 60% of dairy producers in eastern South Dakota and western Minnesota consumed raw milk.

Under the Dairy Products Hygiene Regulations "Pathogenic microorganisms and their toxins shall not be present in quantities such as to affect the consumer health" (Jayarao and Henning, 2001). The Advisory Committee on the Microbiological Safety of Food (ACMSF, 1997) has expressed a concern at the high levels of faecal indicator microorganisms and in some cases pathogenic organisms present in raw milk.

The emergence of multiple antibiotic-resistant strains of *Salmonella* should be of great concern to the public, especially dairy producers, their families, and employees, because this organism is resistant to antibiotics that are commonly used in medical and veterinary practices. An outbreak following the handling of sick calves and consumption of raw milk that contained *Salmonella typhimurium* received national attention (Spake *et al.*, 1997). Clinical and laboratory-based findings have shown that following an episode of severe foodborne illness some individuals tend to develop painful, often debilitating, reactive arthritis caused by sensitization to Salmonella spp. and other gram negative microorganisms (Jayarao and Henning, 2001).

The aim of this study is to investigate the prevalence of some food poisoning bacteria in raw milk collected from different areas' markets in Egypt and to pay attention on the public health hazards associated with its consumption.

MATERIALS AND METHODS

Milk samples: -

One hundred and sixty cow's milk samples (80 each) were collected from retail markets in six Egyptian governorates in winter and summer seasons (Table 1, 3) and examined for their microbial quality and for the presence of food poisoning pathogens including *B.cereus, Salmonella* sp., *E. coli O157:H7, L. monocytogenes*, and *Staphylococcus aureus*. Samples were collected in a separate sterile marked vial. The samples were transferred in ice box to the laboratory and examined immediately.

Foodborne pathogens enumeration, isolation, identification, and characterization

Total bacterial count (using plate count agar and incubation at 37°C), Total coliform count and Faecal coliform count (using VRB agar and incubation at 37 °C and 44 °C respectively), *Staphylococcaus aureus* count (using Baird Parker agar and incubation at 37 °C), *Listeria monocytogenes* (using Listeria Oxford agar as plating media after enrichment) and *Salmonella* isolation (using Salmonella Shigella agar after enrichment) and identification (using biochemical and serological tests) were performed according to Desmasures *et al.*, 1997.

Bacillus cereus count (using Bacillus cereus agar and incubation at 30 °C) and Total Enterobacteriaceae Count (using VRBG agar and incubation at 37 °C) were performed according to NMKL 1997 and 2000, respectively.

Escherichia coliO157:H7 from raw milk were isolated and serologically typed as described by a oarayaJnd Henning 2001.

RESULTS

Data in Table (1) showed that 100% of the examined winter samples exceeded the threshold for milk prior to heat treatment for Total Bacterial Count, Total Coliform Count, and *Staphylococcus aureus* count ($2x10^4$, 10^2 and 10^2 cfu/ml respectively) as prescribed in the Dairy Products' Hygiene Regulations 1995 (yaJdna oara Henning 2001).

Table (1) Differential count of bacterial content of raw milk in winter samples:

Area	No. of exam. samples	TPC	TEC	TCC	FCC	Staph. aureus count
Bani Suif	3	$57x10^6$	$99x10^5$	$244x10^4$	$29x10^2$	$78x10^3$
Cairo	30	$79x10^6$	$160x10^4$	$163x10^4$	$167x10^2$	$56x10^3$
Fayoum	3	$257x10^6$	$114x10^4$	$212x10^4$	$28x10^2$	$129x10^3$
Giza	34	$40x10^6$	$120x10^6$	$220x10^6$	$150x10^4$	$39x10^5$
Kalioubeya	3	$188x10^5$	$31x10^5$	$39x10^4$	$117x10^4$	$38x10^4$
Sharkeya	7	$76x10^5$	$105x10^5$	$55x10^5$	$54x10^4$	$61x10^4$
Total	80	$105x10^5$ *	$116x10^5$ *	$80x10^5$ *	$34x10^4$ *	$93x10^4$ *

TPC = Total Plate Count TEC = Total Enterobacteriaceae count
TCC = Total Coliform Count FCC = Faecal Coliform Count
* = Average number of count

Table (2) showed that *Bacillus cereus, E.coli O157:H7* and *Listeria monocytogenes* were isolated from 26.25%, 5% and 5% of the examined winter samples respectively on the other hand, *Salmonella spp.* were not detected in any of the milk samples indicated in this table.

Table (2) Incidence of food poisoning bacteria in raw milk winter samples:

Area	No. of samples	B. cereus		Salmonella species		E. coli O157:H7		Listeria monocytogenes	
		No. of (+) samples	%	No. of (+) samples	%	No. of +ve samples	%	No. of (+) samples	%
Bani Suif	3	1	33.33	0	0.00	0	0.00	0	0.00
Cairo	30	6	20.00	0	0.00	3	10.00	4	13.33
Fayyoum	3	2	66.67	0	0.00	0	0.00	0	0.00
Giza	34	10	29.41	0	0.00	1	2.94	0	0.00
Kalioubeyya	3	0	0.00	0	0.00	0	0.00	0	0.00
Sharkeyya	7	2	28.57	0	0.00	0	0.00	0	0.00
Total	80	21	26.25	0	0.00	4	5.00	4	5.00

B. cereus = Bacillus cereus E.coli = Escherichia coli

Data in Table (3) indicated that 100% of the examined summer samples failed the legal standards for aerobic plate count, Coliform count and Staphylococcus aureus count ($2x10^4$, 10^2 and 10^2 cfu/ml respectively) as prescribed in the Dairy Product's Hygiene Regulations 1995 (**dna oarayaJ** Henning 2001).

Table (3) Differential count of bacterial content in raw milk Summer samples:

Area	No. of exam. samples	TPC	TEC	TCC	FCC	Staphylococcal count
Bani Suif	4	$212x10^6$	$199x10^5$	$125x10^4$	$137x10^2$	$33x10^4$
Cairo	30	$198x10^6$	$270x10^5$	$173x10^4$	$198x10^3$	$156x10^4$
Fayyoum	4	$287x10^6$	$244x10^4$	$166x10^4$	$133x10^2$	$239x10^3$
Giza	30	$120x10^6$	$228x10^4$	$194x10^4$	$230x10^3$	$229x10^4$
Kalioubeyya	4	$288x10^6$	$281x10^6$	$139x10^5$	$147x10^4$	$138x10^5$
Sharkeyya	8	$276x10^5$	$245x10^5$	$298x10^4$	$157x10^4$	$261x10^4$
Total	80	$189x10^{6*}$	$59x10^{6*}$	$39x10^{5*}$	$58x10^{4*}$	$35x10^{5*}$

TPC = Total Plate Count TEC = Total Enterobacteriaceae count
TCC = Total Coliform Count FCC = Faecal Coliform Count
* = Average number of count

Bacillus cereus, Salmonella sp., E.coli O157:H7 and *Listeria monocytogenes* were detected in 32.50%, 3.75%, 6.25%, and 6.25% of the examined summer samples respectively (Table 4).

Staphylococcus aureus count in summer season samples were more than it's prospective in winter season samples (Table 2 and 4).

Table (4) Incidence of food poisoning bacteria in raw milk summer samples:

Area	No. of samples	B. cereus		Salmonella species		E.coli O157:H7		Listeria monocytogens	
		No. of (+) samples	%	No. of (+) samples	%	No. of (+) samples	%	No. of (+) samples	%
Bani Suif	4	1	25.00	0	0.00	1	25.00	1	25.00
Cairo	30	15	50.00	1	3.33	2	6 67	3	10.00
Fayyoum	4	0	00.00	0	0.00	1	25.00	0	0 00
Giza	3 •	8	26.67	2	6.67	1	3 33	2	6.67
Kalioubeyya	4	0	0.00	0	0.00	0	0.00	0	0.00
Sharkeyya	8	2	25.00	0	0.00	0	0.00	0	0
Total	80	26	32.50	3	3.75	5	6.25	5	6.25

B. cereus = Bacillus cereus E.coli = Escherichia coli

DISCUSSION

As the dairy industry moves towards production of products of extended shelf life and as several cases of food poisoning are associated with consumption of raw cow's milk or raw cow's milk products, greater demand must be placed on the quality of raw milk. The microbial contamination of raw milk originates from three main routes: within the udder, the teats and udder exteriors and handling, storage equipments and transportation of raw milk (Hayes *et al.*, 2001). Incidence of food poisoning bacteria was documented in many previous studies.

In this study, the average number of Total Bacterial Count (10^6 cfu/ml) agree with the results obtained by Bautista *et al.*, 1986 and Godefay and Molla 2000. The average number of Total Coliform Count (10^5 cfu/ml) was higher than that obtained by Godefay and Molla 2000 (10^4 cfu/ml) but agree with the results obtained by Aleksiever and Krushove 1981 who have a similar average number detected in raw cow's milk samples. The mean Faecal coliform Count (10^4 cfu/ml) agree with the results obtained by Desmasures *et al.*, 1997 who has the same count. The increase in the count of Total Coliform Count in milk may be attributed to contamination of teats, utensils and bedding with faecal material or may be due to post milking contamination.

The isolation rates of *Bacillus cereus* bacteria in this study (26.25% and 32.50% in winter and summer season samples respectively) seems to be agreed with Larsen and Jorgensen 1997 who had isolation rate of 25% of the examined samples. The high prevalence of this microorganism in summer season may be attributed to the differences in environmental temperature and also may be due to bad management during summer season.

The low incidence of *Salmonella sp.* in summer season samples (3.75%) nearly agree with McManus and Lanier 1987 who could isolate this type of microorganism from raw cow's milk from 4.7% of the examined samples. The absence of *Salmonella sp.* in winter season samples agrees with the results obtained by Little and Louvois 1999.

E.coli O157:H7 has been associated with human diseases. Foods of animal origin including raw milk have been implicated as important vehicle for this type of bacteria in humans and it is widely documented that, this bacteria can be isolated from faeces of asymptomatic cattle and raw milk (Jayarao and Henning 2001). Seldom, raw milk has been implicated in outbreaks of disease caused by E.coli O157:H7. From 1982 to 1990, only 14 confirmed cases of E.coli O157:H7-associated illness following consumption of raw milk occurred in the United States (Jayarao and Henning 2001). In this study E.coli O157:H7 strain was isolated from 5% and 6.25% of winter and summer seasons' samples respectively. These results nearly agree with the results obtained by Jayarao and Wang 1999 who could isolate this type of bacteria from raw milk in a percentage of 7.3%.

The discrepancy between the rates of isolation of microorganisms in this study with that in the other studies may be attributed to many factors such as differences in used tests and techniques, region, temperature, humidity or management.

Listeria monocytogenes was isolated from 5% and 6.25% of winter and summer season samples respectively. The isolation rate observed was apparently similar to that obtained by Slade *et al.,* 1988 and Desmasures *et al.,* 1997 whose rates were 5.4% and 5.8% respectively.

The rate of isolation of *Staphylococcus aureus* (100%) and the mean count reported in this study ($10^4/10^5$ cfu/ml), agree with results obtained by Adesiyun *et al.* 1998. The high prevalence of this type of bacteria may be attributed to post milking contamination and support the massive investigation and medical examination of milkers to avoid risk factors.

One of the important factors responsible for the presence of pathogenic bacteria in milk may be attributed to the lack of a proper program for isolation of sick animals and complete destruction of milk obtained from them before reassurance of the absence of the disease causing pathogens before being introduced in milking herds.

The isolation of food borne disease causing bacteria has been reported in many countries like United States and Canada (Table 5). The isolation rates reported in the last two decades varies due to many reasons such as isolation and identification, true prevalence, season, geographic area, hygiene and farm managements. These findings truly suggest that, pathogens do occur in raw milk and may have a health hazard if raw milk is consumed. There are many previous data showed the rate of consumption of raw milk in many regions. Jayarao and Cassel (1999) and Jayarao and Henning 2001 have found that 60% of dairy producers how participate in Bulk Tank Milk surveys consumed raw milk. An epidemiological study conducted by Headrick *et al.,* 1997 on raw milk associated food borne disease outbreaks in United States revealed that, 46 raw milk associated outbreaks occurred between1973 and 1992. They concluded that, consumption of raw milk remains a preventable cause of food poisoning outbreaks.

Table (5): Isolation rates of food borne pathogens from raw milk reported in Canada and United States:

Pathogen	Percent of isolation rate	Year	Country	Refrence
E.coli O157:H7	3.8	1997	USA	Jayarao and Henning (2001)
				Steele et al., (1997)
	0.87	1997	Canada	
Listeria monocytogenes	4.2	1987	USA	Lovette *et al.*, (1987)
				Slade *et al.*, (1988)
	5.4	1988	Canada	Liewen and Plautz (1988)
	4.0	1988	USA	Roherbach et al (1992)
				Jayarao and Henning (2001)
	4.1	1992	USA	
	4.6	1997	USA	
Salmonella spp.	4.7	1987	USA	McManus and Lanier (1987)
				McEwen *et al.*, (1988)
	2.9	1988	Canada	Jayarao and Henning (2001)
	6.1	1997	USA	

CONCLUSION

The findings of this study suggest that, new immerging pathogenic bacteria of human health significance like *Listeria monocytogenes* and *E.coli O157:H7* together with the well known food poisoning types are present in raw milk. This observation with previously reported data of consumption of raw milk indicated that dairy producers, consumers and farm families are at great risk of ingestion of pathogenic bacteria when consuming raw milk. Based on the findings of this study, educational programs must be conducted to address issues related to consumption of raw milk. The incidence of food borne pathogens in this study was similar to many previous reports. There was a slight increase in the bacterial counts and rates of isolation of pathogenic bacteria in summer season samples than that of winter season samples. Massive investigation and medical examination must be applied on milk handlers to avoid risk factors. The high percentage of isolation of pathogenic bacteria from certain governorates indicated that there is a need to stress on good management practices in such governorates to overcome these situations. Raw milk must be heat treated just after collection to keep its quality in a good condition before either consumption or processing to avoid food poisoning outbreaks.

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المخاطر الصحية المرتبطة باللبن الخام في الاسواق المصرية

جيهان محمد المغازي – سعيد عبد المنعم حسن
المعمل المركزي للاغذية و الاعلاف – مركز البحوث الزراعية

تمت دراسة الحالة الميكروبية لعدد ١٦٠ عينة لبن تم الحصول عليها من منافذ البيع في ٦ محافظات في جمهورية مصر العربية اثناء فصلي الصيف و الشتاء (٨٠ عينة في كل فصل). تم تقدير العدد الكلي للبكتريا – العدد الكلي لبكتريا القولون – العدد الكلي لبكتريا القولون النموذجية – العدد الكلي لبكتريا الاستافيلوكوكس.

و قد اشارت النتائج ان نسبة ١٠٠% من العينات المختبرة لم تتطابق مع المواصفات الصحية القياسية التي تم تحديدها من الجهات المختصة بالنسبة الى العدد الكلي للبكتريا – العدد الكلي لبكتريا القولون – العدد الكلي لبكتريا الاستافيلوكوكس و هي $١٠^{٤}$ و $١٠^{٢}$ و $١٠^{٢}$ خلية حية / مل على الترتيب.

تم عزل الميكروبات الضارة بالصحة العامة و التي تسبب التسمم الغذائي و هي Listeria monocytogenes, E.coli O157:H7, Bacillus cereus and Salmonella spp. و كانت نسب العزل في العينات المختبرة هي ٢٥و٦% و٢٥و٦% و٥٠و٣٢% و٧٥و٣% على الترتيب من عينات الموسم الصيفي و ٥% و ٥% و ٢٥و٢٦% و صفر% على الترتيب من عينات الموسم الشتوي.

و قد اشارت نتائج هذه الدراسة الي ضرورة زيادة برامج التوعية بالنسبة للبائعين و المستهلكين الى مدى خطورة استخدام الالبان الخام لما تحتوية من بكتريا ضارة تؤثر تاثيرا سلبيا على الصحة العامة و ذلك بعد ظهور العديد من حالات التسمم الغذائي في مختلف انحاء العالم المرتبطة بتناول الالبان الخام.