Alexandria Journal of Veterinary Sciences 2014, 44: 111-118
ISSN 1110-2047, www.alexjvs.com
DOI: 10.5455/ajvs.47762

Detection of Some Pathogenic Organisms from Dairy Farm Milk

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Key words

raw cow milk, dairy farms, milk quality, microbiological properties

ABSTRACT:

This study was conducted to evaluate the quality of raw cow milk at the farm level which considered the first step for production of safe and high quality milk. A total of 150 raw cow milk samples were collected from governmental and private farms at Alexandria Governorate, Egypt. The samples were analyzed for bacteriological properties. The mean values of TBC, coliforms ,Staphylococcus aureus and Aeromonas hydrophila count in positive samples were $3.22 \times 10^4 \pm 7.28 \times 10^3$, $1.4 \times 10^3 \pm 3.9 \times 10^2$, $5.26 \times 10^2 \pm 8.1 \times 10$ and $6.4 \times 10 \pm 1.3 \times 10$ in examined raw cow milk collected from governmental farms while the respective counts in raw cow milk collected from private farms were $1.51 \times 10^5 \pm 2.59 \times 10^4$, $3.28 \times 10^2 \pm 4.7 \times 10$, $2.95 \times 10^2 \pm 7.7 \times 10$ and $6.8 \times 10 \pm 4.1 \times 10$. Also prevalence of some pathogens as E. coli ,Salmonellae and Yersinia enterocolitica organisms were detected .The hygienic as well as the public health significance of raw milk were discussed.

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1. INTRODUCTION

Milk is a highly nutritious food and ideal for microbial growth. The fresh milk deteriorates easily to become unsuitable for processing and human consumption (FAO, 2001). Milk obtained from healthy animal's udder is free from pathogenic bacteria but some of the animals in field condition may be suffering from sub-clinical mastitis and are excreting the causative agent in milk, such milk contaminates the bulk milk. Moreover, fresh milk may get microbial contamination from utensils, animal skin, environment, or water used for cleaning etc. (FAO, 2008). Foodborne diseases are a common and widespread global problem. Several outbreaks have been reported as a result of consuming contaminated milk that may look, taste and smell perfectly normal but are in fact contaminated with large number of harmful bacteria (Centers for Disease Control and Prevention, CDC, 2009). Milk contaminated by high levels of spoilage bacteria usually becomes unsuitable for further processing since it does not meet the consumer's expectations in terms of health (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes) (Nanu et al., 2007).

The presence of Coliforms in food of animal origin indicates environmental and fecal contamination since these micro-organisms are abundant in the environment food (Shojaei and Yadollahi, 2008). E. coli is important as mastitis pathogens and widely distributed in the farm environment (Hogan and Smith, 2003). Amongst the coliforms, Escherichia coli organisms are the most common contaminants of raw and processed milk (Quinn et al., 2002). It is a reliable indicator of fecal contamination of water and food such as milk and dairy products (Todar, 2008).

Staphylococcus aureus is one of the important causes of food-borne diseases in humans, S. aureus is commonly associated with intoxications due to its ability to produce a variety of potent enterotoxin (Balaban and Rasooly, 2000 and Le Loir et al., 2003). Identical *S. aureus* strains have occasionally been isolated from dairy cows and hands of milking persons (Jorgensen et al., 2005 a), but strains originating from bovine mastitis in general represent a genetically different cluster than the human strains, suggesting host specificity (Van Leeuwen et al., 2005).

Aeromonas species are potential food-poisoning agent. A. hydrophila is psychrotrophic and has been associated with the spoilage of refrigerated (5^{o}C) animal products including raw milk (Majeed et al., 1989). Aeromonas microorganism is commonly present in farms, in feeding stuff, water, faces and soil (El-Shenawy and Marth, 1990). The organisms can invade the udder tissues; multiply in mammary tissues and subsequently discharge in milk. Also the contaminated water used for washing milking equipment is considered as a significant source of contamination. So presence of Aeromonas in a high level in raw milk samples is indicative to bad hygienic measures of milk production and distribution (korashy, 2006).

Salmonellae are considered among the most important enteric foodborne pathogens whose presence in the food constitutes a severe health hazard. Many outbreaks of human illness have been associated with the consumption of raw or inadequately heat treated milk or their dairy products (Ellis et al., l998). Contamination of raw milk with Salmonella spp. is mostly due to infected persons and contamination of the environment, since natural infections of the udder are rare and seldom contribute to human food poisoning. Deficient hygiene in dairies, especially those from developing countries, has often been considered as one of the major reasons for contamination of milk with both spoilage and pathogenic bacteria (Mubarack et al., 2010).

Yersinia enterocolitica is the most prevalent Yersinia species connected to disease in human. The organism has received considerable attention as a causative agent of human gastroenteritis (Robins-Browne, 2001). Yersinia enterocolitica is rapidly emerging worldwide as an enteric pathogen and has become a major cause of diarrhea in most of the industrialized world. Any raw animal food including raw milk may carry Yersinia enterocolitica and cause illness when the food is eaten (Mauro et al., 2008).

Milk taken from healthy udder is often free from pathogen germs. Fresh milk (immediately after milking) drawn from healthy cows contains relatively few bacteria (10^{2}-10^{3}/ ml). During milking operation occur changes in the microbial quality that favor microbial growth of milk. These changes may be due to many reasons such as: infection of udder, unsanitary milking utensils, personnel and sanitary status of cows and environment surrounding milking operation. Hence, the present study was designed to assess the hygienic quality of raw cow milk at the farm level through determining bacteriological properties of raw cow milk collected from governmental and private farms at Alexandria Governorate, Egypt.

2. MATERIALS AND METHODS

2.1. Collection of samples:

This study was carried out between January 2013 and July 2013. A total of 150 samples of raw cow milk were collected randomly from governmental dairy farms (No. of samples= 100) and private dairy farms (No. of samples= 50) at Alexandria Governorate. The collected samples were transferred as rapidly and directly as possible to the laboratory. Samples were prepared according to the technique recommended by International Organization for Standardization (ISO 8261:2001).

2.2. Bacteriological evaluation of examined raw cow milk samples:

The samples were examined for Total viable aerobic bacterial count according to ***(ISO 4833:2003)***, Staphylococcus aureus count (ISO 6888-3:2003), Coliforms count (ISO: 4832:2006) and Aeromonas hydrophila count (Oxoid, 1998; Popoff and Veron, 1976). E. coli identification was performed by using the surface spread technique on Eosin Methylene Blue (EMB) agar as recommended by (Atlas et al., 1995). Isolation of Salmonellae was performed according to (ISO: 6579:2002). Isolation of Yersinia was performed by using the method recommended by Schieman and Wauters (1992); Schieman (1982) and Walker and Glimour (1986).

2.3. Statistical Analysis:

Data were analyzed by SPSS programme (Statistical Package for Social Science, version 10.00). This test combines ANOVA with comparison of differences between means of the treatments at the significance level of $P< 0.05$.

3. RESULTS and DISCUAAION

Table (1) Bacteriological properties of examined raw cow milk samples:

Count(cfu/ml)	Governmental farm milk (n=100)			Private farm milk (n=50)		
	No	%	Mean ± SEM	No	%	Mean ± SEM
TBC	100	100	$3.22 \times 10^4 \pm 7.28 \times 10^3$	50	100	$1.51 \times 10^5 \pm 2.59 \times 10^4$
coliforms count	17	17	$1.4 \times 10^3 \pm 3.9 \times 10^2$	3	6	$3.28 \times 10^2 \pm 4.7 \times 10$
Staphylococcus aureus count	9	9	$5.26 \times 10^2 \pm 8.1 \times 10$	4	8	$2.95 \times 10^2 \pm 7.7 \times 10$
Aeromonas hydrophila count	9	9	$6.4 \times 10 \pm 1.3 \times 10$	4	8	$6.8 \times 10 \pm 4.1 \times 10$

No: Number of positive samples **%:** percent of positive samples
SEM: Standard error of the mean.

Table (2) Prevalence of some pathogenic bacteria in examined raw cow milk samples:

Parameter	**Governmental farm milk (n=100)**		**Private farm milk (n=50)**	
	No	**%**	**No**	**%**
E. coli	8	8	0	0
Salmonellae	14	14	1	2
Y. enterocolitica	15	15	7	14

No: Number of positive samples **%:** percent of positive samples

Table (3) Comparison between the results obtained from bacteriological evaluation of raw cow milk samples of governmental farms and the Egyptian Standards (2005) n=100:-

Parameters	**Governmental farms (n=100)**				
	No. of compatible samples	**% compatible samples**	**No. of incompatible samples**	**% incompatible samples**	**EOSQC**
E. coli	92	92	8	8	Nil
Staph. aureus	91	91	9	9	Not more than 100 cell/ml
Salmonellae	86	86	14	14	Nil
Y. enterocolitica	85	85	15	15	Nil
A. hydrophila	91	91	9	9	Nil

Table (4) Comparison between the results obtained from bacteriological evaluation of raw cow milk samples of Private farms and the Egyptian Standards (2005) n=50:-

Parameters	Private farms (n=50)				
	No. of compatible samples	% compatible samples	No. of incompatible samples	% incompatible samples	EOSQC
E. coli	50	100	0	0	Nil
Staph. aureus	46	92	4	8	Not more than 100 cell/ml
Salmonella	49	98	1	2	Nil
Y. enterocolitica	43	86	7	14	Nil
A. hydrophila	46	92	4	8	Nil

Table (1) showed that The mean values of TBC, coliforms, Staphylococcus aureus and Aeromonas hydrophila count in positive samples $3.22 \times 10^4 \pm 7.28 \times 10^3$, $1.4 \times 10^3 \pm 3.9 \times 10^2$, $5.26 \times 10^2 \pm 8.1 \times 10$ and $6.4 \times 10 \pm 1.3 \times 10$ in examined raw cow milk obtained from Governmental farms while the respective count in raw cow milk obtained from Private farms were $1.51 \times 10^5 \pm 2.59 \times 10^4$, $3.28 \times 10^2 \pm 4.7 \times 10$, $2.95 \times 10^2 \pm 7.7 \times 10$ and $6.8 \times 10 \pm 4.1 \times 10$.

This high counts indicated the poor quality of this milk comparing the results with Egyptian standard as showed in Table (3, 4) revealed that this bad quality .TBC, usually represented by spoilage and lactic acid producing bacteria (Nangamso, 2006 and Quinn et al., 2002), and sometimes pathogenic bacteria (Bonsu et al., 2000; Weinhaupl et al., 2000) such counts used as indicators of milk quality because they largely reflect the level of milking hygiene on dairy farms (Grimaud et al., 2009; Shojaei and Yadollahi, 2008).

It has also been established in previous studies that higher levels of bacterial contamination are obtained in milk from hand milked compared to machine milked cows (Filipoviet and Kokaj, 2009) due to less hygienic practices associated with hand milking (Hidayet and Mehmet, 2004 and Millogo et al., 2008).The health and hygiene of the cow, the environment in which the cow housed and milked, hygiene during milking and storage equipment, all influence microbial numbers in milk, also ineffective cleaning, insufficient hot water temperatures and/or the absence of sanitizers tends to select for faster growing of less heat resistant organisms (Murphy and Boor, 2000). Higher TBC value was obtained by Tryness et al., (2012) but similar results were reported by Abid et al., (2009) and Hakem et al., (2012).

The presence of high numbers of coliforms in milk provides an index of hygienic standard used in the production of milk, as unclean udder and teats can contribute to the presence of coliforms from a variety of sources such as manure, soil, food, personnel and even water (Bille et al., 2009). Nearly similar results were obtained by Desmasures et al., (1997) and Lingathurai et al., (2009) while higher results were obtained by Salman and Hamad (2011) and Meshref (2013).

Staphylococcus aureus is a major food borne pathogen due to its capability to produce a wide range of heat-stable enterotoxins (Peles et al., 2005). S. aureus can gain access to milk either by direct excretion from udders with clinical or subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk (Donkor et al., 2007; Peles et al., 2005). Similar results were obtained by Makovec and Ruegg, (2003) while Fatine et al., (2012) find high count which was 1.4×10^5 cfu ml.

There are a number of studies on the occurrence of motile Aeromonas (A. hydrophila, A. sorbia and A. caviae) in milk and dairy products (Abdel-Hadi et al., 1995 and Hafez, 1996). A. hydrophila has become a topic of much recent as a potential cause of food poisoning with long duration of abdominal cramps and diarrhea after ingested contaminated food, perhaps food protects the bacteria from gastric juices and allows the colonization of Aeromonas in the lower gastrointestinal tract (Buchanan and Palumbo, 1985). These low results agreed with Dimitrios et al., (1999) and Enany et al., (2004).

The recovery of some pathogenic bacteria such as E. coli, salmonellae and Yersinia as showed in table (2) is serious public health concern because they are a source of food poisoning which can cause severe illness and even death. Food poisoning is frequently caused by bacteria from food that has been poorly handled, stored or cooked. Symptoms of food poisoning can include nausea, stomach cramps, diarrhea, fever, and headaches within 30 minutes after eating, or a number of hours or days later. The symptoms can occur mild or severe. Some people are more at risk from food poisoning including young children, pregnant women, the elderly and people with other illnesses. The high incidence of E. coli was reported by Sobeih et al., (2002) and Meshref (2013).

Contamination of raw milk and products with Salmonella spp. is mostly due to infected persons and contamination of the environment, since natural infections of the udder are rare and seldom contribute to human food poisoning. Deficient hygiene in dairies, especially those from developing countries, has often been considered as one of the major reasons for contamination of milk with both spoilage and pathogenic bacteria (Grimaud et al., 2009; Mubarack et al., 2010). Van Kessel et al., (2004); Ibtisam and Mahboba (2007) and Hamida et al., (2009) who could also isolate salmonella from raw milk samples.

Both human and animals feces are an important source of contamination for soil, surface and depth waters, vegetation and food. Some authors noted that contact with animal manure and/or sick people or healthy carriers are the most important source of contamination. Numerous studies have highlighted the species Yersinia enterocolitica in surface water (rivers, lakes) and even in the depth, where it can survive longer because of the low concentration of toxic substances (Kattathara and Mandyam 2009).Also the incidence of Yersinia enterocolitica was much higher during winter than summer seasons (Toora et al.,1989).These reasons explained this high result. The high incidence of Yersinia enterocolitica was also reported by Vidon and Delmas (1981).

The present study showed that the quality of raw cow milk produced at the farm level in the study area was poor. Hence, adequate sanitary measures should be taken at all stages from production to consumption. These measures include proper handling of the cow, personnel

hygiene, use of hygienic milking and processing equipment, improving milk and milk handling environment among others. Further investigation of the status of the animals' health is required, especially mastitis and the significance of the effect of containers to ascertain their contribution on microbial quality. So application of good veterinary practice, HACCP and GHP improve the quality of milk.

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