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# INVESTIGATION OF HYGIENIC QUALITY OF FARM MILK IN SHARKIA GOVERNORATE

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# ABSTRACT

80 random bulk milk samples were collected from different dairy farms in Sharkia Governorate. Each sample was divided into two subsamples. The first was used for keeping quality and sanitary tests and the second was examined bacteriologically for determination of its bacterial condition. 8.75 of examined samples were reacted positively with Alizarin precipitation test "APT" and Clot-on-boiling test "C.O.B.". The results of methylene blue reduction test ranged from 2.0-5.54 hours with a mean value of 3.57  $\pm$  0.12. The number of samples graded by methylene reduction test as inferior quality (above 4.5 hours) were 60 with a percentage (75%). The milk samples have graded by resazurin reduction test as 81.82 were in grade A. 12.5% were in grade B and 6.25 were in grade C (inferior quality). Total colony, psychrotrophic, thermoduric and coliform counts (MPN) per ml, ranged from 1.18 x 10<sup>9</sup> - 2.5 x 10<sup>10</sup> with a mean value of 1.35 x 10<sup>10</sup>  $\pm$  0.04 x 10<sup>10</sup>, 1.1 x 10<sup>3</sup> - 2.24 x 10<sup>4</sup> with a mean value of 1.18 x 10<sup>7</sup>  $\pm$  0.61 x 10<sup>7</sup> and 2.30 x 10<sup>7</sup> - 9.3 x 10<sup>8</sup> with a mean value of 1.72 x 10<sup>8</sup>  $\pm$  0.32 x 10<sup>8</sup> respectively.

In conclusion it deems necessary that concerned authorities should impose bacteriological standers for control of milk production and handing.

كلية الطب البيطري

أجريت هذه الدراسة على ٨٠ عينة عشوائية من اللبن الخام المجمع من المزارع المختلفة من محافظة الشرقية وقسمت كل عينة منها إلى جزئين، الأول منها لعمل اختبارات الجودة والثانى تم إختباره بكتريولوچيا لتقدير الظروف الصحية، وجد أن ٧٥ر٨٪ من العينات المختبرة تعطى نتائج إيجابية مع إختبار التخثر بالغليان واختبار الترسيب بإضافة الكحول.

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وتم مناقشة الاحتياطات اللازمة لتحسين الجودة والظروف الصحية لإنتاج اللبن وتناوله ونقله لأقسام الاستقبال بمصانع الألبان.

# **INTRODUCTION**

Milk and milk products are extremely valuable food for people allover the world. The importance of milk as a food needs no emphasis. Most people are aware of the enormous wastage, because of its high perishability, milk is subject unless it is early an effectively processed, and milk provides an admirable culture for microorganisms and can dose serve as a vehicle for these and other disease producing microorganisms (**WHO**, **1962**).

High- quality raw milk should have a normal appearance, flavour and taste; moreover, it should have a low bacterial count and must not contain extraneous matter (**Berg, 1988**).

Even under very hygienic conditions of milk production some contamination of the milk is unavoidable, but in general, relatively few microorganisms will be present in milk immediately after milking. During handling and storage the number may increase considerably, depending on the type of bacteria, their virulence and the surrounding conditions, especially the temperature **(Al-Ashmawy, 1990)**. If the milk has not been properly cooled shortly after milking, preferably within few hours, a large variety of microorganisms will start to reproduce in milk resulting in its spoilage, thus causing economic loss. Moreover, if pathogenic organisms find their way to milk either from lactating animal or milk handlers, the harm has augmented and such milk constitutes a public health hazard (**Donkor et al., 2007**).

As quality improvement progresses, interest goes for beyond rapid rejection tests. Various methods are employed in assessing the bacteriological quality of raw milk. Resazurin and methylene blue reduction tests are the simplest methods for rapid determination of the cleanliness and the hygienic quality of raw milk, throughing the reducing activity of microorganisms (Weinand and Conlin, 2003). For bacteriological evaluation of raw milk, standard plate count (viable count), together with the measure of coliform contamination, were found to be a reliable index of production methods. Also, counting of thermoduric and psychrotrophic bacteria controlling sanitary conditions of production.

# **MATERIAL AND METHODS**

80 random bulk raw milk samples were collected from different dairy farms in Sharkia

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governorate. All samples were transferred directly to the laboratory refrigerated under aseptic condition. They were examined as soon as possible. Each sample was perfectly mixed before being divided into two subsamples. The first was used for keeping quality tests and the other was examined bacteriologically.

## (I) Quality tests:

- (1) Alizarin- alcohol test: according to **APHA**, (1985).
- (2) Clot-on-boiling test (C.O.B): according to **Chalmers (1955)**.
- (3) Methylene blue reduction test : recommended by Wilson et al, (1935).
- (4) Resazurin reduction test: according to **Athertian and Newlander (1977)**.

### (II) Bacteriological studies:

- (1) Total colony count (T.C.C): according to **A.P.H.A.** (1985).
- (2) Thermoduric bacterial count: after laboratory pasteurization of milk according to **A.P.H.A** (1985).
- (3) Psychrotrophic bacterial count (PBC): according to **A.P.H.A.** (1985).
- (4) Coliform count "MPN/ml" according to Thatcher and Clark (1978).

# **RESULTS AND DISCUSSION**

## (I) Quality tests:

(1) Alizarin precipitation (ATP) and clot on boiling (C.O.B) tests:

From the results given in table (1), it is evident that 87.5 of examined samples were reacted positively with APT and C.O.B tests. Alcohol precipitation and clot on boiling tests are the most suitable tests for indicating the end point of keeping quality. Positive results of (ATP) indicate increase acidity in the milk due to bacterial action (up to 0.216% lactic acid) (Jayarao and Wolfgang, 2003).

### (2) Reduction tests:

Results recorded in table (2) reveal that, the minimum time of MBRT of examined samples was 2h., the maximum was 6h., with a mean value of  $3.57 \pm 0.12$ . The highest frequency distribution (83.25%) lies within the range 4 - 6 (Table 3).

The numbers of samples below (4.5 hours) were 20, while the numbers at (4.5 hours) were 56 samples (Table 4).

Grading of examined samples according to methylene blue reduction time (Table 5) indicate that non of samples (0.0%) were graded excellent, 3 (3.75%) belonged to grade good, most of samples (96.25%) were graded fair.

Results are nearly similar to that obtained by Fahmy, (1975); Moustafa, (1978); Lee and Chen, (1987) and Masud et al., (1988).

## (3) Resazurin reduction test (RRT):

The distribution of examined samples according to their grades, given in table (6) points out that most of the samples (66 samples) belonged to grade A, 12.5% of samples were in grade B while 4 samples (6.25%) were of inferior quality (grade C).

The dye reduction tests are considered by several authors to be indicative for the sanitary condition under which milks were produced and handled (Garvie and Rawlands, 1952). It seems evident that the necessary sanitary precautions during production, handling and processing of milk must be applied.

### (II) Bacteriological studies:

(a) Total colony count (T.C.C.):

Obtained values of milk samples were 1.18 x  $10^9$  as a minimum and 2.51 x  $10^{10}$  as a maximum with a mean value of 1.35 x  $10^{10} \pm 0.04 \times 10^{10}$  (Table 7).

Results recorded in table (8) showed that 12.5% of samples had a count ranging from  $10^9 - 10^{10}$ , while the most of samples showed count ranging from  $10^{10} - 10^{11}$ . These results are nearly similar to that obtained by **Morgan** et al., (1989). On the other hand, lower findings were reported by **Sasano et al.**, (1993).

The high counts obtained in this study may be attributed to unsanitary environmental conditions during milk production and lack of cooling that favours the growth and multiplication of initial bacterial load. Also, the role of milkers as well as utensils and equipment should not be overlooked (**Reneau**, **2001; Cook, 2002 and Cook, 2004**).

## (b) Thermoduric count (T.C.):

Data recorded in table (7) revealed that all milk samples examined, were contaminated with thermoduric organisms. The minimum was  $1.15 \times 10^6$ , the maximum was  $2.35 \times 10^8$  with a mean value of  $3.48 \times 10^7 \pm 0.61 \times 10^7$ . The highest frequency distribution (87.5%) lies within the range  $10^7 - 10^8$  (Table 9). In the present study, the incidence of thermoduric organisms in milk samples was higher

than that reported by **Sasano et al.**, (1993).

The high thermoduric count in the examined milk samples are closely associated with persistent improper cleaning and sanitizing of equipment at the dairy farm (**Elmagli and Ibtisarn, 2006**).

## (c) Psyhrotrophic count (PC):

The results reported in table (7) showed that all milk samples were contaminated with psychrotrophic bacteria. The maximum count was  $2.24 \times 10^4$ ; the minimum was 1.10x 10<sup>3</sup>, with a mean value of 1.18 x  $10^4 \pm 0.51$  $x 10^4$ . The highest frequency distribution (82.5%) lies within range  $10^4 - 10^5$  (Table 8). Nearly similar results were reported by Swart et al., (1989) and Sasano et al, (1993). The relatively high count met within this work declare to what extent the raw milk is exposed to contamination during handling in dirty equipment, or produced under undesirable conditions or carelessness of milk, or contact with infected water and the milk is held in a warm place (Lampert, 1975 and Slaghuis, 2002).

## (d) Coliform count (MPN/ ml):

Inspecting the results obtained in table (7), it is evident that all samples were contaminated with coliforms. The minimum count/ml was 2.30 x  $10^7$ , the maximum was 9.30 x  $10^8$ , with a mean value of  $1.72 \times 10^8 \pm 0.32 \times 10^8$ . The highest frequency distribution lies within the range  $10^7 - 10^8$  (Table 8). These results are lower than finding reported by **Moustafa et al, (1988)**.

Presence of coliforms in milk may be

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indicative of fecal contaminations. Their count reflects the inadequate sanitation during milk production and its handling in dirty equipment as well as milk collected from subclinically mastitic animals. Therefore, presence of coliforms in milk may be responsible for development of objectionable taints and flavours rendering it unmarketable, thus causing economic losses beside they may at times constitutes a public health hazard (**Ruegg, 2003 and Cook, 2006**).

*Table (1):* Prevalence of Alizarin Precipitation (APT) and Clot on Boiling (COB) tests of examined samples.

Test	No of samples	Negative	samples	Positive samples		
	No. of samples	No.	%	No.	%	
A.P.T	80	73	90.1	7	8.75	
C.O.B	80	73	90.1	7	8.75	

*Table (2):* Statistical analytical results of methylene blue reduction test (MBRT) of examined samples.

No of grander		Reduction	time (hours)		
No. of samples	Min.	Max.	Mean	± S.E.M	
80	2.0	5.54	3.57	0.12	

*Table (3):* Frequency distribution of examined samples based on their methylene blue reduction test (MBRT).

	Frequency					
Intervals (hours)	No. of samples	%				
0.5 - 2	8	10				
2 - 4	12	15				
4 - 6	57	71.25				
6 - 8	3	3.75				
Total	80	100				

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	Frequency					
Intervals (hours)	No. of samples	%				
0.5 - 2	8	10				
2-3.5	10	12.5				
3.5 - 4.5	20	25				
4.6 - 6	40	50				
6 - 8	2	2.5				
Total	80	100				

 Table (4): Frequency distribution of examined samples based on their legal limits of methylene blue reduction test (MBRT).
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*Table (5):* Grading of samples quality according to methylene blue reduction test (MBRT).

Grade	Reduction time	Frequency				
Graue	(hours)	No. of samples	%			
Excellent	≥ 8	0	0.0			
Good	6 - 8	3	3.75			
Fair	2-6	77	96.25			
Bad	< 2	0	0.0			
Total		80	100			

*Table (6):* Grading of samples quality according to resazurin reduction test (R.R.T).

	Grade								
No. of samples		Α		B	С				
	No.	%	No.	%	No.	%			
80	66	81.25	10	12.5	4	6.25			

*Table (7):* Statistical analytical results of bacteriological tests of examined raw milk samples (N = 80).

Bacteriological tests	Min.	Max.	Mean	I.S.E.M.
T.C.C	1.18 x 10 <sup>9</sup>	$2.5 \ge 10^{10}$	1.35 x 10 <sup>10</sup>	$0.04 \ge 10^{10}$
T.C.	1.19 x 10 <sup>6</sup>	$2.35 \times 10^8$	3.48 x 10 <sup>7</sup>	0.61 x 10 <sup>7</sup>
P.B.C.	$1.1 \ge 10^3$	2.24 x 10 <sup>4</sup>	1.18 x 10 <sup>4</sup>	0.51 x 10 <sup>4</sup>
C.C.	$2.30 \ge 10^7$	9.3 x 10 <sup>8</sup>	1.72 x 10 <sup>8</sup>	0.32 x 10 <sup>8</sup>

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T.C.C. = total colony count T.C. = Thermoduric count P.C. = Psychrotrophic count C.C. = Coliform count.

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Total colony	tal colony count (T.C.C.) Thermoduric count (T.C.)		Psychrotrophic count (P.C.)			Coliform count (C.C.)					
Intervals	Frequency		Intervals	Frequency		Intervals	Frequency			Frequency	
	No.	%	Liver vals	No.	%	intervals :	No.	%	Intervals	No.	%
$10^9 - 10^{10}$	7	12.5	$10^{6} - 10^{7}$	2	5	$10^{7} - 10^{8}$	56	70	$10^{3} - 10^{4}$	14	17.5
$10^{10} - 10^{11}$	73	87.5	$10^7 - 10^8$	72	87.5	$10^8 - 10^8$	24	30	$10^4 - 10^6$	66	82.5
			$10^8 - 10^9$	6	7.5						
Total	80	100		80	100		80	100		80	100

Table (8): Frequency distribution	examined samples based on their total colony count (T.C.C.); thermoduric	count
(T.C.); psychrotrophic	unt (P.C.) and coliform count (C.C.)	

## REFERENCES

**A.P.H.A. (1985):** Standard methods for the examination of dairy products. 15th Ed. American Public Health Associations, New York.

**Al-Ashmawy, A. M. (1990) :** Food Hygienic Handbook. Hygienic of fluid milk, dairy products, fats, oils, and eggs. Fac. of Vet. Med., Zagazig Univ.

Atherton, H. V. and Newlander, J. A. (1977) : Chemistry and testing of dairy products. 4th Ed. Avi Publishing Company, Inc. Westport, Connecticut.

**Berg, J. C. T. Vanden (1988) :** Dairy technology in tropics and subtropics. Pndoc., Wageningen, the Netherlands.

Brito, J. R. F.; Paiva e Brito, M. A. V. and da S Verneque, R. (2000) : Bacterial counts on the surface of the teats of cows milked under different methods of udder preparation, including cows milked by hand and stimulated by suckling a calf. Ciencia Rural, 30 (5): 847 - 850.

**Chalmers, J. D. P. (1955) :** Standard methods for the examination of dairy products. 14th Ed. American Public Health Associations, New York.

**Cook, N. B. (2002) :** The influence of barn design on dairy cow hygiene, lameness and udder health. Pages 97-103 in Proc. of the 35th Ann. Conv. Amer. Assoc. Bov. Pract., Madison, WI. Amer. Assoc. Bov. Pract. Rome, GA.

**Cook, N. B. (2004) :** The cow comfort link to milk quality. Proc. NMC Regional Meeting, Bloomington, Minnesota, June, 29-30. pp. 19-30.

**Cook, N. B. (2006) :** Sand bedding and coliform mastitis, a case report. Proc. 45th Annual NMC Meeting. Tampa, Florida. pp. 308-309.

**Donkor, E. S.; Aning, K. G. and Quaye, J. (2007) :** Bacterial contaminations of informally marketed raw milk in Ghana. Ghana Medical Journal. June, Vol. 41, No. 2.

**Elmagli, A. A. O. and Ibtisarn Ibtisam E. M. El-Zubier (2006) :** Study on the hygienic quality of pasteurized milk in Khartoum State (Sudan). Research Journal of Animal and Veterinary Sciences, 1 (1): 12-17.

**Farag, H. A. M. (1987) :** Bacteriological quality of market raw milk M.V.Sc. Thesis, Fac. Vet. Med., Moshtohor, Zagazig Univ.

**Garvie, E. I. and Rowlands, S. A. (1952):** The role of microorganisms in dye reduction and keeping quality tests II. The effect of microorganisms when added to milk in pure and mixed cultures. J. Dairy Res. 19: 263.

Jayarao, B. M. and Wolfgang, D. R. (2003): Bulk-tank milk analysis. A useful tool for improving milk quality and herd udder health. Vet. Clin. North Am. Food Anim. Pract. 19: 75-92.

**Lampert, L. M. (1975) :** Modern dairy products. Chemical publishing Company Inc. New York.

Lee, S. J. and Chen, M. C. (1987) : Survey of raw milk quality in Taiwan. J. of Taiwan Livestock Res. 20 (2): 123-134.

Lukasona, G. and Dvorak, B. (1983) : Some important indices of microbiological quality of milk processing. Veterinarstri 33 (9): 401. Dairy Sci, Abst., 46: 266.

Masnd, T.; Athar, I. H.; Chishti and Shah, M. A. (1988) : Bacteriological and chemical changes buffalo's milk stored ± t 30 Co. Dairy Sci. Abst. 1990 (052- 03450).

Morgan, S. D.; Hafez, R. S. and Mohamed, H. A. (1989) : Aspects on the sanitary status of raw milk Kaliobia Governorate, Assiut Vet. Med. J. 21 (42): 59-62.

**Moustafa, M. K. (1978) :** Studies on the sanitary condition of market milk in Assiut. Thesis, M.V.Sc. Fac. of Vet. Med., Assiut. Univ.

Moustafa, M. K.; Ahmed, H. A. A. and Abdel-Hakiem (1988): Sanitary condition of market milk in Assiut City. Assiut Vet. Med. J. Vol. 19, 38.

**Reneau, J. K. (2001) :** Somatic cell counts: measures of farm management and milk quality. Pages 29-37 in Proc. Natl. Mastitis Counc., Reno, NV. Natl. Mastitis Counc., Inc., Madison, WI.

**Ruegg, P. L. (3002) :** Investigation of mastitis problems on farms. Vet. Clin. North Am. Food Anim. Pract., 19: 47-73.

Sasano, M.; Aoyama, H. and Arai, Y. (1993) : Standard plate count in raw milk produced in Hokaido, Japan. Japanese J. of Dairy and Food Sci. 42, 5 (a): 181-189, 9 ref.

**Slaghuis, B. A. (2002) :** Personal communication, research Institute for Animal Husbandry (PV), Lelystad, the Netherlands.

Swart, G. J.; Jooste, P. J. and Moster, J. F. (1989) : Occurrence and seasonal distribution of psychotropic and seasonal distribution of psychotropic and certain mesophilic bacterial types in the bulk milk supply of the Pretoria area (Suid- Afrika anse- tydskifvir-Suiwe Ikunde). 21: I, 1-8, 26 ref.

Thatcher, F. S. and Clark, D. S. (1978): Microorganisms in Foods. Their significance and methods of enumeration ICMSF, Academic Press, New York.

Weinand, D. and Conlin, B. J. (2003) : Impacts of dairy diagnostic teams on herd performance. J. Dairy Sci., 86: 1849-1857.

Wilson, G. S.; Twigg, R. S.; Wright, R. C.; Hendry, C. B.; Cowell, M. P. and Maier, I (1935): The bacteriological grading of milk. Special Report Series of the Medical Research Council, No. 206, London: HMSO.

**World Health Organization "W.H.O"** (**1962**) : Milk Hygienic. Hygienic in milk production and distribution. WHO, Geneva.