

STUDIES ON MICROBIAL POLLUTANTS OF MILK PRODUCED IN ASSIUT VICINITY

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Abstract: 309 samples of raw buffalo's and cow's milk were collected over one year from 3 different milk supplies in Assiut city and examined for microbiological quality .

The microbiological evaluation of milk samples was carried out by the determination of total microbiological counts (T.M.C), presence of coliform bacteria (CB), and estimation the incidence of both aerobic spores(AS) and anaerobic spores bacteria (ANAS).

Generally, the average (T.M.C) of investigated milk samples was 8.94×10^{12} C.F.U /ml. The higher microbiological count was detected in cow's milk samples (1.09×10^{13}) as compared with buffalo's milk samples (5.54×10^{12}). In addition , samples collected during cold months appeared to have lower microbiological counts (1.40×10^7) than that during warm months (1.39×10^{13}).

The average (CB) titer was 3.16×10^4 bacterium /ml . Samples of cow's milk

showed lower (CB) titer (2.06×10^3 /ml) than those of buffalo's milk (8.21×10^4 /ml). Furthermore, high incidence of (CB) was found in samples collected during warm months (4.3×10^4 /ml) . The (CB) titer of cold months samples was (0.15×10^4 /ml) .

The mean value of (AS) incidence was 2.21×10^3 /ml. The incidence of (AS) was high in buffalo's milk (3.25×10^3) than in cow's one 1.6×10^3 /ml . Samples collected during cold months appeared contaminated with (AS) in cow level (1.2×10^3 spores /ml) than that found in warm months samples (2.77×10^3).

The mean value of (ANAS) incidence was 26.4 spores /ml. Buffalo's milk contained (ANAS) with high level (39.7) than cow's milk (18.6 spore /ml) .Samples collected during cold months showed low incidence of ANAS (9 spores /ml) compare with 46 spores /ml in samples of warm months.

Key words: microbial pollutants, milk

Introduction

Milk is a complex biological fluid which contains a wide variety of different constituents and posses a unique physical characteristic. In addition of being a nutritious medium, it is also presents a favourable physical environment for the

multiplication of contaminant microorganisms. During production, handling and distributes dairy products may be subjected to contamination with several types of microorganisms from different sources. In general, the bacterial

content of fresh milk is an indication for the hygienic conditions at the time of milk production.

In countries with poor milk production and marketing practices, high initial bacterial counts in the raw milk are to be expected. Such groups of microbial contamination which play an important role in the quality of milk and its products are coliforms, aerobic and anaerobic spore forming bacteria. The presence of coliform is always taken as a definite index of faecal contamination. Aerobic spore formers, being wide spread in nature, they gain entrance into milk during production either from the producing animal or/and from the environment (Mohran and Said, 1991). The present study was carried out to evaluate the microbiological and keeping quality of milk produced in Assiut vicinity.

Materials and Methods

1- Milk samples:

Milk samples used in the present study were fresh milk from morning milking of individual cow or buffalo in Assiut Vicinity. The first group of milk samples obtained from private small farms at Arab El madabigh region near Assiut city, which waste water is used for irrigation and drinking animals. Samples collected from this source were individual milk samples of either cow's or buffalo's milk. The second source studied samples was Assiut University animal production research farm while the

third one was the Friesian cow's station of Assiut governorate at Bany More.

2- Microbiological analysis:

2.1) Total microbial count:

Appropriate dilutions of each milk samples were plated in duplicates using basal medium (EL-Gendy, 1970). Plates were incubated at 33°C for 48 hr. After then the colonies were counted.

2.2) Coliform test:

The presence of coliform bacteria was tested by the inoculation of milk samples or their dilutions into **MacConkey broth (Mohran, 1971)**. Duplicate tubes were used and incubated at 37°C for 24 hours.

2.3) Detection of aerobic and anaerobic spores:

Spores were determined by inoculating litmus milk with appropriate dilutions of milk. The inoculated tubes heated for 10 minutes at 85°C. For facultative and anaerobic spores, the medium was covered immediately after heating with a layer of paraffin wax (vaspar) and cooling, while it was left uncovered under aerobic condition for aerobic spores. The tubes were incubated at 33°C for two weeks. Peptonization of milk after 24 hours under aerobic or anaerobic conditions was taken as a criterion for the presence of aerobic and facultative bacilli, while stormy gas formation after 72 hours under anaerobic conditions indicated butyric acid

fermentation and the presence of anaerobic spores.

3) Statistical analysis:

Data were programmed in a computer using statistical computing software (SAS 1989) for statistical analysis. The analysis of variance and Duncan's multiple range tests had been carried out.

Results and Discussion

Microbiological evaluation of raw milk supplies from Assiut Vicinity

The microbiological evaluation of the foregoing milk samples was carried out by the determination of total microbial count total counts (c.f.u /ml), coliform titre and the estimation of the incidence of both aerobic and anaerobic spore forming bacteria. The obtained results are shown in table 1,2,3 and 4 table 1 shows a comparison microbial content of the three milk supplies.

Generally, studied milk samples had a high microbial content (8.94×10^{12} c.f.u /ml), about 43% of such samples recorded more than 10^8 c.f.u /ml. The general means of the three source are 1.28×10^{13} , 1.27×10^{13} and 1.82×10^8 c.f.u for El-Madabigh, Bany More and Assiut University samples respectively. The data indicate that highest microbial count was found in El-Madabigh samples and the lowest one in Assiut University farm samples. The foregoing indicates the poor hygienic conditions under which the milk was produced in Assiut province. Some

variation in total bacterial count of raw milk according to milk supplies were reported by Yadava et al. (1985) who found that the average plate counts of raw milk samples collected from three different sources were 0.22, 4.4 and 180.2×10^6 /ml. Results of Kiswa et al. (1996) revealed also a wide variation in the bacteriological quality of raw milk, where the highest quality milk was samples taken from the farm as compared with that taken from the collection point and the dairy factories.

Data showed that, cow's milk highly contaminated with microorganisms than buffalo's milk. About 47% of cow's milk supplies contained 10^{10} to 10^{15} c.f.u /ml, while only 17.6% of buffalo's milk samples showed the same range. About 53% of the buffalo's milk samples showed low count less than 10^6 c.f.u /ml. About 30% of buffalo's and 11% of cow's milk samples contained total microbial count ranging from 10^6 to 10^9 c.f.u /ml. This may be attributed to the difference in milk composition as well as the milk buffering action.

The general mean bacteria content of both types of milk is 5.59×10^{12} and 1.09×10^{13} c.f.u /ml for buffalo's and cow's milk respectively.

The difference in microbial counts between buffalo's and cow's raw milk have been also reported by Gary et al. (1977) observed low bacteria count in buffalo's milk ranged from 0.1 – 32×10^6 /ml than of cow's milk ($0.54-40 \times 10^6$ /ml). Some variations in

bacterial count of cow's and goats milk were reported by Abo Elnaga et al. (1985).

A high microbial count of raw milk was also reported by several investigators. Martins et al. (1977) showed that, 35% of raw milk samples had average bacterial count less than 10^8 /ml, 41% had count of 10^8 - 10^9 and 24% with more than 10^9 /ml. Mahari and Gashe (1990) found that, total plate counts of raw milk samples in Addis Ababa ranged from 4×10^7 to 1×10^9 c.f.u. /ml. while kausar et al. (1994) reported a viable count in raw milk of 1.23×10^6 /ml.

Pelczynska and Libelt (1995) pointed out that the average bacterial count in raw milk was 4×10^6 ml and recently Gomah (1999) found that total bacterial count of raw milk ranged from 7.4×10^4 to 88.0×10^6 c.f.u. /ml .

Results concerning the effect of climate temperature, on the microbial load of milk samples collected during warm months had high microbial count than that of cold months. The general mean was 1.4×10^7 and 1.39×10^{13} c.f.u / ml for samples collected during cold and warm months, respectively.

Generally, 75% of cold samples have less than 10^6 c.f.u. /ml and the highest total count was 5.25×10^8 c.f.u./ml. However, 69% of the warm month samples recorded more than 10^6 c.f.u./ml , 80% of them were highly contaminated, and had count

ranging from 10^{10} to 10^{15} c.f.u./ml. Same findings were reported by Abo-Elnaga and Abdel-Mottaleb (1967), Yankov (1967), Tsaregradskaya (1970) and Gaholf et al. (1975) which the bacterial counts of raw milk tended to be higher in summer than in winter. Mohran and Said (1991) , krsyanowski et al (1996) and Gomah (1999) concluded also that the highest bacterial load was found during summer months while the lowest one was in samples tested during the winter months.

Coliform bacteria were present in most of the studied raw milk samples. The obtained mean value was 3.16×10^4 bacterium per ml.

The obtained results showed that, about 26% of the examined samples were free from coliform bacteria or containing less than 10 bacterium per ml. It was also found that 24% of the samples contained 10^3 to 10^5 bacterium per ml and a few samples 7.1% contained from 10^5 to 10^7 .

The present results are in agreement with those of Hofi and Foda (1963), who reported that incidence coliform bacteria was in 73.3 to 99.3% of the examined samples.

Coliform bacteria were present in all raw milk samples examined by pashovskil (1974) with a titer of 10^5 to 10^6 /ml. on the other hand , a high incidence of coliform was found in raw milk samples by farag (1987), Moawad (1988) and Abdel-Hady

(1989) with a mean values of 10^{11} to 10^{13} bacterium per ml.

There were some variations in coliform content of the examined samples owing to their sources. The mean value of coliform bacteria in 88 raw milk samples collected from Arab El-Madabigh region was 1.1×10^5 /ml and 62.5% of these samples contained more than 10^3 bacterium per ml. while 38% contained 10^5 to 10^7 bacterium /ml. On the other hand, only 15% of the 129 samples collected from Bany-Mor contained more than 10^3 bacterium per ml, and 5% of these samples contained 10^5 to 10^6 bacterium per ml. The obtained mean value of milk samples of Bany-Mor station was 1.5×10^3 /ml.

On the same trend, most of the 92 raw milk samples obtained from Assiut University farm were characterized with a low coliform titre, mean value of 5.8×10^2 /ml. About 20% of these samples free or containing less than 10 cell, per ml and 42.4% showed coliform titre ranging from 10^2 to 10^4 /ml. Milk samples with coliform content more than 10^4 was very small (3.3%). Daivies (1977) recorded that, coliform were found in 80.6 and 5.71% of can and bulk milk samples respectively with an average of > 100 /ml.

Among the 195 samples of raw cow's milk 61 samples (31.3%) were free from coliform bacteria or containing less than 10 cell per ml. samples containing coliform bacteria ranging from 10^4 to 10^6 was very

small (4.1%) Buffalo's milk samples showed a high incidence of coliform bacteria than that of cow's milk with a mean value of 8.2×10^4 and 2.06×10^3 respectively. Singh and Ranganathan(1978) reported that the coliform count of cow's milk ranged from 5.0×10^2 to 5.0×10^4 /ml while buffalo's milk was highly contaminated with coliform bacteria ranging from 5×10^4 to 2×10^6 /ml. Abo-Elnaga et al. (1985) reported some variation also in the average of coliform counts of cows, ewe's and goat's milk samples.

The high incidence of coliform bacteria was found in samples obtained during warm months with general mean of 4.27×10^4 . A among 199 samples, a number of 74 samples (37.2%) contained more than 10^3 bacterium per ml, 25.7% of which contained 10^5 - 10^7 bacterium per ml. Samples free or contained less than 10 bacterium per ml were 2.6% of the samples.

On the other the mean value of coliform titre in cold months raw milk samples analyses was 1.15×10^4 bacterium per ml. About 20% of the samples were free or containing less than 10 bacterium per ml. samples with high coliform content ranging from 10^5 to 10^7 were very small (2.73%).

The forgoing results were in good agreement with those reported by Mohran and Said (1991). Who found that higher coliform content was obtained in sterile milk exposed to

milking stall air during summer months and the lower one was at winter months. This finding was also reported by Abo-Elnaga and Abdel-Mottaleb(1967), Boganowiz and Nochiewicz (1973) and Kaloyanov and Gogov (1977). Petrova and Petrova (1984) recorded that the coliform count of samples during the cold months ranged from 6×10^3 to 6×10^4 /ml and during the warm months ranged from 6×10^4 to 6×10^5 /ml. Aleksieva and Krushev (1981) reported that the coliform count of raw milk was correlated with the season, being much higher during the summer months.

Aerobic spore forming bacteria had been detected in most samples of raw milk. In 4.5% of the examined samples such spores were not detected in one ml of milk and 17.5% of these samples contained less than 10 spores per ml. The highly contaminated samples (6.5%) contained 10^4 to 10^6 spores /ml. Generally the mean value of aerobic spores incidence of the examined samples was 2.21×10^3 /ml.

Considerable variation in the presence of aerobic spores in raw milk were also reported by many investigators (Khalfalla et al., 1976., Stewart, 1978., Galli and Franzetti, 1985., Angless Mosso et al.1989., El-Shinawy, 1991 and Giffel et al.,1995). It was concluded that the major contamination sources of raw milk was aerobic spores are soil , faces and using unsanitised dairy equipments in dairy farms.

It could be observed that samples from Arab-Elmadabigh were highly contaminated with aerobic spores followed by those from Bany More station. The lowest aerobic spores counts were found in samples from Assiut University herd. The mean values of aerobic spores counts were 5.20×10^3 , 1.39×10^2 and 5.12×10^2 c.f.u /ml for the three sources respectively.

The distributions of aerobic spores numbers in El-Madabigh samples showed that 36% of these samples contained from 10^3 to 10^6 spore /ml .Samples containing less than 10 spores /ml or free from aerobic spores were 25% of the samples on the other hand raw milk samples from Bany More farm had relatively low number of spores than samples from El Madabigh region. Also spores were not detected in about 28% of 129 samples. Samples with relatively high number of aerobic spores (10^3 to 10^6 /ml) were about 11% of the total samples.

Raw milk samples obtained from Assiut University farm had the lowest number of aerobic spores. Only 15.22% of 92 samples contained 10^3 to 10^5 spores per ml while those free or contained less than 10 spores per ml was about 11% of samples. The present results clearly reveal that incidence of aerobic spores in raw milk depend on the hygienic conditions in the farm during milking. Saubois et al (1991) also reported that the incidence of aerobic spore counts

in raw milk was depended on of the samples source.

The interval distributions indicated that the incidence of aerobic spores was higher in buffalo's milk than in cows one. Generally, the mean values of aerobic spores counts in both buffalo's and cow's milk sample was 3.25×10^3 and 1.6×10^3 spores /ml respectively. The presence of higher number of aerobic spores in buffalo's milk than that of cow's milk possibly due to that most of the examined buffalo's milk sample were taken from El-Madabigh region (62.3%) where milking process was done under dusty air conditions, using unsanitized equipments and very low sanitary condition as well as animal movement during milking process.

Table (3) showed the influence of climatic temperature on the incidence of aerobic spores in raw milk samples collected during cold months of the year appeared to be less contaminated with this group than that obtained during the warm or hot months. The data indicate that, about 30% and 17.14% of the collected samples taken during the cold and warm months contained less than 10 spores/ml. Generally, the mean values of aerobic spores incidence in raw milk samples during cold and warm or hot months were 1.2×10^3 and 2.77×10^3 spores /ml respectively.

The present results are in agreement with those reported by Mohran and said (1991), who found high aerobic spores count in sterile milk exposed to

various air conditions in milking stall during summer and the lowest one was obtained during winter months . Such results also agree with those previously reported by Abo-Elnaga and Abdel-Mottaleb (1967).

The samples from Arab El-Madabigh were highly contaminated with anaerobic spores than those from Bany Mor and Assiut University farms. The mean values of anaerobic spore forming counts in samples of El-Madabigh, Bany- Mor and Assiut University were 43.26 and 11 spores per ml respectively. The foregoing results revealed that anaerobic spores present in about 90% of the investigated raw milk samples. This finding are in agreement with those reported by Abo-Elnaga and Abdel - Mottaleb (1967) who found that more than 65% of the examined raw milk samples contaminated anaerobic spore formers.

The buffalo's milk samples contained high level of anaerobic spores than cow's milk. The mean values of both are 40 and 19 spores /ml respectively. Samples collected during the cold months were low anaerobic spores content with a mean value of a bout 9 spore per ml while these collected during the warm months contained 46 spores /ml.

The present results are in good agreement with those of Mohran and said (1991) who found that in cold monthes coliform and anaerobic spores were absent or detected in few numbers, while the undesirable

bacteria were detected in relatively high levels during the warm and hot months. On the other hand, Abo Elnaga and Abdel-Mottaleb (1967) reported that market difference was observed between raw milk samples examined during summer and winter in their content of anaerobic spores. Statistically analysis of the obtained data demonstrated that there were significant differences in T.M.C., C.B. and AS among the three sources of raw milk samples and

always samples of El_Mandabigh were the poorest microbiological quality followed by that of Bany Mor and the higher microbiological quality were those. Samples obtained from Assiut University herd.

Buffalo's milk showed lower quality than cow's milk especially its coliform content. This may be attributed to the environmental condition during milking.

Table (1): Duncan's Multiple Range test for bacteriological evaluation: as effected by the source of milk samples.

Supplies	N	T.B.C.	CB	AS	ANAS
Arab-El-Madabigh	88	A 1.28X10 ¹³	A 1.08X10 ⁵	A 5.20X10 ³	A 4.31X10
Bany-Mor	129	A 1.27X10 ¹³	B 1.15 X10 ³	B 1.39X10 ³	A 2.58X10
Assiut University	92	B 1.82X10 ⁸	B 5.78X10 ²	B 5.12X 10 ²	A 1.12X10
General	309	2.99X10 ¹²	3.16X10 ⁴	2.21X10 ³	2.64X10

Table (2): Duncan's Multiple Range test for bacteriological evaluation as affected with the type of milk (cows or buffaloes).

Sources	N	T.B.C.	CB	AS	ANAS
Cow	195	A 1.09X10 ¹³	B 2.06X10 ³	A 1.6X10 ³	1.86X10
Buff	114	A 5.59X10 ¹²	A 8.21 X10 ⁴	A 3.25X10 ³	3.97X10

Table (3): Duncan's Multiple Range test for bacteriological evaluation as affected by sampling atmosphere.

Temp	N	T.B.C.	CB	AS	ANAS
Warm	199	A 1.39X10 ¹³	A 4.27X10 ⁴	A 2.77X10 ³	A 3.61x10
Cold	110	B 1.4X10 ⁷	A 1.15X10 ⁴	A 1.2X10 ³	B 8.78

Table (4): F-values for bacteriological quality of studied milk samples.

Sources	D.F	Dependent variable			
		T.B.C.	CB	AS	ANAS
Farms	2	3.76**	7.57**	3.76**	1.10*
Type	1	1.86*	2.94*	6.11 ^{NS}	1.89*
Temp	1	9.28***	5.35**	1.91*	4.03**
Factorial total	4	4.86***	10.16****	2.69**	2.40**

*,** signal 0.05 or 0.01 level, respectively

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دراسات على التلوث الميكروبي للبن الخام المنتج في منطقة أسيوط

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ارتبطت الألبان ومنتجاتها منذ قديم الأزل بحياة الإنسان والحيوان لما تتمتع من قيمة غذائية عالية وهبها الله إياها، وبالرغم من هذا فقد تكون سبباً في إحداهن الأمراض نتيجة لتلوثها ميكروبيولوجياً. ومن هنا تأتي أهمية توفير الشروط الصحية المناسبة لإنتاج لبن ومنتجات ذات مواصفات طبيعية وصحية عالية. وتهدف الدراسة إلى تقييم مدى جودة اللبن الخام المنتج في منطقة أسيوط من الناحية الميكروبيولوجية.

وتم ذلك بتجميع (٣٠٩) عينة لبن خام (أبقار وجاموس) على مدار سنة خلال الأشهر الحارة والباردة من ثلاث مناطق بأسيوط هي: ١. مزرعة كلية زراعة جامعة أسيوط (أبقار وجاموس)

٢. مزرعة بني مر (أبقار الفريزيان)

٣. بعض مربي الماشية (الأبقار والجاموس) بمنطقة عرب المدايغ حيث تستخدم مائة الصنف الصحي في الري وسقاية الحيوانات، وأظهرت النتائج مايلي.

متوسط العدد الكلي للميكروبات في العينات المختبرة $8,94 \times 10^{11}$ خلية / مل حيث تتراوح بين $1,82 \times 10^8$ لعينات قطيع كلية الزراعة جامعة أسيوط ($1,3 \times 10^{13}$) للعينات المجمعة من منطقة عرب المدايغ، وكانت ٤٣% من العينات التي درست ذات حمولة ميكروبية 10^8 خلية /ملي وكانت عينات اللبن البقرى ذات محتوى ميكروبي أعلى من عينات اللبن الجاموسى بمتوسط $1,1 \times 10^{13}$ ، $5,6 \times 10^{11}$ خلية/مل على الترتيب ، وقد وجد أن هناك اختلافاً معنوياً جداً بين متوسط المحتوى الميكروبي للعينات التي جمعت صيفاً وتلك التي جمعت خلال أشهر الشتاء البارد حيث كانت $1,4 \times 10^{13}$ ، $1,4 \times 10^7$ على الترتيب . وكان متوسط تلوث العينات ببكتريا القولون تعادل $3,16 \times 10^4$ حيث تتراوح ما بين ($5,8 \times 10^2$) لعينات قطيع كلية الزراعة ($1,1 \times 10^9$) للعينات المجمعة من منطقة عرب المدايغ.

وأن عينات اللبن البقرى كانت الأقل احتواءً على بكتريا القولون بالمقارنة بعينات اللبن الجاموسى ($2,06 \times 10^7$ بالمقارنة ب $8,2 \times 10^4$) كما أن العينات المجمعة صيفاً كانت الأعلى تلوثاً ببكتريا القولون عن عينات الشتاء ($4,3 \times 10^4$ بالمقارنة ب $1,15 \times 10^4$ خلية /مل).

وكانت معظم العينات المختبرة تحتوى على البكتريا المتجرثة الهوائية بمتوسط قدرة $2,2 \times 10^3$ خلية /مل ومدى يتراوح من $5,20 \times 10^3$ للعينات المجمعة من عرب المدايغ و $5,12 \times 10^2$ لعينات قطيع كلية الزراعة وأن درجة تلوث اللبن الجاموسى بالبكتريا المتجرثة الهوائية أعلى من درجة تلوث اللبن البقرى بمتوسط قدرة $3,25 \times 10^3$ ، $1,6 \times 10^3$ جرثومة /مل على الترتيب كما أن درجة تلوث العينات المجمعة صيفاً بالبكتريا الهوائية المتجرثة أعلى منها في العينات المجمعة شتاء- وبتوسط درجة تواجدتها $2,77 \times 10^2$ و $1,2 \times 10^3$ جرثومة /مل على الترتيب وكانت الجرثيم اللاهوائية توجد في حوالى ١٠% من العينات المختبرة بمتوسط قدرة $26,4$ جرثومة/مل . وكانت درجة التلوث في اللبن الجاموسى أعلى من اللبن البقرى ٤٠ بالمقارنة ب ١٩ جرثومة /مل . كما أن العينات المجمعة صيفاً احتوت على العدد الأكبر من الجرثيم اللاهوائية ٣٦ جرثومة /مل. لوحظ أيضاً وجود اختلافات معنوية جداً في الجودة الميكروبيولوجية من المناطق الثلاثة . كما أن عينات الصيف كانت الأقل جودة من عينات الشتاء.