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SANITARY STATUS OF RAW COW MILK MARKETED IN NORTHERN JORDAN

(With 9 Tables)

By

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الحالة الصحية للحليب الخام المتداول بالأسواق في شمال الأردن

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أجريت هذه الدراسة الكيميائية والميكروبية على (١٦٠) مائة وستين عينة حليب خام استنادا إلى اختبار تواجد أنزيم البروكسيديز ، وقد جمعت العينات بطريقة عشوائية من محلات الألبان بشمال الأردن لبيان مدى حالتها الصحية . وقد تبين أن متوسط معامل الأس الهيدروجيني PH للحليب هو ٦,٥٦ ، ومتوسط الحموضة المعيارية لهذا الحليب هو ١٩٩ ,.% مقدرة كحامض لاكتيك. وأظهرت الدراسة أن نسبة توافق نتائج الفحوصات التالية مع المواصفة القياسية الأردنية للحليب الخام هي: اختبار الحموضه المعياريه ٤٦ %, اختبار التخثر بالغليان ٧٨,٧٥% , اختبار اختزال الأزرق مثيلين ٧٧% واختيار الترسيب بالكحول ٧٣,٢% . وكان متوسط العدد الكلي للميكروبات الهوائية في المليلتر الواحد ١١,٢٠ X ١٠^{٦} مستعمره. وهذه النتيجة أظهرت أن ٣% فقط من العينات التي تم فحصها تتوافق مع المواصفة القياسية الأردنية . ولقد تواجدت ميكروبات القولون في ٨٨% من العينات التي تم فحصها بمتوسط للمليلتر الواحد ٢,٩٥ X ١٠^{٥} مستعمره. هذا ولقد وجدت المكورات العنقودية بمتوسط للمليلتر الواحد قدرة ١,٦٦ X ١٠^{٥} مستعمره وكان ٥٨,٤٧% من هذه المكورات العنقودية إيجابية لاختبار التخثير (Coagulase). وقد أمكن الكشف عن تواجد الميكروبات السبحية في العينات التي تم فحصها بنسبة ٨٦% وكانت ٣٢,٩% من هذه الميكروبات من الميكروبات السبحية البرازية . ولقد أتبعت الدراسة الكيميائية والميكروبية باستبيان متضمنا الأوضاع والأحول الفعلية للحالة التي جمعت عليها العينات من محال الألبان وكذلك من المزارعين والمنتجين لهذا الحليب . وخلصت الدراسة بأن الحالة الصحية للحليب الخام بشمال الاردن تحتاج للمزيد من التدقيق من الاشتراطات الصحية للمنتج ضمانا لسلامته ووصوله للمستهلك بصورة آمنة .

SUMMARY

A total of 160 raw cow milk samples were collected randomly from different dairy shops in northern Jordan at early morning during spring months (March to May) to determine their sanitary status. In addition, questionnaire concerned conditions of milk production, handling, transportation and marketing were answered by one hundred dairy farmers and one hundred milk retailers. The mean value of milk pH was 6.56 while its Titeratable acidity (T.A.) was 0.199%, which did not meet the Jordanian standards (JS). The percentages of compatibility of T.A., Clot On Boiling (COB), Methylene Blue Reduction Time (MBRT) and Alcohol Precipitation Test (APT) with the (JS) were 46%, 78.75%, 77% and 73.2%, respectively.

The mean value of Aerobic Plate Count (APC) was 11.2 x 10^6 cfu/ml which, was above the highest acceptable limit of the J.S. (4 x 10^6 cfu/ml). Only five samples (3%) had acceptable quality in respect to the APC. Coliforms had been isolated from 88% of the samples with a mean count of 2.95 X 10^5 cfu/ml. The mean Staphylococci count was 1.66 x 10^5 cfu/ml and 58.47% of Staphylococci isolates were positive to coagulase test. The incidence of Enterococci in raw milk was 86% from which 32.9% were *Enterococcus feacalis*. These results indicated that milk was highly contaminated with microorganisms and produced as well as marketed under unsatisfactory hygienic measures.

***Key words**: Sanitary, cow raw milk, marketing, Jordan*

INTRODUCTION

Milk is derived from the blood stream, therefore it is supposed to be sterile as the secretory cells in the udder produce it. As it moves through the mammary gland, it becomes contaminated with bacteria that reside within the teat canal and cistern. Most often, these bacteria are non-pathogenic like Micrococci, Streptococci and Bacilli. These types of bacteria are known as commensals, which has no relation with food-borne diseases. However, their presence in high numbers may cause spoilage of milk by means of fermentation, lipolysis and proteolysis (Bushnell, 1985 and Rea *et al.*, 1992).

During milk transportation until being consumed or processed, milk may be exposed to contamination by many types of microorganisms, due to bad handling practices (Anon, 1972, Desai and

Natarajan, 1982 and Anon, 1998). One of the main changes that occur in milk is lactose fermentation. This will affect the TA which, is a valuable guide in manufacturing operations and for measuring the quality of dairy product (Moustafa, 1988).

Microorganisms in milk are classified into two main categories, spoilage and pathogenic microorganisms. Spoilage microorganisms impart off-flavor, increased acidity and subsequent low keeping quality. Some of these are psychrotrophic microorganisms, such as *Pseudomonas fluorescence*, *Pseudomonas fragi*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Arthrobacter*, *Micrococcus* and *Streptococcus species*. (Farber *et al.*, 1988 and Alice, 1997). One of the most important pathogens in milk is *Staphylococcus aureus* that may cause food poisoning (Donnelly, 1990).

Good initial raw milk quality increased the period of keeping quality of pasteurized milk more than 22 days. In some cases, pasteurized milk had acceptable standard APC after 42 days of storage (Quinn *et al.*, 1994 and Desmasures *et al.*, 1997).

Health hazards may originate from ingestion of raw milk contaminated with pathogenic bacteria (Collins and Lyne, 1987, CAMPBELL *et al.*, 1993). The presence of *S. aureus* in milk indicates, beside its role in food poisoning, bad hygiene practice during milking, transportation, handling and retail sale (Richardson, 1989). The pathogenic microorganisms in milk include *Mycobacterium tuberculosis*, *Brucella abortus*, *Brucella melitensis*, *Clostridium botulinum*, *Salmonella spp.*, Entero-pathogenic *E. coli* O157: H7, *Bacillus anthracis* and pathogenic Streptococci (Felice *et al.*, 1999).

The objectives of this study are the evaluation of the conditions under which milk is produced, handled, transported and marketed in Jordan. In addition, the determination of fitness of milk for human consumption, depending on the Jordanian standards (Js4, 1987, Table 7).

MATERIALS and METHODS

Questionnaires concerning cow raw milk production and marketing:

Two forms of questionnaires have been designed, one (form A) for farmers and the other (form B) for retailers. One hundred dairy farmers were chosen according to the capacity of their farms and the other one hundred dairy retailers were chosen randomly to evaluate the condition of milk at production and retail level.

Sampling

A total of 160 raw cow milk samples were collected from supermarkets and retailers in all governorates of northern Jordan (Irbid 40, Ramtha 20, Mafrak 20, Jarash 25, Ajloun 20 and northern Jordan valley 35 samples). The samples were collected during March to May 1999, in the early morning un-cooled from containers of the dairy shops. Collection, transportation and preparation of samples were based on the guideline described by the American Public Health Association (APHA, 1992):

Sanitary and keeping quality methods:

Peroxidase test (APHA, 1992):

Only unheated milk samples were used for the sanitary and microbiological tests. Five milliliter of milk sample were transferred into a test tube, then 1 drop of 0.2% H2O2 and 2 drops of 2% paraphenyline diamine solution were added. The test tube was shaked well before reading the result. Appearance of dark blue color means raw milk while red or no color means heated milk (80 ^{0} C).

Methylene Blue Reduction Time "MBRT" :

Aseptically, 10 ml of thoroughly mixed milk sample were placed in a sterile test tube. Then, 1 ml of standard sterile methylene blue thiocynate solution was added to each tube. Tubes were closed tightly and inverted three times gently to completely spread the blue color, then incubated in water bath at 37^{o} C. The time of reduction of the color (decolorization) from blue to white was recorded after 20 minutes, then every hour for five hours. Tubes inverted gently to spread cream layer since microorganisms accumulate there. Any sample showed four-fifth of its column as white was recorded as decolorized (Hogan *et al., 1989*). Control tubes were incubated with each patch of experimental tubes.

PH value

Measuring the natural acidity of milk was determined by using electrometrical pH meter. Before use soak the glass electrode in a potassium chloride solution for at least 2 hrs. Clean the electrod with distil water and calibrate it daily by using buffer (pH: 7 & 4) and blot by tissue paper. Place 30 ml of milk in a clean beaker (warm milk to 25 ^{o} C) then immerse the electrode directly into the sample for at least 45 seconds until the reading stabilize then read the pH directly.

3.4 Titeratable Acidity (T.A.)(APHA, 1992):

In a porcelain dish, 9 ml of well mixed milk sample was transferred and diluted with 18 ml of carbon dioxide free water. Then, 1 ml of phenolphthalein (1% alcoholic solution) was added. After mixing, by using a burette containing sodium hydroxide solution N/10, drop by drop was added until the appearance of first persistent faint pink color. The amount of alkali used was recorded in titration procedure.

Clot On Boiling "COB" (APHA, 1992):

Clean test tube containing 5 ml of milk sample was placed in boiling water bath for 5 minutes. The tube was removed and rotated by hands without shaking. Clot formation or fine clot on the wall not restricted to the cream line indicated a positive result.

Alcohol Precipitation Test "APT" (APHA, 1992):

In a clean dry test tube, equal amounts (5 ml) of neutral ethyl alcohol 68% (w/v) and milk sample were mixed. Protein precipitate adheres to the wall of the tube was indicates a positive result.

Microbiological methods:

Preparation of samples and testing for; aerobic plates count, Staphylococci count, *S. aureus*, coliform count, Enterococci count and *E. faecalis* were performed according to the instructions of the Standard Methods for the Examination of Dairy Products (SMEDP) compiled by the APHA, Committee for the Microbiological Examination of Foods (Foster *et al.,* 1983 and Umoh, *et al.,* 1990, APHA, 1992, Carter *et al.,* 1995).

RESULTS

Questionnaire:

The results in Table 1 showed that 97% of the farmers did not have cooling system in their farms and all of them did not cool the milk during transportation. Also, the questionnaire indicated that 82% of the farmers did not clean their cows' udder before milking and 98% did not practice teat dipping. The hygienic status and the procedures concerning raw cow milk production in northern Jordan are shown in Table 1. Answers of 100 retailers are presented in Table 2.

Table 1: Answers of 100 farmers about application of hygienic procedures related to milk production in northern Jordan.

Parameter	Number of farmers	
	Yes	No
Cooling system in the farms	03	97
Disposable of mastitic milk	90	10
Periodic detection of subclinical mastitis	0	100
Selling of milk containing antibiotics	03	97
Examination of udder before milking	15	85
Udder cleaning before milking	82	18
Teat dipping practice	02	98
Use of disinfectants	0	100
Availability of separate milking place	02	98
Dairy farms workers certified- in dairy production	0	100
Presence of insects and rodents in farms	92	08

Table 2: Answers of 100 retailers about milk selling process in northern Jordan

Parameter	Number of farmers	
	Yes	No
Shop licensed for selling milk and milk products	100	0
Workers health certificate	100	0
Cooling milk during selling process	15	85
Presence of insects	100	0
Presence of rodents	40	60
Presence of sediment (hair, straw and soil) in milk	59	41
Milk pasteurization	02	98
Milk filtration	80	20
Use of disinfectants in cleaning	0	100

Table 3: Results of pH tests of 160 examined cow raw milk samples collected from northern Jordan.

PH range	No. of samples	Mean ± S.E.
6.2-6.3	7	6.56 ± 0.11
6.3-6.4	15	
6.4-6.5	29	
6.5-6.6	37	
6.6-6.7	40	
6.7-6.8	32	

Sanitary parameters

Milk acidity:

PH test:

Table 3 illustrate the pH values of 160 milk samples examined. The pH range between 6.2-6.8 with a mean value of 6.56 ± 0.11.

Titeratable acidity:

Results of T.A. are demonstrated in Table 4. Only 74 out of 160 milk samples examined had T.A.= 0.18% or less. The mean value of T.A. was 0.199% ± 0.033. The correlation coefficient (r) of pH and T.A. for the examined milk samples was found to be 0.785 at the 0.01 level of 2-tailed analysis.

Table 4: Results of the titeratable acidity tests of 160 examined cow raw milk samples collected from northern Jordan.

T.A. values	No. of samples	Mean ± S.E.
0.16	16	0.199 ± 0.033
0.17	26	
0.18	32	
0.19	30	
0.20	13	
0.21	4	
0.22	5	
0.23	3	
0.24	3	
0.25	5	
0.26	3	
0.27	4	
0.28	2	
0.29	5	
0.30	4	
0.31	3	
0.32	2	

Reduction time of methylene blue:

Results are shown in Table 6. One hundred-twenty three (77%) of the examined milk samples reduced the methylene blue beyond two hours, while 37(23%) samples induced reduction within less than two hours.

Clot on boiling and alcohol precipitation:

Results are demonstrated in Table 5.

Table 5: Clot on boiling test (COB) and alcohol precipitation test (APT) results of 160 raw cow milk samples collected from northern Jordan.

Parameter	Clotted		Unclotted	
	No.	%	No.	%
COB	34	21.25	126	78.75
APT	43	26.8	117	73.2

Table 6: Results of reduction time of methylene blue for 160 examined raw cow milk samples collected from northern Jordan

Reduction time of methylene blue			
More than two hours		Less than two hours	
No. of samples	%	No. of samples	%
123	77	37	23

Table 7: Percentage of agreement of the sanitary and microbiological parameters of 160 cow raw milk samples with the Jordanian standards (JS4:1987)

Parameter	JS4:1987	Percentage of agreement
pH	≥ 6.4	86.3
Titeratable Acidity	0.18%	46
Reduction Time of Methylene Blue	≥ 2 hours	77
Clot On Boiling	Not clotted	78.7
Alcohol Precipitation	Not precipitated	73.2
Aerobic Plate Count	$< 4X10^6$ cfu/ml	41.87
Pathogenic bacteria	Free	*S. aureus* present in 58.4% of samples

Microbiological parameters:

All bacterial parameters are presented in Table 8.

Aerobic plates count:

The total aerobic count ranged between $9.1X10^4$ cfu/ml and $9.3X10^8$ cfu/ml with a mean of $11.2X10^6 \pm 2X10^6$ cfu/ml.

Table 8: Frequency of the bacterial parameters of 160 cow raw milk samples collected from northern Jordan.

Parameters	Min.	Max.	Mean ± S.E.	+ ve samples	
				No.	%
Aerobic plates count	$9.1X10^4$ cfu/ml	$9.3X10^8$ cfu/ml	$11.2X10^6 \pm 2 \times 10^6$	160	100
Staphylococci count	$1X10^3$ cfu/ml	$1.3X10^6$ cfu/ml	$1.66X10^5 \pm 9.8X10^4$	128	80
Coliform count	$2.5X10^4$ cfu/ml	$1.4X10^6$ cfu/ml	$2.95X10^5 \pm 1X10^5$	142	88.7
Enterococci count	$7.3X10^4$ cfu/ml	$8.5X10^6$ cfu/ml	$6.3X10^5 \pm 2.3X10^5$	137	86.6

Coliforms count:

Only 142 (88.75%) milk samples were contaminated by coliforms. The total coliforms count ranged between $2.5X10^4$ cfu/ml and $1.4X10^6$ cfu/ml, while the mean was $2.95X10^5 \pm 1X10^5$ cfu/ml.

Total Staphylococci:

Only 128 (80%) milk samples were contaminated by Staphylococci. The Staphylococci count ranged between $1.0X10^3$ cfu/ml and $1.3X10^6$ cfu/ml. The mean value was $1.66X10^5 \pm 9.8X10^4$ cfu/ml. Results of coagulase test are illustrated in Table 9 and revealed that 75 (58.4%) of the Staphylococcus isolates were positive and 53 (41.6%) was negative.

Enterococcus and *Enterococcus faecalis* counts:

Only 137 (86.6%) milk samples were contaminated by Enterococci. The Enterococcus count ranged between $7.3X10^4$ cfu/ml and $8.5X10^6$ and the mean value was $6.3X10^5 \pm 2.3X10^5$ cfu/ml. Forty-five (32.9%) of the Enterococci isolates were identified as *Enterococcus feacalis*.

Table 9: Results of coagulase tests for Staphylococci isolates obtained from 160 cow raw milk samples collected from northern Jordan

No. of isolates	CPS [1]		CNS [2]	
128	No.	%	No.	%
	75	58.4	53	41.6

CPS: Coagulase positive Staphylococci
CNS: Coagulase negative Staphylococci

DISCUSSION

During dairy retailers' visits, milk was found to be kept in open buckets, which lead to contamination. Retailers usually use plastic cups to fill the Polypropylene bags with milk. These cups were often left on the table where they were exposed to dirt and flies. Most of dairy shops were found in confined places lacking ventilation and cleaning and there were no uses of disinfectants. The mentioned conditions of milk production and handling may be responsible for the increase in the bacterial counts, which affects all values of sanitary tests.

Teat dipping "which is not usually practiced by Jordanian farmers" is highly effective in reducing the occurrence of mastitis and then reducing contamination of raw milk (Farag, 1987). One of the faulty practices was the postponement of delivery of the evening milk to the next day due to lack of transportation.

The mean of T.A. demonstrated in this study was higher (0.199%) than that of *J.S.4* (1987) as demonstrated in Table 7. However, it complies with Walstra *et al.*, *(1984)*, who reported that T.A. of most milk examined ranged from 0.14 to 0.21%.

In this study, compatibility rate (CR) of the pH readings with the JS 4 was 86.3% and the mean value was 6.56, which is acceptable by JS.4 (Table 7). Whereas, the CR of T.A. was merely 46%. The differences in CR between pH and T.A. equals 40%. This difference is due to many factors such as solid-not-fat content, speed of titration, atmospheric temperature and quantity of the diluent. The JS.4 specifications accept wide range of pH readings, ranging from 6.4 to 6.8. This wide range included 86% of the examined milk samples. The data of this study showed that when the value of pH increased towards 6.8, the value of T.A. decreased toward less than 0.18%.

Also, the high T.A. values were comparable with growth of high numbers of bacteria, which has been found higher than that of the JS.4. The correlation of T.A. and pH was found to be 0.785 and for aerobic plate count and pH was 0.735, which means that there were almost linear correlation between T.A. and pH and between APC and pH for the tested milk.

Jordanian specifications, indicated that the reduction time must be not before two hours, which means the presence of not more than $4X10^6$ cfu/ml raw cow milk as a presumptive count. Reduction time is generally inversely proportional to the bacterial content of the milk sample at the start of incubation *(*APHA, 1992*)*. In this study, 23% of the examined milk samples have reduced methylene blue within less than 2

hrs, which indicated that 77% of the tested milk samples have bacterial contents within the accepted level by J.S. The MBRT test is a strong parameter of sanitary status of milk, according to which raw milk can be graded as grade 1 (RT>5hrs), grade 2 (RT>4hrs) and grade 3 (RT> 2hrs).

Clotting of milk by boiling occurs when T.A. is 0.23% or more (APHA, 1992). The J.S. reported that the (COB) positive test indicates unfitness of milk for heat treatment. This investigation demonstrated that boiling clotted 34 samples (21%). The COB results were comparable with the results of alcohol precipitation test where 43 (26%) out of 160 samples were precipitated (Table 7).

Further, the mean value of APC was higher than that of J.S. (<4X 10^6 cfu/ml, Table 7), and higher than that of Das and Nag, 1986; Rea *et al.*, 1992 and Desmasures *et al.* 1997. However, its close to the findings of Aesiyun (1994) where the counts of 507 raw cow milk samples ranged from $5.8X10^5$ to $5.7X10^8$ cfu/ml and close to that of Farag (1987) where the mean value of 100 samples of raw cow milk examined was $1.4X10^7$ cfu/ml.

The achieved results proved that 3% of the samples had low bacterial count and considered as grade A milk. By tracing their source, these samples were found produced from healthy cows, under high hygienic conditions and stored in efficient cooling system. Grade A milk found in this study was low when compared with that in USA, 85% (Horowitz, 1982). However, findings of this study indicated the presence of 35% of the tested samples were of grade C.

Moreover, 88.75% of the tested samples were contaminated with coliform. This indicates that the tested milk was contaminated with *E. coli*, *Klebsiella spp.* and *Enterobacter spp* that may originate from soil, utensils, feed and feces. The mean value of coliform counts was higher than the accepted limit of the J.S. Teat washing and drying of cows, reduced the total aerobic bacterial counts by 40% and Streptococcal and coliform counts by 50% (Lampert, 1975).

The results of this investigation were in agreement with those found in Trinidad by Rea *et al.* (1992). In contrast, it differs from that of Ombui *et al.* (1994a) in Kenya, where 42.2% and 10.3% of the milk samples collected from farmer cans and cooperative cans, respectively.

Raw milk may become contaminated by Staphylococci from; unclean udder, milker hands and mastitic milk (Lancette and Tatini, 1992). Many studies ascertained that *S. aureus* in raw milk might originate from sub-clinical or clinical mastitis and its presence in the raw

milk had been reported in different surveys (Abo-El-Naga *et al.*, 1985 and Ombui *et al.*, 1994b). The pathogenic Staphylococci; *S. aureus*, *S. intermedius* and most of *S. hyicus* are coagulase positive. Its presence in raw milk points to contamination, poor handling and production conditions (Carter *et al.*, 1995), whereas the coagulase negative Staphylococci such as *S. epidermidis* and *S. saprophyticus* occur as commensals and in the surrounding environment (Ombui *et al.*, 1994b). This ascertained that the tested milk was contaminated and carries risk of infection and food poisoning to the consumers.

Enterococci such *as E. faecalis, E. faecium*, and *E. durans,* may cause infection to human and animals and their presence in milk indicates faecal contamination (Farag, 1987; Andrade *et al.*, 1998 and Batish and Rangnathen, 1998). Also, *E. faecalis* is a common inhabitant of the intestinal tract of animals, humans and insects (Carter *et al.*, 1995). Most strains of *E. faecalis* can survive traditional milk pasteurization procedures up to 72^{o} C and resist traditional freezing procedures. It can grow in medium containing 6.5% NaCl and at pH 9.6 (Ravanis and Lewis, 1995). These properties ascertain the necessity of heat treatment to higher than 72^{0} C.

Moustafa (1988) studied Enterococci in raw milk produced in Egypt and found the mean was 1.22×10^{6} cfu/ml, which is two times higher than that found in this study. Also, Moustafa ascertained the presence of Enterococci in 92% of the examined samples, which is close to the findings of this study. Farag (1987) in Egypt examined 100 milk samples and reported Enterococci in 97% of the samples. Further, he found that the mean of Enterococci is 5.3×10^{4} cfu/ml which, is close to the results of this study.

The current results allow to conclude that the raw cow milk produced in northern Jordan was highly contaminated with various bacterial species, including; *S. aureus*, other *Staphylococcus spp.*, coliforms and *E. faecalis*. The presence of such high bacterial counts indicated that milk was produced, handled, transported and stored under unhygienic conditions. A high agreement of correlation between TA, pH and APC of the tested milk was found. The agreement of the results of sanitary tests with that of JS varies from 46 to 78%.

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