Prevalence of *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in raw milk

Mohamed E.A. Nasef
Animal Health Research Institute , Damietta Branch , ARC , A . R . E .

Abstract
A total of 100 random samples of raw milk were collected from cooler tanks in different supermarkets at Damietta Governorate, A. R. E and examined for prevalence of *Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The obtained results revealed that Bacillus cereus was detected in 20% of total raw milk samples and its count ranged from 2.0 x 10^2 to 6.0 x 10^4 cfu/ml with a mean count 9.12 x 10^3 cfu/ml, Pseudomonas aeruginosa was detected in 5% of total examined samples, while Staphylococcus aureus was detected in 24% of total examined samples and its count ranged from 2.2x10^2 to 2.7x10^5 cfu/ml with a mean count 3.94 x 10^4 cfu/ml of raw milk. All isolates of Staph. aureus from raw milk were found coagulase–positive strains and examined for staphylococcal enterotoxins (SE). None of *S. aureus* strains which isolated from raw milk samples produced enterotoxins A, B, C, D or E. Significance of the obtained results on raw milk quality, public health risk, prevention and control as well as recommendations for production of raw milk safe for human consumption were discussed.

Key words:
raw milk , *Bacillus cereus , Pseudomonas aeruginosa, Staphylococcus aureus,* enterotoxins A, B, C, D, E

Introduction

Milk is very rich in nutrients which provide the nutrional needs of human body rather than any other single food in the nature. Unfortunately, it is considered to be an ideal medium for growth of several types of microorganism which contaminate milk from various sources during milking, handling, cooling and distribution. These contaminants impair milk utility and consumption. Moreover, milk from cows with sub-clinical mastitis accidentally mixed into bulk milk enters food chain and poses a threat to human health. Bacillus cereus is a common contaminant of raw milk whose spores survive pasteurization. Bacillus cereus produce two different types of toxin known as an agent of food poisoning and include diarrhoeal type and emetic type **(Eley, 1996).** *Pseudomonas aeruginosa* is a pychrotrophic bacteria that contaminate and grow in raw milk , produce proteinase and lipase, while the pathogenic strains produce heat- labile entertoxins **(Mickova *et al.,* 1989 and Grover *et al.,* 1993).** While Staphylococcus aureus produce thermostable enterotoxins which are serologically distinct types and implicated in staphylococcal food poisoning **(Eley, 1996).** Therefore, this work was planned to throw light on the content of raw milk contamination with *Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus* and their public health significance.

Materials and Methods

1- Collection samples.

A total of 100 random samples of raw milk (500cc each) were collected from cooler tanks in different supermarkets at Damietta Gover-

norate. Each raw milk samples was kept into sterile capped bottle, labeled and sealed separately to avoid contamination. The collected samples were kept in sealed containers with ice cubes and transmitted to the laboratory for examination without delay.

2- Preparation of collected samples. (A.P.H. A., 1992)

Each sample container was mixd thoroughly and vigorsously by shaking before examination. Eleven ml of each raw milk sample were added to 99 ml of peptone water / saline solution into sterile bottles and thoroughly mixed to make a dilution of 1/10, from which decimal serial dilutions were prepared.

3- Enumeration and identification of Bacillus cereus.

Plate count technique using spread plate inoculation method recommended by **(Kim and Geopferet, 1971)**. 0.25 ml from each prepared dilution of raw milk sample was transferred and evently spread onto the surface of Bacillus cerus medium supplemented with polymyxin B and egg yolk emulsion. All plates with inculated media were incubated at 30 ^{o}C for 24 hr aerobically. Three or more suspected turquoise blue colonies of Bacillus cereus which surrounded by precipitated zone of lecithinase activity were purified and streaked onto blood agar plates and incubated at 30 ^{o}C for 24 hr aerobically for further confirmation and identification with biochemical tests according **to (Cowan and Steel, 1974)** and **(Winn *et al.,* 2006).** Total number of Bacillus cereus per milliliter of each raw milk sample was calculated and recorded.

4- Enumeration and identification of *Staph. aureus* (A.P.H.A., 2001)

Plate count technique using spread plate inculation method was used. 0.25 ml from each prepared dilution of raw milk samples was transferred and evently spread onto the surface of Baired –Parker agar plates and incubated at 35 ^{o}C for 24-48 hr aerobically. Appearance of black colonies surrounded by clear halo zone was considered to be presumptive Staph.aureus and used for further purification by subcultivation on Mannitol salt agar according to **(Winn *et al.,* 2006).** Confirmation and identification of the suspected colonies of staph.aureus were done by using various biochemical tests according to the methods described by **(Cruickshank *et al.,* 1975)**, while the confirmation by coagulase test was done according to the method described by **(Monica, 1991)**. Total count of coagulase-positive Staph.aureus per mililiter of each raw milk sample was calculated and recorded.

5- Isolation and identification of *Pseudomonas aeruginosa* (Roberts *et al.,* 1995).

One ml of each raw milk sample was inoculated into 10 ml of sterile nutrient broth and incubated at 37^{o}C for 24 hr aerobically . A loopful from the incubated tubes was transferred and evently streaked onto the surface of Pseudomonas selective agar medium with CFC Pseudomonas supplement. All inoculated plates were incubated at 25-30 ^{o}C for 24-48 hr aerobically. Suspected colonies were small and blue- green in colour. The purified colonies were inoculated onto plates of King's medium **(King *et al.,* 1954)** and incubated at 37^{o}C for 48 hr to enhance pyocyanin production which distinguished as blue-green in colour. All suspected colonies of Ps.aeruginosa were used for further identification with biochemical tests according to **(Krieg and Holt, 1984 and Winn *et al.,* 2006).**

6- Detection of staphylococcal enterotoxins (SE).

Staphylococcus aureus strains which isolated from raw milk samples in present study were examined for production of staphylococcal enterotoxins A,B,C,D and E by using Enzyme-Linked Immuno-Sorbent Assay (ELISA) according to **(Freed *et al.,* 1982 and Lapeyre *et al.,* 1988).**

Results and Discussion

The results recorded in Table (1) revealed the incidence and total cell count of Bacillus cer-

eus in the examined raw milk samples which collected from cooler tanks in different supermarkets at Damietta Governorate during this study. The obtained results showed that Bacillus cereus was isolated from 20 samples which represent 20% of total examined raw milk samples and its count was ranged from $2.0x10^2$ to $6.0x10^4$ cfu/ml with a mean count of 9.12×10^3 cfu/ml. Lower incidence and count of B.cereus in raw milk samples than the obtained results were reported by **(Hahn *et al.,* 1999; Lim *et al.,* 2000; Berthold and Molska, 2002; Kivaria *et al.,* 2006; Vyletelova *et al.,* 2011 and Jackson *et al.,* 2012)**. While, higher incidence and count of B.cereus in raw milk were recorded by **(Larsen and Jorgensen, 1997 and Bedi *et al.,* 2005).** Nearly similar incidence of B. Cereus in raw milk was recorded by **(Ruusunen *et al.,* 2013).**

The sources which may result in contamination of raw milk with (psychrotrophic) Bacillus cereus spores and vegetative cells on farms include air, soil, grass, bedding (used and unused), feed (hay, silage, concentrates, beet), drinking water, faeces, teats, udders, milking equipment and tankers **(Giffel *et al.,* 1995; Huang *et al.,* 1998; Christiansson *et al.,* 1999 and Magnusson *et al.,* 2007).** In addition, bacterial biofilm which formed on surfaces of stainless steel due to inadequate sanitization of dairy equipments and tanks of milking, storage and transporting protect food- borne pathogens against external threats and lead to contamination of raw milk by Bacillus cereus, Pseudomonas aeruginosa and Staphylococcus aureus **(Munsch-Alactossava and Alatossava, 2006; Simoes *et al.,* 2010; Raats *et al.,* 2011 and Bayoumi *et al.,* 2012).** Bacillus cereus is a common contaminant of raw milk whose spores survive pasteurization. Bacillus cereus diarrhoeagenic enterotoxin was detected in both raw and pasteurized milk samples submitted for routine quality analysis and the enterotoxin was less affected by heating in milk **(Baker and Griffiths, 1995 and Odumeru *et al.,* 1997).** While, emetic toxin of B.cereus is a heat stable and can withstand exposure at 121°C for 90 minutes **(Eley, 1996).**

Table (1). Statistical analytical results of total Bacillus cereus count / ml of raw milk samples.

No. of examined samples	Positive samples		Min.	Max.	Mean ±SE
	No.	%			
100	20	20	$2.0X10^2$	$6.0X10^4$	$9.12X10^3 \pm 2.81X10^3$

Bacillus cereus is an environmental microorganism having thermoduric and psychrotrophic properties. B.cereus having proteolytic and lipolytic activities in dairy products stored under refrigeration **(Montanhini and Bersot, 2013).** Acidification is a common method of preservation and as the acidity of fermented milk increased, B. cereus fails to survive **(Hassan *et al.,* 2010).** Presence of Bacillus cereus in raw milk may represent a risk of food poisoning due to B.cereus diarrhoeal enterotoxin **(Baker and Griffiths, 1995 and Odumeru *et al.,* 1997).** In addition, the shelf life of pasteurized dairy products depends partly on the concentration of Bacillus cereus spores in raw milk **(Vissers *et al.,* 2007).**

Results recorded in Table (2) revealed that only five samples of the examined raw milk were contaminated by Pseudomonas aeruginosa and representing 5% of total examined raw milk samples. Nearly similar incidence of Pseudomonas aeruginosa in raw milk samples was reported by **(Uraz and Citak, 1998),** while lower incidence was reported by **(Ahmed *et al.,* 2002).** Higher incidence of Pseudomonas aeruginosa in raw milk samples than the obtained results was recorded by **(Otte *et al.,* 1978; Mickova *et al.,* 1989 and Ezzeldeen *et al.,* 2004).**

The sources which may result in contamination of raw milk with Pseudomonas aeruginosa in farms include milking equipments, moist areas of the cow's body (udder and teats), drinking bowls, feeds, floors and milker's hands **(Otte *et***

***al.,* 1978).** Moreover, apparently healthy lactating cows with subclinical mastitis secrete milk that looks apparently normal for long time and may be contaminated with Pseudomonas aeruginosa when implicated in cases of bovine subclinical mastitis **(Malinowski *et al.,* 2006 and Ibrahim, 2007).** Ps.aeruginosa is a psychrotrophic bacteria that contaminate and grow in raw milk, produce proteinase and lipase, while the pathogenic strains produce heat-labile enterotoxins **(Mickova *et al.,* 1989 and Grover *et al.,* 1993)**. As Ps.aeruginosa is destroyed by HTST pasteurization, it is suggested that raw milk should be pasteurized before consumption **(Otte *et al.,* 1978).**

Table (2). Incidence of Pseudomonas aeruginosa in the examined raw milk samples.

No. of examined samples	Positive samples	
	No.	%
100	5	5

Results recorded in Table (3) revealed that Staphylococcus aureus was isolated from 24 samples which represent 24% of total examined raw milk samples which collected from cooler tanks during this study and its count was ranged from $2.2x10^2$ to $2.7x10^5$ cfu/ml with a mean count of $3.94x10^4$ cfu/ml of raw milk. All isolated strains of Staph.aureus were found coagulase positive. Higher incidence and count of Staph.aureus in raw milk samples than the obtained results were recorded by **(Bainotti *et al.,* 1990; Adesiyun *et al.,* 1995; Gomes and Gallo, 1995; Jorgensen *et al.,* 2005 and Guecuekoglu *et al.,* 2012),** while lower incidence was reported by **(Tsegmed *et al.,* 2007; Zouharova and Rysanek, 2008; Fagundes *et al.,* 2010; Alian *et al.,* 2012 and Gundogan *et al.,* 2012).**

Table (3). Statistical analytical results of total Staphylococcus aureus count / ml of raw milk samples.

No. of examined samples	Positive samples		Min.	Max.	Mean ± SE
	No.	%			
100	24	24	$2.2x10^2$	$2.7x10^5$	$3.94x10^4 \pm 1.17x10^4$

Staph.aureus is a contagious pathogen, causing a wide range of infections in both human and animal. It can be found as a commensal on the skin and in the anterior nares of the nose. This organism may be present in the air , in milk and in sewage. It produce thermostable toxins known as staphylococcal enterotoxins which are serologically distinct types (A, B, C1, C2, C3, D,E and TSST-1) and implicated in staphylococcal food poisoning except TSST-1 **(Eley, 1996).** Staph.aureus was implicated in many cases of both clinical and subclinical mastitis in bovines which consider the main source of bulk tank milk contamination with Staph.aureus **(Workineh *et al.,* 2002; Kalmus *et al.,* 2011** and **Sharma *et al.,* 2012).** Most strains of Staph. aureus isolated from raw milk produce Staphylococcal enterotoxins (SE) which may be transmitted to the dairy products during processing and represent a direct risk to the health of consumers **(Borelli *et al.,* 2006; Joffe and Baranovics, 2006; Arcuri *et al.,* 2010; Hunt *et al.,* 2012 and Valero- Leal, 2012).** When raw milk was subjected to heat treatments equal to or greater than pasteurization, no staphylococci were detected in the resulting heated milk or cheese **(Zottola *et al.,* 1969).**

The obtained results revealed that none of the 24 strains of staph.aureus which isolated from raw milk samples produced enterotoxins A, B, C, D, or E. This obtained result was in an

agreement with the results which reported by **(Castro *et al.,* 1986; Gomes and Gallo, 1995 and Tsegmed *et al.,* 2007).**

Conclusion & Recommendations

Data given by the obtained results from this work indicate that some samples of raw milk which exposed for sale in cooler tanks of supermarkets at Damietta Governorate are of inferior quality due to its contamination with Bacillus cereus, Pseudomonas aeruginosa and Staphylococcus aureus. Contamination of raw milk with these pathogenic and psychrotrophic bacteria may lead at time either to development of objectionable change rendering the milk unfite for consumption or increase the risk of food poisoning and infection with respective pathogenic bacteria. Contamination of raw milk with these types of pathogenic bacteria may be due to improper hygienic measures during milking, handling, transportation , cooling , storage and using unsanitized dairy equipments and cooler tanks for raw milk distribution. Moreover, raw milk of cattle with subclinical mastitis may be accidentally mixed into bulk milk and represent an additional route of raw milk contamination with these pathogenic bacteria. Therefore, to save a lot of raw milk from being contaminated and deteriorated on the markets and safeguard the consumers from being infected, the following suggestions are recommended: routine examination of dairy animals and elimination of both diseased and mastitic cases from milk production until treated. Strict hygienic measures should be applied for healthy udder, mastitic udder, teat dip, milking machine, dairy utensils, bulk tanks, cooler tanks and worker's hands. Application of the proper hygienic practices and periodical medical examination of workers and persons who sharing in production, handling, cold storage and sale of raw milk. Educational programmes should be imposed for milk producers and handlers to improve the quality of raw milk and its products to ensure maximum safty to the consumers. Adequate sanitization of dairy equipments and tanks which used for milking, storage, transporting and sale of raw milk to prevent bacterial biofilm. Pasteurization as well as acidification are common methods of milk preservation.

References

Adesiyun, A.A.; Webb, L. and Rahaman, S. (1995). Microbiological quality of raw cow's milk at collection in Trinidad. Journal of Food Protection, 58 (2): 139-146.

Ahmed, F.; Ismail, A.M. and Tanios, A.I. (2002). Occurrence of Pseudomanas in raw cow's milk and trials to control Ps. aeruginosa in butter and tallaga cheese. Vet. Med. J. Giza, 50 (4): 589-599.

Alian, F.; Rahimi, E.; Shakerian, A., Momtaz, H.; Riahi, M. and Momeni, M. (2012). Antimicrobial resistance of Staphylococcus aureus isolated from bovine, sheep and goat raw milk. Global Veterinaria, 8 (2): 111-114.

American Public Health Association "A.P.H.A." (1992). Standard methods for the examination of dairy products. 16th Ed; Washington, USA.

American Public Helth Association "A.P.H.A." (2001). Compendium of methods for the microbiological examination of foods. 2nd Ed., Washington, D.C., USA.

Arcuri, F.E; Angelo, F.F.; Guimaraes, F.M.M.; Talon, R. and Borges, F.M. (2010). Toxigenic status of Staphylococcus aureus isolated from bovine raw milk and Minas frescal cheese in Brazil. Journal of Food Protection, 73 (12): 2225-2231.

Bainotti, A.; de Mendoza, M.C. and Simonetta, A. (1990). Microbiological quality of raw milk. II. Incidence of Enterobacteriaceae, Coliform and Staphylococcus aureus. Revista Argentina de Lactologia, 2(3): 15-28.

Baker, J.M. and Griffiths, M.W. (1995). Evidence of increased thermostability of Bacillus cereus enterotoxin in milk. Journal of Food Protection, 58(4): 443- 445.

Bayoumi, M.A.; Kamal, R.M.; Abe El Aal, S.F. and Awad, E.I. (2012). Assessment of a regulatory sanitization process in Egyptian dairy plants in regard to the adherence of some food-borne pathogens and their biofilms. International Journal of Food Mi-

crobiology, 158(3): 225- 231.

Bedi, S.K.; Sharma, C.S.; Gill, J.P.S., Aulakh, R.S. and Sharma, J.K. (2005). Incidence of enterotoxigenic Bacillus cereus in milk and milk products. Journal of Food Science and Technology, 42(3): 272-275.

Borelli, B.M.; Ferreira, E.G.; Lacerda, I.C.A.; Santos, D.A.; Carmo, L.S.; Dias, R.S.; Silva, M.C.C. and Rosa, C.A. (2006). Enterotxigenic Staphylococcus spp. and other microbial contaminants during processing of Canastra cheese, Brazil. Brazilian Journal of Microbiology, 37: 545-550.

Berthold, A. and Molska, I. (2002). The occurrence of Bacillus cereus in raw milk. Zywnosc, 9 (3, supplement): 8-17.

Castro, R.; Schoebitz, R.; Montes, L. and Bergdoll, M.S. (1986). Enterotoxigenicity of Staphylococcus aureus strains isolated from cheese made from unpasteurized milk. Lebensmittel - Wissenschaft und- Technologie, 19 (5): 401-402.

Christiansson, A.; **Bertilsson, J. and Svensson, B. (1999).** Bacillus cereus in raw milk : factors affecting the contamination of milk during the grazing period. Journal of Dairy Science, 82(2): 305-314.

Cowan, S.T. and Steel, K.J. (1974). Manual for the identification of medical bacteria. 2nd Ed. Cambridge Univ. Press, London, England.

Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, H.A. (1975). Medical microbiology. The practice of medical microbiology. 12 Edition, Vol. II. Churchill, Edinburgh.

Eley, A.R. (1996). Microbial Food Poisoning.2nd Ed. Chapman & Hall, 2-6 Boundary Row London SE1 8HN, UK.

Ezzeldeen, N.A.; Abdel-Fattah and Soliman, Y.A. (2004). Phenotypic and molecular characterization of Pseudomonas areuginosa of animals and human origin. J. Egypt. Vet. Med. Assoc. 64 (1): 93-104.

Fagundes, H.; **Barchesi, L.; Filho, A.N.; Ferreira, L.M. and Oliveira, C.A.F. (2010).** Occurrence of Staphylococcus aureus in raw milk produced in dairy farms in Sao Paulo state, Barazil. Barazilian Journal of Microbiology, 41 (2): 376-380.

Freed, R.C.; Evenson, M.L.; Reiser, R.F. and Bergdoll, M.S. (1982). Enzyme-Linked Immuno-Sorbant Assay for detection of Staphylococcal enterotoxin in foods. Appl. Environ. Microbiol., 44:1349-1355.

Giffel, M.C.; Beumer, R.R.; Slaghuis , B.A. and Rombouts, F.M. (1995). Occurrence and characterization of (psychrotrophic) Bacillus cereus on farms in the Netherlands. Netherlands milk and Dairy Journsl, 49 (2/3) : 125-138.

Gomes, H. de A. and Gallo, C.R. (1995). Occurrence of Staphylococcus aureus and enterotoxins production by isolated strains from raw milk, pasteurized milk type C and " Minas Frescal" cheese, commercialized in Piracicaba-SP. Ciencia e Tecnologia de Alimentos, 15 (2): 158-161.

Grover, S.; Batish, V.K. and Srinivasan, R.A. (1993). Growth, proteolytic and enterotoxic activities of Pseudomonas aeruginosa CTM-3 in milk, ice-ceram and butter. Archiv fuer Lebensmittelhygiene, 44 (5): 115-117.

Guecuekoglu, A.; Kevenk, T.O.; Uyanik, T.; Cadirci, O.; Terzi, G. and Alisarli, M. (2012). Detection of enterotoxignic Staphylococcus aureus in raw milk and dairy products by multiplex PCR. Journal of Food Science, 77 (11): M620-M623.

Gundogan, N.; Ataol, O. and Gunal, S. (2012). Determination of some virulence factors in staphylococci isolated from milk and meat products. Journal of Food safty and Food Quality / Archiv fuer Lebensmittelhygiene, 63 (6): 182-186.

Hahn, G.; walte, H.G.; Coenen, C.and Teufel, P. (1999). Direct marketing of raw milk : finding and risk consideration. Kieler Milchwirtschaftliche Forschungsberichte, 51 (2) : 105-115.

Hassan, G.M.; Al-Ashmawy, M.A.M.; Meshref, A.M.S. and Afify, S.I. (2010). Studies on enterotoxigenic Bacillus cereus in raw milk and some dairy products. Journal of Food Safety, 30 (3): 569-583.

Huang, C.C.; Lai, S.H.; Liau, S.M.; Huang,

C.H.; Wang, S.L. and Yue, S.J. (1998) : The contamination of the equipment for raw milk handling by Bacillus cereus. Journal of the Chinese Society of Animal Science, 27 (3): 409-419.

Hunt, K.; Schelin, J., Radstroem, P.; Butler, F. and Jordan, K. (2012). Classical enterotoxins of coagulase – positive Staphylococcus aureus isolates from raw milk and products for raw milk cheese production in Ireland. Dairy Science & Technology. 92 (5, special issue: a selection of papers from Eighth Cheese symposium, Moorepark, 28-29 september 2011): 487- 499.

Ibrahim, H.S. (2007) : Bacterial study on the bovine mastitis in Assiut Governorate with special reference to virulence of some isolates. J. Egypt. Vet. Med. Assoc., 67 (2): 57 – 74.

Jackson, E.E.; Erten, E.S.; Neeraj Maddi; Graham, T.E; Larkin, J.W.; Blodgett, R.J.; Schlesser, J.E. and Reddy, R.M. (2012). Detection and enumeration of four foodborne pathogens in raw commingled silo milk in the United States. Journal of Food Protection, 75 (8): 1382 – 1393.

Joffe, R. and Baranovics, E. (2006). Bovine mastitis as the primary contamination source of milk and milk products with S. aureus entertoxins. Veterinarijair Zootechnika, 36 (58): 21- 26.

Jorgensen, H.J.; Mork, T.; Hogasen, H.R. and Rorvik, L.M. (2005). Enterotoxigenic Staphylococcus aureus in bulk milk in Norway. Journal of Applied Microbiology, 99 (1): 158- 166.

Kalmus, P.; Aasmae, B.; Karssin, A.; Orro, T. and Kask, K. (2011). Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. Acta Veterinaria Scandinavica, 53:4.

Kim,H.U. and Goepfert, J.M. (1971). Enumeration and identification of Bacillus cereus in foods. Applied Microbiology 22:581-587.

King, E.O.; Waed, M.K. and Raney, D.E. (1954). Two sample media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med.; 44: 301-307.

Kivaria, F.M.; Noordhuizen, J.P.T.M. and Kapaga, A.M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. Tropical Animal Health & Production, 38 (3): 185-194.

Krieg, N.R. and Holt, J.G. (1984). Bergy's Manual of Systematic Bacteriology. The Williams and Wilkins, Baltimore/ London.

Larsen, H.D. and Jorgensen, K. (1997). The occurrence of Bacillus cereus in Danish pasteurized milk. International Journal of Food Microbiology, 34 (2) : 179-186.

Lapeyre, C.; Janine, F. And Kaveri, S.V. (1988). Indirect double sandwich ELISA using monoclonal antibodies for detection of Staphylococcal enterotoxins A,B,C1 and D in food samples. Food Microbiol., 5: 25-31.

Lim, J.H.; Kim, Y.H.; Ahn, Y.T. and Kim, H.U. (2000). Studies on the contamination and inhibition of Bacillus cereus in domestic raw milk and milk products. Korean Journal of Animal science, 42 (2): 215- 222.

Magnusson, M.; Christiansson, A. and Svensson, B. (2007). Bacillus cereus spores during housing of dairy cows : factors affecting contamination of raw milk. Journal of Dairy Science, 90 (6): 2745-2754.

Malinowski, E.; Lassa, H.; Hossowska, A., Smulski, S.; Markiewicz, H. and Kaczmarow, S.M. (2006). Etiological agents of dairy cow's mastitis in Western part of Poland. Pol. J.Vet. Sci., 9 (3): 191-194.

Mickova, V.; Lukasova, J. and Konecny, S. (1989). Pseudomonas aeruginosa in raw and pasteurized milk. Veterinarni Medicina, 34 (7): 411-419.

Monica, C. (1991). Medical Laboratory Manual for Tropical Countries. Vol. ll. ELBS, 60-63.

Montanhini, M.T.M. and Bersot, L.D.S. (2013). Evaluation of psychrotrophic behavior and lipolytic and proteolytic activity of Bacillus cereus isolated from refrigerated dairy products. Maringa, 35 (1): 163-167.

Munsch-Alatossava, P. and Alatossava, T. (2006). Phenotypic characterization of raw

milk- associated psychrotrophic bacteria. Microbiological Research, 161: 334-346.

Odumeru, J.A.; Toner, A.K.; Muckle, C.A.; Griffiths M.W. and Lynch, J.A. (1997). Detection of Bacillus cereus diarrheal enterotoxin in raw and pasteurized milk. Journal of Food Protection, 60 (11): 1391-1393.

Otte, I.; Hahn, G. and Tolle, A. (1978). Occurrence, detection and significance of Pseudomonas aeruginosa in raw milk and in the environment of dairy cattle. Milchwissenschaft, 33 (12): 737-739.

Raats, D.; **Offek, M.; Minz, Dror and Halpern, M. (2011).** Molecular analysis of bacterial communities in raw cow milk and impact of refrigeration on its structure and dynamics. Food Microbiology, 28:465-471.

Roberts, D.; Hooper, W. and Greenwood, M. (1995). Practical food microbiology methods for the examination of food for microorganisms of public health significance. 2nd Ed. Public health laboratory service. London.

Ruusunen, M.; Salonen, M.; Pulkkinen, H.; Huuskonen, M.; Hellstrom, S.; Revez, J.; Hanninen, M.L.; Fredriksson-Ahomaa, M. and Lindstrom, M. (2013). Pathogenic bacteria in Finnish bulk tank milk. Foodborne Pathogens and Disease, 10 (2): 99-106.

Sharma, A., Singh, R. and Beigh, S.A. (2012). Antibiogram profile of bacterial isolates from mastitic crossbred cattle of Jammu region. Veterinary Practitioner, 13 (1): 84-85.

Simoes, M.; Simoes, L.C. and Vieira, M.J. (2010). A review of current and emergent biofilm control strategies. LWT-Food Science and Technology, 43: 573-583.

Tsegmed, U.; Normanno, G.; Pringle,M. and Krovacek, K. (2007). Occurrence of enterotoxic Staphylococcus aureus in raw milk from yaks and cattle in Mongolia. Journal of Food Protection, 70 (7): 1726-1729.

Uraze, G. and Citak, S. (1998): The isolation of Pseudomonas and other Gram-negative bacteria in raw milk. J. Basic Microbiol., 38 (2): 129-134.

Valero-Leal, K.; Rivera-Salazar,J.; Valbuena, E.; Boscan, L.; Valeris, R.; Castro, G. and Brinez, W. (2012). Biochemical characterization and enterotoxins production by Staphylococcus aureus strains isolated from raw milk and traditional fresh cheese in dairy farms of Zulia state. Revista Cientifica, 22 (4): 303- 314.

Vissers, M.M.M.; Giffel, M.C.; Driehuis, F., Jong, P. and Lankveld, J.M.G. (2007). Predictive modeling of Bacillus cereus spores in farm tank milk during grazing and housing periods. Journal of Dairy Science, 90(1): 281 -292.

Vyletelova, M.; **Roubal, P.; Karpiskova, R., Vlkova, H., Hanus, O. and Bubikova, M. (2011).** Microbiological quality of milk from milk venfing mschines in Jesenik. Mlekarake Listy, (No. 126, Suppl., Veda Vyzkum): XVIII-XXI.

Winn, W.; Allen, S.; Janda,W.; Koneman, E.; Procop, G.; Schreckenberger P. and WooG. (2006). Konemana's color Atlas and Textbook of Diagnostic microbiology. 6th Ed., Lippincott Williams & Wilkins. London and New York.

Workineh, S.; Bayleyegn, M.; Mekonnen, H. and Potgieter, L.N.D. (2002). Prevalence aetiology of mastitis in cows from two major Ethiopian dairies. Tropical Animal Health and Production, 34(1): 19-25.

Zottola, E.A.; Jezeski, J.J. and Al-Dulaimi, A.N. (1969): Effect of short-time subpasteurization treatments on the destruction of Staphylococcus aureus in milk for cheese manufature. Journal of Dairy Science, 52 (11): 1707-1714.

Zouharova, M. and Rysanek, D. (2008). Multiplex PCR and RPLA identification of Staphylococcus aureus enterotoxigenic strains from bulk tank milk. Zoonoses and Public Health, 55 (6): 313-319.

مدى تواجد ميكروب باسيلس سيرس وميكروب سدومونس إيروجينوزا وميكروب المكور العنقودي الذهبي في اللبن الخام

محمد السعيد عبد الحميد ناصف

معهد بحوث الصحة الحيوانية (فرع دمياط) – مركز البحوث الزراعية

الملخص العربى

أجريت هذه الدراسة للوقوف على مدى تواجد كل من ميكروب باسيلس سيرس وميكروب سدومونس إيروجينوزا وميكروب المكور العنقودي الذهبي في عدد مائة عينة عشوائية من اللبن الخام الموجود بالمبردات في منافذ البيع المختلفة بأسواق قرى ومدن محافظة دمياط . وقد أظهرت النتائج وجود ميكروب باسليس سيرس في عدد 20 عينة (20%) وميكروب سدومونس إيروجينوزا في عدد 5 عينة (5%) وميكروب المكور العنقودي الذهبي في عدد 24 عينة (24%) من إجمالي عدد عينات اللبن الخام . كما أظهرت النتائج أن العدد الكلي لميكروب باسيلس سيرس تراوح من 2x 10^{2} إلي 6x 10^{4} خلية / سم3 ومتوسط العد 9.12 x 10^{3} خلية / سم3 وأن العد الكلي لميكروب المكور العنقودي الذهبي تراوح من 2.2 x 10^{2} إلي2.7 x10^{5} خلية / سم3 ومتوسط العد 3.94 x10^{4} خلية / سم3 من عينات اللبن الخام الإيجابية .كما أظهرت النتائج أن جميع عترات المكور العنقودي الذهبي المعزولة من العينات كانت إيجابية لإنزيم التجلط وثم اختبارها لإفراز سموم المكورات العنقودية . هذا وقد تم مناقشة النتائج وتوضيح الأهمية الصحية والاقتصادية لتواجد مثل هذه الميكروبات في اللبن الخام ووضع التوصيات والمقترحات اللازمة للحد من تواجد تلك الميكروبات وإنتاج لبن صحي آمن للاستهلاك الآدمي .