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Microbial species of safety concern in milk from informal processors in Harare, Zimbabwe

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In this study, the bacteriological quality and the presence of *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Salmonella* species, and *Pseudomonas* species were studied in raw milk, cultured milk, milk handlers and packaging containers. A total of 36 samples were collected over 3 months from three different farmers. Samples were analyzed for means of counts per milliliter of milk for total bacterial count (TBC), total coliform count (TCC), total *E. coli* count (TEC), *S. aureus*, *B. cereus, Salmonella* spp. and *Pseudomonas* spp. Microbial load ranged between 0.81 and 7.6 $\log_{10}$ cfu/ml for various critical sampling locations. Isolates of *E. coli*, *S. aureus* and *B. cereus* were taken for simple polymerase chain reaction (PCR) to investigate the presence of virulent genes, *rfB*, *sei*, and *cytK* with amplicon sizes of 1.0 kb, 500 bp and 320 bp, respectively. The *sei* gene was detected in 19% of the samples and 2.8% were found to have the *cytK* gene. The *rfB* gene could not be picked in *E. coli*. The results show poor hygienic practices at the processors and potential risk to the consumers.

Key words: Fresh milk, fermented milk, packaging container, milk handler, pathogens.

INTRODUCTION

The occurrence of several serious food safety problems in the last decade has put higher demands on the control and assurance of food safety and quality by all actors in the agro-food chain (Jacxsens et al., 2010). Although other dairies have made significant efforts and investments in designing and implementing good hygiene practices, using hazard analysis and critical control point (HACCP) based systems (CAC, 2003) the dairy industry is still faced with the challenge of implementing good hygiene practices to ensure the production of safe products especially in the developing countries (Milios et al., 2012; Kussaga et al., 2014). While it has been reported that fresh drawn milk from a healthy cow normally contains a low microbial load (<3 $\log_{10}$ cfu/ml), the load may increase by 2 $\log_{10}$ cfu/ml, or more, once it is stored at room temperature (Walstra et al., 2005;

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Bytyqi et al., 2011). Several studies have reported the presence of both gram positive and gram negative pathogens in the dairy industry (Amagliani et al., 2012; De Vliegher et al., 2012; Gurol et al., 2012; Lee et al., 2012, 2014; Ranieri et al., 2012; Barancelli et al., 2013). Control activities should therefore be instituted to prevent microbial contamination and growth in production areas thereby reducing pathogens (Luning et al., 2008; Jacxsens et al., 2010; Milios et al., 2012).

Consumption of unprocessed milk and its by-products is potentially hazardous and has been associated with several types of infections including brucellosis (Ramos et al., 2008), tuberculosis (Doran et al., 2009), salmonellosis (Poppe, 2011), yersinosis (Greenwood and Hooper, 1990), *Escherichia coli* O157:H7 (Anand and Griffiths, 2011) and staphylococcal enterotoxins poisoning (Baylis, 2009; Ostyn et al., 2010). Despite modern dairy ensuring that consumers get processed safe milk, consumption of unprocessed milk is still common in Zimbabwe especially in the rural areas with the two main types being fresh liquid milk and naturally fermented milk. Although the food regulations in Zimbabwe impose a general responsibility to ensure safety of any food brought to the market on the producer, this has not always been the case due to a number of factors including, cultural, economic and poor legislation enforcement among others.

The Zimbabwean dairy industry can be easily categorised into 2 groups, the formal and the informal sector with the latter posing greater safety concerns. The informal dairy sector mainly produces two products, raw milk termed “fresh milk” and the naturally fermented/cultured milk (Amasi/Hodzeko). The latter product taking greater preference, because it is used as a relish which provides proteins at a low cost for the lower class while it is a delicacy for the affluent that they consume as part of the main meal or dessert. Due to the faltering economy of Zimbabwe, many dairy farmers have been downsizing operations and ultimately preferring to sell their milk product directly to consumers without supplying any processor (Gadaga et al., 1999; Gran et al., 2003).

Fermentation of milk is important as it is a cheap traditional way of improving nutritional properties as well as sensory properties (Gadaga et al., 1999). It is not only effective on flavour development but has been used as a preservation method for many years; hence, fermented foods are regarded as microbiologically safe. The low pH is effective in inhibiting the growth of many microbes (Jeevartnam and Jamuna, 2004). *Staphylococcus aureus* among other pathogens is destroyed by pH lower than 5 (Medveďová and Valík, 2012). However, emerging research has shown that some strains of *E. coli* can survive the low pH (Gran et al., 2003; Bore et al., 2007). A combination of harsh conditions and prolonged exposure time has been said to result in microbes building some adaptation mechanism (Lucking et al., 2013). The ability of pathogens such as *E. coli* to survive low pH emerges as a potential health hazard to consumers of the naturally fermented milk, which is generally regarded as a safe product in Zimbabwe due to its low pH. The aim of this research was to determine the incidence of pathogens (*E. coli, S. aureus,* and *Bacillus cereus*) in fresh milk, naturally fermented/cultured milk, milk handlers as well milk packaging containers used at farm level in the informal sector in Zimbabwe.

MATERIALS AND METHODS

Three farms (FD 1, FD 2 and FD 3) around Harare province were selected for the research. The microbial assessment scheme (MAS) methodology developed by Jacxsens et al. (2011) was used to determine the food safety status in the informal milk processing farms. A total of 4 critical sampling locations (milk handler, milk container, fresh milk, and cultured milk) were selected. A total of 4 samples (milk handler swab, milk container swab, 50 ml fresh and cultured milk) from each dairy farm were collected during each visit and the sampling was done 4 times over a three month period. The samples were transported at 4°C to Government Analyst Laboratory for analysis within 3 h of collection. Samples were analyzed for total aerobic bacteria count (TBC), total coliform count (TCC), *E. coli, Salmonella* species, *Pseudomonas* species, *S. aureus*, and *B. cereus*. Methods of microbial analysis are summarized in Table 1. After enumeration, isolates were, purified and stored at -20°C in 30% sucrose solution awaiting polymerase chain reaction (PCR).

PCR Reactions

B. cereus, S. aureus and *E. coli* isolates were resuscitated and DNA was extracted according to procedures described in Medina-Acosta and Cross (1993). All PCRs were carried out in 10 µl reaction tubes in a Bio-Rad T100™ Thermal Cycler (Singapore). The reaction mix consisted of 1 µl bacterial DNA template, 5 µl KAPA BIOSYSTEMS 2X KAPA Taq Ready Mix (Cape Town, South Africa), 3.4 µl PCR-grade water, and 0.6 µl primers. The primers and annealing temperatures used in the reactions are listed in Table 2. The PCR conditions for *cytK* are described in Swiecicka and Mahillon (2006), while conditions for *rfB* and *sei* are described in another study (Thapa et al., 2012). PCR products were separated by Gel electrophoresis using a 2% Agarose gel ULTRAPURE™ (USA) and the image was viewed using a Bio-Rad Gel Doc™ EZ (USA).

Statistical analysis of data

Descriptive statistics and other explanatory analysis were used as well as statistical techniques: univariate analysis (one way analysis of variance [ANOVA]) using SPSS version 16.0 and STATA 12 adjusted for multiple comparisons to detect significance difference between means. The significance test was at alpha level 0.05 or 95% confidence level.

RESULTS

Total bacterial count (TBC)

The average (TBC) for three batches was found to be low

Table 1. Microbiological analysis methods used to enumerate and isolate bacteria.

Organism	Media	Manufacturer	Incubation temperature (°C)	Incubation time (h)	References
Total bacterial count	Plate Count Agar (PCA)	Oxoid Basingstoke Hampshire, England	25, 30, 55	24	Duncan et al. (2004)
Total coliform	MUG Violet Red Bile (VRB)	Oxoid Basingstoke Hampshire, England	37	24	ISO 4832:2006
B. cereus	Tryptone Soya Agar (TSA)	Biolab, South Africa	37	24	ISO 7932:2004
E. coli	Tryptone Bile X-glucuronide (TBX)	Oxoid Basingstoke Hampshire, England	44	24	Aijuka et al. (2015)
S. aureus	Baird-Parker	Biolab, South Africa	37	24	Aijuka et al. (2015)

Table 2. PCR primers for *B. Cereus*, *S. aureus* and *E. coli*.

Microorganism	Target gene	Sequence	References
B. cereus	*cytK*	GAAACGGGCGCTGTTATCTT TGCTGCTTACGCTCAAACGA	Swiecicka and Mahillon (2006)
S. aureus	*Sei*	CAGGCAGTCCATCTCCTGTA AAAGGCGTCACAGATAAAAACC	Thapa et al. (2012)
E. coli	*rfB*	TAAGTAATGGAACGGTTGCTCT CCCCACTCGTAAAATCCATC	Thapa et al. (2012)

at FD 1 where it was ranging from 3.61 to 5.57 $\log_{10}$ cfu/ml in packaging container and fresh milk, respectively. Generally, the TBC in packaging containers was found to be lower than in milk handler, fresh milk and cultured milk in all the three dairies. The highest TBC was recorded in cultured milk from FD 2 and 3 which had 7.3 and 7.6 $\log_{10}$ cfu/ml, respectively. There was no change in levels of TBC from batch 1 to batch 3 during the sampling period. This is supported by an average of 3.49 $\log_{10}$ cfu/ml in milk handler batch 1 to 5.73 $\log_{10}$ cfu/ml in milk handler batch 3. Batch 3 had the highest average of TBC in all the three CSLs, ranging from 5.0 to 8.3 $\log_{10}$ cfu/ml.

Total coliforms

FD 1 had the highest coliforms ranging from 1.56 to 6.22 $\log_{10}$ cfu/ml in packaging container and fresh milk, respectively. FD 2 had the lowest coliforms, ranging from not detected in milk handler to 3.94 $\log_{10}$ cfu/ml in fresh milk. When comparing the growth pattern of coliforms in fresh milk and cultured milk, fresh milk had the highest number of coliforms.

Total *E. coli*

The milk handler from FD 2 and 3 had no *E. coli* as well as the packaging container in FD 1. The highest number of *E. coli* was recorded in fresh milk collected from FD 1 and milk handler of FD 1. There was a gradual increase in *E. coli* in the fresh milk to cultured milk from FD 3. Fresh milk had 1.78 $\log_{10}$ cfu/ml and cultured milk had 2.21 $\log_{10}$ cfu/ml.

S. aureus

All three dairies were found to have *S. aureus* at all CSLs. The highest number of *S. aureus* was recorded in milk handler of FD 3 (4.4 $\log_{10}$ cfu/ml). The lowest was in the same dairy in cultured milk

with log 1.98 $\log_{10}$ cfu/ml. Packaging containers of FD 1 had high levels of *S. aureus* (4.18 $\log_{10}$ cfu/ml).

Pseudomonas spp.

The incidence of *Pseudomonas* spp. was generally low in all three dairies. However, the fresh milk collected from FD 1 had high levels of *Pseudomonas* (4.16 $\log_{10}$ cfu/ml) and the minimum recorded was 0.68 $\log_{10}$ cfu/ml. No incidence of *Pseudomonas* spp. was recorded in FD 3 in cultured milk and milk handler from FD3.

B. cereus

The highest incidence of *B. cereus* in all CSLs was recorded in FD3 followed by FD1. The least incidence was recorded in FD 2. Fresh milk in all three dairies had higher levels of *Bacillus* species when compared with cultured milks of all the three dairies.

PCR reactions

The genomic DNA for *S. aureus, B. cereus,* and *E. coli* were subjected to PCR to investigate the presence of virulent genes, *rfB*, *sei*, and *cytK* with amplicon sizes of 1.0 kb, 500 bp and 320 bp, respectively. The *sei* gene was detected in 19% of the samples and 2.8% were found to have the *cytK* gene. The *rfB* gene could not be picked in *E. coli*.

DISCUSSION

TBC results were not in agreement with previous studies (Al-Tarazi et al., 2003) who reported considerably lower levels of TBC in fresh milk with mean values of 1.1×10^7 cfu/ml. However, they concurred with previous studies in Zimbabwe that showed high TBC in raw milk (Gran et al., 2003). While it is reported that raw milk TBC should be less than 100 000 cfu/ml (Gosta, 1995), Zimbabwean food regulations require that raw milk should not exceed 20 000 cfu/ml (Food and Food Standard Act, 2001). High bacterial load in raw milk has been attributed to poor hygienic practices that include failure to properly clean the udder and use of contaminated water among others (Gran et al., 2003). The bacteria in milk handler are also likely to find its way into the raw milk and other subsequent stages of milk processing (Gran et al., 2003). In food safety, implementation of prerequisites and application of HACCP guarantee the control of processes (Domenech et al., 2013). The absence of documented hygienic practice followed by the processors in this study might also have contributed to high levels of contaminants. Cusato et al. (2014) also included diagnosis of prerequisites programs (PRPs), implementation of good manufacturing practices (GMP), standard sanitation operating procedures (SSOP) and training of the food as the major steps in the implementation of food safety system. However, such conditions are likely to be found in formal organizations which are well established mostly with food safety management systems. Out of the fourteen PRPs (CAC, 2003; Jacxsens et al., 2010; Luning et al., 2008) informal processors in this study can concentrate on cleaning and disinfection, personal hygiene of the milk handlers, and temperature control as recommended by Kussaga et al. (2014) as a way of improving the microbiological quality of the milk. This concurs with Lee et al. (2012) who pointed out that high frequency of *S. aureus* isolates in dairy farms highlights the need for constant improvement of hygiene and quality assurance.

While fermentation results in increased acidity that discourages the growth of most pathogens (Gran et al., 2003), our results showed the presence of potential pathogens in both fresh milk and fermented milk. Our findings on coliforms and *E. coli* were in agreement with previous studies (Saudi and Moawad, 1990; Ahmed and Sallam, 1991; Sobeih et al., 2002; Al-Tarazi et al., 2003; Chye et al., 2004; Korashy and Mohammed, 2008; Altalhi and Hassan, 2009) who reported that all examined fresh milk samples were contaminated with coliforms. These results were in violation of both European and Zimbabwean standards for raw milk intended for processing and milk for consumption (Gran et al., 2003). These findings concurred with a previous study in Zimbabwe that concluded on poor hygiene and sanitary practices during milking and further handling (Gran et al., 2003).

In this study, fresh milk with 4.16 $\log_{10}$ cfu/ml of *Pseudomonas* spp. was found to be the most prevalent. The presence of *Pseudomonas* spp. in both fresh and fermented milk indicates that *Pseudomonas* spp. can survive in fermented foods. This is in violation of food regulations (Dairy Regulations, 1977; Food and Food Standards Act, 2001; Public Health Act, 2001; CAC, 2003) where the food is not supposed to have either pathogenic or spoilage organisms.

In spite of generally low *E. coli* counts, its presence is suggestive of poor hygienic indicators, faecal contamination and implies a risk that other enteric pathogens may be present in the product (Gran et al., 2003). The findings concur with previous studies which indicated that poor hygiene was evidenced by the presence of *E. coli* (Ghafir et al., 2008; Jacxsens et al., 2009; Sampers et al., 2010). The presence of *E. coli* violates regulations which prohibit the presence of pathogenic and spoilage microorganisms in any food item intended for human consumption (Dairy Regulations, 1977; Food and Food Standards Act, 2001; Public Health Act, 2001; CAC, 2003). The results in this study indicated that the *E. coli* survives in fermented milk. This concurs with another study which reported that acid adapted cells

showed a marked increase in levels of resistance to lactic acid even though the level of resistance varied among strains (Gregory et al., 1995). Our results showed that none of our strains belonged to O157:H7. This contradicted with a previous study that suggested the presence of the pathogenic *E. coli* in a number of food items (Prasad et al., 2012).

The presence of *S. aureus* at all the CSLs in all the dairies contravening various food regulations was in agreement with a previous study (Gran et al., 2003). The isolation rate observed in this study was higher than was reported by Abdel-Hammed and El Malt (2009) who reported that 24% were contaminated with *S. aureus.* Dissemination of *S. aureus* from humans to food can occur by direct contact, indirectly by skin fragments, or through respiratory tract droplet nuclei (Jablonski and Bohach, 1997). *S. aureus* is also commonly found in mastitis udder (Wellenberg et al., 2002), hence common in milk from animals suffering from mastitis. The minimum amount of *S. areus* required to produce intoxication in human is estimated to be about 5 $\log_{10}$ cfu/g (Rørvik and Granum, 1999). To produce sufficient enterotoxin, the pH should be higher than 4.6 and the temperature should be above 15°C for more than 3 to 4 h (Rørvik and Granum, 1999). If *S. aureus* gains access to the milk before fermentation, the pH would have been higher than 4.6 for longer than 6 h and therefore pose a definite risk of toxin production during the early part of the fermentation. Our findings on the presence of the toxin producing *sei* gene in *S. aureus* was in line with a previous study (Nazari et al., 2014), although we could not distinguish between the most toxic *sei* 1 and *sei* 2. Similar to previous studies (Larsen and Jogernsen, 1997), *B. cereus* was isolated in milk and other sampling locations. The prevalence of the *cytK* in *B. cereus* from our study was in line with previous studies that concluded that its distribution is low in *B. cereus*.

Conclusion

Fresh and fermented milk from informal processors in Harare pose a potential threat to consumers and this is in violation of both the Zimbabwean and international food regulations. Poor hygiene practices by the milk handlers maybe the greatest link of milk contamination. The presence of virulent genes (19% *S. aureus* and 2.8% *B. cereus*) confirms that consumers of fermented milk are at safety risk.

Conflicts of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Abdel-Hameed KG, El-Malt LM (2009). Public health hazard of *Staphylococcus aureus* isolated from raw milk and ice cream in Qena Governorate. Assiut Vet. Med. J. 55(121):191-200.

Ahmed AM, Sallam SS (1991). Public health significance of coliforms in raw milk and domiati cheese. Assiut Vet. Med. J. 25(50):89-92.

Aijuka M, Charimba G, Hugo CJ, Buys EM (2015). Characterization of bacterial pathogens in rural and urban irrigation water. J. Water Health 13(1):103-117.

Altalhi AD, Hassan SA (2009). Bacterial quality of raw milk investigated by *E. coli* and isolates analysis for specific virulence -gene markers. Food Control 20:913-917.

Al-Tarazi Y, Al-Zamil A, Shaltout F, Abdel-Samei H (2003). Sanitary status of raw cow milk marketed in northern Jordan. Assiut Vet. Med. J. 49:180-194.

Amagliani G, Petruzzelli A, Omiccioli E, Tonucci F, Magnani M, Brandi G (2012). Microbiological surveillance of a bovine raw milk farm through multiplex real-time PCR. Foodborne Path. Dis. 9(5):406-411.

Anand SK, Griffiths MW (2011). In: Fuquay JW, Fox PF and McSweeney PL. Encyclopedia of dairy sciences. Academic Press. pp. 67-71.

Barancelli GV, Camargo TM, Gagliardi NG, Porto E, Souza RA, Campioni F, Falcão JP, Hofer E, Cruz AG, Oliveira CA, (2014). Pulsed-Field Gel Electrophoresis characterization of *Listeria monocytogenes* isolates from cheese manufacturing plants in São Paulo, Brazil. Int. J. Food Microbiol. 173:21-29.

Baylis CL (2009). Raw milk and raw milk cheeses as vehicles for infection by Verocytotoxin-producing *Escherichia coli*. Int. J. Dairy Technol. 62(3):293-307.

Bore E, Langsrud S, Langsrud Ø, Rode TM, Holck A (2007). Acid-shock responses in *Staphylococcus aureus* investigated by global gene expression analysis. Microbiology 153(7):2289-2303.

Bytyqi H, Bigler S, Muji S, Jahja A, Zaugg U (2011). Survey on raw milk quality in Kosovo. Food Nutr. Sci. 2(5):414.

CAC-Codex Alimentarius Commission (2003). Hazard analysis and critical control point (HACCP) system and guidelines for its application. ANNEX to recommended international code of practice/ general principles of food hygiene. CAC/RCP 1969. Rev 4. FAO/ WHO

Chye FY, Abdullah A, Ayob MK (2004). Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol. 21:535-541.

Cusato S, Gameiro AH, Sant`Ana AS, Corassin CH, Cruz AG, de Oliveira CAF (2014). Assessing the costs involved in the implementation of GMP and HACCP in a small dairy factory. Qual. Assur. Saf. Crops Foods 6(2):135-139

Dairy Regulations in Zimbabwe (1977). Part 1 Section 1 (c).

De Vliegher S, Fox LK, Piepers S, McDougall S, Barkema HW (2012). Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control. J. Dairy Sci. 95(3):1025-1040.

Domenech E, Amoros JA, Escriche I (2013). Effectiveness of prerequisites and HACCP Plan in the control of Microbial Contamination in Ice Cream and Cheese Companies. Foodborne Path. Dis. 10(3):222-228.

Doran P, Carson J, Costello E, More SJ (2009). An outbreak of tuberculosis affecting cattle and people on an Irish dairy farm in 2005, following the consumption of raw milk from a cow with tuberculous mastitis. Ir. Vet. J. 62:390-397

Duncan SE, Yaun BR, Sumner SS, Bruhn SE (2004). Microbiological methods for dairy products. Standard methods for the examination of dairy products 17:261-263.

Food and Food Standards Act of Zimbabwe (2001). Food Additives and Prohibited Substances Regulations. Section 4(b).
Gadaga TH, Mutukumira AN, Narvhus JA, Feresu SB (1999). A review of traditional fermented foods and beverages of Zimbabwe. Int. J. Food Microbiol. 53(1):1-11.
Ghafir Y, China B, Dierick K, Dezutter L, Daube G (2008). Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. J. Food Prot. 71:35-45.
Gosta B (1995). Cheese. Tradition and basic knowledge W: Handbook–milk and dairy products. Tetra Pak Processing Systems. Lund. Pp.328-329.
Gran HM, Wetlesen A, Mutukumira AN, Rukure G and Narvhus JA (2003). Occurrence of pathogenic bacteria in raw milk, cultured pasteurised milk and naturally soured milk produced at small-scale dairies in Zimbabwe. Food Control 14(8):539-544.
Greenwood MH, Hooper WL (1990). Excretion of *Yersinia* spp. associated with consumption of pasteurized milk. Epidemiol. Infect. 104:345-350.
Gregory JL, Wang L, Johnson EA (1995). Acid adaptation of *E.coli* O157:H7 increases survival in acidic foods. Appl. Environ. Microbiol. 61(10):3752-375.
Gurol C, Ekinci FY, Aslan N, Korachi M (2012). Low temperature plasma for decontamination of *E. coli* in milk. Int. J. Food Microbiol. 157(1):1-5.
ISO 4832:2006. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique
ISO 7932:2004. Microbiology of food and animal feeding stuffs—horizontal method for the enumeration of presumptive *Bacillus cereus*-Colony-count technique at 30 ^{o}C.
Jablonski LM, Bohach GA (1997) *Staphylococcus aureus*. In. Microbiología de los alimentos: Fundamentos y fronteras. Acribia. Pp. 371-393
Jacxsens L, Kussaga J, Luning A, Van der Spiegel M, Devlieghere F, Uyttendaele M A (2009). Microbial Assessment Scheme to measure microbial performance of Food Safety Management Systems. Int. J. Food Microbiol. 134:113-125.
Jacxsens L, Luning PA, Marcelis WJ, Van Boekel T, Rovira J, Oses S, Kousta M, Drosinos E, Jasson V, Uyttendaele M (2011). Tools for the performance assessment and improvement of food safety management systems. Trends Food Sci. Technol. 22:S80-S89.
Jacxsens L, Uyttendaele M, Devlieghere F, Rovira J, Gomez SO, Luning PA (2010). Food safety performance indicators to benchmark food safety output of food safety management systems. Int. J. Food Microbiol. 141:S180-S187.
Jeevartnam K, Jamuna M (2004). Isolation and partial characterisation of bacteriocins from *Pediococcus* species. Appl. Microbiol. Biotechnol. 65(4):433-439.
Korashy E, Mohamed N (2008). Chemical and microbiological analysis to evaluate the sanitary condition of raw milk in Assiut. Assiut Vet. Med. J. 54(118):38-50.
Kussaga JB, Luning PA, Tiisekwa BP, Jacxsens L (2014). Challenges in performance of food safety management systems: a case of fish processing companies in Tanzania. J. Food Prot. 77(4):621-630.
Larsen HD, Jørgensen K (1997). The occurrence of *Bacillus cereus* in Danish pasteurized milk. Int. J. Food Microbiol. 34(2):179-186.
Lee SHI, Camargo CH, Gonçalves JL, Cruz AG, Sartori BT, Machado MB, Oliveira CAF (2012). Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. J. Dairy Sci. 95(12):7377-7383.
Lee SHI, Mangolin BLC, Gonçalves JL, Neeff DV, Silva MP, Cruz AG, Oliveira CAF (2014). Biofilm-producing ability of *Staphylococcus aureus* isolates from Brazilian dairy farms. J. Dairy Sci. 97(3):1812-1816.
Lucking G, Stoeckel M, Atamer Z, Hinrichs J, Ehling-Schulz M (2013). Characterization of aerobic spore-forming bacteria associated with industrial dairy processing environments and product spoilage. Int. J. Food Microbiol. 166(2):270-279.
Luning PA, Bango L, Kussaga J, Rovira J, Marcelis WJ (2008). Comprehensive analysis and differentiated assessment of food safety control systems: A diagnostic instrument. Trends Food Sci. Technol. 19:522-534.
Medina-Acosta E, Cross GA (1993). Rapid isolation of DNA from trypanosomatid protozoa using a simple 'mini-prep'procedure. Mol. Biochem. Parasitol. 59(2):327-329.
Medveďová A, Valík Ľ (2012). *Staphylococcus aureus*: characterisation and quantitative growth description in milk and artisanal raw milk cheese production. Structure and function of food engineering. Rijeka: InTech.
Milios K, Drosinos EH, Zoiopoulos PE (2012). Factors influencing HACCP implementation in the food industry. J. Hell. Vet. Med. Soc. 63(4):283-290.
Nazari N, Gondarzi H, Rahmi Baghi F, Moeinrad M (2014). Enterotoxin gene profiles among *S. aureus* isolates from raw milk. Iran. J. Vet. Res. 15(4):409-412.
Ostyn A, De Buyser ML, Guillier F, Groult J, Felix B, Salah S, Delmas G, Hennekinne JA (2010). First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E. Euro Surveill. 15(13):1-4.
Poppe C (2011). In. Fuguay JW (Ed.) Pathogens in Milk, Salmonella spp., Encyclopedia of Dairy Sciences, 2nd Edition. pp. 93-98.
Prasad ST, Han AR, Cho JM, Hur JH (2012). Multiplex PCR and DNA array for the detection of *B. cereus*, *S. aureus, L. monocytogenes*, *E. coli* O157H:7 and *Salmonella* spp. targeting virulence related genes. Ann. Microbiol. 63:725-731.
Public Health Act of Zimbabwe (2001). Sale of unwholesome, diseased or contaminated articles of food prohibited. Section 69 (1).
Ramos JM, Bernal E, Esguevillas T, Lopez-Garcia P, Gaztambide MS, Gutierrez F (2008). Non-imported brucellosis outbreak from unpasteurized raw milk in Moroccan immigrants in Spain. Epidemiol. Infect. 136:1552-1555.
Ranieri ML, Ivy RA, Mitchell WR, Call E, Masiello SN, Wiedmann M, Boor KJ(2012). Real-Time PCR Detection of *Paenibacillus* spp. in Raw Milk to Predict Shelf Life Performance of Pasteurized Fluid Milk. Appl. Environ. Microbiol. 78:5855-5863.
Rørvik LM, Granum PE (1999). *Staphylococcus aureus*. In: Granum P.E (Ed.), Infectious Diseases from Food. Oslo.
Sampers I, Jacxsens L, Luning PA, Marcelis MJ, Dumoulin A, Uyttendaele M (2010). Performance of food safety management systems in poultry meat preparation processing plants and relation to *Campylobacter* spp. contamination. J. Food Prot. 73(8):144-157.
Saudi AM, Moawad AA (1990). Incidence of Enterobacteriaceae in market milk in Cairo and its and its suburbs. Assiut Vet. Med. J. 24(47):134-139.
Sobeih AMK, Al-Hawary II, Aman IM (2002). Microbiological quality of milk and ice cream sold in Kafr El-Sheikh and El-Gharbia governorates. Minufyia Vet. J. 2:79-88.
Swiecicka I, Mahillon J (2006). Diversity of commensal *Bacillus cereus* sensu lato isolated from the common sow bug (Porcellio scaber, Isopoda). FEMS Microbiol. Ecol. 56(1):132-140.
Thapa SP, Park DH, Wilson C, Hur JH, Lim CK (2012). Multiplex PCR assay for the detection of *Pantoea stewartii* subsp. *stewartii* using species-specific genetic markers. Australas. Plant Pathol. 41(5):559-564.
Walstra P, Walstra P, Wouters JT, Geurts TJ (2005). Dairy science and technology. CRC Press.
Wellenberg GJ, Van Der Poel WHM, Van Oirschot JT (2002). Viral infections and bovine mastitis: a review. Vet. Microbiol. 88(1):27-45.