Research Article
Influence of Some Hygienic Measures on the Prevalence of Subclinical Mastitis in a Dairy Farm

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Abstract

Background and Objective: The hygienic measures of the farm especially during milking and the storage condition are all key agents in the count and kind of pathogen contamination present in the bulk milk, so the influence of some hygienic measures (bedding, silage, equipment swabs, worker hand swabs and SCM cow's milk samples) of the farm on the prevalence of subclinical mastitis (SCM) pathogens was evaluated in this study. **Material and Methods:** Swabs (workers hands and milking equipment) silage, bedding, SCM samples were collected from dairy farm located in Fayoum district, Egypt and the different types of SCM pathogens were isolated. Bulk milk tank and water samples were subjected to count (total colony and coliforms), respectively. The significant ($p \leq 0.05$) relationship between hygienic condition of environmental samples and prevalence of different subclinical mastitis pathogens was calculated. **Results:** In this study, 51 BTM (bulk tank milk) samples were examined through the 4 seasons of the year for the TCC, the highest mean value was $4.8 \times 10^4 \pm 3.6 \times 10^3$ CFU mL^{-1} in the spring. The results of coliforms count of the examined 24 water samples from trough showed that, the highest mean value $8.3 \times 10 \pm 3.45$ MPN/100 mL was observed at the winter. **Conclusion:** There was a significant relation between the hygienic condition of the farm and microorganisms causing subclinical mastitis which could enter the food chain and accidently mixed with bulk tank milk which may induce health hazards to human, so adequate sanitary measures should be taken by improving the personnel, cow, equipment and farm hygiene. Producers should well know the influence of each hygienic measure on a load of microbes in raw milk and how they can control to prevent the occurrence of clinical form of mastitis.

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INTRODUCTION

Specific cells in mammary gland are responsible for milk synthesis (sterile in udder). After this phase, the microbial contamination may occur from three main sources, interior or exterior of the udder and during handling, storage of the milk[1]. Bovine mastitis is highly significant disease of dairy cows from an economic, social and public health point of view[2]. It is an inflammation of the mammary gland of differ etiology. The disease can sitting in a clinical and subclinical form. In SCM, there is no detectable change in the udder and there are no remarkable abnormalities in the milk but the production of milk reduced[3]. However, mastitis is caused by multiple bacterial species; it is the result of contest between the pathogen and defense mechanisms of host. Abundant environmental causes favor the bacterial invasion of the mammary gland as; incorrect milking procedures, unhygienic condition of milking machines, inadequate sanitary level of the farm and cows[4]. Mastitis is caused by a broad spectrum of microbes and, assorted into contagious pathogens are those for which udders of infected animals serve as the main reservoir. They spread from cow to cow, primarily during milking and tend to result in sub-clinical infections with flare-ups of clinical form. Contagious microbes include: *Staphylococcus aureus* and *Streptococcus agalactiae*[5]. Environmental mastitis can be defined as those Intra-Mammary Infections caused by pathogens whose primary barrage is the environment. Pathogens of environment include *E. coli* and *Klebsiella* spp., infection of these microbes was characterized by short duration[6]. An administrative ability of the dairy farm is a key and critical points for mastitis control or for prevention the existing infections[7]. Therefore, this study was carried out for monitoring the influence of some of hygienic measures of the farm on the prevalence of SCM and its causing microorganisms that had a highly potential hazard on the milk consumers.

MATERIALS AND METHODS

Study area: Private dairy farm located in Fayoum district, Egypt was chosen for this study all over 4 seasons during the period of December, 2016 to November, 2017. The farm was depending on automatic Afi milking machine system where the cows were milked 3 times daily and the milk was collected automatically in large milk tanks. The water source was running water supply from municipal pipes, while the cows obtained their daily water needs from common water trough.

Collection and preparation of samples: A total of 284 different environmental samples were obtained from bedding, silage, swabs from worker hands, milking equipment in some selected points in the farm in addition water samples from troughs and pipes (24 each) and fifty one samples of Bulk Tank Milk (BTM) were subjected to bacteriological analysis for detection of influence of some hygienic measures on the prevalence of subclinical mastitis (SCM) applied on the farm.

Hands of workers and milking equipment swabs: The swabs were removed from the sterile wrapping and moisten the tip by immersing it in a tube containing sterile buffered peptone water (Oxoid, CM1049). The swabs were pressed against the wall of the tube to remove excess liquid. Moistened sterile swabs were rubbed onto the palm of both hands, area between fingers, finger tips and streaked an estimated area of 10 cm^2 on the surface of the equipment to be investigated as teat cups, pipelines as well as collecting tanks.

Each swab was inserted in the tubes (labeled with date) and transported to the laboratory immediately in an ice box. The tubes containing swabs were mixed and homogenized using shaker (Fisher scientific vortex mixer)[8].

Silage and bedding samples: Fifty grams of bedding were collected from the area in which the udder of recumbent cow was resting and 50 g of silage also were collected from different areas were placed into sterilized bags, labeled, kept in an ice box and transported to the laboratory immediately. The bedding and silage samples were mixed with two hundred ml of Peptone Water (Oxoid, CM0009) in 2-chamber filter bags and stomached (Seward 400 scientific lab blender) for 1 min[9].

SCM cow's milk samples: The 444 SCM cow's milk samples were collected from 695 lactating Holstein Friesian cows according to Radostits *et al.*[10] and prepared for bacterial investigation according to Carter and Cole[11], where the percentage of SCM in the farm was found to be at cows and quarter level 63.88 and 41.18%, respectively by California mastitis test (CMT) according to Schalm *et al.*[12].

The initial suspension of all prepared samples was incubated aerobically at 37°C (24-48 h) for aerobic bacterial growth. A loopful from the initial suspension was sub-cultured on 10% sheep blood agar (Oxoid, CM0055), MacConkey agar (Oxoid, CM0115), *Pseudomonas* specific agar media (Oxoid, CM0559), *Bacillus* specific agar media (Oxoid, CM0617), Edwards media (Oxoid, CM0027), EMB agar media (Oxoid, CM0069), Baird parker agar media

(Oxoid, CM1127+SR0054) and Mannitol salt agar plates (Oxoid, CM0085) and incubated aerobically at 37°C (24-48 h) for isolation of some microorganisms. The suspected colonies were isolated on nutrient slope agar (Lab M, LAB008) and incubated at 37°C/24-48 h for further identification. The bacterial isolates were identified by the *Vitek 2* compact system according to BioMerieux[13].

The significant (p≤0.05) relationship between hygienic condition of environmental samples and prevalence of different subclinical mastitis pathogens was calculated using Chi-square value and also p-value was calculated between the mean values of TCC of examined BTM samples and MPN for water samples (SPSS software; version 25).

Total colony count of BTM samples: Sixty milliliters of bulk tank milk samples were collected after proper turn on the sterile agitator from the top of the bulk tank using clean sanitized dipper. Samples were immediately labeled and transported to the laboratory in an ice box. Tenth fold serial dilutions were prepared for the collected samples and the total colony count was performed within 24 h using standard plate count agar medium (Oxoid, CM0463)[14].

Coliforms count in water samples: A water sample of 100 mL was collected from water troughs in the yard or pipes of the examined dairy farm in sterile screw capped bottles of 150 mL capacity by following strict aseptic procedures. Collected samples were identified and kept in an ice box and

transported immediately to the laboratory. Five tubes were prepared for three serial dilutions of a water sample and incubated at 37°C for 24-48 h. The MPN index is determined by comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical tables. The tabulated value is reported as MPN/100 ml of sample[15].

RESULTS

Bacterial species isolated from environmental samples: Table 1 reveals that the most often microorganisms isolated from the bedding samples (n = 38) were *Escherichia coli* 29 (76.30%), *Enterococcus* species 18 (47.40%), *Staphylococcus aureus* 16 (42.10%), *Pseudomonas aeruginosa* 11 (28.94%), *Proteus species* 3 (7.90%), while *Enterobacter aeruginosa*, *Serratia* and *Klebseilla* species were isolated in a percentage of 5.30% for each.

Bacterial species isolated from SCM cow's milk samples: A total of 444 SCM cow's milk samples were subjected to bacteriological investigation. The data obtained showed that the most isolated microorganisms from the samples were *Staphylococcus aureus, Enterococcus* species, *Escherichia coli* and *Streptococcus agalactiae* in a percentage of 66.66, 51.80, 47.29 and 23.87, respectively. While *S. chromogenes, Klebsiella oxytoca*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Bacillus cereus* were found in the examined samples in a percentage of 17.34, 13.73, 10.13, 7.20 and 6.08, respectively.

Table 1: Prevalence of bacterial species isolated from different environmental samples and SCM cow's milk samples

Samples microorganism	Bedding (n = 38)		Silage (n = 38)		Equipment swabs (n = 90)		Worker hand swabs (n = 118)		SCM cow's milk (n = 444)	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Enterococcus species	18	47.40	8	21.10	6	6.70	10	8.50	230	51.80
Staphylococcus aureus	16	42.10	20	52.60	26	28.90	36	30.50	296	66.66
Bacillus cereus	0	0.00	4	10.50	0	0.00	0	0.00	27	6.08
Pseudomonas aeruginosa	11	28.90	2	5.30	3	3.30	0	0.00	32	7.20
Proteus species	3	7.90	0	0.00	0	0.00	0	0.00	0	0.00
Serratia species	2	5.30	0	0.00	0	0.00	0	0.00	0	0.00
Klebsiella oxytoca	2	5.30	0	0.00	0	0.00	0	0.00	61	13.73
Enterobacter aeruginosa	2	5.30	2	5.30	0	0.00	2	1.70	13	2.92
Citrobacter amalanaticus	0	0.00	2	5.30	0	0.00	0	0.00	3	0.67
Enterobacter cloaca complex	0	0.00	1	2.60	0	0.00	0	0.00	5	1.12
Escherichia coli	29	76.30	10	26.30	7	7.80	8	6.80	210	47.29
Streptococcus agalactiae	0	0.00	0	0.00	0	0.00	0	0.00	106	23.87
Staphylococcus chromogenes	0	0.00	0	0.00	0	0.00	0	0.00	77	17.34
Bacillus subtilis	0	0.00	0	0.00	0	0.00	0	0.00	45	10.13
Sphingomonas paucimobilis	0	0.00	0	0.00	0	0.00	0	0.00	17	3.82
Raoultella ornithinolytica	0	0.00	0	0.00	0	0.00	0	0.00	12	2.70
Pantoea species	0	0.00	0	0.00	0	0.00	0	0.00	10	2.25
Other *Bacillus* species	0	0.00	0	0.00	0	0.00	0	0.00	9	2.02

n: Number of examined samples, Number: Number of isolated microorganisms (Bedding = 83, silage = 49, equipment = 42, worker hand = 56, SCM milk = 1153)

Relation between hygienic conditions of environmental samples and SCM pathogens: There is a significant (p<0.05) relation between hygiene of environmental samples and prevalence of (*Enterococcus* species, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) with a chi-square value 41.63, 8.58, 32.68 and 101.19, respectively (Table 2).

Results of total colony count (CFU mL^{-1}) of the examined BTM samples (n = 51): In the present study, 51 BTM samples were examined through the four seasons of the year for the TCC, the data presented in the Table 3 revealed that the highest mean value of the TCC of the BTM was observed in the spring ($4.8 \times 10^4 \pm 3.6 \times 10^3$ CFU mL^{-1}), followed by summer, winter and autumn seasons with a mean values $4.4 \times 10^4 \pm 3.4 \times 10^3$, $4.0 \times 10^4 \pm 3.1 \times 10^3$ and $2.7 \times 10^4 \pm 2.3 \times 10^3$ CFU mL^{-1}, respectively and absence of significant difference between all seasons of year (p>0.05).

Results of coliforms count (MPN/100 mL) of the examined water samples (n = 48): The data presented in Table 4 demonstrate that the mean value of coliforms count of the

examined 24 water samples from pipes is <3 MPN/100 mL all over the year, while results of coliforms count of the examined water samples from trough showed that the highest mean value ($8.3 \times 10 \pm 3.45$ MPN/100 mL with a percentage of 83.3) was observed at the winter, followed by summer and autumn with a mean values of ($4.3 \times 10 \pm 1.79$ and $4.1 \times 10 \pm 1.71$ MPN/100 mL with a percentage of 83.3 and 50.0), respectively.

DISCUSSION

Milk is a perfect medium for the propagation of various microorganisms, particularly bacterial pathogens. The SCM is the most prevalent disease that affects milk hygiene in dairy animals[16]. Choosing the environment in which lactating cows will spend most of their time has great effect on the productivity, health, reproduction of cows as well as milk quality and farm profitability. Dirty housing, unsuitable hygiene and the incorrect cow preparation for milking are all prerequisite factors that can potentially lead to SCM[17]. The degree of cleanliness of the milking system significantly influences the TCC and coliforms count. Environmental contaminants from bedding, manure and feeds as well as the less efficient cleaning and the absence of sanitizers support the growth of variety of microbes[18].

The most often microorganism could be isolated from the environmental samples and SCM cow's milk samples was *Staphylococcus aureus* in a percentage of 42.10, 52.60, 28.90, 30.50 and 66.66 from bedding, silage, equipment swabs, worker hand swabs and SCM cow's milk samples, respectively, while *Escherichia coli* was 76.30, 26.30, 7.80, 6.80 and 47.29%, respectively. In this study, the highest mean value of TCC of BTM was observed in the spring, while the Coliforms count of the examined trough water samples was $8.3 \times 10 \pm 3.45$ MPN/100 mL observed in the winter.

The results in Table 1 are nearly similar to data recorded by Edberg *et al.*[18], Hogan *et al.*[19] and Kristula *et al.*[20] for *Klebseilla* and *Enterobacter* species, while the *Staphylococcus aureus* incidence was nearly similar to those reported by Hassan[21] and Saied[22], higher incidence were recorded by Nahed *et al.*[23], however, lower incidence reported by Elbably *et al.*[24] and Mcauley *et al.*[25].

Table 2: Relationship between hygienic conditions of environmental samples and prevalence of the different SCM pathogens

Microorganism (MO)	Chi-square value	p-value
Enterococcus species	41.63	<0.05*
Staphylococcus aureus	8.58	<0.05*
Bacillus cereus	12.79	<0.05*
Pseudomonas aeruginosa	32.68	<0.05*
Proteus species	9.48	<0.05*
Serratia species	6.57	<0.05*
Klebsiella oxytoca	6.57	<0.05*
Enterobacter aeruginosa	5.87	>0.05
Citrobacter amalanaticus	6.57	<0.05*
Enterobacter cloaca complex	4.57	>0.05
Escherichia coli	101.19	<0.05*

*Significance difference (p<0.05)

Table 3: Statistical analytical results of total colony count (CFU mL^{-1}) of the examined bulk tank milk samples (n = 51)

Seasons	n	Minimum	Maximum	Mean±SE
Winter	13	2.9×10^4	5.7×10^4	$4.0 \times 10^4 \pm 3.1 \times 10^{3a}$
Spring	13	1.8×10^4	8.4×10^4	$4.8 \times 10^4 \pm 3.6 \times 10^{3a}$
Summer	13	1.6×10^4	7.5×10^4	$4.4 \times 10^4 \pm 3.4 \times 10^{3a}$
Autumn	12	1.8×10^4	4.2×10^4	$2.7 \times 10^4 \pm 2.3 \times 10^{3a}$

n: Total number of examined samples, [a]Non-significant difference (p>0.05)

Table 4: Statistical analytical results of coliforms count (MPN/100 mL) of the examined water samples (n = 48)

Seasons	Water from pipes				Water from trough			
	n	Minimum	Maximum	Mean	n	Minimum	Maximum	Mean±SE
Winter	6	<3	<3	<3	6	<3	2.4×10^2	$8.3 \times 10 \pm 3.45^a$
Spring	6	<3	<3	<3	6	<3	<3	$<3^b$
Summer	6	<3	<3	<3	6	<3	2.4×10^2	$4.3 \times 10 \pm 1.79^a$
Autumn	6	<3	<3	<3	6	<3	2.4×10^2	$4.1 \times 10 \pm 1.71^a$

n: Total number of examined samples, [a,b]Significant difference (p<0.05)

In addition, the microorganisms which isolated from 38 samples of silage were *S. aureus*, *Escherichia coli*, *Enterococcus* species and *Bacillus cereus* in a percentage of 52.60, 26.30, 21.10 and 10.50, respectively. While *Pseudomonas aeruginosa*, *Enterobacter aeruginosa* and *Citrobacter* species were isolated in a percentage of 5.30 for each, However, *Enterobacter cloaca* complex was detected in a percentage of 2.60.

Staphylococcus aureus, *Escherichia coli*, *Enterococcus* species and *Pseudomonas aeruginosa*, were isolated from the 90 equipment swab samples in a percentage of 28.90, 7.80, 6.70 and 3.30, respectively. These results are nearly similar to those reported by Nahed *et al.*[23], higher incidence were recorded by Saied[22].

One hundred eighteen samples of worker hand's swabs were positively for *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* species and *Enterobacter aeruginosa* in a percentage of 30.50, 8.45, 6.80 and 1.70, respectively. These data are nearly similar to results reported by Elbably *et al.*[24], higher incidence was recorded by Saied[22], Nahed *et al.*[23] and Mohamed *et al.*[26], however, lower incidence was reported by Lee *et al.*[27], Parmar *et al.*[28] and Zeinhom and Abdel-Latef[29]. *Escherichia*, *Klebsiella* and *Enterobacter* species are considered to be an indicative of fecal contamination[30].

Bacteria involved in bovine mastitis are classified as either contagious or environmental pathogens based on their epidemiological association with the disease. The SCM is a multietiologic disease, many microorganisms is implicated as causes. Contagious pathogens are those organisms transmitted from animal to animal where the primary reservoir harboring the pathogens is the cow. The SCM dominant contagious pathogens are *S. aureus* and *Streptococcus agalactiae*. Environmental pathogens are transmitted during milking from the environment serving as the primary source of these organisms. The main pathogens in this set are Coliforms as *Escherichia coli*, *Klebsiella* and *Enterobacter* species, these organisms are usually found in the animal environment and can be contracted by udder[31,32].

If *Staphylococcus aureus* persists in mammary gland, the milk comes into contact with teats of infected animals, the infection becomes contagious[33]. The results were showed in (Table 1) reveal that the nearly similar percentage of the isolated *S. aureus* from the SCM cow's milk samples was obtained by Michael *et al.*[34], Alemu *et al.*[35] and Zenebe *et al.*[36]. The presence of *S. aureus* may be due to lack of effective udder washing, post milking teat as well as improper disinfection in the milking area[37].

Enterococcus deserves widely distributed and makes up a large proportion of the saprophytes related with the gastrointestinal tracts of humans and animals[38,39]. The nearly similar percentage of isolation of the *Enterococcus* species was reported by Giraffa[40], Domig *et al.*[41] and Scheidegger *et al.*[42].

Streptococcus agalactiae is considered one of the major economic losses in dairy farms, that don't apply strict sanitary measures. It breaks the natural udder barriers, enters the teat canal and ascends in the milk through the quarter. In later phase, the acini become filled with scars resulting in quarter fibrosis[43]. The percentage of *Streptococcus agalactiae* in this study was found to be in accordance with that reported by Alemu *et al.*[35] and Biressaw and Deme[44]. On the other hand the higher prevalence was recorded by Abdel-Rady and Sayed[45], Ramirez *et al.*[46] and Momtaz *et al.*[47], however, the lower prevalence was reported by Gao *et al.*[48], Mpatswenumugabo *et al.*[49] and Mulate *et al.*[50].

Coagulase negative staphylococci (CNS) have become the main SCM pathogens in several countries and its prevalence may be a consequence of poor hygienic practices in farm[51]. These results are supported by the data obtained from previous researches[52-54].

Coliforms cause up to 25% of cows mastitis in proper managed animals[55]. The high percentage of SCM caused by Coliforms indicates to unsanitary production[56]. According to Mellenberger *et al.*[57], Coliforms may multiply in polluted water, contaminated bedding and manure. Coliforms invade the udder through the teat sphincter when teat-ends come into contact with an environment that is contaminated with these microorganisms[58].

The relatively near percentage of the isolated *E. coli* in this study is obtained by Ahmed *et al.*[59] and Barbour *et al.*[60], while lower incidence was obtained by Biressaw and Deme[44], Darbaz *et al.*[54] and Mia *et al.*[61].

Klebsiella mastitis has become a trouble in sand bedding farms, this may be attributed to fecal shedding of *Klebsiella* by healthy animals[62]. Incidence of *Klebsiella oxytoca* in this study is agreed to results that reported by Chander *et al.*[63] and Saidi *et al.*[64]. While the higher prevalence recorded by Oluchi[65] and Ali and Ali[66], but lower findings were observed by Shahid *et al.*[67], Kateete *et al.*[68] and Ahmed *et al.*[69]. Mouth ingestion of *Klebsiella* spp. could be due to the presence of the organism in and/or on crops, or due to fecal contamination of water and the fecal shedding of *Klebsiella* lead to contamination of dairy animals and their environment[70]. *Klebsiella* mastitis causes a considerable decrease in milk production, with average losses of 7.6 kg/day after infection[71]. Thus, prevention of exposure is the main strategy for control of this type of mastitis[20].

Pseudomonas aeruginosa subclinical mastitis has increased in large dairy farms, causing significant problems. The origin of SCM due to *P. aeruginosa* has been traced to contaminated water sources. Contamination of the parlor floor by fore-milking also facilitated its transmission[72]. The low prevalence of *P. aeruginosa* species in this study could be attributed to the intermittent shading nature of this organism from the udder to milk. The same prevalence of *P. aeruginosa* in this study is in agreement with the results reported by Ayano *et al.*[73].

Sewage, water, soil and food are mostly sources of *Citrobacter amalonaticus*. It produces enterotoxins acting on intestinal mucosa[74]. The incidence of *Citrobacter* species in this study was nearly similar to that isolated from mastitic cow's milk by Kateete *et al.*[68] and Turutoglu *et al.*[75], while the higher incidence was reported by Oluchi[65].

Bacillus cereus mastitis has a lower incidence than *Staphylococcus* spp. and *Streptococcus* spp.[61]. *Bacillus* species are environmental and also found among normal flora of the teat skin. So, *Bacillus* spp. can infect mammary gland when animals have incoming to pasture[76]. The prevalence of *Bacillus* in this study was nearly similar to that reported by Mulate *et al.*[50] and Abera *et al.*[77]. While the higher incidence reported by Mpatswenumugabo *et al.*[49], Darbaz *et al.*[54] and Vasquez-Garcia *et al.*[78].

In this study, a Chi square test of independence was performed to examine the relation between hygienic conditions of environmental samples and prevalence of the different SCM pathogens. There was a significant (p<0.05) relation between hygiene state of (bedding, silage, equipment swabs, worker hand swabs) and all investigated SCM pathogens expect *Enterobacter aeruginosa* and *Enterobacter cloaca* complex. The results presented in Table 2 are nearly similar to the data that recorded by Abera *et al.*[77], DeVries *et al.*[79], Nwankwo *et al.*[80], Azevedo *et al.*[81], Suleiman *et al.*[82] and Sanotharan *et al.*[83].

Low bacterial count milk production involves multiple factors related to cow health and udder hygiene, hygiene of the milking environment in which the animals are lived. Contamination of BTM occurs by different types of microorganism[84,85]. Collected milk from clean, healthy animals has TCC value ($<1 \times 10^3$) CFU mL^{-1}. Higher count suggest that contaminating bacteria are entering the milk from a variety of possible sources and poor cleaning and sanitizing during milking. Counts of 10×10^3 CFU mL^{-1} or lower should be achievable by most dairy farms[86].

The obtained results (Table 3) are nearly similar to those reported by Hassan[21], Saied[22], Axelsson[87], Al-Tahiri[88], Perkins *et al.*[89], Mhone *et al.*[90] and El-Leboudy *et al.*[91]. The cooler condition, the more bacteria are found within the Bulk Tank Milk because of an increase in bacteria from the animal directly. The difference in count of bacteria between the seasons was larger, with increasing total milk production; the effect of season on bacterial count in milk was the least[89,92,93]. Coliforms count of water is necessary to determine sanitary quality. According to the Egyptian Standard[94] the water should be free from coliforms, while Oshiro[95] recorded that for animal consumption (young calves), coliforms should be <1/100 mL. For adult animals, coliforms should be <10/100 mL.

This result (Table 4) is similar to data reported by Rice and Johnson[96]. significant difference (p<0.05) could be obtained between the mean values of coliforms count in (spring-summer, spring-autumn and spring-winter) season, but the higher coliforms count in the winter may be due to that the water troughs were near feed bunks, or they were exposed to improper cleaning and sanitizing. This data is nearly similar to those reported by LeJeune *et al.*[97], while higher mean value recorded by Sanderson *et al.*[30]. Cattle may contaminate the troughs with fecal material as well as extraneous matter (dust, feed or bedding) may also contaminate the trough[98].

CONCLUSION

From this present study, there is a significant association between the hygienic condition of the farm environment and some of SCM pathogens (*S. aureus*, *B. cereus*, *E. coli* and *pseudomonas aeruginosa*). This significant association indicates the efficiency degree (level) of hygienic measures which applied to environment of the farm. Lack of maintenance of strict hygiene and good sanitary environment may be a contributory factor in the cause of SCM. To achieve high raw milk quality, producers should be aware of the all previous factors that influence contamination of raw milk and how they can be controlled.

SIGNIFICANT STATEMENT

This study discover the strong relationship between some of inadequate hygienic measures which applied on this dairy farm and the prevalence of SCM pathogens, that can be beneficial for the farm to pay more attention during applying of these measures. This study will help the researcher to uncover the critical areas of zoonotic pathogens of SCM and the demand for more investigation concerning the SCM.

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