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Evaluation of biosecurity practices applied on some dairy cattle farms in egypt and their impact on milk quality and production

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ABSTRACT

Biosecurity is a set of practices applied to prevent the introduction and spread of diseases in animal farms. Mastitis is one of the most important diseases affecting dairy cows worldwide.This work aimed to evaluate the impact of biosecurity practices, environmental hygiene, and sanitation on milk quality, quantity, and prevalence of subclinical mastitis in some Egyptian dairy cattle farms. Along six months, Daily milk yield (DMY) was recorded and the total average milk production (TAMP) was calculated for six randomly selected cows of each of three farms (in Giza, Ismailia, and Alexandria Governorates, Egypt). A detailed questionnaire was also designed and filled out to detect the different biosecurity practices and hygienic levels. Milk, water, feed, and bedding samples were collected. In addition, swabs from workers' hands, cows' teats (before and after sanitation with Iodine 1% solution), equipment, and milking parlour surfaces were collected to assess the milk quality and environmental hygiene. Total bacterial count (TBC) and total coliform count (TCC) were determined. Subclinical mastitis (SCM) was detected using the White Side Test (WST). The obtained data revealed variable biosecurity practices in the investigated farms with different levels of environmental hygiene. A negative correlation was detected between the produced quantity and microbial quality of produced milk, besides the prevalence of SCM. Data reflected the negative impact of poor environmental hygiene on milk production and highlighted the role of following proper sanitary measures and biosecurity practices for improving milk production in dairy farms.

Introduction

Disease agents can be introduced to dairy farms through various sources such as people, replacement cattle, water, feedstuffs, manure, farm equipment, drugs, environmental sources, and vehicles that resemble the most critical biosecurity risks (Villarroel *et al*., 2007). Different ways are available to prevent or control cattle diseases. Some of them have some restrictions on use. Some of them have some restrictions on use due to the development of antimicrobial resistance or tissue residues, including chemical drugs or antibiotics. However, traditional management practices to prevent and manage diseases have proven to be the best strategy (Wells, 2000).

Biosecurity in dairy operations refers to a strategy of management practices designed to control and prevent animal and public health-related losses through avoiding the introduction of diseases and pathogens to the farm (bio-exclusion) and limiting the spread of disease agents within the farm (bio-confinement), mainly those focus on sick cow management, calving area management, and manure management (Sischo, 1998; Wells, 2000). Dairy farm biosecurity aims at maintaining and improving the health, welfare, productivity, and profitability of herds and flocks by utilising a strategy of management practices performed at different levels of action: national, regional, and local (Villarroel, 2007; Baraitareanu and Vidu, 2020).

In dairy farms, biosecurity measures can be categorised into structural, sanitary, animal, feed, and manure management (Baraitareanu and Vidu, 2020). An effective biosecurity program needs to be more than a to-do list. It needs to be flexible to adapt to the unique situations of individual farms, which requires an understanding of biosecurity principles, disease prevention goals, and specific information about the biology and epidemiology of particular pathogens (Wells, 2000).

Milk is the main product of dairy farms. Milk and dairy products are rich in nutrients essential to the growth of infectious and spoilage microorganisms (Fernandes, 2009). Milk from a sub/clinically mastitic cow commonly contains etiological agents, while milk from non-mastitic cows is contaminated by extraneous sources or unclean processing water. The microbial milk contamination originates from poor herd hygiene in milking parlours and milk-conserving practices, besides the health status, prevalence of mastitis, and production environment in dairy farms (Velazquez-Ordonez *et al*., 2019). Hence, good milk quality is obtained through sufficient feed, good environmental sanitation, proper manure disposal, and reasonable milking procedures (Suranindyah *et al*., 2015). The swab method is commonly used for the quantitative assessment of bacterial loads before cleaning and disinfection procedures of surfaces in animal houses. This method can be used to examine and compare the effectiveness of sanitation in terms of reduction rates before and after hygiene measures in livestock housing systems (Mateus-Vargas *et al*., 2022). The water used during milk handling or processing can be a potential source of microbial contamination and affect its quality and safety (Amenu *et al*., 2016). So, an assessment of water quality used for cleaning equipment and milk processing should be applied, and quality standards should be similar to those used for drinking water (Terplan, 1980).

Mastitis is a disease of concern affecting dairy animals. Clinical and subclinical mastitis (SCM) are prevalent according to the signs of inflammation. Clinical mastitis has visible signs. In contrast, subclinical mastitis has no visible signs; however, it decreases the daily milk yield and increases the somatic cell count (Abebe *et al*., 2016). So, SCM is the most economically important type of mastitis and is one of the most persistent and widely spread diseases of dairy cattle, affecting milk quality and quantity. Bovine SCM can directly or indirectly affect the world's economy as it accounts for over 90% of the total loss in milk production (Schepers and Dijkhuizen, 1991; Mungube *et al*., 2005). Mastitis also has a public health significance; its treatment leaves drug residues in milk, aside from its zoonotic risk of transmitting pathogens to humans (Sharma *et al*., 2010). The dairy herd needs to be tested periodically for the prevalence of SCM using screening field tests such as the White Side Test (WST) and California mastitis test (CMT). WST is considered relatively inexpensive, available, and feasible to be applied at the herd level (Tanni *et al*., 2021).

There are two types of mastitis: contagious and environmental mastitis. The dairy environment is the source of pathogens causing the second type, including manure, bedding, and corral surfaces. Biosecurity principles for controlling environmental mastitis should include clean, dry teats and udders, disinfecting teats before milking, and vaccination with *E. coli* core antigen vaccines (Smith and Hogan, 1993). On the other hand, control of contagious mastitis requires preventing the introduction of infected cattle to a herd and within-herd biosecurity principles to reduce the number of new infections.

The microbial assessment of milk is a significant feature in determining its quality and safety and reflects farm hygiene (Dehinenet *et al*., 2013). Understanding the role of farmhouses and management in milk contamination can help dairy practitioners establish corrective actions to reduce bacterial contamination of milk at the farm level and improve their production. Therefore, this work aimed to evaluate the impact of biosecurity practices and environmental hygiene on the microbial quality of milk and the prevalence of subclinical mastitis that reflects directly on milk production yield level and economic investment in dairy cattle farms.

Materials and methods

Ethical approval

Authors confirm that ethical approval to conduct this research was obtained from the Faculty of Science and Biotechnology, MSA University. We pledge to adhere to all ethical principles and standards set by the university to ensure the safety and rights of all research animals. Regarding the participation of workers in the study, an oral consent was obtained from them before collection of hand swabs.

Study design, location, and duration

A cross-sectional study was carried out over six months, from November 2022 to April 2023, in three dairy cattle farms that belong to three governorates in Egypt: farm (1) at Giza, farm (2) at Ismailia, and farm (3) at Alexandria. Farm visits were conducted to detect the impact of environmental hygiene and different biosecurity practices on the prevalence of subclinical mastitis and the quantity and microbial quality of the milk produced. Farm (1) is a small private farm with about 25 cows, but farms (2) and (3) are more intensive with 250 cows. Six cows were randomly selected from each farm, where the daily milk yield (DMY) was recorded twice daily. Then, each farm's average daily milk production was calculated and collated as total average milk production (TAMP) during the study duration. During farm visits, a designated questionnaire was filled out, and an in situ screening of SCM was conducted using WST to collect milk, water, feed, bedding samples, surfaces, and workers' hand swabs. The samples were transported to the laboratory using an ice box, where the microbial assessment was conducted, and bacterial log reduction was calculated with minimum delay.

Questionnaire

A structured questionnaire was prepared to assess the biosecurity practices conducted on each farm. The farm owner or executive manager filled out the form that involved the following points: (1) environmental sanitation, (2) visitors, (3) employees, (4) diseases, (5) milking, machinery and tool assessment, (6) cow care, (7) sick cows, (8) purchased cows, and (9) disposal of carcasses.

Samples collection

Milk samples

According to Mason (2006), 18 milk samples were collected from apparently healthy dairy cows (6 samples/farm).

Swab samples

A total of sixty-three (63) swab samples were collected (20 swabs/farm and one control negative swab). Four swabs were obtained from each surface: floor, walls, workers' hands, equipment, and cows' teat skin (Collins *et al*., 1991). All surfaces were swabbed before and after cleaning, disinfection, or sanitation. After the application of disinfectants, swabs were received into neutralising solution tubes composed of a combination of 3% Tween 80 (BiomedicalisTM), 0.3% Lethcine, 1% Histidine, 0.5% Sodium thiosulphate, and 3% Saponine (Fisher chemicals TM) prepared according to Douglas and Kampf (2010) to stop the disinfectant action.

The four teats of each animal were sampled using the modified wet-dry swab technique before and after pre-milking sanitation (Hohmann *et al*., 2020). All sampled cows had no clinical mastitis, visible udder lesions, or trauma, and their skin appeared normal.

Workers' hands were swabbed, and examined bacteriologically, as Lambrechts *et al*. (2014) described.

Water samples

Water samples were collected from the water source (tap) used for watering animals or cleaning surfaces, utensils, and udders. According to Amenu *et al*. (2016), samples were collected directly from taps in sterile capped plastic cups with 100 ml capacity.

Feed samples

Samples were collected from the feed troughs using gloved hands and received in sterile plastic bags (Tabib *et al*., 1981).

Bedding samples

Bedding samples were collected from the areas between animals away from faecal matter and caked bedding using gloved hands, received in sterile plastic bags, and then transported in an ice box to the laboratory for bacteriological examination (Bradley *et al*., 2017).

Sample preparation

The tubes containing swabs were vortexed for 30 seconds; however, water samples were shaken 25 times using back-and-forth movement to ensure uniform distribution of microorganisms. Each feed sample was mixed, and then ten grams were suspended in 100 ml of 0.85% saline and shaken to mix the sample for one minute. Then, large particles were left to settle down for 1 minute. For bedding samples, 30 g of thoroughly mixed bedding materials were added to 270 mL of sterile saline solution 0.85% and mixed for 1 minute, followed by preparation of tenfold serial dilutions. In the laboratory, teat-skin swabs were vortexed for 20 seconds, and samples of each animal were pooled before tenfold serial dilutions were prepared.

Microbiological examination of samples

It was assessed after determining the TBC and TCC of collected milk samples, teats, hands, and farm surface swabs. The examination of the milk samples included the WST and determination of the total aerobic plate count (TBC) and coliform count. White Side test is an indirect screening test for subclinical mastitis (SCM) detection. It was performed as described by Kahir *et al.* (2008). Five ml of milk sample were mixed with 2 ml of WST reagent (4% NaOH solution). The results were interpreted as strong (+++), distinct (++), weak (+), trace (±), and negative (-) based on the formation of coagulation or milk precipitation.

In the laboratory, the total aerobic plate count of milk samples (TBC) CFU/ml was carried out using standard plate count agar (OxoidTM) according to ISO (2003) and APHA (2004). However, the coliform content of milk (MPN/ml) was adopted according to FDA (2013) using the 3-tube technique containing lauryl sulfate tryptose broth (OxoidTM).

For determination of TBC and TCC of swab samples of farm surfaces, hands, teat skin and also, water, feed and bedding, plate count and MacConkey agar plates were inoculated each with aliquots of 0.1 ml of original samples and their tenfold dilutions. The inoculated plates were then incubated under aerobic conditions at 37°C for 24 hrs. After incubation, plates with countable colonies (30- 300 numbers) were counted using the illuminated colony counter. The total count obtained was multiplied by the dilution factor according to ISO (2009). Counts were expressed as CFU/cm^2 (swabs), CFU/ml (water), CFU/g (feed or bedding), and CFU/ml (teat skin and workers' hands). For assessing the pre-milking hygienic procedures, the bacterial log reduction was calculated by subtracting the log number of CFU/cm^2 (floor and walls swabs) and CFU/ml (teat and hand swabs) from those before cleaning and disinfection.

Statistical analysis

Farm Milk Daily records were collected and computed using Microsoft® Excel® for Microsoft 365 MSO (Version 2403). Means and least significant differences of milk production ± standard errors (SEM) were calculated and compared using a one-way analysis of variance using PASW Statistics, Version 24.0. software (SPSS Inc., USA). For bacterial counts, the colony-forming units were all converted to log_{10} colony-forming units (log CFU), and then the differences in log reduction after the action of the disinfectants were obtained and presented in tabular and graphic forms for surface, hand, and teat swabs. Regression biostatistics and correlation were analysed between the quantity and the microbial quality of produced milk and the prevalence of SCM. Values were considered significant at P< 0.05.

Results

Table 1 illustrates the answers to the designated questionnaire for environmental, hygiene, and biosecurity evaluation in each farm. Where Farm (2) and (3) achieved most of the points under study, while Farm (1) showed the worst results; concerning the environmental assessment, the data showed that Farm (2) applied moderately to excellent cleaning procedures using hot water and 3% sodium hydroxide; however, Farm (3) used hot water only. On the other hand, Farm (1) used tap water for washing manual milking equipment (buckets), udder, and workers' hands, and no walls or floor cleaning was applied.

Regarding milk production, our data revealed a significant difference in the amount of produced milk (p-value ≤0.05), among the three farms located in Giza, Ismailia, and Alex., where the total average milk production was the lowest in Farm 1 and the highest in Farm 3 (546.73 ±2.07 and 761.48±2.4 kg/6 months/6 cows, respectively) (Table 2).

In Table 3, subclinical mastitis was detected in the three investigated farms using the WST, where the prevalence was 50%, 33%, and 50%, in Farms 1, 2 and 3 respectively. Concerning the microbiological quality of the milk produced, the TBC and TCC of milk samples were highest on farm (1), followed by farm (3), and the lowest were counted on farm (2). Moreover, regression biostatistics revealed a negative correlation between the

Table 1. Questionnaire data.

Assessment	Farm (1)	Farm (2)	Farm (3)
Location	Giza	Ismalia	Alexandria
Environmental Assessment	Cleaning and sanitation: • Poor cleaning • Use water only Wild animals: • Donkeys, horses, dogs, cats, rodents, and birds) Manure collection: • Once daily	Cleaning and sanitation: • Moderate to Excellent • Use hot water +3% sodium hydroxide for surfaces and equipment • Teat dip using 1% iodine preparation) Wild animals: • No Animals, birds only Manure collection: • Once daily	Cleaning and sanitation: • Moderate • Use hot water for equipment • Teat dip using 1% iodine preparation) Wild animals: • Dogs, cats, rodents, and birds) • Manure collection: • Once daily
Visitors	• No visitor records. • No Protective clothing	• Visitors recorded. • Protective clothing	• Visitors recorded. • Protective clothing
Employees	• No Clothing • No Medical or Training Certificates	• Clothing: overall (sanitised) • Medical & Training Certificates	• Clothing: Boots only • Medical & Training Certificates
Disease	Acute, Clinical and sub-clinical Mastitis & Gastrointestinal Diseases	• Subclinical Mastitis	• Acute-Clinical & Sub-Clinical Mastitis
Milking, Machinery & Tool Assessment	• Manual milking • No Worker hygiene • Animals are milked directly on the floor of the pens.	• Automatic milking • Pasteurised Milk Worker hygiene	• Automatic milking • Worker hygiene
Sick Cows	• Veterinarian not called at once • Animals not quarantined, and if mastitis occurs, milk is disposed of until signs disappear.	• Veterinarian resident on the farm • Sick animals isolated • If mastitis, milk is disposed of until antibiotic residue is removed	• Veterinarian resident • Sick animals isolated • If mastitis, milk is disposed of until antibiotic residue is removed
Purchased Cows	The veterinarian called for an animal evaluation.	The resident veterinarian called, and the animals were quarantined for two weeks	The resident veterinarian called, and the animals were quarantined for two weeks
Cow Carcasses	• Unhygienic disposal	• Incinerated, then buried	• Buried

total average daily amount of produced milk over the investigated duration of 6 months in the three farms (TAMP) and the prevalence of SCM (r= - 0.3) (Figure 1). Also, a reverse correlation was revealed between TAMP and the microbial quality of milk, TBC (r= - 0.7) (Fig. 2), and TCC (r= - 0.6) (Fig. 3).

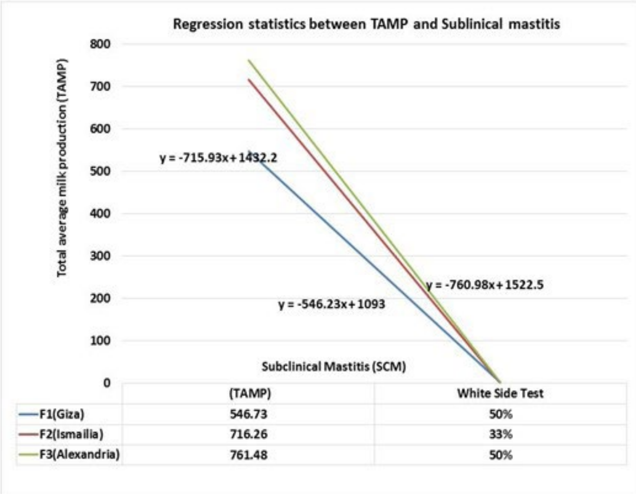

Fig. 1. Reverse Regression statistics indicate the negative correlation between the total average milk production and the prevalence of subclinical mastitis (SCM).

Data in Table 4 illustrates the assessment of the microbial quality of the dairy environment: aerobic and coliform counts in bedding, feed, and water samples. Both the TBC and TCC were high in samples of all farms except for water samples; the log_{10} of TBC was 3.5, 3.4, and 2.7 for farms 1, 2, and 3, respectively; however, no coliform bacteria were detected in all samples.

Concerning the cleaning of farms, the microbial load of walls, floor, and milking equipment was the highest and log ten reductions were the lowest on the farm (1), with the highest counts on the floor (Fig 4&5). After cleaning and disinfection in Farms 2&3, data showed that the highest log_{10} reductions were for walls and teat skin, followed by milking equipment, and the lowest reductions were on the other surfaces (floor and workers' hands) (Fig. 6).

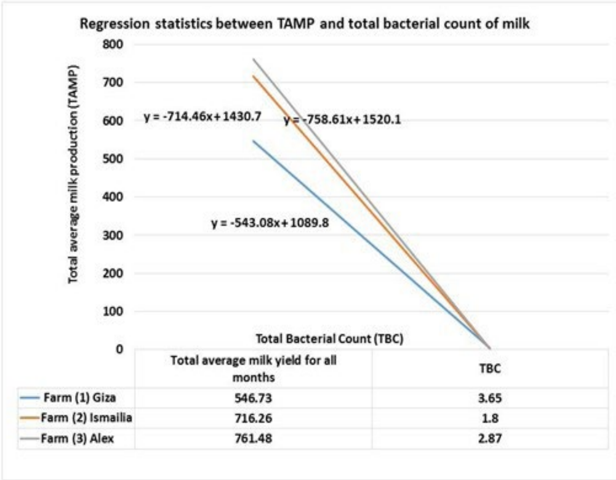

Fig. 2. Reverse Regression statistics indicate the negative correlation between the total average milk production and the total bacterial count of milk.

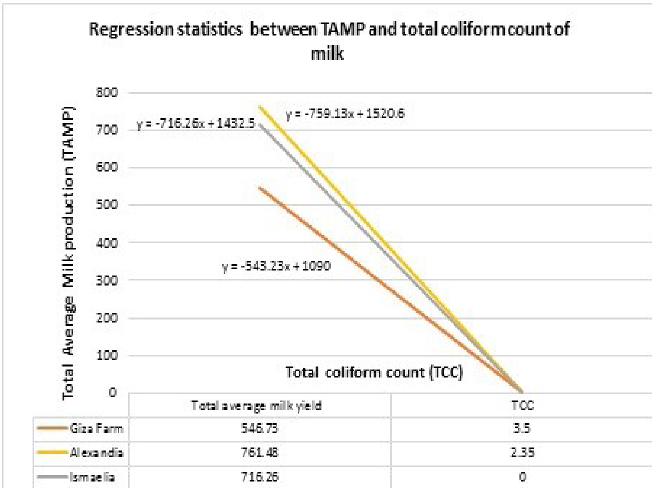

Fig. 3. Reverse Regression statistics indicate a negative correlation between total average milk production and the total coliform count of milk.

Table 2. Total average Milk Production (TAMP) for each farm.

Farm no./ milk production	Average milk production (AMP)/six cows/ month (kg)						TAMP/ 6 months/6cows (kg
	20-Nov	20-Dec	21-Jan	21-Feb	21-Mar	21-Apr	
Farm 1	93.78	93.38	98.42	84.21	89.23	87.69	546.73±2.07[b]
Farm 2	121.47	118.69	118.69	113.26	127.99	116.16	716.26±2.05[a]
Farm 3	123.15	133.55	134.79	125.62	124.29	120.08	761.48±2.4[a]

TAMP is the total average milk production/6 cows/6 months.
[a, b] Mean values of different superscripts indicate significant differences (ANOVA, single factor test; P≤0.05 with LSD = 6.59 kg), and data were presented as means ± SEM.

Table 3. The prevalence of SCM, TBC, and TCC in collected milk samples.

Farm no. /Microbial investigations	White Side Test (+ve %)	TBC (log_{10} CFU/ml)	TCC (MPN/ml)
Farm 1	50%	3.65	3.5
Farm 2	33%	1.8	0
Farm 3	50%	2.87	2.35

Table 4. log10 of Total bacterial counts (TBC) and total coliform count (TCC) of the examined bedding, feed, and water samples.

Sample/ farm no./ microbial index/	F1		F2		F3	
	TBC	TCC (MPN/ml)	TBC	TCC (MPN/ml)	TBC	TCC (MPN/ml)
Bedding (log_{10} CFU/g)	9.7	9	8.6	8	8.9	8.3
Feed (log_{10} CFU/g)	8.9	8.6	9	5.3	9.6	7.5
Water (log_{10} CFU/ml	3.5	0	3.4	0	2.7	0

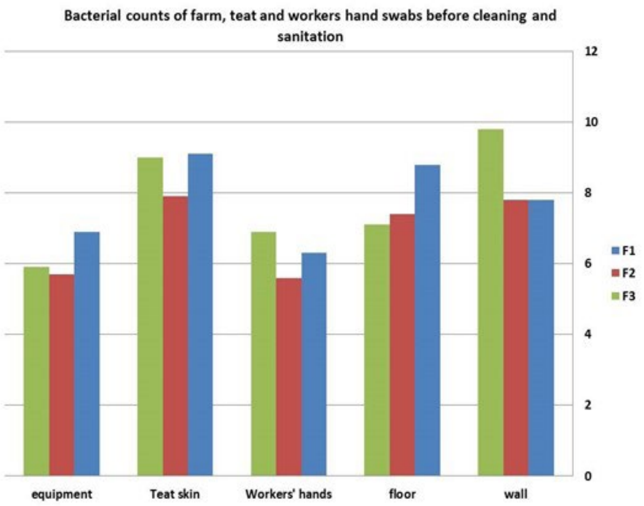

Fig. 4. Log_{10} of TBC of the farm equipment, surfaces, teat skin and workers' hands swabs before cleaning and sanitation.

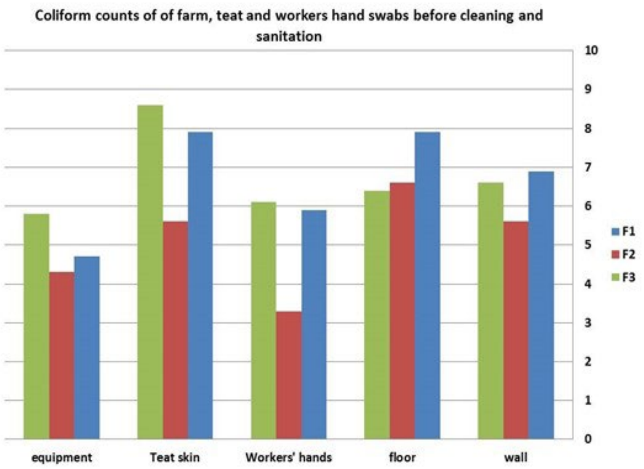

Fig. 5. Log_{10} of TCC of the farm equipment, surfaces, teat skin and workers' hands swabs before cleaning and sanitation.

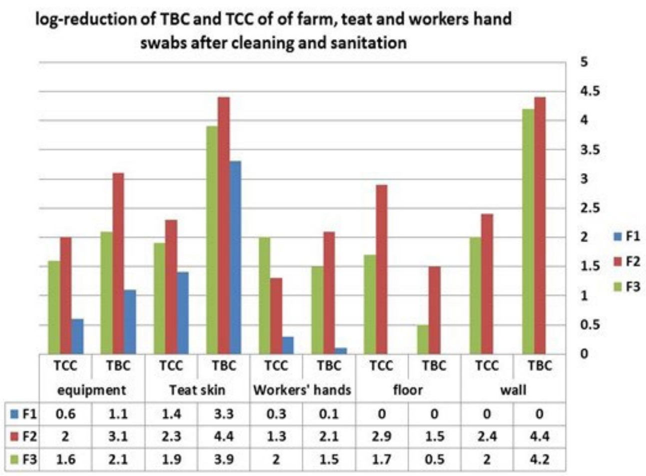

Fig. 6. Log_{10} reduction of TBC and TCC of the farm equipment, surfaces, teat skin and workers' hands swabs after cleaning and sanitation.

Regarding the evaluation of the pre-milking cleaning and sanitation procedures, data showed that the farm sanitation method was reflected on the log reduction of bacterial counts of milk, teat skin and the percentage of positive subclinical mastitis cases (Table 3) and Figures 5&6. Generally, log_{10} Reductions in TBC were mostly higher than those in TCC. Besides, the highest log_{10} reductions were for Farm (2), followed by Farm (3), and the lowest reductions were for Farm (1). Although four-log_{10} reductions were obtained on the walls of Farms 2&3 and teat skin of farm (2) samples, they could not be achieved on milking equipment.

Discussion

Biosecurity measures should be respected in dairy farms to maintain disease-free herds and sustain maximum production. Questionnaire data revealed that the cleaning and sanitation procedures were nearly similar in Farms 2 and 3; however, they were the worst in Farm (1). Thorough cleaning and disinfection can decrease the pathogen level and break the disease cycle, improving the health of dairy cows raised in modern systems under high intensity to obtain high productivity (Baraitareanu and Vidu, 2020). Manure, water, and bedding are pathogen reservoirs of significant concern to dairy farms. Dairy farm manure is the most problematic waste, resembling a biological risk material due to its substantial bacterial load (Villarroel *et al.*, 2007; Stanković, 2011). Good manure management practices, including frequent collection and cleaning of manure alleys accompanied by manure storage in areas inaccessible to cattle, can improve dairy farm hygiene and reduce the coliform bacteria count on teats and milk (Oliver *et al.*, 2005; Lowe *et al.*, 2015; Firth *et al.*, 2019). In our study, solid manure was collected once daily and removed outside the barn.

Farms 2 and 3 inhibited access to wild animals. Several studies reported that wildlife and wind could affect and facilitate disease transmission between nearby production units (Mikkelsen *et al.*, 2003; Woodroffe *et al.*, 2006).

The risk of disease introduction by personnel (employees and visitors) should be considered when evaluating farm biosecurity measures. Answers about visitors showed a lack of visitor recording, and sterile clothing was offered to visitors on the farm (1). Wallace (2003) advised limited access of visitors to farms, visitor recording in a logbook, separate visitor parking, and warning signs to limit direct contact of visitors with farm feed and animals. On the other hand, results about employees showed that those on the farm (1) had no unique clothing for work and did not have health or training certificates. Villarroel (2007) recommended that employees and hired persons for dairy farms should be regularly trained and educated on hygiene principles, disinfection, and methods of prevention of disease agent introduction and spreading from outside and inside sources. Pankey (1989) suggested that milking parlour personnel should wear latex gloves to reduce the spreading potential of contagious mastitis pathogens and recommended limited access for visitors.

Introducing new cattle is one of the most critical biosecurity risks for dairy farms as it is considered the main route of between-farm transmission of many infections (Gilbert *et al.*, 2005). Sayers *et al.* (2014) stated that a quarantine facility is essential to sequestrate newly purchased cows before they join the main herd. In our study, farms 2&3 had a quarantine facility to isolate purchased animals for two weeks before they joined the farm herd. However, farm (1) failed to fulfil this point. Wallace (2003) mentioned that infectious diseases can enter a herd through purchased additions or be carried onto a farm by other animal species or humans. Cullor (2004) stated that strict quarantine procedures, more thorough sanitation, increased testing for pathogens, and less contact between animals are essential to prevent infections.

The rapid disposal of dead animals according to national regulations and farm possibilities can reduce the risk of disease transmission in farm animals. The disposal can be done by a licensed dead stock collector, burial, or composting and should be located away from a stable feed storage bin or silo (Payne, 2015; Baraitareanu and Vidu, 2020). In our study, farm (2) disposed of carcasses using both incineration and burial, farm (3) used burial only, while farm (1) applied unhygienic disposal.

Concerning the microbial quality of the produced milk, our data clarified the effect of the dairy environment and the applied hygienic measures on the quantity and microbial quality of produced milk. We reported a reverse regression between milk quantity and microbial counts. Standard specifications of total bacterial count in raw milk vary with countries. Unfortunately, there is no definite value for the standard permissible TBC or TCC in the Egyptian standards ES: 154-1/2005 for raw milk. However, In Zimbabwe, the bulk tank TBC limits for raw milk acceptable at dairy processing plants are 100,000 CFU/ml (Katsande *et al*., 2010).

Many factors cause milk contamination and affect its quality. Our data revealed that contamination of the milking parlour surfaces, workers' hands, and water sources were positively related to the microbial load of teat skin and milk contamination. Milk production conditions are the primary sources of bacteriological contamination of milk and its products (Kelly *et al*., 2009). Elmoslemany *et al*. (2010) and Olofsson (2013) defined milk contamination sources as the animal environment, milking equipment, feeds, soil, dung, farm personnel, and housing. Paraffin *et al*. (2019) stated that housing system compartments like doors, floors, walls, and windows can be sources of bacteria if they are not constructed and managed correctly.

Teat skin is a significant source of milk contamination, acting as a reservoir of microorganisms arising from farming practices. These organisms can gain entrance to the udder through the teat orifice and canal and consequently cause intramammary infection (Monsallier *et al*., 2012; Hohmann *et al*., 2020). Newbould (1970) found a positive relationship between teat-contaminating bacteria and intramammary infection. Also, Múnera-Bedoya *et al*. (2017) concluded that high TBC in milk had been mainly blamed on contamination from dirty teats, udders and tails of cows, dirty workers' hands, dirty clothes, and milking equipment.

Thorough cleaning and disinfection are essential compartments of a biosecurity program as they can decrease the pathogen level, break the disease cycle and improve the health of dairy cows raised under high intensity in modern houses. In our study, different hygienic measures (cleaning and sanitation) were used in the milking parlour in the three farms under study based on knowledge and cost availability. Farm (1) was a traditionally managed farm that did not apply cleaning of walls or floor, and equipment cleaning was applied using water only; farm (2) used hot water with 3% sodium hydroxide, and farm (3) used hot water alone for cleaning surfaces and milking equipment (cups). The sanitation method was reflected in the log, which showed a reduction of bacterial counts of teat skin and the percentage of positive subclinical mastitis cases in the investigated farms. Questionnaire data about the farm (1) revealed that udder and teats were washed with water only. Milking was applied manually, and milk was received using traditional equipment (buckets) inside the barn on the contaminated floor. However, in farms (2&3), udder and teats were washed before milking using 1% iodine preparation solution as a sanitiser. Our data showed that cleaning and sanitation methods impacted the microbial reduction obtained differently. These data agree with Ingawa *et al*. (1992); Miller *et al*. (1993), and Myllys and Rautala (1995), who found that poor milking hygiene has been associated with inferior milk quality. We also agree with Grindal and Bramley (1989); Ingawa *et al*. (1992) and Hutchison *et al*. (2005), who concluded that machine milking is a significant cause of bacterial cross-contamination from cow to cow. They added that an appropriate pre-milking hygiene routine can decrease the cow infection ratio by reducing udder bacterial contamination from the environment and other infected animals.

Additionally, Smith *et al*. (1985) found that reducing the environmental pathogen contamination of the teat end is a method for controlling environmental mastitis. The workers' hand examination showed that the highest counts were obtained in farms (3&1) and the lowest for farms (2). Workers of all farms did not apply any hand sanitation. They just washed their hands with water. Wallace (2003) recommended that milking parlour personnel wear latex gloves to reduce the spreading potential of contagious mastitis pathogens and milk contamination (Elmoslemany *et al*., 2010; Olofsson, 2013).

Bedding is considered a primary source of environmental pathogens. Cows usually spend most of the day lying down; this allows contaminated bedding to stick to the teat-end skin. Zdanowicz *et al*. (2004) and Rowbotham and Ruegg (2016) concluded that both the type of bedding material and its microbial load (constituents and count) could affect the bacterial load of teat skin and so facilitate mastitis occurrence. Also, water is considered a possible source of milk contamination and could increase milk bacterial counts (Ingawa *et al*., 1992; Vissers *et al*., 2007; Amenu *et al*., 2016). Terplan (1980), Villarroel (2007), and Villarroel *et al*. (2007) stated that the quality and potability of water used for milk handling and processing should be tested regularly and should have quality standards similar to those of drinking water. They added that samples from each feedstuff batch or lot should be stored for possible laboratory analyses until that batch is consumed without incidents. Crump and Griffin (2002) mentioned that food-producing animals are the major reservoirs for many organisms. This contributes to the infection and colonisation of food-producing animals with these pathogens that can then be transmitted through the food chain to humans and cause human foodborne illness.

Cleaning included walls, floor, and milking equipment. The floors of all farms were made of concrete; they were rougher and cracked more on the farm (1). Walls were made of glazed tiles on all farms (smooth, non-porous surfaces). Paraffin *et al*. (2019) stated that surfaces broken or made from rough material are difficult to clean and, thus, can keep bacteria due to dust accumulation and moisture. They added that poor drainage, urine, and manure accumulation could contribute to increased TBC of the milking unit. Rutala and Weber (2008) enumerated some factors that can affect cleaning and disinfection processes, which are the number and location of microorganisms, equipment surface (crevices are more challenging to disinfect than flat surface equipment), and organism's resistance to chemical germicides. These results agreed also with the reports of Weise and Levetzow (1976); Gracey (1981), and Schütt *et al*. (1992), who pointed out that water lacks germicidal and antimicrobial properties, and its high surface tension facilitates poor contact between surfaces and water. Skaarup (2003), mentioned that detergent has more excellent germicidal properties than cold or hot water. In addition to water, it lowers the surface tension, thus facilitating good contact between the surface and water, and, in the process, detergent exerts its germicidal effect. Other researchers explained the difficulty in removing soiled, dry materials from instruments. They mentioned the need for efficient manual cleaning using friction (rubbing/scrubbing the soiled area with a brush) and fluidics (fluids under pressure) (Reichert and Young, 1997; Rutala and Weber, 2008). Roberts (2001) stated that manual and mechanical cleaning of instruments can achieve approximately a 4-$\log_{10}$ reduction of contaminating organisms count. In our results, 4 $\log_{10}$ reductions were obtained on walls of farm 2&3 and teat samples of farm (2) and could not be achieved on milking equipment. Additionally, reductions in TBCs were mostly higher than in those in TCC, which agrees with Awosanya *et al*. (2011) who found that washing with pipe-borne water has more effect in reducing total aerobic microbes than coliforms.

Conclusion

Results revealed that high contamination of the dairy farm environment is reflected directly on daily milk production, microbial quality of milk, and the prevalence of subclinical mastitis. Therefore, following biosecurity practices in dairy farms, including cleaning, pre-milking sanitation of teats, workers' hands, milking equipment, and farm premises, is critical for the improvement of milk quantity, quality and minimising subclinical mastitis.

Conflict of interest

The authors declare that they have no conflict of interest.

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