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Biosecurity Measures, Bacterial Prevalence and Economic Implications of Environmental Mastitis and Hygienic Milking Practices on an Egyptian Dairy Farm

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ABSTRACT

Hygiene and biosecurity on dairy farms reduce the incidence of mastitis and other infectious diseases. Bovine mastitis, a common infectious condition, causes cattle culling and reduces milk output and quality, causing significant economic loss. This study studied the association between environmental mastitis, hygienic milking practices, and dairy cattle milk output. In addition, a thorough microbiological examination to detect the most important environmental indicator bacteria that cause mastitis, such as *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *E. coli* spp., helps determine the best mastitis control protocols. Three visits to a dairy farm in Qalioubia governorate, Egypt, yielded 285 samples (186 environmental, 27 workers, 72 milk samples, and swabs). *Staphylococcus* spp., *Streptococcus*, *Pseudomonas* spp., and *E. coli* were the farm's most common bacteria, with an average hygiene score of 59%. Cow milk output peaked in May at 4252L. With clinical mastitis incidence in June and July, it steadily decreased, reaching 3343L in August in cows with the condition. Bovine Ephemeral Fever (BEF) complicated clinical mastitis during this decrease. Due to farm workers' lack of biosecurity awareness, several biosecurity and personal hygiene processes in the dairy farm were flawed, so the farm was infected with BEF, which complicated the losses, as it was $9,348.86/100 cows because of clinical mastitis and became $53,561.29 after a complication with viral infection, these exorbitant losses draw the need for training on the importance and the use of these measures. Overall, the results highlighted the critical role of hygiene and biosecurity measures in reducing mastitis and other infectious diseases on dairy farms, as it identified a significant link between poor hygiene milking practices and environmental mastitis caused by *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *E. coli*, which adversely affects milk output and quality. These findings emphasize the need to enhance biosecurity and hygiene awareness among farm workers to mitigate environmental mastitis-causing bacteria to improve farm management and productivity.

Key words: Environmental Mastitis, Hygienic Milking Practices, Biosecurity, Bacterial Prevalence, Economic Losses.

INTRODUCTION

The dairy industry plays a pivotal role in global agriculture and nutrition, contributing significantly to human health and economic stability. Milk, the main product of the dairy industry, is a rich source of essential nutrients such as proteins, vitamins, and minerals. It serves as a fundamental dietary component for people of all ages,

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supporting bone health, muscle building, and overall well-being (Lambrini et al. 2021). In healthy mammary glands, milk is thought to be relatively sterile due to the strong inherent defense mechanisms in the udder; however, once milk is secreted from the udder, the potential for exposure to different sources of contamination is increased. Spoilage bacteria and pathogens can infiltrate milk from various sources, including, encompassing the dairy farm environment, feed, water sources, udder and teat surfaces, milking equipment, raw milk tanks, and even personnel involved in the milking process. Contamination risks are particularly higher during the milking process, where direct contact with hands, clothing, and milking equipment can introduce microorganisms. Moreover, dairy farms pose additional challenges as airborne particles and general environmental conditions can contribute to the presence of microorganisms (Ruegg 2017).

Mastitis, a prevalent disease in dairy cattle, is a significant challenge to the global dairy industry. Public health is potentially at risk because mastitis may transmit zoonoses and sicknesses associated with food toxins (Blum et al. 2008; Zouharova and Rysanek 2008). This is often related to factors such as suboptimal hygiene practices and inadequate farm management; contribute to the inflammation of mammary glands (AL-bayati et al. 2023). In addition, the multifactorial nature of mastitis results in varying prevalence and transmission rates among different farms. This variation is influenced by the effectiveness of the udder health control programs implemented within individual farms (Hogeveen et al. 2011). This inflammatory condition has direct adverse impacts on milk production by decreasing its quantity, quality, and shelf life, increasing milk somatic cell count (SCC), flocculation, or unfavorable chemical, physical, and usually bacteriological changes in the milk (Constable et al. 2017). These alterations are attributed to the inflammatory processes that damage the epithelial cells of the mammary gland, which are responsible for the synthesis of milk components. In addition to the presence of mastitis pathogens, depending on the type of bacteria present in milk, which can invade the milk-secreting tissues of the mammary gland and cause severe food poisoning. Thereby all these changes directly influence the economic performance of dairy farms worldwide (Murphy et al. 2016; Tancin et al. 2017). Additionally, other indirect costs associated with mastitis arise from other sources such as veterinary services, reproductive failure, culling and replacement, elucidating the significant economic impact of mastitis on the dairy industry (Vissio et al. 2015; Hogeveen et al. 2019).

Being a complex multi-etiological disease, mastitis results from the interaction of host, environmental, and pathogenic factors, increasing the risk of zoonotic transmission through contaminated milk or direct contact with infected cows depending on the type of microorganism causing mastitis (Kibebew 2017; Maity and Ambatipudi 2021). A Variety of pathogens, including viruses, bacteria, and fungi can cause mastitis, with certain species like *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *E. coli* spp. being the most prominent environmental and contagious pathogens causing mastitis (AL-bayati et al. 2023; Abd-Elfatah et al. 2023). Furthermore, Bovine ephemeral fever (BEF) is an immune-related acute febrile viral infection affecting cattle (Abo-Sakaya and Bazan 2020). It is also known as a three-day sickness in tropical and subtropical regions. In dairy herds, it adversely impacts reproduction and reduces resistance against mastitis. This results in significant economic loss due to reduction or even complete cessation of milk production, so some lactating cows can dry up completely. As well as abortion, loss of condition, and prolonged recovery for some animals (Lunagariya et al. 2015). For early detection of mastitis, it is important to regularly check the milk density and color of suspected animals. Although there are various diagnostic methods, the bacteriologic culture of milk samples is considered the standard for accuracy (Dohoo et al. 2011). The hygiene of the farm environment plays an important role in preventing the transmission and spread of dangerous pathogens in dairy farms, with a strong correlation between the prevalence of mastitis and farm hygiene (Quintana et al. 2020). The close contact between milkers` hands, animals, and equipment facilitates the spread of disease. Detection is challenging due to the subclinical nature of most cases, requiring special attention for diagnosis, prevention, and control (Kibebew 2017). Hygiene improvement, sanitation, disinfection, hygienic feeding, water provision, isolation of diseased cows, routine screening, and isolating and identifying prevalent bacteria to determine the proper treatment are being the key steps for effective mastitis prevention and control (Adkins and Middleton 2017; Bari et al. 2022). Understanding the causative pathogens and risk factors is crucial for planning control strategies, emphasizing the need for research on hygiene-related factors (Cobirka et al. 2020). Biosecurity measures, including disease prevention and environmental hygiene, are essential to reduce disease risks and economic losses. Despite recommendations, gaps exist between effective biosecurity and actual practices, highlighting the importance of information about farm hygiene and biosecurity levels for disease prevention and identifying areas for improvement (Harun et al. 2022).

This study aims to assess the level of awareness about farm hygienic milking practices and biosecurity in dairy farms, and to assess the relationship between these factors, and the prevalence of environmental bacteria causing mastitis, such as *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *E. coli* spp., in addition to their impact on the milk production and farm profitability. Ultimately, this study provides valuable insights for developing effective mastitis control strategies.

MATERIALS AND METHODS

Study location

This study was conducted on a El Qanater El Khairyah dairy farm in the Qalioubia Governorate, Egypt. The farm comprised 12 yards, each measuring 500m² and housing 50 cows, totaling 632 cows. It employed a loose housing system with a milking parlor (Fig. 1a). The farm was approximately 500m² from the main road and 5km from the nearest neighboring farm. Potential sources of contamination included a nearby slaughterhouse and agricultural fields.

Fig. 1: a) the loose housing system with a milking parlor for the dairy farm under study. **b)** Udder edema and redness in addition to bloody milk clots indicating clinical mastitis.

Farm hygiene and biosecurity scoring system

The farm's hygiene and biosecurity measures were assessed monthly from June to August 2023 using a scoring system based on the methodologies of Damiaans et al. (2020) and Harun et al. (2022). This involved evaluating external and internal environmental hygiene, farm constructions, milking hygiene practices, animal management and handling, and worker awareness. Scores were calculated as follows (Dewulf and Immerseel 2019):

Farm score = (Scores of applied measures / Scores of total measures) x 100

Hygienic measures taken on the farm before the 2nd farm visit: firstly, during animal hygiene practices, udders were disinfected with chlorhexidine or iodophors. Hygienic disposal of dead animals was also part of the process. Solid manure was removed monthly using tractors, and sick animals were quarantined in separate isolation pens. Secondly, during worker hygiene practices, Hand sanitizers such as TH7 Nano plus® and Veticon-S® were used. Additionally, foot baths containing formalin or copper sulfate were changed weekly.

Physical examination of udder and milk

Cow's udder and teat were examined for the signs of clinical mastitis. The udders of the study cows were examined visually and by palpation for the presence of clinical mastitis. During examination, attention was given to cardinal signs of inflammation (i.e., redness and edema) of udder quarters, in addition, the fore streams of milk were collected and examined visually on a routine basis for any abnormalities in the milk as in color or consistency (i.e., presence of bloody milk clots) (Fig. 1b). In this study, mild mastitis cases included changes were observed only in the milk including the presence of flakes, clots and blood, watery consistency (as apparently healthy, recovery, recurrent and antibiotic treated cows).

On the other hand, severe mastitic cows had visible changes in the milk characters, swollen udders with loss of appetite as in case of fibrosis in which cows suffered from a hard fibrotic bigger mass diffused in whole one or four udder quarters and others with localized fibrotic nodules or pea like lesions near the base of the teats were selected after strict manual physical palpation to the udder (Kumar 2020).

Milk yield determination

Data on milk output was gathered from cows that appeared to be in good health. The total monthly milk production divided by the average number of cows free of mastitis each month yielded the average daily milk yield for healthy cows.

Sampling

Collection of environmental samples

A total of 213 environmental samples and swabs were collected from various farm locations including the roof, walls, floors, feeders, manure areas, milking parlor, milk storage tanks, and worker contact points.

Milk samples collection

Following the protocol described by Carter and Cole (2012), briefly, before sampling, teat ends were disinfected with a 0.5% iodine solution and dried with disposable towels before collection. The first streams of foremilk were discarded, and 72 milk samples and swabs were collected from clinical mastitis-positive cows (Only udder quarters showed visible signs). Milk tank samples were agitated for 10 min and collected from the top using a clean, sanitized dipper and all samples were collected into labeled sterile bottles for bacteriological analysis. Ethical approval was granted by the Ethical Approval Committee of the Faculty of Veterinary Medicine, Benha University, Egypt (BUFVTM 15-11-23).

Bacteriological examination of collected samples

enrichment of samples

Samples were incubated in Buffer Peptone Water (BPW) at 37°C for 24h.

Isolation of indicator bacteria

Isolation of *Staphylococcus* spp.

The enriched swabs and samples were cultured on Baird-Parker agar (BP) supplemented with egg yolk telluride emulsion, incubated at 37˚C for 48h. Colonies are showing characteristic phenotypes of *Staphylococcus* spp.(circular, black, convex with or without light halo on BP agar) according to Sudershan et al. (2012).

Isolation of *Streptococcus* spp.

The enriched samples and swabs were cultured on Kenner fecal (KF) Streptococcal agar and incubated aerobically at 37°C for 24h. According to Yashoda et al. (2001), colonies are small pinpoint yellowish-brown colonies.

Isolation of *Pseudomonas* spp.

A loop full of prepared enriched samples was streaked onto Cetrimide agar and incubated aerobically for 24 h at 37°C. This gave a large yellow colony with irregular growth and was examined for pigment production (green fluorescent) and odor (fruity) according to Sule et al. (2019).

Isolation of *E. coli*

The enriched samples were streaked on Eosin Methylene Blue agar (EMB) plates and incubated aerobically at 37°C overnight. Single metallic sheen colonies on the EMB agar plates were considered as indicative of *E. coli* then the typical colony was confirmed by morphological study by Gram staining according to Ievy et al. (2020).

Biochemical identification

Biochemical identification of *Staphylococcus* spp.

The fresh separate colony was taken for biochemical tests such as Mannitol fermentation (positive), Coagulase (negative), Catalase (positive), Nitrate reduction (positive), Oxidase (negative). All biochemical test tubes were incubated for 24h at 37°C according to Quddoumi et al. (2006).

Biochemical identification of *Streptococcus* spp.

Subculture separated fresh colonies were taken for making Catalase test (negative), Simmon citrate test (positive), Indole test (negative), Urease test (negative), Methyl red test (positive), Nitrate reduction test (negative), H_2S production test (negative) and Gelatin hydrolysis test (positive) then all tubes were incubated aerobically for 24h at 37°C according to Yashoda et al. (2001).

Biochemical identification of *Pseudomonas* spp.

A typical fresh separate colony was taken for Oxidase test (positive), Catalase test (positive), Urease test (positive), Simmon Citrate test (positive), Indole test (negative), Triple Sugar Iron test (negative), Methyl red test (negative), Voges–Proskauer test (negative), growth of all at 37°C for 24h then take the result according to Sule et al. (2019).

Biochemical identification of *E. coli*

Separate colony subculture on EMB and incubated aerobically at 37 °C for 16h then take fresh colony for performance of TSI (Triple Sugar Iron) test (positive), Simmon citrate test (negative), Methyl red test (positive), Voges–Proskauer test (negative) and Indole test (positive) according to Ievy et al. (2020).

Statistical analysis

Statistical analysis for impact of mastitis on milk production was done by Friedman test using GraphPad Prism version 10.1.1 (GraphPad Prism 10.1.1 ©1992-2023 GraphPad software, LLC). Multiple comparisons were done using Dunn's multiple comparisons test with the significance value set at $P<0.05$. Statistical procedures were performed using the computer programs SPSS/PC$^+$ "version 23" (SPSS 2015). Descriptive statistics such as frequency distribution and percentages were used to determine the biosecurity scores of different applied hygiene measures in the tested dairy farm and bacterial prevalence associated with different samples sources. Chi-square was used to check for the statistical significance of the variation in the prevalence of bacterial species isolated from various sample types over the course of three visits to the dairy farm under investigation. Economic impacts are estimated descriptively based on the collected data; we calculated the losses per 100 cows per $. Direct and indirect costs of BEF associated with mastitis are considered to give a comprehensive estimation of economic total losses.

RESULTS

Assessment of dairy farm biosecurity measures

The biosecurity questionnaire aimed to comprehensively evaluate the cleanliness and hygienic status of the examined dairy farm. Four main hygiene categories were assessed: farm constructions, animal hygiene, worker hygiene, and milk hygiene, each with specific subcategories (Fig. 2). The results of the assessment demonstrate that the milk-related hygienic measures at the dairy farm being examined received the highest biosecurity score (80%), whereas farm construction hygiene received a little lower score (72%). Nevertheless, the hygienic precautions implemented by the worker received the lowest biosecurity score, amounting to only 16.67% (Fig. 3).

Main hygiene category	Hygiene Subcategory	Yes	No
1-Farm constructions	1- Distance from other farms is legal	√	
	2- Distance from the main road is legal	√	
	3- Presence of fence	√	
	4- External pollution sources	√	
	5- Wheel dip		√
	6- Pets control		√
	7- Pest control	√	
	8- Regular manure disposal	√	
	9- Regular disinfection of concrete drinker	√	
	10- Disinfection of farm construction		√
	11- Suitable ventilation system	√	
	12- Suitable cooling system	√	
	13- Suitable number of milk parlor and milk room		√
	14- Good disinfection program to milk parlor	√	
	15- Good disinfection program to milk room	√	
	16- Presence of incinerator		√
	17- Documentation and recording system to the farm	√	
	18- Visitors control to the farm		√
2-Animal hygiene	1- Sick animal isolation	√	
	2- Hygienic disposal of dead animals		√
	3- Monitoring of subclinical mastitis		√
	4- Good source of purchased animal	√	
	5- Quarantine of newly introduced cow		√
	6- Culling strategy	√	
	7- Presence of artificial insemination	√	
	8- Bull care		√
	9- Dry cow care	√	
	10- Pre-weaned calf care	√	
	11- Presence of a veterinarian for regular observation	√	
	12- Suitable stocking density		
	13- Using teat dip	√	
3- Worker hygiene	1- Presence of specific uniform		√
	2- Foot dip to worker shoes		√
	3- Hand sanitizer using		√
	4- Worker does not contact with other flocks	√	
	5- Separation between workers dealing with flock and others dealing with milk		√
	6- Knowledge about biosecurity		√
4- Milk hygiene	1- Presence of automatic milking process	√	
	2- Good disinfection process for milk equipment	√	
	3- Discard of abnormal physical milk character	√	
	4- Regular examination of milk sample	√	
	5- Good milk room hygiene		√

Fig. 2: Biosecurity questionnaire for evaluation of hygienic status of tested dairy farm.

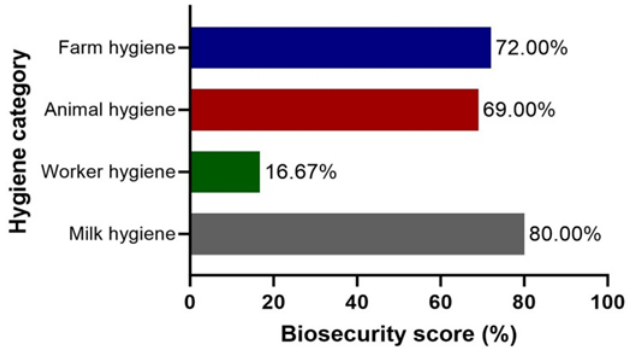

Fig. 3: Biosecurity score of different applied hygiene measures in the tested dairy farm.

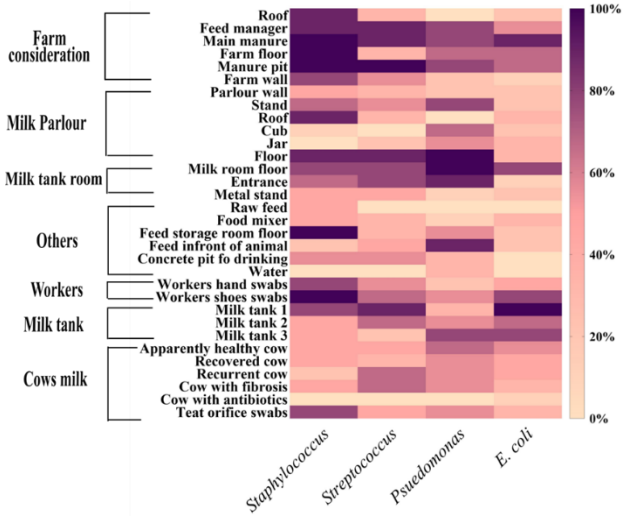

Fig. 4: Heat map showing the prevalence rates of isolated bacterial species from various environmental and milk samples. The dark-colored cells show low prevalence rates, and the light-colored cells show high prevalence rates of different bacterial spp. within three visits.

Bacterial prevalence in different farm compartments

The results revealed distinct bacterial prevalence pattern within different farm environments and milk samples (Fig. 4). *Staphylococcus* spp. showed higher prevalence in primary manure, farm floor, and manure pit, while being absent in water, milk jars, and antibiotic-treated cow milk. *Streptococcus* spp. predominated the manure pit, feed manager, main manure, and farm floor, but was absent in cubs, water, and jars. *Pseudomonas* was detected in farm and milk facility floors, while it was notably absent from farm and milk parlor roofs, raw feed, and milk tank rooms. Interestingly, *E. coli* was prevalent in major manure samples but absent in raw feed, concrete pits for drinking, water samples, farm walls, and milk tank room entrance swabs. Additionally, workers' hands and shoes emerged as significant carriers of *Staphylococcus* spp., *Streptococcus* spp., and *E. coli* spp. Milk samples from tanks exhibited the highest prevalence of *E. coli* spp., *Streptococcus* spp., and *Staphylococcus* spp. *Streptococcus* spp. was more prevalent in recurrent cows and those with fibrosis, whereas *Pseudomonas* was found in apparently healthy cows. Antibiotic-treated cow milk samples were free of all bacteria except *E. coli*. Statistical analyses using Chi-square (X2) revealed significant variations in the prevalence of isolated bacterial species throughout various sample types over the course of three visits to the dairy farm under investigation, with only *Staphylococcus* spp. for the milk samples that obtained showing a significant difference (P=0.05) between various farm visits (Table 1). Furthermore, only *Streptococcus* showed a significant difference (P<0.05) between worker's hand and shoe swabs (Table 1). Surprisingly, all the recovered bacterial species (including *Staphylococcus, Streptococcus, Pseudomonas,* and *E. coli spp.*) for the environmental swabs exhibited a significant (P<0.05) variation between different farm visits (Table 1).

Impact of clinical mastitis on milk production

Throughout the course of this study, there was a decrease in the overall number of healthy and lactating cattle on the farm that was the subject of this study (Fig. 5a) over the four months (May, June, July, and August). Additionally, there was a consistent decrease in daily milk production over the four months (May, June, July, and August). In May, cows exhibited the highest average milk yield, peaking at more than 4252L. However, the production gradually decreased in June and July with the onset of clinical mastitis, reaching its lowest in August (Fig. 5b). Notably, this decline coincided with the emergence of Bovine Ephemeral Fever (BEF) disease, complicating clinical mastitis, and contributing to the observed reduction in milk production.

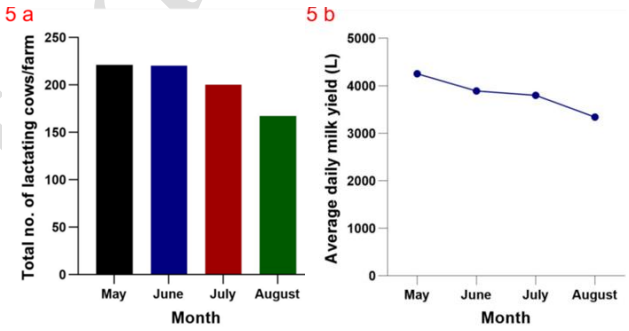

Fig. 5: a) the overall number of healthy and lactating cattle/farm that was the subject of this study. **b)** Average daily milk production for all lactating cattle/farm over the four months (May, June, July, and August).

Adverse impact of clinical mastitis and Bovine Ephemeral Fever complicated with clinical mastitis on holstein dairy farm

The cows inside the farm of our study suffered from clinical mastitis of 18.1% during the 1st and 2nd visits. Before the 3rd visit, the farm showed the emergence of Bovine Ephemeral Fever (BEF) disease, complicating the clinical mastitis with discarding the milk of diseased cows for five days. The BEF showed a 100% morbidity rate, alongside a mortality rate of 16.5% and a culling rate of 8%. Reproductive complications were evident, with a 28.17% incidence of abortion and an 11.27% occurrence of stillbirths. Clinical Mastitis affected 30.5% of the cows compared with 18.1% before viral infection as shown in Table 2. BEF complicated with Mastitis showed huge losses in comparison with clinical mastitis alone as shown in Table 3. The losses represented in mortality, culling, abortion, still birth, viral (treatment, vaccination and disinfection) cost, mastitis treatment cost and losses of

Table 1: Prevalence of some bacteria isolated from milk samples, workers hand and shoes swabs and environmental swabs during three visits

Bacterial spp.	Farm visit	milk samples			workers hand and shoes swabs			environmental swabs		
		Prevalence (%)	Chi-square (X^2)	P-value	Prevalence (%)	Chi-square (X^2)	P-value	Prevalence (%)	Chi-square (X^2)	P-value
Staphylococcus spp.	1st visit	37.5	5.85	0.05	39.1		0.17	31.4	8.2	0.017
	2nd visit	18.8			30.4	3.5		28.0		
	3rd visit	43.8			30.4			40.7		
Streptococcus spp.	1st visit	39.5	5.46	0.06	53.3	8.9	0.01	47.3	16.4	0.00
	2nd visit	21.1			13.3			24.7		
	3rd visit	39.5			33.3			28.0		
Pseudomonas spp.	1st visit	28.2	2.35	0.31	50.0	2.72	0.26	38.8	5.9	0.05
	2nd visit	30.8			25.0			25.5		
	3rd visit	41.0			25.0			35.7		
E. coli spp.	1st visit	40.5	4.46	0.11	40.5	4.46	0.11	53.3	17.14	0.00
	2nd visit	23.8			23.8			18.3		
	3rd visit	35.7			35.7			28.3		

Table 2: Incidence and adverse effect of clinical mastitis before and after Bovine Ephemeral Fever (BEF) infection for Holstein dairy farm

Item	Number/total	Percentage
Clinical mastitis Incidence	40/221	18.1
Mortality	5/221	2.26
Culling	16/221	7.23
Abortion	0/71	0
Stillbirth	0/71	0
Viral infection		
BEF incidence	611/611	100
Clinical mastitis Incidence	61/200	30.5
Mortality	33/200	16.5
Culling	16/200	8
Abortion	20/71	28.17
Stillbirth	8/71	11.27

Table 3: Economic losses of clinical Mastitis and Bovine Ephemeral Fever (BEF) complicated with clinical Mastitis for Holstein dairy farm

Item	Percentage (%)	Losses/1 cow (EGP)	Losses/100 cows (EGP)	Losses/100 cows ($)=35EGP
Before viral infection				
Mortality	2.26	60,000	(60,000*2.26)=135600	3,874.29
Culling	7.23	40,000	(40,000*7.23)=289,200	8,262.86
Mastitis treatment cost	18.1	400	(400*18.1)=7,240	206.86
Discarded milk (5d)	100	(20kg*17EGP*5d)	(1,700*18.1)=30,770	879.14
Total losses	-	-	327,210	9348.86
After viral infection				
Mortality	16.5	60,000	(60,000*16.5)=999,000	28,542.86
Culling	8	40,000	(40,000*8)=320,000	9,142.86
Abortion	28.17	9,000	(9000*28.17)=253,530	7,243.71
Stillbirth	11.27	9,500	(9500*11.27)=107,065	3,059.0
Treatment cost	100	1,000	100,000	2,857.14
Vaccination cost	100	260	26,000	742.86
Disinfection cost	100	50	5000	142.86
Mastitis treatment cost	30.5	400	(400*30.5)=12,200	348.57
Discarded milk (5d)	100%	(20kg*17EGP *5d)	(1700*30.5)=51,850	1,481.43
Total losses	-	-	1,874,645.0	53,561.29

discarding milk, they were about $28,285.71, $4,571.43, $7,243.71, $3,059, $2,857.14, $742.86, $142.86,$348.57, and $1,481.43, respectively) per 100 cows. Concerning the losses of clinical mastitis before viral infection, the treatment cost was about $206.86 per 100 lactating cows with $879.14 from discarding milk. The total estimated losses before and after viral infection per 100 cows were approximately ($9091.71 and $48,989.86, respectively).

DISCUSSION

Still, one of the biggest challenges dairy farms face is the introduction of pathogenic microbes. Mastitis is one of the numerous dangerous diseases that are more likely to spread due to poor hygiene and inadequate biosecurity measures (Baraitareanu and Vidu 2020). Mastitis still has a major influence on milk yield, quality, and dairy economics even with advancements in quality control and cleanliness in the milk production process (Ruegg 2017; Kim et al. 2023). A clinical examination that includes palpating and visually evaluating the afflicted area can determine whether there is clinical mastitis (Min et al. 2007). According to this study, a physical examination that notes important symptoms such as changes in milk and inflammation in the udders makes it simple to diagnose clinical mastitis. Among these modifications are obvious abnormalities in milk, such flakes, clots, pus, bloody or watery consistency. Feverish symptoms, decreased milk supply, and appetite loss accompany this. Gross inflammation of the udder is evident in its swelling, elevated warmth, redness, and pain. This study examined the

workers' perceptions of the importance of hygienic milking and biosecurity measures on the farm, as well as the hygienic standards of a dairy farm in Qalioubia Governorate, Egypt. The biosecurity scoring system-based questionnaire was used to gauge the employees' understanding and implementation of these crucial precautions. Additionally, the study examined the prevalence of environmental bacteria through microbiological analysis of a variety of samples taken from various farm compartments, workers, and milk. These bacteria are known to cause environmental mastitis and include *Streptococcus*, *Staphylococcus*, *Pseudomonas*, and *E. coli* spp.

The results showed that the average hygiene score of the dairy farm under evaluation was 59%, indicating a "Good" hygienic state based on the evaluation technique proposed by Harun et al. (2022). The highest score (80%) was attributed to sanitation practices related to milk; whereas the lowest score (16.67%) was for hygiene practices related to workers. Although the farm implemented the most recommended hygiene protocols for milk handling, infrastructure, and animal care, it fell short in implementing key hygiene practices for workers. These neglected practices include the provision of specialized farm clothing, use of foot dips and hand sanitizers, maintaining separate personnel for flock and milk handling, and ensuring workers have adequate biosecurity knowledge. This lack of implementation shows the workers' inadequate understanding of the significance of maintaining proper hygiene. A previous study in Southwest Delhi showed that the dairy farmworkers had insufficient knowledge. Therefore, it is crucial to establish guidelines for the workers to adhere, while also ensuring that officials educate them and regularly monitor their performance to uphold hygienic practices (Ahmed et al. 2020). Yilmaz and Koyuncu (2022) reported comparable results while evaluating the biosecurity protocols of Bursa's dairy farms. They stressed how crucial it is for laborers and farmers to comprehend the variables that lead to the spread of infectious diseases, how to prevent and control them, and how to employ good hygiene and biosecurity measures.

To track the improvement or decline in the farm's level of hygiene, the dairy farm was visited three times as part of this study. During each visit, various environmental samples including farm construction, animal samples, milk samples, and worker swabs were collected to identify various environmental bacteria species. According to the findings, *E. coli*, *Pseudomonas* spp., *Streptococcus* spp., and *Staphylococcus* spp. had the greatest average incidence rate. According to a prior study done in China, the prevalence rates of E. coli, Pseudomonas, Streptococcus, and Staphylococcus species were 30.3%, 68.4%, less than 1.0% and 9.1%, respectively (He et al. 2020). Furthermore, a study conducted in southern Ethiopia found that the prevalence rates of *E. coli* spp., *Streptococcus*, and *Staphylococcus* were 17.3%, 18.6% and 57.3%, respectively (Abebe et al. 2023).

The findings of our study indicate that the highest prevalence of *Staphylococcus*, *Streptococcus*, *Pseudomonas*, and *E. coli spp*. were observed in manure, farm floor, and manure pit. Therefore, the primary origin of these bacteria in dairy farms is the contamination of the environment through animal's manure. This finding aligns with the findings given by Sobur et al. (2019), Alegbeleye and Sant'Ana (2020), Schauer et al. (2021) and Casey et al. (2013). Moreover, the findings of our study indicate a significant presence of *Staphylococcus*, *Streptococcus*, *Pseudomonas*, and *E. coli* in swabs taken from workers' shoes and hands. This suggests that there is a notable issue with the workers' hygiene practices, which in turn plays a crucial role in the transmission of infections among cows on the farm (Ahmed et al. 2020; Quintana et al. 2020; Youssef et al. 2021). This could be attributed to inadequate management practices, such as the utilization of shared towel fabric and the use of a milking machine without proper sanitation between and after each milking session. *Staphylococcus*, *Streptococcus*, and *E. coli* were not found in any of the water samples. This could be attributed to the use of public tap water, which is typically treated with chlorine. Chlorine treatment effectively eliminates these bacteria from water (Oziegbe et al. 2023).

Bulk tank milk culture may be used as a monitoring tool for estimating herd-level prevalence of contagious mastitis pathogens in both clinical and subclinical mastitis cases. This tool may be useful while investigating potential milk quality problems on a dairy farm (Jayarao and Wolfgang, 2003). During the first visit, the prevalence of *E. coli* in milk samples was higher than the finding of Li et al. (2018) who reported high prevalent of *E. coli* isolated from mastitis milk samples. The greatest prevalence of *E. coli* mainly in milk tank may be associated with poor hygienic condition and dirty bedding surrounding dairy animal, in addition to poor personal hygiene of dairy workers (Metz et al. 2020). There may be a connection between the high *E. coli* prevalence in milk and the unclean living conditions of dairy animals, as well as the lack of personal hygiene among dairy employees (Metz et al. 2020). *E. coli* can enter milk via the teat canals, which are also accessible to the workers hands and cups. Moreover, presence of *E. coli* in milk and milk products act as a good indicator of fecal contamination, in addition *E. coli* led to complete loss of milk production in dairy farm and its negative effect on milk quality can persist for weeks after the eradication of the bacteria (Gomes and Henriques 2016).

Streptococcus spp. is the second dominant isolate mainly in milk tank. This finding was higher than that of Kassa et al. (2014). Most *Streptococcus spp.* can form a strong of biofilm that increase their resistance during the production process of some dairy products (cream and cheese) (Ali 2020). One possible explanation for the elevated *Streptococcus* spp. isolation rate is that Penstrip was not widely used to treat mastitis on the farm under investigation. This medicine is extensively utilized to reduce Streptococcus infection in dairy farms because it is regarded an effective antibiotic. The high prevalence of *Staphylococcus* spp. mainly in milk tank could be associated with the absence of improper post milking teat dipping, poor udder washing and drying before milking (Gemechu et al. 2019). Also, *Staphylococcus* spp. may be transmitted from teat orifices and hands of dairy workers which showed the highest prevalence of these pathogens. *Staphylococcus* and *E. coli* spp. enhance the production of urokinase which induces an increase in plasminin bovine epithelial cells that survive the heat treatment and hydrolyze milk casein decreasing the quality of dairy product and curd formation (Ma et al. 2000).

The prevalence of *Pseudomonasspp.*in milk samples may be attributed to the psychotropic nature of theses bacteria which can survive on the milk bulk tanks, milking machines, and animal production environment and can produce heat stable lipolytic and proteolytic enzymes in raw milk (Simões et al. 2010).These enzymes remain active even after pasteurization(at 71.5-74°C for 15 to 30s), and ultra-high temperature treatment (at 135-145°C for 2–3s), causing deterioration of refrigerated milk and dairy products while also altering milk coagulation characteristics (Oliveira et al. 2002).

The isolation of all bacterial species from milk samples agreed with Mahamad and Mohammed (2023) who isolated *Staphylococcus* spp. (48.21%), *E. coli* (19.64%), *Streptococcus* spp. (12.5%) and *Pseudomonas* spp. (11.61%), which were the most dominant species in mastitis milk. On the other hand, all studied bacteria (in cows treated with antibiotics) were unable to be isolated from milk samples, except for *E. coli*, which was isolated at a low prevalence; this finding can be explained by drugs' capacity to kill these bacteria. Eliminating existing infections decreases the exposure of sensitive quarters, which can be accomplished through therapy during lactation or during dry off. However, there has been an increase in *E. coli* antibiotic resistance, according to the Food and Drug Administration (FDA, 2014). Schukken et al. (2011) noted that there is still a lack of clarity regarding the characteristics and virulence factors that are unique to *E. coli* strains that cause mastitis. The variation in prevalence of these infections across studies could be attributed to changes in geographical location, management systems, sample size, and hygienic practices used in farms and milk collection locations. Moreover, the results showed that milking equipment and feed samples had a low prevalence of isolated bacteria, and this is due to disinfection process of milking equipment which cause killing of the bacteria (Pacheappan et al. 2022). On the other hand, the thermal and chemical treatments during feed manufacturing and processing kill or reduce the survival of bacteria in feed (Shurson et al. 2022).

The dairy farm showed the highest frequency of isolated bacteria on the first visit, which was primarily caused by poor hygiene procedures and a lack of implementation of several biosecurity measures. As a result, there was a considerable degree of contamination on the property. This discovery is consistent with the findings reported by Nyokabi et al. (2023). Furthermore, a major contributing reason to the increased spread of diseases across the farm is the disregard for human hygiene practices. Depending on the owners' and employees' level of awareness on the significance and mode of implementation, different approaches are taken while implementing biosecurity measures on farms. Dairy farms implement low levels of biosecurity controls due to the seldom implementation of certain techniques (Harun et al. 2022). Despite dairy producers' belief that biosecurity is vital, previous research conducted in the UK and Ireland has revealed a low rate of adoption of biosecurity policies (Brennan and Christley 2013).

Because of this, the problem was found on the farm, and the people responsible were told what steps needed to be taken to stop the germs from spreading on the farm. The result was clear during the second visit, when most of the cleanliness measures for workers and animals were put into place. However, during the third visit, even though the farm was cleaner, the spread of bacteria started up again. This time, the real reason was that Bovine Ephemeral Fever got into the farm, mostly through hematophagous insects. As a result, the animals' immune systems were weakened (Lavon et al. 2023). Bacteria like *Staphylococcus*, *Streptococcus*, *Pseudomonas*, and *E. coli* spp. grow and spread more quickly when viruses attack people (Rezzoagli et al. 2020; Rossi et al. 2020; Sora et al. 2021; Pilarczyk-Zurek et al. 2022). In relation to the economic impacts of clinical mastitis and Bovine ephemeral fever complicating mastitis, our findings revealed significant losses. Mastitis is regarded as the most common disease of dairy cows and causes great economic loss to the dairy industry as udder health is considered one of the most important reasons for culling in dairy herd, it costs $8,262.86/100 cows our result in the same line with Down et al. (2013)who showed that the economic costs of clinical mastitis is one of the foremost important reasons for culling. In responding to discarding the milk and treatment cost, we estimated average losses per 100 cows, they were ($879.14 and $206.86, respectively) which varied from cow to another one depending on the severity of mastitis, and milk yield of this cow, as acute cases characterized by general illness, so they require more time for treating (Petrovski et al. 2006). Regarding the viral infection, BEF exhibited a morbidity rate of 100%, with 28.17% of cases resulting in abortion and 11.27% resulting in stillbirth. The issue of mastitis, which resulted in a complete elimination of all milk production during this period, and the milk output, does not return to pre-illness levels following recovery. Our result in the same line with Akakpo (2015) who indicated that BEF has significant economic value due to its influence on reproduction and milk production. When mastitis is associated with bovine ephemeral fever (BEF), it further hampers the sustainable growth of the dairy industry. The economic losses incurred due to this combination are substantial, amounting to approximately $53,561.29 per 100 cows.

The total milk output was measured in May, June, July, and August, whereas milk loss was estimated in August, when clinical mastitis associated with BEF illness occurrence. This study found a negative connection between clinical mastitis and milk yield. Larger monthly clinical mastitis rates mean larger monthly milk production losses. Mastitis reduced milk supply and rejected poor-quality milk (Gomes and Henriques 2016). Mastitis' economic losses are largely due to decreased milk production due to mammary gland tissue damage (Zhao and Lacasse 2008). This is supported by Kandeel et al. (2018) who found that severe mammary gland inflammation in dairy cows reduced milk output. A week before clinical signs, Wilson et al. (2007) reported that a rapid reduction in milk output. Mastitis severely affects milk production, especially in early lactation or before peak production (Sharma et al. 2011). This supported by Le Maréchal et al. (2011), who found that gram-negative pathogens like *E. coli* caused the highest milk output losses compared to gram-positive pathogens like *Streptococcus* and *Staphylococcus* spp. Daily milk production loss from *E. coli* infections is estimated at around 30% per cow during the 305 days of lactation (Blum et al. 2014). The

largest average monthly milk loss due to clinical mastitis was 5% of the average total monthly milk production, according to (Ameni et al. 2022). They also found significant milk output losses in the second, third, and fourth weeks after clinical mastitis.

Conclusion

Prevention is preferable to control, thus implementing effective management, biosecurity, and hygienic milking practices are essential for reducing bacterial, viral, and other kinds of contamination, especially those known to cause environmental clinical mastitis, such as *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* sp., and *E. coli* spp. This would result in the avoidance of the enormous economic losses that are a consequence of mastitis, which are estimated to be around $9348.86 per hundred cows before viral infection and reach about $53,561.29after viral infection according to this study.

Conflicts of interest statement

The authors have no conflicts of interest to disclose.

Author contributions

Practical and laboratory work: All authors contributed equally. Data investigation: Hala El Daous, Manar Elkhayat,Nehal Alm El Din, Eman Nafei, Eman Hafez, Amira M. Abd-El Hamed.Writing original draft: Nehal Alm El Din and Amira M. Abd-El Hamed.Statistical analyses and editing manuscript: Manar Elkhayat and Hala El Daous. All authors revised and edited the final version of the manuscript.

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