CONTENTS

RESUMO: Um total de 695 vacas leiteiras Holstein Friesian lactantes foram examinadas para a prevalência de mastite subclínica (SCM) e sua associação com fatores de risco selecionados em uma fazenda privada de leite no distrito de Fayoum, Egito. 444 vacas em lactação com 1145 quartos foram consideradas positivas para SCM com prevalência de 63.88% e 41.18% no nível de vacas e quartos, respectivamente com base no California Mastitis Test (CMT), Condutividade Elétrica (EC) que teve um valor médio de 6.27 ± 0.066 mS/cm e Contagem de células somáticas (SCC) com um valor médio de 8.8×10^5 ± 9.2×10^3 células/ml. Os fatores de risco investigados neste estudo revelaram que o maior percentual de SCM foi observado no inverno, em vacas no final da lactação com um percentual de 32.21 e 59.91 respectivamente. Os resultados concluíram que as vacas jovens foram mais expostas ao SCM do que as vacas velhas. Enquanto a ocorrência de SCM foi maior nas vacas com 2°, 3° e 1° do que nas com 4°, 5°, 6° paridade número. Os resultados analíticos também revelaram que existe uma diferença altamente significativa (p <0.05) entre os números de paridade. No entanto, não há diferença significativa (p > 0.05) entre os diferentes estágios de idade, lactação e estação do ano como fatores de risco.

PALAVRAS-CHAVE: Mastite subclínica, Fatores de risco, Idade, Paridade, Estágio de lactação, Estação

INTRODUCTION
Bovine mastitis is a global problem that negatively affects the animal health and quality & quantity of milk. In addition, it causes huge financial losses to the dairy industry (Amenu; Shitu; Abera, 2016; Youssif et al., 2019).

Subclinical mastitis (SCM) is characterized by no obvious signs either within udder or milk. However, the milk production diminishes and the somatic cell count (SCC) elevates. SCM is characterized by changes in milk composition, milk pH and ion concentration without any udder reaction marks (Baştan et al., 2015; Nesma et al., 2020).

The inflammatory process is usually initiated when bacteria enter the mammary gland through the teat canal and multiply in the milk. Therefore, leukocytes are released into the mammary gland in response to the bacterial invasion. The few amount of the somatic cells that are normally present in cow’s milk attempt to overcome the infection immediately. Both bacteria and leukocytes in the infected parts

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Received: 10/03/2020. Accepted: 12/30/2020
of the udder liberate chemo-attractive products like prostaglandins, histamine, serotonin, Tumor Necrosis Factor and interferon that may be directly involved in the pathogenesis of the disease through the teat canal (Samad, 2008; Gonsalves et al., 2016).

The ordinary symptoms of inflammation comprise the dilation of blood vessels, the increased of permeability, edema, high blood flow, neutrophil migration, mammary activity reduction, pain and fever. Cow’s mastitis is a multifocal condition and its manifestation based on many variables that may be related to the animal, environment or to the pathogen. The realization of risk factors is conclusive for the oversight schema for mastitis in dairy cows (Qayyum et al., 2016).

‘Risk Factor’ is mentioned as any characteristics that elevates the probability of a disease occurrence. Risk factors such as breed, age, parity, herd size, stage of lactation, hygiene and weather have a great attention on cows subclinical mastitis (FRANDSON et al., 2009). BIFFA; DEBELA; BEYENE (2005); ALGAMMAL et al. (2020) mentioned that the subclinical mastitis is the main cause of cows mastitis in different countries in the world and Egypt. This investigation was achieved to study the association of selected factors of risk with the prevalence of subclinical mastitis in a dairy farm.

MATERIALS AND METHODS

Study area

This study was carried out to investigate the prevalence and associated risk factors for bovine subclinical mastitis. The harvest period took place from December 2016 to November 2017 in dairy herds located in Fayoum district, Egypt. The study duration was sectioned into the 4 year seasons.

Dairy animals

Six hundred and ninety five lactating cows (2780 quarters) from 2300 dairy cows herd were examined for the prevalence of SCM. The farm uses milking machine supported by Afimilk system, which is a milk market that measures milk yield and milk Electrical Conductivity (EC) during milking to monitor cow health and milk production. The milking machine can detect the SCM automatically and gives warning alarm when the daily milk yield decreases and electrical conductivity increases than normal level.

Description of the dairy farm and some constant risk factors

The data was collected with the help of the farm manager concerning the farm, surroundings environment and the lactating cows. The data includes the following: Cow breed: Holstein Friesian; land type: cement; age average: 2-12 years; weight average: 550-700 kg; automatically milked; water source: municipal and running water supply in the milk parlor; pest control: exist and the cows were sprayed (once/week); insemination artificially; not applied oxytocin; a common water trough was available. The farms’ cows were automatically milked three times daily and the milk was collected in big milk containers.

Aseptic milk samples collection

1145 quarter milk samples from 444 apparently healthy cows which were suspected to harbor SCM during milking by Afimilk system and did not show any visible abnormalities or any sign of clinical mastitis were collected according to RADOSTITS et al. (2007) and were examined as follows:

A- California Mastitis Test (CMT) based on Schalm; Carroll; Jain (1971); Anri (2012): Lactating cows were tested using Schalm reagent (Sigma-Aldrich, 23608888) to detect SCM in four quarters of the udder.

B- Electrical Conductivity (EC) according to Linzell; Peaker (1971): Electrical Conductivity was measured using Waterproof EC/TDS Tester (AD31 & AD 32), on a scale up to 200 mS/cm (milliSiemens per centimeter). The meter was calibrated with a specific standard solution before measuring the EC of milk samples. The meter electrode was washed with Distilled Water and dried with paper towels after each measurement.

C- Somatic cell counts (SCC) according to Gonzalo et al. (2006): The Somatic Cell Counts were estimated using Bentley soma count 150 (115 Volts/ 60Hz/ 2Amps) (Chaska, MN 55318, United States). The device measured the total number of milk SCC per ml of milk samples (MSCC/ml). The analysis of variance (ANOVA) test was conducted to test the possible significance \( P \leq 0.05 \) among different mean values using SPSS, version 25. Typical EC of IMI to be \( \geq 5.5 \) mS/cm, cow with CMT score \( \geq +1 \) for at least one quarter was identified as a subclinical mastitis cow. A milk SCC concentration exceeding 200,000 cells / ml generally indicates that cows have an underlying IMI (KASIKCI et al., 2012; PANCHAL et al., 2016; VIKOVA et al., 2017).

RESULTS AND DISCUSSION

The data presented in (Table 1) reveals that the total number of animals inside the farm was 2300 dairy cows, including seven hundred and fifty (32.61%) from them were classified as a dry group, 855 (37.17%) were heifer and before weaning, and the lactating group was 695 cows with a percentage of 30.22.

| Group                          | No. | %   |
|-------------------------------|-----|-----|
| Dry group                     | 750 | 32.61|
| Lactating group               | 695 | 30.22|
| Heifer and before weaning     | 855 | 37.17|

No. = Number of dairy cows
In this study, subclinical mastitis was found to affect 444 dairy cows out of a total of 695 dairy cows with 1145 affected quarters based on CMT, EC and SCC investigation (Table 2).

The results illustrated in (Table 3) showed that the highest prevalence of SCM was recorded in CMT score (+1) at a percentage of 60.00 and the lowest prevalence was reported in CMT score (+3) at a percentage of 8.00.

The mean values of the SCC and EC in this study for the examined SCM quarter milk samples were $8.8 \times 10^5 \pm 9.2 \times 10^3$ cell/ml and $6.27 \pm 0.066$ mS/cm respectively (Table 4).

Inspection of the data presented in (Table 5) revealed that there is a significant difference between both seasons: winter and autumn ($P < 0.05$) based on CMT, EC, and SCC test results, but there were no significant differences of the same variables between other seasons.

The highest percentage of SCM (Table 6) was observed in 4-5 years cows with a percentage of 48.42, while winter was the season of the highest percentage (32.21%) of the SCM inside the farm. On the other hand, the cows with 2<sup>nd</sup>, 3<sup>rd</sup> parity number and cows at the late stage of lactation were exposed to SCM more than other cows.

The data presented in (Table 7) illustrated the significant difference between seasons in each risk factor based on SCM cows. The results revealed that there is a significant difference ($P <0.05$) between winter and summer on one hand and autumn and summer on the other in parity and age as risk factors. However, there is no significant difference ($P >0.05$) in the stage of lactation between the seasons.

Subclinical mastitis is related to changeable origin, seriousness and consequence depending on various agents. Reliable diagnostic methods are needed to detect subclinical mastitis such as California Mastitis Test, SCC and EC.

**Table 2.** SCM quarters and SCM cows’ percentages in the examined farm based on CMT, EC and SCC results.

| Positive samples | Number | %   |
|------------------|--------|-----|
| SCM quarters     | 1145/2780 | 41.18 |
| SCM cows         | 444/695   | 63.88 |

**Table 3.** Total number of SCM quarters in the farm based on CMT score ($n= 1145$).

| CMT score | No. of the affected quarters | %   |
|-----------|------------------------------|-----|
| +1        | 687                          | 60.00 |
| +2        | 367                          | 32.00 |
| +3        | 91                           | 8.00  |

**Table 4.** Statistical analytical results of SCC (cell/ml) and EC (mS/cm) of the examined SCM milk samples.

| Parameter | No. | Min | Max    | Mean ± SE     |
|-----------|-----|-----|--------|---------------|
| SCC       | 1145| 2.5×10^5 | 8.8×10^5 | 8.8×10^5 ± 9.2×10^3 |
| EC        | 1145| 5.50 | 10.83  | 6.27 ± 0.066     |

**Table 5.** Significant differences between seasons based on each SCM diagnostic test result.

| Diagnostic test | Seasons     | Sig. difference |
|-----------------|-------------|-----------------|
| CMT             | Winter      | 0.108 0.021 *   |
|                 | Spring      | 0.108 0.021 *   |
|                 | Summer      | 0.108 0.021 *   |
|                 | Autumn      | 0.108 0.021 *   |
| EC              | Winter      | 0.190 0.034 *   |
|                 | Spring      | 0.190 0.034 *   |
|                 | Summer      | 0.190 0.034 *   |
|                 | Autumn      | 0.190 0.034 *   |
| SCC             | Winter      | 0.204 0.089 *   |
|                 | Spring      | 0.204 0.089 *   |
|                 | Summer      | 0.204 0.089 *   |
|                 | Autumn      | 0.204 0.089 *   |

* Significant difference, $P$-value $< 0.05$.
Our study exhibited that the highest prevalence of SCM was observed in 143 lactating cows in winter than in other seasons at 32.21%, followed by summer, autumn and spring at percentage of 31.31, 21.84 and 14.64 respectively. There was no significant difference between the winter and other seasons. The findings were in line with the data recorded by Ellis et al. (2006); Rahman et al. (2009); Tiwari et al. (2013); Litwińczuk; Król; Brodziak (2017); Getaneh; Gebremedhin (2015). This also indicates the ability of used SCM diagnostic tests to clear this statistical significant difference.

Based on SCM diagnostic test results; the P-value (<0.05) between winter and autumn, might be due to the variation in the period and location of the study, animals number, parity number, lactation stage, and administration practices. Therefore, a combination of above parameters would give more precise information on SCM as compared to a single parameter. The detection of the SCM at different stages would improve significantly (Panchal; Sawhney; Dang, 2016).

The presented results showed that; the minimum value of the EC of the examined SCM milk samples was 5.50 mS/cm and the maximum value was 10.83 mS/cm with a mean value of 6.27 ± 0.066 mS/cm. These results are consistent with Yoshida (2005).

This change in EC may be due to altered concentration of Na+, K+ and Cl- in the SCM milk which causes the increase in EC. The EC of milk may be changed due to interaction of factors other than subclinical mastitis, such as environmental state, temperature, season, animal breed and stages of lactation. Therefore, a combination of above parameters would give more precise information on SCM as compared to a single parameter. The detection of the SCM at different stages would improve significantly (Panchal; Sawhney; Dang, 2016).

Therefore, any indicative test faults will happen when relying on a single test (Jaeger et al., 2017).

The total number of positive SCM quarters, depending on the three tests of CMT, SCC and EC were 1145 from 2780 examined quarters at a percentage of 41.18. The results of this work are nearly similar to those reported by DASOHARI et al. (2017) - India; SARBA; TOLA (2017)- Ethiopia; TRAJCEV et al. (2017) - Macedonia; SULEIMAN; KARIMURIBO; MDEGELA (2018)- Tanzania. Lower data were registered by Mir; Bansal; et al. (2017) - India; Suleiman; Karimuribo; Mdegela et al. (2017) - India; Sarba; Tola (2017)- Ethiopia; Trajchev et al. (2017) exhibited the highest prevalence of SCM in winter with 32.21% and the lowest was in spring with 14.64%.

Table 7. Significant differences between seasons in each risk factor based on subclinical mastitis cows.

| Risk factor       | Seasons   | Winter | Spring | Autumn |
|-------------------|-----------|--------|--------|--------|
| Parity            | Summer    | 0.013* | 0.187  | 0.004* |
| Age               | Summer    | 0.049* | 0.298  | 0.039* |
| Stage of lactation| Summer    | 0.854  | 0.826  | 0.824  |

* Significant difference, *P*-value < 0.05.
that the high temperature and relative humidity encourage the propagation of different bacteria, and the exhibition of animals to rising temperature can elevate the stress of the animal (reduced its immunity).

Moreover, the results exhibit that the highest percentage of SCM (59.91%) was observed in the late stage of lactation (266 cows), followed by mid stage and early stage of lactation with a percentage of 23.20 and 16.89 respectively, but there was not any significance ($p > 0.05$) statistical differences between each stage of lactation. These results are in accordance with those reported by Syridion et al. (2013); Tancin (2013); Getaneh; Gebremedhin (2017); Patel; Trivedi (2018). The late lactation especially before drying is considered a critical period for udder health and the farmers should take more care of cows during this period. The explanation of the high prevalence of SCM at this stage may be due to the repeated and cumulative exposure to infection with contagious pathogens during milking practice or due to the removal of teats “plug” (Dingwell et al., 2004; Hussain et al., 2012), or due to increasing to the SCC that is linked with the defense improved of the mammary gland at calving time. Hence the importance of microbiological analyzes are important to clarify the cause of MSCC elevation.

During the stages of early and late lactation; the amount of the neutrophils is liable to increase, while the percentage of lymphocytes tends to reduce. A parturition period, the SCC is of the neutrophils is liable to increase, while the percentage of lymphocytes tends to reduce. A parturition period, the SCC is of the neutrophils is liable to increase, while the percentage of lymphocytes tends to reduce. A parturition period, the SCC is of the neutrophils is liable to increase, while the percentage of lymphocytes tends to reduce.

The data recorded in our study revealed that the young cows were exposed to SCM more than old age ones, the cows of 4-5 years had the highest percentage, followed by 2-3 years, 6-7 years, 8-9 years and ≥10 years, with a percentage of 48.42, 26.35, 15.09, 9.23 and 0.91 respectively. This result may be attributed to high stress of nutrition, elevated milk production and maximum immunity level not occurring (SHELKE et al., 2019). Moreover, Oliver et al. (2005); Hussain et al. (2012); Kline; Flores; Joyce (2018) reported that the older cows did not display more bacteria in their milk, or more somatic cells that fight off those bacteria and that they have a lower chance of contracting an Intra-mammary infection than low age animals.

Moreover, the results presented in this study showed that the occurrence of mastitis was higher in cows with 2nd, 3rd and 1st parity number than those with 4, 5, 6th parity number at a percentage of 37.61, 22.30, 14.64, 11.94, 7.66 and 5.85 respectively. This result may be due to possible lower resistance of first parity cows (mammary system not exposed earlier to the environmental pathogens). The mammary gland of primiparous cows is characterized by a smaller cisternal portion compared to pluriparous ones. Hence, it is useful to perform efficient mammary gland stimulation before teat cup attachment in order to induce a proper oxytocin release and excellent milk let down (Patel; Trivedi, 2018). The data reported by Zucali et al. (2009); Elbably; Emeash; Asmaa (2013) was consistent with these results.

These findings revealed that there is a significant difference ($p < 0.05$) between numbers of parity. This result is in agreement with those reported by QUADERI et al. (2013); Biressaw; Deme (2015); Prabhu (2015); Mureithi ; Njuguna (2016); Singh et al. (2017); Zeryehun ; Abera (2017); Seyoum et al. (2018). However, there are no significance differences ($p > 0.05$) between different stages of age, lactation and season. This result was supported by results mentioned by Bitew; Tafere; Tolosa (2010); AHMED; Abdelhamid; Mohammed (2015).

Based on parity and age as risk factors, there is a significant difference ($P < 0.05$) between summer and winter; summer and autumn. This may be due to the variation in parity number and age of the examined dairy cows. Moreover, the occurrence of SCM varies from season to another; because the growth and multiplication of organisms depend on some factors, like temperature and humidity (Sudhan; Sharma, 2010). Thus, the season influences the appearance of SCM (Shathele, 2009). Training scheme of dairy farm workers and perfection of (HACCP) program are requested for safe milk production (Hafiz et al., 2016; Ibrahim; Saad; Hafiz, 2020; Ibrahim; Saad; Hafiz, 2021).

CONCLUSIONS

The occurrence of subclinical mastitis in a dairy farm varies depending on complex interaction among age, season, parity, and lactation stage. Therefore, it is recommended to give more attention to young age, primiparous cows’, particularly in winter and late stage of lactation.

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