Prevalence and risk factors of clinical mastitis in Holstein cows under subtropical Egyptian conditions

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ABSTRACT

The current study was built on 1015 dairy records of Holstein Friesian cows, aimed to determine the prevalence and potential risk factors of clinical mastitis (CM). Logistic regression models were conducted to determine the risk factors associated with the onset of CM. The average age at 1st calving was 1.98 years in mastitic cows, and 2.01 years in healthy ones. Daily milk yield (DMY) was shown to be somewhat lower in mastitic cows than in healthy (31.9 and 32.3 kg, respectively). Older dairy cows >5 years had a higher incidence of mastitis (53.66%). The prevalence of CM was higher in multiparous cows, either 2nd parity (48.24%) or ≥ 3rd parity (47.23%). Regarding the calving season, cows that calved in winter showed a lower percentage of CM (38.36%). Pregnant cows were found to have a greater incidence of CM than non-pregnant cows (59.35% and 32.89%, respectively). Concerning the history of the previous mastitis, cows that were infected previously showed a higher prevalence of CM (51.66%). The greatest percentage of CM was observed in cows in the early and mid-stages of lactation (44.59% and 52.69%, respectively). The final multivariate logistic regression model revealed that age at 1st calving had a significant positive correlation with the incidence of CM (β=1.19, P-value= 0.003). Older cows >5 years (odds ratio (OR)=1.79), cows calved during the summer season (OR=1.84), Pregnant cows (OR=7.27), cows with a history of previous mastitis (OR=1.81), and lactating cows at early lactation stage (1-90 days) were significant risk factors associated with an increased incidence of CM.

1. INTRODUCTION

Mastitis is considered an endemic disorder in the dairy sector all over the world, causes massive economic losses (El-Tarabany andAli, 2015). Generally, mastitis is an inflammatory reaction of the mammary gland that could be infectious (caused by pathogen infection) or non-infectious (caused by physical, chemical, or traumatic factors) (Abd-El Hamed and Kamel, 2020). Clinical mastitis (CM) is considered as one of the extremely poisonous disease problems in the dairy industry as it causes great economic loss and has a major impact on dairy cows’ productivity and welfare (Nakov et al., 2014). There are series of pathogens that can induce mastitis in dairy cattle, these causative agents were allocated into two broad categories: one of them causes contagious mastitis such as Staphylococcus aureus, Streptococcus agalactiae, etc. that can be contagiously widespread from the infected udder mainly during milking. While the other category causes environmental mastitis such as Str. dysgalactiae, Str. Uberis, coliforms, etc. (Elbably and Asmaa, 2013). Mastitis can also be categorized into clinical and subclinical. CM is characterized by sudden occurrence, changes in milk content and appearance, a decline in milk production, and the appearance of the clinical signs of inflammation in the infected quarter. It is easily detected and diagnosed. While no visible clinical signs are observed either in the milk or on the udder of cows with sub-clinical mastitis, but the somatic cell count (SCC) increases, and the milk production reduces. This type is more serious and frequent (Khan and Khan, 2006). Costs due to mastitis are resulted from milk contamination due to antibiotic residues, diminished milk production, chronically infected cows culling, veterinary costs, and occasional mortalities. Moreover, mastitis has significant zoonotic importance through the shedding of pathogens and their toxins in the human consumed milk (Abebe et al., 2016). Mastitis occurrence in dairy herds occurs because of a complex interface between the agent, cow, and environment. Generally, the most popular risk factors for CM incidence in dairy herds are categorized into two groups: cow-level risk factors and environmental or managerial risk factors (Nakov et al., 2014). A large number of individual cow-level risk factors for CM incidence in dairy herds have been studied, including breed, parity, stage of lactation, the morphology of udder and teat, udder edema, age at first calving, milk production, milk leakage, milk somatic cell count and reproductive disorders by (Nyman et al., 2007) and (Peeler et al., 2000). The current study aimed to estimate the prevalence of CM and to study the individual cow-specific risk factors that affecting the prevalence of CM in Holstein Friesian (HF) dairy cattle in Egypt, such as age at 1st calving, average daily milk yield (DMY), age,
number of parties, calving season, reproductive status, history of the previous mastitis and lactation stage.

2. MATERIAL AND METHODS

2.1. Study area:
A cross-sectional longitudinal survey was conducted to assess and evaluate the cow-level non-genetic risk factors for CM in Holstein dairy cows during the period from January 2018 to December 2019.

2.2. Animals and management:
Data used in this study were obtained from the records of a private dairy cattle farm located at 80° Km of Cairo-Alexandria desert road. About 1015 Holstein dairy cows’ records were collected and analyzed. Cows on the farm are housed in a shaded open yard with free stalls, lined with a sand floor, and furnished in the summer with a cool spraying system thus reducing heat stress during the summer months. The farm applies the total mixed ration method with computerized calculating systems that control feeding portions according to the productivity and the reproductive status of animals. Water was supplied freely all day long. Pre-milking and post-milking udder hygiene measures were practiced through dipping the teats in iodine solution. The milking process was performed in a herringbone rapid exit milking parlor three times a day using machine milking, and milk parameters for each individual cow were recorded in a computerized database. Detection of CM depending on the presence of clinical signs on the udder such as hotness, redness, swelling, painful reaction, and hardness of udder tissues.

2.3. Data variables and classification:
The data variables are the outcome variable which is a dichotomous variable coded as (0= Healthy, and 1= Mastitic), and the independent variables included the age at 1st calving and the average daily milk yield (DMY) as continuous variables, and other categorical variables as the age classified into two categories: ≤5 years old and >5 years old. Number of parties divided into primiparous, 2nd parity, and multiparous (≥3 parties), calving season classified into winter season which extends from 21 September to 20 March, and summer season that extend from 21 March to 20 September, reproductive status coded as (0= Non-pregnant, and 1= Pregnant), History of the previous mastitis coded as (0= No, and 1= Yes), and lactation stage classified into early-stage (1-90 days), mid-stage (91-180 days), and late-stage (>180 days).

2.4. Statistical analysis:
All Statistical analysis practices were performed by the SPSS statistical software package (SPSS version 25). Firstly, the data were analyzed using the univariate logistic regression to determine the potential risk factors associated with CM incidence. All variables with (P-values < 0.05) in the initial univariate logistic regression analysis were examined for multicollinearity using tolerance and variance inflation factor (VIF). Multicollinearity is considered if tolerance <0.04 and VIF >10 (Miles, 2014). Then, the multivariate logistic regression using the enter method was conducted,  

\[ \log \left( \frac{P}{1-P} \right) = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k \]

Where, \( \frac{P}{1-P} \) is the odds, \( P \) is the probability of the outcome occurrence, \( \beta_0 \) is the Y-intercept, \( \beta_i \) is the beta coefficient of the independent variable \( X_i \) and \( \beta_k \) is the beta coefficient of the independent variable \( X_k \) (Boateng and Abaye, 2019). The results of the logistic regression were expressed as the (OR) of the associated predictor at a confidence interval of 95% and the p-value. Finally, the overall goodness of fit of the multivariate model was assessed using the receiver operating characteristics (ROC) curve.

3. RESULTS

The clinical mastitis (CM) prevalence concerning the age, parity, calving season, reproductive status, history of the previous mastitis, lactation stage, age at 1st calving, and daily milk yield (DMY) were presented in (Table 1). Holstein Friesian (HF)cows aged > 5 years old showed a higher prevalence of CM (53.56%) than those of ≤ 5 years old. The prevalence of CM varied according to the number of parties, 2nd parity, and multiparous cows revealed a higher prevalence (48.24% and 47.23%, respectively) compared to the primiparous cow (36.08%). Regarding the calving season, cows calved in summer showed a prevalence of CM greater than those that calved in winter (50.24% and 38.36%, respectively). Non-pregnant cows had a lower CM prevalence (32.89%) than pregnant cows (59.35%). According to the history of the previous mastitis, the CM prevalence was higher among cows with previous mastitis history (51.66%) than cows that did not show CM previously (37.98%). Also, lactating cows within the mid-stage (52.69%) and early-stage (44.59%) of lactation had higher CM prevalence than cows within the late lactation stage (32.08%).

As described in table (2), the mean age at 1st calving of the mastitic cows was 1.98 years while the mean age at 1st calving of the healthy cows was 2.01 years. Also, mastitic cows had relatively lower DMY compared with their healthy contemporaries (31.9 and 32.3 kg, respectively).

Table 1 Prevalence of Clinical mastitis in Holstein cows:

| Factors                          | Class   | No. of total animals | No. of mastitic animals | Prevalence of clinical mastitis (%) |
|----------------------------------|---------|----------------------|-------------------------|-----------------------------------|
| Age (<5 years)                   | 776     | 351                  | 40.08%                  |
| Parity                           | >5 years| 239                  | 53.56%                  |
| Calving season                   | Primiparous | 388                  | 36.08%                  |
|                                  | 2nd parity | 284                  | 48.24%                  |
|                                  | Multiparous | 343                  | 47.23%                  |
| History of the previous mastitis | Winter  | 597                  | 38.36%                  |
|                                  | Summer  | 418                  | 50.24%                  |
| Reproductive status              | Not pregnant | 614                  | 32.89%                  |
|                                  | Pregnant | 401                  | 59.35%                  |
| Lactation stage                  | No      | 624                  | 37.98%                  |
|                                  | Yes     | 391                  | 51.66%                  |
| Period (days)                    | 1-90    | 241                  | 52.69%                  |
|                                  | >180    | 265                  | 32.08%                  |

As described in table (2), the mean age at 1st calving of the mastitic cows was 1.98 years while the mean age at 1st calving of the healthy cows was 2.01 years. Also, mastitic cows had relatively lower DMY compared with their healthy contemporaries (31.9 and 32.3 kg, respectively).

Table 2 Descriptive statistics of the continuous independent variables expressed as the mean and standard error:

| Age at 1st calving (years) | DMY (kg) |
|----------------------------|----------|
| Healthy                    | 2.01 ± 0.008 | 32.3 ± 0.57 |
| Clinical Mastitis          | 1.98 ± 0.007 | 31.9 ± 0.65 |

Table (3) showed the results of the univariate logistic regression analysis. The univariate logistic regression models showed that the age at 1st calving, parity, calving season, reproductive status, history of the previous mastitis, and lactation stage were all significantly associated with the occurrence of CM. All the initial risk factors were checked for the absence of multicollinearity and none of them showed a high correlation with each other. So, except for DMY which was not significant in the univariate analysis,
The following step was conducting the multivariate logistic regression analysis with the enter method. The age at 1st calving showed a significant direct relationship with the prevalence of CM in Holstein dairy cows (β=1.19 at P-value= 0.003). Regarding the age category of the lactating cow, cows aged ≥ 5 years had a higher odds of CM 1.79 times higher than cows of ≤ 5 years old (OR=1.79, 95% CI=1.12-2.84). The odds of CM for cows calved in winter were estimated to be greater than the reference group (winter calving season) by 1.84 times (OR=1.84, 95% CI=1.36-2.49). It was also revealed that the odds of CM were 7.27 times greater for the pregnant cows aged >5 years than those with no history of the previous mastitis (OR=7.27, 95% CI=5.00-10.6). In accordance with the history of the previous mastitis, the previously infected cow with CM had a higher odds of 1.81 times than those with no history of the previous mastitis (OR=1.81, 95% CI=1.22-2.69). Dairy cows within the mid-stage and the late-stage of lactation showed lower odds of CM by (0.59 and 0.18 times, respectively) than cows within the early stage of lactation (OR= 0.59, 95% CI=0.39 –0.88 and OR= 0.18, 95% CI= 0.12-0.29, respectively) (Table 4).

Finally, the overall goodness of fit of the final multivariate regression model was proved in the ROC curve (Figure 1). The area under the curve (AUC) was 0.75 at a 95% confidence interval (0.72 – 0.78) which significantly differed from 0.05 at P-value= 0.001, indicating the very good predictive ability of the final multivariate logistic regression model.

4. DISCUSSION

Mastitis is a complex disease of multifactorial etiology that causes massive economic losses in the dairy industry worldwide. Whereas the control of mastitis in dairy herds depends on the identification and elimination of risk factors associated with the environment, management, and the cows, we tried to study the effect of different non-genetic factors on the occurrence of the CM in the Holstein Friesian dairy cow such as the age at 1st calving, DMY, age, parity, calving season, reproductive status, history of the previous mastitis, and lactation stage.

Table 4 The odds ratio for different factors affecting the prevalence of CM in the Holstein cows using multivariate logistic regression model

| Factor at 1st calving | OR (95% CI) | p-value |
|-----------------------|-------------|---------|
| Age                   |             |         |
| 1st                  | 1.19 (0.39 – 3.3) | 0.52 |
| 2nd                  | 1.22 (0.34 – 4.9)  | 0.78 |
| 3rd                  | 1.35 (0.43 – 4.8)  | 0.29 |

β= regression coefficient, S.E. = standard error, OR= odds ratio, CI= confidence interval, * = significant at p-value <0.05, ** = significant at p-value <0.001, *** = significant at p-value <0.0001 and NS= non-significant.
factor for the introduction of the infection from the surrounding skin or the external environment (Shittu et al., 2012). Such results were in agreement with Shittu et al. (2012) and Abebe et al. (2016). Concerning the calving season, it was revealed that CM was more likely in cows calved during the summer season than the winter season. Consistent with our findings, Boujeneane et al. (2015) reported that the increased cases of mastitis during summer might be due to the proliferation of pathogens during the hot weather in the surrounding environment, especially around the water troughs. On the contrary, Kerro Dego and Tareke (2003) revealed that bovine mastitis was frequent in cows calved in the wet season than in the dry season.

The current study revealed that pregnant dairy cow has a probability to CM infection 7.27 times higher than non-pregnant ones. This might be due to the stress of the pregnancy, in addition to the milk production causing immune suppression and pregnant cow become sensitive to the infection like mastitis. Dairy cows with a history of the previous mastitis had a higher risk of CM than those that did not expose previously that might be associated with persistent bacteria in the mammary gland and reinfection after treatment. These results were in agreement with Tezera and Aman Ali (2021), who found that cows that suffered from CM previously (66.3%), were more susceptible to mastitis than non-exposed cows (31.6%). Finally, regarding the lactation stage, it was revealed that the prevalence of CM was higher during the early stage of lactation than in the mid and late stages. This might be attributed to the marked sensitivity of the udder to infectious agents and the higher physiological demands after calving (Boujeneane et al., 2015). Also, it might be due to the delay of the neutrophils diapedesis into the mammary gland in recently calved cows (Hagnostam et al., 2007). On the other hand, the reduced antioxidant defense mechanisms and increased oxidative stress due to lipid peroxidase due to the high demand in the early stage of lactation are predisposing factors to mastitis and other productive diseases (Sharma et al., 2011). These results came in agreement with Koeck et al. (2012), who found that the majority of CM cases were in the first lactation stage by a ratio of 32.7% at the 1st month, 9.4% at 2nd month, and 8.6% at 3rd month of lactation. Also, Chegini et al. (2016) attributed the cause to the high tension of peak milk production at the early lactation stage.

5. CONCLUSION

In conclusion, CM is a widely spread multifactorial complex disease in dairy farms. The majority of the cow-level non-genetic risk factors such as age at the first calving, age, calving season, reproductive status, history of the previous mastitis, and lactation stage were shown to affect the prevalence of CM, hence the control and the elimination of these factors will help a lot in reducing the CM rates. Older multiparous cows and during the early stage of lactation and pregnant cows are more susceptible to udder infections, and hence they should be supplied with a balanced ration with all essential supplements to face the high demands of pregnancy and lactation without affecting the immune response. Also, during the summer season, efficient cooling systems should be used to reduce the heat stress in the hot weather but with caution to avoid the dampness of the bedding and the proliferation of pathogens in the surrounding environment.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data.

AUTHORS’ CONTRIBUTION

All authors contributed equally.

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