

Journal of the Hellenic Veterinary Medical Society

Vol 70, No 4 (2019)



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doi: [10.12681/jhvms.22237](https://doi.org/10.12681/jhvms.22237)

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To cite this article:

THEMISTOKLEOUS, K., KARAGIANNIS, I., BOSCOS, C., PANOUSIS, N., & KIOSSIS, E. (2020). Epidemiological evaluation of subclinical mastitis of dairy cows in Greece. *Journal of the Hellenic Veterinary Medical Society*, 70(4), 1865–1874. <https://doi.org/10.12681/jhvms.22237>

Epidemiological evaluation of subclinical mastitis of dairy cows in Greece

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ABSTRACT. Subclinical mastitis, diagnosed by elevated somatic cell count (SCC) in milk, is an important monitoring parameter of dairy cows' udder health, related to their productivity and welfare. The present retrospective study aims to evaluate the epidemiology of subclinical mastitis (SCM) among the 37 herds of the Holstein Association of Greece participating in the milk quality recording system "ΙΩ", from the start of 2015 until the end of 2018. The herds' inclusion criterion was the consistency of monthly SCC recording throughout at least one full year between 2015 and 2018, with a maximum interval of 61 days between two consecutive monthly SCC recordings. Twenty-six herds (8630 cows) in 2015, thirty herds (10763 cows) in 2016, thirty herds (10945 cows) in 2017 and twenty-six herds (9597 cows) in 2018 were included. The prevalence of SCM and chronic SCM, the incidence rate of new cases of SCM, as well as the average somatic cell score and bulk tank milk SCC were determined for each of the four years. The results indicate a progressive deterioration of udder health from the onset of the cow's productive life until culling. A year-over-year increase in the number of cows with subclinical mastitis led to an overall SCM prevalence of 34.5%, chronic SCM prevalence of 26.9% and a bulk tank milk SCC of 463000 cells/mL, in 2018. The average somatic cell score, a base 2 logarithm of individual cow's SCC, was found persistently above the subclinical mastitis indicative cut-off in all four years, with a peak in 2018. At herd level, the incidence rate of new SCM cases was 12 new cases / 100 cows / month; the highest incidence rate was observed in the early lactation stage group (1-60 days-in-milk), in all four years, reaching a peak of 31 new cases / 100 cows / month, in 2018. In 2018, prevalence of heifers' SCM and chronic SCM was 23.4% and 16.9%, respectively. Despite the adequate average 305-days milk yield (9608 kg in 2018), the results were indicative of poor udder health status, pointed out by reduced duration of cows' productive life (less than 3 lactations) and lower milk quality (elevated SCC). The severity and wide spreading of subclinical mastitis in Greek dairy herds highlights the necessity of a national mastitis control program, aiming to improve the productive efficacy, management decisions accuracy and quality of produced milk.

Keywords: subclinical mastitis; somatic cell count; dairy cows; epidemiology; udder health

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Date of initial submission: 17-05-2019
Date of revised submission: 03-06-2019
Date of acceptance: 05-06-2019

INTRODUCTION

Udder health status of dairy cows is related to their productivity and welfare. Thus, it is crucial both for the consumer and for the dairyman, regarding the production of high-quality milk, duration of productive life, reduction of antibiotic residues, as well as the profitability of the livestock business. A study of the National Animal Health Monitoring System (NAHMS) in USA revealed that udder health problems is the number one reason for culling a dairy cow (26.9% ±0.5%) (USDA / NAHMS, 2002). The importance of this subject has led to a worldwide increase of the attention paid to the establishment and development of udder health monitoring programs (Schukken et al., 2003). Subclinical mastitis, new and chronic, is an important monitoring parameter of udder health (Schukken et al., 2008), as this disease leads to the reduction of milk yield (Archer et al., 2013), higher risk of clinical mastitis (Rupp et al., 2000), higher chances of premature culling (De Vliegher et al., 2005) and, consecutively, economic losses (Hamann, 2005).

Subclinical mastitis (SCM) is the presence of an infection without apparent clinical changes (condition of the udder or milk secretion) (Blowey and Edmondson, 2010). The diagnosis of SCM is based on the recognition of the mammary inflammatory response against the infection (Shook et al., 2017). A widely used tool for this purpose is somatic cell count (SCC) (Dohoo and Leslie, 1991; Schukken et al., 2003; Ruegg and Pantoja, 2013). Somatic cells take part in the udder defence mechanisms (Pillai et al., 2001) and their presence reflects the inflammatory response to an intra-mammary infection (IMI) or some other trigger of the immune system (Schukken et al., 2003). Longitudinal data of bulk tank milk SCC (BTSCC) over time can be an indicator of the udder health status at herd level. BTSCC is highly correlated with 305-days milk yield and it can be associated with the prevalence of cows that produce milk with elevated SCC (Smith, 1996; Barkema et al., 1998). A more appropriate parameter to summarize herd's average SCM situation is the arithmetic average test-day SCC from individual cow milk samples, as well as a base 2 logarithmic conversion of SCC, the somatic cell score (SCS), which is providing statistical superiority (Shook, 1993; Lievaart et al., 2007).

Various thresholds have been suggested for the classification of infected and non-infected cows. It is repeatedly shown that a cut-off of approximately 200000 to 250000 cells per mL is optimal to reduce

diagnostic error (Dohoo and Leslie, 1991; Schepers et al., 1997). Many studies used a cut-off of 200000 cells/mL (Schukken et al., 2003; De Vliegher et al., 2004; Svensson et al., 2006; Fouz et al., 2010; Madouasse et al., 2012; Lam et al., 2013; Fauteux et al., 2014; Santman-Berends et al., 2016), while especially for heifers, IMI cut-offs of 150000 cells/mL (Santman-Berends et al., 2016) and 100000 cells/mL (Bludau et al., 2014) have been used. In our study, due to the available data, a preset threshold of 250000 cells/mL was utilized. These cut-offs are a practical threshold under field conditions and not the ultimate goal for udder health and best quality milk production (Schukken et al., 2003).

This four-year retrospective study aims to introduce an epidemiological evaluation of SCM among Holstein dairy cows in Greece, from 2015 until 2018. Utilizing the available data from "IQ" recording system, provided by the Holstein Association of Greece (HAOG), our study presents: subclinical mastitis prevalence, chronic subclinical mastitis prevalence, subclinical mastitis incidence rate, as well as somatic cell score (SCS), bulk tank milk somatic cell count (BTSCC) and some overall observations regarding the productive efficacy of the population of dairy cows belonging to HAOG, during the study period.

MATERIALS AND METHODS

Study population

Among the 84 herd-members of HAOG, 37 took part in the monthly milk quality recording system, called "IQ". From those 37 herds, the ones included in the current study met the following criterion: they were consistently recording monthly SCC for at least one full year between 2015 and 2018, having a maximum interval of 61 days between two consecutive monthly SCC recordings. Based on the above criterion, 26 herds (8630 cows) were included in the study in 2015, 30 herds (10763 cows) in 2016, 30 herds (10945 cows) in 2017 and 26 herds (9597 cows) in 2018. In total, 39424 test-month recordings and 1568 annual recordings were utilized from "IQ" during this four-year study.

Available data

On a monthly basis, milk SCC on test-day was calculated from individual cow milk samples, as well as bulk tank milk samples collected from each of the aforementioned herds. The following data were obtained from "IQ":

- Monthly, the number of milked cows per herd, per number [1st (“heifers”), 2nd and 3rd lactation] and per stage of lactation [1-60 (“early lactation”), 61-120, 121-180, ≥181 days-in-milk], provided as a pre-set grouping by “IQ”.

- Monthly, the total number of infected (SCC above the cut-off on test-day) and new cases of infected cows per herd, per number and per stage of lactation.

- Monthly, the average individual SCC per herd, per number and per stage of lactation, as well as mean bulk tank milk SCC (BTSCC) of the herd on test-day.

- Annually, the total number of milked cows per herd, the herd average age at first calving (months), the calving interval (days) and the number of lifetime lactations per cow. Also, the average individual 305-days milk yield (kg) and duration of lactation period (days) per herd, per number and per stage of lactation.

Definitions and epidemiological analysis

Diagnosis of subclinical mastitis

Subclinical mastitis diagnosis was based on individual cow milk SCC results at monthly test-day. “Infection” was defined as SCC value above the threshold for normal milk SCC concentration (Schukken et al. 2008), on test-day. The threshold was preset by “IQ” at 250000 cells/mL.

Somatic cell score

A base 2 logarithmic transformation of the SCC calculated as $\log_2(\text{SCC}/100) + 3$.

Average subclinical mastitis prevalence

Subclinical mastitis prevalence was calculated as the monthly average percentage of infected cows on test-day, per herd, per number and per stage of lactation, for each of the years 2015-2018. This monthly percentage is the ratio of infected (SCC above the cut-off) lactating cows to the total number of lactating cows participating in the test-day recording of a certain month. (Santman-Berends et al., 2016).

Average chronic subclinical mastitis prevalence

The number of chronically infected cows of a certain month was calculated by the subtraction of the new cases of infected cows from the total number of infected cows on the monthly test-day, both provided by “IQ” system. Herd average chronic SCM prevalence

(%) was calculated as the average monthly percentage of chronically infected cows on test day, per herd, per number and per stage of lactation, for each of the years 2015-2018. This monthly percentage is the ratio of chronically infected lactating cows to the total number of lactating cows participating in the test-day milk recording of a certain month (Santman-Berends et al., 2016).

Subclinical mastitis incidence rate

Subclinical mastitis incidence rate was calculated per 100 cows at risk / month, and was the number of new SCM cases divided by days at risk (DAR), multiplied by 30 days and 100 cows, as follows:

$$(\text{new infections} / \text{DAR}) \times 30 \text{ days} \times 100 \text{ cows}$$

DAR refers to the days of a certain month, during which the lactating cow was exposed to an intra-mammary infection risk. Cows that maintained a SCC below the threshold all month long, despite the exposure to the risk, had 30 DAR. Given the fact that the only available data was monthly (and not daily) recordings, the approximate method was used (Dohoo et al., 2003), assuming that a new infection happened in the middle of each month, i.e. 15 DAR for newly infected cows.

Annual data

Provided by “IQ” system, herd annual data were utilized for the calculation of the annual number of milked cows participating in the study during each year from 2015 until 2018, as well as the annual average age at 1st calving (months), calving interval (days), number of lifetime lactations / cow and annual herd average BTSCC (cells/mL). The annual average 305-days milk yield / cow, SCS and duration of lactation period (days) were calculated per herd and per number of lactations. Furthermore, annual average SCS was calculated per stage of lactation, from 2015 to 2018.

The aforementioned udder health evaluation parameters were processed and presented via descriptive statistics, using Stata 13.1[®] (StataCorp LLC, College Station, Texas, 2014) and Microsoft Excel[®] (Microsoft Office 365, Microsoft[®]).

RESULTS

Annual data

The average annual herd data evaluated in this

four-year study are presented in Table 1. Average age at first calving ranged between 27.5 (± 2.2) and 28.2 (± 2.4) months and remained above the 27 months (upper optimal threshold) during all four years, progressively decreasing from 2015 to 2018. A minor decrease of 9 days was observed for herd average calving interval, with a nadir of 447 (± 34) days in 2018. Herd average BTSCC in 2015 was 385000 cells/mL, although there was a considerable year over year increase up to 463000 cells/mL in 2018. Average number of lifetime lactation periods / cow was increased by 0.21 compared to 2015, peaking in 2017 (2.91

± 0.35) and remaining stable in 2018 (2.91 ± 0.32), but still lower than the minimum target of 3 lifetime lactations. Herd average 305-days milk yield / cow, presented in Table 2, increased year over year with a peak of 9608 (± 1609) kg in 2018, 645 kg higher than 2015. Interestingly, during the four-year period, in 46% of the herds the average 305-days milk yield / cow of the 3rd lactation was lower than that of the 2nd lactation. Finally, herd average duration of lactation was decreased by 14 days from 2015 to 2016, then increased by 10 days from 2016 to 2017 and remained unaltered in 2018 at 347 (± 35) days (Table 2).

Table 1. Annual herd data of milked cows, average age at first calving, average number of lifetime lactations, average calving intervals and average bulk tank milk somatic cell count (BTSCC), during 2015-2018 in Greece.

| year (number of herds) | number of milked cows | average age at 1st calving (months) | average number of lifetime lactations | average calving interval (days) | average BTSCC (x1000 cells/mL) |
|---------------------------|-----------------------|-------------------------------------|---------------------------------------|---------------------------------|--------------------------------|
| 2015 (n=26) | 8630 | 28.2 (± 2.4) | 2.70 (± 0.28) | 456 (± 24) | 385 (± 142) |
| 2016 (n=30) | 10763 | 28.1 (± 2.3) | 2.74 (± 0.32) | 449 (± 28) | 396 (± 180) |
| 2017 (n=30) | 10945 | 27.6 (± 2.3) | 2.91 (± 0.35) | 448 (± 29) | 416 (± 178) |
| 2018 (n=26) | 9597 | 27.5 (± 2.2) | 2.91 (± 0.32) | 447 (± 34) | 463 (± 165) |

(\pm): standard deviation

Table 2. Average somatic cell score (SCS), 305-days milk yield (kg) and duration of lactation period (days), per herd & per lactation period (1st, 2nd, 3rd LP) groups, during 2015-2018 in Greece

| year (number of herds) | | SCS | average 305-days milk yield (kg) | average duration of lactation period (days) |
|---------------------------|--------|-------------------|----------------------------------|---|
| 2015 (n=26) | Herd | 4.8 (± 0.5) | 8963 (± 1308) | 351 (± 25) |
| | 1st LP | 4.2 (± 0.5) | 8461 (± 1269) | 365 (± 36) |
| | 2nd LP | 4.7 (± 0.7) | 9284 (± 1361) | 359 (± 27) |
| | 3rd LP | 5.6 (± 0.6) | 9527 (± 1279) | 343 (± 41) |
| 2016 (n=30) | Herd | 4.8 (± 0.6) | 9131 (± 1346) | 337 (± 24) |
| | 1st LP | 4.4 (± 0.8) | 8573 (± 1217) | 358 (± 41) |
| | 2nd LP | 4.6 (± 0.8) | 9429 (± 1489) | 337 (± 36) |
| | 3rd LP | 5.5 (± 0.6) | 9350 (± 1795) | 338 (± 38) |
| 2017 (n=30) | Herd | 4.9 (± 0.7) | 9429 (± 1404) | 347 (± 27) |
| | 1st LP | 4.4 (± 0.9) | 8807 (± 1245) | 377 (± 69) |
| | 2nd LP | 4.8 (± 0.8) | 9856 (± 1626) | 354 (± 27) |
| | 3rd LP | 5.6 (± 0.5) | 9935 (± 1491) | 351 (± 59) |
| 2018 (n=26) | Herd | 5.1 (± 0.6) | 9608 (± 1609) | 347 (± 35) |
| | 1st LP | 4.5 (± 0.6) | 9190 (± 1386) | 366 (± 47) |
| | 2nd LP | 5.0 (± 0.8) | 9849 (± 1941) | 359 (± 44) |
| | 3rd LP | 5.8 (± 0.5) | 9973 (± 1538) | 344 (± 45) |

(\pm): standard deviation

Somatic cell score

Herd average SCS, presented in Table 2, was steadily above the 4.0 SCM-indicative threshold during all four years, both at herd level and at number and stage of lactation levels. Average SCS appeared elevated even from the first lactation (4.2 in 2015, 4.4 in 2016, 4.4 in 2017 and 4.5 in 2018), increased in the second and peaked in the third lactation (5.8 in 2018). Early lactation SCS, presented in Table 3, ranged from 4.7 (2015, 2016) to 5.1 (2018), exceeding the aforementioned threshold during all four years of the study. Average SCS remained above the threshold throughout the whole lactation and it reached its highest score in the last stage of lactation (≥ 181 days-in-milk).

Subclinical mastitis and chronic subclinical mastitis prevalence

Average subclinical mastitis prevalence was consistent with the SCS results at herd and at number and stage of lactation levels, as seen in Table 4. In the four-year period, the average herd SCM prevalence was between 29.6% and 34.5%, increasing from 2016 until 2018. Herd average chronic SCM prevalence was between 22.1% and 26.9% during the whole study period, showing an increase during the last 3

years. Interestingly, both SCM prevalence and chronic SCM prevalence were noticeably high from 1st lactation, reaching 23.4% and 16.9%, respectively, in 2018. This situation deteriorated as lactation number increased, reaching its' peak within the 3rd lactation (average SCM prevalence 46% and average chronic SCM prevalence 37% in 2018). Average SCM prevalence in all four years was elevated even from early lactation (29.6% in 2018) and reached its highest level in the last stage of lactation (39.2% in 2018). Finally, average chronic SCM prevalence during 2015-2018 ranged between 5.3 and 6.6 in early lactation and increased with the progress of lactation, reaching a peak at the last stage of lactation (35.6% in 2018).

Subclinical mastitis incidence rate

The highest SCM incidence rate was observed in early lactation, also increasing from 27 to 31 new cases / 100 lactating cows / month in the last three years. In the later lactation stages, this rate was reduced by almost three times. Regarding the number of lactations, the SCM incidence rate gradually increased from 1st to 3rd one and peaked in the 3rd lactation. Overall herd average SCM incidence rate in 2018 was 12 new cases / 100 lactating cows / month (Table 4).

Table 3. Average somatic cell score (SCS) per lactation stage groups (≤ 60 , 61-120, 121-180, ≥ 181 days-in-milk, DIM) during 2015-2018 in Greece

| | Lactation stage (DIM) | SCS |
|----------------|-----------------------|-------------------|
| 2015 (n=26) | ≤ 60 | 4.7 (± 0.6) |
| | 61-120 | 4.6 (± 0.7) |
| | 121-180 | 4.8 (± 0.6) |
| | ≥ 181 | 5.2 (± 0.5) |
| 2016 (n=30) | ≤ 60 | 4.7 (± 0.6) |
| | 61-120 | 4.6 (± 0.8) |
| | 121-180 | 4.7 (± 0.6) |
| | ≥ 181 | 5.1 (± 0.6) |
| 2017 (n=30) | ≤ 60 | 4.8 (± 0.6) |
| | 61-120 | 4.8 (± 0.8) |
| | 121-180 | 4.8 (± 0.8) |
| | ≥ 181 | 5.2 (± 0.7) |
| 2018 (n=26) | ≤ 60 | 5.1 (± 0.6) |
| | 61-120 | 5.0 (± 0.7) |
| | 121-180 | 5.2 (± 0.6) |
| | ≥ 181 | 5.3 (± 0.6) |

(\pm): standard deviation

Table 4. Subclinical mastitis prevalence (SCMP - %), chronic subclinical mastitis prevalence (chronic SCMP - %) and subclinical mastitis incidence rate (SCMIR - number of new cases per 100 lactating cows per month), per herd, number of lactation period (1st, 2nd, 3rd LP) and lactation stage (≤ 60 , 61-120, 121-180, ≥ 181 days-in-milk, DIM) groups, during 2015-2018 in Greece

| | 2015 (n=26) | | | | | | 2016 (n=30) | | | | | | 2017 (n=30) | | | | | | 2018 (n=26) | | | | | | |
|------------|-------------|------------|--------------|------------|-------|----------|-------------|------------|--------------|------------|-------|---------|-------------|------------|--------------|------------|-------|----------|-------------|------------|--------------|------------|-------|----------|--|
| | SCMP | | chronic SCMP | | SCMIR | | SCMP | | chronic SCMP | | SCMIR | | SCMP | | chronic SCMP | | SCMIR | | SCMP | | chronic SCMP | | SCMIR | | |
| | % | SD | % | SD | # | SD | % | SD | % | SD | # | SD | % | SD | % | SD | # | SD | % | SD | % | SD | # | SD | |
| Herd | 30.2 | ± 7.9 | 23.7 | ± 7.5 | 10 | ± 2 | 29.6 | ± 9.2 | 22.1 | ± 8.2 | 11 | ± 4 | 32.4 | ± 12.6 | 25.1 | ± 11.6 | 11 | ± 4 | 34.5 | ± 11.6 | 26.9 | ± 11.1 | 12 | ± 4 | |
| LP: | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1st | 19.4 | ± 5.4 | 14.3 | ± 5.2 | 6 | ± 2 | 20.8 | ± 9.5 | 14.6 | ± 9.1 | 8 | ± 3 | 23.3 | ± 13.4 | 17.0 | ± 11.7 | 9 | ± 6 | 23.4 | ± 10.3 | 16.9 | ± 9.6 | 9 | ± 3 | |
| 2nd | 27.7 | ± 10.0 | 21.0 | ± 9.2 | 10 | ± 3 | 26.5 | ± 12.0 | 19.3 | ± 10.9 | 10 | ± 4 | 28.9 | ± 12.6 | 22.1 | ± 12.0 | 11 | ± 7 | 32.6 | ± 16.5 | 25.3 | ± 15.5 | 12 | ± 7 | |
| 3rd | 41.8 | ± 10.2 | 33.7 | ± 10.1 | 14 | ± 4 | 40.5 | ± 12.2 | 31.4 | ± 11.7 | 15 | ± 6 | 42.0 | ± 14.2 | 33.6 | ± 13.5 | 15 | ± 6 | 46.0 | ± 12.7 | 37.0 | ± 12.3 | 17 | ± 7 | |
| DIM: | | | | | | | | | | | | | | | | | | | | | | | | | |
| ≤ 60 | 26.7 | ± 6.9 | 6.1 | ± 3.1 | 27 | ± 11 | 26.7 | ± 7.8 | 5.6 | ± 3.7 | 27 | ± 9 | 27.9 | ± 11.7 | 5.3 | ± 2.7 | 29 | ± 11 | 29.6 | ± 9.3 | 6.6 | ± 3.7 | 31 | ± 12 | |
| 61-120 | 25.6 | ± 10.5 | 18.1 | ± 9.0 | 10 | ± 6 | 23.5 | ± 8.9 | 16.5 | ± 7.9 | 9 | ± 4 | 26.7 | ± 11.4 | 19.0 | ± 10.2 | 10 | ± 5 | 28.4 | ± 10.9 | 21.1 | ± 9.7 | 10 | ± 5 | |
| 121-180 | 26.9 | ± 9.4 | 22.3 | ± 9.9 | 7 | ± 4 | 26.9 | ± 9.8 | 21.7 | ± 9.5 | 8 | ± 4 | 29.1 | ± 13.7 | 24.4 | ± 13.0 | 8 | ± 5 | 31.9 | ± 10.8 | 26.0 | ± 10.4 | 9 | ± 4 | |
| ≥ 181 | 34.6 | ± 9.0 | 31.5 | ± 9.5 | 5 | ± 2 | 33.3 | ± 9.9 | 29.3 | ± 9.7 | 6 | ± 3 | 37.1 | ± 14.7 | 33.3 | ± 14.9 | 6 | ± 2 | 39.2 | ± 13.8 | 35.6 | ± 14.2 | 6 | ± 3 | |

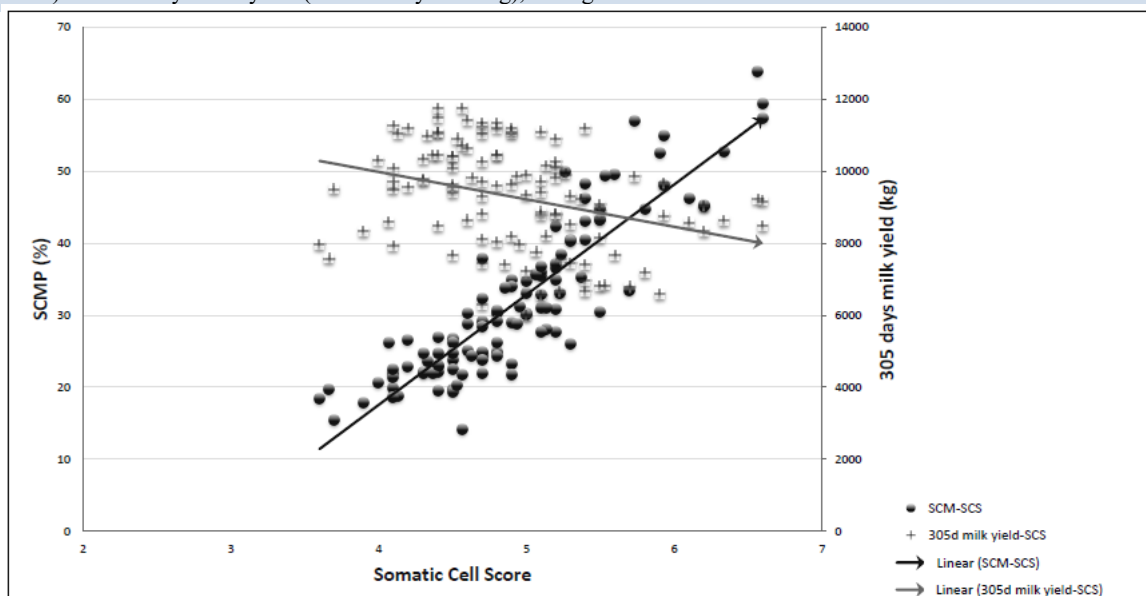
SD (\pm): Standard Deviation

Subclinical mastitis: year over year epidemiology

The epidemiology of subclinical mastitis presented a year over year deterioration. Therefore, the results of 2018 appeared worse than 2015. More precisely, herd average BTSCC and herd average SCS were higher by 78000 cells/mL (Table 1) and by 0.3 points (Table 2), respectively, in 2018 compared to 2015. Herd average SCM prevalence in 2018 was 4.9% higher than 2016 and herd average chronic SCM prevalence was 4.8 % higher than 2016. Finally, according to herd average SCM incidence rate in 2018, 12 new cases per 100 lactating cows (2 more new cases than 2015) were added to the existing chronic cases each month (Table 4).

DISCUSSION

The aim of this study was to evaluate the epidemiology of subclinical mastitis among dairy herds of the Holstein Association of Greece, during the period 2015-2018. The necessary inclusion criterion of consistent data recording could be a selection bias, firstly because precise and meticulous dairymen are related to better herd performance and, secondly, because low milk SCC can be associated with farmer's management style (Barkema et al., 1999; Barnouin et al., 2004). The subclinical mastitis epidemiology presented in the results of this study could be summarized as high overall subclinical mastitis prevalence, high chronic subclinical mastitis prevalence and high subclinical mastitis incidence rate, as well as consequently elevated SCS and BTSCC.

Figure 1. Scatter diagram with linear trend lines of the relationship between somatic cell score (SCS), subclinical mastitis prevalence (SCMP - %) and 305 days milk yield (305d milk yield – kg), during 2015-2018 in Greece

Regarding the relationship between herd average SCM prevalence and herd average SCS (Figure 1), including all herds that took part in the study during the four-year period, the increasing linear trend line of “SCM prevalence – SCS” confirmed the expectedly positive relationship between SCC and intramammary infection. Elevated average SCS of individual cows at herd level, as well as at number and stage of lactation groups (Tables 2, 3) is closely related with the elevated SCM prevalence, as each 1-point increase in SCS can be associated with a 9.1% increase in the prevalence of intra-mammary infection (Shook et al., 2017).

The decreasing linear trend line of “305-days milk yield – SCS” (Figure 1) shows that high somatic cell score was negatively related to milk production. It is known that cows with high somatic cell count (and, consequently, SCS) produce a lower milk volume than cows with low SCC, and that there is a negative correlation between total milk volume produced and the somatic cell count per milliliter of milk produced. Intramammary infections (leading to high SCC) may reduce milk yield through chronic damage to mammary secretory cells, but even in short-duration infections with no permanent damage, metabolic resources

may be diverted from milk production to immune defense (Green et al., 2006).

Interestingly, 46% of the herds during the four-year period of our study had a lower 305-days milk yield / cow in the 3rd than in the 2nd lactation period. This result is contrary to the normal milk production pattern, according to which milk production is increasing from 1st to the 3rd lactation (Clark, 1924; Michel, 1994). This abnormality can be attributed to the deterioration of udder health from 1st to 3rd lactation groups, as observed by the elevated subclinical mastitis prevalence, especially the chronic one, and somatic cell score above the SCM-indicative threshold throughout all four years of the study (Tables 2, 4). Other factors that can lead to decreased milk production as the number of lactations increases are: high prevalence of lameness, poor reproduction, nutritional and managerial errors. However, due to the retrospective nature of the study, there were no data available for those factors and, therefore, their co-effect cannot be evaluated. Table 5. National levels of subclinical mastitis prevalence (%), heifers subclinical mastitis prevalence (%) and 305-days milk yield (kg), compared to the results of 2018 in Greece.

Table 5. National levels of subclinical mastitis prevalence (%), heifers subclinical mastitis prevalence (%) and 305-days milk yield (kg), compared to the results of 2018 in Greece

| Subclinical Mastitis Prevalence | | | | Heifers Subclinical Mastitis Prevalence | | | | 305-days Milk Yield †† | |
|------------------------------------|-----------------|---------------|--------------|---|---------------|---------------|--------------|------------------------|----------------------|
| Author | Country | SCC cut-off | SCMP | Author | Country | SCC cut-off | Heifers SCMP | Country | 305d milk yield (kg) |
| <i>Skrzypek et al., 2004</i> | Poland | 400000 | 21.3% | <i>Fox et al., 1995</i> | USA | 200000 | 36% | Germany | 9219 |
| <i>Madouasse et al., 2010</i> | England & Wales | 200000 | 25% | <i>De Vliegher et al., 2004b</i> | Belgium | 200000 | 27.5% † | France | 9042 |
| <i>Lam et al., 2013</i> | Netherlands | 200000 | 22% | <i>Svensson et al., 2006</i> | Sweden | 200000 | 18.1% † | Sweden | 10325 |
| <i>Fauteux et al., 2014</i> | Canada | 200000 | 26% | <i>Parker et al., 2007</i> | New Zealand | 200000 | 13.3% | Italy | 9980 |
| <i>Shook et al., 2017</i> | USA | - | 37.6% * | <i>Fouz et al., 2010</i> | Spain | 200000 | 21.7% § | Spain | 10152 |
| | | | | <i>Santman-Berends et al., 2012</i> | Netherlands | 150000 | 25.5% II | Netherlands | 9958 |
| | | | | <i>Bludau et al., 2014</i> | Switzerland | 100000 | 20.6% | Switzerland | 8813 |
| Themistokleous et al., 2019 | Greece | 250000 | 34.5% | Themistokleous et al., 2019 | Greece | 250000 | 23.4% | Greece | 9608 |

*: % of intramammary infection (pathogen specific tests), †: 5-14 days in milk (DIM), †: first milking, §: 5-37 DIM, II: first 100 DIM, ††: Data obtained from the European Holstein & Red Holstein Confederation, except Greece (Themistokleous et al., 2019).

Despite the satisfactory, in comparison with other European countries (E.H.R.H.C. 2017), average 305-days milk yield / cow (Table 5), the results discussed below indicate a progressive deterioration of udder health from the onset of productive life until culling. It was observed here that the average BTSCC in 2018 was 463000 cells/mL, approximately 78000 cells/mL higher than in 2015. The BTSCC results found in the present study are also noticeably higher compared to those of the U.S. (Schukken et al., 2003), the Netherlands (Santman-Berends et al., 2016) and Canada (Aghamohammadi et al., 2018). In Finland, a mastitis control program resulted in the reduction of BTSCC from 330000 (in 1988) to 170000 (in 1995) cells/mL within seven years (Honkanen-Buzalski and Myllys, 1996).

The overall SCM prevalence in Greece (increased from 2016 to 2018, 34.5% in 2018) was higher compared to countries like the Netherlands, Canada, England and Wales (Table 5), affecting one in three dairy cows every year. Furthermore, in 2018, heifers' SCM prevalence (23.4%) in Greece was 5% higher than in 2015, comparatively lower than the Netherlands and Belgium and higher than Spain, Sweden and Switzerland. However, it is important to consider that in all of these studies the SCC cut-offs used to define subclinical mastitis were lower than the cut-off used in the "IQ" system. The lower cut-off could be responsible for the higher SCM prevalence observed in heifers of the Netherlands and Belgium, compared to Greece.

Average age at first calving was above the upper optimal threshold in all four years of the study (Table 1). Belated first calving has been associated with increased first lactation SCC and lower lifetime milk production, as well as longer calving intervals (also observed in the present study) and worse reproductive performance (Eastham et al., 2018).

In all four years of our study, the highest SCM incidence rate was observed in early lactation, accompanied by elevated SCS and SCM prevalence (Table 4). Many cows were probably already infected before or became infected at calving, implying a high possibility of either ineffective dry cow therapy or errors in dry and fresh cows' management. Dry period is a critical time in the lactation cycle (Bradley et al., 2010), as it is the optimum time to cure existing intramammary infections (Wilson et al., 1972) and a high-risk period for new intramammary infections (Smith et al., 1985). The probability of cows to develop new intramammary infections during the dry

period has been related with high milk yield before drying-off, longer duration of the dry period, housing of dry cows in tie-stall barns, as well as with number of parity and SCS above 4.0 on last test-day before drying-off (Dingwell et al., 2002, Madouasse et al., 2012). Research has shown that cows from herds with high chances of maintaining or having newly elevated SCC over the dry period in the previous year had a higher probability of elevated SCC at first recording after calving (Madouasse et al., 2012).

The risk of new intramammary infections during the dry period is elevated during: i) the first weeks after drying-off, when involution of the udder occurs (Neave et al., 1950), and ii) the weeks preceding calving, when colostrogenesis takes place in the udder (Oliver et al., 1983). Therapeutic levels of antibiotics for dry cow therapy may be achieved only for the first 14 to 28 days after infusion, thus, failing to protect the udder during the last trimester of the dry period (especially for long ones); this can lead to new quarter intramammary infections during the dry period, which rate can reach 17.44% (Rindsig et al., 1978; Robert et al., 2006; Petzer et al., 2009). As a result, at the end of the dry period untreated and treated cows would stay at the same risk of new intramammary infections (Robert et al., 2006). In Greece, although not supported by data due to the retrospective nature of the study, it can be presumed that unsuccessful dry cow therapy and/or dry cow management errors could be involved into the current problem of high SCM incidence rate and SCM prevalence after calving and in early lactation. A focused research should be conducted to investigate the association between the applied dry period management practices in Greece and udder health.

Regarding heifers, the results indicated bad udder health from the very first lactation, even from the early stages (Tables 2, 3 and 4). Intramammary infections in dairy heifers may already occur at breeding age (Trinidad et al., 1990), but the risk is greater in the last trimester of pregnancy and at calving (Fox et al., 1995; Piepers et al., 2009). Heifers' intramammary infections during (late) gestation and early lactation are crucial not only for first lactation, but also for future milk production. That is because they are associated with impaired development of the mammary gland, negatively affecting both first lactation and future udder health, while can additionally lead to elevated risk of culling in first lactation (De Vliegher et al., 2005; Piepers et al., 2009; Santman-Berends et al., 2012). First lactation somatic cell count (especially in ear-

ly lactation) is negatively associated with both first lactation and lifetime milk yield (De Vliegher et al., 2005; Archer et al., 2014). Subclinical mastitis on first test-day after calving led to a higher risk of developing chronic mastitis or early culling and, moreover, isolation of major pathogens from first calving day up to the 5th day-in-milk was associated with 60% increased culling risk in first lactation (Compton et al., 2007; Bludau et al., 2014).

Apart from the negative effects of intramammary infections on the secretory tissue, suboptimal production has been associated with permanently elevated SCC throughout the whole lactation (De Vliegher et al., 2005). The longer the intramammary infections exist and the longer they persist into lactation (as observed in the present study by the elevated chronic SCM prevalence in first lactation), the larger the impact on heifers' production potential (Piepers et al., 2009). Given the fact that prevention rather than cure of early lactation elevated SCC is needed (De Vliegher et al., 2005), a series of preventive interventions are crucial for the improvement of the potential dairy herd productivity.

CONCLUSION

The results of this study indicated high prevalence of SCM and poor udder health in Greek Holstein dairy herds, leading to shorter productive life and lower milk quality (elevated somatic cell count). Moreover, the average 305-days milk yield of 3rd lactation was lower than 2nd lactation cows, in 46% of the herds. These findings underline the necessity of implementing a series of actions in order to control subclinical mastitis among Greek dairy herds. The investigation of the applied herd management practices, accompanied by the development of a national mastitis monitoring and prevention program is considered important.

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflicts of interest to declare.

ACKNOWLEDGEMENTS

The authors would like to sincerely thank the Holstein Association of Greece for the data records from 2015 to 2018, provided by "IQ" system, as well as for the flawless collaboration and assistance.

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