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Somatic cell count as a decision tool for selective dry cow therapy in Italy

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ABSTRACT

The application of selective dry cow therapy is one of the measures currently suggested to reduce the use of antibiotics in dairy herds. However, the application of selective dry cow therapy will have a profound impact on Italian dairy herds, very likely affecting both milk yield and quality. Identifying cows to be treated at drying off is crucial for farmers and health authorities, therefore it is necessary the definition of a consistent and certified procedure. This article reports the results of a study aiming to identify which SCC threshold would be the most appropriate to identify cows to be treated and the potential consequences of different selection protocols on udder health after calving under field condition. Last milk test record before drying off and the average of lactation milk test records were considered on a database including 45,682 cow from 709 herd. Five different threshold were considered (50,000; 100,000; 150,000; 200,000; and 250,000 cells/mL). The statistical analysis of the database and a rational evaluation of the results suggest to define thresholds of 100,000 cells/mL for primiparous cows and 200,000 cells/mL for pluriparous cows measured either before drying-off or as the average of all the milk tests of the lactation. The criteria proposed will be useful to manage herd health and, specifically, dry-cows in an efficient and sustainable way, decreasing the use of antimicrobials without increasing the risk of affecting milk yield and quality after calving.

HIGHLIGHTS

- The definition of a consistent and approved procedure to identify cow to be treated in a selective dry cow therapy approach is crucial.
- SCC from milk test records are a convenient, accurate and certified method. SCC values obtained before drying off or calculated as the average of lactation records can be used.
- The thresholds of 100,000 cells for primiparous cows and of 200,000 cells for pluriparous cows are suggested as an efficient and sustainable decision tool.

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Introduction

The problem of an increased frequency of antimicrobial resistance (AMR) affects both human and veterinary medicine. In this latter area the use of antimicrobials in food producing animal is often considered as one of the major source of AMR for pathogens affecting human beings (Tang et al. 2017).

The scientific evidences of an the association of antibiotic usage in food-production animals and AMR is still controversial (Erskine et al. 2004; Tang et al. 2017). However, the problem of AMR could not be underestimated also in the area of food-producing animals (Trevisi et al. 2014); the initiatives based on an One Health approach (Cipolla et al. 2015) both at European level (Guidelines for the prudent use of

antimicrobials in veterinary medicine, 2015/C 299/04) and at Italian level (Italian National Plan to reduce Antimicrobial Resistance 2017–2018) are strongly influencing herd health management.

In the area of milk production, the application of selective dry cow therapy (SDCT) is one of the measure currently suggested to reduce the use of antibiotics. This procedure very likely will be compulsory when EU will implement the new regulations on veterinary drugs. Currently, the large majority of Italian dairy farmers apply blanket dry cow therapy (BDCT), but there is an increasing pressure to apply SDCT to fulfil the request of the Health Authorities to reduce the antibiotic usage by 20%, compared to the levels of 2016.

However, the application of SDCT will have a profound impact on Italian dairy herds, very likely affecting both milk yield and quality. Indeed, several studies showed as SDCT will increase the new infection rate after calving and reduce the cure rate at drying off (Halasa, Nielsen, et al. 2009; Halasa, Osteras, et al. 2009). Consequently, a decrease in herd efficiency is expected not only due to the decrease in milk quality and quantity (Cinar et al. 2015), but also to a decrease in reproductive performances (Rahman et al. 2012). There are also other studies suggesting that SDCT is cost-effective, despite an increase of intramammary infection and clinical cases (Scherpenzeel, den Uijl et al. 2016; Scherpenzeel et al. 2018).

The discrepancy among results can be related also to the different selection protocols in identifying cows to be treated at drying off, which is very different among studies, countries and, sometimes, within countries (Scherpenzeel et al. 2014; Cameron et al. 2015; Godden et al. 2017; Wittek et al. 2018).

The definition of a consistent and approved procedure to identify cows to be treated at drying off is crucial for both the farmers, who need to reduce the risk of new infection after calving, and the health authorities having to verify the compliance of farmers and veterinarians with the regulations on antimicrobial use in dairy farms.

A procedure to identify cows to be treated at drying-off should fulfil several criteria: it should be sufficiently accurate, easy to perform and interpret, cheap, relatively safe (low risk to have new intramammary infection after calving), certifiable, and applicable in the different area of the country. In our opinion, there are only two methods that are close to fulfil all these criteria: the microbiological analysis of milk before drying-off and the evaluation of somatic cell counts (SCC). The first one is the most accurate, it is relatively easy to perform and interpret, it is safe, but it could be relatively expensive and not applicable in some Italian areas, due to the lack of laboratories devoted to milk microbiological analysis. SCC fulfil all the criteria out of the accuracy, because this latter one is strictly dependent on the threshold applied to define the cow to be treated.

Based on the previous considerations, our intention was to meet the need of information and protocols to use SCC as a method to identify udder health status at drying off, in order to have a practical and sustainable decision tool to identify cows to be treated at drying off within a SDCT approach, under field condition in Italy. This approach is crucial for dairy milk producers to maintain the efficiency of the herds, without

compromising cow health, and the compliance with current or incoming regulations.

This article reports the result of a study simulating a SDCT approach based on SCC, aiming to identify which threshold would be the most appropriate to identify cow to be treated and the potential consequences of the different selection protocols on udder health after calving under field condition.

Materials and methods

Data collection

The study considered a database including 45,682 cow milk test records performed in 2017 (MTR) from 709 dairy herds located in several provinces of Lombardy Region (Como, Lecco, Lodi, Milano, Monza-Brianza, Pavia, Varese). These provinces include all the different production areas of the Region (from Alpine area to Po river valley) and all the entire regional herds' size range.

More than 95% of the cows included in the study were Italian Holstein Friesian, most of the remaining ones were Italian Brown Swiss, while other breeds or cross breeds represented less than 1% of the sample.

SCC were performed by certified methods, currently applied by Italian Breeders Association (A.I.A.) at the laboratories of Regional Breeders Association of Lombardy (ARAL) on Fossomatic FC (Foss DK). Cow and MTR were supplied by A.I.A. through ARAL and they were: herdID, cowID, number of lactations, SCC and milk yield at last milk test before drying off, first milk test record after calving; lactation SCC milk test record average before drying-off, drying-off date, calving date. Only records comprising data before and after calving were included in the study, without any exclusion criteria.

SCC was also used to define udder health status. A value of SCC $>200,000$ cells/mL was used to define subclinical mastitis (Piccinini et al. 2005), and by Italian Breeder Association nationwide to define subclinical mastitis in MTR.

Statistical analysis

Data collected in a database were analysed by the appropriate procedures of a statistical analysis software (SPSS 24, IBM USA) and by the procedures of an epidemiological analysis software (Open Epi, www.openepi.com). In details, a very simple ANOVA model with parity as factor was applied to assess difference between SCC mean values.

Chi square test on binomial was applied to assess the differences in subclinical mastitis frequency between two different thresholds (i.e. 50,000 cell/mL vs. 100,000 cell/mL).

Ethical statement

All the experimental procedures are in compliance with the art. 2 of EU regulation 2010/63/UE about the protection of experimental animals.

Results

The study considered 45,682 cow records from 709 dairy herds. Herd size was in the range of 5-473 lactating cows with a mean of 64 cows/herd and a median of 46 cows/herds. The lowest 25th percentile of the herds had less than 25 cows, and the upper one had more than 85 lactating cows. The distribution of cows by lactations showed as 45.7% of them were in their first lactation, while 27.9% were in second lactation, and all the other ones were in third or higher lactation.

The last milk test record (MTR) was collected on average 21 days before drying off (median 20 days), with 25% of tests executed <11 days before drying off, and the upper 25th percentile of the tests executed >29 days before drying off (max 139 days).

Table 1 describes the mean values and standard deviation of individual SCC (log₁₀ cell/mL) based on parity. Mean values were calculated for both last milk

Table 1. Analysis of variance of somatic cell count (log₁₀ cell/mL) distribution based on parity and on type of milk test (last one before drying off or average of lactation tests).

| Parity | N | Last milk test | | Lactation average | | Mean difference p |
|---------------|--------|-------------------|-----------|-------------------|-----------|----------------------|
| | | Mean | Std. dev. | Mean | Std. dev. | |
| One | 20,584 | 5.00 ^a | .51 | 5.04 ^a | .45 | <.0001 |
| Two | 12,745 | 5.19 ^b | .50 | 5.18 ^b | .48 | Not significant |
| Three or more | 12,353 | 5.37 ^c | .51 | 5.35 ^c | .50 | .0018 |
| Total | 45,682 | 5.16 | .53 | 5.16 | .49 | Not significant |

Different letters are statistically different ($\alpha < .05$).

Table 2. Proportion of cows (%) below the four defined SCC threshold values measured at drying off (LMT) or as lactation average (AMT) by parity.

| Parity | Threshold value, cells/mL | | | | | | | | | |
|------------------|---------------------------|------------------|---------|-------|---------|-------|---------|-------|-----------------|-------|
| | 50,000 | | 100,000 | | 150,000 | | 200,000 | | 250,000 | |
| | LMT ^a | AMT ^b | LMT | AMT | LMT | AMT | LMT | AMT | LMT | AMT |
| One | 29.60 | 25.50 | 54.70 | 51.70 | 67.90 | 65.60 | 76.00 | 73.50 | 81.10 | 78.80 |
| Two | 14.20 | 16.80 | 37.00 | 40.90 | 53.00 | 55.10 | 63.80 | 63.40 | 70.60 | 69.30 |
| Three or more | 7.00 | 9.00 | 22.50 | 26.90 | 36.80 | 40.00 | 47.80 | 49.00 | 56.00 | 55.90 |
| Total | 19.20 | 18.60 | 41.00 | 42.00 | 55.30 | 55.70 | 65.00 | 64.00 | 71.40 | 70.00 |
| P, χ^2 test | <.0001 | | <.0001 | | <.0001 | | .0023 | | Not significant | |

^aLast milk test record before drying-off.

^bAverage of milk test records performed during the lactation before drying-off.

test before drying off (LMT) and average of all the MTR performed during lactation (AMT). Despite the overall SCC means of LMT and AMT were equal, the statistical analysis showed as the mean SCC between LMT and AMT were statistically different ($\alpha \leq .05$) between cows at the end of first lactation (primiparous) and pluriparous cows. Both methods of SCC calculation (LMT and AMT) gave mean SCC values always statistically different when cows were classified by number of lactation.

The data were also analysed after the application of five different thresholds (50,000; 100,000, 150,000, 200,000, and 250,000 cells/mL) and reported in Table 2. A statistical difference between LMT and AMT proportions were observed for all the thresholds out of the highest one (250,000 cells/mL). Primiparous cows showed a higher frequency of records below the thresholds in LMT versus AMT, out of 50,000 cells/mL threshold, while the difference was less consistent in older cows.

Drying-off period was on average 62.4 (± 24.53) days with significant differences among age-classes. Indeed, primiparous cows average was 58.37 ± 20.08 days, while in secondiparous cows it was 64.6 ± 26.74 days and 67.0 ± 27.60 days in older cows.

Table 3 reports data and statistical analysis of MTR performed after calving. Only 75 cows (0.16%) were lost during drying-off, suggesting the absence of external confounding factors affecting the population under study. First MTR was collected on average 26.5 ± 13.83 days after calving (median 25 days) with 25% of tests carried out <15 days after calving, and

Table 3. Analysis of variance of somatic cell count distribution at first milk test after calving, classified by parity.

| Parity | N | SCC, log ₁₀ cell/mL | |
|---------------|--------|--------------------------------|-----------|
| | | Mean | Std. dev. |
| One | 20,552 | 4.86 ^a | .63 |
| Two | 12,725 | 4.97 ^b | .67 |
| Three or more | 12,330 | 5.10 ^c | .71 |
| Total | 45,607 | 4.96 | .67 |

Different letters are statistically different ($\alpha < .05$).

Table 4. Frequency of cows with subclinical mastitis (95% confidence limits) at first milk record after calving in relation to the five thresholds defined for the last milk record before drying-off classified by parity.

| Parity | Threshold, cells/mL | | | | |
|---------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| | 50,000 | 100,000 | 150,000 | 200,000 | 250,000 |
| One | 14.27 ^a (13.42–15.80) | 16.49 ^b (15.82–17.19) | 17.66 ^b (17.03–18.28) | 18.47 ^b (17.87–19.08) | 19.04 ^b (18.45–19.65) |
| Two | 19.02 ^a (17.27–20.89) | 20.36 ^a (19.23–21.53) | 21.6 ^a (20.64–22.6) | 22.63 ^a (21.73–23.55) | 23.52 ^a (22.66–24.41) |
| Three or more | 22.26 ^a (19.61–25.15) | 24.4 ^a (22.83–26.03) | 26.34 ^a (25.08–27.64) | 27.34 ^a (26.22–28.49) | 28.61 ^a (27.56–29.69) |
| Total | 16.05 ^a (15.29–16.83) | 18.63 ^b (18.08–19.2) | 20.27 ^b (19.78–20.77) | 21.37 ^c (20.91–21.84) | 22.31 ^d (21.86–22.76) |

Frequencies with different letters are statistically different ($\alpha < .05$).

Table 5. Frequency of cows with subclinical mastitis (95% confidence limits) at first milk record after calving in relation to the five thresholds defined for the average of lactation milk records classified by parity.

| Parity | Threshold, cells/mL | | | | |
|---------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| | 50,000 | 100,000 | 150,000 | 200,000 | 250,000 |
| One | 13.03 ^a (12.14–13.96) | 15.20 ^b (14.53–15.90) | 16.76 ^c (16.13–17.40) | 17.61 ^c (17.01–18.22) | 18.35 ^c (17.76–18.95) |
| Two | 15.07 ^a (13.61–16.65) | 17.57 ^b (16.56–18.63) | 19.36 ^c (18.45–20.30) | 20.66 ^d (19.79–21.56) | 21.78 ^e (20.93–22.65) |
| Three or more | 15.22 ^a (13.22–17.46) | 20.06 ^b (18.73–21.46) | 22.17 ^c (21.04–23.36) | 23.92 ^d (22.86–25.01) | 25.67 ^e (24.65–26.71) |
| Total | 13.89 ^a (13.17–14.64) | 16.69 ^b (16.17–17.22) | 18.52 ^c (18.05–19.01) | 19.76 ^d (19.30–20.22) | 20.93 ^e (20.48–21.38) |

Different letters are statistically different ($\alpha \leq .05$).

the upper 25th percentile of the tests carried out >35 days after calving (max 90 days).

As expected primiparous cows had the lowest SCC, and the mean values increased accordingly to the number of parturitions (Table 3).

Frequency of subclinical mastitis in relation to the five threshold considered and applied at LMT (Table 4) resulted in significant differences in proportions among thresholds only in primiparous cows, and specifically between 50,000 and 100,000 cells/mL.

The same analysis applied to AMT showed the presence of significant differences among threshold for all the classes of age (Table 5). The comparison of proportion of subclinical mastitis between LMT and AMT thresholds, by age classes, did not showed any statistical difference ($\alpha = .05$).

Discussion

The need to reduce antibiotic treatment in dairy herd, as well as in other food-producing animals, requires to develop new or improved control practices aiming to decrease antimicrobial treatments, meanwhile maintaining or at least increasing the herd health status, cow welfare, and herd sustainability.

Moreover, the highly probable introduction in EU of restrictions on the use of BDCT requires the development of criteria to identify cows to be treated sustainable in the different European countries in relation to

their economic and social characteristics (Scherpenzeel, den Uji, et al. 2016; Scherpenzeel, Tijs, et al. 2016; Pulina et al. 2017). Indeed, despite the epidemiology of mastitis has very similar characteristics worldwide, herd characteristics (size, management, hygiene, efficiency) and diagnostic services offered are broadly different among countries and, often, within countries.

A diagnostic method, accurate and certified, and the availability of diagnostic laboratories, offering these services in a quick and sustainable way, are essential to apply a SDCT approach within a country.

The microbiological analysis showed to be the most accurate method to define the mammary gland status (Dohoo, Andersen, et al. 2011; Dohoo, Smith, et al. 2011). However, the method is relatively expensive, requires accurate sampling, proper delivery to the laboratory and the availability of diagnostic laboratories with sufficient expertise in mastitis diagnosis. This latter characteristic represents very often an obstacle in the application of microbiological diagnosis, at least in some areas of Italy.

The use of SCC showed to be a potential useful and practical alternative (Sargeant et al. 2001; Ferronato et al. 2018). Unfortunately, the criteria proposed by several studies are different and the results obtained are not consistent (Cameron et al. 2014; Scherpenzeel et al. 2014), whereas the different herd characteristics support the definition of criteria fitting the specific conditions of the country.

In order to contribute in defining criteria applicable to the current Italian dairy herd situation, a large database of milk test records of Italian dairy cows was analysed. Individual SCC included in MTR were selected being the most convenient and consistent sampling procedure applied in Italian dairy herds. SCC measured at quarter level were not considered because this type of sampling requires as much work as microbiological analysis without a comparable accuracy.

This analysis allows to define methods and criteria to make decision on antimicrobial treatment of cows at drying-off. Indeed, the data and the statistical analysis showed as MTR performed just before drying-off or the lactational average of MTR can be useful in defining thresholds. Moreover, these criteria will be acceptable by local health authorities, being objective consistent and certified. Indeed, by current Italian legislation, these authorities have to assess the compliance of the producer to the current or incoming regulations on antimicrobial treatments in veterinary medicine.

We considered five different thresholds to identify the most sustainable for Italian dairy herds. This means that threshold applied should result in a sensible reduction of antimicrobial treatments at drying-off, without inducing a significant increase in subclinical mastitis risk after calving.

Among the factors to be considered, age of cow (measured as number of lactation), as expected, shows to have a significant influence on SCC, independently of the milk record considered. This latter parameter can be used to define the thresholds selecting cows to be treated at drying-off, by taking in consideration age of the cow.

Age of the cows affected subclinical mastitis frequency after calving, and conditioned the selection of thresholds. The analysis of frequency of subclinical mastitis applying LMT or AMT thresholds suggests a value of 100,000 cells/mL for primiparous cows, because any other value resulted in a significant increase in subclinical mastitis frequency after calving. The 50,000 cells/mL threshold gave also a significant increase in subclinical mastitis after calving, when compared to 100,000 cells/mL. However, the application of this threshold will result in a treatments reduction of only 30%, value very close to the current requirements of Italian health authorities. Therefore, it could be insufficient to fulfil current and incoming legal requirements.

For older cows, the selection of a threshold is more controversial. Indeed, in LMT there are no significant differences in subclinical mastitis frequencies among thresholds, while the frequencies of subclinical mastitis

were always statistically different among AMT thresholds. Therefore, for secondiparous and older cows, a reasonable threshold of the value of 200,000 cell/mL can be suggested. This value is generally applied to define the presence of a subclinical mastitis, and represents a threshold under which about 45% of cows are classified in the treatable group, frequency very similar to the one estimated for primiparous cows with the threshold of 100,000 cells/mL.

The criteria described in this article are, in our knowledge, the first ones defined after analysis of a MTR database in Italy, involving herds with different characteristics and a large number of cows. This criteria can be applied in most of Italian dairy herds, however, in peculiar areas where cows population is largely different from the one considered in this study (95% Italian Holstein Friesian), also breed effect can be considered. These approach based on SCC can be integrated with other ones such as the occurrence of a clinical mastitis, an antimicrobial treatment during lactation or the presence of teat alteration, all factors increasing the risk of recurrent mastitis (Tamburini et al. 2010; Vanhoudt et al. 2018; Zecconi et al. 2018). However, these latter factors are strictly dependent by the accuracy of herd records and, therefore, less consistent when compared to the ones based on SCC, and applicable only to some herds.

The criteria proposed, in our opinion, will be useful to manage herd health and, specifically, dry-cows in an efficient way, decreasing the use of antimicrobials without increasing the risk of affecting milk yield and quality after calving, as shown by a recent study (Vanhoudt et al. 2018). It must be noted that the cows considered in this study were all treated at drying off; therefore, the values observed should be considered as the best result attainable if SDCT will be applied. If all the cows below the selected threshold would be untreated, an increase of subclinical mastitis should be expected. To reduce this negative outcome, it must be emphasised that this approach should be go together with an evaluation of herd health and hygiene management, to identify critical issues on the management of cows (Savignano et al. 2008; Trevisi et al. 2010) and avoid increasing the risk of poor performances after calving.

Conclusions

The results of this study confirm that SCC is a practical method to select cows to be treated at drying-off. The identification of thresholds, as performed in this study, is the necessary starting point to apply this approach under field conditions. Ongoing field trial based on the threshold defined, will allow to

assess the effect of selective dry cow therapy on milk production and cow health in Italian dairy herds.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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