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## Observational study on application of a selective dry-cow therapy protocol based on individual somatic cell count thresholds

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### ABSTRACT

Selective dry cow therapy (SDCT) is one measure suggested to reduce the use of antibiotics on dairy farms. This procedure may have a negative impact on dairy herds, affecting both milk yield and quality. The aim of this observational study was to evaluate the implementation of a SDCT protocol based on the treatment only of cows with SCC at last individual milk test before drying-off >100,000 cells/mL (primiparous cows) or >200,000 cells/mL (pluriparous cows) on udder infection status. We also assessed the association between cow and management factors with the likelihood of cure or of new IMI across the dry period. The study considered 516 dairy cows (2064 quarters), and only 53% of the cows were treated with antibiotics. Before drying-off, 999 quarters (49.1%) were bacteriological negative at two consecutive samples. After calving the negative quarters were 1004 (49.3%). The likelihood of a cure across the dry period was significantly associated with parity, drying-off length and teat sealant application, while the likelihood of new IMI across the dry period was associated to all the risk factors considered (parity, dry period length, teat sealant application, infections status at drying-off and type of long-acting antibiotic applied). The application under field conditions of the proposed SDCT protocol showed to be feasible, and to largely reduce the use of antimicrobials at drying-off, with a relatively small impact on cow health.

### HIGHLIGHTS

- First observational study in of Italian dairy herds on the application of a selective dry-cow therapy protocol based on SCC.
- The study showed as a reduction of about 50% in antimicrobial usage at drying off can be achieved.
- Management and cow factors affect the likelihood of a cure of a new intramammary infection across the dry period.

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## Introduction

The problem of an increased frequency of antimicrobial resistance (AMR) affects both human and veterinary medicine, and should not be underestimated also in food-producing animals (Trevisi et al. 2014). In the dairy industry, the application of selective dry cow therapy (SDCT) is one of the measures suggested to reduce the use of antibiotics. This procedure may have a negative impact on dairy herds, very likely affecting both milk yield and quality. Several studies showed as SDCT will increase the new infection rate

and reduce the cure rate across dry period (Halasa, Nielsen, et al. 2009; Halasa, Osteras, et al. 2009), even if other studies suggest that SDCT is cost effective, despite an increase of intramammary infection (IMI) and clinical cases after calving (Scherpenzeel et al. 2016, 2018). It should be also emphasised that these latter problems are not always observed (Cameron et al. 2015; Vasquez et al. 2018).

Furthermore, 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

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 Supplemental data for this article can be accessed [here](#).

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reduce the health risk for cows after calving, and the health authorities having to verify the compliance of farmers and veterinarians with the regulations on antimicrobial use in dairy farms.

To help in tackling these problems, we recently proposed a protocol for SDCT based on SCC at the last individual milk test before drying-off (Zecconi et al. 2018b). Individual cow milk SCC measured at the last milk test day before drying-off were selected being the most convenient and consistent sampling procedure applied in Italian dairy herds. However, to verify the feasibility of this protocol, it was necessary to test it under field conditions.

We designed an observational study to evaluate the effects of implementing SDCT in five Italian dairy herds based on the protocol suggested (Zecconi et al. 2018b). More precisely, quarter milk samples were collected at drying-off and after calving to assess the prevalence of intramammary infection (IMI). Based on these data, cure rate and new IMI rate were assessed comparing untreated and treated cows, and we considered in these latter ones also the effect of two different antimicrobial treatments. The associations of cow and management factors with the likelihood of cure or of new IMI across the dry period were also assessed, to gain information useful to increase the feasibility and efficacy of the protocol proposed.

## Material and methods

### Study herds

Five herds, that volunteered to take part to the study, were involved. The herds are located in 4 Lombardy provinces (Cremona, Como, Pavia, Sondrio) and the herd sizes were in the range 80–485 lactating cows, milk yield were in the range 30–35 Kg/day and average herd somatic cell counts (SCC) were in the range 175–220.000 cells/mL. All the cows were housed in cubicle barns (including cows during drying-off period) and two of them have rotary parlours, while the other three have herringbone parlours. Milking procedure followed the current best practices (Tamburini et al. 2007). All five herds are members of the national milk quality improvement (NMQ) program managed locally by Associazione Regionale Allevatori Lombardia (ARAL) which supplied SCC values from individual milk test (IMT). All the samplings full complied with all relevant codes of experimentation and legislation.

### Microbiological analyses

To assess infection status at drying off and after calving in these herds, quarter milk samples (QMS) were

taken two times (7 days before and the day of drying-off) before drying-off and two times after calving (5–7 days and 12–17 days). Sampling was scheduled weekly, and the samples were delivered refrigerated to the Dept. of Veterinary Medicine laboratories where bacteriological analyses were performed by streaking 0.01 mL of QMS on blood agar plate with 5% (v/v) bovine blood according to Hogan et al. (1999). A selection of the isolates was furthermore identified by Vitek-2™ (bioMérieux, Lyon, F). This is a fully automated system that performs bacterial identification and antibiotic susceptibility testing (Barry et al. 2003; Meyer et al. 2008). The selection of isolates to be furthermore identified as follows: all the suspect major pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Echerichia coli*, *Klebsiella* spp) independently from the number of colonies and only pure cultures of CNS and other pathogens were selected. When these outcomes were observed in more than one quarter of the same cow, we randomly selected a colony from a single quarter for further identification.

### Selective dry-cow therapy protocol

The protocol applied was based on the results of the SCC from the last IMT prior to drying-off cow; primiparous cows were treated with antibiotics only when SCC was >100.000 cells/mL, whereas for pluriparous cows the threshold was 200.000 cells/mL. When cows should be treated with antibiotics two different products were applied (cefalonium 250 mg Cefravin™, MSD Italy treatment A, and cefalexin 504.7 mg Rilexine HL™, Virbac Italy – treatment B). The sensitivity to the antimicrobials was pre-emptively estimated by random quarter milk sampling of 24–36 cows and assessment of minimal inhibitory concentration on isolated bacteria by Vitek™ (bioMérieux, Lyon, F). In this latter case, the breakpoints automatically applied by the software were the most recent ones suggested by Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing for bovine isolates. None of the isolates showed to be resistant to the two antimicrobials applied.

The antimicrobial treatment was applied to the four quarters and the application of treatments was sequential within each herd: the first cow to be treated received treatment A and the next one product B, and so on until the end of the study. All the data concerning cow characteristics, SCC and

treatments were recorded in a data sheet delivered together with QMS weekly at the lab.

Cows below the thresholds previously described did not receive any treatment (antimicrobial or others) out of teat sealant application as described in the next section.

### Teat sealant application

Teat sealant was applied in herds C, D and E on all cows and quarters before these herds were enrolled into the study as a management practice to prevent new IMI after calving. In the other two herds, teat sealant was not used in any cow before and during the study. We avoid interfering with farmers decisions on this aspect, but we recorded if teat sealant was used or not.

### Infection status definition

We collected all the data in a database; the assessment of infection status at quarter level, before drying-off and after calving, based on the interpretative scheme reported in [Supplementary Table S1](#). The evaluation of the outcome of the three treatments applied (no therapy, treatment A, treatment B) were based on the interpretative scheme reported in [Supplementary Table S2](#).

### Statistical analyses

Data description and  $\chi^2$  test were calculated by XLSTAT 2019 1.1 (Addinsoft, Boston, MA, USA). A binary logistic analysis was performed by Procedure Logistic of SAS 9.4 (SAS Institute, Cary, NC, USA) to identify the risk factors associated with the likelihood of new and cured IMI across the dry period by

calculating odds ratio. The risk factors considered were parity, drying-off length, teat sealant application, therapy and infection status at drying-off. The final models were described in terms of odds ratios and 95% confidence intervals.

## Results

### Herd and cow characteristics

The study considered 516 dairy cows (2064 quarters), and the distribution of treatments in relation with herd and cow characteristics were reported in [Table 1](#).

Samples distribution among herds was relatively uniform, only herd A showed relative lower proportion of samples (10.5%). Over 50% of the cows were in the first lactation, and 71% of them were treated with teat sealant at drying-off. On average, drying-off period was  $61.1 \pm 19.1$  days long; 10% of cow had a drying-off  $<45$  days and 25%  $>68$  days. The length of drying-off period was significantly correlated to parities. Indeed, drying-off period was  $58.2 \pm 14.0$  days in primiparous cows,  $60.5 \pm 16.3$  days in secondiparous cows and  $63.6 \pm 16.3$  days in pluriparous cows.

### Bacteriological analyses

The results of bacteriological analyses showed as 999 quarters (49.1%) were bacteriological negative at samples taken before drying-off ([Table 2](#)). Among the positive quarters, 442 (41.5%) were selected for a more detailed identification and the results were reported in [Supplementary Table S3](#). Coagulase-negative staphylococci (CNS) represented the largest proportion of isolates (72.1%), environmental Streptococci 7.1%, *S. aureus* 5.1%, while coliforms represented only

**Table 1.** Description of cow population characteristics included into the study.

Factor	Class	N, %	Frequency, %		
			No treatment	Treatment A <sup>1</sup>	Treatment B <sup>2</sup>
Herd	A	216 (10.5)	79.6	11.1	9.3
	B	384 (18.6)	27.1	51.0	21.9
	C	496 (24.0)	49.2	20.2	30.6
	D	536 (26.0)	44.8	17.2	38.1
	E	432 (20.9)	49.1	20.4	30.6
Parity (n)	1	1072 (51.9)	54.5	17.2	28.3
	2	652 (31.6)	44.2	26.4	29.4
	$\geq 3$	340 (16.5)	29.4	42.4	23.2
Dry period length, days	1–45	266 (12.9)	54.5	19.9	25.6
	46–60	966 (46.7)	51.2	17.4	31.4
	61–75	592 (28.7)	40.4	29.2	30.4
	$\geq 75$	240 (11.6)	38.8	44.2	17.1
Teat sealant	Yes	1643 (70.9)	47.5	19.1	33.4
Total		2064	47.1	24.2	28.7

<sup>1</sup>Treatment A: cefalonium 250 mg, Cepravin<sup>TM</sup>, MSD Italy.

<sup>2</sup>Treatment B: cefalexin 504.7 mg Rilexine HL<sup>TM</sup>, Virbac Italy.

**Table 2.** Distribution of quarters by their infection status and treatment at drying-off.

Drying-off status <sup>1</sup>	N (%)	Frequency, %		
		No treatment	Treatment A <sup>5</sup>	Treatment B <sup>6</sup>
Negative	999 (49.1)	54.9 <sup>a,2</sup>	19.3 <sup>b</sup>	25.8 <sup>b</sup>
IMI <sup>4</sup>	531 (26.0)	20.6 <sup>a</sup>	31.4 <sup>b</sup>	29.4 <sup>b</sup>
Transient IMI	506 (24.8)	41.5 <sup>a</sup>	27.7 <sup>b</sup>	30.8 <sup>a,b</sup>
Total	2036 <sup>3</sup>	47.1	24.2	28.7

<sup>1</sup>Infection status defined as reported in Table S1 (Supplementary Data).

<sup>2</sup>Column means with different letters are statistically different at  $\chi^2$  test ( $\alpha < 0.05$ ).

<sup>3</sup>28 blind quarters were not reported in the table.

<sup>4</sup>Intramammary infection.

<sup>5</sup>Treatment A: cefalonium 250 mg. Cepravin<sup>TM</sup>, MSD Italy.

<sup>6</sup>Treatment B: cefalexin 504.7 mg Rilexine HL<sup>TM</sup>, Virbac Italy.

**Table 3.** Distribution of quarters by their infection status and treatment after calving.

Post-calving status <sup>1</sup>	N (%)	Frequency, %		
		No treatment	Treatment A <sup>5</sup>	Treatment B <sup>6</sup>
Negative	1004 (49.3)	53.4 <sup>a,2</sup>	20.2 <sup>b</sup>	26.4 <sup>b</sup>
IMI <sup>4</sup>	477 (23.5)	37.9 <sup>a</sup>	30.6 <sup>b</sup>	31.4 <sup>b</sup>
Transient IMI	553 (27.3)	25.9 <sup>a</sup>	31.4 <sup>a</sup>	30.9 <sup>a</sup>
Total	2034 <sup>3</sup>	47.1	24.2	28.7

<sup>1</sup>Infection status defined as reported in Table S1 (Supplementary Data).

<sup>2</sup>Column means with different letters are statistically different at  $\chi^2$  test ( $\alpha < 0.05$ ).

<sup>3</sup>30 blind quarters were not reported in the table.

<sup>4</sup>Intramammary infection.

<sup>5</sup>Treatment A: cefalonium 250 mg. Cepravin<sup>TM</sup>, MSD Italy.

<sup>6</sup>Treatment B: cefalexin 504.7 mg Rilexine HL<sup>TM</sup>, Virbac Italy.

2.1% of the isolates. In all the other cases, we detected several unrelated bacteria species.

After calving the bacteriological results were shown in Table 3. The negative quarters were 1004 (49.3%); 288 (30.0%) of bacteriological positive quarters were identified by Vitek<sup>TM</sup> (bioMérieux, Lyon, France) as reported in Supplementary Table S3. Overall, 59.9% of the isolates were CNS, 8.7% *Str. Uberis*, 2.4% other environmental Streptococci, 6.8% coliforms and only 2.7% *S. aureus*.

### Infectious status across dry period

Following the interpretative scheme reported in Supplementary Table S1, 531 positive quarters (26.1%) harboured an IMI (TIMI), 506 (24.8%) a transient IMI (LIMI) and 28 (1.4%) were blind. Only 47 quarters were classified as having mixed infections and, therefore, they were included in LIMI group (Table 2). After calving the proportions were relatively similar with 23.5% of quarters with TIMI and 27.3% with LIMI, while 49.3% were negative (Table 3).

The distribution of treatments among quarters at drying-off was reported in Table 4. The cure rate was overall 68.5% in TIMI quarters and 67.5% in LIMI, while

**Table 4.** Distribution of quarters by their infection status and treatment after calving.

Drying-off status <sup>1</sup>	Outcome <sup>2</sup> , %				
	Negative	Cured	Cured/New IMI	NO cure	New IMI
Negative	64.6 <sup>a,3</sup>	–	–	–	35.4 <sup>b</sup>
IMI <sup>4</sup>	–	35.0 <sup>a</sup>	33.5 <sup>a</sup>	31.5 <sup>a</sup>	–
Transient IMI	–	38.9 <sup>a</sup>	28.6 <sup>b</sup>	32.5 <sup>b</sup>	–

<sup>1</sup>Infection status defined as reported in Table S1 (Supplementary Data).

<sup>2</sup>Outcome defined as reported in Table S2 (Supplementary Data).

<sup>3</sup>Column means with different letters are statistically different at  $\chi^2$  test ( $\alpha < 0.05$ ).

<sup>4</sup>Intramammary infection.

**Table 5.** Distribution of quarters by their infection status and treatment after calving.

Drying-off status <sup>1</sup>	Treatment	Outcome <sup>2</sup> , %				
		Negative	Cured	Cured/New IMI	NO cure	New IMI
Negative	No	69.7 <sup>a,3</sup>	–	–	–	28.3 <sup>a</sup>
	A <sup>5</sup>	46.6 <sup>b</sup>	–	–	–	53.4 <sup>b</sup>
	B <sup>6</sup>	62.0 <sup>a</sup>	–	–	–	38.0 <sup>a</sup>
IMI <sup>4</sup>	No	–	32.6 <sup>a</sup>	34.2 <sup>a</sup>	33.2 <sup>a</sup>	–
	A <sup>5</sup>	–	45.2 <sup>b</sup>	29.3 <sup>a</sup>	25.5 <sup>a</sup>	–
	B <sup>6</sup>	–	28.7 <sup>a</sup>	36.2 <sup>a</sup>	35.1 <sup>a</sup>	–
Transient IMI	No	–	43.3 <sup>a</sup>	29.3 <sup>a</sup>	27.4 <sup>a</sup>	–
	A <sup>5</sup>	–	28.8 <sup>b</sup>	34.7 <sup>a</sup>	36.5 <sup>a</sup>	–
	B <sup>6</sup>	–	36.3 <sup>a,b</sup>	37.6 <sup>a</sup>	26.1 <sup>a</sup>	–

<sup>1</sup>Infection status defined as reported in Table S1 (Supplementary Data).

<sup>2</sup>Outcome defined as reported in Table S2 (Supplementary Data).

<sup>3</sup>Row means with different letters are statistically different at  $\chi^2$  test ( $\alpha < 0.05$ ).

<sup>4</sup>Intramammary infection.

<sup>5</sup>Treatment A: cefalonium 250 mg. Cepravin<sup>TM</sup>, MSD Italy.

<sup>6</sup>Treatment B: cefalexin 504.7 mg Rilexine HL<sup>TM</sup>, Virbac Italy.

35.4% of negative quarters at drying-off developed a new IMI after calving.

The differences in frequencies observed for cured, cured with new IMI and uncured quarters were not statistically significant among TIMI quarters, while significant differences in those frequencies were observed among LIMI quarters. Indeed, cured quarters had a higher frequency when compared both with quarters cured but having a new IMI due to a different bacteria species and with uncured ones.

When data were analysed by type of treatment (Table 5), we observed as among negative quarters, the new IMI rate was significantly higher in quarters treated with product A, when compared with untreated ones and quarters treated with product B. The presence of a treatment in negative quarters should not be surprising because the application of treatment was based only on SCC and it is well known that cows with low SCC may harbour IMI (Zecconi and Piccinini 2002; Zecconi et al. 2020).

In TIMI quarters, treatment A resulted in a significantly higher cure rate, while we observed no significant differences in all the other result categories. Similarly, in LIMI quarters a significant higher



**Table 6.** Logistic regression analysis: factors associated to the odds of a cure across the dry period.

Factor ( <i>p</i> for overall effects)	Levels	<i>p</i> Value	OR	95% Lower limit	95% Upper limit
Parity (reference 1) ( <i>p</i> = .089)	2	0.03	0.72	0.53	0.97
	≥3	0.36	0.85	0.59	1.21
Drying-off length (reference 46–60 days) ( <i>p</i> = .027)	1–45	0.01	0.59	0.41	0.86
	61–75	0.21	0.81	0.58	1.13
	≥75	0.11	0.71	0.46	1.08
Teat sealant (reference = NO)	YES	<.01	3.12	2.35	4.19
Infection status (reference = transient IMI <sup>1</sup> )	IMI	0.32	1.15	0.88	1.50
Therapy (reference = NO)	Product A <sup>2</sup>	0.83	1.04	0.74	1.47
( <i>p</i> = .102)	Product B <sup>3</sup>	0.07	0.74	0.54	1.02

<sup>1</sup>Intramammary infection.<sup>2</sup>Product A: cefalonium 250 mg. Cepravin<sup>TM</sup>, MSD Italy.<sup>3</sup>Product B: cefalexin 504.7 mg Rilixine HL<sup>TM</sup>, Virbac Italy.**Table 7.** Logistic regression analysis: factors associated to the odds of a new infection across the dry period.

Factor ( <i>p</i> for overall effects)	Levels	<i>p</i> Value	OR	95% Lower limit	95% Upper limit
Parity (reference 1) ( <i>p</i> < .01)	2	0.75	0.96	0.73	1.25
	≥3	<0.01	0.49	0.34	0.72
Dry period length (reference 46–60 days) ( <i>p</i> < 0.01)	1–45	<0.01	0.45	0.32	0.66
	61–75	0.27	0.85	0.64	1.13
	≥75	<0.01	0.24	0.16	0.38
Teat sealant (reference = NO)	YES	.02	0.72	0.55	0.94
Infection status (reference = Healthy) ( <i>p</i> < .01)	transient IMI <sup>1</sup>	<0.01	4.09	2.85	5.86
	IMI	<0.01	5.85	4.13	8.29
Therapy (reference = NO) ( <i>p</i> < .01)	Product A <sup>2</sup>	<0.01	1.95	1.40	2.70
	Product B <sup>3</sup>	0.18	1.23	0.91	1.63

<sup>1</sup>Intramammary infection.<sup>2</sup>Product A: cefalonium 250 mg. Cepravin<sup>TM</sup>, MSD Italy.<sup>3</sup>Product B: cefalexin 504.7 mg Rilixine HL<sup>TM</sup>, Virbac Italy.

frequency of new IMI was observed for treatment A, and none in all the other result categories.

### **Risk factors affecting cure rates and new IMI rates**

To assess the association among the different risk factors considered (parity, drying-off length, teat sealant application, infection status at drying-off and type of treatment) and the frequency of IMI after calving, two logistic models were applied, one having cure as response variable (cured/not cured) and a second one having new IMI (presence/absence) as response variable. The results were reported, respectively, in Tables 6 and 7. Risk factors associated with the likelihood of new and cured IMI across the dry period were parity, dry period length and teat sealant, while the likelihood of new IMI rate was associated to all the risk factors considered.

Teat sealant application showed to significantly increase the chance of a cure, Odds Ratio (OR) was 3.1 (2.3–4.2) and to significantly decrease new IMI rate (OR 0.7; 0.6–0.9).

Dry period length also had a consistent significant association with both cure and new IMI rates. When dry period length was shorter than the reference value of 46–60 days a significant decrease of cure rate was observed (OR 0.6; 0.4–0.9); we also observed a reduction of the odd of new IMI when dry period was <45 days or when it was longer than 75 days. Parity

showed a similar pattern with a decreased chance of cure in second lactation cows.

Infection status at drying-off was associated to an increased odds of new IMI with an OR of 4.1 (2.8–5.7) and 5.8 (4.1–8.3) respectively in LIMI and TIMI quarters.

When antimicrobial treatment was considered, we need to report that a shortage in the supplying product A caused a departure from the treatment sequence for a temporary period of time, which explain the difference in treatment frequencies, even if the distribution of the treatments among the overall samples is close (Table 1). During the period of product A shortage (three months) all the cows were treated with product B; when it became available again, the sequential treatment was re-applied. The statistical analysis showed as treatment A showed to be associated to an increased odds of a new IMI (OR 1.9; 1.4–2.7).

### **Discussion**

The application of the SDCT protocol based on SCC of the last IMT prior to drying-off (Zecconi et al. 2018b) showed to be feasible under field conditions and to give the expected results. Indeed, only 53% of the cows were treated with antibiotics.

The proportion of bacteriologically negative quarters was also close to 50% supporting the approach based on SCC, which has the advantage to be cheaper than microbiological analysis, being included in the annual fee paid by a farmer for the NMQ program, thus increasing its sustainability and promoting to the compliance by the farmers. The high proportion of negative quarters and, on the other side, the relatively low proportion of TIMI (26%) confirms the good level of herd hygiene observed in the enrolled herds and support the application of selective dry-cow therapy (Robert et al. 2008; Scherpenzeel et al. 2014).

Apparently, the application of the SDCT did not affect the frequency of IMI. Indeed, the proportion of negative, LIMI and TIMI was very similar at drying-off and after calving. However, when data were analysed based on bacteria recovered from quarter milk samples, the pattern was different. In both cases, CNS represented the most frequently isolated bacteria species both at drying-off and after calving with, respectively, a frequency of 72% and 60%. Environmental Streptococci frequency grew from 7.1% at drying off to 11.1% after calving as well as coliforms, which grew from 2.1% at drying-off to 6.8% after calving. These changes represent a potential risk for the development of clinical and subclinical mastitis in the initial months of lactation (Smith et al. 1985). In the present study, for ethical reasons, farmers and veterinarians were informed of all the positive samples for contagious or environmental pathogens and the information was used to reduce such risk (i.e. treating infected quarter), resulting a very low number of reported clinical cases (data not shown). The changes in epidemiological pattern after calving, when SDCT is applied, suggest that cows should be carefully clinically monitored and/or milk samples should be taken routinely about 15 days after calving to decrease the risk of developing clinical or subclinical mastitis.

The results of the treatment protocol applied in relation to the infection status at drying-off gave very similar results. These data were not unexpected, and confirms that SDCT increases the risks for IMI after calving (Halasa, et al. 2009; Halasa, et al. 2009; Scherpenzeel, et al. 2014). We should add that that 95% of uncured cases are due to CNS (data not shown), the number of species involved, their high prevalence in dairy herds (Zecconi and Piccinini 2002; Dufour et al. 2012; Vanderhaeghen et al. 2014) and their peculiar reservoir on teat skin (Zecconi et al. 2003a; Zecconi and Hamann 2006) highly increases the chances to have IMI due to these bacteria in post-calving cows leading to an overestimation of uncured

infections. Moreover, new IMI in negative quarters, in infected quarters and in uncured quarters had a frequency in the range 29–35%.

Since SDCT was applied based on SCC, we could compare the treatments, since we have IMI within untreated cows as well as we have bacteriologically negative quarters in treated cows. Significant differences were observed either when cows were treated or not and between treatments. The new IMI rate was significantly lower in negative untreated cows when compared with treated ones, and we also observed a similar pattern in LIMI. The pathogenesis of IMI can explain these results, apparently contradictory. Indeed, treated quarters, even if negative, came from a cow with relative high SCC, therefore with an active inflammatory process due to an IMI. These cows (and their quarters) showed to be more susceptible to have an infection when compared to healthy ones (Barkema et al. 1997; Zecconi and Piccinini 2002; Zecconi et al. 2004; Piccinini et al. 2005).

### **Risk factors and cure rate**

Drying-off, including dry-cow therapy, is a peculiar and very critical period for dairy cows. The application of SDCT introduces new challenges in an already complex process. The evaluation of the entire process is not easy, and it should also include aspects related to nutrition, welfare and hygiene which are out of the scope of this paper. We focussed our analyses on the relationship among five risk factors related to the cows (parity, drying-off length, teat sealant application, therapy and infection status at drying-off), and the infection status after calving.

Two of these risk factors were associated to the cure rate: application of teat sealant, and dry-period duration. The application of teat sealant showed to be one with the highest OR value, supporting its use to improve both self-cure rates, in untreated cows and cure rate in treated cows (Huxley et al. 2002; Winder et al. 2019). On the contrary with dry period <45 days showed to have a decrease in cure rate, respectively, of 29% and 41%. The debate on the effect of shortening dry period has been wide in recent years, and our data are consistent with the evidences that shortening drying-off period impairs udder health in the next lactation (Zecconi et al. 2003b; Church et al. 2008).

### **Risk factors and new infection rate**

When new IMI rate was considered all the five risk factors showed to be statistically associated to it. Teat

sealant application showed, as for cure rate, a positive effect decreasing the odds of about 28. On the opposite, infected cows at drying-off have a higher risk for new infections, because of the probable higher susceptibility as previously discussed. Dry period length shorter or longer than the reference interval of 46–60 days showed to be associated to a decrease in new IMI risk. Since the largest proportion of IMI was due to CNS, these results may be explained hypothesising the presence of an udder environment which would not support the development of new IMI, for the higher concentration of natural antimicrobial substances in the case of a short dry period (Piccinini et al. 2007; Zecconi et al. 2018a), or the absence of milking that reduce the risk of infection mainly due to CNS (Rajala-Schultz et al. 2009; Frey et al. 2013). Similarly, we observed a significant reduction of the odds for a new IMI in older cows. This result may be explained by the higher frequency of subclinical IMI at drying off in these group of cows when compared to younger cows (respectively 43% and 25%).

We observed a statistical difference in the new infection rate between the two antimicrobial treatments, which was unexpected, since the active principle is in the same class (1<sup>st</sup> generation cephalosporins). The difference observed may be explained by the different composition of the products which in the case of product A led to a longer persistence in milk at a concentration below minimal inhibitory one, (product A: withdrawal time 168 h post-calving; while it is 12 h for product B) suggesting a potential selection of bacteria leading to the development of new IMI mainly due to CNS (Rajala-Schultz et al. 2009; Frey et al. 2013).

## Conclusions

The result of this observational study on the application under field conditions of the proposed SDCT protocol showed to be feasible from practical point of view, to largely reduce the use of antimicrobials at drying-off, and to have a relatively small impact on cow health. These results strictly depend on a rigorous compliance with the protocol, and to a careful monitoring of udder health after calving, including microbiological and/or cytological assays. The application of a teat sealant at drying-off seems to improve the likelihood of a cure and to decrease the likelihood of new IMI during dry period. The management of the whole drying-off process is not easy, and it should also include aspects related to nutrition, welfare and herd

hygiene that should be implemented together with a rational and sustainable SDCT protocol.

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