J. Dairy Sci. 102:11670–11680
https://doi.org/10.3168/jds.2019-16281

Efficacy of cabergoline in a double-blind randomized clinical trial on milk leakage reduction at drying-off and new intramammary infections across the dry period and postcalving

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ABSTRACT

The abrupt cessation of milking at dry-off may induce milk leakage, which may increase the risk of new intramammary infections (IMI). This study assessed the efficacy of 1 i.m. injection of 5.6 mg of cabergoline (Velactis, Ceva Santé Animale, Libourne, France) at drying-off on milk leakage after dry-off and new IMI across the dry period and postcalving compared with a placebo (negative control) and an intramammary antibiotic treatment (positive control) under field conditions. The study was a double-blind, randomized, 3-arm, multicenter, clinical trial performed under Good Clinical Practice conditions. Data from 900 dairy cows of various breeds from 63 farms in France, Germany, and Hungary were analyzed. Only quarters with no bacterial growth at drying-off and a cow somatic cell count ≤200,000 cells/mL were included. Quarters infected with major or minor pathogens or cows with high somatic cell count at time of inclusion were excluded. Cows that qualified for the study were visited 7 times in total before and after drying-off and after calving. Presence (yes/no) of milk leakage was recorded on the day after dry-off. A new infected quarter (new IMI) was defined as one with a major pathogen present in any one of the 2 postcalving samples. Two mixed logistic regression models were fitted to the data to evaluate the efficacy of cabergoline in the reduction of milk leakage and new IMI. One i.m. injection of cabergoline at drying-off significantly reduced the incidence of milk leakage the day after dry-off compared with both placebo and antibiotic treatment. Cabergoline-treated cows significantly reduced the risk of new IMI by major pathogens across the dry period and postcalving by 21% when compared with placebo cows (20.5 vs. 26.0%, respectively). However, when milk leakage was added to the model, the significance of cabergoline was reduced. We interpreted this to show that milk leakage is an intervening variable between treatment with cabergoline and lower risk of new IMI. The antibiotic treatment significantly decreased the odds of new IMI compared with both cabergoline and placebo. However, because several countries are currently disallowing the preventive use of antibiotics at dry-off in noninfected quarters, the dry-off facilitator cabergoline may therefore be of particular value to reduce the risk of new IMI across the dry period.
Key words: cabergoline, milk leakage, intramammary infection, dry-off

INTRODUCTION

A nonlactating (dry) period before an impending parturition is necessary to optimize milk production in the following lactation (Steeneveld et al., 2014). At drying-off, the mammary gland still produces milk for some days that is no longer removed from the gland. The abrupt cessation of milking at drying-off induces udder engorgement, which may lead to discomfort for the cow and possible milk leakage (**ML**) in the early dry period.

Milk leakage facilitates the entry of microorganisms through the teat canal into the mammary gland (Oliver and Sordillo, 1989; Schukken et al., 1993; Dingwell et al., 2004). Additionally, during the involution of the mammary gland, there is a reduction in the recruitment of immune cells and antibacterial proteins such as immunoglobulin and lactoferrin (Monks et al., 2002), whereas mammary gland secretions contain high concentrations of fat, casein, lactose, and citrate (Oliver and Sordillo, 1989; Collier et al., 2012). These physiological events can inhibit the defense capacity of the mammary gland and offer a favorable medium for bacterial growth (Oliver and Sordillo, 1989; Collier et

Received January 10, 2019.
Accepted July 14, 2019.
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al., 2012). Accordingly, during the first 3 wk of the dry period, which is the mammary gland involution period, the susceptibility to new IMI is high (Eberhart, 1986).

Schukken et al. (1993) demonstrated that cows leaking milk after dry-off had a 6.1 times higher risk of developing an IMI with a major pathogen during the dry period compared with cows that did not leak milk. Rajala-Schultz et al. (2005) reported that this risk increases with the level of milk production. Thus, when milk production immediately before dry-off equals 12.5 kg of milk or more, every additional 5 kg of milk produced increased the odds of having an IMI at calving by at least 77%.

A reduction in the level of milk production before dry-off and, consequently, in the risk of ML and new IMI, can be achieved in a few ways (Ollier et al., 2013). A dietary intake reduction in the days before the dry period limits milk production, but it also creates a temporary state of negative energy balance and may lead to metabolic problems (Odensten et al., 2005) and, consequently, to health disorders such as mastitis (Ingvartsen et al., 2003). Another method that has been reported to lower milk yield is reduced milking frequency (Bushe and Oliver, 1987). However, its association with reduced IMI postcalving has not been clearly proven (Newman et al., 2010).

In an experimental setting, reducing milk production by pharmacologically decreasing the lactogenic signals that drive milk production was researched by, among others, Lacasse et al. (2011) and Ollier et al. (2013, 2014, 2015). Prolactin (**PRL**), a mammogenic and lactogenic hormone, plays a major role in the maintenance of lactation (Boutinaud et al., 2012). Ollier et al. (2013, 2014, 2015) showed that inhibiting PRL secretion using the dopamine agonist quinagolide sharply decreased milk production in cows in late lactation within 24 h. They also showed the protective effect of PRL-release inhibition against IMI (Ollier et al., 2015). However, this effect requires daily injections of quinagolide for between 7 and 18 d (Ollier et al., 2013, 2015).

Data from several experiments in cows have shown that the potent dopamine D2 receptor agonist, cabergoline (**CAB**), decreases plasma PRL concentration, udder pressure, and ML, and increases lactoferrin concentrations (Bach et al., 2015; Bertulat et al., 2017; Boutinaud et al., 2016, 2017). These scientists hypothesized that the use of CAB may reduce ML and hence the risk of new IMI after dry-off.

Additionally, there is a need to reduce the use of antibiotics for preventative purposes (EMA, 2014; EMA and EFSA Joint Scientific Opinion, 2017) because their use in dairy cows and other food-producing animals is believed to contribute to increased antimicrobial resistance (Tacconelli, 2009; Oliver et al., 2011; Landers et al., 2012). However, the reduction in the use of dry cow antibiotic therapy requires alternatives to protect the mammary gland from new IMI at the time of dry-off.

Therefore, the objective of this study was to assess the efficacy of 1 i.m. injection of 5.6 mg of CAB at drying-off on ML reduction the day after the dry-off and new IMI across the dry period and postcalving period compared with a placebo (**PLA**, negative control) and an intramammary antibiotic treatment (**AB**, positive control) under field conditions.

MATERIALS AND METHODS

The study was a multicenter, double-blind clinical trial using a randomized 3-armed design with a negative and positive control group; it was conducted in accordance with EEC Directive 2004/28 and Good Clinical Practice (**GCP**) Guideline VICH GL9 (VICH, 2001).

Selection of Farms and Cows

Commercial dairy farms in France, Germany, and Hungary were selected on the basis of history of a high new IMI (at least 5%) across the dry period. The maximum number of enrolled animals was set at 90 cows per farm or 135 cows per site in cases where a site consisted of 2 or more farms.

Dairy cows were enrolled in the study if (1) they were at the end of lactation with a planned dry-off period of at least 5 wk, (2) they had a good overall health status (animals without evidence of apathy or signs of suffering from any infectious or metabolic diseases, (3) had no evidence of an IMI, (4) had an SCC of $\leq$200,000 cells/mL at all milk recording tests during the last 3 mo and immediately before dry-off, and (5) had an estimated daily milk production of at least 13 kg on the day before drying-off, recorded from in-line milk meters (when available on the farm) or manually measured by weighing the milk. This threshold for daily milk production ($\geq$13 kg/d) was chosen based on previous studies (Rajala-Schultz et al., 2005) and ensured a wide breed and a large milk production distribution among the included cows. Cows were excluded if (1) they had received systemic or intramammary antibiotics or anti-inflammatory treatment (local treatments, including intrauterine treatments, excepted) in the last 30 d before dry-off, (2) they were treated with a teat sealant, (3) they had clinical or sub-clinical mastitis (>200,000 cells/mL) during the last 3 mo before the dry-off or immediately before dry-off, or (4) they were infected with minor pathogens in more than one quarter or major pathogens in any quarter. Minor pathogens were considered CNS and *Corynebacteriun bovis*, and major

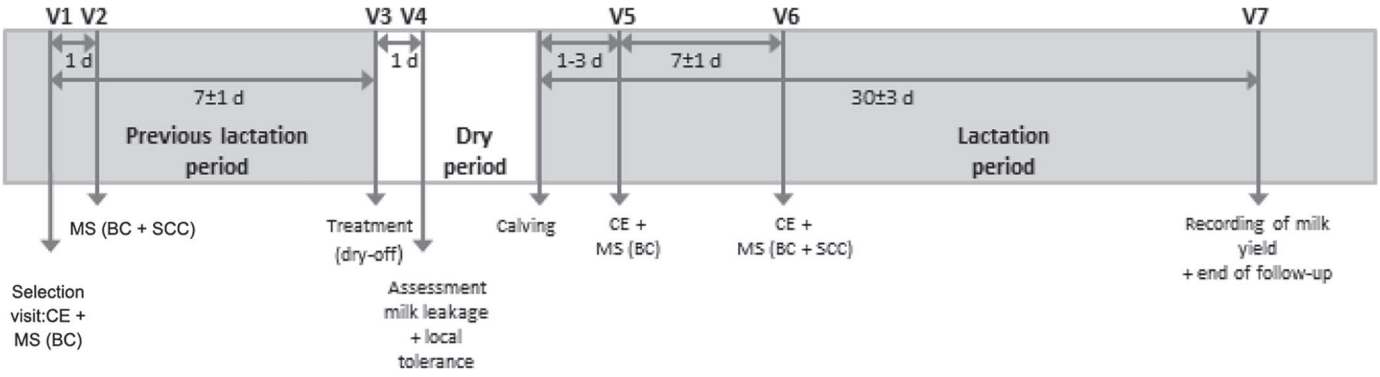

Figure 1. Study protocol with 7 farm visits (V1–V7) before and after drying-off, and after calving. CE = clinical examination; MS = milk sampling; BC = bacteriological culture.

pathogens were considered all other pathogens (i.e., *Staphylococcus aureus*, *Staphylococcus hyicus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*, *Enterococcus* spp., *Pseudomonas*, *Arcanobacterium* (*Actinomyces*) *pyogenes*, *Mycoplasma bovis*, *Mycoplasma agalactiae*, *Prototheca* spp., *Bacillus* spp., *Candida* species, *Escherichia coli*, and *Klebsiella* spp.).

Study Design and Quality Assurance

Sample size was calculated using the following assumptions: an assumed percentage of new IMI of 12% in the PLA group, a 1-sided significance level (α = 0.05) and 80% power to detect a 33% reduction in the relative risk of new IMI. The estimated sample size per treatment group was 932 quarters (233 cows), and considering a 20% loss due to (for example) sale, abortion, culling, and death, the number of necessary cases per treatment group was 280 cows.

Cows that qualified for the study were visited 7 times in total (**V1**–**V7**) before and after drying-off and after calving (Figure 1). All the visits except V3 were performed by an examining veterinarian. However, to keep the study blind, V3 was performed by a different person of the clinic staff (dispenser) who administered the drugs according to the random treatment allocation plan (**RTAP**). There were standard operating procedures for milk sampling, labeling, sending the samples to the laboratory, recording data, and treatment procedures. Following GCP guidelines, people involved from each investigational site were trained on the protocol, observational procedures, and standard operating procedures before starting the trial. During the selection visit (V1), 7 ± 1 d before dry-off, the cow, udder, and milk were clinically examined and milk samples were collected from each quarter and cultured. The next day (V2), a second milk sample from each quarter was collected for bacteriology to ensure reliable results. A pooled sample of the 4 quarters from a cow was performed in the laboratory to determine SCC. On the day of drying-off (V3), cows that met all inclusion criteria (including the microbiology data) were divided into 2 groups based on their daily milk yield (milk yield on the day before drying-off between ≥13 kg and ≤18 kg (group 1) or >18 kg (group 2) and then allocated to 1 of 3 treatments according to RTAP. The RTAP was prepared by a biostatistician and was followed in order of cow inclusion in the trial. Two randomization lists per farm were present due to the 2 milk yield groups, and randomization was performed in blocks of 3 due to 3 different treatments. If a full block of 3 cows was not allocated to treatment at one visit, the allocation recommenced at the next available random treatment at the next visit.

After the last milking before drying-off, cows received a single i.m. injection of 5.6 mg of CAB (Velactis, Ceva Sante Animale, Libourne, France) in a solution of 5 mL/cow in the neck using strict hygiene procedures, or a PLA with an equivalent volume of the same excipient used in the CAB treatment group in the neck using strict hygiene procedures, or an intramammary infusion (150 mg of cefquinome/quarter) of an AB (Cobactan DC, Virbac, Carros, France), following the guideline of the SPC. Milk leakage and possible local reactions at the injection site were assessed the next day (V4). To observe ML, cows that were located in the dry pen were put in a headlock and leakage observations lasted for at least 5 min. Cows were not allowed to be touched before or during the ML observation. Milk leakage was observed by looking at milk coming from the teats, milk on the lower part of the hind legs of the cow, or milk on the ground underneath the udder. Presence or absence of ML was recorded at cow level. Up to 72 h after calving (V5), the cow, udder, and milk were clinically examined and milk samples from each of the 4 quarters

were collected and cultured for microbial growth. Six to 8 d later (V6), the cow, udder, and milk were clinically examined again and a second milk sample from each quarter was collected and cultured for microbial growth and SCC. The end of the follow-up period was scheduled 30 (±3) d postcalving (V7), during which the daily milk yield on the day before V7 was recorded from in-line milk meters (if available on the farm) or manually measured by weighing the milk. Abnormal health statuses and concomitant treatments were recorded during the follow-up period. The enrollment phase lasted between June 2010 and September 2011.

Masking treatment was done in 2 ways. First, CAB and PLA were both provided in identical vials (product blinding) to be identified by a label code (A or B) and an individual number (4 digits). Second, masking was ensured by using different individuals, one acting as the examining veterinarian and another one only as the treatment dispenser. This ensured masking the particular intramammary dry-cow treatment. The dispenser randomly assigned the 3 treatments and treated animals in the absence of the examining veterinarian. Treatment details were not revealed to the examining veterinarian until the end of the animal phase of the study. The personnel at the laboratory performing bacteriological analyses or carrying out SCC tests were also masked to treatments.

All data collected on clinical forms, including laboratory results, were checked for correctness and completeness, and were entered into an Access database (Microsoft Office Access 2007, Microsoft Corp., Redmond, WA). The laboratory staff verified all raw data before entering into the database. The verification of data was done by using a double-data entry technique. Entry of the laboratory data was done via Excel spreadsheets. The data were supplied in electronic form for the statistical analyses. All observations and activities were recorded on case report forms or on provided study documents. The investigators completed the data for each animal included in the study on case report forms.

Microbiological investigations and SCC were carried out in accordance with the National Mastitis Council's *Laboratory Handbook on Bovine Mastitis* (NMC, 1999). Milk samples from each of the 4 quarters collected during V1 and V5 (for bacteriology analysis) were frozen immediately at ≤−18°C until shipment. Milk samples from each of the 4 quarters collected during V2 and V6 (for bacteriological and SCC analyses) were chilled immediately and transported to the laboratory the same day (preferably) or the day after. The V1 sample was shipped together with V2 sample and V5 sample together with the V6 sample. The date of receipt and the condition of the samples were recorded by the laboratory staff. Microbiological culture for udder pathogens was performed at the arrival to the laboratory for samples collected at V1, V2, V5, and V6. A sample was considered contaminated if 3 or more pathogens were cultured. The SCC was assessed based on pooled samples of the 4 quarter samples collected at V2 and V6.

Statistical Analysis

The efficacy of CAB relative to PLA (negative control) and an intramammary AB (positive control) was tested. For the analyses, the intention to treat (**ITT**) population was used. This population included all cows as originally allocated after randomization (Sargeant et al., 2010). Two mixed logistic regression models were fitted to the data to evaluate the efficacy of CAB in the prevention of ML and new IMI. The initial model for ML was defined as

$$\text{Logit (MilkLeak)} = \text{Treatment} + \text{Breed} + \text{Lactation} + \text{Milk18} + \text{Farm [random]} + \text{e}, \quad [1]$$

where MilkLeak was a binary variable indicating the presence or absence of ML at V4. Treatment was an indicator variable indicating CAB, PLA, or AB. Breed was a binary variable indicating Holstein Friesians or other breeds. Lactation was a categorical variable indicating first, second, or third and higher lactation number. Milk18 was a binary variable indicating milk production on the day before dry-off either above or below 18 kg. Farm was an indicator variable for farm of origin of the included cows and was treated as a random variable. Finally, e was a binomial error term. First-order interaction terms were evaluated in the model fitting process, but none of the interactions were significant. A P-value of 0.05 was used for decisions on significance.

New IMI were defined as the presence or absence of a new IMI in a quarter. Only quarters with no bacterial growth (V1 and V2) and a cow level SCC ≤200,000 cells/mL in the last 3 mo and at V2 were analyzed, thus excluding quarters infected with major or minor pathogens at V1 or V2. Treatment failure was defined as (1) IMI with a major pathogen present in any one of the 2 postcalving samples (V5 and V6), (2) a case of clinical mastitis up to V6 (including the day of V6), and (3) treatment with antibiotics or other supportive treatments associated with mastitis during the dry period or postcalving (up to V6). Bacteria were grouped into 2 main groups: environmental streptococci spp. (*Streptococcus uberis*, *Streptococcus dysgalactiae*, and enterococci) and coliforms (*Escherichia coli*, *Klebsiella, Enterobacter*, and others) to be presented descriptively.

If there was no information about the quarter(s) given, all quarters were considered as treatment failure according to the worst-case philosophy. Data collected from quarters that were treated with AB or anti-inflammatory drugs due to diseases other than mastitis or treated for mastitis in another quarter of the same cow between V1 up to V6 were excluded from the final analysis and coded as missing samples. The initial model for new IMI was defined as

$$\text{Logit (NewIMI)} = \text{Treatment} + \text{Breed} + \text{Lactation} + \text{Milk18} + \text{MilkLeak} + \text{Farm [random]} + \text{Re}, \quad [2]$$

where NewIMI was a binary variable indicating the presence or absence of a new IMI. Treatment, Breed, Lactation, Milk18, MilkLeak, and Farm [random] were as defined in model [1]. Re was a complex error term consisting of within-cow correlation (R) and a random binomial error term (e).

Model 2 was run first without and subsequently with MilkLeak as a covariate. Including MilkLeak in the model was done to identify whether ML is an intermediate variable in the causative pathway between treatment and the outcome of a new IMI. In both mixed logistic regression models, confounding and biologically relevant 2-way interactions were tested and included in the models where significant.

Milk production at approximately 30 d postcalving was evaluated using linear regression:

$$\text{Milk production (kg)} = \text{Treatment} + \text{Breed} + \text{Lactation} + \text{Milk18} + \text{Farm [random]} + \text{e}, \quad [3]$$

where the variables in this model were as defined above.

To test whether the 3 treatment groups were homogeneous, homogeneity analysis was performed. The 3 treatment groups were compared with regard to the individual selection and inclusion criteria (i.e., lactation rank, SCC at V2, daily milk production at drying-off, breed, interval between last recorded AI date and drying-off, duration of the dry period, number of DIM at dry-off, and number of mastitis cases during the last lactation). The multivariate Wilcoxon-Mann-Whitney test was used to check for homogeneity. This test calculates Mann-Whitney (**MW**) estimators per individual criterion. The benchmark for noninferiority was defined as MW measure = 0.36 as medium-sized inferiority, 0.44 as small inferiority, 0.50 as equality, 0.56 as small superiority, 0.64 as medium-sized (relevant) superiority, and 0.71 as large superiority based on Cohen (1969) and Colditz et al. (1988). Additionally, the Wilcoxon-Mann-Whitney test applies the Wei-Lachin procedure (Wei and Lachin, 1984; Thall and Lachin, 1988; Lachin, 1992) on all variables simultaneously. The baseline comparability was checked with this omnibus test. The analysis was performed with either Testimate Version 6.5 from IDV Gauting (validation of software, hardware, and user according to FDA 21 CFR Part 11) or with SAS version 9.3 (SAS Institute Inc., Cary, NC).

Table 1. Farms per country and characteristics of the farms (intention to treat population) involved in the study (all sites, n = 80)

Item	No. of farms/ country	No. of dairy cows/ farm (minimum/mean/ maximum or %)
Selected farms		
France	26	37/81.5/250
Germany	45	25/173.8/1,520
Hungary	9	370/759.6/1,870
Housing		
Loose housing	48	60
On pasture	16	20
Cubicles	14	17.5
Tiestalls	2	2.5
Feeding		
TMR	32	40
Silage (maize and grass)	48	60
Dry-off management		
Antibiotic use		
Blanket	65	81.25
SDCT[1]	11	13.75
No antibiotics	4	5
Postdipping disinfection		
Yes	60	75
No	20	25
Type of milking parlor		
Herringbone	45	56.25
Parallel	16	20
Tandem	5	6.25
Rotary	4	5
Robot	4	5
Other	6	7.5

[1]SDCT = selective dry cow therapy.

RESULTS

Selected Farms and Cows

Information about the 80 individual farms involved in the study are presented in Table 1.

On 17 of the 80 selected farms, no cows were eligible for inclusion in the study, particularly due to a high IMI prevalence. In total, 1,845 dairy cows of various breeds met the selection criteria, of which 917 were enrolled in the study. Due to severe protocol deviations (missing data due to sale of study animals, missing visits, or no milk samples collected), 17 cows were excluded from the ITT population (ITT population = 900 animals). Of these 17 animals, 6 were in CAB, 8 in PLA, and 3 in AB. About 70% of the ITT population was Holstein Friesian, 8.4% was Simmental, 8.1% was Montbéliard, 5.2% was Red Holstein, 5.1% was Angler, and the remaining animals were Abondance,

Table 2. Mean (SD) characteristics of 900 cows (intention to treat population) enrolled, treated with cabergoline (CAB), a placebo (PLA), or an antibiotic (AB)

Parameter	CAB (n = 298)	PLA (n = 298)	AB (n = 304)	Total (n = 900)
Lactation rank	1.8 (1.21)	1.8 (1.19)	1.7 (0.99)	1.76 (1.13)
Missing (no.)	0	0	0	0
SCC at visit 2 (geometric mean × 1,000 cells/mL)	84.6 (188.59)	71.5 (56.08)	76.0 (58.55)	77.4 (118.22)
Missing (no.)	2	2	1	5
Daily milk production (kg) at drying-off	18.37 (4.29)	18.77 (4.15)	18.47 (4.26)	18.54 (4.23)
Missing (no.)	0	0	0	0
Breed (no.)				
Holstein Friesian	209	210	208	627
Simmental	26	25	25	76
Montbéliard	23	21	29	73
Other	40	42	42	124
Interval between last recorded AI date and drying-off (d)	226.4 (12.50)	227.1 (12.74)	225.6 (16.86)	226.35 (14.18)
Missing (no.)	0	0	0	0
Actual duration of the dry period (d)	54.1 (13.15)	53.5 (12.36)	54.4 (12.42)	54.00 (12.64)
Missing (no.)	0	0	0	0
No. of DIM	326.5 (69.37)	318.4 (58.48)	329 (65.11)	324.76 (64.53)
Missing (no.)	0	0	0	0
No. of mastitis cases during the last lactation (mean)	0.1 (0.41)	0.1 (0.42)	0.1 (0.46)	0.1 (0.43)
Missing (no.)	0	0	0	0

Brown Swiss, Red Dual purpose, Normande, and others. About 56.8% of the ITT population was in the first lactation at enrollment, a quarter (24.9%) of the animals was in the second lactation, and 18.3% was in the third and higher lactation. Table 2 presents an overview of cow characteristics per treatment group. As the randomization was performed at farm level, the distribution of cows among treatment groups was balanced (CAB = 298 cows and 1,192 quarters, PLA = 298 cows and 1,192 quarters, and AB = 304 cows and 1,216 quarters). The 3 treatment groups were homogeneous (Wei-Lachin MW = 0.5 for CAB vs. PLA, CAB vs. AB, and PLA vs. AB) with regard to individual selection and inclusion criteria, except for the number of DIM. A small superiority (higher number of DIM) of CAB (326.5 DIM) and AB (329.2 DIM) to PLA (318.4 DIM) was shown (MW = 0.55 for CAB vs. PLA and AB vs. PLA).

ML and New IMI

The percentage of cows for which ML recorded on the day after dry-off (V4) was 2.0% (6/298), 10.7% (32/298), and 14.8% (45/304) in CAB, PLA, and AB, respectively. Logistic regression analysis on ML (Table 3), corrected for farm and milk production on the day before dry-off (Milk18), showed a significantly decreased odds of ML for cows treated with CAB (OR = 0.13; 95% CI = 0.05–0.34; $P < 0.0001$; PLA as baseline). Compared with the intramammary AB, cows treated with CAB also showed a significantly decreased odds of ML (OR = 0.08; 95% CI = 0.03–0.20; $P < 0.0001$). Compared with PLA, treatment with an intramammary AB was not significantly different with regard to ML ($P = 0.073$).

Cows with a daily milk yield of >18 kg on the day before dry-off showed an increased odds of ML (OR = 3.19; 95% CI = 1.77–5.75; $P = 0.0001$) compared with cows with a daily milk yield between ≥13 and ≤18 kg on the day before dry-off. The risk of ML did not differ significantly for the variables breed and lactation number.

In the ITT population, 46.9% (1,689/3,600) of the quarters was interpretable with regard to new IMI: quarters infected with minor pathogens at inclusion (1,103/3,600 quarters) and contaminated or missing (509 and 299/3,600 quarters, respectively) were excluded according to the GLP guidelines. The percentages of new IMI caused by major pathogens were 20.5% (113/551), 26.0% (147/566), and 14.3% (82/572)

Table 3. Results of logistic regression analysis of milk leakage[1]

Parameter	Coefficient (±SD)	*P*-value	OR (95% CI)
Intercept	−4.72 (0.59)		
Treatment[2]			
CAB	−2.02 (0.48)	<0.000	0.13 (0.05; 0.34)
PLA	Baseline		
AB	0.52 (0.29)	0.073	1.67 (0.95; 2.94)
Milk18[3]	1.16 (0.30)	0.000	3.19 (1.77; 5.75)
Milk production <18 kg	Baseline		

[1]The random effect of farm was estimated at 1.3 (SD 0.52).

[2]CAB = cabergoline; PLA = placebo (negative control); AB = intramammary antibiotic (positive control).

[3]Milk18 = milk production on the day before dry-off either above or below 18 kg.

in CAB, PLA, and AB, respectively. Thus, new IMI in CAB and AB were relatively reduced by 21 and 45%, respectively, compared with PLA. Logistic regression analysis on new IMI (Table 4), corrected for farm, within cow correlation, and lactation number, showed a decreased odds chance of new IMI for quarters treated with CAB (OR = 0.75; 95% CI = 0.56–0.99; P = 0.0407) or intramammary AB (OR = 0.47; 95% CI = 0.35–0.63; $P < 0.0001$; PLA as baseline). Adding ML to this model changed the originally significant association between quarters treated with CAB and new IMI into a nonsignificant one (OR = 0.78; 95% CI = 0.59–1.04; $P = 0.092$). Including ML also decreased the chance of new IMI for quarters treated with AB (OR = 0.45; 95% CI = 0.33–0.61; $P < 0.0001$). Quarters treated with AB had a decreased odds chance of new IMI compared with CAB (OR = 0.57; 95% CI = 0.41–0.79; $P = 0.0009$; variable ML included in the model; results not shown in Table 3). In the CAB, PLA, and AB groups, when a quarter had ML, IMI risk increased from 20.1 to 50.0%, from 25.5 to 30.4%, and from 14.1 to 15.5%, respectively. Logistic regression analysis showed a lower odds of new IMI for cows in the first (OR = 0.63; $P < 0.05$) or second (OR = 0.56; $P < 0.05$) lactation, compared with cows in the third or higher lactation. The number of new IMI quarters did not differ significantly for the variables breed and Milk18 and were, therefore, not included in the final models. No first-order interactions were significant in the regression models.

Figure 2 presents the percentage of new IMI at quarter level per major pathogen per treatment group. No differences were found at species level among treatments. Descriptive results about the species detected indicated that the percentage of environmental streptococci spp. (*Streptococcus uberis*, *Streptococcus dysgalactiae*, and enterococci) was lowest in the AB group, followed by the CAB and the PLA groups [4.7% (27/572), 8.9% (49/551), and 13.1% (74/566), respectively]. The same trend was observed for coliforms (*Escherichia coli*, *Klebsiella*, *Enterobacter*, and others): 0.5% (3/572), 2.0% (12/551), and 3.5% (20/566) in AB, CAB, and PLA, respectively. The rate of new IMI caused by fungus, yeast, *Prototheca*, and *Bacillus* spp. was lowest in CAB: 1.5% (8/551) compared with 3.0% (17/566) in PLA and 4.2% (24/572) in AB. In total, major pathogens were detected in 295 samples. This number is lower than the number of new IMI (342 quarters) as, in the event that the concerned quarter was not recorded, all 4 quarters of animals suffering from clinical mastitis were considered to be infected according to the worst-case philosophy. Additionally, in some of the mastitis milk samples, major pathogens were not detected during the laboratory analysis. Coagulase-negative staphylococci and *Corynebacterium bovis* were classified as minor pathogens and were, therefore, not included in the analysis of new IMI.

Linear regression analysis on milk yield recorded at 30 (±3) d after calving (V7) did not show a significant treatment effect (Table 5). The average daily milk production (kg) at V7 was 38.2 (±8.8), 38.4 (±8.2), and 38.3 (±8.5) in CAB, PLA, and AB, respectively. The geometric means of SCC (× 1,000 cells/mL) at V6 were 150.4 (±5.0), 163.6 (±5.7), and 113.4 (±4.4) in CAB, PLA and AB, respectively. A statistical difference was only found between AB and PLA ($P < 0.05$). During the follow-up period [from dry-off up to 30 (±3) d after calving], health events that were not the focus of this study happened in 20.39, 21.24, and 16.61% of cows in CAB, PLA, and AB, respectively, with no significant difference between the 3 treatment groups. The most important disease events were hypocalcemia, clinical mastitis, metritis, milk fever, displacement abomasum, and retained placenta. The main difference between the groups was a lower percent of clinical mastitis in the

Table 4. Results of logistic regression analysis of new IMI; the final models are shown with and without the variable milk leakage[1]

	Without milk leakage			With milk leakage		
Parameter	Coefficient (±SD)	P-value	OR (95% CI)	Coefficient (±SD)	P-value	OR (95% CI)
Intercept	−0.62 (0.18)			−0.63 (0.18)		
Treatment[2]						
CAB	−0.29 (0.14)	0.04	0.75 (0.56; 0.99)	−0.25 (0.15)	0.09	0.78 (0.59; 1.04)
PLA	Baseline			Baseline		
AB	−0.76 (0.15)	<0.00	0.47 (0.35; 0.63)	−0.80 (0.16)	<0.00	0.45 (0.33; 0.61)
Lactation						
1	−0.42 (0.17)	0.01	0.66 (0.47; 0.92)	−0.45 (0.17)	0.01	0.63 (0.45; 0.89)
2	−0.54 (0.19)	0.00	0.58 (0.4; 0.85)	−0.57 (0.19)	0.00	0.56 (0.39; 0.82)
≥3	Baseline			Baseline		
Milk leakage	Not included			0.54 (0.23)	0.02	1.71 (1.08; 2.71)

[1]Within cow, the correlation was 3 times larger compared with the variance component for farm effects, 0.97 (SD 0.034) and 0.32 (SD 0.17), respectively. OR = odds ratio.

[2]CAB = cabergoline; PLA = placebo (negative control); AB = intramammary antibiotic (positive control).

Table 5. Results of linear regression analysis of milk production recorded at 30 (±3) d postcalving (V7)[1]

Parameter	Coefficient (±SD)	*P*-value	Odds ratio (95% CI)
Intercept	32.0 (1.26)		
Treatment[2]			
CAB	−0.28 (0.62)	0.647	0.75 (0.22; 2.54)
PLA	Baseline		
AB	0.22 (0.61)	0.715	1.25 (0.38; 4.13)
Milk18[3]	1.27 (0.56)	0.024	3.56 (1.18; 10.78)
Milk production <18 kg	Baseline		
Breed			
Holstein Friesian	3.01 (1.03)	0.003	20.30 (2.72; 151.66)
Other breeds[4]	Baseline		
Lactation			
1	0.34 (0.73)	0.636	1.41 (0.34; 5.85)
2	2.07 (0.80)	0.009	7.96 (1.67; 38.04)
≥3	Baseline		

[1]The random effect for farm was estimated at 15.2 (SD 4.30).
[2]CAB = cabergoline; PLA = placebo (negative control); AB = intramammary antibiotic (positive control).
[3]Milk18 = milk production on the day before dry-off either above or below 18 kg.
[4]Non-Holstein Friesian.

AB group compared with PLA ($P < 0.05$) and CAB ($P = 0.06$) (mastitis/health events: 20/55, 34/73, and 31/71 in AB, PLA, and CAB, respectively).

DISCUSSION

This paper aimed to assess the efficacy of 1 i.m. injection of CAB at drying-off on the ML incidence one day after dry-off and new IMI across the dry period and postcalving compared with PLA and an intramammary AB under field conditions.

Across selected farms and countries, cows in the 3 treatment groups did not differ significantly in lactation rank, SCC at V2, daily milk production at drying-off, breed, interval between last AI date and drying-off, duration of the dry period, and number of mastitis cases during the last lactation. Although the numerical differences were small, cows in CAB and AB had a sta-

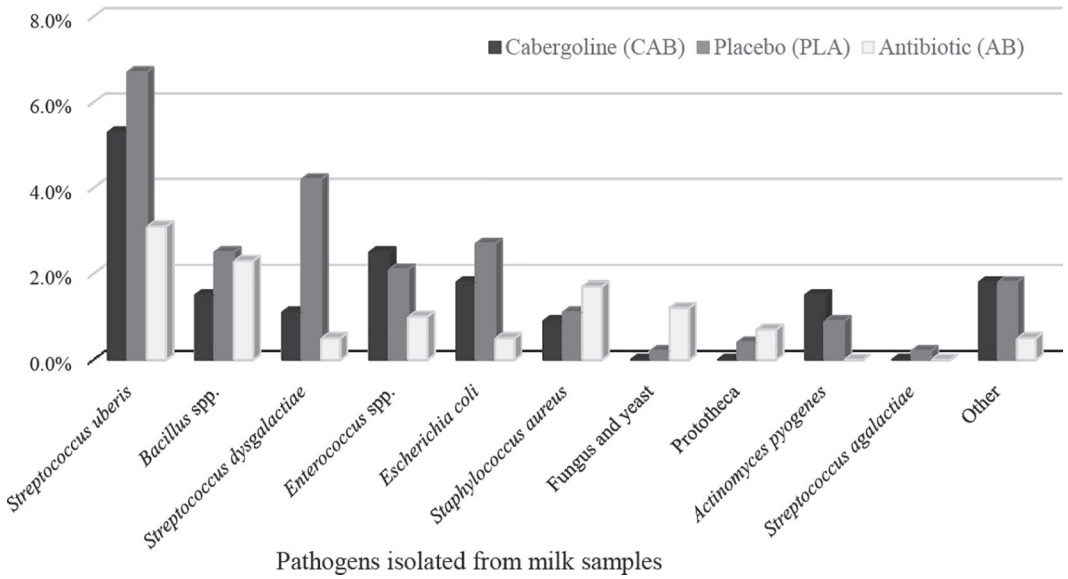

Figure 2. Percentage (%) of new IMI at quarter level per major pathogen per treatment group. The pathogen category "other" includes *Staphylococcus hyicus*, *Lactobacillus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella* spp., and other species not classified as minor. Coagulase-negative staphylococci and *Corynebacterium bovis* were classified as minor pathogens. Quarters infected with minor pathogens were not included in the analysis of new IMI.

tistically significant higher number of DIM compared with PLA. It is not likely that this difference had an effect on the key outcomes of ML and new IMI, especially since milk production was not different between the treatment groups.

Cows treated with CAB showed a significantly lower incidence of ML compared with PLA and the AB treatment. The incidence of ML was 2.0% in CAB-treated animals and 10.7 and 14.8% in PLA- and AB-treated cows, respectively. Similarly, the overall incidence of ML in CAB cows (3.1 ± 0.88%) was 73.5% of that obtained in PLA cows (11.7 ± 1.64%); and CAB cows had a 0.2 lower ($P < 0.001$) odds (95% CI = 0.09–0.46) to incur ML than PLA cows (Bach et al., 2015). In another study, the occurrence of ML was 21.0 and 11.3% ($P < 0.001$) in PLA and CAB groups, respectively (Bertulat et al., 2017).

The objective of the study was to detect a 33% reduction in the risk of new IMI between CAB and PLA, as we expected to detect at least a 4% point difference between PLA (12%) and CAB (8%). However, the percentage of new IMI caused by major pathogens was 20.5, 26.0, and 14.3% in CAB, PLA, and AB, respectively. Thus, we obtained a 5.5% point difference between PLA and CAB, was a reduction of 21% due to CAB. Data showed that a single injection of CAB at drying-off significantly decreased the odds of new IMI caused by major pathogens in the first weeks after calving (V5 and V6), even if the targeted number of included quarters was not fully reached due to missed or contaminated samples (which had no effect on this study). From the modeling results, it became clear that the effect of CAB appears to be acting via a reduction in ML (Table 4), although the odds ratios of CAB on IMI in both models are similar. Quarters that had ML had more infected quarters regardless of the treatment group. The difference was smaller in the AB group [ML no: 14.1% (67/475), ML yes: 15.5% (15/97)] as the effect of AB likely ameliorated the risk of acquiring new IMI. In the CAB-treated group, very few quarters had ML (2%), but when ML was observed, the risk of IMI increased [ML no: 20.1% (109/543), ML yes: 50% (4/8)]. Most likely these few remaining leaking quarters had a poor teat end closure, possibly due to a suboptimal sphincter canal or a teat condition. In the PLA group infected quarters were 25.5% (130/510) when no ML and 30.4% (17/56) when ML was present. At cow level, the effect of ML was present in the CAB, AB, and PLA groups in so far as IMI risk increased from 26.4 to 33.3%, from 16.2 to 20.0%, and from 29.3 to 38.6%, respectively. Thus, CAB reduced ML, this in turn was associated with an important reduction in IMI, with −7, −4, and −9% in the CAB, AB, and PLA groups, respectively. The models with and without ML indicated that ML is an intervening variable (i.e., CAB reduces the incidence of ML and, as a result, decreases the risk of new IMI). In addition to this, it has been proven that CAB accelerates involution and mammary gland remodeling in dairy cows after dry-off (Boutinaud et al., 2016, 2017) based on changes in the composition of mammary secretions after the abrupt cessation of milking. The CAB also increased and accelerated lactoferrin content in the mammary tissue and in mammary secretions (Boutinaud et al., 2016; 2017). This may enhance local mammary gland immunity against mastitis-causing pathogens, suggesting a better capacity to resist new IMI during mammary gland involution.

These results support the theoretically and experimentally expected efficacy of CAB in inhibiting PRL secretion as shown by Bach et al. (2015) and Boutinaud et al. (2017), where CAB cows had significantly lower PRL concentrations than PLA cows.

In the current study, the protective effect of PRL-release inhibition against new IMI is supported. The latter is in line with results reported for other dopamine agonists that suppress PRL release, such as quinagolide (Ollier et al., 2015). Studies on the less potent dopamine agonist bromocriptine only reported small or no reductions in milk yield (Knight, 1993; Akers, 2002). Although the inhibitory effect of CAB on PRL release and milk production was strong, no treatment effects were observed on milk yield recorded at 30 (±3) d after calving (V7). Bach et al. (2015) showed that serum PRL levels did not differ between the CAB and PLA groups in blood samples from cows 5 and 3 d before the expected calving date.

Cows with a daily milk yield of >18 kg on the day before dry-off showed a higher probability of ML compared with cows that produced between ≥13 and ≤18 kg. The number of new IMI quarters, however, did not differ significantly between these 2 milk yield groups. These findings contradict several studies that have reported an increased susceptibility to new IMI during the dry period when milk production at drying-off was higher (Dingwell et al., 2004; Rajala-Schultz et al., 2005; Newman et al., 2010). Compared with these published studies, the population that was studied in this efficacy trial differed as only known noninfected quarters were included in the analysis of new IMI. This may have biased the study population to animals with a relative high risk of new infections, unrelated to other known risk factors.

Not unexpectedly, intramammary AB use significantly decreased the odds of new IMI compared with both CAB and PLA. Across most bacteriological species, AB was most effective and PLA was least effective in preventing new IMI. Exceptions were new IMI caused

by fungus, yeast, *Prototheca*, and *Bacillus* spp., which were lowest in the CAB group and highest in the AB group. The increased incidence of IMI with these predominantly nonbacterial isolates may have resulted as a side effect of nonhygienic application of AB through intramammary infusion during mastitis treatments in lactation. The use of antimicrobials may affect the mammary gland microbiome allowing overgrowth of microorganisms present in the mammary gland. The AB treatment showed a numerically higher incidence of ML compared with the PLA treatment (14.8 vs. 10.7%), but this difference was not statistically significant ($P = 0.073$). We hypothesize that ML following intramammary AB treatment may result in a lower observed treatment efficacy due to a lower concentration of the molecule in the mammary gland. As a result, the observed treatment efficacy of intramammary AB may be somewhat lower than what might be expected from AB per se. Several countries are now prohibiting the preventive use of AB at dry-off in quarters that are not infected (Barkema et al., 2013), especially with third- and fourth-generation cephalosporins. Under these circumstances, CAB may be of particular value to reduce the risk of new IMI across the dry period.

The lower number of environmental streptococci spp. and coliforms in the CAB group compared with the PLA group was expected given the ML incidence results. After all, ML prevents or reduces the formation of a keratin plug in the teat canal (Dingwell et al., 2002; Odensten et al., 2007), which in turn facilitates the entry of microorganisms through the teat canal into the mammary gland (Oliver and Sordillo, 1989; Dingwell et al., 2004). These findings are in line with those by Schukken et al. (1990, 1991), who found that ML was associated with an increased incidence rate of clinical mastitis caused by *E. coli* and *S. aureus* in herds with low SCC. In addition, leaked milk also offers a favorable medium for bacterial growth when dripped on litter, contributing to an active multiplication of pathogens in the cow's immediate environment. This situation could result in increased concentrations of bacteria such as *E. coli* and *S. uberis*, which are particularly difficult to control (Zadoks et al., 2001). Since major pathogens are for obvious reasons the more pathogenic infections, the data presented regarding preventing new IMI after dry-off (Table 4) represent the key findings of this efficacy study.

CONCLUSIONS

This double-blind, randomized, 3-arm, multicenter, clinical trial, performed under GCP conditions on 63 farms in France, Germany, and Hungary, showed that 1 i.m. injection of CAB (Velactis, Ceva Sante Animale) at drying-off significantly reduced the risk of ML during the mammary involution period compared with PLA and AB treatments. The CAB significantly decreased the risk of new IMI across the dry period and postcalving. However, when ML was added to the model, the significance of CAB was reduced. We interpreted this to show that ML is an intervening variable between treatment and the lower risk of new IMI. The percentage of new IMI caused by major pathogens was 20.5% (113/551) in the CAB group and 26.0% (147/566) in the PLA group, a reduction of 21%. The AB treatment significantly decreased the odds of new IMI compared with both CAB and PLA. However, because several countries are now prohibiting the preventive use of antibiotics at dry-off in quarters that are not infected, the dry-off facilitator CAB may therefore be of particular value to reduce the risk of new IMI across the dry period.

ACKNOWLEDGMENTS

The authors thank the participating veterinarians and all the staff of the 63 dairy farms involved in the research. In particular, we acknowledge the staff of the Centre d'Elevage Lucien Biset, Poisy, France, and the Institute for Veterinary Medical Research, Budapest, Hungary, and its director Tibor Magyar. We extend particular thanks to Johan Küsters for study monitoring and all the staff of Milk Control Centre Flanders for milk analyses. This study was supported by CEVA Santé Animale (Libourne, France).

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