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Influence of an Intramammary Infusion at Drying-Off of Combined Penethamate Hydriodide, Benethamine Penicillin, and Framycetin Sulfate on Intramammary Infections and Somatic Cell Counts in Dairy Sheep

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ABSTRACT

The dynamics of intramammary infection (IMI) during the dry period were studied in 435 half-udders of 229 Assaf ewes, belonging to 2 flocks with high and medium IMI prevalences. Ewes were randomly assigned to 2 lots: 1) treated lot (TL) with 223 half-udders (118 ewes), which received complete dry therapy (1 syringe/teat) of an antibiotic combination containing 100 mg of penethamate hydriodide, 280 mg of benethamine penicillin, and 100 mg of framycetin sulfate, and 2) control lot (CL) with 212 nontreated half-udders (111 ewes). Two samplings per half-udder were carried out on 2 different days in the 5 d preceding drying-off, and 2 other samplings were again carried out in the 5 first d of the postpartum period. The length of the dry period averaged 109.0 d. Cure, persistent infections, reinfection, and new infection rates were 81.7, 12.8, 5.5, and 7.9%, respectively, for TL and 13.3, 70.4, 16.3, and 22.8%, respectively, for the CL. The prevalence of IMI decreased significantly from 48.9% at drying-off to 13.0% at lambing for the TL, but it did not vary for the CL (46.2 and 52.4%, respectively). Within the TL, IMI prevalence significantly diminished for *Staphylococcus* (41.3 to 9.9%) and *Streptococcus* (5.8 to 1.8%) genera, and more specifically this decrease was most evident for *Staphylococcus epidermidis* and *Streptococcus agalactiae* species. Log somatic cell count (SCC) diminished significantly between drying-off (5.68) and lambing (5.33) in the TL, whereas log SCC did not vary in the CL (5.61 vs. 5.66). This SCC reduction was very significant in the flock with the greater IMI prevalence. As a conclusion, the antibiotic formulation used as dry therapy drastically diminished IMI prevalence and SCC during the dry period in dairy ewes as a result of greater IMI cure rates and lower reinfection and new infection rates in the TL compared with the CL.

Key words: antibiotic treatment, dry therapy, mammary pathogen, somatic cell count

INTRODUCTION

Mastitis is the most costly disease in cattle dairy herds when adequate control procedures are not used (Natzke, 1981; Halasa et al., 2007). One of the most effective tools in mastitis control programs is antibiotic dry therapy (**DT**), the aim of which is to get as few infected quarters as possible at calving (Eberhart, 1986). Complete and selective DT have been assessed in dairy cows (Rindsig et al., 1978; Natzke, 1981), and their effect on IMI in that species is well known (Eberhart, 1986; Berry and Hillerton, 2002; Dingwell et al., 2002).

In dairy sheep, IMI caused by coagulase-negative staphylococci and other mammary pathogens elicit high SCC (Pengov, 2001; Ariznabarreta et al., 2002), cause damage to udder tissue (Burriel et al., 1997) and important losses of milk yield (Gonzalo et al., 1994, 2002, 2004), and persist in a high percentage of glands from one lactation to the next (Watson and Buswell, 1984; Marco, 1994). These facts, together with the high IMI prevalence in flocks in traditional milk-producing areas (González-Rodríguez et al., 1995; Gonzalo et al., 2002; Contreras et al., 2007), make DT necessary to control IMI in this species, although DT effects on IMI dynamics during the dry period and its relationship with SCC at lambing have not been studied extensively in dairy ewes (Gonzalo et al., 2004). Indeed, the key aspects to reducing IMI prevalence during the dry period are to ensure the IMI is cured, thus avoiding persistence into the next lactation, and to prevent new infections from occurring in half-udders that are bacteriologically negative at the time of drying-off.

In addition, very few intramammary formulations are registered for use in dairy sheep in the European Union (**EU**), and an attempt should be made to evaluate the effectiveness of other antibiotic combinations within the mastitis control programs in this species. Parenteral treatment with penethamate hydriodide has

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been shown to be effective against streptococcal infections during lactation in dairy cattle (Rose et al., 2003), and an intramammary antibiotic dry cow preparation containing procaine penicillin, penethamate hydriodide, and framycetin sulfate decreased gram-negative clinical mastitis and was suitable for maintenance of low-milk SCC in the subsequent lactation (Bradley and Green, 2001). A first experiment designed to study the depletion of antibiotic residues in the postpartum period using this last antibiotic combination as dry ewe therapy demonstrated that antibiotic residues were not detected ≥54 h postpartum (Linage and Gonzalo, 2006), but its effectiveness against IMI during the dry period is not known in dairy sheep.

The objective of the present study was to determine the effect of an antibiotic combination containing penethamate hydriodide, benethamine penicillin, and framycetin sulfate, used as DT, on IMI dynamics and SCC variation during the dry period in dairy sheep.

MATERIALS AND METHODS

Ewe Lots, Antibiotic Treatment, and Sampling

A total of 435 useful half-udders of 229 Assaf ewes belonging to 2 flocks with high (56.80%) and medium (28.37%) IMI prevalences were randomly assigned to 2 lots: 1) control lot (CL) with 212 half-udders (111 ewes) that received no treatment and 2) treated lot (TL) with 223 half-udders (118 ewes), which received a complete DT (1 syringe per teat) consisting of 100 mg of penethamate hydriodide, 280 mg of benethamine penicillin, and 100 mg of framycetin sulfate (Mamyzin Secado, Benestermycin, Boehringer Ingelheim Spain S.A., Barcelona). This intramammary formulation is licensed in many EU countries, in Mexico, and in New Zealand for the treatment and prevention of IMI at dry-off in dairy cows; it was used off-label in sheep following the recommended instructions for use in dairy cattle. The number of ewes per lot in the high- and medium-prevalence flocks were 77 and 41 for TL and 78 and 33 for CL, respectively. The total number of ewes in the high- and medium-prevalence flocks was approximately 300 and 150, respectively, and both herds were machine-milked. Half of the ewes from each flock were randomly sampled for this study (155 and 74 ewes from the high- and medium-prevalence flocks, respectively).

For the bacteriological study, teats were aseptically sampled twice, on different days, in the 5 d preceding abrupt drying-off of the ewes, the second sampling coinciding with the last milking of the lactation. Teats were carefully cleaned using cotton wool soaked in 96% ethanol. After the first streams of milk were discarded, 5 to 10 mL from each half-udder were collected in sterile containers. Samples were kept at 4°C until the bacteriological analysis, which was carried out immediately after arrival in the laboratory. After lambing, all half-udders were again sampled twice, on different days, in the first 5 d of the postpartum period, the first sampling being carrying out <72 h postlambing. The sampling procedure was the same as that described for drying-off. All samplings were performed immediately before the morning milking (0800 h). The average duration of the dry period was 109.0 ± 7.9 d. Ewes with clinical mastitis or another clinical disorder presenting in the 3 wk preceding drying-off and those that had received any antibiotic or antiinflammatory treatment in the 3 wk preceding drying-off were excluded from this study. Three animals with clinical disorders were excluded: 2 in CL and 1 in TL.

Bacteriological Procedures

An inoculum of 0.01 mL of each milk sample was plated onto 5% sheep blood agar (bioMérieux S.A., Marcy l'Etoile, France). Plates were incubated at 37°C and examined for bacterial growth at 24 and 48 h. Similarly to Contreras et al. (1997) and Gonzalo et al. (2002, 2004), half-udder IMI was defined as the growth of 3 or more identical colonies (≥300 cfu/mL). The growth of 2 different types of colonies with ≥300 cfu/mL per type was considered as a mixed culture. A sample was considered contaminated and rejected if 3 or more colony types were present on a plate. Bacteria were identified according to the recommendations of the National Mastitis Council (Harmon et al., 1990). Briefly, colonies were tentatively identified based on colony growth, morphology and appearance, pattern of hemolysis, and gram staining. Preliminary assays of catalase for the gram-positive organisms and of the oxidase (Becton, Dickinson and Company, Hunt Valley, MD) and fermentation pattern in triple sugar iron agar (Oxoid Ltd., Basingstoke, UK) for the gram-negative ones were carried out in all cases. Gram-positive, catalase-negative cocci were identified as belonging to the Streptococcaceae family and subjected to the CAMP (Christie-Atkins-Munch-Petersen) test and esculin hydrolysis. The CAMP-positive, esculin-negative strains were subjected to agglutination of latex particles sensitized by Lancefield group B specific rabbit immunoglobulins (Slidex Strepto B, bioMérieux S.A.); the positive strains were identified as *Streptococcus agalactiae*. The gram-positive, pleomorphic, and catalase-positive coccobacilli were classified within the *Corynebacterium* genus, whereas the catalase-negative ones were identified as *Arcanobacterium pyogenes*. Definitive identification for gram-positive and gram-negative (enteric/nonfermenter) organisms was carried

out using the BBL Crystal ID system (Becton, Dickinson and Company). Confirmation of *Staphylococcus aureus* was carried out with a rapid agglutination test with blue latex particles coated with porcine fibrinogen and rabbit IgG (Staphytect Plus, Oxoid Ltd.). The presence of *Mycoplasma agalactiae* was examined in the bulk tank milk samples and in half-udders with SCC $\geq 1{,}000 \times 10^3$ cells/mL, which did not present any bacterial growth following the procedure described by Gonzalo et al. (2002). A half-udder was considered infected at drying-off if the same pathogen was present in the 2 samples obtained before drying-off, and a half-udder was considered infected at parturition if the same pathogen was present in the 2 samples obtained immediately after lambing. Thirteen half-udders with inconsistent bacteriological results were excluded from this study, as well as 10 dry half-udders that could not be sampled. The final number of useful half-udders used was 435.

The dynamics of IMI during the dry period were studied by examining cure, persistent infection, cure-reinfection, and new infection rates. Cure rate was the percentage of half-udders infected at drying-off and bacteriologically negative at lambing. Persistent infection rate was the percentage of half-udders infected with the same pathogen at drying-off and at lambing. Mixed infections in which one of the organisms persisted until lambing were also considered as persistent infections. Cure-reinfection rate was the percentage of half-udders that were infected with a specific pathogen at drying-off but showed a different pathogen at lambing. New infection rate was the percentage of half-udders that were healthy at drying-off but infected at lambing.

SCC

After bacteriological analysis, SCC was determined for each milk sample using the Fossomatic method (Fossomatic 90, A/S N Foss Electric, Hillerød, Denmark). This was done 24 to 48 h after collection, as described by Gonzalo et al. (1993).

Statistical Analyses

Variation factors of the IMI dynamics and prevalence during the dry period were studied according to 2 categorical models, using the SAS CATMOD procedure (SAS Institute, 1998).

The categorical model used to study IMI dynamics throughout the dry period was:

$$Y \cong T + F + D + P,$$

where Y = the cure, persistent infection, cure-reinfection, and new infection rates; T = the treatment group (2 levels: CL and TL); F = the flock (2 levels: flock 1 and 2); D = the duration of the dry period (2 levels: ≤120 d and >120 d); and P = the parity (2 levels: 2nd parity and >2 parities). The interactions treatment group × flock, treatment group × dry period duration, and flock × dry period duration were also studied, but they were not statistically significant and were excluded in the final mathematical model.

The IMI prevalence was also studied with the SAS CATMOD procedure (SAS Institute, 1998), using the following categorical model:

$$Y \cong T + F + S + P + TF + TS + FS,$$

where Y = the IMI prevalence; T, F, and P = the same effects as in the above-mentioned model; S = the period of lactation (2 levels: drying-off and lambing); TF = the treatment group × flock interaction; TS = the treatment group × lactation period interaction; and FS = the flock × lactation period interaction.

Differences in infection rates and prevalence were tested with a χ^2 analysis, using the SAS FREQ procedure (SAS Institute, 1998). The unit of study for rates and global prevalence was the half-udder, but when organism prevalence and isolates were analyzed, the unit of study was the organism because of mixed cultures.

Somatic cell count variations were studied by mixed model using the SAS MIXED procedure (SAS Institute, 1998):

$$Y_{ijklm} = \mu + F_i + T_j + G_{k(ij)} + S_l + P_m + FT_{ij} + TS_{jl} + FS_{il} + FTS_{ijl} + e_{ijklm},$$

where Y_{ijklm} = log SCC; $G_{k(ij)}$ = the effect half-udder within flock × treatment group interaction; F_i, T_j, S_l, and P_m = the same effects as in the previous analyses; FT_{ij}, TS_{jl}, FS_{il}, and FTS_{ijl} = the flock × treatment, treatment × lactation period, flock × lactation period, and flock × treatment × lactation period interactions; and e = the residual effect. All effects were fixed, except $G_{k(ij)}$, which was random. In this procedure, random effect $G_{k(ij)}$ was absorbed in the analysis, and only the significance of fixed effect was shown. Least squares means and test of significance were obtained for the fixed effects of the mixed model.

RESULTS AND DISCUSSION

In the present study, 94.5% of infected half-udder observations were pure cultures, and 5.5% were mixed cultures. Mixed cultures were more frequent in the

Table 1. Statistical analysis and χ^2 statistical significance of cure, persistent infection, cure-reinfection, and new infection rates during the dry period for the factors of variation studied

Factors of variation	df	Cure[1]	Persistent infection[1]	Cure-reinfection[1]	New infection[1]
		χ^2			
Treatment	1	73.11***	58.52***	6.02**	8.13**
Flock	1	0.03^{NS}	1.54^{NS}	1.76^{NS}	0.40^{NS}
Dry period duration	1	0.46^{NS}	0.05^{NS}	0.26^{NS}	0.08^{NS}
Parity	1	2.84†	1.70^{NS}	0.03^{NS}	1.06^{NS}

[1]Total number of observations for cure, persistent infection, cure-reinfection, and new infection rates = 207, 207, 207, and 228.
^{NS}Nonsignificant; †$P < 0.10$; **$P < 0.01$; ***$P < 0.001$.

flock with high IMI prevalence (6.6%) compared with the flock with low prevalence (1.4%), in accordance with results described by other authors in dairy sheep (Marco, 1994; Ariznabarreta et al., 2002).

The ANOVA for categorical model used to study the variation of IMI dynamics during the dry period (Table 1) showed that only the treatment had a significant effect ($P < 0.01$ to $P < 0.001$) on all cure, persistent infection, cure-reinfection, and new infection rates. Other factors, such as dry period duration or flock effects, did not contribute significantly to variations in the studied rates.

Table 2 shows the values of the variables studied in the 2 treatment groups. Differences between lots were very important ($P < 0.001$) for cure rates: 81.7% in the TL vs. 13.3% in the CL. Conversely, persistent infections were greater in the CL compared with the TL (70.4 vs. 12.8%). Cure rates were similar to those obtained by other authors in dairy sheep using intramammary infusions containing cloxacillin and ampicillin (86.0%) (Marco, 1994), or penicillin and novobiocin (82.6 to 84.4%; Tardáguila, 1999), and greater than cure rates obtained by Chaffer et al. (2003) in Assaf ewes dry-treated with a combination of penicillin, nafcillin, and dihydrostreptomycin (64.9%). In the TL, bacteriological cure involved 89 half-udders infected at drying-off with *Staphylococcus epidermidis* (69.7% of total cured half-udders), *Strep. agalactiae* (10.1%), *Corynebacterium* spp. (5.6%), *Staphylococcus simulans* (4.5%), other CNS (5.5%), *Staph. aureus* (3.4%), *Arc. pyogenes* (2.2%), *Micrococcus kristinae* (1.1%), *Enterococcus faecalis* (1.1%), and *Pseudomonas* spp. (1.1%). These percentages total up to 104.3% due to the fact that some samples had 2 isolates (mixed infections). Auto-cure in the CL was only evidenced in 13 half-udders, 9 of which were infected at drying-off with *Staph. epidermidis*.

Reinfection and new infection rates were also lower ($P < 0.01$) in the TL (5.5 and 7.9%, respectively) vs. the CL (16.3 and 22.8%, respectively), which may indicate a preventive effect of DT against IMI during the dry period, particularly in dairy sheep flocks with medium-high IMI prevalences. This prophylactic use of DT to prevent new infections during dry period has also been described in dairy cattle (Dingwell et al., 2002). In a very extensive study in 24 dairy herds, these authors demonstrated that the probability of quarters developing new IMI during the dry period was greater in the cows that had longer dry periods. However, the dry period duration effect was not significant in our study, in which the 35 new IMI recorded (*Staph. epidermidis*: 22; *Staph. simulans*: 4; *Staph. aureus*: 3, of which 1 was clinical mastitis; *Streptococcus uberis*: 3; *Corynebacterium* spp.: 2; and *Aspergillus fumigatus*: 1) were mainly caused by contagious pathogens. New infection caused by *Asp. fumigatus* could be the result of iatrogenic

Table 2. Cure, persistent infection, cure-reinfection, and new infection rates in control and treated ewe lots, showing the statistical significance for the differences between lots

Rate (half-udder level)	Control lot	Treated lot	*P*-value
Cure (%)	13.27	81.65	<0.001
(n^1/N^2)	(13/98)	(89/109)	
Persistent infection (%)	70.41	12.84	<0.001
(n/N)	(69/98)	(14/109)	
Cure-reinfection (%)	16.33	5.50	<0.01
(n/N)	(16/98)	(6/109)	
New infection (%)	22.81	7.89	<0.01
(n/N)	(26/114)	(9/114)	

[1]Number of half-udders concerned in each rate.
[2]Total number of half-udders.

Table 3. Statistical analysis and χ^2 statistical significance of intramammary infection prevalence for the factors of variation studied[1]

Source of variation	df	χ^2
Treatment	1	30.14***
Flock	1	29.40***
Lactation period	1	16.34***
Parity	1	1.80^{NS}
Treatment × flock	1	0.60^{NS}
Treatment × lactation period	1	47.71***
Flock × lactation period	1	2.23^{NS}

[1]Total number of observations of categorical model = 870.
^{NS}Nonsignificant; ***$P < 0.001$.

contamination at the time of DT application (Pérez et al., 1998; Gonzalo et al., 2004), although the teats were disinfected with fast-acting iodine before syringe insertion, and syringes were under strict hygiene conditions during application. Syringes were not reinserted.

The causes of variation in IMI prevalence during the dry period are shown in Table 3. Antibiotic treatment, flock, lactation period, and treatment × lactation period were statistically significant effects. Similar IMI prevalences ($P > 0.05$) were found at drying-off both in the CL (46.2%) and the TL (48.9%). However, the IMI prevalences at lambing were very different ($P < 0.001$) between both lots: 52.4% for the CL and 13.0% for the TL after DT using the antibiotic combination studied. This reduction in IMI prevalence (75.2%) evidenced in the TL compared with the CL was slightly greater than that found with other antibiotic preparations such as penicillin-novobiocin (61.5 to 64.9%) or cloxacillin-ampicillin (69.5%) in dairy sheep (Marco, 1994; Tardáguila, 1999) and summarized the global effectiveness of the antibiotic DT used in this study. Although the IMI prevalences at drying-off were very different between the 2 flocks involved in the experiment (56.8 and 28.4%), the antibiotic treatment effect was similar in both flocks, because IMI prevalence was reduced significantly in both flocks over the studied time period (Table 4). Furthermore, there was a trend toward increased IMI prevalence in the CL of both flocks during the dry period (Table 4), probably due to an increase in new infections (i.e., *Staphylococcus* spp. other than *Staph. epidermidis*) as shown in Table 5, although these differences in prevalence were not statistically significant for the CL.

The greatest prevalences were observed for the *Staphylococcus* and *Streptococcus* genera (Table 5), according with the results of Gonzalo et al. (2002) and Contreras et al. (2007) in dairy sheep. For the TL, the prevalence of staphylococci drastically diminished ($P < 0.001$) between drying-off (41.3%) and lambing (9.9%), as did the prevalence of streptococci (5.8 to 1.8%, respectively, $P < 0.05$). More specifically, the decrease in prevalence during the dry period was very significant for *Staph. epidermidis* (34.1 to 7.6%) and *Strep. agalactiae* (5.8 to 0.9%). These results were consistent with those of a study reporting bacteriological cure in 59% of penethamate-treated quarters with *Strep. uberis* or *Streptococcus dysgalactiae* infection in dairy cattle during lactation (Rose et al., 2003). In the present study, the bacteriological cure for *Strep. agalactiae* during the dry period was 84.6% after antibiotic treatment. For the CL, however, variations in prevalence were not significant except for *Staphylococcus* spp. (other than

Table 4. Intramammary infection prevalence during the dry period for each flock, treatment lot, and for the total, indicating the χ^2 test and the statistical significance for the differences between drying-off and lambing

Treatment	Drying-off	Lambing	χ^2
Flock1			
Control lot	53.02	57.05	0.49^{NS}
(n^1/N^2)	(49/149)	(85/149)	
Treated lot	60.69	15.17	63.80***
(n/N)	(88/145)	(22/145)	
Flock 2			
Control lot	30.16	41.27	1.69^{NS}
(n/N)	(19/63)	(26/63)	
Treated lot	26.92	8.97	8.53**
(n/N)	(21/78)	(7/78)	
Total			
Control lot	46.23	52.36	1.59^{NS}
(n/N)	(98/212)	(111/212)	
Treated lot	48.88	13.00	67.16***
(n/N)	(109/223)	(29/223)	

[1]Number of half-udders infected.
[2]Total number of half-udders.
^{NS}Nonsignificant; **$P < 0.001$; ***$P < 0.001$.

Table 5. Number of isolates and prevalences of bacterial species and groups found in each treatment lot and lactation period

	Control lot					Treated lot				
	Drying-off		Lambing			Drying-off		Lambing		
Bacterial species and groups	N	%	N	%	χ^2	N	%	N	%	χ^2
Staphylococcus genus	80	37.73	90	42.45	0.98^{NS}	92	41.26	22	9.86	57.74***
Staphylococcus epidermidis	70	33.02	65	30.66	0.27^{NS}	76	34.08	17	7.62	47.29***
Other *Staphylococcus* spp.	10	4.72	25	11.79	7.01**	16	7.17	5	2.24	6.05*
Streptococcus genus	13	6.13	19	8.96	1.22^{NS}	13	5.83	4	1.79	4.95*
Streptococcus agalactiae	10	4.72	14	6.60	0.71^{NS}	13	5.83	2	0.90	8.35**
Other *Streptococcus* spp.	3	1.41	5	2.36	0.51^{NS}	0	0.00	2	0.90	2.01^{NS}
Other organisms	11	5.19	5	2.36	2.34^{NS}	11	4.93	6	2.69	1.53^{NS}
Total isolates	104	49.06	114	53.77	0.94^{NS}	116	52.01	32	14.35	71.35***
Noninfected half-udders	114	53.77	101	47.64	1.59^{NS}	114	51.12	194	87.00	67.16***

^{NS}Nonsignificant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Staph. epidermidis), which increased ($P < 0.01$) during the dry period (Table 5). Other organisms had very low prevalences. The prevalence of half-udders with clinical mastitis at lambing was also low (2 isolates in the CL and 0 in the TL) and corresponded to *Staph. aureus* isolates from the flock with the greater IMI prevalence. *Mycoplasma agalactiae* was not isolated from bulk tank milk or from half-udders.

Several studies have revealed the predictive value of the SCC and its efficacy in diagnosing infection in dairy ewes (González-Rodríguez et al., 1995; Gonzalo et al., 2002). Consequently, the SCC can be used as a suitable tool for monitoring mammary health and as part of mastitis control strategies in dairy sheep. A test of significance for all SCC variation factors studied is shown in Table 6. The half-udder was the most important factor influencing log SCC variation, because it accounted for 40.7% of total variance. Flock, treatment, lactation period, flock × treatment, lactation period × treatment, flock × lactation period, and flock × lactation period × treatment contributed significantly to the variation of log SCC. Thus, as shown in Table 7, log SCC decreased ($P < 0.001$) between drying-off (5.68) and lambing (5.33) for the TL but did not vary ($P > 0.05$) for the CL (5.61 vs. 5.66). Overall, these results showed an improvement in SCC during the beginning of the subsequent lactation in treated ewes, which concurs with IMI dynamics and the reduction in IMI prevalence found for the TL in this study. These results were consistent with lower bulk tank milk SCC found in dairy ewe flocks implementing dry-ewe therapy (Gonzalo et al., 2005). Nevertheless, SCC variation was not significant in the flock with the lowest IMI prevalence, where log SCC was similar at drying-off (5.26) and at lambing (5.23) in treated ewes (Table 7). This was probably due to a low IMI prevalence of major pathogens (3 isolates of *Staph. aureus* and 1 isolate of *E. faecalis* at drying-off and 1 isolate of *Staph. aureus* at lambing) eliciting high SCC in this flock. Thus, the prevalence decrease in treated ewes corresponded mainly to minor pathogens (23.1% at drying-off to 8.9% at lambing). The improvement in the hygienic quality of milk, particularly in the flock with a greater IMI prevalence, demonstrated improved mammary health in the TL and showed the particular importance of antibiotic DT in attaining the ewe milk quality standards for SCC demanded of producers by the milk industry.

Regarding antibiotic depletion in the postpartum period, the presence of residual antibiotics in 50 Assaf ewes (100 glands) dry-treated with the same antibiotic formulation (1 syringe/teat) was measured in colostrum and milk in the first 7 milkings postpartum previously to the present study (Linage and Gonzalo, 2006). The detection method was the Blue-Yellow screening test (Charm Sciences Inc., Lawrence, MA) recently adapted for ovine milk (Linage et al., 2007), the detection limits (3 to 4 μg/kg for penicillin and 704 to 781 μg/kg for framycetin) being lower than maximum residue limits established by EU in ovine milk (4 μg/kg and 1,500 μg/kg, respectively). Antibiotic residues were not detected

Table 6. Statistical analysis of log SCC for the factors of variation studied, indicating their test of significance[1]

Source of variation	df	*F*
Treatment	1	5.86**
Flock	1	134.96***
Lactation period	1	48.63***
Parity	1	1.91^{NS}
Flock × treatment	1	4.63*
Lactation period × treatment	1	48.22***
Flock × lactation period	1	58.35***
Flock × lactation period × treatment	1	22.58***

[1]Half-udder within flock × treatment: random factor absorbed in the model. Number of half-udders = 435. Number of observations = 1,740.

^{NS}Nonsignificant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 7. Least squares means of log SCC (LSM), standard errors (SE), and SCC geometric means ($\times 10^3$/mL; GM) in control and treated lots by lactation period, for each flock and for the total

	Control lot		Treated lot	
Flock	Drying-off	Lambing	Drying-off	Lambing
Flock 1				
LSM	6.05[a]	5.98[a]	6.07[a]	5.46[b]
SE	0.06	0.06	0.06	0.06
GM	1,122	955	1,174	288
Flock 2				
LSM	5.23[a]	5.29[a]	5.26[a]	5.23[a]
SE	0.08	0.08	0.08	0.08
GM	170	195	182	169
Total				
LSM	5.61[a]	5.66[a]	5.68[a]	5.33[b]
SE	0.06	0.06	0.06	0.06
GM	407	457	478	213

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

≥54 h postpartum; consequently, this delay may be considered as relevant to respect maximum residue limits as established by the EU in dairy ewes. Nevertheless, in the present experiment, the withdrawal time was 7 d as stated in the case of exceptional prescription according to Spanish rules (R.D. 109/1995, B.O.E. No. 53, 03/March/1995).

CONCLUSIONS

The original combination of penethamate hydriodide, benethamine penicillin and framycetin sulfate evaluated in this study as dry ewe therapy was very efficient in reducing IMI prevalence and improving milk SCC at lambing. These effects were related to greater IMI cure rate and lower reinfection and new infection rates during the dry period in treated ewes compared with nontreated ones.

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