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Prevalence of intramammary infections by major pathogens at parturition in dairy cows after intramuscular antibiotic therapy at drying-off: a preliminary report

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In dry cow therapy (DCT), antibiotics are usually injected direct into the mammary gland after the last milking of the lactation. Most products are designed to eliminate existing intramammary infections (IMI) caused by *Staphylococcus aureus* and *Streptococcus agalactiae* and to prevent the establishment of new IMI caused by major pathogens during the early non-lactating period (Nickerson et al. 1999). Systemic therapy has been attempted as a way of improving the cure rates of intramammary treatments of clinical and subclinical mastitis during lactation (Ziv, 1980a; Calvinho et al. 1988; Owens et al. 1988). For systemic therapy to be useful, effective passage of the drug from blood to the foci of infection must be achieved. Maintaining an effective antibacterial concentration in the udder depends on the physicochemical properties of the drug, the dose, the bioavailability of the injected formulation, and the sensitivity of the pathogen (Ziv et al. 1980a, b). The theoretical pharmacokinetic and pharmacodynamic basis for systemic DCT has been reported (Soback, 1988). When used in an attempt to improve cure rates of IMI, subcutaneous injection of tilmicosin at drying-off was ineffective against *Staph. aureus* IMI (Nickerson et al. 1999) and intramuscular (i.m.) oxytetracycline, in combination with intramammary cephapirin dry-cow treatment did not improve the cure rate for *Staph. aureus* mastitis (Soback et al. 1990a; Erskine et al. 1994). Nevertheless, i.m. tylosin treatment 2 weeks before the expected day of calving decreased IMI after calving (Zecconi et al. 1999) and subcutaneous norfloxacin nicotinate applied at the cessation of milking proved useful in controlling *Staph. aureus* infections (Soback et al. 1990a). Moreover, the incidence of this pathogen after systemic DCT may be significantly lower compared with untreated control groups (Soback et al. 1990a, b).

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Intramammary spiramycin and neomycin sulphate at drying-off significantly reduced the incidence of IMI by major mastitis pathogens during the dry period (Tarabla et al. 2000). Systemic spiramycin also has been administered to enhance the therapeutic success of intramammary infusions as a treatment for clinical mastitis (Calvinho et al. 1988). Spiramycin is a macrolide antibiotic that is active against Gram-positive bacteria. Its mean residence time after i.m. administration is significantly longer in milk than in plasma and its concentration up to 50-times higher in the mammary gland than in plasma (Ziv & Rasmusen, 1975; Franklin et al. 1986; Sanders et al. 1992). In lactating cows, it has a good distribution throughout the udder after i.m. administration and its minimum inhibitory concentrations in milk for sensitive isolates occurs at around 48 h (Ziv, 1980a). Efficacious treatment with bacteriostatic drugs requires the persistence of effective concentrations at the site of action, to allow the elimination of pathogens by tissue defence mechanisms. Mean residence time of spiramycin in milk after i.m. administration of a single dose of 9·37 mg/kg was 37·6 h. Mean times to maintain milk minimum inhibitory concentrations of 2·5, 1·25 and 0·625 ug/ml were 50·5, 61·4 and 86·5 h respectively (Sanders et al. 1992), reflecting the potential clinical efficacy of the drug (Ziv et al. 1974; Cester et al. 1990). Meanwhile, streptomycin is an aminoglycoside antibiotic that is active against Gram-negative pathogens. It is commonly used in conjunction with other drugs for mastitis therapy and its concentration in mastitic milk is high after i.m. administration (Ziv, 1980b). In lactating cows, effective free streptomycin concentrations for sensitive isolates after i.m. administration can be maintained from 12–24 h (Ziv, 1980a). The objective of this study was to determine the association between i.m. administration of a commercially available preparation containing spiramycin and streptomycin at cessation of lactation as DCT, and the prevalence of IMI with major pathogens after parturition.

Materials and Methods

Forty-four Holstein cows free of IMI with major pathogens, and from a single herd, were paired according to their lactation number and were randomly allocated to one of two experimental groups (G1 and G2). Sample size was restricted owing to previous bacteriological screening and matching. Cows in G1 were injected i.m. with a single dose of 2500 mg spiramycin adipate and 5000 mg streptomycin sulphate on the last day of lactation, while cows assigned to G2 remained untreated as control animals. Cows were kept on the same pasture with the same management throughout the trial period. Duplicate milk samples from each quarter, obtained 24–48 h apart, were aseptically collected within 7 d prior to drying-off and 7 d after parturition. Samples were transported to the laboratory on ice, refrigerated at 4 °C and cultured for bacterial isolation within 24 h of collection. Samples (0·01 ml) were plated on aesculin-blood agar plates and incubated at 37 °C for 24 h. Pathogens were identified according to standard procedures (Hogan et al. 1999).

A cow at drying-off was considered non-infected when no pathogens were isolated at any sampling. Conversely, at parturition, a cow was considered infected when the same pathogen was isolated from the same quarter at both samplings. Prevalences of cows having IMI with major pathogens at calving were compared by means of the McNemar test and Odds Ratio (OR). Confidence intervals (95% CI) were calculated for prevalences and OR.

Results and Discussion

The antibiotic preparation was tested as systemic DCT for various reasons: (a) the macrolide antibiotics such as spiramycin show a clear potential advantage over other antimicrobial drugs for the parenteral treatment of IMI due to Gram-positive bacteria (Ziv, 1980a), (b) spiramycin has proved to be effective *in vivo* for clinical mastitis therapy (Calvinho et al. 1988), (c) the relatively long pharmacokinetic half-life of spiramycin allows good maintenance of effective concentrations in mammary tissue (Sanders et al. 1992), (d) the combination is commercially available for both dry cow treatment and clinical mastitis therapy although its 4-d withdrawal period makes it inconvenient for use in lactating cows, (e) the cost per cow in Argentina can be less than for some intramammary preparations.

No cow from either group had any IMI at drying-off. The frequency of cows having IMI with major pathogens after parturition was associated with the application of systemic DCT ($P<0·05$). Cows from G1 showed less risk of acquiring IMI with major pathogens than did those from G2 (Table 1).

The only cow with IMI in G1 had two quarters infected with *Str. dysgalactiae*. Meanwhile, in G2, five cows had just one quarter infected with *Str. uberis* ($n=3$), *Str. dysgalactiae* ($n=1$) or *Staph. aureus* ($n=1$), one cow had two quarters infected with *Str. uberis* and one cow had two quarters infected with *Str. uberis* and one infected with *Staph. aureus*. Infections with minor pathogens were not considered in this exploratory trial. Since these bacteria may have a protective effect against subsequent infection with major pathogens (Lam et al. 1997), infection with minor pathogens should be taken into account in future trials.

Table 1. Prevalence of infections with major pathogens after parturition in Group 1 (spiramycin adipate and streptomycin sulphate) and Group 2 (untreated control)

	Intramammary infections				
	Yes	No	*n*	Prevalence % (95% CI)	Odds Ratio (95% CI)
Group 1	1†	21†	22	4·5 (0·24–24·9)	0·10 (0·01–0·92)
Group 2	7	15	22	31·8 (14·7–54·9)	Not applicable

† Significant association between treatment at drying off and intramammary infections at parturition ($P<0·05$)

At drying-off, culture results were interpreted in parallel (i.e. if a pathogen was isolated from any quarter at any sampling the cow was considered infected) to enhance sensitivity and negative predictive value in order to minimize false negative results. Conversely, at parturition, results were interpreted in series (i.e. if a pathogen was isolated from the same quarter at both samplings the cow was considered infected) to enhance specificity and positive predictive value, minimizing false positive results (Galen & Gambino, 1975). The experimental unit was the cow because it comprised the smallest grouping that could receive a treatment and provided one observation in the statistical analysis (Thorburn, 1990). Moreover, the most practical figure for decision making is the prevalence of infected cows and not that of infected quarters (Erskine, 1994). The odds ratio of 0·10 (0·01–0·92) showed that, in this trial, cows that had received i.m. spiramycin adipate and streptomycin sulphate had approximately 10-times less risk of getting IMI than untreated cows. Although prevalence of IMI with major pathogens after parturition and systemic antibiotic treatment were not independent events ($P<0·05$), 95% CI of the prevalences did overlap. A larger sample size may be needed to narrow the CI. Clinical trials involving systemic DCT have so far reported inconsistent results (Soback et al. 1990a, b; Erskine et al. 1994; Nickerson et al. 1999; Zecconi et al. 1999). Our results, however, agree with previous reports using a macrolide antibiotic, tylosin, 2 weeks before the expected day of calving (Zecconi et al. 1999). Not surprisingly, *Str. uberis* was the most prevalent pathogen at parturition. Incidence of *Str. uberis* IMI is very high in the last 2 weeks of the dry period (Oliver & Sordillo, 1989), when it was unlikely that the i.m. treatment could still prevent new IMI (Ziv, 1980b). However, not even intramammary DCT in all quarters eliminates the high incidence of IMI by environmental streptococci in the 2 weeks prior to calving (Smith & Hogan, 1998).

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Systemic administration could simplify DCT routine. It would also eliminate the risk of introducing infections through non-sterile intramammary injection (Soback, 1988) and the risk of reducing the natural resistance to infection owing to removal of the protective keratin layer after complete insertion of the cannula into the teat canal (Boddie & Nickerson, 1986). Effective DCT, however, is the result of a favourable resolution of several factors, such as the prevalence and aetiology of IMI present at drying-off and the pharmacokinetics of the antimicrobial drug. In this trial, i.m. administration of spiramycin adipate and streptomycin sulphate was associated with reduced prevalence of IMI after calving. However, these kinds of trials should be replicated elsewhere to ensure consistency. Moreover, more research is needed to address the subjects of pharmacokinetics in the dry period and efficacy of treatment of existing IMI at drying-off.

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