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Short communication: Drug residues in goat milk after prophylactic use of antibiotics in intravaginal sponges for estrus synchronization

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ABSTRACT

The aim of this study was to determine whether the prophylactic use of antibiotics in intravaginal sponges used for estrus synchronization in goats may result in the presence of inhibitors in milk and, therefore, of positive results by microbial screening tests. Ninety-eight Murciano-Granadina goats were used, divided into 7 groups of 14 animals. Intravaginal sponges were placed in 6 groups using 2 concentrations of 3 different antibiotics: doxycycline, oxytetracycline, and sulfathiazole-framycetin. The sponges of the control group were placed without antibiotics. Milk samples were collected daily until 7 d posttreatment and analyzed using 3 microbial tests. Positive samples were retested by specific receptor-binding assays to confirm the positive results. Vaginal status was evaluated by visual assessment of the external aspect of the sponges after removal. The microbial test response was not affected by either day posttreatment or dose of antibiotic used, except for oxytetracycline at the higher concentration. Moreover, no positive results were obtained using receptor-binding assays, suggesting that residues, if present in milk, did not exceed the regulatory (safety) levels established for these drugs. The occurrence of soiled sponges was higher in the control group. With respect to the dose of antibiotics used, no significant differences were found for the lower dose administered. However, a significant increase in the percentage of clean sponges was observed for the higher dose of doxycycline. We conclude that the prophylactic use of low doses of doxycycline, oxytetracycline, or sulfathiazole in intravaginal sponges used for synchronization of estrus helps to reduce clinical vaginitis in dairy goats and does not seem to be the cause of positive results in microbial inhibitor tests used to detect antibiotics in goat milk.

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Short Communication

Intravaginal sponges impregnated with progesterone or synthetic progestogens during 6 to 16 d before AI are typically used for estrus synchronization in goats (Menchaca and Rubianes, 2004; Rowe et al., 2009). However, this practice is related to the occurrence of clinical vaginitis in goats (Motlomelo et al., 2002; Penna et al., 2013), which could negatively affect fertility rates on farms (Scudamore, 1988).

To avoid these negative effects, some authors recommend sprinkling the sponges with antibiotics before their insertion in the vagina (Guerra et al., 2002; Suárez et al., 2006; Gatti et al., 2011), with oxytetracycline being the drug commonly used for this purpose in practice (Manes et al., 2013). Such prophylactic use of antibiotics in the placement of intravaginal sponges is an unregulated management practice for which there are no approved recommendations with regard to the dose and withdrawal period required to avoid the presence of drug residues in milk. In goats, estrus synchronization using intravaginal sponges takes place during lactation and, therefore, the inclusion of antibiotics on sponges may pose a risk of contamination of the milk. Some researchers have found antibiotic residues in cow milk 24 to 48 h after intrauterine administration of suppositories, infusions, and tablets of penicillin, streptomycin, and tetracycline (Miller and Bergt, 1976; Black et al., 1979; Bishop et al., 1984). In ewes, drug residues in milk after the inclusion of intravaginal sponges impregnated with benzylpenicillin procaine were evaluated by Berruga et al. (2008) by using different microbial inhibitor tests for screening antibiotics. Positive and inconclusive results were obtained at the time of the first milking, and occasionally later, due to the presence of drug residues in milk. In dairy goats, related information is rather limited.

Microbial inhibitor tests are widely used for screening for antibiotics in milk above maximum residue

limits (**MRL**) established by legislation (European Union, 2010). The performance of these screening tests (specificity and detection capability) allows the detection of a large number of substances in milk at or below the MRL to guarantee the safety of milk and related products.

Thus, the aim of this study was to evaluate whether the prophylactic use of antibiotics on intravaginal sponges used in the synchronization of estrus in goats may explain the presence of inhibitors in milk and, therefore, of positive results in the microbial screening tests.

Experimental animal procedures were approved by the Ethics Committee of Universitat Politècnica de València (UPV, Valencia, Spain). Ninety-eight Murciano-Granadina goats in the fourth month of lactation, from the herd of Diputación de Castellón de la Plana (Ares del Maestrat, Spain) were used. The animals were healthy and did not receive any drug treatment before the experiment.

Goats were randomly divided into 7 groups of 14 animals each. A polyurethane sponge containing 30 mg of flugestone acetate (Sincropart, Ceva Salud Animal, Barcelona, Spain) was inserted into the vagina for an 11-d period. Before insertion, the sponges were impregnated with 2 concentrations of 3 veterinary drugs usually applied in dairy goats: doxycycline (Hipradoxi, Laboratorios Hipra, S.A., Gerona, Spain), oxytetracycline (Terramycin, Pfizer S.L.U., Madrid, Spain), and sulfathiazole-framycetin (96%/4%; Framicas, Laboratorios Ovejero S.A., León, Spain). Consistent with veterinary practice, antibiotics were added into a bag containing 25 sponges and mixed by shaking. In the control group, the sponges did not contain any antibiotic. Two doses of antibiotics were used: dose 1 was equivalent to 1 g of commercial product corresponding to a concentration of 100 mg (active agent) of doxycycline, 550 mg of oxytetracycline, or 960 mg of sulfathiazole; dose 2 was equivalent to 2 g (i.e., twice the amount of active compound of dose 1).

According to the estrus synchronization protocol of hormonal treatment, on d 9 after the placement of the intravaginal sponge, an i.m. administration of 300 IU of equine chorionic gonadotropin (Sincropart PMSG, Ceva Salud Animal, Barcelona, Spain) and 0.5 mL of Enzaprost (synthetic analog of $PGF_{2\alpha}$, Ceva Salud Animal) was applied. Vaginal status was evaluated through visual assessment of the sponge's external aspect after removal. Sponges were classified as follows: 0 = clean sponge (without bloody, purulent or foul-smelling discharges), 1 = soiled sponge (presence of bloody secretions and vaginal mucus but no abnormal odor), and 2 = very soiled sponge (presence of bloody secretions, high quantity of vaginal mucus, and foul odor).

The animals were milked once a day (0700 h) in a milking parlor. For 7 d after sponge insertion, individual goat milk samples (100 mL) were collected and transported under refrigeration (<10°C) to the laboratories of the Universitat Politècnica de València (Valencia, Spain).

Milk samples were analyzed in triplicate by using 3 microbial inhibitor tests: BRT MRL (AiM Analytik in MilchProduktions-und Vertriebs-GmbH, Munich, Germany), Delvotest SP-NT MCS (DSM Food Specialties, Delft, the Netherlands), and Eclipse 100 (Zeulab S.L., Zaragoza, Spain) according to each manufacturer's instructions. In all cases, negative (antimicrobial-free milk) and positive (milk spiked with 4 μg/kg of benzylpenicillin) controls were included in each test. Interpretation of the test results was carried out independently by 3 trained technicians by visually assessing the color change after incubation and classifying milk samples as positive (blue) or negative (yellow). Positive samples were retested by specific receptor-binding assays for tetracycline (SNAP Tetracycline test, Idexx Laboratories, Westbrook, ME) and for sulfonamides (Sulfasensor test, Unisensor, Liege, Belgium) following each manufacturer's instructions. The test results were classified as positive or negative by instrumental readers (SNAP shot reader, Idexx Laboratories and Readsensor, Unisensor).

The detection limits (**DL**) of microbial inhibitor tests for doxycycline, oxytetracycline, and sulfathiazole were calculated according to the International Dairy Federation recommendations (IDF, 2003) and are summarized in Table 1. The DL of the receptor-binding assays provided by the manufacturers were also included (Table1).

A logistic regression model was applied to evaluate both the effect of the dose of antibiotic administered and the days after treatment on the occurrence of positive outcomes in the microbial screening tests:

$$L_{ijk} = \text{Logit}\,[P_{ijk}] + \beta_0 + \beta_1\,[PD]_i + \beta_2\,D1 + \beta_3\,D2 + \varepsilon_{ijk},$$

where L_{ijk} = Logit model; $[P_{ijk}]$ = probability for the response category (positive or negative); β_0 = intercept; β_1, β_2, β_3 = parameters estimated for the model; $[PD]_i$ = effect of the days posttreatment (n = 7); D1 and D2 = effect of dose 1 and dose 2, respectively, in dummy variable (without antibiotic: D1 = 0 and D2 = 0; dose 1: D1 = 1 and D2 = 0; dose 2: D1 = 0 and D2 = 1); and ε_{ijk} = residual error.

To evaluate the status of the sponges as an indicator of the antibiotic's effectiveness to prevent clinical vaginitis, the χ^2 test was applied. Statistical analyses